

**PROCESS STANDARDIZATION, CHARACTERIZATION AND
SHELF-LIFE ANALYSIS OF READY-TO-EAT PRODUCTS
MADE FROM PIGMENTED *BAO* RICE OF ASSAM**

**A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

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MADE FROM PIGMENTED *BAO* RICE OF ASSAM**

**BY
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Submitted

**In partial fulfilment of the Degree of Doctor of Philosophy in Food
Technology of Mizoram University, Aizawl**



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This is to certify that the thesis entitled “PROCESS STANDARDIZATION, CHARACTERIZATION AND SHELF-LIFE ANALYSIS OF READY-TO-EAT PRODUCTS MADE FROM PIGMENTED *BAO* RICE OF ASSAM” submitted to Mizoram University for the award of the degree of **Doctor of Philosophy in Food Technology** by **Gunjana Deka**, Ph.D. Registration No. MZU/Ph.D./1797 of 06.09.2021. She is a Ph.D. scholar in the Department of Food Technology, under my guidance and supervision. The thesis or any of its part has not been previously submitted for the award of any degree in any Indian or foreign University. She has fulfilled all criteria prescribed by the UGC (Minimum Standard and Procedure governing Ph.D. Regulations). She has fulfilled the mandatory publications (Publications enclosed) and completed Ph.D. coursework. It is also certified that the scholar was admitted to the Department through an entrance test, followed by an interview as per the UGC Regulations of 2018.

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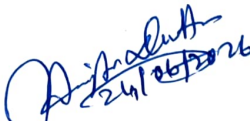
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I **GUNJANA DEKA**, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to do the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

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List of abbreviations

AAC	Apparent amylose content
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
ACE	Angiotensin converting enzyme
ALOX5	Arachidonate-5-lipoxygenase
ANOVA	Analysis of variance
ANS	Anthocyanidin synthase
AOAC	Association of Official Analytical Chemists
Apo	Apolipoprotein
BDV	Breakdown viscosity
bHLH	Basic-helix-loop-helix
BMI	Body mass index
BRAE	Black rice anthocyanin extract
CCFC	Central composite face centered
CF	Crosslinked flour
CFR	Code of Federal Regulations
CFU	Colony forming unit
CIE	Commission internationale de l'éclairage
DEC	Decelerator of internode elongation
DFR	Dihydroflavonol 4-reductase
DPPH	2,2-diphenyl-1-picrylhydrazyl
DRSA	Dpph radical scavenging activity
DSC	Differential scanning calorimetry
DUS	Distinctness, uniformity and stability
F3H	Flavanone 3-hydroxylase
FAE	Ferulic acid equivalent
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization corporate Statistical database
FCR	Folin-ciocalteu reagent
FFA	Free fatty acid
FRAP	Ferric reducing antioxidant power/potential
FSSAI	Food Safety and Standards Authority of India
FTIR	Fourier transform infrared (spectroscopy)
FV	Final viscosity
GABA	Gamma aminobutyric acid
GAE	Gallic acid equivalents

GF	Gluten free
GFAT	Glutamine fructose-6-phosphate amidotransferase
GI	Glycemic index
GLUT	Glucose transporter
GWAS	Genome-wide association studies
H-B	Herschel Bulkley
HDL	High density lipoprotein
HTST	High temperature-short time
IDE	Insulin-degrading enzyme
IGF	Insulin like growthfactor
IRS	Insulin receptor substrate
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LAR	(Leucoanthocyanidin reductase)
LDL	Low density lipoprotein
LLDPE	Linear low density polyethylene
MYB	Myeloblastosis
NC	Negative control
OAA	Overall acceptability
OSA	Octenyl succinic anhydride
PC	Positive control
PCA	Principal component analysis
pH	Potential of hydrogen
PP	Polypropylene
PT	Pasting temperature
PV	Peroxide value
PV	Peak viscosity
QE	Quercetin equivalents
QTL	Quantitative trait loci
RDS	Rapidly digestible starch
RE	Rutin equivalent
RH	Relative humidity
RPC	Residual phosphorus content
RPM	Revolutions per minute
RS	Resistant starch
RSM	Response surface methodology
RTC	Ready-to-cook
RTE	Ready-to-eat
RTH	Ready-to-heat

RTP	Ready-to-prepare
RTS	Ready-to-serve
SBV	Setback viscosity
SCFA	Short chain fatty acid
SD	Semidwarf
SDS	Slowly digestible starch
SEM	Scanning electron microscopy
SHMP	Sodium hexametaphosphate
SMP	Skimmed milk powder
SP	Swelling power
STMP	Sodium trimetaphosphate
STPP	Sodium tripolyphosphate
TAPC	Total aerobic plate count
TBARS	Thiobarbituric acid reactive substances
TE	Trolox equivalent
TFC	Total flavonoid content
TPA	Texture profile analysis
TPC	Total polyphenolic content
TV	Trough viscosity
UDP	Uridine diphosphate
UGT	UDP-glucosyl transferase
UV	Ultraviolet
UV-Vis	UV-Visible
WAI	Water absorption index
WHO	World health organization
WSI	Water solubility index
XRD	X-ray diffractometry
YMC	Yeast and mold count

Chapter 1

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops globally. Rice serves as a staple food for more than half of the world's population, contributing significantly to global food security. Recent estimates indicate that global rice production reached approximately 532.87 million metric tonnes during 2024-2025, with cultivation extending across more than 120 countries, predominantly within tropical and subtropical regions (FAOSTAT, 2025; Evangelista and Schönlechner, 2025). Asia remains the dominant producer and consumer, accounting for a substantial proportion of global output and consumption, where rice contributes nearly 60-70% of the daily caloric intake for more than 2 billion people (Tiozon et al., 2023; Mbanjo et al., 2020). On a global scale, rice provides more than one-fifth of the total caloric intake, underscoring its central role in human nutrition and dietary patterns (Evangelista and Schönlechner, 2025).

The origin and domestication of rice provide important insight into the evolution of pigmented rice varieties. Archaeological findings and genomic analyses indicate that rice was first domesticated about 9,000 years ago in the Yangtze River basin of China, followed by its spread across Asia through migration and trade (Sweeney and McCouch, 2007). Parallel domestication in the Indian subcontinent has also been suggested. Over time, rice diversified into major subspecies such as *indica* and *japonica* under the influence of ecological and climatic adaptation (Evangelista and Schönlechner, 2025). Taxonomically, rice belongs to Kingdom *Plantae*, Division *Magnoliophyta*, Class *Liliopsida*, Order *Poales*, Family *Poaceae*, Genus *Oryza* and Species *sativa*.

Within this evolutionary framework, pigmented rice emerged as distinct landraces due to the accumulation of flavonoid pigments in the bran layer, resulting in red, purple and black grain types. Pigmented rice does not represent a separate taxonomic category but comprises genetically diverse varieties within *Oryza sativa*, characterized by colored pericarp and aleurone layers (Punia and Kumar, 2022). From a historical perspective,

pigmented rice held cultural and medicinal importance. Ancient Indian texts and Ayurvedic literature recognized red rice for its nutritional and therapeutic value. Black rice was reserved for royalty in China (Ahuja et al., 2007; Sweeney and McCouch, 2007). However, the Green Revolution led to the dominance of high-yielding white rice varieties, resulting in a decline in pigmented rice cultivation and utilization.

Modern rice utilization is predominantly centered on polished white rice. The preference for polished rice is largely driven by its desirable sensory attributes, including softer texture, improved palatability, reduced cooking time and longer shelf life. However, the milling process that produces polished rice involves removal of its bran and aleurone layers, which are rich in essential nutrients and bioactive compounds. This results in significant losses of vitamins, minerals, dietary fiber, lipids and phytochemicals, thereby reducing the nutritional density and functional value of the grain (Reddy et al., 2017). Consequently, although rice is a major energy source, its contribution to micronutrient intake is relatively limited in its polished form, raising concerns regarding nutritional adequacy and hidden hunger in rice-dependent populations.

In contrast to polished white rice, pigmented rice varieties represent a nutritionally superior alternative. Pigmented rice, including red, black and purple rice types, constitutes a minor fraction of global rice production, accounting for less than 1% of total rice output (Mbanjo et al., 2020; Tiozon et al., 2023). These varieties are characterized by the accumulation of bioactive compounds in the pericarp and aleurone layers, contributing to enhanced antioxidant activity, improved nutritional quality and potential health benefits (Evangelista and Schönlechner, 2025). The distinct functional properties of pigmented rices are governed by their pigment composition and genetic regulation. Red rice primarily contains proanthocyanidins which are polymeric and structurally stable compounds that interact strongly with starch and digestive enzymes (Sudan et al., 2023; Zhang et al., 2023). In contrast, black and purple rice are rich in anthocyanins such as cyanidin-3-glucoside which exhibit strong antioxidant activity but are sensitive to processing conditions (Mbanjo et al., 2020). These pigments are synthesized through the

phenylpropanoid pathway where metabolic regulation determines pigment type (Lang et al., 2025; Mackon et al., 2021). Genetic control further differentiates these varieties with *Rc* gene regulating red pigmentation and *Kala* gene complexes controlling anthocyanin accumulation in black rice (Mbanjo et al., 2020; Sudan et al., 2023).

In addition to pigment composition, pigmented rice is particularly valued for its diverse bioactive composition of phenolic acids, flavonoids, anthocyanins, proanthocyanidins, γ -oryzanols, tocopherols, carotenoids and essential minerals concentrated in the bran layer (Evangelista and Schönlechner, 2025; Mbanjo et al., 2020). Phenolic acids such as ferulic, p-coumaric and caffeic acid exist predominantly in bound forms linked to cell wall components, which influence their bioaccessibility (Sudan et al., 2023). Flavonoids including tricetin, apigenin and quercetin contribute to antioxidant and anti-inflammatory activities, while anthocyanins and proanthocyanidins determine pigmentation and functional properties (Bhat et al., 2020; Oliveira et al., 2026). These compounds exhibit strong radical scavenging capacity, metal chelation and modulate enzymatic pathways. Studies have consistently shown that pigmented rice possesses significantly higher total phenolic and flavonoid content compared to white rice, leading to enhanced antioxidant activity and reduced risk of chronic diseases such as diabetes, cardiovascular disorders and obesity (Tiozon et al., 2023). Additionally, their interaction with dietary fiber results in delayed release and microbial metabolism in the colon, thereby contributing to gut health and metabolic regulation (Evangelista and Schönlechner, 2025). The variation in composition among red black and purple rice further determines their specific functional applications in food systems.

However, processing of pigmented rice plays a decisive role in determining its functional performance in food systems as well as the retention of nutritionally important bioactive compounds. Inherently occurring phenolics, flavonoids and anthocyanin compounds are highly sensitive to processing conditions such as temperature, moisture and mechanical shear. Milling remains the most critical step affecting quality as it removes the bran layer where most bioactive compounds are concentrated. Studies have shown that even minimal

milling results in drastic reductions in anthocyanins, phenolics and vitamins, thereby significantly lowering antioxidant potential (Laokuldilok et al., 2013; Paiva et al., 2014). Thermal processing further modifies the physicochemical and nutritional properties of pigmented rice. Conventional cooking methods such as boiling lead to extensive leaching of water-soluble compounds with anthocyanin losses reported up to 90% (Colasanto et al., 2021). In contrast, controlled processing techniques such as steaming, microwave cooking and extrusion have shown improved retention depending on processing parameters. Extrusion cooking, in particular, has been widely explored due to its ability to produce ready-to-eat products with desirable texture and improved digestibility. Studies have reported that extrusion at moderate temperatures preserves anthocyanins while enhancing starch gelatinization and product expansion (Kumar and Murali, 2020). Similarly parboiling has been shown to increase free phenolic content due to compound migration from bran to endosperm, although excessive heat may degrade these heat-sensitive compounds (Paiva et al., 2016; Min et al., 2014).

Starch plays a central role in determining the functional properties of rice-based products. However, native starch exhibits limitations such as retrogradation, syneresis and instability under processing conditions. Chemical modification, particularly crosslinking, has emerged as an effective approach to overcome these limitations by enhancing structural integrity, thermal stability and resistance to enzymatic degradation (Gałkowska et al., 2023; Compart et al., 2023). Chemical modification of starch can be broadly classified into substitution, crosslinking, acid hydrolysis, and oxidation. Substitution (acetylation, hydroxypropylation) introduces functional groups that improve swelling and reduce retrogradation, whereas crosslinking forms covalent bonds between starch chains, thereby strengthening granule integrity and improving resistance to shear, heat, and acidic conditions. Acid hydrolysis reduces molecular weight, altering viscosity and digestibility, while oxidation introduces carbonyl and carboxyl groups, enhancing paste clarity and reducing viscosity (Compart et al., 2023). Therefore, modified starches exhibit improved gelatinization behavior, viscosity and water retention, making them suitable for application in structured food systems. These modifications are particularly relevant for

pigmented rice, where the interaction between starch and bioactive compounds further influences functional behavior.

Building on these processing aspects, incorporation of pigmented rice into different food systems has consistently been associated with improvements in nutritional and functional quality (Melini et al., 2019; Kumar and Murali 2020). Across various product categories, inclusion of pigmented rice has generally enhanced bioactive composition and contributed to improved health-promoting properties. However, these benefits are often accompanied by modifications in structural and textural attributes. Pigmented rice lacks gluten and exhibits a different starch-protein matrix compared to many conventional cereal products (Melini et al., 2019). Product characteristics such as volume, texture and cooking behavior are altered depending on the level of incorporation and processing conditions. Moderate inclusion levels have been typically found to provide a balance between nutritional enhancement and acceptable product quality. Excessive substitution may negatively affect structural integrity and sensory attributes of the products (Kumar and Murali 2020). In thermally processed and extruded products, pigmented rices have been found to contribute to enhanced antioxidant potential and visual appeal, although their impacts on expansion and texture depend on formulation and processing parameters (Sukumar et al., 2023). Overall, these findings indicate that pigmented rice has strong potential as a functional ingredient across diverse food systems provided the processing techniques and formulation strategies are carefully optimized to achieve desirable product characteristics.

Parallely, in recent years increasing consumer demand for convenience foods has created new opportunities for utilization of pigmented rice. Convenience foods are broadly classified into ready-to-eat, ready-to-cook, ready-to-serve and instant products, depending on the level of processing and preparation required. Ready-to-eat products include extruded snacks, breakfast cereals and baked goods, while ready-to-cook products include pasta, noodles and porridge mixes and likewise products. Instant foods such as dehydrated rice products and quick-cooking formulations require minimal preparation time and are particularly relevant for urban consumers. These products are designed to reduce time,

effort and skill required for food preparation, while maintaining acceptable sensory and nutritional quality (Brunner et al., 2010; Bogard et al., 2024). Pigmented rice offers a unique advantage in this segment due to inherent nutritional richness and functional properties. However, pigmented rice integration in convenience foods requires addressing challenges related to color, stability, texture, cooking quality, and shelf-life through process optimization.

Mathematical approaches such as Response Surface Methodology and Mixture Design are extensively used in food process optimization studies to evaluate the combined effects of multiple variables and to identify optimal processing conditions. These techniques enable systematic modeling and prediction of responses by considering interactions between ingredients and process parameters. In addition to these conventional statistical tools, fuzzy logic has also emerged as an effective approach for handling complex systems characterized by uncertainty and non-linear relationships. Unlike traditional methods, fuzzy logic allows for integration of qualitative and quantitative data, making it particularly useful in food formulation and process optimization where multiple attributes such as texture, sensory quality and nutritional parameters need to be balanced simultaneously.

Deepwater rice represents a specialized ecotype adapted to prolonged flooding through rapid internode elongation mediated by ethylene-responsive genes such as SNORKEL1 and SNORKEL2 (Hattori et al., 2011; Rohilla et al., 2020). This adaptive mechanism allows their survival under water depths exceeding several meters and is widely observed in flood-prone regions of South and Southeast Asia. In India, deepwater rice occupies approximately 2.4 million hectares and supports millions of livelihoods (Rohilla et al., 2025). The growth strategy of deepwater rice influences grain composition including amylose content, protein levels and mineral distribution which are critical for food processing applications. Within this broader ecological framework, *bao* rice of Assam represents a unique regional form of deepwater rice cultivated in the flood-prone Brahmaputra valley. These landraces are characterized by rapid elongation, aerenchyma

formation and kneeing ability, enabling their survival under prolonged submergence (Rajkhowa and Borgohain 2024).

Bao rice of Assam offers unique advantages due to its adaptation to flood-prone ecosystems and its rich bioactive composition. Pigmented deepwater *bao* rice varieties exhibit high nutritional and functional value with reported total phenolic content ranging from approximately 752.89 to 2223 mg/100 g and flavonoid content from 252.12 to 1000.75 mg QE/100 g (Mudoi and Das, 2019). Reportedly, their anthocyanin content ranges from 76.05 to 159.42 µg/100 g with DPPH scavenging activity between 81.5% and 96.0% indicating strong antioxidant potential. These varieties also contain significant levels of protein (9-13%) and minerals such as iron (2.12-54.40 mg/100 g) and zinc (2.42-26.57 mg/100 g) (Kalita and Handique 2020; Mudoi and Das 2019). Such compositional richness highlights their potential for functional food applications. However, despite these advantages *bao* rice remains largely confined to traditional consumption systems with limited studies on their processing behavior, product development and storage stability. Lack of systematic studies on its processing behavior and product development have limited their utilization. Addressing these gaps through targeted research on processing techniques, starch modification and product formulation can facilitate the development of nutritionally enriched, shelf-stable and consumer-acceptable *bao* rice based food products. Such efforts will not only enhance the economic value of *bao* rice but also contribute to sustainable agricultural practices and improved livelihoods in flood-prone regions.

In line with this rationale, the present study encompassed three pigmented *bao* rice varieties namely *Amona bao*, *Badal bao* and *Kenkowa bao* for product development. Initially, prior to processing, a preliminary evaluation of their physical and chemical properties was conducted to understand compositional variability and functional behavior. The results presented in Table 1.1 indicate significant differences among the varieties. The morphological characteristics of the selected pigmented *bao* rice varieties are presented in Fig. 1.1. *Kenkowa bao* exhibited the highest length-to-breadth ratio (3.21) indicating slender grains while *Amona bao* showed shorter grains (2.75). 1000 kernel weight was

highest in *Kenkowa bao* (26.50 g) suggesting higher grain density. Cooking time also varied significantly with *Badal bao* requiring the longest duration (36 min) followed by *Amona bao* (33 min) and *Kenkowa bao* (28 min), reflecting differences in their starch structure and water absorption behavior. Apparent amylose content ranged from 25.87% in *Amona bao* to 30.20% in *Kenkowa bao*, indicating high amylose classification. Further compositional analysis showed that *Amona bao* had the highest protein content (12.05%) while *Kenkowa* and *Badal bao* showed similar values of about 10.67% and 10.86%, respectively. Total carbohydrate content remained relatively similar across all three varieties, indicating starch dominance. Notably significant variation was observed in bioactive composition. *Amona bao* exhibited the highest total phenolic content (1853.19 µg GAE/g) and flavonoid content (756.19 µg QE/g) followed by *Badal bao* and *Kenkowa bao*. Antioxidant activity measured through DPPH scavenging remained high across all varieties exceeding 85%, while ferric reducing antioxidant power (FRAP) was highest in *Badal bao* (3.76 mM FeSO₄ eq/g). These findings clearly demonstrated the compositional diversity among the selected varieties which is expected to influence their processing behavior, functional properties and suitability for product development.

Table 1.1. Physicochemical properties of the raw rice and flour samples

Parameters	<i>Kenkowa bao</i>	<i>Amona bao</i>	<i>Badal bao</i>
L/B ratio	3.21±0.24 ^a	2.75±0.19 ^c	2.83±0.12 ^b
Weight of 1000 kernels	26.50±0.23 ^a	23.46±0.17 ^c	25.00±0.22 ^b
Cooking time (min)	28.00±2.00 ^a	33.00±4.00 ^c	36.00±3.00 ^b
Apparent amylose content (%)	30.20±0.69 ^a	25.87±0.27 ^c	29.14±0.21 ^b
Moisture content (% w.b)	12.13±0.78 ^a	11.87±0.92 ^a	11.92±0.63 ^a
Total carbohydrate (% d.b)	72.56±2.14 ^a	70.97±1.65 ^a	70.72±1.72 ^a
Total protein (% d.b)	10.67±0.34 ^b	12.05±0.53 ^a	10.86±0.25 ^b
Total fat (% d.b)	1.99±0.08 ^a	2.15±0.07 ^a	1.66±0.08 ^a
Total ash (% d.b)	1.94±0.06 ^a	1.68±0.07 ^b	1.59±0.06 ^c
Total phenolic content (µg GAE /g)	1522.90±2.33 ^c	1853.19±2.92 ^a	1753.49±4.33 ^b
Total flavonoid content (µg QE/g)	629.28±3.55 ^c	756.19±2.72 ^a	732.89±2.45 ^b
DPPH radical scavenging activity (%)	87.29±0.92 ^a	86.95±1.09 ^a	85.10±1.03 ^b
Ferric reducing antioxidant power (mM FeSO ₄ eq/g)	2.72±0.03 ^c	3.18±0.03 ^b	3.76±0.02 ^a

Means with different superscript letters between the rows are significantly different at p≤0.05 as indicated through Duncan's multiple range test



Fig. 1.1. Comparative morphology of the selected pigmented *bao* rice varieties.
(a-c) Paddy grains of *Amona*, *Badal* and *Kenkowa*, respectively; (d-f)
corresponding dehusked single brown rice kernels; (g-i) dehusked brown rice

In view of these limitations, this research was designed to systematically explore the potential of the three pigmented *bao* rice types for developing ready-to-eat value-added products. The objectives of the study were as follows:

1. To standardize processes for three *bao* red rice-based products, namely porridge mix, rice gel and pasta
2. To analyze the physicochemical, digestibility and phytochemical status of the developed products
3. To study the storability and shelf-life of the developed products

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CHAPTER 2

Review of literature

Pigmented rice exhibits considerable genetic and compositional diversity, encompassing a wide range of traditional landraces that differ in grain color, morphology, nutritional composition and functional properties. Nevertheless, white polished rice dominates global consumption and processing due to its widespread acceptance, ease of cooking and well-established value chains. In contrast, pigmented rice varieties remain underutilized despite their superior nutritional profile and health promoting properties. Among these, the traditional pigmented *bao* rice landraces of Assam represent a unique group, low-input and submergence-tolerant rice varieties adapted to flood-prone ecosystems. These landraces possess distinct physicochemical and functional attributes, making them promising candidates for value addition and food product development. However, their utilization remains largely confined to conventional household consumption, with limited scientific exploration in terms of processing and commercialization. This underutilization is further evident from the absence of systematic studies on the processing behavior, product standardization and storage stability of *bao* rice-based products, with existing research primarily limited to their physicochemical properties, bioactive composition, mineral content, genetic characteristics and cooking behavior. In view of the limited availability of scientific studies specifically on pigmented *bao* rice, the present chapter reviews the broader body of literature on pigmented rice to provide a comprehensive understanding of its potential for value-added applications. It is anticipated that insights derived from studies on pigmented rice in general will offer a useful basis for understanding the characteristics and processing behavior of pigmented *bao* rice types, as well as their suitability for product development.

The chapter comprehensively reviews pigmented rice with emphasis on its classification, pigment composition, genetic regulation and diverse bioactive profile, along with associated health benefits. It critically examines physicochemical and functional

properties, particularly starch behavior and evaluates the impact of processing techniques in improving functionality. The review further situates *bao* rice of Assam within the deepwater rice ecotype, highlighting its unique adaptive traits, compositional diversity and nutritional potential, while noting the limited research on its processing and utilization. By integrating these aspects, this chapter aims to identify existing research gaps and support the effective utilization of pigmented *bao* rice in the development of nutritionally enriched, value-added and shelf-stable ready-to-eat products.

2.1. Historical background of pigmented rice

The historical background of pigmented rice is closely intertwined with the origin, domestication, and diversification of *Oryza sativa*, highlighting its deep-rooted role in early agricultural systems. Archaeological and genomic evidence suggests that rice was initially domesticated in the Yangtze River basin of China approximately 9,000 years ago, followed by its gradual dissemination across South, Southeast, and East Asia and later to other parts of the world through migration and trade. Some hypotheses also propose an independent or parallel origin in the Indian subcontinent, where rice cultivation has long been associated with early civilizations such as the Indus Valley (Sweeney and McCouch, 2007). Over time, domesticated rice diverged into the major subspecies *indica* and *japonica*, with further diversification influenced by climatic shifts and ecological adaptation (Evangelista and Schönlechner, 2025). Within this evolutionary framework, pigmented rice types including red, black and purple rice emerged as distinct landraces due to the accumulation of natural pigments in their bran layer.

Historical evidence indicates that pigmented rice, particularly red rice, held considerable cultural, dietary and medicinal importance in ancient societies. Early Indian texts such as the *Taittiriya*, *Satapatha*, *Samhita* and *Brahmana* document the use of colored rice in ritual offerings, reflecting its sacred status. Classical Ayurvedic literature further classified red rice (*rakta shali*) as highly beneficial, attributing to it superior nutritional and therapeutic properties, especially in maintaining physiological balance (Ahuja et al.,

2007). References in later religious and historical texts also highlight its continued relevance in diet and traditional medicine. Across Asia, pigmented rice has been consumed for more than three millennia. With distinct regional preferences, black rice has been historically associated with royalty in China, while red rice remained a staple among communities in India and neighboring regions (Sweeney and McCouch, 2007). Despite this historical prominence, the large-scale adoption of high-yielding white rice varieties during the Green Revolution led to a significant decline in pigmented rice cultivation, further compounded by its perception as a contaminant or “red menace” in modern agriculture (Ahuja et al., 2007).

2.2. Taxonomy and types of pigmented rice

Taxonomically, rice is classified within the kingdom *Plantae* and falls under the division *Magnoliophyta* (angiosperms), class *Liliopsida* (monocots), subclass *Commelinidae*, order *Poales*, family *Poaceae*, genus *Oryza*, and species *Oryza sativa* L., reflecting its position among the cultivated cereal crops with diverse morphological and biochemical characteristics (Punia and Kumar, 2022). The genus *Oryza*, belonging to the tribe *Oryzeae* within the subfamily *Oryzoideae*, comprises two cultivated species, *Oryza sativa* L. and *Oryza glaberrima* Steud., along with more than 20 wild species distributed across tropical and subtropical regions. Among these, *O. sativa* is predominantly cultivated worldwide, whereas *O. glaberrima* is confined to limited regions in Africa. Within this species, three principal subspecies are recognized, namely *indica*, *japonica* and *javanica* which differ in grain type, cooking behavior, and ecological adaptation. *Indica* is the most widely grown, followed by *japonica*. These subspecies exhibit distinct morphological and agronomic characteristics. *Indica* types are typically long-grained with a less sticky texture, *japonica* types are short-grained and more glutinous in nature and *javanica* are large thick grained (Evangelista and Schönlechner, 2025). Pigmented rice does not constitute a separate taxonomic category but represents a group of genetically diverse varieties within *Oryza*

sativa distinguished by the presence of colored bran layers. Pigmented rice is broadly classified into red, black and purple rice types based on the colour of the pericarp, which is primarily determined by the accumulation of bioactive compounds, namely anthocyanins and proanthocyanidins (Mbanjo et al., 2020).

Table 2.1. Different pigmented rice varieties and their distribution

Pericarp Colour	Name of Varieties	Growing Location	Reference
Some pigmented rice varieties reported from other countries			
Red	Hongnuo, Bianduhongmi, Hongcaomi, Hongxiangmi, Yueyahongmi, Jingganghongmi, Sikoutianyoujihongmi	China	Chen et al., 2022a
Black	Lianjian33, Heizhenmi, Okunomurasaki, Yunanheixiangnuo, Chongxueheimi	China	
Black	BLR (Liangyou 695; moisture 12.16%, starch 65.80%, protein 7.60%, fat 2.40%)	China	Hu et al., 2024
Red	RR (Huiliangyou 1898; moisture 10.71%, starch 72.20%, protein 9.40%, fat 1.50%)	Thailand	Yamuangmorn et al., 2021
Purple	Kum Doi Saket, Kum Akha, Pieisu, Kum Hom CMU, KJ CMU 107, Riceberry, Hom Nil, Sang Yod		
Red	YMCB 3 CMU	Indonesia	Sholikhah et al.; Jati et al., 2014
Black	Black Purwokerto, Black Banjarnegara, Black Purbalingga, Black Bantul, Black Melik, Here Lahok, Black Lumajang, Black Blitar, Black Toraja		
Black	<i>Oryza sativa</i> var. jowo	Indonesia	Jati et al., 2014
Red	<i>Oryza sativa</i> var. mandel	Some pigmented rice varieties reported from India	
Red	Amana <i>Bao</i> , Dal <i>Bao</i> , Hurupi <i>Bao</i> , Jul <i>Bao</i> , Kenkowa <i>Bao</i> , Kotia <i>Bao</i> , Negheri <i>Bao</i>	Assam	Mudoi and Das, 2019
Red	Betu, Biroi, Bogaguni, Burali, Ixojoy, Kolaguni, Kopouguni, Ronga Chokua, Ronga Dariya	Manipur Nagaland Arunachal Pradesh Mizoram Meghalaya Arunachal Pradesh	Sahewalla et al., 2023
Black	Chakhao akupi, Chakhao poireiton, Chak-hao amubi		
Red	Lumre, Tsulu tsuk		
Red	Aamda, Tasung		
Red	Kawnglawng, Fazu	Arunachal Pradesh	Singh et al., 2022
Red	Menil mibabaret		
Red	Lingkang taker ame (LA), Umling ame (UA)	Tamil Nadu	Samyor et al. 2016
Red	Maapillai Samba		
			Rathna Priya et al. 2019

Different pigmented rice varieties and their distribution are presented in Table 2.1. Red rice is often associated with traditional landraces and in some cases, wild relatives such as *Oryza punctata* (Kotzchy ex Steud.), whereas purple rice is commonly classified as *Oryza*

sativa L. var. *glutinosa*. Black rice, frequently considered a form of purple rice, is generally linked to *indica* or tropical *japonica* backgrounds and includes several glutinous types (Punia and Kumar, 2022). Despite sharing a common species origin, these pigmented varieties exhibit significant differences in physicochemical, nutritional, and functional properties, largely governed by their genetic makeup and pigment composition.

2.2.1. Comparison of pigment, biosynthesis and genetics in red, purple and black rice

2.2.1.1. Pigment composition and chemical characteristics

The primary distinction between red and purple/black rice lies in the nature of the flavonoid pigments accumulated in the grain pericarp and aleurone layers. Red rice derives its characteristic coloration mainly from proanthocyanidins, which are condensed tannins composed of polymerized flavan-3-ol units such as catechin and epicatechin (Sudan et al., 2023). These compounds are high molecular weight, chemically stable and poorly soluble in water. They contribute to the red to brown appearance of their pericarp and to their persistence during processing and digestion (Zhang et al., 2023). Proanthocyanidins tend to remain localized within the pericarp and are not readily degraded, making them functionally distinct from other flavonoid pigments (Mbanjo et al., 2020).

In contrast, purple and black rice accumulate anthocyanins, a class of glycosylated flavonoids derived from anthocyanidins such as cyanidin, peonidin and pelargonidin. Anthocyanins are smaller, water-soluble molecules with conjugated double-bond systems and multiple hydroxyl groups, which give rise to purple, dark purple, or black coloration, depending on concentration and molecular structure (Zhang et al., 2023). The most abundant anthocyanins in black and purple rice are cyanidin-3-O-glucoside and peonidin-3-O-glucoside, along with several acylated and diglycosylated derivatives. Compared to proanthocyanidins, anthocyanins are more reactive and biologically potent as antioxidants, but they are also more sensitive to milling, thermal processing and pH changes (Mbanjo et al., 2020).

2.2.1.2. Flavonoid biosynthetic pathways

Although both red and purple/black rice pigments originate from the common phenylpropanoid flavonoid pathway, the pathway splits at a key step involving leucoanthocyanidin intermediates (Sudan et al., 2023). In red rice, the pathway is directed toward proanthocyanidin biosynthesis (Oliveira et al., 2026). Leucoanthocyanidins are reduced by leucoanthocyanidin reductase to form flavan-3-ols, which subsequently polymerize into proanthocyanidins. This metabolic route limits the conversion of intermediates into anthocyanins and favors the accumulation of condensed tannins in pericarp (Lang et al., 2025).

In purple and black rice, the same intermediates are instead oxidized by anthocyanidin synthase to form anthocyanidins, which are inherently unstable. These anthocyanidins are rapidly glycosylated by uridine diphosphate glycosyltransferases, producing stable anthocyanins that accumulate in the aleurone and pericarp layers (Sudan et al., 2023). Transcriptomic analyses have demonstrated that genes encoding anthocyanin biosynthetic enzymes are strongly upregulated in black rice, whereas red rice shows enhanced expression of genes associated with proanthocyanidin formation (Mackon et al., 2021). This confirms that pigment differences arise from pathway level regulation rather than differences in precursor availability.

2.2.1.3. Genetic regulation

Red rice pigmentation represents the ancestral state of cultivated rice and is genetically simpler than purple/black pigmentation. It is primarily regulated by the *Rc* (red pericarp) gene, which encodes a basic helix-loop-helix (bHLH) transcription factor (Mbanjo et al., 2020). *Rc* activates downstream structural genes involved in proanthocyanidin biosynthesis, thereby promoting the accumulation of condensed tannins in the pericarp. The effect of *Rc* is enhanced by the *Rd* (red determinant) gene, which encodes dihydroflavonol 4-reductase and increases the availability of leucoanthocyanidin

substrates for proanthocyanidin synthesis (Lang et al., 2025). When both *Rc* and *Rd* are active, rice grains exhibit a red pericarp. However, if *Rc* gene is damaged due to mutations such as small deletions or early stop signals, pigment production stops and results in white rice. During rice domestication, the non-functional *Rc* gene was often selected because it was linked to traits like reduced seed dormancy and less grain shattering, which made harvesting easier. As a result, white rice became more common. Modern red rice varieties largely retain ancestral alleles that favor metabolic stability and proanthocyanidin accumulation (Sudan et al., 2023).

Purple and black rice pigmentation is controlled by a more complex, polygenic regulatory network. Central to this regulation are loci collectively referred to as *Kala* genes, particularly *Kala4*, *Kala1* and *Kala3*. *Kala4* encodes a bHLH transcription factor whose promoter region undergoes structural rearrangements, leading to strong expression in the pericarp (Mbanjo et al., 2020; Mackon et al., 2021). This activation triggers coordinated upregulation of multiple anthocyanin biosynthetic genes. *Kala1* contributes to pigment intensity through its association with dihydroflavonol 4-reductase, while *Kala3* corresponds to an R2R3 type Myeloblastosis (R2R3-MYB) transcription factor that further amplifies anthocyanin accumulation (Sudan et al., 2023). Together, these genes form part of a Myeloblastosis basic helix-loop-helix-WD40 (Tryptophan-Aspartic acid repeat) transcriptional complex (MYB-bHLH-WD40 transcriptional complex), which precisely regulates the spatial and temporary expression of flavonoid pathway genes (Lang et al., 2025). This regulatory architecture enables high-level anthocyanin synthesis and explains the intense pigmentation observed in purple and black rice. Unlike red rice, where pigmentation depends largely on the presence or absence of *Rc*, purple/black rice pigmentation is strongly influenced by promoter variation, gene dosage and transcriptional synergy (Mackon et al., 2021).

Table 2.2. Comparative biochemical, genetic and functional characteristics of pigmented rice varieties (red, purple and black rice)

Feature	Red Rice	Purple Rice	Black Rice
Dominant pigment	Proanthocyanidins (condensed tannins)	Anthocyanins	Anthocyanins (highly condensed)
Major biosynthetic branch	Proanthocyanidin pathway	Anthocyanin pathway	Anthocyanin pathway (upregulated)
Key regulatory genes	<i>Rc, Rd</i>	Pb/Pp, MYB–bHLH complex	<i>Kala4, Kala1, Kala3, MYB–bHLH–WD40</i>
Key structural enzymes	LAR, DFR	DFR, ANS, UGT	F3H, DFR, ANS, UGT
Molecular size of pigments	High (polymeric)	Low-moderate	Low (glycosylated)
Enzyme inhibition	Strong α -amylase and amyloglucosidase inhibition	Moderate α -glucosidase inhibition	Strong β -glucosidase, moderate α -glucosidase inhibition
Glycemic index behaviour	Consistently low	Moderate	Variable (depends on amylose and milling)
Resistant starch (RS) formation	High	Moderate	Low–moderate
Antioxidant intensity	Moderate, sustained	High	Very high
Antioxidant mechanism	Enzyme modulation, metal chelation	ROS scavenging	Rapid ROS scavenging
Sensitivity to milling	Low	High	Very high
Best health relevance	Diabetes management, gut health, metabolic regulation	Antioxidant & anti-inflammatory	Antioxidant, anticancer, anti-aging

Reference: Sudan et al., 2023; Mbanjo et al., 2020; Oliveira et al., 2026; Chen et al., 2024

2.2.1.4. Functional implications of pigment and genetic differences

The biochemical and genetic differences between red and purple/black rice have important functional consequences (Table 2.2). Proanthocyanidins in red rice are structurally robust and interact strongly with starch and digestive enzymes, contributing to improved glycemic regulation and gut-related health benefits. Their resistance to processing and digestion makes red rice particularly suitable for functional foods targeting metabolic health. In contrast, anthocyanins in purple and black rice confer superior antioxidant, anti-inflammatory and cytoprotective effects due to their rapid free-radical scavenging capacity (Mbanjo et al., 2020). However, their sensitivity to milling and thermal treatments necessitates careful processing to preserve functionality. Consequently, red and purple/black rice should be viewed as distinct functional phenotypes shaped by

differential gene regulation and metabolic flux, each offering unique advantages depending on dietary application and food system design.

2.3. Bioactive compounds in pigmented rice

Pigmented rice is characterized by a diverse array of bioactive constituents that are primarily concentrated in the outer bran and aleurone layers of the grain (Table 2.3). These compounds belong to multiple chemical classes, including phenolic acids, flavonoids, anthocyanins, proanthocyanidins, and other minor bioactives such as γ -oryzanols, tocopherols, and carotenoids, each differing in chemical structure, solubility, and spatial distribution within the grain matrix. The variability in their molecular forms plays a crucial role in determining their stability, bioaccessibility, and functional behavior during processing and digestion. Collectively, these bioactive compounds contribute to the enhanced antioxidant capacity, metabolic regulatory effects and nutraceutical potential of pigmented rice.

2.3.1. Phenolic compounds

Pigmented rice varieties, including red, black and purple rice are well recognized for their rich composition of phenolic compounds, which are primarily concentrated in the bran and aleurone layers of the grain. These phenolics exist in three major forms, namely free, soluble conjugated, and insoluble-bound phenolics, with the latter constituting the predominant fraction. The insoluble-bound phenolics are mainly esterified or ether-linked to cell wall components such as hemicellulose, lignin, and structural polysaccharides, thereby contributing to the rigidity and integrity of plant cell walls (Evangelista and Schönlechner, 2025). Among the various phenolic acids identified, hydroxycinnamic acid derivatives such as ferulic acid, caffeic acid, *p*-coumaric acid and sinapic acid, along with hydroxybenzoic acid derivatives including gallic acid, vanillic acid, protocatechuic acid and syringic acid are the most abundant (Mbanjo et al., 2020). Ferulic acid, protocatechuic acid, and *p*-coumaric acid are predominantly present as bound phenolics, whereas sinapic, vanillic, and a portion of ferulic acid occur in soluble forms, particularly in black rice

(Sudan et al., 2023). Additionally, cinnamic acid serves as a key precursor in the phenylpropanoid pathway, leading to biosynthesis of other hydroxycinnamic acids. The distribution of phenolic compounds follows a gradient within the grain, increasing from endosperm to bran, with the aleurone layer acting as the principal site of accumulation. Total phenolic content (TPC), determined by UV visible spectroscopy, was significantly lower in white rice than in red and black rice varieties (Chen et al., 2022b).

The strong association of phenolic compounds with dietary fiber through covalent and non-covalent interactions significantly limits their bio-accessibility in the upper gastrointestinal tract. As a result, a large proportion of these compounds reaches the colon, where they are released by microbial fermentation, thereby exerting localized physiological effects (Evangelista and Schönlechner, 2025). In red and black rice, several phenolic acids have been identified. Caffeic acid occurs as a minor component in red rice, while syringic acid is present in both red and black rice. Pinellic acid has been also reported in red rice, whereas black rice contains hydroxybenzoic acid. Additionally, gallic acid has been detected in certain red rice extracts (Mbanjo et al., 2020). These phenolic compounds exhibit strong antioxidant properties, enabling them to scavenge reactive oxygen species and reduce oxidative stress, thereby contributing to the prevention of chronic diseases such as cardiovascular disorders and type-2 diabetes (Tiozon et al., 2023).

2.3.2. Flavonoids

Flavonoids represent another major class of bioactive compounds in pigmented rice, characterized by their polyphenolic structure consisting of a C6-C3-C6 carbon skeleton (Wongsa, 2020). These compounds are highly diverse and include subclasses such as flavonols, flavones, isoflavones, and anthocyanins (Evangelista and Schönlechner, 2025). Pigmented rice varieties generally contain significantly higher flavonoid content compared to non-pigmented counterparts, primarily due to enhanced expression of genes involved in flavonoid biosynthesis (Punia and Kumar, 2022). The concentration of

flavonoids is closely associated with the intensity of grain pigmentation, with darker varieties such as black rice exhibiting the highest levels. Among the identified flavonoids, tricin is considered the predominant compound in rice bran, accounting for a substantial proportion of total flavonoid content. Other important flavonoids include apigenin, kaempferol, catechin and quercetin, which are present in varying concentrations depending on the genotype and environmental conditions (Mbanjo et al., 2020). Flavonoids play multiple biological roles, including free radical scavenging, anti-inflammatory, anticancer activities and modulation of cell signaling pathways (Bhat et al., 2020). Furthermore, they contribute to plant defense mechanisms and protect against ultraviolet radiation, while also enhancing the functional and nutraceutical value of pigmented rice (Oliveira et al., 2026). Anthocyanins and proanthocyanidins are the principal flavonoids responsible for pigmentation in rice.

2.3.2.1. Anthocyanins

Anthocyanins are water-soluble pigments responsible for the characteristic purple, and black coloration of pigmented rice. Structurally, they are glycosylated derivatives of anthocyanidins such as cyanidin, peonidin, malvidin and delphinidin (Mbanjo et al., 2023). Among these, cyanidin-3-O-glucoside is the most dominant in black rice, accounting for a major proportion of the total anthocyanin content. Other identified anthocyanins include cyanidin-3-galactoside, peonidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3,5-diglucoside, pelargonidin-3,5-diglucoside and malvidin derivatives (Chen et al., 2024). The structural diversity of anthocyanins arises from variations in glycosylation, acylation and methylation patterns, which influence their stability, color intensity and biological activity. Anthocyanins are primarily localized in the pericarp and aleurone layers of the grain, with black rice containing significantly higher concentrations compared to red rice. These compounds exhibit strong antioxidant activity and are known to neutralize free radicals, reduce oxidative stress and protect cellular components from damage (Bhat et al., 2020). Additionally, anthocyanins have been associated with improved cardiovascular health, modulation of inflammatory

pathways and enhancement of immune responses. However, their stability is highly sensitive to environmental factors such as pH, temperature, light and enzymatic activity, which pose challenges during processing and storage (Oliveira et al., 2026).

2.3.2.2. Proanthocyanidins (Condensed tannins)

Proanthocyanidins, also known as condensed tannins, are polymeric flavonoid compounds. The red coloration of rice is primarily attributed to proanthocyanidins, which are oligomers and polymers of flavan-3-ols such as catechin and epicatechin linked through carbon-carbon or ether bonds (Sudan et al., 2023). Based on their linkage patterns, proanthocyanidins are classified into A-type and B-type structures, with the latter being the more common in nature. Proanthocyanidins are synthesized through intermediates such as dihydroquercetin and leucoanthocyanidins, ultimately forming catechin units that polymerize into complex structures (Zhang et al., 2023). The degree of polymerization plays a crucial role in determining their biological activity, with lower oligomers exhibiting anticancer properties and higher polymers contributing to immune regulation. In addition to their antioxidant potential, proanthocyanidins interact with starch molecules to form complexes that reduce enzymatic digestibility, thereby influencing glycemic response. The concentration of these compounds varies widely depending on genotype, environmental conditions, and storage factors, with higher levels typically observed in the bran (Oliveira et al., 2026). Comparative studies among pigmented rice varieties indicate significant variation in bioactive composition, with black rice generally exhibiting higher levels of total phenolics and flavonoids than red rice (Chen et al., 2022b; Singh et al., 2022).

2.3.3. Other bioactive constituents

Apart from phenolics and flavonoids, pigmented rice contains a wide range of other bioactive compounds, including γ -oryzanols, tocopherols, sterols, carotenoids, vitamins and essential minerals (Sudan et al., 2023). γ -oryzanols and tocopherols function as lipid-

soluble antioxidants, contributing to membrane stability and cholesterol regulation. Carotenoids such as lutein and β -carotene are present in appreciable amounts, particularly in black rice, where they contribute to provitamin A activity (Kodape et al., 2025). Additionally, pigmented rice varieties are reported to contain higher concentrations of micronutrients such as iron and zinc compared to conventional polished rice, enhancing their nutritional value. The tocol content, including tocopherols and tocotrienols, is significantly higher in the bran fraction, further emphasizing the importance of minimal processing in retaining nutritional quality (Oliveira et al., 2026).

Table 2.3. Classification and distribution of bioactive compounds in pigmented rice

Class of Compounds	Sub-class / Type	Major Compounds Identified	Form (Free/Bound)	Location in Grain
Phenolic Compounds	Hydroxycinnamic acids	Ferulic acid, p-coumaric acid, caffeic acid, sinapic acid	Mostly bound	Bran, aleurone
	Hydroxybenzoic acids	Gallic acid, protocatechuic acid, vanillic acid, syringic acid	Free + bound	Bran
Flavonoids	Flavonols, flavones	Tricin, apigenin, kaempferol, quercetin	Mostly free	Bran
	Flavanols	Catechin, epicatechin	Free	Bran
Anthocyanins	Anthocyanidins	Cyanidin-3-glucoside, peonidin-3-glucoside, delphinidin derivatives	Glycosylated	Pericarp, aleurone
Proanthocyanidins	Condensed tannins	Catechin polymers (A-type, B-type)	Polymerized	Bran (red)
Other Bioactives	γ -oryzanols	Ferulate esters	Lipid-soluble	Bran
	Tocopherols and Tocotrienols	Vitamin E compounds	Lipid-soluble	Bran
	Carotenoids	Lutein, β -carotene	Free	Endosperm + bran
	Minerals	Iron, Zinc	—	Whole grain

Reference: Evangelista and Schönlechner, 2025; Mbanjo et al., 2020; Sudan et al., 2023; Bhat et al., 2020; Oliveira et al., 2026; Chen et al., 2022b; Zhang et al., 2023

2.4. Health benefits of pigmented rice

Traditional knowledge systems highlight the long-standing therapeutic relevance of pigmented rice, which has been used across cultures to manage inflammatory conditions, gastrointestinal disorders, fever, circulatory problems, and for wound healing and organ-

related ailments. In Asia, rice bran-based preparations such as “tiki tiki” have been used to treat thiamine deficiency (Mbanjo et al., 2020). In India, pigmented rice is recommended for lactating mothers and conditions such as jaundice and paralysis. Specific landraces such as *Laicha* and *Kavuni* have been associated with antioxidant, anti-diabetic and gastrointestinal protective effects (Hemamalini et al., 2018, Mbajo et al., 2020).

These ethnomedicinal uses are supported by modern scientific evidences recognizing pigmented rice as a functional cereal, rich in bioactive compounds. Unlike polished white rice, it retains the bran and aleurone layers, which are abundant in phenolic acids, flavonoids, anthocyanins or proanthocyanidins, γ -oryzanol, tocopherols, dietary fiber and resistant starch (Tiozon et al., 2021). The synergistic action of these compounds contributes to antioxidant, anti-inflammatory, and metabolic regulatory effects, including improved glucose and lipid homeostasis. Consequently, regular consumption has been associated with reduced risk of chronic diseases such as diabetes, cardiovascular disorders, obesity and certain cancers (Oliveira et al., 2026). Importantly, the magnitude and nature of these health effects vary among red, purple and black rice, reflecting differences in pigment composition, molecular structure, bioaccessibility and genetic regulation.

2.4.1. Antioxidant properties

Pigmented rices demonstrate significantly higher antioxidant potential than non-pigmented rices, largely attributable to their elevated levels of phenolic acids, flavonoids, anthocyanins or proanthocyanidins, tocopherols, and γ -oryzanol. These bioactive compounds function as effective hydrogen and electron donors, stabilizing reactive species and interrupting oxidative chain reactions through resonance mechanisms. Evidence from bioavailability studies further indicates that these antioxidants are not only released during digestion but are also absorbed and capable of exerting systemic physiological effects.

Experimental findings consistently support the enhancement of endogenous antioxidant defenses following pigmented rice consumption. An *in vivo* study by Shen et al. (2027) showed that inclusion of 51% whole grain purple rice in high-fat diet-fed mice significantly increased total antioxidant capacity (T-AOC) and elevated the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), while concurrently reducing malondialdehyde (MDA) levels in serum and liver, indicating reduced oxidative stress under hypercholesterolemic conditions. In a human intervention study, Callcott et al. (2019) reported that purple rice consumption increased antioxidant activity by 70.5% and significantly reduced inflammatory markers, including TNF- α (-21.9% at 1 h and -25.4% at 4 h) and IL-6 (-11.7% at 1 h), whereas red rice reduced oxidative stress, reflected by a decrease in MDA (-9.2% at 30 min) and IL-6 (-14.1% at 2 h), with negligible effects observed for brown rice. Complementary *in vitro* evidence from Gong et al. (2020) demonstrated that phenolic- and flavonoid-rich extracts from red and black rice (50- 200 μ g/ml) effectively attenuated ABAP-induced oxidative stress in HepG2 cells, further confirming their cellular protective capacity compared to non-pigmented rice.

2.4.2. Anti-inflammatory properties

Inflammation is a tightly regulated physiological defense mechanism. However, its persistent low-grade activation is a defining feature of obesity, insulin resistance and metabolic syndrome. Bioactive compounds in pigmented rice exert significant anti-inflammatory effects by modulating cytokine production and suppressing pro-inflammatory signaling pathways, thereby attenuating metabolic inflammation. Evidence from clinical and experimental studies supports these effects. Seesen et al. (2020) reported that supplementation with black rice germ and bran, combined with exercise in older adults, significantly reduced inflammatory biomarkers (CRP and IL-6) and improved endocrine response, as reflected by increased IGF-1 levels, alongside enhanced physical performance and lower-body muscle strength, with no change in grip strength. Complementary findings were observed by Joo et al. (2020), where roasted black rice

consumption in adult subjects reduced systemic inflammation, indicated by decreased hs-CRP, without significant effects on body weight or waist circumference. Further supporting this, Anuyahong et al. (2022) demonstrated that Riceberry rice drink intake in overweight and obese individuals improved antioxidant capacity and reduced glucose, insulin, triglycerides, MDA, and inflammatory cytokines (IL-1 β , IL-6, TNF- α), without affecting appetite. Corroborating evidence from animal models strengthens these observations. Zhao et al. (2021) demonstrated that diets supplemented with 40% whole grain anthocyanin-rich black rice (*Heishuai*) significantly reduced ear edema and downregulated inflammatory gene expression in mice compared to an anthocyanin-deficient variety (*AM22*), highlighting the critical role of anthocyanins in mediating anti-inflammatory effects and preventing chronic disease progression.

2.4.3. Anti-obesity properties

Pigmented rice exhibits pronounced anti-obesity effects through regulation of lipid metabolism, adipocyte differentiation and inflammatory signaling. Its phenolic constituents enhance lipid oxidation, suppress lipogenesis and promote cholesterol efflux, thereby improving lipid homeostasis. Mechanistic evidence is supported by multi-level studies. An *in vitro* study by Krishnan et al. (2021) using biochemical assay systems showed that proanthocyanidins from black rice (*Chakhao Amubi*) and red rice (*Njavara*) significantly reduced hyperglycemia. In high-fat diet-induced insulin-resistant mice, Munkong et al. (2022) reported that red rice bran extract (0.5-1 g/kg) improved glucose–insulin homeostasis by modulation of insulin, IDE, IRS and GLUT gene expression. Supporting *in vitro* studies using adipocyte cell models further demonstrate anti-obesity potential through inhibition of adipocyte formation by anthocyanin-rich pigmented rice extracts (Kongthitlerd et al., 2020). Consistent *in vivo* evidence further reinforces these findings. Wang et al. (2020) showed that whole-grain black rice anthocyanin extract (0.12-0.48 g/kg) reduced body weight, triglycerides, total cholesterol and non-HDL cholesterol in hypercholesterolemic mice, while increasing fecal sterol excretion and cecal short-chain fatty acids. Extending these observations, human studies further supported these

effects. A clinical trial by Jung et al. (2021) in obese postmenopausal women reported that supplementation with black rice extract (BRE) significantly reduced trunk fat, total fat and body fat percentage, although body weight, BMI and waist-to-hip ratio remained unchanged. Therefore, pigmented rice and its derivatives primarily improved adiposity distribution and metabolic health rather than inducing marked weight loss. Additional evidences indicate that anthocyanin-rich pigmented rice products improve lipid metabolism in human subjects, including a reduction in the glycemic index of wheat bread upon fortification with black rice anthocyanin extract (BRAE), along with significant improvements in lipid profiles such as increased HDL, apolipoprotein A1 (Apo-A1) and apolipoprotein B (Apo-B), and favorable modifications in LDL and HDL subfractions (Ou et al., 2023).

2.4.4. Antidiabetic properties

A key mechanism underlying the antidiabetic effects of pigmented rice is the inhibition of carbohydrate-hydrolyzing enzymes, which delays starch digestion and glucose release. Extracts from red and purple rice inhibit α -glucosidase, while red rice additionally suppresses α -amylase activity. Anthocyanins from black rice also inhibit β -glucosidase, collectively contributing to reduced postprandial glucose levels (Boue et al., 2025). *In vivo* studies in diabetic animal models demonstrated that supplementation with black or red rice extracts significantly reduced fasting blood glucose levels, improved glucose tolerance, lowered glycated hemoglobin and promoted regeneration of pancreatic β -cells (Wahyuni et al., 2016; Tantipaiboonwong et al., 2017; Posuwan et al., 2013). Cyanidin-3-O-glucoside was identified as a key bioactive compound enhancing insulin sensitivity, glucose uptake and adiponectin secretion (Matsukawa et al., 2016). Anthocyanin-rich black rice, particularly cyanidin-3-glucoside, has demonstrated significant antidiabetic potential through multiple mechanisms. In an *in vivo* study using streptozotocin-induced diabetic rats, Qi et al. (2020) reported that cyanidin-3-glucoside improved glycemic control, enhanced renal function and reduced markers of renal fibrosis, indicating protective effects against diabetes-associated complications. Mechanistically, Bhuyan et

al. (2020) showed that black rice anthocyanins inhibit glutamine: fructose-6-phosphate amidotransferase (GFAT), a key enzyme in glucose metabolism, thereby improving insulin sensitivity and reducing insulin resistance. Additionally, the glycemic index (GI) of black rice has gained attention due to its role in modulating postprandial glucose response. Black rice varieties from the Philippines and Vietnam have been reported to exhibit relatively low GI values (Pipil et al., 2024). Variations in GI among black and red rice flours have been reported (Waewkum and Singthong, 2021). These findings highlight the potential of pigmented rice as a dietary strategy for glycemic control and diabetes management.

2.4.5. Antimicrobial properties

Pigmented rice exhibits antimicrobial activity through both direct inhibitions of pathogens and indirect modulation of gut microbiota. Its anthocyanins and other phenolic compounds can disrupt microbial growth while simultaneously promoting a favorable intestinal environment. An *in vivo* study in *Helicobacter pylori*-infected Mongolian gerbils by Kim et al. (2019) demonstrated that black rice extract (50, 100 and 200 mg/kg) significantly reduced bacterial infection, enhanced antimicrobial activity and improved gastric health.

Beyond direct antimicrobial effects, pigmented rice influences intestinal microbiota composition, which contributes to host defense. Both *in vitro* and *in vivo* studies have shown that pigmented rice consumption increases microbial diversity and selectively enriches beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* (Owolabi et al., 2020; Wang et al., 2020). The fermentation of its dietary fiber, resistant starch and phenolic compounds leads to increased production of short-chain fatty acids (SCFAs), which lower intestinal pH, inhibit pathogen growth and strengthen gut barrier function (Makki et al., 2018, Li et al., 2023a). Mechanistic insights further indicate that anthocyanins are not fully absorbed in the upper gastrointestinal tract but reach the colon, where they are metabolized by microbiota into bioactive compounds (Verediano et al., 2021). This process results in increased *Bacteroides*, reduced *Firmicutes*, enhanced SCFA

production, decreased intestinal permeability and improved tight junction integrity and villus morphology. Supporting this, anthocyanins from purple-red rice bran were shown in animal models to restore intestinal barrier structure, increase beneficial microbes, suppress harmful bacteria and enhance SCFA levels (Chen et al., 2022c). Therefore, pigmented rice exerts antimicrobial effects both directly against pathogens such as *H. pylori* and indirectly by modulating gut microbiota and intestinal barrier function, thereby enhancing overall gastrointestinal and immune health.

2.4.6. Other health-promoting properties

Pigmented rice exhibits diverse biological activities beyond metabolic functions, including hypouricemic, anticancer, immunomodulatory, cardioprotective, neuroprotective and anti-ageing effects across *in vitro*, *in vivo* and human models. In mice, Feng et al. (2022) showed that black rice anthocyanins (0.3-0.8 mg/ml) inhibited xanthine oxidase and uric acid-producing enzymes, reducing uric acid levels, while Li et al. (2023b) reported that black rice bran (1-15%) in high-fat/high-cholesterol diet-fed mice reduced weight gain and lipid levels, improved hepatic function, insulin sensitivity and gut microbiota. Anticancer effects were observed in dextran sodium sulfate/azoxymethane-induced mice, where 10% black rice bran (21.68 g/100 g) reduced tumors and enhanced antioxidant enzymes (Budijanto et al., 2023). It was also reported in diethylnitrosamine-induced rats, where purple rice bran extract (500 mg/kg) suppressed GST-P foci and inflammatory genes (Dokkaew et al., 2019). At the cellular level, HCT-116 cells treated with black rice anthocyanins (0.01-0.05 mg/ml) showed increased apoptosis (Liang et al., 2019), while cyanidin-3-glucoside (10-100 μ M) inhibited proliferation and invasion in human prostate carcinoma cells (Jongsomchai et al., 2020). Immunomodulatory effects were evident in cyclophosphamide-immunosuppressed mice, where purple rice bran (50-200 mg/kg) enhanced macrophage activity and restored immune function (Chen et al., 2022c), and in leukemia rats, where black rice anthocyanins (200-100 mg/kg) increased T and B cells and macrophage activity (Fan et al., 2017). Cardioprotective effects were reported in animal models, where black rice extract (200-

400 mg/kg) improved antioxidant status, lipid profile and reduced inflammation (Chakraborty et al., 2023). A human study in Norwich found no significant changes in lipid or glycemic markers (Aboufarrag et al., 2022). However, fermented glutinous black rice improved antioxidant intake, fiber, and lipid profile in dyslipidemic patients (Syarief et al., 2020). Neuroprotection was demonstrated in SH-SY5Y neuron-like cells, where black rice bran extract (10-50 µg/ml) provided 38–94% protection against oxidative stress (Vargas et al., 2018). Anti-ageing effects were studied in human skin fibroblast cells, where purple glutinous rice extract (0.01-10 mg/ml) showed antioxidant, anti-tyrosinase and proliferative effects (Poomanee et al., 2021). Additionally, red rice proanthocyanidin extract (25-2500 µg; 2-200 mg/kg/day; 50 µl/ear/day) inhibited ALOX5 and improved psoriatic inflammation in mice (Toda et al., 2020). Collectively, these findings suggest the broad therapeutic potential of pigmented rice across multiple biological systems.

2.5. Deepwater rice

Deepwater rice represents a unique ecological and physiological adaptation of *Oryza sativa* L. in which survival under prolonged flooding is achieved through active growth regulation rather than stress avoidance. Unlike flash-flood tolerant rice, which relies on metabolic suppression and carbohydrate conservation, deepwater rice survives by sustaining aerobic metabolism through continuous vertical growth that maintains leaf emergence above the water surface. This strategy evolved in riverine floodplains where water depth increases gradually and remains stagnant for extended durations, imposing selection pressure favoring growth plasticity rather than dormancy (Rohilla et al., 2020). Deepwater rice is cultivated in highly specialized ecosystems characterized by prolonged flooding, where water depth may range from several centimeters to several meters and persist for months, making it one of the few cropping systems where no other crops can survive. Globally, more than 100 million people across South and Southeast Asia depend on deepwater rice for their livelihoods, particularly in floodplains of major river systems such as the Ganges, Brahmaputra Mekong and Irrawaddy. In India, deepwater rice occupies approximately 2.4 million hectares, representing a significant component of

rainfed lowland ecosystems (Rohilla et al., 2025). This growth-dependent survival strategy has profound downstream consequences, because the same genetic and hormonal systems that regulate elongation under flooding also influence plant architecture, grain filling, and ultimately grain physicochemical properties that are relevant for food processing.

2.5.1. Genetic regulation of flood-induced growth

At the molecular level, deepwater rice adaptation is primarily governed by ethylene-responsive growth regulation, mediated by genes distinct from those controlling flash-flood tolerance. Under submerged conditions, restricted gas diffusion leads to ethylene accumulation within plant tissues, where it acts as a key positive regulator of shoot elongation. Ethylene triggers transcriptional programs that enhance gibberellin biosynthesis and signaling, while suppressing abscisic acid-mediated growth inhibition (Rajkhowa et al., 2025). Central to this response are the *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) genes, which encode ethylene-responsive transcription factors that activate internode elongation pathways (Hattori et al., 2011). These genes promote rapid cell elongation and division in internodal meristems, enabling growth rates sufficient to match rising floodwater levels. In contrast, the *SUB1A* gene, which governs quiescence under flash flooding, restricts elongation and therefore offers limited adaptive advantage in deepwater ecosystems (Phukon et al., 2024).

Deepwater adaptation is inherently polygenic, involving multiple QTLs and interconnected regulatory networks that coordinate flood-induced growth responses. Key genes such as *SD1* (semi-dwarf1), *ACE1* and *DECI* play crucial roles in modulating internode elongation through complex transcriptional interactions involving transcription factors, microRNAs and long non-coding RNAs (Rohilla et al., 2025). Collectively, these multilayered regulatory mechanisms enable precise coordination of elongation dynamics, carbohydrate mobilization and post-flood recovery, thereby ensuring effective adaptation to deepwater environments.

2.5.2. Genes, morphological and physiological traits

The activation of *SNORKEL*-mediated elongation pathways manifests as distinct morphological traits, including exceptionally tall culms, increased internode number, flexible zig-zag stem architecture and pronounced kneeing ability that allows stems to reorient vertically after flood recession (Rajkhowa and Borgohain, 2025). Internally, deepwater rice develops extensive aerenchyma networks within culms and roots, facilitating oxygen diffusion from aerial tissues to submerged organs and sustaining aerobic respiration during prolonged flooding. A key anatomical feature is the formation of lysigenous aerenchyma, produced through programmed cell death, which creates interconnected air spaces that enhance internal oxygen transport and mitigate hypoxic stress (Rohilla et al., 2025). One of the most remarkable features of deepwater rice is its rapid elongation capacity, with growth rates reaching 20-25 cm per day, enabling plants to keep pace with rising water levels. This rapid growth is supported by enhanced cell division, increased expression of cyclins and histone proteins and cell wall loosening mediated by expansins (Hattori et al., 2011).

A unique adaptation for survival under complete submergence is the formation of a thin air layer on leaf surfaces, which facilitates gas exchange under waterlogged conditions (Hattori et al., 2009). Since gas diffusion in water is approximately 10,000 times slower than in air, this air layer acts as a low-resistance pathway for CO₂ uptake and O₂ release, thereby sustaining photosynthesis and respiration. Additionally, deepwater rice exhibits tolerance to anaerobic germination, allowing seeds to germinate under oxygen-deficient conditions. Certain genotypes have demonstrated germination rates as high as 82% under anoxic environments, indicating significant genetic variability for this adaptive trait (Rohilla et al., 2025).

2.5.3. Impact of growth strategy on grain development and composition

The growth strategy of deepwater rice has important consequences on grain characteristics. Sustained elongation and prolonged vegetative growth under flooded

conditions often result in moderate grain yields but relatively stable grain filling under submergence stress (Hattori et al., 2011). Traditional deepwater cultivars exhibit variation in grain size, amylose content, protein fractions and mineral composition, reflecting genotype-specific allocation between vegetative growth and reproductive development. This compositional variability is further supported by studies on traditional Northeast Indian landraces, which demonstrate significant diversity in starch (~75 g/100 g), amylose (~22 g/100 g), protein (~9.8 g/100 g) and dietary fiber (~4.67 g/100 g), highlighting their functional and nutritional heterogeneity (John et al., 2023). Deepwater rice is also recognized for relatively higher nutritional quality, including elevated protein content (~12-13 g/100 g), dietary fiber, minerals such as iron and zinc and bran oil (~5.5 g/100 g), often exceeding other cereal crops (Rohilla et al., 2025). Notably, the dominance of glutelin as the major storage protein fraction and the relatively higher amylose content reported in several deepwater rice cultivars contribute to distinct functional properties compared to modern lowland rice varieties (Rohilla et al., 2024).

2.6. *Bao* rice

Bao rice represents the regional manifestation of the deepwater rice ecotype, specifically adapted to the unique flood dynamics of the Brahmaputra valley in Assam, India. These ecosystems are characterized by prolonged water stagnation exceeding 50-100 cm for 2 to 6 months, often accompanied by a gradual but continuous rise in water level. Under such conditions, survival depends on sustained growth responses rather than stress avoidance, making the escape strategy a defining feature of *bao* rice cultivation. *Bao* rice exhibits key adaptive traits such as progressive internode elongation, ethylene-mediated growth regulation, aerenchyma development and post-flood kneeing ability, which collectively enable persistence under prolonged submergence (Rohilla et al., 2025). In Assam, these flood-prone systems support large agrarian communities, where *bao* rice often represents the only reliable crop during the monsoon season, underscoring its socio-economic significance. Morphological characterization based on Distinctness, Uniformity and Stability (DUS) descriptors has revealed extensive phenotypic variability among *bao*

rice accessions, including differences in culm length, elongation patterns, tillering behavior, panicle structure, awn presence, grain size and seed coat coloration (Rajkhowa and Borgohain, 2025). This diversity reflects both the underlying polygenic control of flood adaptation and long-term farmer-driven selection across heterogeneous environments. While sharing the core adaptive framework of deepwater rice, *bao* rice represents a localized expression shaped by regional selection pressures. Traits such as rapid elongation and kneeing ability are functionally linked to *SNORKEL*-mediated pathways, reinforcing its classification within the deepwater ecotype rather than as a separate agronomic category (Rajkhowa and Borgohain, 2025). At the genetic level, *bao* rice relies predominantly on elongation-associated regulatory mechanisms rather than *SUB1*-mediated quiescence. Genome-wide association studies (GWAS) on Northeast Indian rice germplasm, including *bao* types, have identified multiple Quantitative Trait Loci (QTLs) across chromosomes 2, 4, 6 and 9, encompassing genes encoding zinc-finger and C3HC4-type domain proteins involved in transcriptional regulation and signal integration (Phukon et al., 2024). These findings highlight the complex genetic architecture underlying its adaptive performance under prolonged flooding.

2.6.1. Pigmented and non-pigmented *bao* rice

Within the *bao* rice group, both pigmented and non-pigmented varieties exist, reflecting genetic diversity superimposed on the deepwater adaptation background. A large proportion of *bao* rice landraces possess red or dark-colored kernels due to accumulation of anthocyanins and other phenolic compounds in the pericarp and aleurone layers. These pigmented *bao* rice types are widely reported to contain high levels of phenolics (up to ~900 mg GAE/100 g), flavonoids (~286 mg QE/100 g) and anthocyanins (~93 mg/100 g), contributing to strong antioxidant activity (Singh et al., 2022). However, studies on indigenous *bao* rice germplasm from Assam reveal an even broader compositional range. For instance, total phenolic content in brown pigmented rice has been reported to vary from 752.89 to 2223 mg/100 g, while total flavonoid content ranges from 252.12 to 1000.75 mg QE/100 g and anthocyanin content from 76.05 to 159.42 µg/100 g (Mudoi

and Das, 2019). Correspondingly, antioxidant activity (2,2-Diphenyl-1-picrylhydrazyl, DPPH radical scavenging) in these varieties remains remarkably high, ranging between 81.5% and 96.0%, highlighting their strong free radical scavenging potential (Mudoi and Das, 2019). Additionally, they are rich in antioxidant enzymes such as catalase, superoxide dismutase and ascorbate peroxidase, which further enhance their oxidative stress mitigation potential (Singh et al., 2022).

Deepwater pigmented *bao* rice also contains appreciable levels of protein (9-13%) and essential micronutrients such as iron, zinc and calcium, making them nutritionally superior compared to non-pigmented counterparts (Kalita and Handique, 2020). Detailed mineral profiling of indigenous red *bao* rice indicates that iron content can vary widely from 2.12 to 54.40 mg/100 g, zinc from 2.42 to 26.57 mg/100 g and manganese up to 25.13 mg/100 g in brown rice forms (Mudoi and Das, 2019). Such variability reflects both genetic diversity and environmental influences, particularly soil conditions in flood-prone ecosystems. Non-pigmented *bao* rice varieties, despite their lower phenolic content, exhibit comparable flood survival and are widely cultivated for household use. Their coexistence with pigmented types indicates that grain pigmentation is independent of flood tolerance, with both groups sharing similar adaptive mechanisms while differing mainly in nutritional and functional properties (Rohilla et al., 2024).

2.6.2. Performance of *bao* rice in flood-prone ecosystems

Field studies on *bao* rice cultivars such as *Amona*, *Maguri bao*, *Dal bao*, *Rangoli* and others demonstrated substantial variations in plant height, internode number, panicle length, spikelet number, grain filling percentage and yield under deepwater conditions. *Maguri bao*, in particular, exhibited superior physiological performance, combining rapid elongation with high spikelet number and grain filling, illustrating how effective escape growth translates into relatively stable yield under prolonged flooding (Rajkhowa and Borgohain, 2025). Although yields of *bao* rice are lower than those of irrigated lowland rice, typically ranging between 1.5 and 2.0 t ha⁻¹, these yields represent successful crop production in environments where other rice ecotypes fail completely (Mondal, 2025).

2.6.3. Food functionality of *bao* rice

The gene-regulated growth strategy and resulting grain composition of *bao* rice have direct implications on food functionality. Notably, the relatively high amylose content enables processability in starch-based systems, resulting in improved functionality. This higher amylose fraction is also associated with slower starch digestibility, supporting the development of low-glycemic index foods. In parallel, the presence of abundant bioactive compounds particularly phenolics, flavonoids and anthocyanins in pigmented *bao* rice significantly enhances the nutritional and functional quality. These compounds contribute to increased antioxidant activity and confer added phytochemical richness, thereby improving the nutraceutical value of processed foods (Kalita and Handique, 2021). Recent studies on extrusion processing of red rice (*bao dhan*) demonstrated that processing conditions critically influence the retention of functional attributes. Conventional high-temperature extrusion led to degradation of heat-sensitive bioactive compounds, whereas vacuum-assisted extrusion significantly improved phytochemical retention while maintaining desirable physical properties. For instance, extruded products retained anthocyanins (~3.89 mg/l), along with cyanidin-3-glucoside and peonidin-3-glucoside, highlighting their potential for nutritionally enriched ready-to-eat foods (Ramchiary and Das, 2020). However, absence of standardized processing strategies tailored to the unique starch-protein-bioactive matrix of *bao* rice has limited its wider application. Importantly, these challenges are not inherent drawbacks but reflect the need for targeted processing interventions that leverage the intrinsic functional and nutraceutical potential. Processing parameters significantly influence functional properties. Optimized conditions, therefore, promote desirable product characteristics.

2.6.4. Economic significance and developmental gaps

Bao rice is integral to the subsistence economy of flood-prone districts of Assam, including Dhemaji, Lakhimpur, Majuli and Sibsagar, where it serves as the primary crop during the monsoon. In the Brahmaputra valley, prolonged flooding (2-6 months) creates

conditions unsuitable for conventional crops, necessitating cultivation of deepwater ecotypes such as *bao* dhan (Gogoi et al., 2020). Its cultivation is typically low-input or organic due to water stagnation and minimal fertilizer use, enhancing sustainability (Rohilla et al., 2020). Despite its agronomic resilience and nutritional potential, especially in pigmented varieties, *bao* rice remains largely underutilized beyond traditional consumption. Research has primarily focused on genetic diversity and flood tolerance, with limited emphasis on processing, product development, or value addition. This gap has restricted its integration into modern food systems and highlights the need for targeted research. Expanding value-added applications and functional food development from *bao* rice could significantly enhance market opportunities, thereby improve farmers' income and contribute to rural economic growth in flood-prone regions.

2.7. Processing of pigmented rice and their effects

Processing of pigmented rice encompasses a sequence of primary operations such as dehulling, milling, parboiling and drying, followed by secondary treatments including thermal processing (baking, roasting, extrusion), cooking and biobased approaches such as germination and fermentation. Each of these processes induce structural, physicochemical and biochemical transformations in the grain matrix, starch gelatinization and ultimately determine the retention, transformation or degradation of nutrients and bioactive compounds such as phenolics, flavonoids, anthocyanins, vitamins and minerals. The extent of these changes is governed by processing conditions (temperature, moisture, pressure), grain characteristics (waxy/non-waxy, genotype) and environmental factors.

Dehulling and milling are the first major steps in rice processing, primarily aimed at removing the husk and bran layers. However, since pigmented rice stores most of its bioactive compounds in the bran, these processes significantly influence nutritional quality. Early studies by Luh (1975) and Dexter (1980) demonstrated that milling drastically reduces essential nutrients, including thiamine (68-82%), niacin (64-79%),

riboflavin (57%), folic acid (60-67%), pantothenic acid (51-67%), pyridoxine (43-86%), biotin (86%), vitamin E (82%), proteins (10-16%), lipids (77-82%) and dietary fibre (63-78%). In pigmented rice, this effect is even more pronounced. A short milling duration of only 10 s resulted in decreases of 74.49% in anthocyanins, 55.35% in γ -oryzanol and 70.36% in α -tocopherol (Laokuldilok et al., 2013). Even minimal polishing intensified these losses. For instance, a 4% degree of milling reduced total flavonoids by 58.6% in black rice and 71.2% in red rice, while anthocyanin losses reached 53% and 91% at 4% and 7% milling, respectively (Paiva et al., 2014). Further polishing to around 9% resulted in nearly 90% depletion of total phenolics along with substantial mineral loss (Reddy et al., 2017). Progressive milling from 0 to 25% showed that even mild treatment retained only about 30% anthocyanins and 69% phenolics, with further losses occurring as milling intensity increased (Choi et al., 2018, 2021). However, structural disruption during milling occasionally led to a slight increase in certain polar phenolics such as protocatechuic and syringic acids due to redistribution effects (Paiva et al., 2016). Plate milling further illustrated this complexity, where insoluble polyphenols and γ -oryzanol increased, whereas tannins, flavonoids and antioxidant activity declined (Sapna et al., 2019). Thus, milling not only reduces nutritional quality but also alters starch composition, increasing digestibility by removing the phenolic barriers that otherwise inhibit enzymatic hydrolysis.

Parboiling is a hydrothermal treatment involving soaking (to 30-40% moisture), steaming and drying (<14% moisture), which facilitates nutrient migration from the bran into the endosperm. During this process, starch undergoes gelatinization and subsequent retrogradation, forming more ordered crystalline structures leading to increases in slowly digestible starch (SDS) and resistant starch (RS) (Oliveira et al., 2026). Parboiling also modifies bioactive compounds, although results vary depending on conditions and genotype. Under conditions involving soaking at 60 °C for 4 h, autoclaving at 108 °C for 10 min and drying to 13% moisture, parboiled pigmented rice exhibited a 4.3-9.9-fold increase in free phenolic content compared to non-parboiled rice, indicating migration of bound phenolics into the endosperm (Paiva et al., 2016). However, when more severe

conditions were applied, such as autoclaving at 121 °C for 20 min followed by drying at 200 °C for 15 min, reductions in total phenolics, anthocyanins and antioxidant activity were observed, although an increase in vitamin E homologues and low-degree polymerized proanthocyanidins was noted (Min et al., 2014). The effect of parboiling is therefore highly dependent on treatment severity and duration. While some studies reported reductions in dietary fiber, total phenolics and DPPH antioxidant activity without significant changes in protein and ash (Pinta et al., 2014), others demonstrated that parboiling for more than 5 min increased free phenolic content and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) antioxidant activity despite a decrease in bound phenolics (Hu et al., 2019). Paiva et al. (2016) reported higher protein migration in red rice compared to black rice, along with reduced ash content. Structural changes during parboiling influenced physicochemical properties. Parboiling promotes the formation of amylose double helices leading to increased crystallinity and a significant rise in resistant starch content. Corroborative studies by Hu et al. (2019) reported increased pasting viscosity (except breakdown) and higher thermal parameters (T_o , T_p , T_c), while enthalpy decreased, indicating disruption of crystalline regions. These transformations illustrated that parboiling simultaneously facilitates nutrient migration, structural reorganization and selective degradation of heat-sensitive compounds.

Drying, which follows parboiling or is applied independently, further governs the stability and transformation of bioactive compounds while ensuring storage stability. Drum drying resulted in a significant reduction in total phenolic content (TPC) and total antioxidant capacity (TAC), although anti-inflammatory activity remained unaffected, indicating selective preservation of biological functionality (Qiu et al., 2021). Fluidized bed drying caused reductions in cyanidin-3-O-glucoside and caffeic acid, yet improved starch digestibility and enhanced the bioaccessibility of ferulic acid, suggesting conversion of bound phenolics into more available forms (Ziegler et al., 2020). When drying was performed across a temperature range of 20-100 °C followed by 12 months of storage, substantial degradation of free phenolics such as ferulic, p-coumaric, caffeic and gallic acids was observed, particularly under conventional atmospheric conditions, whereas

storage under nitrogen significantly preserved these compounds (Lang et al., 2020a). The importance of packaging conditions was further emphasized when hot air drying at 40 °C resulted in greater losses of anthocyanins and γ -oryzanol compared to sun drying, but the use of nylon/LLDPE packaging filled with nitrogen effectively preserved total anthocyanin content, total phenolics and the key aroma compound 2-acetyl-1-pyrroline (Norkaew et al., 2017). High-temperature drying (40-100 °C) followed by storage led to reductions in proanthocyanidins, phenolics and protein, while increasing hardness and grain defects (Ramos et al., 2022). High-intensity drying using superheated steam at 250-300 °C for 1 min under 145 kPa pressure reduced phenolic content and antioxidant activity but decreased cooking time by 1-5 min due to structural weakening of the grain (Piyawanitpong, 2018). Drying also induces important functional changes. High-temperature fluidization decreased glycemic index and increased resistant starch content without affecting antioxidant activity, while prolonged exposure at 100-150 °C increased free fatty acids, reflecting lipid hydrolysis, with optimal conditions identified at 150 °C for 3 min (Junka et al., 2017). Thus, drying represents a balance between preservation and transformation, strongly influenced by temperature, duration and storage environment.

Thermal processing methods such as baking, roasting and extrusion further modify the rice matrix. Baking reduces anthocyanins due to enzymatic oxidation and air incorporation during dough preparation. However, it can increase free phenolic acids by releasing bound forms (Melini et al., 2019). Extrusion cooking, a high-temperature short-time (HTST) process, enhances starch gelatinization and protein digestibility while maintaining nutritional quality. Temperatures of 90-110 °C show minimal anthocyanin degradation, whereas temperatures above 120 °C cause slight losses (Kumar and Murali, 2020). Process optimization using higher moisture and screw speed improves anthocyanin retention.

Cooking represents the final stage of processing, where the combined effects of heat and water determine the actual nutritional profile. Water-based cooking methods are particularly detrimental due to leaching of water-soluble compounds. Boiling reduced anthocyanin content from 6.99 $\mu\text{g/g}$ in raw rice to 0.94 $\mu\text{g/g}$, corresponding to nearly 90%

loss (Colasanto et al., 2021). Similarly, soaking followed by wet cooking resulted in losses ranging from 73% to 93% of total anthocyanins in purple rice (Yamuangmorn et al., 2018). Significant quantities of phenolics are lost into cooking water, which contained 614-711 mg cyanidin-3-O-glucoside and 240-545 mg catechin (Fracassetti et al., 2020). The extent of degradation varies with cooking method, with anthocyanin loss following the order roasting > steaming > pan-frying > boiling (Surh and Koh, 2014; Ryu and Koh, 2017). Microwave cooking presents contrasting outcomes; while some conditions resulted in more than 90% loss of phenolics (Chatthongpisut et al., 2015), others showed significant increases in total phenolic content (1.2-2.0 fold), total anthocyanin content (2.0-3.2-fold), DPPH (1.3-2.5 fold) and FRAP (1.5-2.4 fold) compared to steaming (Thuengtung and Ogawa, 2020), indicating that processing parameters such as power and duration are critical determinants. Cooking methods that minimize water usage, such as risotto cooking, preserved higher levels of phenolics, anthocyanins and procyanidins (Zaupa et al., 2015), while electric rice cookers and water bath techniques resulted in lower losses compared to pressure cooking (Fracassetti et al., 2020). Additionally, cooking in the presence of lipids such as rice bran oil enhanced resistant starch formation and reduced glycemic response through amylose-lipid complex formation (Krishnan et al., 2020).

Biobased processing methods such as germination and fermentation improve nutritional and functional properties through enzymatic and microbial activity. Germination enhances water absorption, dough stability, phenolic content and γ -aminobutyric acid (GABA), while improving bread volume and reducing hardness (Mehmood et al., 2023). Fermentation further enhances bioactive compounds. Lin et al. (2024) reported increases in phenolic acids such as protocatechuic, syringic, caffeic and ferulic acids, although anthocyanins decreased by 10.80-50.38%. Jan and Kumar (2025) observed increases in total phenolics (8.16 to 10.73 mg GAE/g), flavonoids (125.51 to 187.21 mg RE/100 g) and antioxidant activity (28.91%), along with reductions in tannins (50.47%) and phytic acid (65.65%). Zhu et al. (2024) reported a 1.7-fold increase in protein and 1.4 fold increase in phenolics in red rice. However, some fermentation processes caused significant losses of flavonoids (83.16%) and carotenoids (Janarny and Gunathilak, 2020).

Therefore, processing of pigmented rice results in a complex interplay of degradation and enhancement mechanisms. While milling and high-temperature treatments lead to substantial losses of bioactive compounds, processes such as parboiling, controlled cooking, germination and fermentation improve bioavailability and functional properties. The variability among studies highlights the importance of optimizing processing conditions to preserve nutritional quality, while enhancing technological and functional attributes.

2.8. Chemical modification and effect on starch functionality

While hydrothermal and mechanical processing methods such as parboiling, steaming, drying and milling influence the functional properties of pigmented rice, their effectiveness is largely governed by starch, which constitutes more than 70% of the rice grain. Despite processing, starch still exhibits inherent limitations such as retrogradation, syneresis and instability under thermal, shear and freeze-thaw conditions; factors that are particularly critical in systems requiring structural integrity and water retention. To address these limitations, chemical modification is widely employed to tailor starch functionality. By introducing functional groups into starch molecules, properties such as gelatinization behavior, viscosity, swelling and environmental stability can be effectively controlled (Gałkowska et al., 2023; Compant et al., 2023).

Chemical modification of starch often involves the substitution or transformation of hydroxyl groups in amylose and amylopectin chains, resulting in controlled changes in physicochemical and functional properties such as gelatinization behavior, viscosity and stability under processing conditions (Gałkowska et al., 2023). Among these, cross-linking is one of the most widely utilized modification techniques due to its ability to significantly enhance structural integrity and resistance under processing conditions. It is achieved through the formation of inter and intramolecular covalent bridges between starch chains that restrict granule swelling and molecular mobility. As a result, cross-linked starch exhibits increased gelatinization temperature and enthalpy, decreased

swelling capacity, higher paste viscosity, improved cohesiveness, enhanced gel strength and greater thermal stability of pastes and gels, along with altered susceptibility to amylolytic enzymes (Compart et al., 2023). Esterification, including acetylation and Octenyl succinic anhydride (OSA) modification, involves the substitution of hydroxyl groups with acyl groups, reducing intermolecular hydrogen bonding and introducing steric hindrance. Acetylated starch shows increased swelling and water absorption capacity, reduced gelatinization temperature, increased peak viscosity and decreased retrogradation and syneresis, along with improved paste clarity and higher susceptibility to enzymatic hydrolysis. In the case of Octenyl succinic anhydride (OSA)-modified starch, the reduction in gelatinization temperature and enthalpy is accompanied by the development of surface-active properties, enabling reduction of surface tension, stabilization of emulsions and encapsulation of bioactive compounds (Tian et al., 2018). In etherification, particularly hydroxypropylation, bulky substituents are introduced into the starch structure, disrupting chain interactions. This results in reduced gelatinization temperature, increased paste clarity, improved freeze-thaw stability, enhanced resistance to extreme pH, high temperatures and shear forces, and reduced retrogradation, making such starches highly suitable for systems subjected to harsh processing conditions (Pooremaeil et al., 2021; Musarurwa et al., 2020). In contrast, oxidation converts hydroxyl groups into carbonyl and carboxyl groups, leading to partial depolymerization of starch chains. Consequently, oxidized starch exhibits reduced gelatinization temperature and enthalpy, lower paste viscosity, improved thermal stability and clarity, reduced gelling ability and retrogradation and enhanced adhesion and film-forming properties, supporting its application in coatings and structured food systems (Vanier et al., 2017). Acid hydrolysis modifies starch by cleaving glycosidic linkages, producing lower molecular weight fractions characterized by reduced paste viscosity but increased gel strength and solubility, which are advantageous in applications such as gelling agents and fat replacers (Chen et al., 2017). Additionally, grafting introduces polymer chains onto the starch backbone, enhancing viscosity, thermal stability and biodegradability, thereby enabling applications

in pharmaceuticals, agriculture, wastewater treatment and biodegradable materials (Compart et al., 2023).

2.9. Convenience foods

Convenience foods have become a dominant component of modern food systems, driven by rapid urbanisation, time scarcity, changing household structures, increased workforce participation and shifting domestic labour patterns. Across global food environments, convenience is recognized as a multidimensional construct encompassing reduced time, physical effort, mental effort and skill requirements across food planning, acquisition, preparation, consumption and post-meal activities (Bogard et al., 2024; Brunner et al., 2010). These characteristics have positioned convenience foods as practical solutions for contemporary lifestyles, contributing to their widespread adoption across diverse population groups. From a market perspective, the convenience food sector has shown sustained growth over the past two decades, supported by urbanization, technological advancements and expansion of modern retail and digital food systems (Sequeira et al., 2026). Ready-to-eat, ready-to-cook and related food categories constitute a significant share of this market, particularly among young adults, working populations and urban consumers seeking time-efficient meal solutions. Technological innovations such as microwave cooking, high-pressure processing, modified atmosphere packaging and advanced preservation techniques have further enhanced shelf-life, safety and accessibility of these products (Al-Mansoori et al., 2025; Cui et al., 2024).

The primary advantages of convenience foods include time savings, reduced cooking burden, standardized quality, extended shelf life and ease of storage (Jackson and Viehoff, 2015). At the household level, they also enable time reallocation from cooking to work, leisure or caregiving activities, reinforcing habitual consumption patterns (Woy et al., 2024). However, despite these benefits, concerns regarding nutritional quality persist, as many convenience foods particularly ultra-processed variants contain high levels of refined carbohydrates, added sugars, saturated fats, and sodium, which are associated with

obesity, cardiovascular diseases and type-2 diabetes (Al-Mansoori et al., 2025; Agarwal, 2023).

2.9.1. Categories based on degree of preparation

Based on the extent of processing and consumer involvement prior to consumption, convenience foods are commonly classified into ready-to-eat (RTE), ready-to-heat/serve (RTH/RTS), ready-to-cook (RTC) and ready-to-prepare (RTP) products. The concept of “3R foods” (ready-to-eat, ready-to-heat and ready-to-cook) further illustrates the spectrum of convenience foods that cater to varying consumer needs and preferences (Cui et al., 2024). This framework highlights the gradation of convenience levels, ranging from fully processed foods requiring no preparation to partially processed products requiring minimal cooking, thereby aligning with diverse consumer expectations for time efficiency, ease of preparation and freshness perception.

Ready-to-eat (RTE) foods are fully processed products that can be consumed directly without further preparation. These foods are designed to ensure microbiological safety, extended shelf life, and consistent quality through technologies such as thermal processing, dehydration and retort processing (Temgire et al., 2021). They represent the highest level of convenience and are widely consumed in urban environments. Ready-to-heat or ready-to-serve (RTH/RTS) foods require minimal reheating before consumption and provide a balance between convenience and freshness perception. These products are commonly associated with microwaveable meals and chilled or frozen prepared foods and are particularly relevant in institutional and urban food systems (Cui et al., 2024). Ready-to-cook (RTC) foods require short cooking times but eliminate extensive preprocessing steps such as soaking, grinding or prolonged boiling. RTC foods are defined as pre-processed meals that can be prepared quickly using simple cooking methods, making them highly suitable for modern consumers seeking both convenience and perceived freshness (Cui et al., 2024; Roy and Khatun, 2024). Another similar category is ready-to-prepare (RTP) foods represent semi-processed ingredients that reduce preparation effort while

allowing consumer control over final cooking. These include pre-processed cereal flours, mixes and partially treated ingredients that simplify cooking without fully replacing traditional preparation methods. However, ready-to-eat foods constitute a subset of the broader category of convenience foods, and the terms RTS, RTP and RTC are often used interchangeably with ready-to-eat products. Such products align with the broader definition of convenience as reducing time, effort and skill requirements across food-related activities (Bogard et al., 2024).

2.9.2. Nutritional, functional and value-added convenience foods

Convenience foods are increasingly differentiated not only by their level of preparation but also by their nutritional value, functional attributes and suitability for specific dietary needs. Functional convenience foods are formulated to provide health benefits beyond basic nutrition, often incorporating bioactive compounds, dietary fibre and antioxidant-rich ingredients, reflecting growing consumer awareness of diet-related health issues and demand for healthier food options (Baker et al., 2022). In parallel, fortified and enriched convenience foods are being developed to address micronutrient deficiencies through the addition of vitamins and minerals, particularly in developing regions where improving nutritional adequacy remains a priority (Al-Mansoori et al., 2025).

The rising prevalence of lifestyle disorders and food intolerances has further driven the development of specialized convenience foods tailored to specific dietary requirements, including gluten-free, low-glycemic, low-fat and low-sodium formulations. Consumer acceptance of these products is influenced by factors such as perceived health benefits, product characteristics and psychological attitudes, emphasizing the importance of aligning nutritional improvements with consumer expectations (Baker et al., 2022). In addition, value-added convenience foods have gained prominence, where processing technologies are employed to enhance functionality, stability, nutritional quality and sensory attributes. Techniques such as extrusion, high-pressure processing, and modified atmosphere packaging have enabled the development of innovative products with

improved shelf life, safety and overall acceptability (Temgire et al., 2021; Al-Mansoori et al., 2025). These advancements facilitate the transformation of traditional staples into modern, high-value convenience foods. However, alongside these developments, there is an increasing emphasis on ensuring that technological innovations are aligned with sustainability considerations, particularly in relation to packaging waste and environmental impact (Woy et al., 2024).

2.10. Pigmented rice-based food products

The application of pigmented rice has expanded beyond traditional consumption to include a wide range of food and non-food products due to its rich bioactive composition and functional properties. It is widely utilized in products such as extruded snacks, pasta, bakery items, fermented foods and traditional formulations, where it enhances nutritional quality and antioxidant potential (Table 2.4). In addition, pigmented rice-derived compounds are increasingly explored in non-food applications, including biodegradable films, natural colorants and functional materials with antioxidant and UV-protective properties. However, processing conditions significantly influence product quality and retention of bioactive compounds, presenting important challenges for product development.

2.10.1. Extruded products

Extrusion processing has been extensively explored for developing pigmented rice-based breakfast cereals and snack products, primarily due to its ability to induce starch gelatinization, modify structure and enhance digestibility. Under optimized conditions of high temperature and low feed moisture, pigmented rice formulations generally produced desirably expanded products with low density. For example, multigrain extrudates containing red rice processed at 130 °C, 350 rpm and 10% feed moisture showed low bulk density ($\sim 1.5 \text{ g/mm}^3$) and good expansion (~ 1.16), indicating efficient structural transformation and product stability (Sukumar et al., 2023). A similar trend was observed when whole pigmented rice flours were processed at 110-160 °C, 15-21% moisture and

250 rpm, where increased temperature and reduced moisture enhanced expansion (up to 3.30) and specific volume (~5.91 ml/g), along with increased water absorption (~5.47-7.77 g/g), reflecting extensive starch gelatinization (Meza et al., 2019). However, these thermal conditions also resulted in reductions in phenolics (~68-76%) and antioxidant activity (~56-70%), although residual levels remained higher than conventional cereals (Meza et al., 2021). Modification of formulations further influenced product quality. Incorporation of fruit components, such as passion fruit powder, into red rice-based extrudates processed at ~97.5 °C, 250 rpm and ~25.2% moisture improved expansion (~8.05) and antioxidant activity (~65.79% DPPH), with total phenolics reaching ~129.49 mg GAE/100 g (Samyot et al., 2018). Likewise, co-extrusion of pigmented rice at 40-120 °C with 10% moisture and 100 rpm retained considerable antioxidant capacity (ABTS ~17.8 mmol TE/kg), although anthocyanins were highly sensitive and decreased by up to 90% (Blandino et al., 2022). Overall, extrusion improved structural and functional properties while partially compromising thermolabile bioactive compounds.

2.10.2. Pasta and noodle products

In pasta and noodle systems, pigmented rice has been incorporated primarily to enhance nutritional quality, although structural modifications were consistently observed due to gluten dilution. Moderate inclusion levels generally maintained acceptable quality while significantly improving nutritional attributes. For instance, substitution of semolina with black rice bran (5-25%) showed optimal acceptability at 15%, where protein, fibre and anthocyanin content increased, while higher levels reduced cohesiveness and chewiness (Sethi et al., 2020). This behavior was further supported by studies reporting increased water absorption and solubility due to the high swelling capacity of pigmented rice flours (Laishram and Das, 2017; Kumar and Murali, 2020). In gluten-free systems, pasta prepared with 20% red rice flour and processed through extrusion and drying showed improved fibre and antioxidant content but exhibited higher cooking loss and reduced firmness, reflecting weakening of the structural matrix (Cappa et al., 2021). Similarly, pasta based on black glutinous rice flour processed through mixing, extrusion and drying

showed improved cooking quality and texture at moderate fortification (20%), whereas higher levels reduced cohesiveness and antioxidant content (Masavang et al., 2026). As substitution levels increased (20-60%), composite pasta systems processed through extrusion showed reduced cooking time (10-25%) and elasticity (15-30%), but increased cooking loss (20-40%) and stickiness (15-25%), indicating disruption of the gluten network. Despite these changes, nutritional improvements were substantial, with fibre (1.5-2.5 fold), phenolics (2-3 fold) and antioxidant activity (~30-60%) significantly increasing (Subanmanee et al., 2018). Comparable gluten-free pasta formulations also demonstrated acceptable cooking loss (~4.8%) and high consumer acceptance (~67-89%), supporting their practical applicability (Chan et al., 2019). In noodle production, pigmented rice flours were processed through dough formation, sheeting and hydrothermal cooking, where substitution levels strongly influenced quality. Incorporation of black rice bran (2-15%) increased protein, minerals and anthocyanins but resulted in increased hardness and reduced cohesiveness (Kong et al., 2012). Similarly, substitution with purple rice (10-40%) reduced cooking time (5-15%) and elasticity (15-25%), while increasing cooking loss (20-35%) due to weakening of the gluten network (Sirichokworakit et al., 2015). In gluten-free noodles produced through extrusion and cooking, red rice incorporation improved tensile strength (15-25%) and antioxidant content (~2-3 fold), although water absorption (15-30%) and cooking loss (20-40%) increased (Kraithong et al., 2018).

2.10.3. Bakery products

The incorporation of pigmented rice into bakery products significantly enhanced nutritional and functional properties, although structural limitations associated with gluten absence were frequently observed. In bread systems, optimization of formulation and processing was critical. Composite formulations prepared through mixing, fermentation and baking (180 °C) showed reduced hardness (~30-40%) and improved springiness (~15-20%) when appropriate ratios of rice flour and starch were used (Ronie et al., 2017). Further improvements were achieved through fermentation (60-100 min) and enzymatic

treatment (transglutaminase 0.5-1.5%) in red rice, which increased bread volume (~ 3.8 cm³/g) and improved sensory acceptability ($\sim 6.8/9$) (Gusmão et al., 2019). Similarly, breads prepared from pigmented rice flours through conventional baking (180 °C for 40 min) showed higher protein (8.9-10%) and fibre (2.5-7.0%), improved springiness (75-82%) and reduced hardness (~ 5.1 -12.7 N), although defects such as stickiness and cracking were observed (Burešová et al., 2023). Advanced processing techniques further enhanced product quality. Ohmic heating improved bread volume (~ 2.66 -3.45 cm³/g) and antioxidant content (black rice ~ 228.51 mg FAE/100 g) (Evangelista and Schönlechner, 2025), while incorporation of extruded black rice flour increased water absorption (5-12%) and reduced crumb firmness (15-30%) (Ma et al., 2019). Purple rice-based breads also showed increased phenolics (2-4 fold), anthocyanins (50-150 mg/100 g) and reduced glycaemic index (55-65) (Thiranusornkij et al., 2019), while black rice extract incorporation reduced starch digestibility (14-20%) (Sui et al., 2016).

In cake systems, batter-based processing followed by baking showed that increasing pigmented rice substitution (10-100%) enhanced phenolic and antioxidant content but led to increased hardness and reduced volume, with optimal sensory acceptance at moderate levels (Mau et al., 2016). Similar trends were observed in gluten-free cakes, where reduced starch digestibility and improved functional properties were reported (Lang et al., 2020b). Germination further modified product quality, increasing GABA (~ 3.66 mg/100 g after baking) but reducing total phenolics due to enzymatic oxidation (Müller et al., 2021). Brownie formulations also benefited from red rice incorporation, showing improved volume (~ 10 -18%) and reduced hardness (~ 15 -30%) (Penjumras et al., 2014).

In cookies, biscuits and crackers processed through baking (~ 190 °C), pigmented rice incorporation consistently increased fibre (1.5-2.5 fold), phenolics (2-4 fold) and antioxidant activity (~ 40 -70%), while reducing starch digestibility (~ 15 -30%) and glycaemic index (~ 55 -60). However, higher substitution levels resulted in darker colour and altered texture (Soares de Magalhães et al., 2023; Uivarasan et al., 2024).

2.10.4. Fermented products

Fermentation played a crucial role in enhancing the nutritional and functional properties of pigmented rice products by improving bioavailability and inducing biochemical transformations. Fermented beverages using red rice, barley and buckwheat produced through lactic acid fermentation (24 h) showed rapid acidification (pH 7 to 3.9-4.2) and high microbial viability ($\sim 8\text{-}9$ log CFU/ml), contributing to improved stability and digestibility (Cardinali et al., 2021). Similarly, beer prepared through fermentation of pigmented rice with barley showed enhanced antioxidant activity ($\sim 47.68\%$ DPPH), anthocyanin content (~ 35.12 mg/l) and polyphenols (~ 0.410 mg/ml), while maintaining acceptable alcohol levels ($\sim 3.85\text{-}4.17\%$) (Mehra et al., 2020). Traditional fermented foods such as idli, prepared through soaking, grinding, fermentation (12 h) and steaming, showed increased protein ($\sim 12.74\text{-}15.53\%$), phenolics ($\sim 0.25\text{-}0.49$ mg/g) and antioxidant activity ($\sim 0.16\text{-}0.46$ mg/g), although slight reductions in cohesiveness were observed (Susmitha et al., 2022). Instant fermented mixes from black rice processed through fermentation (18 h at 30°C), drying ($\sim 60^\circ\text{C}$) and steaming after reconstitution also showed improved nutritional quality and shelf stability (Dhyani, 2025).

2.10.5. Traditional and functional foods

Pigmented rice has been widely utilized in traditional and functional foods, where hydrothermal processing improved both palatability and bioactive retention. In *kheer*, cooking red rice with milk significantly increased phenolic content (~ 356.24 mg GAE/100 g) compared to conventional formulations (~ 28.21 mg GAE/100 g), while maintaining high sensory acceptability (~ 8.11) (Kumari and Poonia, 2021). Infant cereals prepared from purple wheat and red rice through boiling (100°C) and toasting ($\sim 120^\circ\text{C}$ with enzymatic hydrolysis) showed significantly higher phenolic content ($532\text{-}8626$ $\mu\text{g/g}$) and antioxidant activity ($5.9\text{-}87.1$ $\mu\text{mol TE/g}$), although anthocyanins were partially degraded during processing (Hirawan et al., 2011). In addition, antioxidant beverages developed through hot water extraction ($\sim 87\text{-}97^\circ\text{C}$ for 30-50 min) showed improved phenolic and flavonoid content, with optimal extraction conditions enhancing antioxidant potential

(Handayani, 2015). Functional porridge (red rice) prepared through heating (~95 °C), shear processing, or steaming showed improved digestibility, enhanced polyphenol release and reduced glycaemic response (~63.25), particularly when ultrasonication or steaming was applied (Luangsakul and Ngo, 2025; Nitikornwarakul et al., 2022).

2.10.6. Snack and novel products

Processing techniques such as flaking, roasting and popping further expanded the application of pigmented rice in ready-to-eat snacks. Flake products prepared from orange sweet potato and red rice through heating and flaking showed reductions in phenolics (~49%) and antioxidant activity (~16-23%), although composite formulations improved overall functionality (Jati et al., 2022). In contrast, popped pigmented rice prepared through high-temperature roasting (~250 °C) retained a substantial proportion of bioactive compounds, including γ -oryzanol (~70%), phenolics (~14-28%), flavonoids (~48-65%) and anthocyanins (~38%), while also improving mineral content (Itagi et al., 2023).

2.10.7. Non-food applications

While pigmented rice has been extensively explored for its nutritional and functional roles in food systems, its rich reservoir of anthocyanins and phenolic compounds has also enabled its utilization beyond the food domain. In recent years, these bioactive constituents, particularly from black and red rice, have gained significant attention in the development of non-food materials such as intelligent packaging films, hydrogels, textiles and energy devices, owing to their inherent antioxidant, antimicrobial, UV-protective and pH-responsive properties (Oliveira et al., 2026; Tiozon et al., 2023). In intelligent packaging applications, incorporation of black rice anthocyanins into biopolymer matrices through blending and dispersion techniques resulted in multifunctional films with improved performance. For example, Ge et al. (2020) developed a pectin-chitosan composite film incorporated with black rice anthocyanins, which exhibited enhanced mechanical resistance and a distinct colorimetric transition from red to blue during meat spoilage, enabling real-time freshness monitoring. However, the study also reported

limited moisture resistance and sensitivity of anthocyanins to environmental factors such as light, temperature and pH. In a similar direction, Wu et al. (2019) fabricated a pH-sensitive antioxidant film via dispersion, which demonstrated strong UV–Vis barrier properties, high antioxidant activity and clear pH-dependent color changes, along with improved oxygen and moisture barrier properties despite reduced tensile strength and effectively delayed spoilage in shrimp and fish. Using a casting approach, Yong et al. (2019) developed chitosan-based films incorporating black and purple rice extracts, where black rice extract showed higher phenolic and anthocyanin content, resulting in superior antioxidant activity. However, increasing extract concentration beyond 1wt% negatively affected film homogeneity, indicating the importance of optimized incorporation levels. Further advancements were reported by Zeng et al. (2022), who incorporated black rice extract into soybean protein isolate/chitosan (SPI/CS) films, leading to improved water barrier properties, enhanced antioxidant capacity and better membrane structural stability, although color sensitivity was lower compared to other anthocyanin sources. More recently, Hao et al. (2023) developed an antibacterial intelligent packaging film using black rice extract, which exhibited good mechanical properties, strong antioxidant and antibacterial activity, UV-barrier characteristics and distinct pH-sensitive color changes, ultimately contributing to improved pork preservation through reduced microbial spoilage and delayed odor formation. Beyond film-based systems, pigmented rice extracts have also been utilized in hydrogel matrices. Thanyacharoen et al. (2017) reported that a composite hydrogel loaded with black rice extract exhibited high total phenolic content and antioxidant activity, with a controlled *in vitro* release of 60-80% of the extract within 60 min, demonstrating its potential for controlled delivery applications. In textile applications, Hong (2015) showed that cotton and wool fabrics dyed with black rice extract exhibited antibacterial and antioxidant properties, which were further enhanced through repeated dyeing cycles, indicating improved functional durability. In addition to biomaterial applications, black rice anthocyanins have also been explored in renewable energy systems. Al Qibtiya et al. (2016) utilized black rice anthocyanin extract as a natural dye in dye-sensitized solar cells, where photovoltaic performance improved with changes

in anthocyanin pH, reflecting the sensitivity of pigment structure to environmental conditions.

Table 2.4. Food and non-food applications of pigmented rice

Rice Type	Product Developed	Processing Method	Reference
Extruded Products			
Red rice	Extruded breakfast cereal	Extrusion	Sukumar et al., 2023
Black & Red rice	Breakfast cereal	Extrusion	Meza et al., 2019, 2021
Red rice	Extruded snack	Extrusion	Samyot et al., 2018
Pigmented rice	Co-extruded snacks	Extrusion	Blandino et al., 2022
Pasta and Noodle Products			
Black rice bran	Pasta	Extrusion	Sethi et al., 2020
Red rice flour	Gluten-free pasta	Extrusion	Cappa et al., 2021
Black glutinous rice	Pasta	Extrusion	Masavang et al., 2026
Purple rice (Riceberry)	Noodles	Dough formation + sheeting (hydrothermal cooking)	Sirichokworrakit et al.
Red rice	Gluten-free noodles	Extrusion + hydrothermal cooking	Kraithong et al., 2018
Bakery Products			
Red rice flour	Bread	Fermentation + baking	Gusmão et al., 2019
Black rice extract	Functional bread	Baking (thermal treatment)	Sui et al., 2016
Black rice flour	Cake	Baking (thermal treatment)	Mau et al., 2016
Pigmented rice	Gluten-free cake	Baking (thermal treatment)	Lang et al., 2020
Red rice flour	Cookies	Baking (dry heat)	Soares de Magalhães et al., 2023
Purple rice	Biscuits	Baking (dry heat)	Klunklin and Savage, 2018
Black rice	Crackers	Baking (dry heat)	Uivarasan et al., 2024
Fermented Products			
Red rice	Fermented beverage	Fermentation	Cardinali et al., 2021
Red rice	Beer	Fermentation	Mehra et al., 2020
Black & Red rice	Idli	Fermentation + steaming	Susmitha et al., 2022
Traditional and Functional Foods			
Pigmented rice	Antioxidant drink	Hydrothermal extraction (cooking)	Handayani et al., 2015
Red rice	Infant cereal	Cooking + toasting	Hirawan et al., 2011
Red rice	Kheer	Hydrothermal cooking	Kumari & Poonia, 2021
Red rice	Porridge	Hydrothermal cooking	Luangsakul & Ngo, 2025
Non-food applications			
Black rice	Intelligent film from anthocyanin extract	Blending into pectin-chitosan matrix	Ge et al., 2020
Black rice	Intelligent film from anthocyanin extract	Dispersion in a biopolymer film system	Wu et al., 2019
Black rice	Intelligent film from extract	Casting in chitosan based film	Yong et al., 2019
Black rice	Natural dye in dye-sensitized solar cells	Anthocyanin Extraction	Al Qibtiya et al., 2016

2.11. Conclusion

The body of literature reviewed in this chapter collectively demonstrates that pigmented rice is a nutritionally and functionally distinct cereal system shaped by genetic regulation, biochemical composition and ecological adaptation. Unlike polished white rice, pigmented rice retains its bran and aleurone layers, which serve as reservoirs of diverse bioactive compounds including phenolic acids, flavonoids, anthocyanins and proanthocyanidins. These compounds are not only structurally diverse but also differ in their stability, bioaccessibility and interaction with starch, thereby influencing both nutritional functionality and processing behavior. The distinction between red and black/purple rice, governed by differential activation of flavonoid biosynthetic pathways and regulatory genes such as *Rc*, *Rd* and *Kala* loci, further reinforces that pigmented rice represents multiple functional phenotypes rather than a single category. From a health perspective, the literature provides consistent and multi-level evidence highlighting the role of pigmented rice in mitigating oxidative stress, inflammation, hyperglycemia and lipid dysregulation. These effects are linked to enzyme inhibition, modulation of signaling pathways, improvement of gut microbiota and enhancement of endogenous antioxidant defense systems.

Within this broader framework, deepwater rice systems introduce an additional dimension where environmental adaptation intersects with grain functionality. *Bao* rice, as a regional expression of deepwater rice in the flood-prone ecosystems of Assam, exemplifies this relationship. Its survival strategy is driven by ethylene-mediated elongation. Pigmented *bao* rice, exhibits exceptionally high levels of phenolics, flavonoids and antioxidant activity, along with appreciable mineral content, making it nutritionally superior. Despite these advantages, its utilization remains largely restricted to traditional forms and scientific exploration has been predominantly limited to compositional and genetic characterization, with minimal focus on processing, product development and shelf-life stability.

Processing emerges as a critical factor governing the transformation of pigmented rice from a nutritionally rich raw material into functional food systems. The literature clearly shows that conventional operations such as milling lead to substantial losses of bioactive compounds due to removal of the bran layer, while hydrothermal treatments like parboiling induce complex changes involving nutrient migration, starch gelatinization and formation of resistant starch. Drying and thermal processing further modulate these properties, often causing degradation of heat-sensitive compounds such as anthocyanins, but in some cases enhancing the release of bound phenolics and improving bioaccessibility. Cooking methods, particularly those involving excess water, result in significant leaching losses, whereas controlled methods can help retain nutritional quality. Bioprocessing approaches such as germination and fermentation offer additional advantages by enhancing phenolic content, reducing antinutritional factors and improving functional properties, although they may also lead to selective degradation of certain compounds.

In parallel, the inherent limitations of rice starch such as susceptibility to retrogradation, syneresis and instability under thermal and mechanical stress necessitate targeted interventions for product development. Chemical modification, particularly cross-linking, acetylation, hydroxypropylation and oxidation, has been widely reported to improve structural stability, rheological behavior and resistance to processing stresses. These modifications are especially relevant in designing structured food systems such as gels, extruded products and ready-to-eat formulations, where textural integrity, water retention and storage stability are critical.

The literature on product development further indicates that pigmented rice has been successfully incorporated into a variety of food systems, along with their non-food applications. These studies demonstrate its potential to enhance nutritional quality and antioxidant capacity of food products. However, they also highlight significant challenges related to color retention, textural optimization, cooking quality and stability of bioactive compounds during processing and storage. Notably, while such product-oriented studies

are available for pigmented rice in general, there is a clear absence of systematic research focusing specifically on pigmented *bao* rice, particularly in terms of standardized processing conditions, formulation optimization and shelf-life evaluation of developed products.

Therefore, the literature collectively reveals a critical gap between the inherent potential of pigmented *bao* rice and its practical utilization in modern food systems. While its nutritional richness, functional properties and ecological resilience are well established, the lack of integrated studies linking raw material characteristics with processing behavior, product development and storage stability remains a major limitation. Addressing these gaps is essential for the development of scientifically standardized, nutritionally enriched and shelf-stable ready-to-eat products. Such advancements would not only facilitate the incorporation of *bao* rice into contemporary convenience food systems but also create avenues for value addition, thereby enhancing farmer income and supporting sustainable agricultural practices in flood-prone regions.

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CHAPTER 3

Development of red *bao* rice instant porridge mix by optimizing a hydrothermal process, product characterization and storage stability study

3.1. Introduction

Porridge is a popular ready-to-eat (RTE) product, relished by consumers of all age-groups. Cereals are widely used in RTE and rehydratable porridge industry due to their high starch content, which contribute to the desired thick consistency of porridge. Hydrothermal processes involving heat treatments under added moisture improves hydration and modify crystallinity, thermal, pasting properties, digestibility and content of antinutritional factors of starchy cereal flours, (Deka et al., 2023). Rheology and sensorial properties of rehydrated porridge depend largely on starch granule behavior. Pregelatinization accompanied by severe leaching of simpler glycosidic components in a reconstituted slurry, produce the characteristic viscous texture of porridges contributing to the desired oral lubrication and mouthfeel (Tanjor et al., 2024). For developing instant dry porridge powder, starch is pregelatinized by hydrothermal processes followed by dehydration (Goyat et al., 2019). The extent modification influences storage stability by affecting starch retrogradation and structural integrity, impacting the fat stability, texture and sensory acceptability of instant porridge mixes. Flour hydration properties depend on processing temperature and time, which influence pregelatinized starch behaviour. Swelling power (SP) and water absorption index (WAI) determine moisture uptake and retention, crucial for texture of reconstituted porridge. Water solubility index (WSI) reflects degree of starch conversion and dissolution behaviour. Higher processing temperatures enhance WAI by disrupting hydrogen bonds and increasing hydroxyl group availability for water binding (Sotelo et al., 2023). This modification promotes higher SP. These properties directly impact bulk density (ρ_b) which governs packing efficiency, storage and flow properties, impacting end-use functionality. Hence, WAI,

SP, WSI and ρ_b can be identified as key parameters to consider for developing instant flour. Precise control of these parameters ensures ideal reconstitution, structural integrity and overall performance of an instant mix.

Rice (*Oryza sativa*, L.) with over 70% starch content is ideal for developing rehydratable porridge powder. Northeast India is a hub of rice germplasm, including unique submergence-tolerant deepwater *bao* rice varieties, which are well-adapted to the flood-prone Brahmaputra plains. These rice types require minimal agricultural inputs and exhibit superior resilience. While most *bao* rice varieties yield non-pigmented grains, a select few, are red which are rich in phenolic compounds (1145-2215 mg/100 g) and possess strong antioxidant activities (80-96%) (Mudoi and Das, 2019). Although several pigmented rice varieties from this region have been explored for their nutritional and functional properties (John et al., 2023; Mudoi and Das, 2019; Singh et al., 2022), their application in value-added products remains underdeveloped. Rich in carotenoids and anthocyanins these rice varieties offer potential nutraceutical benefits, making them suitable for functional food development. Leveraging physicochemical and functional properties of the red *bao* rices, can help cater to the currently growing demands for convenient foods and boost the local and global demand of this nascent produce.

This study aimed to develop an instant dry porridge powder base by suitably optimizing hydrothermal processing conditions for three red *bao* rice varieties, namely *Amona*, *Badal* and *Kenkowa*. The developed porridge was evaluated for physical, physicochemical, nutritional, digestibility and bioactive properties. An RTE porridge mix formulated by blending the base powder with caramelized sugar and skim milk powder, reconstituted with water was investigated for sensorial characteristics. Retainment of fat stability, texture and sensorial characteristics over storage was also investigated.

3.2. Materials and methods

3.2.1. Materials

Freshly harvested, pure-line paddy samples of pigmented *bao* rice landraces, namely *Amona*, *Badal* and *Kenkowa*, were procured from the river basin regions of Lakhimpur, Assam, India (Latitude: 27.14° N, Longitude: 94.11° E) in December, 2022. These varieties belong to the high-amylose category, with apparent amylose content ranging from 25-30% (Chapter 1, Table 1.1). The paddy was dehusked (A-Grain, India), dried to 12±1% moisture content under ambient air and stored in airtight containers. Commercial packaged skim milk powder (SMP, Nestle) was purchased. Caramelized sugar powder was freshly prepared by heating white sugar to golden brown, cooling until hard and grinding with corn starch (3%). Analytical grade chemicals were purchased from SRL chemicals, Lobachemie, Himedia, Merck and Sigma-Aldrich.

3.2.2. Hydrothermal processing

Rice (15-30 g) was steeped in water (70-85 g, 100 g total mass), followed by pressure cooking at 15 psig (121 °C) for 5-15 min for *Kenkowa bao* and 10-15 min for *Amona bao* and *Badal bao* in an autoclave. The cooking time range was based on a pretrial study assessing the desired cooked texture of the three rice varieties. Optimization process followed the mentioned range of cooking time and rice concentration for each variety separately (Fig. 3.1). Samples were then dried to a moisture content of <6±2% and ground (150-100 µm).

The rice varieties were coded as K, A and B for *Kenkowa*, *Amona* and *Badal* respectively. K, A and B suffixed with R indicated raw rice resulting in KR, AR and BR. Hydrothermally optimized rice flours were designated with suffix O, giving KO, AO and BO. Porridge mix formulations were indicated by suffix M, where KRM, ARM and BRM represented raw mix samples and KOM, AOM and BOM optimized mix samples prepared from the respective varieties.

3.2.3. Process optimization of dry porridge base

The central composite face-centered (CCFC) model of response surface methodology (RSM) was employed to optimize hydrothermal process conditions. Rice concentration and cooking time were selected as independent variables and evaluated at five levels. The model included 13 experimental runs, comprising 5 center, 4 axial and 4 factorial points. Water absorption Index (WAI), swelling power (SP), water solubility index (WSI) and bulk density (ρ_b) of the developed flours were chosen as response variables. The data were fitted to a second-order polynomial equation (Eq. 3.1).

$$Y = a_0 + a_1X_1 + a_2X_2 + a_{11}X_1^2 + a_{22}X_2^2 + a_{12}X_1X_2 \quad \text{Eq. 3.1}$$

where, Y is the predicted response for the dependent variables, a_0 the fitted response at the center point, a_1 and a_2 the linear points of predicted responses, a_{12} the interaction effect and a_{11} and a_{22} the squared effects. X_1 and X_2 are the independent variables. Data were analyzed by ANOVA at $p \leq 0.05$ significance.

3.2.4. Preparation of reconstituted instant porridge mix

Each optimized base flour (80%, w/w) was thoroughly blended with caramelized sugar powder (10%) and milk powder (10%) all within 150-100 μm particle size. The mixes were packaged in airtight containers until used. For reconstitution, the dry mix was combined with hot water (80 ± 5 °C) at 1:10 ratio.

3.2.5. Proximate composition

Proximate composition of the samples was assessed by standard AOAC (2019) protocols.

Moisture content was determined by the hot-air oven drying method. Approximately 5 g of sample was dried at 105 ± 2 °C until a constant weight was attained, and the loss in weight was recorded as moisture content.

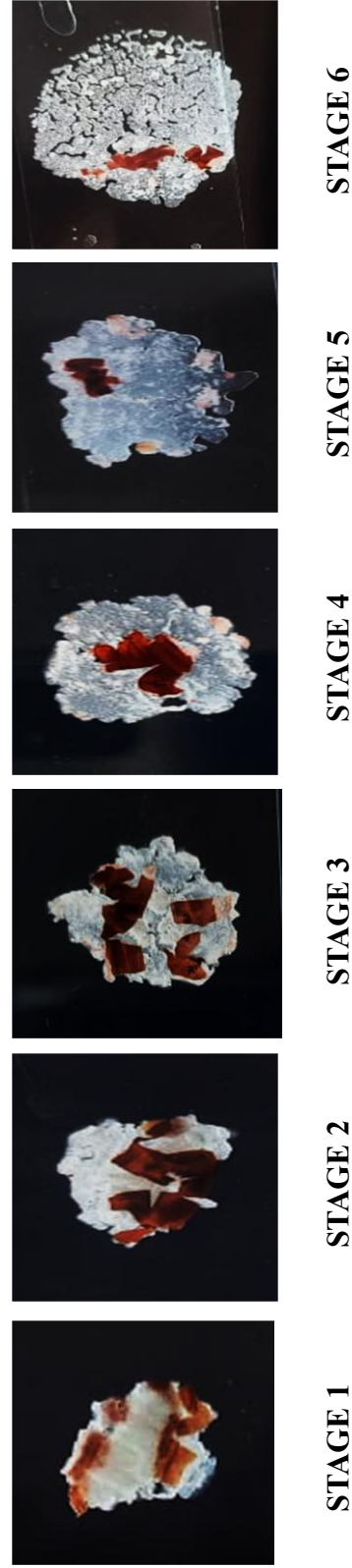


Fig. 3.1. Images of cooked rice and stage of selection

Selection of the cooking time ranges for optimization of the three varieties of rice were determined by the stages of cooked rice as depicted in the figure above. This is a depiction of *Amona bao* variety. Stage 4 and 5 were selected for this variety due to uniform cooking and texture. Stages 1, 2 and 3 shows visible white opaque uncooked portions, while stage 6 depicts overcooked rice, therefore, not considered. Likewise, the ranges of the other two rice were determined.

The dried sample was subsequently used for crude fat determination using a Soxhlet extraction apparatus (SOCS-PLUS, Pelican Equipments, Chennai, India). Samples were extracted with petroleum ether for 1 h. Following extraction, the solvent was evaporated, and the extracted fat fraction was quantified gravimetrically and expressed as percentage fat content.

The defatted sample obtained after Soxhlet extraction was used for crude protein and ash determinations. Crude protein content was estimated by the Kjeldahl method (KEL PLUS Pelican Equipments, Chennai, India), wherein the sample was digested with concentrated sulfuric acid in the presence of potassium sulfate and copper sulfate catalysts, followed by distillation and titration. The nitrogen content obtained was multiplied by a conversion factor of 5.95 to calculate crude protein content.

Ash content was determined by dry ashing in a muffle furnace at 550 ± 50 °C until a light grey or white residue was obtained. The ash was cooled in a desiccator and weighed to determine the ash content.

Total carbohydrate content was calculated through difference method. The energy content of the samples was estimated using Atwater general factors (4 kcal/g for protein, 4 kcal/g for carbohydrates, and 9 kcal/g for fat), following the method described by Merrill and Watt (1973).

3.2.6. Hydration properties

Water absorption Index (WAI), water solubility index (WSI) and swelling power (SP), were determined following modified method of Pradipta et al. (2020). Flour (1 g) was vortexed with 10 ml distilled water, incubated in shaking water bath at 30 °C for 30 min and centrifuged (1308 g, 10 min). The supernatant (dried at 40 °C, 24 h) and the sediment gel was weighed. WAI, WSI and SP were calculated using the following equations (Eq. 3.2, 3.3, 3.4) respectively.

$$WAI(g/g) = \frac{\text{Weight of gel}}{\text{Dry weight of flour taken}} \quad \text{Eq. 3.2}$$

$$WSI (\%) = \frac{\text{Weight of dried supernatant}}{\text{Dry weight of flour taken}} \times 100 \quad \text{Eq. 3.3}$$

$$SP(g/g) = \frac{WAI}{1 - \frac{WSI}{100}} \quad \text{Eq. 3.4}$$

3.2.7. Bulk density

For determination of bulk density (ρ_b), a known mass of flour was allowed to fall in a measuring cylinder from a constant height. The top level of flour was adjusted by gently tapping the base three times on the floor. ρ_b was calculated by the following equation (Eq. 3.5)

$$\rho_b (g/ml) = \frac{\text{mass of flour}}{\text{volume occupied}} \quad \text{Eq. 3.5}$$

3.2.8. Texture

Consistency and cohesiveness of the reconstituted porridge mixes were measured using a texture analyzer (TA.XT. Plus, Stable Microsystems, UK). fitted with a 50kg load cell, a back extrusion rig (piston) operating at a distance of 20mm with an open top cylinder uniformly filled with sample.

3.2.9. Color values

The color characteristics in terms of CIE parameters, namely L^* (lightness or darkness), a^* [(greenness (-) to redness (+))] and b^* [(blueness (-) to yellowness (+))] of the samples were determined using a Color Quest spectrophotometer (Hunter Associates Laboratory, USA). The machine was calibrated using white and black reference tiles prior to the measurements.

3.2.10. Bioactive properties

3.2.10.1. Extraction

Phytochemicals from dry flour samples were extracted in 80% ethanol (1:10, w/v) at 40 °C for 24 h. The extracts were then collected after centrifugation (845 g, 60 min), drying (Evaporator, Equitron Medica) and reconstitution (1:10 ratio, 80% ethanol).

3.2.10.2. Total phenolic content (TPC)

An aliquot (500 µl) of the ethanolic extract was mixed with 2.5 ml of 10% (w/v) Folin–Ciocalteu reagent, followed by the addition of 2 ml of 7.5% (w/v) sodium carbonate. The mixture was vortexed thoroughly and incubated in the dark for 30 min at room temperature. A reagent blank was prepared by substituting the extract with ethanol. The absorbance was measured at 765 nm using Orion AquaMate 8100 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Germany), and total phenolic content was quantified from a gallic acid standard calibration curve. The results were expressed as micrograms of gallic acid equivalents (µg GAE/g) (Pradipta et al., 2020).

3.2.10.3. Total flavonoid content (TFC)

A method described by Pradipta et al. (2020) was slightly modified. Briefly, 0.5 ml extract was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After vortexing and allowing to stand for 5 min, 1 ml of 1 M sodium hydroxide was added. Absorbance was measured at 510 nm and results expressed as quercetin equivalents (µg QE/g).

3.2.10.4. DPPH radical scavenging activity (DRSA)

DRSA was determined as per Pradipta et al. (2020) with slight modifications. Sample extract (200 µl) was mixed with 0.1mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) and

incubated in the dark for 30 min. Absorbance was measured at 517 nm against a blank and results were expressed as DRSA (Eq. 3.6).

$$\text{DRSA (\%)} = \frac{(A_0 - A_s)}{A_0} \times 100 \quad \text{Eq. 3.6}$$

where, A_0 is the absorbance of the blank, A_s is the absorbance of the sample extract

3.2.10.5. Ferric reducing antioxidant power (FRAP)

FRAP was determined as per Zhang et al. (2022). 1 ml sample extract was mixed with 3 ml of freshly prepared FRAP solution and incubated at 37 °C for 4 min. Absorbance was determined at 593nm. A calibration curve was prepared using a concentration range of 0.1 to 1 mM aqueous ferrous sulphate. Values were expressed as ferrous sulphate equivalents (Fe(II)) (mM FeSO₄ /g).

3.2.11. Differential scanning calorimetry

Flour slurries were prepared by dispersing 3 mg of each sample in deionized water (1:2 flour-to-moisture ratio, d.b) in aluminum pans and incubated (4 °C for 1 h), the pans crimped and heated (25 °C-180 °C, 5 °C/min). The onset (T_o), peak (T_p) and conclusion (T_c) temperatures and gelatinization enthalpy (ΔH , J/g) were analyzed using a DSC (DSC60, Shimadzu, Japan).

3.2.12. X-ray diffraction

An X-ray diffractometer (Empyrean, Malvern P analytical, UK) was used to obtain wide angle X-ray diffractograms (XRD) of the samples at 10-40° 2 θ (0.05 steps). Percentage crystallinity was calculated as follows (Eq. 3.7).

$$\% \text{ Crystallinity} = \left(\frac{\text{area under peaks}}{\text{total area under the curve}} \right) \times 100 \quad \text{Eq. 3.7}$$

3.2.13. Fourier transform infrared spectroscopy

Fourier Transform Infrared (FTIR) spectra were obtained using a spectrometer (Bruker Alpha, Germany). Vacuum dried samples were mixed with KBr (1:10 w/w), pelleted and scanned (500 to 4000 cm⁻¹).

3.2.14. Scanning electron microscopy

High-resolution (500X and 10,000X) images were captured using a Scanning electron microscope (SEM, Sigma 300, Zeiss, Germany). Vacuum dried porridge flour base were adhered to carbon tape on an aluminum stub and subjected to gold sputtering before observation.

3.2.15. Digestive properties

A modified Roy et al. (2019) method was used to quantify enzyme-resistant starch (RS), slowly digestible starch (SDS) and rapidly digestible starch (RDS) in the samples. At 37 °C, the flour was first deproteinized with pepsin in HCl-KCl buffer (pH 1.5) for 1 h. Then, pH was adjusted at 5.4 with 7 ml acetate buffer and 3 ml of an enzyme mixture containing 220 U/ml invertase, 3000 U/ml pancreatic α -amylase and 15 U/ml amyloglucosidase was applied. Aliquots were collected on the 20th and 120th minutes of digestion and measured for glucose. The obtained values converted to RDS and SDS, respectively by multiplying the glucose value by a factor of 0.9 and expressed as a percentage ratio to that of total starch (TS) in the sample (AOAC, 2019) (Eq. 3.8, 3.9). Undigested starch remaining after 120 min was considered as RS (Eq. 3.10).

$$RDS(\%) = \left[\frac{G_{20} \times 0.9}{TS} \right] \times 100 \quad \text{Eq. 3.8}$$

$$SDS(\%) = \left[\frac{(G_{120} - G_{20}) \times 0.9}{TS} \right] \times 100 \quad \text{Eq. 3.9}$$

$$RS(\%) = [TS - (RDS + SDS)] \times 100 \quad \text{Eq. 3.10}$$

3.2.16. Sensory evaluation

Sensory evaluation study was conducted with approval by the institutional ethics committee (Clearance No. MZU/HEC/2024/013, dated 17/06/2024). Informed consents were obtained from the sensory panelists. A sensory semi trained panel of 30 members (aged 20-45 years, healthy, non-smoking) with prior experience of sensory evaluation was selected. Panelists underwent two training sessions to get familiarized with the evaluation protocol. Sensory assessment was carried out in three sequential cycles, four samples per cycle and a 1-hour interval between consecutive cycles. Food and beverages, except water, were restricted 30 min before evaluation to minimize sensory fatigue and bias. Reconstituted and anonymized samples (flour-to-water ratio of 1:10) were presented. Samples were rated using the 9-point hedonic scale (1 = dislike extremely; 9 = like extremely) for appearance, aroma, flavor, taste, texture, mouthfeel and overall acceptability (OAA).

3.2.17. Storage study

The samples were packed in air tight containers and stored (26 ± 2 °C; RH=50±5%) for 12 months. Samples were analyzed at 4-month intervals pertaining to retention of fat stability, texture and sensorial characteristics.

3.2.17.1. Water activity

Water activity of the stored porridge samples was estimated using a calibrated water activity meter (METER Aqualab PRE, USA).

3.2.17.2. Free fatty acid (FFA)

A 2 g flour sample was dispersed in a neutral solvent mixture of petroleum ether and ethanol (1:1, v/v) and titrated against 0.1 N potassium hydroxide using phenolphthalein as the endpoint indicator, following AOAC (2019) procedures. The acid value was determined from the titration (Eq. 3.11), and free fatty acid (FFA) content was calculated and expressed as oleic acid equivalents (Eq. 3.12).

$$Acid\ value\ (mg\ KOH/g) = \frac{TV \times N \times 56.1}{W} \quad \text{Eq. 3.11}$$

where, TV: titer value, N: normality of KOH, W: weight of the sample

$$FFA\ (\% \text{ oleic acid}) = Acid\ value \times 0.5 \quad \text{Eq. 3.12}$$

3.2.17.3. Peroxide value (PV)

For PV determination, 5 g sample was dissolved in a 10 ml mixture of acetic acid and chloroform (3:2 v/v). To it, 0.5 ml of saturated potassium iodide (KI) solution was introduced, and the mixture was dark incubated for 1 min. Then, 30 ml distilled water was added and the solution was titrated with 0.01 N sodium thiosulfate using a 1% starch solution as indicator. A stable blue color indicated reaction endpoint (AOAC, 2019). Peroxide value was calculated using the following formula (Eq. 3.13).

$$PV\ (meq/kg) = \frac{TV \times N \times 1000}{W} \quad \text{Eq. 3.13}$$

where, TV: titer value, N: normality of $Na_2S_2O_3$, W: weight of the sample

3.2.17.4. Thiobarbituric acid reactive substances (TBARS)

TBARS of the stored porridge flour samples were assessed following the protocol described by Houketchang Ndomou et al. (2023), with minor modifications. Briefly, 0.25 g sample was combined with aqueous 0.1% trichloroacetic acid (TCA) solution and vortexed. Subsequently, 1 ml each of 0.375% thiobarbituric acid, 15% TCA, and 0.25 N HCl were sequentially added. After vortexing, the mixture was incubated at 95 °C for 30 min and cooled down to room temperature. Absorbance was recorded at 532 nm against a reagent blank. TBARS was calculated using the following equation (Eq. 3.14).

$$TBARS\ (mg\ MDA/kg) = \frac{Abs \times VTCA \times 2 \times M \times 10^{-2}}{1.56 \times W} \quad \text{Eq. 3.14}$$

where, Abs: absorbance, VTCA: volume of TCA solution, M: molar mass of malonaldehyde (72g/mol), W: weight of the sample

3.2.17.5. Texture

The back extrusion test protocol as described previously (Section 3.2.8.) was used.

3.2.17.6. Sensory analysis

Sensory evaluation for taste and OAA was conducted using a 9-point hedonic scale as described in Section 3.2.16.

3.2.18. Statistical analysis

Optimization was conducted using Design Expert 12.0 (Stat-Ease, USA). All the analyses were performed in triplicates and reported as mean $\pm$ standard deviation. Significance of differences among the results were assessed using Duncan's multiple range test ($p\leq 0.05$) using SPSS 21 (IBM, USA). DSC, XRD and FTIR spectra PCA were carried out using Origin 9.0 (OriginLab, USA).

3.3. Results and Discussions

3.3.1. Modelling and fit summary

Experimental layouts of independent and response variables from the CCFC design are reported in Table 3.1, 3.2 and 3.3 for *Kenkowa*, *Amona* and *Badal bao* respectively. The effects of rice concentration and cooking time on WAI, WSI, SP and ρ_b in the developed porridge mix base are analyzed for each rice variety. Quadratic model was the most suitable for describing relationships between the independent variables and responses (Table 3.4, 3.5 and 3.6). The response regression models were statistically significant ($p\leq 0.05$, $R^2>0.9$), suggesting a strong correlation between the predicted and observed data. Agreements between predicted and adjusted R^2 values further validated the model adequacy while non-significant lack of fit ($p>0.05$) confirmed the reliability of the models in capturing the variation in experimental data.

Table 3.1: Experimental layout of the independent variables and their responses as given by the CCFC model for *Kenkwa bao*

Run	Rice g	Cooking time min	Water absorption index (WAI) g/g	Water solubility index (WSI) %	Swelling power	Bulk density g/ml
1	15	10	5.32	1.15	5.38	0.729
2	30	10	5.09	1.41	5.16	0.809
3	30	7.5	4.98	1.58	5.05	0.858
4	15	5	5.15	1.4	5.22	0.762
5	22.5	10	5.37	1.23	5.43	0.744
6	15	7.5	5.29	1.32	5.36	0.745
7	22.5	7.5	5.23	1.36	5.3	0.776
8	22.5	7.5	5.22	1.4	5.29	0.778
9	30	5	4.74	1.6	4.81	0.902
10	22.5	5	5.04	1.49	5.11	0.799
11	22.5	7.5	5.25	1.39	5.32	0.775
12	22.5	7.5	5.21	1.38	5.28	0.778
13	22.5	7.5	5.24	1.37	5.31	0.774
R²						
			0.9897	0.9837	0.9906	0.9983
Adjusted R²			0.9823	0.9721	0.9838	0.9971
Predicted R²			0.9289	0.9031	0.9374	0.9878

Table 3.2. Experimental layout of the independent variables and their responses as given by the CCFC model for *Amona bao*

Run	Rice	Cooking time	Water absorption index (WAI)	Water solubility index (WSI)	Swelling power	Bulk density
	g	min	g/g	%		g/ml
1	15	15	5.99	1.11	6.05	0.736
2	22.5	15	6.01	1.25	6.08	0.754
3	15	10	5.18	1.46	5.25	0.778
4	22.5	12.5	5.42	1.4	5.49	0.784
5	22.5	12.5	5.39	1.43	5.46	0.789
6	30	10	4.69	1.72	4.77	0.911
7	22.5	12.5	5.32	1.37	5.39	0.78
8	30	15	5.29	1.49	5.37	0.821
9	15	12.5	5.62	1.3	5.69	0.759
10	30	12.5	5.01	1.65	5.09	0.874
11	22.5	12.5	5.52	1.35	5.59	0.78
12	22.5	12.5	5.42	1.39	5.49	0.781
13	22.5	10	5.13	1.52	5.2	0.808
R²			0.9656	0.9850	0.9640	0.9955
Adjusted R²			0.9410	0.9743	0.9383	0.9923
Predicted R²			0.8140	0.9570	0.8027	0.9735

Table 3.3. Experimental layout of the independent variables and their responses as given by the CCFC model for *Badal bao*

Run	Rice g	Cooking time min	Water absorption index (WAI) g/g	Water solubility index (WSI) %	Swelling power	Bulk density g/ml
1	30	10	4.82	1.7	4.9	0.889
2	22.5	15	6.02	1.22	6.09	0.732
3	22.5	12.5	5.69	1.4	5.77	0.755
4	30	15	5.24	1.49	5.32	0.796
5	15	15	5.97	1.12	6.04	0.704
6	15	12.5	5.7	1.34	5.77	0.719
7	22.5	12.5	5.66	1.39	5.74	0.753
8	22.5	12.5	5.78	1.42	5.86	0.747
9	15	10	5.22	1.44	5.29	0.737
10	22.5	12.5	5.57	1.43	5.65	0.748
11	22.5	10	5.18	1.56	5.26	0.787
12	22.5	12.5	5.59	1.45	5.67	0.758
13	30	12.5	5.13	1.61	5.21	0.846
R²						
			0.9665	0.9823	0.9674	0.9949
Adjusted R²						
			0.9426	0.9697	0.9441	0.9912
Predicted R²						
			0.8228	0.8928	0.8333	0.9764

Table 3.4. Analysis of variance showing the quadratic interaction and lack of fit of the response variable for *Kenkwa bao*

Source of variation	df	Response variable							
		Water absorption index (WAI) (g/g)		Water solubility index (WSI) (%)		Swelling power		Bulk density (g/ml)	
		Sum of squares	F value	Sum of squares	F value	Sum of squares	F value	Sum of squares	F value
Regression	5	0.3366	134.2*	0.1774	84.55*	0.3299	146.84*	0.0270	312.96*
Residual error	7	0.0035		0.0029		0.0031		0.0000	
Lack of fit	3	0.0025	3.35^{ns}	0.0019	2.58^{ns}	0.0021	2.80^{ns}	0.0000	3.52^{ns}
Pure error	4	0.0010		0.0010		0.0010		0.0000	
Cor total	12	0.3401		0.1803		0.3330		0.0271	

* significant at $p \leq 0.05$; ^{ns} not significant

Table 3.5. Analysis of variance showing the quadratic interaction and lack of fit of the response variable for *Amona bao*

Source of variation	df	Response variable							
		Water absorption index (WAI) (g/g)		Water solubility index (WSI) (%)		Swelling power		Bulk density (g/ml)	
		Sum of squares	F value	Sum of squares	F value	Sum of squares	F value	Sum of squares	F value
Regression	5	1.52	39.30*	0.3030	37.48*	1.49	41.54*	0.0279	310.73*
Residual error	7	0.0542		0.0046		0.0555		0.0001	
Lack of fit	3	0.0335	2.16^{ns}	0.0009	2.24^{ns}	0.0348	0.9945^{ns}	0.0001	1.52^{ns}
Pure error	4	0.0207		0.0037		0.0207		0.0001	
Cor total	12	1.58		0.3076		1.54		0.0281	

* significant at $p \leq 0.05$; ^{ns} not significant

Table 3.6. Analysis of variance showing the quadratic interaction and lack of fit of the response variable for *Badal bao*

Source of variation	df	Response variable							
		Water absorption index (WAI) (g/g)		Water solubility index (WSI) (%)		Swelling power		Bulk density (g/ml)	
		Sum of squares	F value	Sum of squares	F value	Sum of squares	F value	Sum of squares	F value
Regression	5	1.48	40.38*	0.2723	77.92*	1.46	41.54*	0.0318	271.35*
Residual error	7	0.00513		0.0049		0.0494		0.0002	
Lack of fit	3	0.0230	1.09^{ns}	0.0026	1.53^{ns}	0.0211	0.9945^{ns}	0.0001	1.19^{ns}
Pure error	4	0.0283		0.0023		0.0283		0.0001	
Cor total	12	1.53		0.2722		1.53		0.0319	

* significant at $p \leq 0.05$; ^{ns} not significant

Regression Equations for *Kenkowa bao*

Water absorption index (WAI)

$$Y = 5.23 - 0.1583 X_1 + 0.1417 X_2 - 0.1119 X_1^2 - 0.0419 X_2^2 + 0.0450 X_1 X_2 \quad \text{Eq. 3.15}$$

Water solubility index (WSI)

$$Y = 1.39 + 0.1200 X_1 - 0.1167 X_2 + 0.0507 X_1^2 - 0.0393 X_2^2 + 0.0150 X_1 X_2 \quad \text{Eq. 3.16}$$

Swelling power

$$Y = 5.30 - 0.1567 X_1 + 0.1383 X_2 - 0.1107 X_1^2 - 0.0457 X_2^2 + 0.0475 X_1 X_2 \quad \text{Eq. 3.17}$$

Bulk density

$$Y = 0.7757 + 0.0555 X_1 - 0.0302 X_2 + 0.0271 X_1^2 - 0.0029 X_2^2 - 0.0150 X_1 X_2 \quad \text{Eq. 3.18}$$

Regression Equations for *Amona bao*

Water absorption index (WAI)

$$Y = 5.44 - 0.3000 X_1 + 0.3817 X_2 - 0.1876 X_1^2 + 0.0674 X_2^2 - 0.0525 X_1 X_2 \quad \text{Eq. 3.19}$$

Water solubility index (WSI)

$$Y = 1.39 + 0.1650 X_1 - 0.1417 X_2 + 0.0740 X_1^2 - 0.0160 X_2^2 + 0.0300 X_1 X_2 \quad \text{Eq. 3.20}$$

Swelling power

$$Y = 5.51 - 0.2933 X_1 + 0.3800 X_2 - 0.1838 X_1^2 + 0.0662 X_2^2 - 0.0500 X_1 X_2 \quad \text{Eq. 3.21}$$

Bulk density

$$Y = 0.7832 + 0.0555 X_1 - 0.0310 X_2 + 0.0322 X_1^2 - 0.0033 X_2^2 - 0.0120 X_1 X_2 \quad \text{Eq. 3.22}$$

Regression Equations for *Badal bao***Water absorption index (WAI)**

$$Y = 5.66 - 0.2833 X_1 + 0.3350 X_2 - 0.2645 X_1^2 - 0.0795 X_2^2 + 0.0825 X_1 X_2 \quad \text{Eq. 3.23}$$

Water solubility index (WSI)

$$Y = 1.42 + 0.1500 X_1 - 0.1450 X_2 + 0.0524 X_1^2 - 0.0326 X_2^2 + 0.00275 X_1 X_2 \quad \text{Eq. 3.24}$$

Swelling power

$$Y = 5.74 - 0.2783 X_1 + 0.3333 X_2 - 0.2671 X_1^2 - 0.0821 X_2^2 + 0.0825 X_1 X_2 \quad \text{Eq. 3.25}$$

Bulk density

$$Y = 0.7533 + 0.0618 X_1 - 0.0302 X_2 + 0.0263 X_1^2 - 0.0033 X_2^2 - 0.0150 X_1 X_2 \quad \text{Eq. 3.26}$$

3.3.2. Impact of independent variables on responses

The fitted regression equations for each response across the three rice varieties are presented in Eq. 3.15-3.26. For *Kenkowa* and *Badal bao*, rice concentration showed negative linear (X_1), positive quadratic (X_1^2) while cooking time showed positive linear (X_2), negative quadratic (X_2^2) relations. Positive interaction effects (X_1X_2) were observed for WAI and SP. Additionally, positive linear (X_1), positive quadratic (X_1^2) for rice concentration and negative linear (X_2) and negative quadratic (X_2^2) for cooking time with positive interaction effect (X_1X_2) were observed for WSI and ρ_b . In *Amona bao*, rice concentration showed negative linear (X_1) and quadratic (X_1^2) and cooking time showed positive linear (X_2), quadratic (X_2^2) relations with negative interaction effects (X_1X_2) for WAI and SP. However, for WSI and ρ_b , positive linear and positive quadratic relations with rice concentration and negative linear and negative quadratic with cooking time were obtained. The relations implied that on shorter cooking times and increasing rice concentration WAI and SP reduced due to limited water availability per grain. This effect further intensified at higher concentrations. Longer cooking times enhanced WAI and SP by improving starch granule hydration. However, prolonged heating could degrade glycosidic bonds, reducing water absorption. At optimal rice concentration and

extended cooking time (22.5 g;10 min for *Kenkowa* and 22.5 g;15 min for *Amona* and *Badal*), WAI was seen to progressively increase. Immediate starch degradation results in higher soluble fractions. In prolonged process, retrogradation occurs, leading to lower solubility of resultant fractions (Zainab et al., 2025).

Effective control of processing parameters ensures optimal gelatinization, enhancing functional properties such as low ρ_b and WSI and high WAI and SP. Contour plots (Fig. 3.2, 3.3, 3.4) illustrate these effects. WAI exhibited an increasing trend with cooking time. However, as rice concentration increased, WAI initially rose before decreasing, likely due to the gradual reduction in available water with increasing rice concentration. Adequate hydration, temperature and cooking time, are critical for achieving sufficient gelatinization. WSI in contrast, demonstrated an inverse relationship with cooking time. Once retrogradation occurred, further cooking had minimal impact on solubility. SP positively correlated with WAI, increasing over prolonged cooking time under increased water levels. ρ_b decreased with both cooking time and higher WAI, as higher water absorption expanded volume, lowering density. For instant porridge mixes, low ρ_b is desirable as it ensures a light, fluffy texture. The relationship between ρ_b and WAI is inversely proportional, wherein higher WAI corresponds to lower ρ_b . Flours with high WAI expand in volume, reducing density. Conversely, a higher ρ_b indicate reduced porosity, due to interactions between gelatinized starch and coagulated proteins during hydrothermal processing resulting in compact structures. However, excessive heating disrupts this network, creating a more open structure with increased porosity, thereby lowering ρ_b . Powders characterized by low ρ_b thus exhibit high porosity facilitating interaction with water molecules and improving the wettability of hydrothermally treated flour (Pismag et al., 2024).

3.3.3. Optimization

Design Expert-12 predicted rice concentration and cooking time (Table 3.7, 3.8 and 3.9) as 18.68 g, 10 min; 22.5g, 12.5 min and 22.5 g, 12.5 min for *Kenkowa*, *Amona* and

Badal bao respectively, as the optimum process variables to develop the instant porridge mix base. The desirability values were 0.935, 0.988 and 0.998, respectively. The WAI, WSI, SP and ρ_b obtained with the optimized process variables for each variety strongly correlated with the predicted values of responses (Table 3.10, 3.11, 3.12). Fig 3.5 depicts images of optimized dry and reconstituted porridge mix samples.

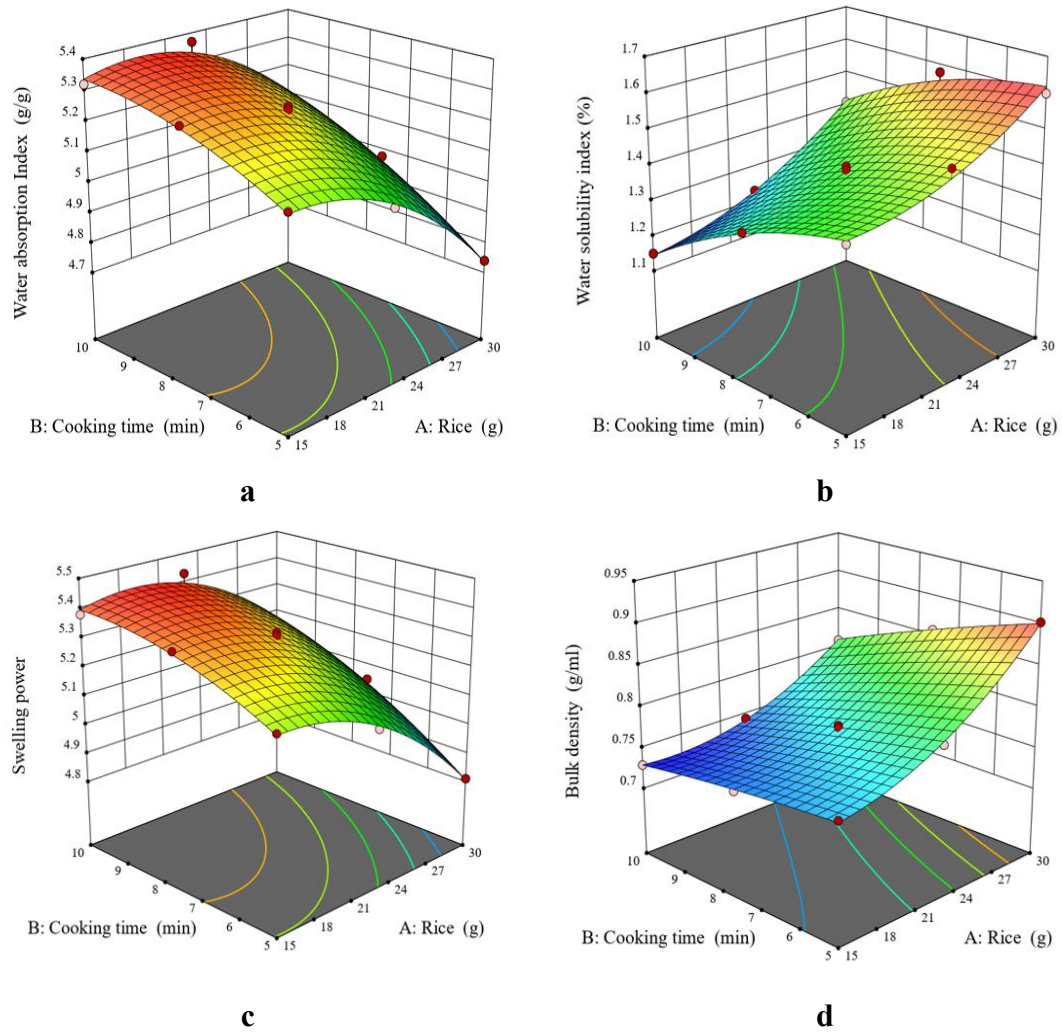
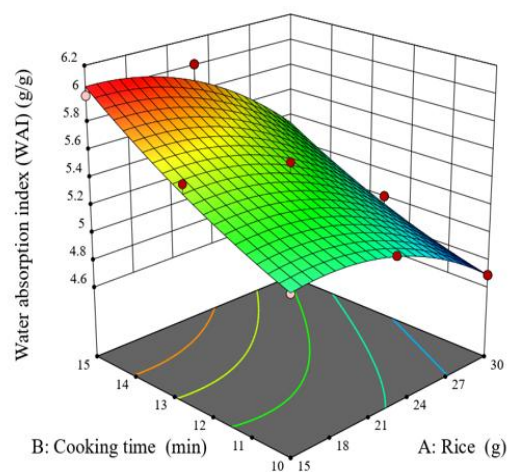
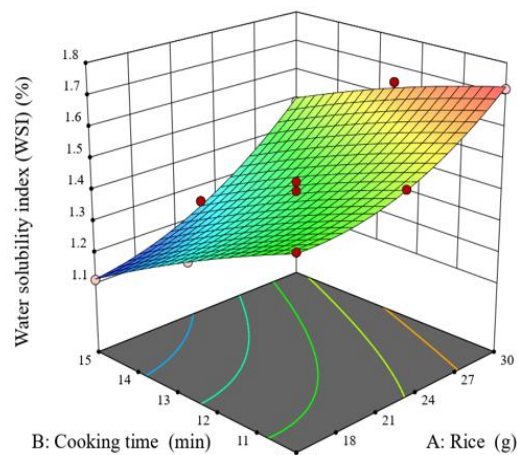


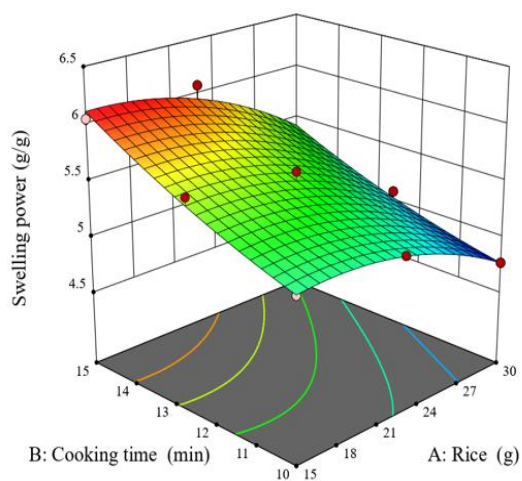
Fig. 3.2. Variation of response variables (a) WAI, (b) WSI, (c) SP and (d) ρ_b due to interaction between independent variables for *Kenkowa bao*



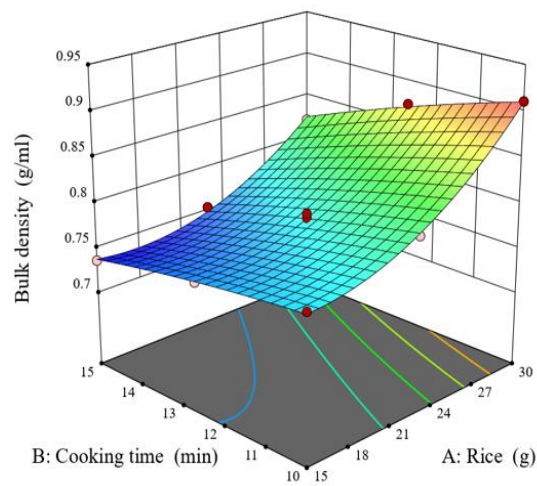
a



b

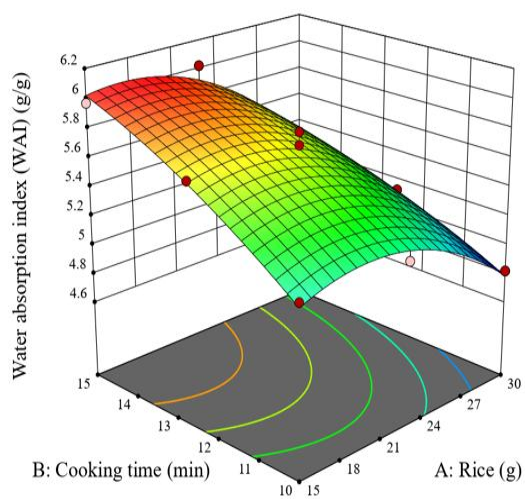


c

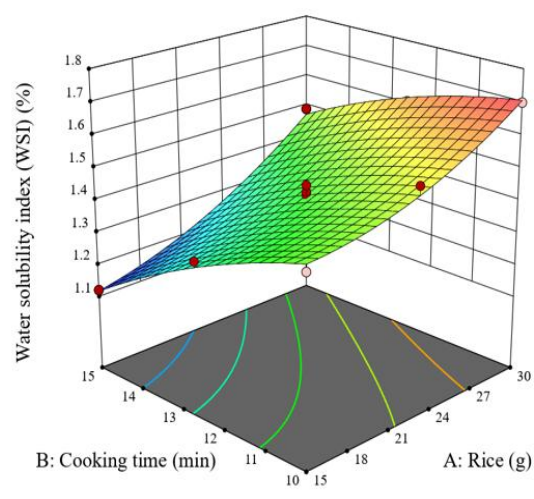


d

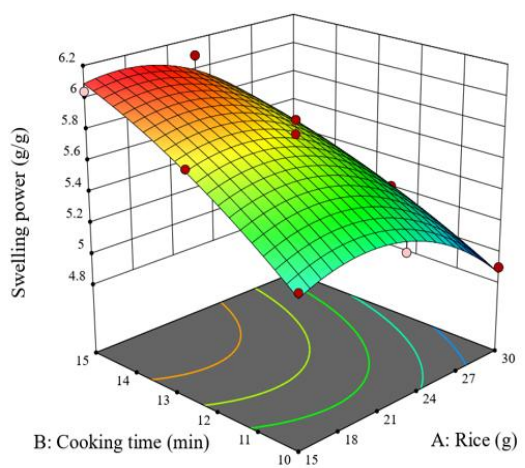
Fig. 3.3. Variation of response variables (a) WAI, (b) WSI, (c) SP and (d) ρ_b due to interaction between independent variables for *Amona bao*



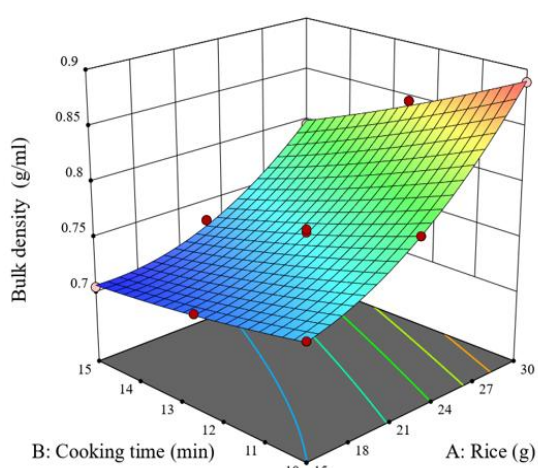
a



b



c



d

Fig. 3.4. Variation of response variables (a) WAI, (b) WSI, (c) SP and (d) ρ_b due to interaction between independent variables for *Badal bao*



Fig. 3.5. (a-c, g-i) Images of dry optimized porridge mix samples and (d-f, j-l) images of corresponding reconstituted porridge mix samples

Table 3.7. Estimated optimal conditions of the independent variables for *Kenkowa bao*

Factor	Low level	High level	Optimal level
Rice (g)	15	30	18.62
Cooking time (min)	5	10	10

Table 3.8. Estimated optimal conditions of the independent variables for *Amona bao*

Factor	Low level	High level	Optimal level
Rice (g)	15	30	22.5
Cooking time (min)	10	15	12.5

Table 3.9. Estimated optimal conditions of the independent variables for *Badal bao*

Factor	Low level	High level	Optimal level
Rice (g)	15	30	22.5
Cooking time (min)	10	15	12.5

Table 3.10. The predicted and experimental values of the responses for *Kenkowa bao*

Responses	Predicted value	Experimental value
Water absorption index (g/g)	5.36	5.38
Water solubility index (%)	1.17	1.18
Swelling power	5.42	5.43
Bulk density (g/ml)	0.724	0.733

Table 3.11. The predicted and experimental values of the responses for *Amona bao*

Responses	Predicted value	Experimental value
Water absorption index (g/g)	5.43	5.45
Water solubility index (%)	1.39	1.37
Swelling power	5.50	5.51
Bulk density (g/ml)	0.783	0.784

Table 3.12. The predicted and experimental values of the responses for Badal *ba*

Responses	Predicted value	Experimental value
Water absorption index (g/g)	5.66	5.65
Water solubility index (%)	1.41	1.43
Swelling power	5.73	5.74
Bulk density (g/ml)	0.753	0.751

3.3.4. Proximate composition

Proximate compositions of the samples are presented in Table 3.13. The lower fat contents in optimized base flours than their respective raw flours could be due to thermal

Table 3.13. Proximate composition and energy values of the raw and optimized porridge mix samples

Samples	Moisture (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Ash (%)	Energy kcal/100g
KR	5.02±1.02 ^a	1.99±0.08 ^{bc}	10.67±0.34 ^{dc}	72.56±1.02 ^a	1.94±0.06 ^a	332.29±1.67 ^d
KO	5.22±0.98 ^a	1.77±0.03 ^{de}	10.37±0.29 ^f	71.67±0.67 ^{abc}	1.56±0.02 ^{def}	317.65±2.12 ^g
KRM	5.09±0.56 ^a	2.05±0.09 ^{bc}	11.56±0.31 ^{bc}	71.92±1.12 ^{ab}	1.95±0.03 ^a	336.61±1.88 ^b
KOM	5.03±0.82 ^a	2.02±0.05 ^{bc}	11.59±0.19 ^{bc}	70.97±0.45 ^{bcd}	1.71±0.02 ^b	328.10±1.19 ^e
AR	5.56±0.56 ^a	2.15±0.07 ^b	11.05±0.45 ^{cd}	71.45±0.77 ^{abc}	1.68±0.05 ^b	332.31±2.05 ^d
AO	5.11±0.62 ^a	1.55±0.11 ^f	10.89±0.12 ^{de}	70.33±0.49 ^{cd}	1.54±0.04 ^{ef}	316.69±1.79 ^g
ARM	5.66±0.78 ^a	2.88±0.02 ^a	12.55±0.56 ^a	71.23±0.58 ^{abcd}	1.72±0.03 ^b	347.90±2.02 ^a
AOM	5.43±0.67 ^a	2.87±0.14 ^a	12.33±0.49 ^a	70.67±0.49 ^{bcd}	1.61±0.01 ^{cd}	335.19±1.27 ^{bc}
BR	5.08±0.42 ^a	1.66±0.06 ^{ef}	10.86±0.33 ^{de}	71.33±1.02 ^{abcd}	1.59±0.02 ^{cde}	326.82±1.33 ^f
BO	5.67±0.62 ^a	1.89±0.16 ^{cd}	10.89±0.46 ^{de}	70.04±0.46 ^d	1.44±0.06 ^g	315.45±1.73 ^h
BRM	5.40±0.51 ^a	2.09±0.11 ^b	11.55±0.11 ^{bc}	70.66±0.78 ^{bcd}	1.62±0.01 ^c	333.61±2.01 ^b
BOM	5.72±0.78 ^a	1.99±0.07 ^{bc}	11.72±0.19 ^b	70.29±0.56 ^{cd}	1.53±0.05 ^f	326.21±1.45 ^f

Means with same superscript letters within the columns are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test

lipid degradation during processing. High heat might have altered the starch-protein matrix of rice, potentially encapsulating fats within the modified structure, thereby lowering their quantified values (Mohamed, 2023). A slightly higher fat and protein contents in the mix flours was contributed by the added SMP. Compared to raw samples the optimized samples exhibited lower energy values.

3.3.5. Texture

Consistency of the reconstituted (1:10) optimized flours increased by ~30% than raw rice flours across all three rice varieties, while cohesiveness rose by ~75%. No significant difference in consistency were observed between raw flours and raw mixes. However, cohesiveness in raw mixes increased by ~15% (Table 3.14). BO exhibited the highest consistency (99.52 g.s.) among base flours, while KOM had the highest consistency (97.67 g.s.) among mixes. Similarly, BO showed the highest cohesiveness (82.56 g.s.) among base flours, and BOM had the highest cohesiveness (89.98 g.s.) among mixes. The optimized mix samples displayed consistency and cohesiveness comparable to the optimized flours with no significant difference ($p \leq 0.05$), indicating optimal gelatinization as suited for their instant preparation. A similar study by Mandge et al. (2014) on multigrain porridge, processed through soaking (5 h at 50 °C, 3.5 h at 65 °C), steaming (15 psi for 10, 15 and 20 min), followed by drying (40 °C) demonstrated that extensive treatment produced a more consistent, less firm and more cohesive porridge due to complete starch gelatinization and reduced grain stickiness.

Table 3.14. Textural parameters, color values and bioactive properties of the raw and optimized porridge mix samples

Samples	Textural parameters			Color values			Bioactive properties				
	Consistency (g.s)	Cohesiveness (g.s)	L*	a*	b*	TPC (µg GAE/g)	TFC (µg QE/g)	DRSA (%)	FRAP (mM FeSO ₄ /g)		
KR	78.26 ± 6.67 ^{bc}	49.12 ± 7.45 ^{cde}	60.89 ± 1.85 ^a	19.78 ± 0.64 ^f	19.89±0.78 ^{bc}	1526.69±9.76 ^c	614.11±11.12 ^c	89.18±1.05 ^a	2.52±0.12 ^c		
KO	98.08 ± 10.67 ^a	81.42 ± 5.09 ^{ab}	58.17 ± 1.12 ^b	22.67 ± 0.22 ^d	18.56±0.67 ^{de}	384.52±11.56 ^g	366.23±10.23 ^e	46.98±0.98 ^f	1.16±0.08 ^e		
KRM	78.92 ± 9.22 ^{bc}	59.72 ± 6.51 ^c	61.07 ± 0.92 ^a	16.67 ± 0.66 ^h	17.62±0.56 ^{ef}	1532.45±2.45 ^c	618.56±12.45 ^c	90.07±0.96 ^a	2.68±0.11 ^c		
KOM	97.67 ± 6.89 ^a	83.78 ± 5.76 ^{ab}	58.98 ± 0.77 ^{ab}	20.12 ± 0.11 ^f	15.78±0.87 ^g	405.34±13.87 ^f	371.67±10.19 ^e	49.48±1.45 ^e	1.43±0.07 ^d		
AR	72.73 ± 8.62 ^c	40.86 ± 4.97 ^e	60.82 ± 1.01 ^a	22.56±0.58 ^d	25.98±0.23 ^a	1752.26±4.67 ^{ab}	718.67±14.19 ^a	86.61±1.11 ^b	2.87±0.02 ^b		
AO	95.13 ± 9.54 ^a	75.45 ± 6.65 ^b	54.89 ± 1.56 ^{cd}	28.63 ± 0.04 ^b	19.82±1.03 ^{bc}	626.62±7.67 ^d	245.04±12.76 ^f	50.78±0.78 ^{de}	1.04±0.05 ^e		
ARM	73.56 ± 7.52 ^c	54.67 ± 6.23 ^{cd}	60.34 ± 0.82 ^a	18.98 ± 0.29 ^g	19.75±0.92 ^c	1766.89±8.77 ^a	721.34±8.44 ^a	87.15±0.77 ^b	2.90±0.08 ^b		
AOM	92.64 ± 7.89 ^{ab}	80.43 ± 9.01 ^{ab}	53.45 ± 0.95 ^d	21.45 ± 0.62 ^e	17.62±1.05 ^{ef}	598.66±9.89 ^e	252.22±17.52 ^f	52.79±0.94 ^e	1.11±0.11 ^e		
BR	72.89 ± 7.65 ^c	45.02 ± 3.98 ^{de}	60.76 ± 0.89 ^a	25.45 ± 0.19 ^c	20.86±0.68 ^b	1752.29±5.89 ^{ab}	682.29±18.03 ^b	87.06±1.33 ^b	3.47±0.13 ^a		
BO	99.52 ± 8.40 ^a	82.56 ± 6.08 ^{ab}	55.72 ± 0.63 ^c	29.55± 0.78 ^a	18.67±1.12 ^{de}	634.38±8.02 ^d	397.45±15.88 ^d	53.06±1.19 ^c	2.54±0.08 ^c		
BRM	74.12 ± 7.56 ^c	51.90 ± 6.78 ^{cde}	59.67 ± 1.03 ^{ab}	19.76 ± 0.77 ^f	19.21±0.45 ^{cd}	1746.38±7.88 ^b	680.23±13.88 ^b	88.21±1.01 ^{ab}	3.55±0.09 ^a		
BOM	94.09 ± 8.91 ^a	89.98 ± 5.29 ^a	56.02 ± 1.18 ^c	21.34 ± 0.33 ^e	17.18±1.22 ^f	640.45±7.97 ^d	403.12±9.97 ^d	52.12±0.93 ^{cd}	2.58±0.08 ^c		

Means with same superscript letters within the columns are not significantly different at p≤0.05 as indicated through Duncan's multiple range test

3.3.6. Color values

Table 3.14 summarizes the color parameters of raw, optimized and mix flours. A notable decrease of 5-7% in L^* values was observed in optimized flours compared to raw flours, indicating reduced whiteness. Conversely, a^* values showed a significant ($p \leq 0.05$) increase of 18%, 30% and 20% in KO, AO and BO, respectively, reflecting enhanced redness post-optimization. The intensification is likely due to anthocyanin release and the formation of intermediate Maillard reaction products (MRPs) during the cooking process that stabilized red hues (Octavia Yusuf et al., 2022). Similar trends were observed in pre-cooked sorghum flours, where increased temperature and moisture led to lower L^* and higher a^* and b^* values due to Maillard reactions and pigment oxidation (Jafari et al., 2017). Additionally, anthocyanin polymerization and the degradation of heat-sensitive carotenoids may have further amplified red tones (Devi and Badwaik, 2022). Mix samples displayed increased L^* and decreased a^* due to dilution with SMP and caramelized sugar. Among the optimized flours, BO exhibited highest redness (a^*) (29.55), followed by AO (28.63) and KO (22.67). In the mix samples, AOM and BOM displayed elevated a^* values, with no significant difference ($p \leq 0.05$) among them. Devi and Badwaik (2022) reported similar trend post cooking treatment in *Chakhao* rice varieties.

3.3.7. Bioactive properties

Optimized flours and mix samples exhibited lower bioactive properties (TPC, TFC, DRSA, FRAP) than raw samples (Table 3.14) primarily due to thermal degradation of phenolics during pretreatment. Heat and moisture induced oxidation, forming quinones and breaking down proanthocyanidins, the major contributors to TPC in red rice. Proanthocyanidins decompose to simpler phenolics like catechin or epicatechin, which may further degrade reducing their bioavailability (Indrakumar et al., 2019). At higher temperatures, flavonoids may undergo autooxidation. Flavonoid glycosides lose their sugar moiety forming intermediate aglycones and other breakdown products. In gelatinized matrices, phenolics bind to the starch gels and proteins with hydrogen

bonding and hydrophobic interactions, reducing their apparent activity (Lang et al., 2019). Optimized flours showed a significant reduction in bioactive compounds with TPC decreasing by ~75%, ~64% and ~64% and TFC by ~40%, ~65% and ~40% for KO, AO and BO respectively. Antioxidant activity also decreased with DRSA reducing by 45%, 40% and 40%, respectively in KO, AO and BO along with a ~55% decrease in FRAP across all varieties. However, mix samples exhibited no significant change in these parameters. Thuengtung and Ogawa (2020) reported similar decrease in bioactivity in black nonwaxy (cv. Hom Nin), red (cv. Red Hommali) and purple waxy rice (cv. Kum Luempua) upon steam and microwave cooking. The inclusion of SMP and caramelized sugar had no significant impact on biochemical activity.

3.3.8. Differential scanning calorimetry

A pronounced increase in onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) along with a narrowing of the endotherm in the optimized samples, was observed due to hydrothermal treatment (Table 3.15; Fig. 3.6 a, b, c). The reduction in enthalpy (ΔH) indicates that less energy was required to disrupt the crystalline regions of starch, directly correlating with the lower crystallinity observed in optimized samples.

Table 3.15. Thermal properties of raw and optimized samples of *Kenkowa*, *Amona* and *Badal bao*

Samples	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	DG (%)
KR	60.23±1.92 ^a	79.14±1.45 ^a	96.94±2.21 ^a	9.84± 1.11 ^a	62.09±1.97 ^a
KO	74.19±1.12 ^b	83.28±1.63 ^b	92.02±1.42 ^b	3.73±0.77 ^b	
AR	55.13±1.32 ^c	74.43±2.22 ^c	96.30±1.30 ^a	7.77±1.42 ^c	42.98±1.42 ^b
AO	79.72±1.61 ^d	89.04±2.40 ^d	98.43±1.20 ^c	4.43±1.60 ^d	
BR	55.63±2.36 ^c	70.56±1.42 ^e	97.34±2.32 ^d	5.02±0.61 ^d	42.28±2.11 ^b
BO	74.54±1.92 ^b	83.14±1.89 ^b	91.13±1.19 ^b	2.89±0.22 ^e	

Means with same superscript letters within the columns are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test

Hydrothermal treatment induces structural modifications altering the balance between crystalline and amorphous regions, promoting partial gelatinization and increased starch molecular mobility. During cooling, recrystallization forms stable double helices while disrupting weaker hydrogen bonds. This reduces overall starch network rigidity, making the granules more susceptible to disintegration during gelatinization, thereby lowering ΔH required for phase transition. This confirms the decline in crystallinity, as supported by XRD analysis. In contrast, raw samples retained a higher degree of crystallinity, necessitating greater ΔH for gelatinization, whereas the optimized samples, with their lower crystallinity, required less energy for this transition (Jin and Xu, 2020).

3.3.9. X-ray diffraction

The raw rice flours exhibited A-type X-ray diffraction pattern of starch with major peaks at diffraction angle (2θ) of 15.2° , 17° , 18.8° and 23.02° (Fig. 3.6 d, e, f). Processing resulted in major losses of relative crystallinity (Table 3.16). The A-type peaks at 15.2° and 17° feebly retained in the optimized flours, suggested ungelatinized or reorganized fractions. The emergence of the $\sim 13^\circ$ peak suggested that amylopectin chains, upon partial recrystallization, adopted a B-type or mixed A+B configuration, possibly due to extended hydration and molecular mobility during processing, leading to the formation of structurally modified starch. Formation of the major diffraction peak at 20° , corresponding to V-type diffraction pattern suggested significant formation of starch-lipid complexes during the process treatments. Moreover, intermediate V-type complexes, such as V6 or V8, may be inferred from the peak near $\sim 13^\circ$ (2θ), which arises due to interactions between high-amylose starch and fatty acids. Optimized flours showed decreased crystallinity compared to raw flours due to gelatinization and subsequent retrogradation.

Table 3.16. Percentage crystallinity of raw and optimized samples of *Kenkowa*, *Amona* and *Badal bao*

Samples	% Crystallinity
KR	42.11±1.42 ^a
KO	7.19±1.02 ^b
AR	47.53±1.32 ^c
AO	9.82±1.46 ^d
BR	43.63±2.36 ^e
BO	9.84±0.92 ^b

Means with same superscript letters within the columns are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test

3.3.10. Fourier transform infrared spectroscopy

FTIR analysis of raw and optimized samples showed characteristic band with minor changes in intensities, indicating changes in molecular bonding parameters upon cooking (Fig. 3.6 g, h, i). The 1300-1400 cm^{-1} region corresponded to bending vibrations of O-C-H, C-O-H and C-C-H. The broad band at 3200-3273.71 cm^{-1} reflected hydroxyl (-OH) stretching in starch. The ratio of intensities of 1047 cm^{-1} :1022 cm^{-1} representing crystallinity showed irregular and dissimilar patterns in the optimized samples compared to raw, indicating altered helical and crystalline arrangements. Changes in the ratio of band intensities at 2855 cm^{-1} and 2925 cm^{-1} (H-C-H stretching) correlated with 1047:1022 cm^{-1} indicating shift of stretching modes of CH_2 due to retrogradation (Xiang et al., 2022). The reduction in ΔH by DSC confirms lower gelatinization energy attributed to smaller, imperfect crystallites formed during reorganization. FTIR further supports these findings with reduced intensity at 1047 cm^{-1} indicating a shift from native crystalline to an amorphous structure. Additionally, shifts in CH_2 and -OH stretching ($\sim 3200\text{-}3273\text{cm}^{-1}$) aligned with amylose-lipid interactions, consistent with the V-type peak in XRD reinforcing that lower crystallinity in optimized samples directly reduced enthalpy for gelatinization.

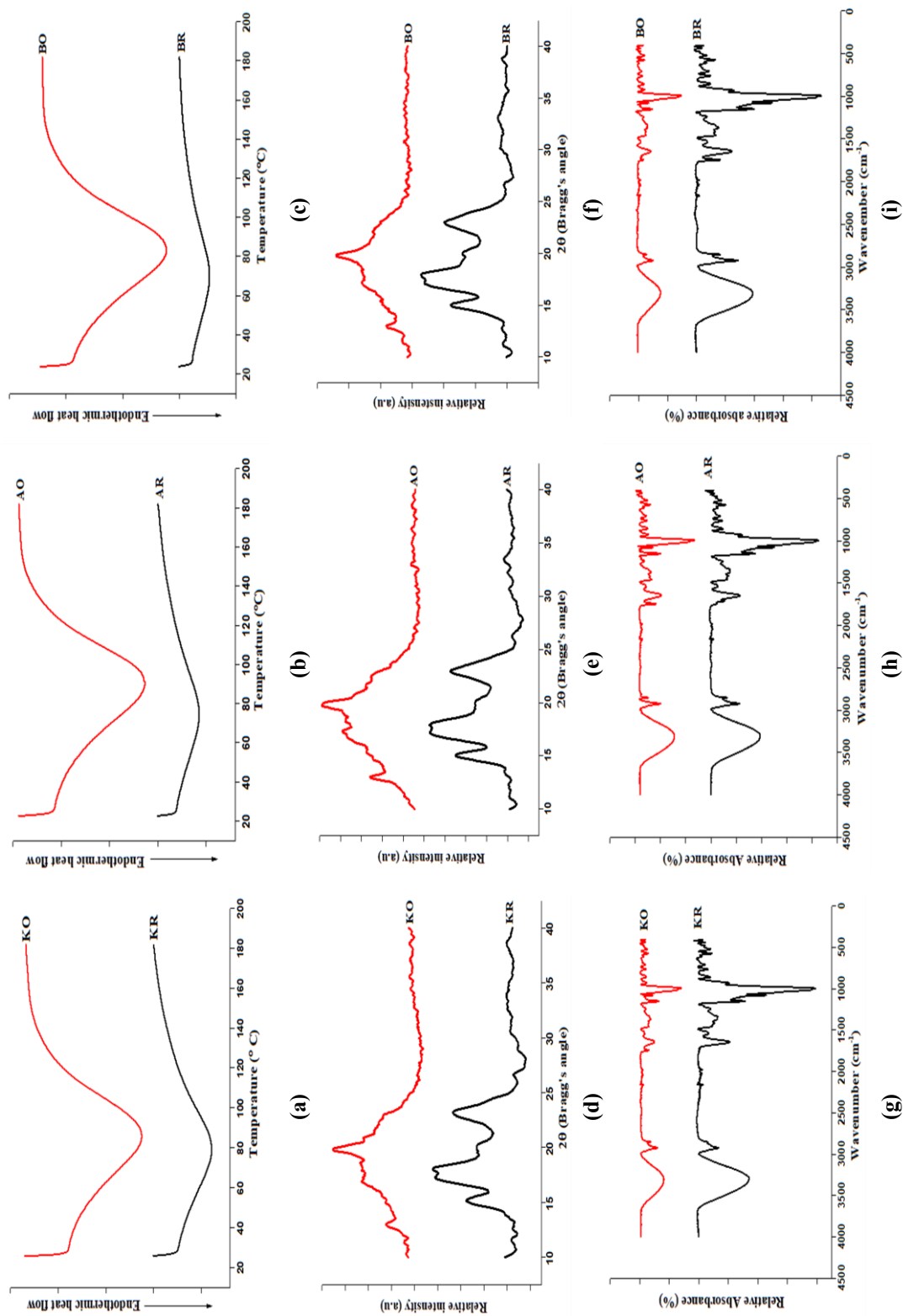


Fig. 3.6. (a-c) DSC thermograms, (d-f) X-ray diffractograms and (g-i) FTIR spectra of raw and optimized samples of *Kenkowa bao* (KR, KO), *Amona bao* (AR, AO) and *Badal bao* (BR, BO) rice varieties respectively.

3.3.11. Scanning electron microscopy

Scanning electron micrographs (Fig. 3.7) revealed distinct morphological differences between raw and optimized samples. The raw samples (KR, AR, BR) exhibited a heterogeneous distribution of discrete loosely packed starch granules with polygonal and oval shapes. Granule surfaces varied from smooth to slightly indented, with some occasional fractured granules from mechanical stresses during grinding. In contrast, the optimized samples (KO, AO, BO) showed significant morphological transformation, along with starch granules losing distinct boundaries and forming a compact, smooth, dense and continuous mass. This transformation is attributed to starch gelatinization, during which heat and moisture disrupt internal hydrogen bonding, leading to granule swelling, loss of crystallinity, and leaching of amylose, followed by reassociation upon cooling to form an amorphous mass. Such structural reorganization enhances water absorption, improves textural uniformity, and increases enzyme accessibility, thereby influencing functional and digestibility characteristics of the porridge mixes. A study by Kumar et al. (2020) revealed similar granule deformation upon cooking in proso and little millet flours.

3.3.12. Digestive properties

The optimized samples demonstrated a decrease in RDS and an increase in SDS and RS content than their respective raw samples (Table 3.17). Hydrothermal treatments initially increase starch susceptibility to enzymatic digestion, however, subsequent cooling and drying, induce partial recrystallization, forming a starch resistant to rapid digestion. This decreases the RDS fraction while increasing SDS and RS content. At higher temperatures, starch granules degrade and tend to reassociate with proteins and lipids, forming starch-lipid V-type complexes, as confirmed by XRD (Amin et al., 2020). KO exhibited the highest RS, followed by BO and AO. This was followed by KOM, BOM and AOM. In comparison to optimized flours, optimized mix samples exhibited higher RDS values and lower SDS and RS content. The ~15% reduction in RS content in mix

samples, relative to raw and optimized flours, likely resulted from dilution effect caused by the inclusion of SMP and caramelized sugar.

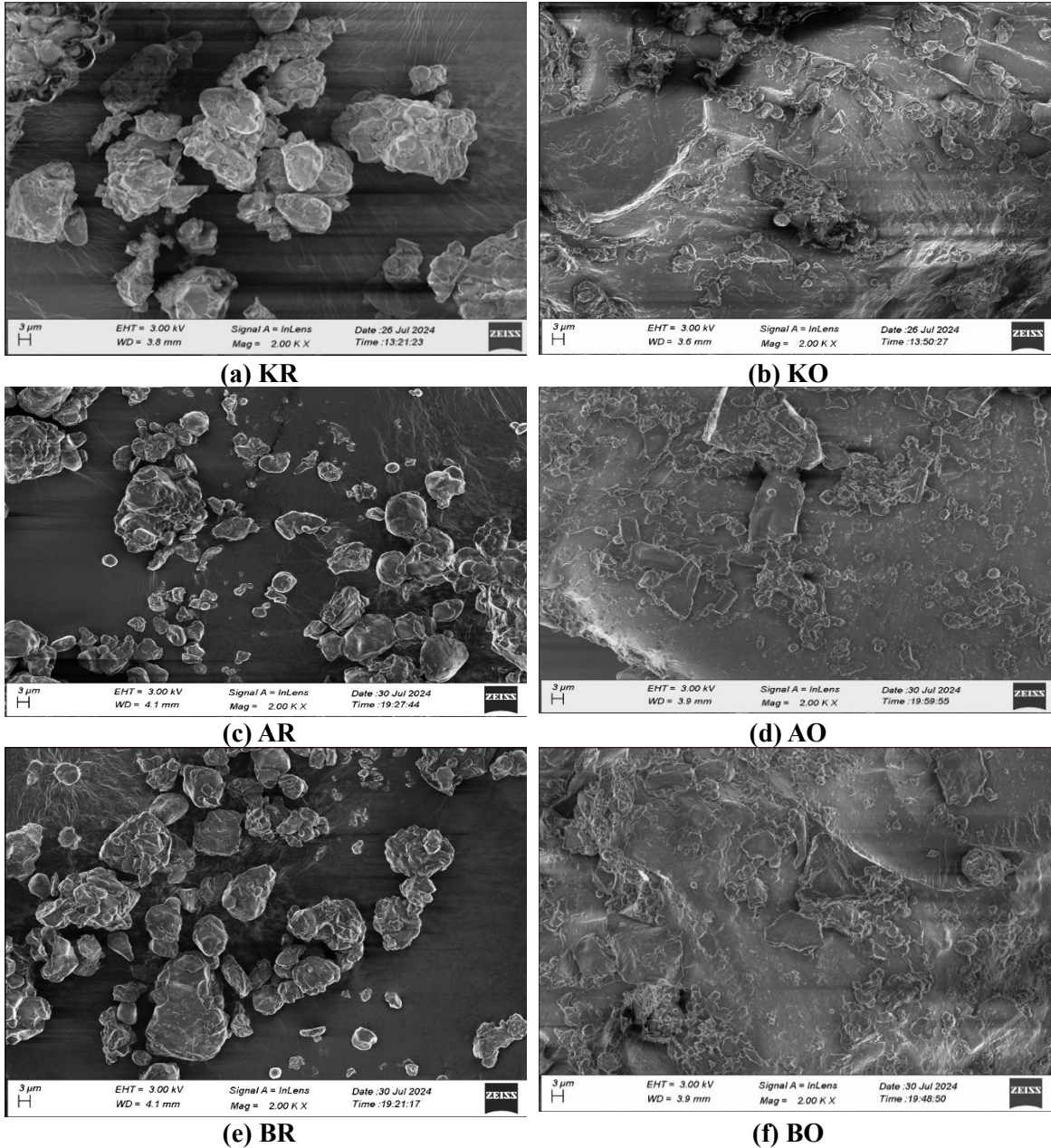


Fig. 3.7. SEM images of (a, c, e) raw (KR, AR and BR) and (b, d, f) optimized (KO, AO and BO) samples of *Kenkowa bao*, *Amona bao* and *Badal bao* rice varieties respectively.

Table 3.17. Digestive properties of the raw and optimized porridge mix samples

Samples	Digestive properties		
	RDS (%)	SDS (%)	RS (%)
KR	59.02±0.22 ^c	21.62±0.22 ^d	9.27±0.11 ^f
KO	51.44±0.17 ^h	24.64±0.34 ^a	13.22±0.09 ^a
KRM	62.78±0.23 ^a	17.45±0.11 ^f	7.88±0.08 ⁱ
KOM	56.44±0.11 ^d	22.49±0.34 ^c	10.16±0.14 ^d
AR	50.56±0.15 ⁱ	19.86±0.27 ^e	8.52±0.27 ^h
AO	47.23±0.25 ^k	23.55±0.17 ^b	11.07±0.11 ^c
ARM	53.45±0.17 ^g	14.12±0.12 ^g	6.57±0.12 ^k
AOM	55.12±0.44 ^e	19.98±0.26 ^e	8.82±0.06 ^g
BR	56.44±1.03 ^d	22.12±0.45 ^c	8.44±0.07 ^h
BO	48.67±0.13 ^j	24.93±0.21 ^a	12.44±0.16 ^b
BRM	59.87±0.22 ^b	17.66±0.24 ^f	7.02±0.09 ^j
BOM	54.34±0.15 ^f	19.87±0.17 ^c	9.87±0.12 ^e

Means with same superscript letters within the columns are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test

3.3.13. Principal component analysis of sensory properties

The PCA biplot explained 96.55% of the total variance, with PC1 explaining 80.23% and PC2 contributing 16.32% (Fig. 3.8). Taste, texture, mouthfeel, flavor, aroma and OAA were the primary drivers of differentiation along PC1, while appearance varied independently along PC2. KOM and BOM were strongly associated with PC1, indicating superior ratings, followed by AOM with moderate scores. KO and BO aligned more with appearance, reflecting strong visual appeal but average sensory performance. AO, positioned separately, exhibited a balanced yet less distinctive profile. KR, AR, BR and KRM, BRM, ARM clustered together, but farthest from key sensory attributes, indicated lower acceptability. KOM scored the highest OAA (8.10), followed by BOM

(8.05) and AOM (7.95), while AR (4.60), followed by BR (4.68) and KR (4.70) scored the lowest.

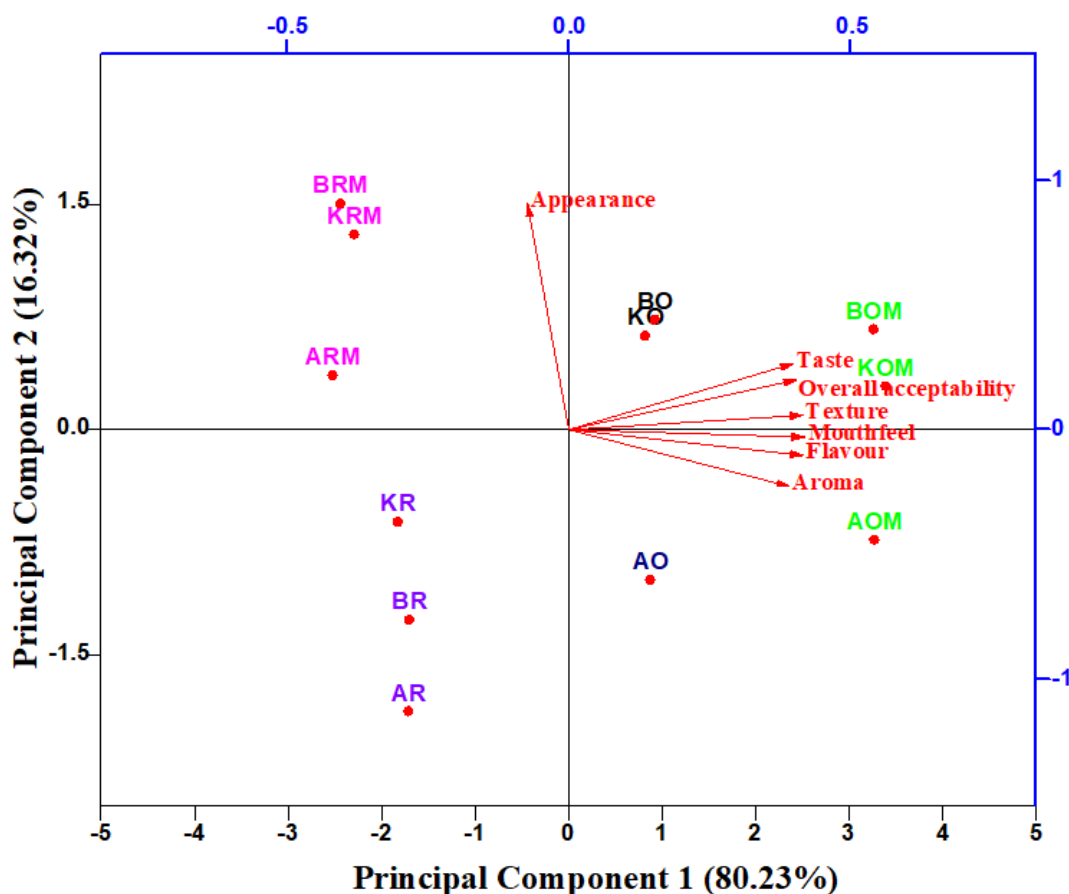


Fig. 3.8. Principal Component Analysis (PCA) biplot of sensory profile of samples based on panellists' preference and rating

3.3.14. Storage study

A gradual increase in water activity (a_w) was observed across all samples during the 12-month storage period (Fig. 3.9). This increase plays a crucial role in accelerating deteriorative reactions, as higher a_w enhances molecular mobility, thereby facilitating lipid hydrolysis and oxidation. Samples containing SMP (KRM, ARM, BRM and KOM, AOM, BOM) exhibited comparatively higher water activity which contributed to their increased susceptibility to deterioration.

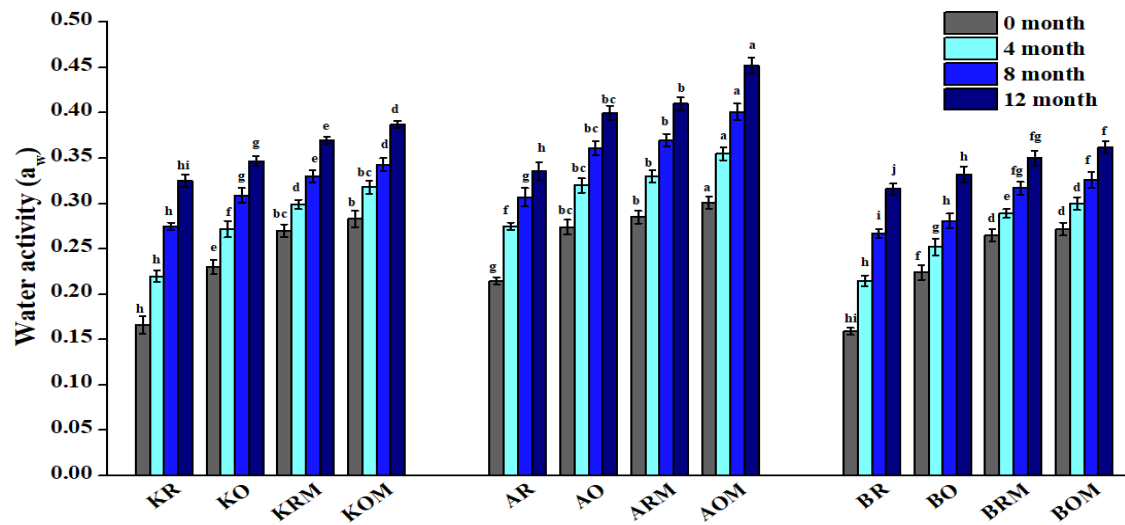


Fig. 3.9. Water activity of the porridge mix samples over a period of 12 months
 Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within each sample across storage months are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

A progressive increase in FFA, TBARS and PV across samples indicated lipid oxidation and hydrolytic rancidity over the storage period (Fig. 3.10). The rise in FFA suggested increased enzymatic or non-enzymatic lipolysis, possibly due to altered lipid-protein interactions post-cooking, making lipids more susceptible to hydrolysis. Caramelized sugar, containing reactive carbonyls, might have further promoted oxidation-lipolysis interactions. Although SMP has negligible fat, its proteins could influence oxidation through Maillard reactions, generating ROS and catalyzing oxidation through metal ions. KRM, ARM, BRM and KOM, AOM, BOM exhibited greater oxidative deterioration than KR, AR, BR and KO, AO, BO samples, attributing to increased water activity from SMP which enhanced molecular mobility for hydrolysis and oxidation. Additionally, MRPs in caramelized sugar acted as pro-oxidants, accelerating peroxide breakdown into secondary oxidation products. Storage temperature, humidity and packaging also influenced oxidation rates. Naseer et al. (2021) similarly reported rising FFA and PV with declining OAA in instant phirni mix over 90 days.

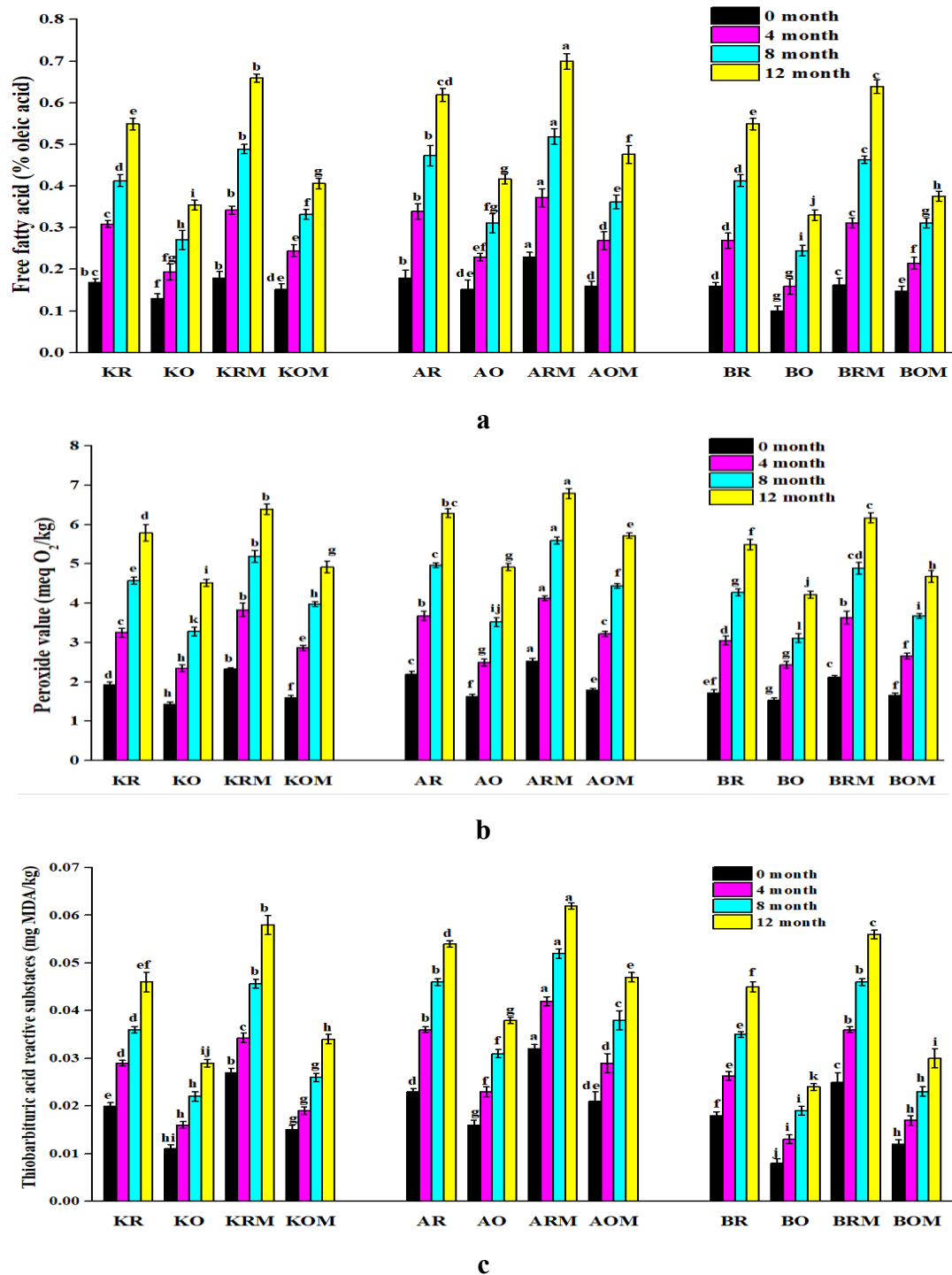


Fig. 3.10 (a) FFA value, (b) PV and (c) TBARS of the porridge mix samples over a period of 12 months

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within each sample across storage months are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

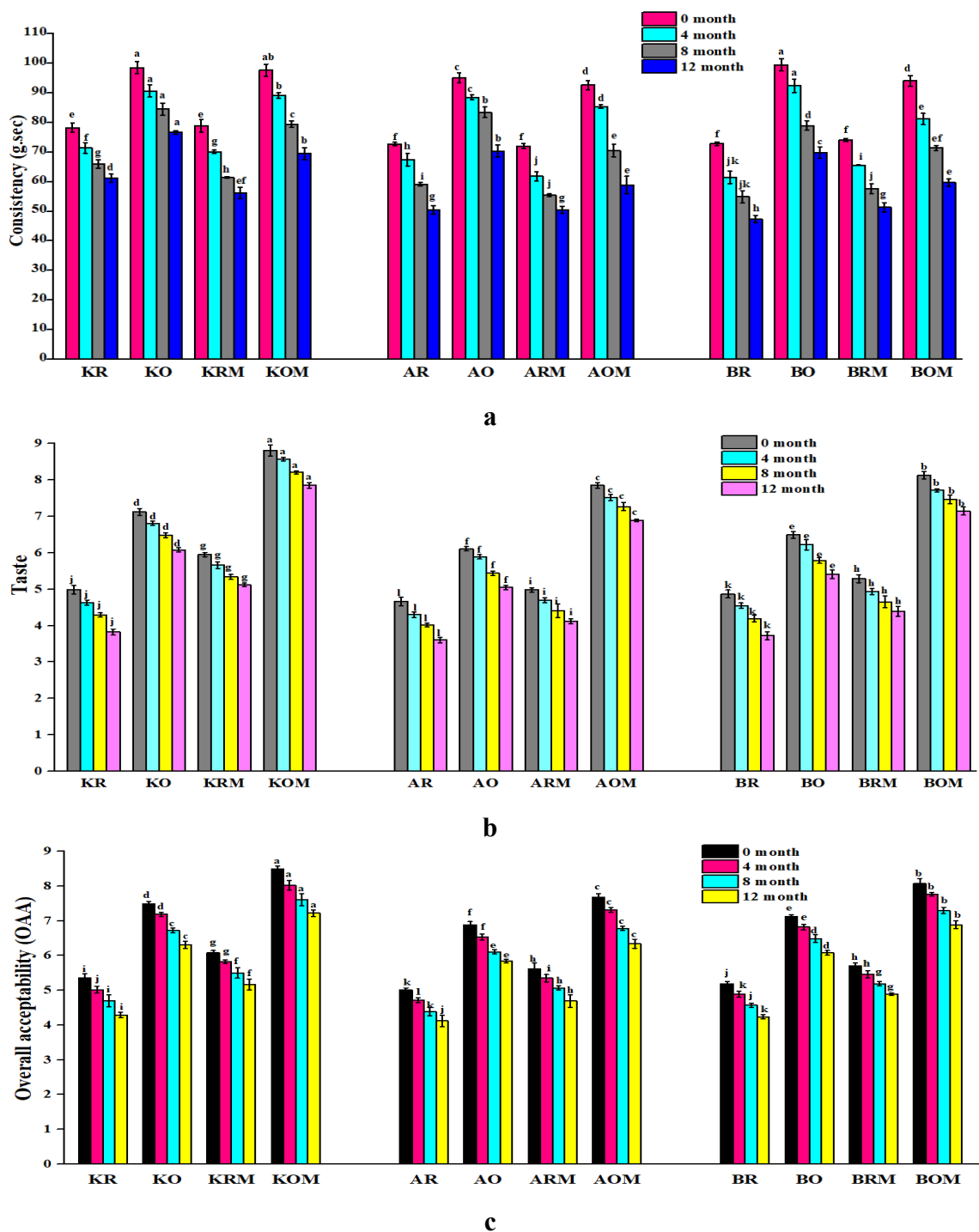


Fig. 3.11 (a) Consistency, (b) Taste and (c) Overall acceptability of the porridge mix samples over a period of 12 months

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within each sample across storage months are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

Loss of consistency over the storage period was observed (Fig. 3.11), which could be attributed to starch retrogradation, protein denaturation and phase separation. Partial gelatinization in hydrothermally treated rice flour leads to amylose-amylopectin reassociation, reducing viscosity over time. SMP proteins, particularly casein, may have interacted with starch, altering hydration and gelation, while caramelized sugar potentially disrupted the starch-protein matrix. Sensory degradation, including taste and OAA (Fig 3.11), likely resulted from lipid oxidation and textural changes. Oxidized byproducts- volatile aldehydes and ketones resulted in rancid flavors, while MRPs' interaction with proteins and starch negatively altered the flavor profile thereby reducing the overall sensory appeal during storage.

3.4. Conclusion

This study successfully evaluated the effect of process variables, rice concentration and cooking time on the key functional properties of the dry instant porridge. Optimization improved techno-functional properties, leading to improved product characteristics. An increase in WAI and SP, along with a decrease in WSI and ρ_b , indicated enhanced packing efficiency, retention behavior and desirable textural attributes for commercial application. Bioactivity was reduced post-optimization, while resistant starch content increased. Addition of caramelized sugar and SMP improved the sensorial properties of the porridge mix. Storage studies confirmed the stability of the dry porridge mix for up to 12 months at ambient temperature. *Kenkowa bao*, followed by *Badal bao*, showed the highest potential for porridge mix development using the optimized methodology. The nutritional, digestive, functional and sensorial attributes of all the three *bao* paddy cultivars highlighted their strong potential for the instant food industry. This approach could contribute to the promotion of local agricultural economy of Northeast India by integrating regional food resources into global markets.

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CHAPTER 4

Modulating structural, functional and rheological properties of pigmented *bae* rice whole flours by sodium trimetaphosphate crosslinking

4.1. Introduction

The expanding demand for structurally stable and functionally tailored food ingredients has constantly accelerated researches on starch modification. Native starches, although widely used in food systems due to high abundance, biodegradability and ability to form viscous pastes and gels, often exhibit limitations during industrial processing. Being susceptible to excessive swelling, shear thinning, thermal degradation and rapid retrogradation during storage are few noteworthy drawbacks associated with native starches. These further lead to undesirable changes in viscosity, gel structure and water retention when subjected to processing stress (Majzoobi and Farahnaky, 2021; Sinhmar et al., 2023). To overcome such challenges, several starch modification techniques, classified as physical, enzymatic and chemical methods have been developed (Nawaz et al., 2020). Physical modifications, such as heat-moisture treatment, annealing, extrusion and pre-gelatinization induce molecular alterations and rearrangements within starch granules. These lead to improved thermal stability and swelling restrictions, without altering chemical structure (Compart et al., 2023). Enzymatic modifications use amylolytic and starch-debranching enzymes such as α -amylase, β -amylase, pullulanase and isoamylase. They selectively hydrolyze glycosidic linkages and modify branching architecture, thereby influencing chain length distribution, impacting rheological behavior and digestibility of the starch (Bangar et al., 2022). In contrast, chemical modification includes reactions such as crosslinking, substitution (acetylation, hydroxypropylation), oxidation and esterification, which introduce covalent changes in starch molecules. These suitably alter the starch's physicochemical behavior, including

anticipated resistance to thermal, mechanical and storage stresses (Gałkowska et al., 2023).

Crosslinking is considered as one of the most effective approaches towards enhancing structural stability of starches. It targets at formation of covalent bonds between hydroxyl groups of adjacent starch chains, producing a three-dimensional molecular network that reinforces the internal structure of starch granules. This molecular reinforcement limits excessive swelling and molecular disintegration during heating and mechanical processing, thereby improving paste stability and gel strength (Compart et al., 2023; Bangar et al., 2024). Chemically crosslinked starches typically exhibit higher resistance to thermal and mechanical shear and reduced syneresis during storage. These functional improvements make crosslinked starches highly suitable for food systems exposed to harsh processing conditions such as cooking, freezing or prolonged storage. Food-grade crosslinking agents such as sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), sodium hexametaphosphate (SHMP), phosphorus oxychloride (POCl_3), citric acid, adipic acid and epichlorohydrin are commonly employed (Min et al., 2023). These can be broadly categorized into phosphate-based and organic acid-based crosslinkers. Phosphate-based agents such as STPP and SHMP form diester phosphate bridges between starch chains, enhancing granule rigidity, although their reactivity is relatively limited. POCl_3 forms strong phosphate ester linkages by nucleophilic substitution. But its application is restricted due to safety concerns. Organic acids such as citric and adipic acid induce crosslinking through esterification reactions, improving stability. However, their crosslinking efficiency are lower (Bangar et al., 2024). Most widely used among these is STMP, owing to its relatively higher reaction efficiency and regulatory acceptance for food applications. During the crosslinking reaction, STMP interacts with the hydroxyl groups of glucose units in starch molecules to form phosphate ester linkages. These phosphate bridges connect adjacent starch chains and create a compact and reinforced granule structure. Its cyclic metaphosphate structure enables efficient formation of stable phosphate diester linkages under mild conditions, allowing better control over crosslinking density. In addition, STMP offers a

safer alternative to highly reactive agents like POCl_3 and provides stronger and more uniform crosslinking than organic acids (Bodjrenou et al., 2023). As a result, STMP-crosslinked starches demonstrate superior paste stability, shear resistance and freeze-thaw stability (Sinhmar et al., 2023).

However, the degree of crosslinking is a critical factor. Insufficient crosslinking may result in only minor improvements in functional properties, whereas excessive crosslinking can produce overly rigid structures with restricted swelling capacity and reduced paste viscosity development (Compart et al., 2023). Also, although crosslinking improves textural stability, it can concurrently influence the nutritional profile, functional attributes and biochemical activity of the starch matrix (Khadsai et al., 2024; Gu et al., 2024). Moreover, the use of higher levels of STMP may result in an increased residual phosphorus content in the modified starch, potentially exceeding the permissible limits for food applications. Regulatory frameworks established by the Code of Federal Regulations (CFR) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) restrict the phosphorus content in crosslinked starches to a maximum of 0.4% (w/w, expressed as phosphorus) (CFR, 2022; JECFA, 2023). Therefore, careful control and optimization of crosslinking conditions are essential in tailoring the desired functional properties for food usage, without compromising regulatory compliance. Systematic evaluation of different crosslinking levels is necessary to understand how modification intensity influences structural and functional characteristics.

Like the other starch modification techniques, crosslinking has been always carried out on native pure starch granules isolated from the plant cells (Gong et al., 2024, Bangar et al., 2024, Kalu et al., 2025). Starch extraction and purification involve wet milling and sodium salt, alkali, or enzymatic treatments to remove the minor non-starch components. However, it could be readily hypothesized that low particle sized flour fractions of popular starch sources like rice and other cereal grains, tubers and roots would have sufficient exposure of their starch granules for surface as well as surface-to-core chemical modifications. Using flours instead of purified starch would significantly lower

the extraction costs and improve material handling. This would also retain the naturally occurring health-promoting compounds. Investigating crosslinking directly on flour matrices would also provide an understanding of how modification affects ingredients intended for practical food product development.

Pigmented rice varieties have recently attracted attention as promising functional food ingredients due to their high levels of bioactive compounds such as phenolics, flavonoids and proanthocyanidins. These compounds contribute to strong antioxidant activity and a range of health-promoting effects (Tiozon et al., 2023). Red-colored, flood-resistant *bao* rice landraces have been reported for their high bioactive and ready-to-eat product forming properties (Deka et al., 2026; Rajkhowa and Borgohain, 2024). Being high in starch content (>70%) and possessing high amylose (25-30% apparent amylose content) in their starches, flours of all these rice grains could potentially serve as effective substrates for chemical modification.

Therefore, the present study aimed to evaluate the effects of STMP-induced starch-crosslinking in the whole flours of three red-pigmented *bao* rice landraces. Different levels of crosslinking were applied to determine how modification intensity influenced the physicochemical, structural and functional characteristics of the flours.

4.2. Materials and methods

4.2.1. Materials

Freshly harvested pigmented *bao* rice types, namely *Amona*, *Badal* and *Kenkowa* were procured for the study. The paddy was dehusked using a laboratory-scale huller, milled to flour with a grinder (Phillips HL7707/00, India) and particle size adjusted at 75-150 μ m using British standard sieves (BSS 200 and 100). The flour moisture was adjusted at 12 $\pm$ 1% and stored in airtight containers until further use. Reagents were of analytical grade and procured from SRL Chemicals, Lobachemie, HiMedia, Merck, and Sigma-Aldrich.

4.2.2. Starch crosslinking and sample coding

Crosslinking was conducted with sodium trimetaphosphate (STMP), using a slightly modified method of Kumar et al. (2022). Rice flour (50 g) was dispersed in 100 ml of distilled water at 45 °C and stirred continuously. To it, STMP was added at concentrations of 3% (1.5 g), 6% (3.0 g), 9% (4.5 g), and 12% (6.0 g). The pH of the mixture was adjusted at 11.0 using 0.5 mol/l sodium hydroxide. The reaction was maintained for 3 h. Following this, the suspension pH was readjusted to 6.5 using 1.0 mol/l HCl and centrifuged to separate the modified crosslinked flour (CF). The obtained precipitate was washed six times with distilled water to remove any residual reagent and subsequently dried at 40 °C to 12±1% moisture content.

Sample codes of CFs were structured according to rice name (*Amona* = A, *Badal* = B, *Kenkowa* = K) and crosslinking level (0-12% STMP) as ACL3, ACL-6, ACL9 and ACL12, BCL3, BCL6, BCL9 and BCL12, and KCL3, KCL6, KCL9 and KCL12, respectively. Native flours (0% crosslinking) were designated as AR, BR and KR, respectively.

4.2.3. Residual phosphorus content

RPC of thoroughly washed native and crosslinked flour samples was determined using a spectrophotometric method (Malik et al., 2023). Briefly, 0.5 g of dried flour sample was digested using 10 ml of a diacid mixture (HNO₃: HClO₄, 3:1 v/v) until a clear solution was obtained. The digest was cooled and diluted to 50 ml with distilled water and filtered. An aliquot (5 ml) of this extract was treated with 1 ml ammonium molybdate solution (2.5% w/v in 5 N H₂SO₄) followed by reduction with 0.4 ml freshly prepared ascorbic acid solution (10% w/v). The mixture was then vortexed and incubated at room temperature (27±3 °C) for 30 min to allow the development of a blue-colored phosphomolybdenum complex. Its absorbance was measured at 880 nm. A standard calibration curve was prepared using KH₃PO₄ solutions (0-5 mg/l phosphorus), and RPC values were expressed as % phosphorus (w/w, d.b.).

4.2.4. Fourier transform infrared spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was employed to verify STMP-induced crosslinking in the rice flours by identifying phosphate functional groups and associated structural modifications relevant to its functional and rheological behavior. Vacuum dried samples were mixed with KBr (1:10 w/w) and pelleted before scanning (500 to 4000 cm^{-1}) in a spectrometer (Bruker Alpha, Germany).

4.2.5. Apparent amylose content

STMP-induced crosslinking at molecular level can have direct effect on quantifiable amylose content of starch, which would have direct implications on the consequent modified starch behavior. Apparent amylose content (AAC) of the native and CF samples was quantified using an iodometric assay procedure described by Govindaraju et al. (2022), with slight modifications. In brief, 0.1 g flour was treated with 1 ml of 95% ethanol and 9 ml of 1 N sodium hydroxide, incubated in a boiling water bath for 10 min, followed by cooling to ambient temperature. The final volume was adjusted to 100 ml with distilled water. Subsequently, a 5 ml aliquot of the prepared solution was transferred to a test tube, to which 1 ml glacial acetic acid and 2 ml iodine solution (0.2% I_2 in 2% KI) were added. The mixture was then diluted to a final volume of 100 ml using distilled water. After incubation at room temperature for 10 min, absorbance was measured at 620 nm using a UV-Visible spectrophotometer (Orion AquaMate 8100, Thermo Fisher Scientific, Germany). AAC was determined by comparing the absorbance values with a standard calibration curve of purified potato amylose (Sigma-Aldrich).

4.2.6. Scanning electron microscopy

Micro-morphological characteristics of the samples were examined using a scanning electron microscope (SEM; Sigma 300, Zeiss, Germany). Prior to analysis, the flours

samples were vacuum dried, mounted onto aluminum stubs using double-sided carbon adhesive tapes and sputter-coated with gold. 1500X magnified images were captured.

4.2.7. Swelling power and solubility

Swelling powers and solubility indices of the flour samples were determined following a modified method of Govindaraju et al., (2025). Briefly, 1 g (dry basis) of flour sample was accurately weighed and dispersed in 20 ml of distilled water in a pre-weighed centrifuge tube. The dispersion was incubated in a thermostatically controlled shaking water bath at 60 °C for 30 min. After incubation, the samples were cooled to room temperature and subsequently centrifuged at 3500 rpm for 15 min. The supernatant was carefully decanted in a pre-weighed petri dish and dried at 105 °C until constant weight. The swelling power was calculated as the weight of the sediment gel per gram of initial flour (Eq. 4.1). Solubility was determined by calculating the ratio of the dry weight of the supernatant to the initial dry weight of the flour sample, and expressed as a percentage (Eq. 4.2).

$$\text{Swelling power (g/g)} = \frac{\text{Weight of swollen sediment (g)}}{\text{Initial dry flour weight (g)}} \quad \text{Eq. 4.1}$$

$$\text{Solubility (\%)} = \left(\frac{\text{Weight of dried supernatant}}{\text{Initial dry flour weight}} \right) \times 100 \quad \text{Eq. 4.2}$$

4.2.8. Pasting properties

Pasting behaviors of the native and CF samples were analyzed using a Rapid Visco Analyzer (RVA; TechMaster, Perten Instruments, Australia). A starch suspension was prepared by dispersing 3 g (dry basis) of starch in 25 ml distilled water. The slurry was initially equilibrated at 50 °C for 1 min, followed by heating to 95 °C at a rate of 12.2 °C/min. The temperature was maintained at 95 °C for 2.5 min before cooling to 50 °C at 11.8 °C/ min and holding for an additional 2 min. Key pasting parameters, including pasting temperature (PT, °C), peak viscosity (PV, cP), hot paste viscosity (HPV, cP),

final viscosity (FV, cP), breakdown viscosity (BDV, cP), and setback viscosity (SBV, cP) were recorded.

4.2.9. Thermal properties

All flour samples were analyzed for their thermal profiles using a differential scanning calorimeter (DSC60, Shimadzu, Japan). Flour slurries were prepared in aluminum pans by dispersing 3 mg of each sample in deionized water (1:2 flour-to-moisture ratio, d.b). The slurries were incubated at 4 °C for 1 h, the pans crimped, and then subjected to heating from 25 °C to 180 °C at intervals of 5 °C/min. The onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy of gelatinization (ΔH , J/g) were obtained from the generated thermograms.

4.2.10. Starch digestibility

Rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) contents of the native and CF samples were estimated by following the procedure of Roy et al. (2019) with slight adjustments. Briefly, the samples were first deproteinized with pepsin in HCl-KCl buffer (pH 1.5) at 37 °C for 1 h. The pH was then raised to 5.4 by adding 7 ml acetate buffer. Starch hydrolysis was then initiated by incorporating 3 ml of an enzyme solution (invertase 220 U/ml + pancreatic α -amylase 3000 U/ml + amyloglucosidase 15 U/ml). Aliquots were withdrawn at 20th and 120th min to determine glucose release. The respective measured glucose concentrations (G_{20} and G_{120}) were converted to corresponding starch values using a conversion factor of 0.9 and expressed as a percentage of total starch (TS). The starch hydrolyzed within 20 min was categorized as RDS, the fraction digested between 20 and 120 min as SDS, and the undigested portion remaining after 120 min as RS and calculated using Eq. 4.3, 4.4 and 4.5 respectively.

$$RDS(\%) = \left[\frac{G_{20} \times 0.9}{TS} \right] \times 100 \quad \text{Eq. 4.3}$$

$$SDS(\%) = \left[\frac{(G_{120} - G_{20}) \times 0.9}{TS} \right] \times 100 \quad \text{Eq. 4.4}$$

$$RS(\%) = [TS - (RDS + SDS)] \times 100 \quad \text{Eq. 4.5}$$

4.2.11. Development of gel

Starch and starchy flours are commonly used in hydrothermally processed food systems. To understand the effect of crosslinking on functionality of the modified flours, their gels were prepared at first (Fig. 4.1). For this, 10 g of each flour was dispersed in 100 ml distilled water in a beaker and heated at 85 ± 5 °C for 5 min under continuous stirring. The cooked gel was then poured into molding trays, cooled down to ambient temperature, followed by storage at 4 ± 2 °C for 12 h to permit complete gel setting. The set gel blocks ($30 \times 30 \times 30$ mm) were carefully removed from the molds and evaluated further.

The suffix “G” was used to denote gel samples. Accordingly, the developed gels were coded as AGCL-0, AGCL-3, AGCL-6, AGCL-9 and AGCL-12 for Amona; BGCL-0, BGCL-3, BGCL-6, BGCL-9 and BGCL-12 for *Badal*; and KGCL-0, KGCL-3, KGCL-6, KGCL-9 and KGCL-12 for *Kenkova*.

4.2.12. Gel color

The color characteristics of the freshly prepared native and CF gels were evaluated in terms of CIE parameters using a Color Quest spectrophotometer (Hunter Associates Laboratory, USA). The machine was calibrated using white and black reference tiles prior to the measurements. In this system, L^* represents lightness (0 = black, 100 = white), a^* denotes the chromatic transition from green (–) to red (+), and b^* indicates the range from blue (–) to yellow (+). The total color difference (ΔE) was calculated using the following equation (Eq. 4.6).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \text{Eq. 4.6}$$

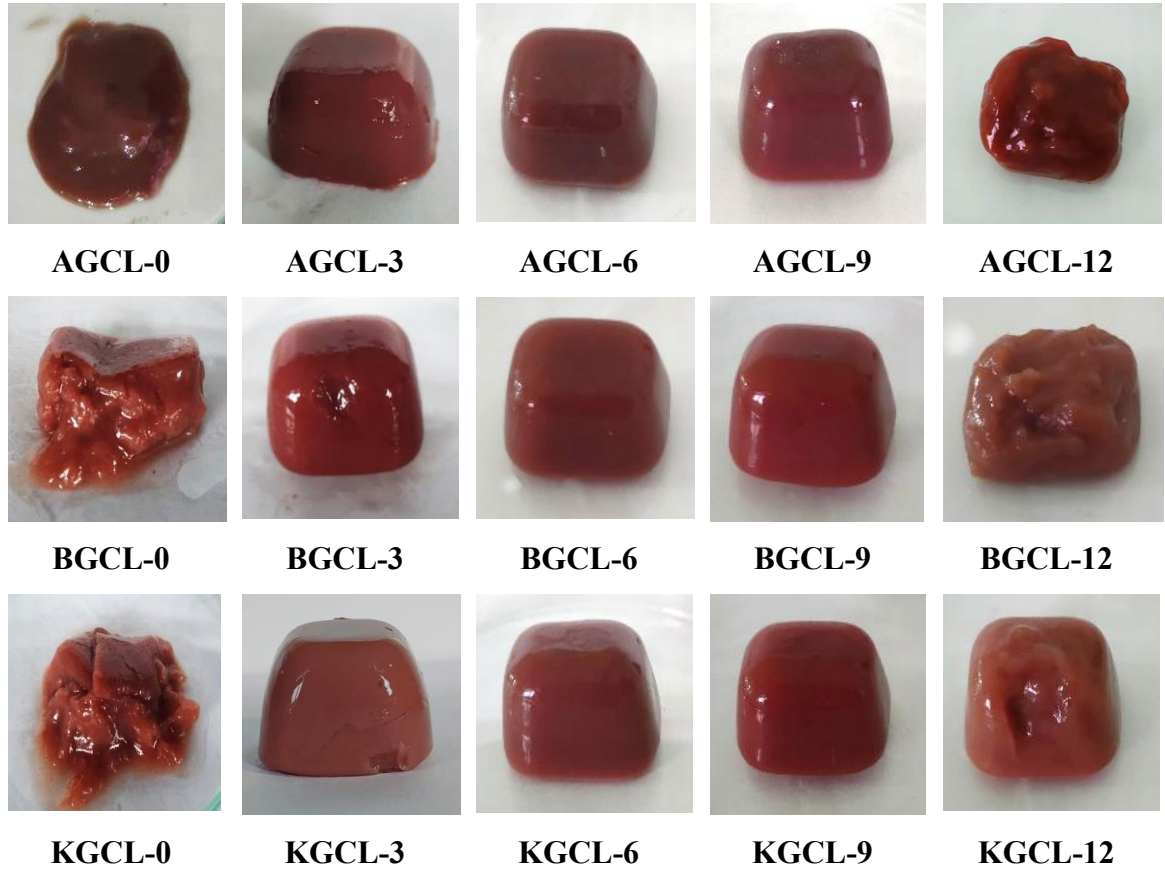


Fig. 4.1. Crosslinked flour gels of *Amona*, *Badal* and *Kengkowa bao*

4.2.13. Syneresis

Stability of the gel samples to temperature fluctuations was assessed by determining the percentage of syneresis over two freeze-thaw cycles, following a modified procedure of Anjali et al. (2025). To evaluate syneresis, 20 g of the freshly prepared gel was placed into a pre-weighed centrifuge tube, tightly sealed, cooled to room temperature, and stored at 4 ± 2 °C. At 24 h intervals, the samples were equilibrated at ambient temperature for 4 h and then centrifuged at 6300 g for 5 min. The released supernatant was carefully decanted and weighed to quantify syneresis value (Eq. 4.7). The procedure was conducted on the 7th and 14th days of storage to assess the progression of syneresis over time.

$$\text{Syneresis (\%)} = \left(\frac{\text{Weight of decanted liquid (g)}}{\text{Initial weight before centrifugation (g)}} \right) \times 100 \quad \text{Eq. 4.7}$$

4.2.14. Texture

Texture profile analysis (TPA) of the native and CF gels were evaluated using a TA.XT Plus Texture Analyzer (Stable Micro Systems, UK). Measurements were carried out through a two-cycle compression test applied to the central portion of gel cubes with a 20 mm cylindrical probe connected to a 10 kg load cell. The operational parameters were set at a pre-test speed of 3 mm s⁻¹, a test speed of 1 mm s⁻¹, and a post-test speed of 1 mm s⁻¹, with a compression distance of 2 mm. A trigger force of 5 g was maintained to standardize sample contact, allowing the probe to penetrate 5 mm into the gel matrix. The resultant force–time curves were analyzed using the instrument’s software (Exponent Connect) to obtain hardness, adhesiveness, cohesiveness, springiness, chewiness, and resilience values.

4.2.15. Rheological properties

Rheological characterization of the crosslinked gels was performed using a Physica MCR 101 rheometer (Anton Paar, Austria) equipped with a cone-and-plate geometry (50 mm diameter, 1° cone angle, 0.1 mm gap). All measurements were conducted at 27 °C. Prior to analysis, each sample was carefully loaded onto the measuring system and allowed to equilibrate for 2 min to ensure thermal stability and structural relaxation (Bora et al., 2023).

4.2.15.1. Steady shear measurements

Steady shear behavior of the crosslinked gel systems was evaluated by applying a controlled shear rate sweep from 0 to 300 s⁻¹ over 2 min, followed by a reverse sweep back from 300 to 0 s⁻¹ over the next 2 min. The apparent viscosity (η) was recorded as a function of shear rate, with particular emphasis on the viscosity at 50 s⁻¹, corresponding

to oral shear conditions. The apparent viscosity at this shear rate was designated as η_{50} (Pa·s).

The flow behavior of the gels was described using the Herschel-Bulkley model, which accounts for yield stress and non-Newtonian characteristics (Eq. 4.8):

$$\tau = \tau_0 + K\dot{\gamma}^n \quad \text{Eq. 4.8}$$

where, τ is shear stress (Pa), τ_0 is yield stress (Pa), K is the consistency coefficient (Pa·s^{*n*}), $\dot{\gamma}$ is shear rate (s⁻¹) and n is the flow behavior index (dimensionless).

4.2.15.2. Dynamic oscillatory measurements

The viscoelastic properties of the crosslinked gels were further investigated under small-amplitude oscillatory shear conditions. Frequency sweep tests were carried out over an angular frequency range of 0.1-100 rad·s⁻¹ at a constant strain of 1%, within the linear viscoelastic region. The storage modulus (G') and loss modulus (G'') were recorded as functions of angular frequency (ω), representing the elastic and viscous components of the material, respectively. The damping factor ($\tan \delta$) was calculated to assess the relative contribution of viscous and elastic behavior (Eq. 4.9):

$$\tan \delta = \frac{G''}{G'} \quad \text{Eq. 4.9}$$

The overall viscoelastic response was further quantified through the complex modulus (G^*) and complex viscosity (η^*), calculated as (Eq. 4.10 and Eq. 4.11):

$$G^* = \sqrt{(G')^2 + (G'')^2} \quad \text{Eq. 4.10}$$

$$\eta^* = \frac{G^*}{\omega} \quad \text{Eq. 4.11}$$

where, ω represents angular frequency

4.2.15.3. Frequency dependence modeling

To describe the structural response of the crosslinked gel network under oscillatory deformation, the dependence of G' and G'' on angular frequency was analyzed using power-law relationships (Eq. 4.12 and Eq. 4.13):

$$G' = K'(\omega)^{n'} \quad \text{Eq. 4.12}$$

$$G'' = K''(\omega)^{n''} \quad \text{Eq. 4.13}$$

where K' and K'' represent the intercepts associated with elastic and viscous contributions, respectively, while n' and n'' denote the frequency sensitivity of the respective moduli. The goodness of fit was evaluated using the coefficient of determination (R^2).

4. 2.16. Bioactivity

4.2.16.1. Sample extraction

Freeze-dried gel samples were initially obtained by pre-freezing cut gel cubes ($10 \times 10 \times 10$ mm) at -40°C for 24 h, followed by primary drying at -35°C under negative pressure of 100 mTorr for 24 h. The dried gel cubes were ground into a fine powder ($75\ \mu\text{m}$) and extracted with absolute ethanol at a sample-to-solvent ratio of 1:10 (w/v) at 40°C for 24 h under continuous agitation. The mixture was subsequently centrifuged at 845 g for 60 min and the supernatant was collected for further analyses.

4.2.16.2. Total phenolic content (TPC)

500 μl of the ethanolic extract was mixed with 2.5 ml of 10% (w/v) Folin-Ciocalteu Reagent (FCR), immediately followed by 2 ml of 7.5% sodium carbonate. The mixture was vortexed and dark incubated for 30 min. A blank was prepared by replacing extract with ethanol. Absorbance was measured at 765 nm and compared with a standard curve

prepared using gallic acid. Value of TPC was expressed as gallic acid equivalent (μg GAE/g) of sample (Kalpan et al., 2021).

4.2.16.3. Total flavonoid content (TFC)

A method described by Norhazlini et al. (2021) was slightly modified. Briefly, 0.5 ml extract was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After vortexing and allowing to stand for 5 min, 1 ml of 1 M sodium hydroxide was added. Absorbance was measured at 510 nm and plotted against a standard curve prepared using quercetin. Result was expressed as quercetin equivalent (μg QE/100 g) of sample.

4.2.16.4. DPPH radical scavenging activity (DRSA)

DRSA was determined as per Kusumawardani and Luangsakul (2024) with slight modifications. A blank solution was prepared by mixing 200 μl ethanol with 2.8 ml of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH). Simultaneously, 200 μl ethanolic sample extract was mixed with 2.8 ml DPPH solution and dark incubated for 30 min. Absorbance was measured at 517 nm and results expressed as percentage DRSA (Eq. 4.14).

$$\% \text{ DRSA} = \frac{(A_0 - A_s)}{A_0} \times 100 \quad \text{Eq. 4.14}$$

where, A_0 is the absorbance of the blank, A_s is the absorbance of the sample extract

4.2.16.5. Ferric reducing antioxidant potential (FRAP)

FRAP was determined as per Zhang et al. (2022). 1 ml sample extract was thoroughly mixed with 3 ml of freshly prepared FRAP solution and incubated at 37 °C for 4 min. Absorbance was determined at 593 nm, taking distilled water as blank. A calibration curve was prepared using a concentration range of 0.1 to 1 mM aqueous ferrous

sulphate. Values were expressed as milimole of ferrous equivalent Fe(II) per g (mM/g) of sample.

4.2.17. Statistical analysis

All the analyses were performed in triplicates, with results reported as mean $\pm$ standard deviation. Significance of differences among the results were assessed using Duncan's multiple range test at a 95% confidence level ($p \leq 0.05$) using SPSS 21 (IBM, USA). Graphs and model fitting were performed using Origin 9.0 (OriginLab Corporation, USA).

4.3. Results and discussions

4.3.1. Residual phosphorus content

The RPC (%) values of native and CFs are presented in Table 4.1. Native samples exhibited low RPC levels (0.011%-0.013%), reflecting inherent phosphate groups. A significant increase ($p < 0.05$) in RPC was observed with increasing STMP-crosslinking levels across CFs of all varieties, reaching highest values of 0.063%, 0.077% and 0.079% in ACL-12, BCL-12 and KCL-12, respectively. This confirmed formation of the distarch phosphate linkages upon modification (Heo et al., 2017; Malik et al., 2023). Slightly higher values in *Badal* and *Kenkowa* suggested varietal influence, possibly due to differences in AAC and granule structure. Importantly, RPC of all samples remained well below the JECFA permitted maximum limit of 0.4%, confirming their safety and suitability for food applications.

4.3.2. Fourier transform infrared spectroscopy

Molecular-level structural modifications induced by STMP-crosslinking were elucidated from the FTIR graphs (Fig. 4.2). The crosslinked samples exhibited noticeable changes in the spectral regions corresponding to O-H stretching (~ 3200 - 3400 cm^{-1}) and C-O-C stretching vibrations (~ 1000 - 1150 cm^{-1}), indicating alterations in hydrogen bonding

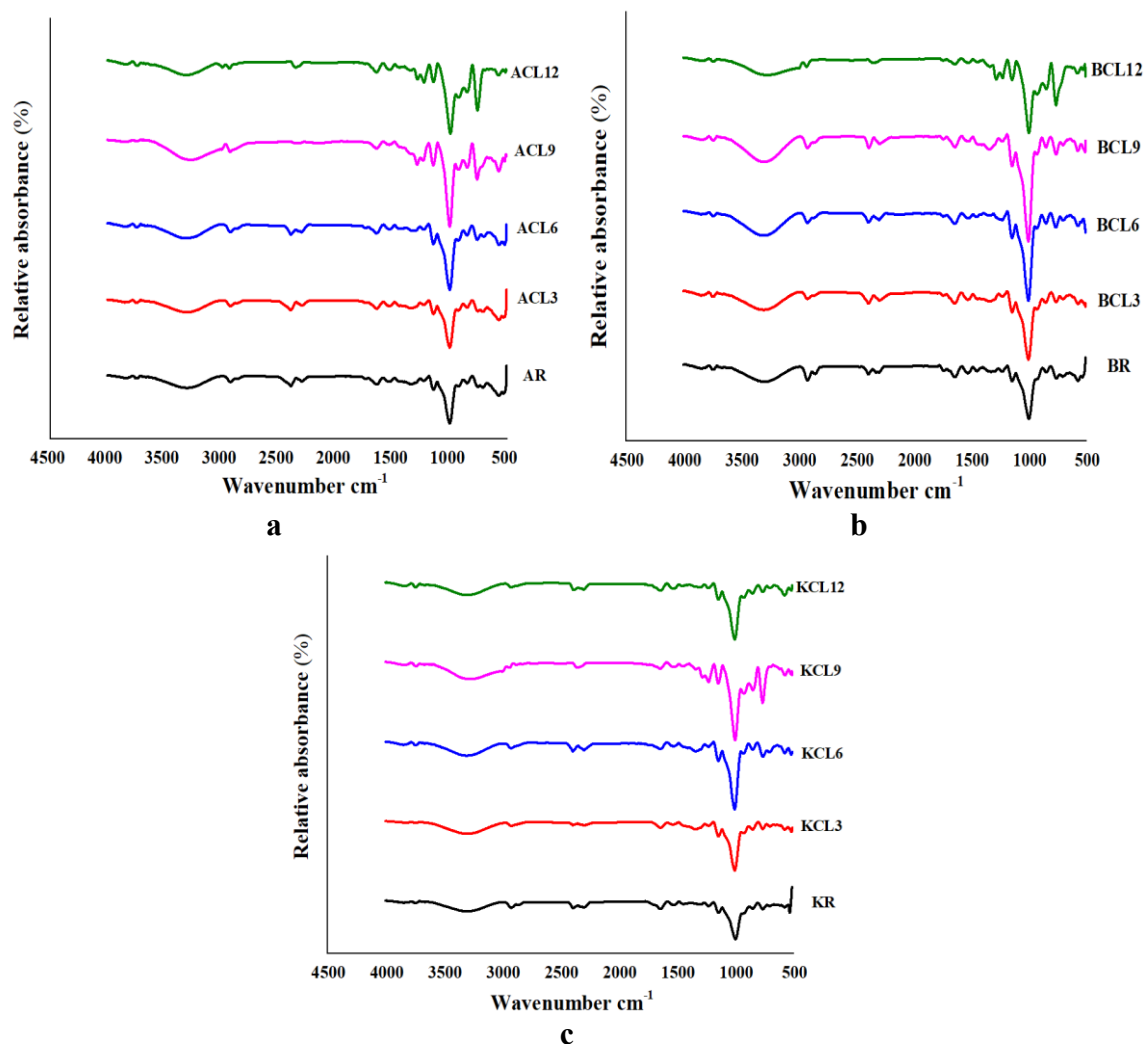


Fig. 4.2. FTIR behavior of the native and crosslinked flours

patterns and starch chain interactions following modification. These changes clearly suggested modifications in the intermolecular associations within the starch matrix, resulting from phosphate-mediated crosslink formation (Malik et al., 2023). Appearance and progressive increase in intensity of absorption bands associated with phosphate ester linkages confirmed successful starch crosslinking in the samples. The shoulder peak at around 1022-1026 cm^{-1} suggested phosphate substitution primarily at the C₂/C₃ hydroxyl groups of the glucose units (Ma et al., 2025). Additionally, characteristic bands at 850-950 cm^{-1} evidenced in the CF graphs corresponded to P=O and P-O-C vibrations (Iweajunwa et al., 2023; Zhao et al., 2024). These bands, absent in the native flour

graphs and weakly detectable in 3% crosslinked CFs, became clearly distinguishable from 6% to 12% CFs of each rice, with a marked increase in intensity at 12%. At the highest crosslinking level of 12%, peaks at $\sim 1024\text{ cm}^{-1}$ and $\sim 1085\text{ cm}^{-1}$ appeared sharper and more intense, whereas bands around $\sim 3300\text{ cm}^{-1}$ (O-H stretching) and $\sim 1640\text{ cm}^{-1}$ (bound water bending) showed noticeable broadening. This behavior suggested heterogeneous crosslink distribution, where highly substituted regions coexist with less modified domains. This suggested a possibly structurally heterogeneous starch network in the 12% CFs.

Table 4.1. Percentage AAC and RPC values of the native and crosslinked flours

Samples	AAC (%)	RPC (%)
AR	25.87 $\pm$ 0.14 ^f	0.011 $\pm$ 0.001 ^k
ACL3	24.84 $\pm$ 0.09 ^g	0.023 $\pm$ 0.001 ^h
ACL6	24.07 $\pm$ 0.18	0.047 $\pm$ 0.003 ^{ef}
ACL9	22.59 $\pm$ 0.11 ^j	0.056 $\pm$ 0.002 ^d
ACL12	21.18 $\pm$ 0.19 ^k	0.063 $\pm$ 0.001 ^b
BR	29.14 $\pm$ 0.05 ^b	0.013 $\pm$ 0.001 ^j
BCL3	27.88 $\pm$ 0.28 ^d	0.028 $\pm$ 0.001 ^g
BCL6	25.33 $\pm$ 0.11 ^f	0.048 $\pm$ 0.001 ^e
BCL9	24.62 $\pm$ 0.19 ^h	0.062 $\pm$ 0.002 ^{bc}
BCL12	23.87 $\pm$ 0.13 ⁱ	0.077 $\pm$ 0.002 ^a
KR	30.20 $\pm$ 0.19 ^a	0.015 $\pm$ 0.001 ⁱ
KCL3	29.11 $\pm$ 0.15 ^b	0.029 $\pm$ 0.003 ^g
KCL6	28.18 $\pm$ 0.06 ^c	0.049 $\pm$ 0.001 ^e
KCL9	26.61 $\pm$ 0.13 ^e	0.060 $\pm$ 0.001 ^e
KCL12	24.78 $\pm$ 0.17 ^{gh}	0.079 $\pm$ 0.002 ^a

Means with same superscript letters within the columns are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test

4.3.3. Apparent amylose content

Values of AAC (%) in native and crosslinked flours exhibited progressive and significant ($p < 0.05$) decline with increasing crosslinking levels across all three varieties (Table 4.1). Phosphate crosslink formation between starch chains restricts amylose mobility. This

reduces its ability to form iodine complexes. Additionally, this results in enhanced granule integrity, that further limits amylose leaching and accessibility for iodine (Bangar et al., 2024). Among the CFs, *Kenkowa* exhibited the highest absolute reduction. This was likely due to its initial higher AAC, providing more reactive sites for crosslinking. However, the relative trend remained consistent for all rice flours. Comparable trends have been reported in earlier studies on rice (Ashwar et al., 2017), mandua (Malik et al., 2023) and millet (Sandhu et al., 2021) starches upon crosslinking.

4.3.4. Morphology

The electron microscopic images of native flours and CFs are presented in Fig. 4.3. As hypothesized, native AR, BR and KR exhibited well-defined, intact polygonal starch granules with smooth surfaces and clear separation between particles. Upon 3% STMP-crosslinking, the granules largely retained their native morphology, and slight clustering and closer packing of particles were observed. The granule boundaries, however, remained distinguishable. Structural integrity of the starch granules was therefore mostly preserved at this lowest crosslinking level. At 6% STMP, the granules appeared more compactly arranged with the formation of small aggregates, indicating increased interactions among them. Granular aggregation became markedly pronounced in the 9% CFs with particles showing close clustering, suggesting strong intermolecular associations within the flour matrix (Kumar et al., 2022). Further increase in crosslinking to 9% STMP resulted in more pronounced aggregation and tighter packing of granules, partial fusion and diminishing of granule boundaries. Although individual granules were still recognizable, they appeared closely associated in irregularly sized dense clusters, suggesting very strong intermolecular associations within the flour matrix (Kumar et al., 2022). Similar binding and clustering in STMP-crosslinked tapioca and corn starches were earlier reported by Zhao et al. (2024) and Ge et al. (2022), respectively. Notably, the clustering effect was observed to be lesser pronounced in the CFs of *Amona* than *Badal* and *Kenkowa*, which could be due to lower degree of crosslinking in the former.

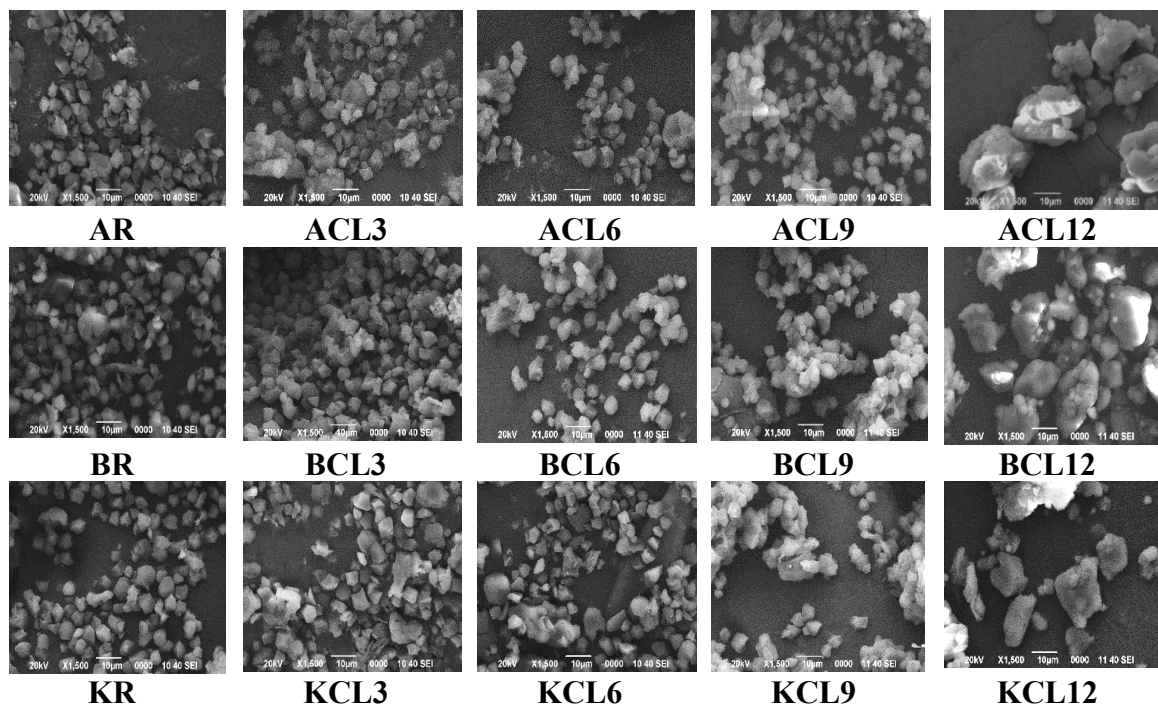


Fig. 4.3. SEM images of the native and crosslinked flours

4.3.5. Swelling power and solubility

Crosslinking resulted in an overall reduction in the swelling power of the flours (Fig. 4.4 a), accompanied by a progressive decline ($p < 0.05$) in solubility (Fig. 4.4 b). At lower to moderate crosslinking levels (3-9%), the formation of phosphate-mediated intermolecular linkages strengthened the internal structure of starch granules, restricting their ability to hydrate and expand. This reinforcement limits granule swelling by reducing molecular mobility and preventing water from freely penetrating the amorphous regions (Agvaandorj et al., 2025). Similar effects of crosslinking have been reported in rice starch (Kumar et al., 2022), *Arenga pinnata* starch (Liu et al., 2024) and pearl millet starch (Siroha and Sandhu, 2018). Varietal differences were also evident. Interestingly, at the highest crosslinking level (12%), a slightly higher swelling power was observed compared to 9% (2.18 to 2.32 g/g for *Amona*; 2.09 to 2.24 g/g for *Badal* and 1.92 to 2.20 g/g for *Kenkora*). This may be attributed to partial disruption or loosening of the granular architecture under intensified chemical treatment, which could

facilitate limited water ingress despite the presence of crosslinks. Overall, *Amona* CFs exhibited the highest swelling powers, followed by *Badal*, while *Kenkowa* showed the lowest values. This trend could be explained by differences in AAC and related crystalline organization. *Amona*, with relatively lower amylose content, would possess a less ordered structure (Flores-Garcia et al., 2024), allowing greater water uptake. In contrast, *Kenkowa*, having higher amylose content and a more compact crystalline arrangement, exhibited restricted swelling due to reduced chain mobility and tighter packing within the granule. The induced phosphate bridges limited molecular mobility, reducing solubility. A slight increase in solubility ($p \geq 0.05$) at 12% crosslinking could be linked with the structural heterogeneity observed under SEM and probable micro-fracturing during heating, leading to the release of small fragments, despite the overall rigidity of the network (Ge et al., 2022). Decreased solubility, attributed to limited granule swelling and the formation of stronger intermolecular crosslinks, has likewise been observed in barnyard millet (Sharma et al., 2021), lotus seed starch (Chandak et al., 2022) and tigernut starch (Xiaofan et al., 2022). Among the CFs, crosslinked *Amona* flours demonstrated the lowest solubility, likely due to the formation of a more cohesive stabilized structure relative to *Badal* and *Kenkowa*.

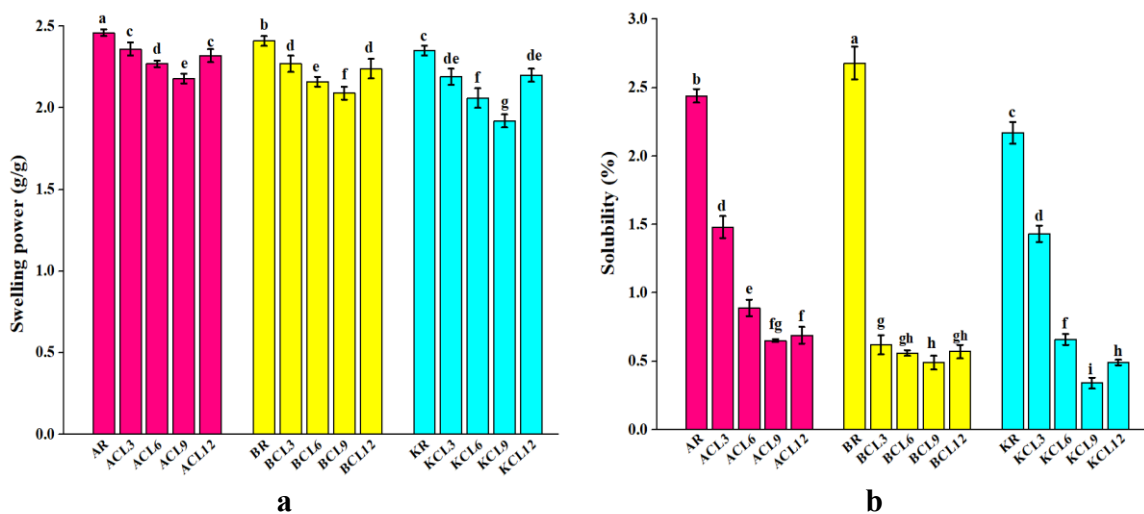


Fig. 4.4. (a) Swelling power and (b) Solubility of the native and crosslinked flours
Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within a parameter are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

4.3.6. Pasting properties

Differences in pasting behavior among the native flours could be attributed primarily to variations in amylose content. *Kenkowa* exhibited higher FV and SBV compared to *Amona* and *Badal*, reflecting a greater tendency to retrograde. Crosslinking had clear influence on the pasting properties (Table 4.2). A gradual increase in PT with increasing crosslinking level indicates enhanced thermal stability of the starch granules. Moderate levels of crosslinking (3-9% STMP) resulted in a gradual but significant increase in PV and FV ($p < 0.05$), indicating improved molecular integrity and controlled swelling, as also seen from the test results on swelling power. Reduced BDV values suggested sustenance of developed molecular integrity at high temperature of 95 °C and mechanical shearing. This is known to be typical to pasting patterns of phosphate crosslinked starches. Phosphate bridges reinforce the internal structure of starch granules and limit their susceptibility to rupture during pasting (Chandak et al., 2022). 9% crosslinking resulted in the highest PVs, indicating an optimum modification level at which the starch granules in the CFs were sufficiently stabilized while still retaining the ability to swell and develop viscosity. Also, the significant increase in FV and SBV ($p < 0.05$) in samples crosslinked up to 9% further indicated enhanced reassociation tendency of disintegrated starch chains during cooling, resulting in the formation of a stronger retrograded gel network. However, 12% CFs exhibited substantial reductions in PVs as well as FVs. For these, excessive crosslinking likely resulted in overly rigid starch matrices that restricted moisture penetration, granule swelling and limited amylose leaching during heating, thereby reducing viscosity development, and finally resulting in a weak gel (Sudheesh et al., 2020).

Table 4.2. Pasting properties of the native and crosslinked flours

Samples	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak Time (min)	Pasting Temperature (°C)
AR	2880.10±16.78 ⁱ	2040.09±12.51 ^l	840.06±2.13 ^d	4012.25±7.83 ^l	1970.19±2.13 ^j	5.81±0.12 ^{ef}	78.52±0.39 ^k
ACL3	3150.08±10.56 ^j	2520.12±9.34 ^h	630.17±1.72 ^h	4620.07±10.67 ^k	2100.33±4.56 ⁱ	6.07±0.09 ^{de}	80.20±0.72 ⁱ
ACL6	3520.13±11.45 ^h	3010.26±17.43 ^f	510.41±1.33 ^j	5250.15±16.52 ^j	2240.45±6.12 ^h	6.11±0.06 ^d	82.09±1.12 ^g
ACL-9	3860.21±6.82 ^f	3375.09±7.82 ^d	485.22±0.82 ^k	5907.51±12.82 ⁱ	2525.12±7.73 ^g	6.25±0.08 ^c	83.62±1.29 ^e
ACL12	2100.10±9.45 ^o	1850.23±10.55 ^m	252.45±1.19 ^o	2650.11±7.43 ^o	800.67±5.56 ^m	6.56±0.11 ^b	86.08±0.67 ^c
BR	3920.12±6.72 ^e	2095.08±8.42 ^k	1825.27±5.89 ^a	6334.78±12.67 ^h	4235.44±18.45 ^d	5.78±0.17 ^{efg}	79.25±0.25 ^j
BCL3	4050.25±7.44 ^c	3050.12±8.11 ^e	1003.23±3.78 ^c	6890.19±18.68 ^f	3841.09±12.83 ^f	5.92±0.04 ^{ef}	81.55±0.19 ^h
BCL6	4320.22±3.58 ^b	3520.17±6.08 ^b	800.05±1.89 ^e	7452.18±10.56 ^d	3932.12±16.36 ^e	6.01±0.07 ^e	83.10±0.47 ^{ef}
BCL9	4550.06±9.12 ^a	3890.56±9.41 ^a	660.72±2.42 ^f	7826.12±8.34 ^c	3930.17±11.45 ^e	6.28±0.12 ^c	84.62±1.12 ^e
BCL12	2400.61±10.22 ⁿ	2101.04±5.82 ^j	305.66±3.54 ⁿ	3120.23±12.51 ⁿ	1020.65±10.52 ^l	6.63±0.06 ^{ab}	87.36±0.45 ^b
KR	3100.27±6.88 ^k	1980.23±8.92 ^l	1120.19±4.55 ^b	6681.15±15.45 ^g	4705.72±12.48 ^b	5.94±0.08 ^e	80.11±0.55 ⁱ
KCL3	3340.09±8.22 ^j	2700.55±9.71 ^g	642.55±6.91 ^g	7210.42±11.92 ^e	4510.16±9.82 ^c	6.04±0.06 ^{de}	82.07±0.36 ^g
KCL6	3620.04±4.78 ^g	3050.41±12.56 ^e	571.04±2.34 ⁱ	7922.45±18.47 ^b	4870.24±11.72 ^a	6.12±0.08 ^d	83.83±1.10 ^e
KCL9	3950.15±10.27 ^d	3500.33±8.92 ^c	450.34±2.78 ^l	8257.12±12.73 ^a	4861.56±15.06 ^a	6.26±0.03 ^c	85.29±0.23 ^d
KCL12	2550.12±8.05 ^m	2200.17±10.17 ⁱ	350.02±1.82 ^m	3408.67±10.86 ^m	1207.33±10.88 ^k	6.69±0.13 ^a	88.02±0.42 ^a

Means with same superscript letters within the columns are not significantly different at p≤0.05 as indicated through Duncan's multiple range test

4.3.7. Thermal properties

Progressive crosslinking levels resulted in an increase in onset (T_o), peak (T_p) and conclusion (T_c) temperatures (Fig. 4.5), accompanied by a marked reduction in gelatinization enthalpy (ΔH) (Table 4.3). The results were in agreement with enhanced PT values recorded from RVA. The elevation in gelatinization temperatures indicates enhanced thermal stability of the starch granules to phase change, suggesting that higher energy input was required to initiate melting of the crystalline regions strengthened by STMP-induced phosphate crosslinks (Ge et al., 2022). A lowering ΔH could be due to incomplete gelatinization owing to lower water mobility inside the modified starch matrices. At higher crosslinking levels (12%), a partial reversal in trends was observed, characterized by a decrease in T_o , T_p , and T_c along with a slight increase or stabilization in ΔH . This uncertainty in physicochemical behavior clearly suggested that the excessing crosslinking treatment resulted in non-uniformity in molecular bonding patterns and localized aggregations (Sukhija et al., 2016). Phosphate crosslinking predominantly occurs within the amorphous regions of starch granules, where hydroxyl groups are more accessible. The incorporation of bulky phosphate groups and the formation of inter- and intramolecular bridges impose steric hindrance, which interferes with the regular alignment and packing of amylopectin chains. This disruption leads to imperfect or fragmented crystalline domains, thereby lowering the total energy required for gelatinization (Sharma et al., 2021).

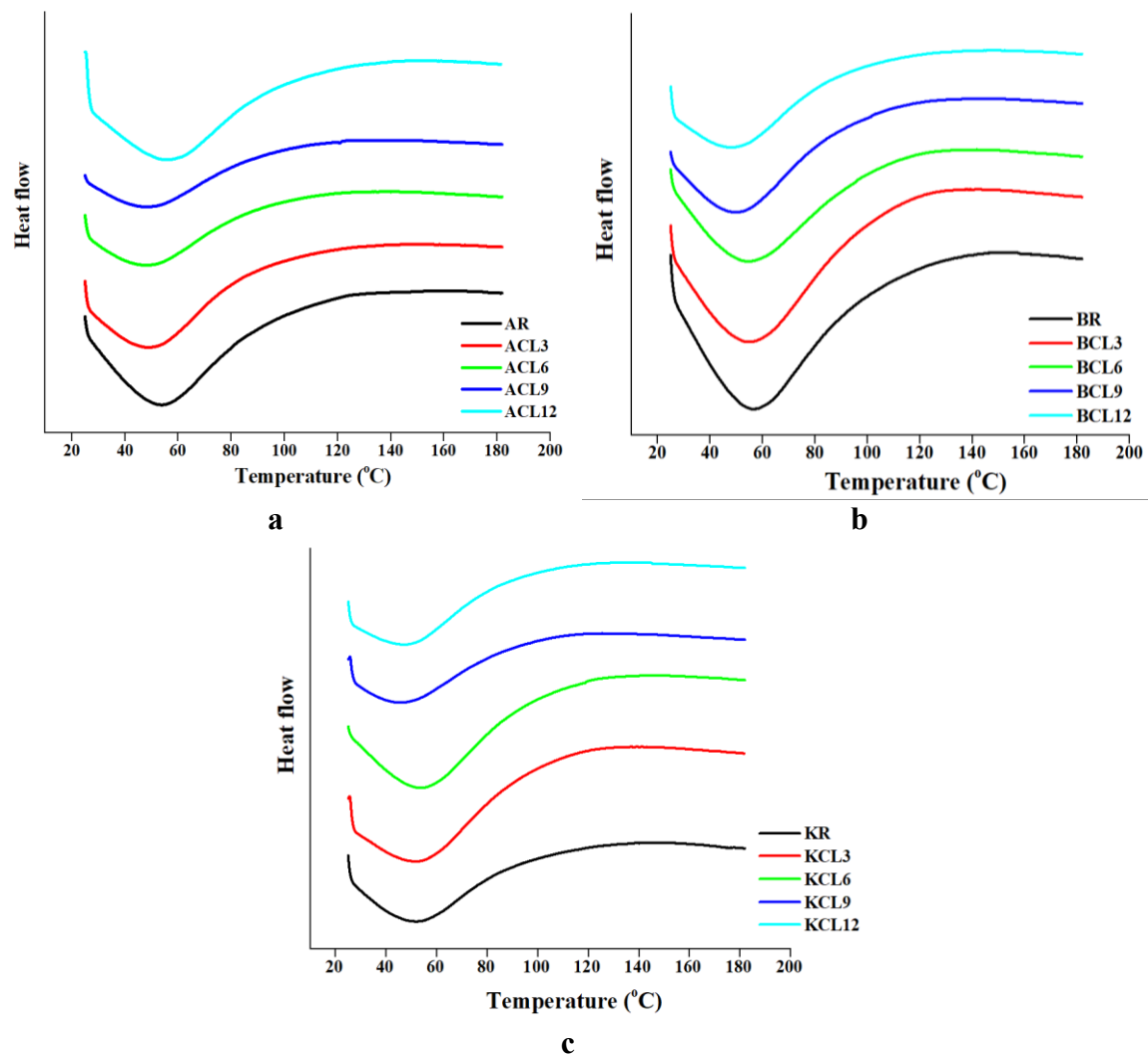


Fig. 4.5. DSC thermograms of the native and crosslinked flours

Table 4.3. Thermal properties of the native and crosslinked flours

Samples	T _o (°C)	T _p (°C)	T _c (°C)	ΔH (J/g)
AR	55.03±0.22 ^g	58.43±0.29 ⁱ	82.30±0.42 ^m	7.77±0.17 ^b
ACL3	55.63±0.08 ^f	59.17±0.18 ^h	85.22±0.13 ^l	3.55±0.22 ^d
ACL6	57.33±0.22 ^{de}	60.67±0.81 ^e	91.81±0.41 ^g	2.90±0.19 ^f
ACL9	58.28±0.31 ^c	63.31±0.67 ^a	93.43±0.18 ^c	2.08±0.16 ^{gh}
ACL12	55.69±0.26 ^f	59.22±0.31 ^h	89.20±0.22 ^j	3.93±0.03 ^d
BR	56.63±0.11 ^c	60.06±0.18 ^f	90.34±0.11 ⁱ	5.02±0.89 ^c
BCL3	58.22±0.29 ^c	60.39±0.11 ^e	92.41±0.45 ^f	2.32±0.12 ^f
BCL6	59.93±0.17 ^b	60.86±0.32 ^d	97.22±1.02 ^{bc}	2.04±0.06 ^h
BCL9	60.26±0.22 ^a	61.27±0.06 ^c	99.18±0.66 ^a	1.89±0.07 ⁱ
BCL12	57.92±0.18 ^c	59.82±0.10 ^g	91.83±0.42 ^g	2.28±0.05 ^j
KR	57.23±0.46 ^{de}	59.14±1.07 ^a	86.94±0.12 ^k	9.84±0.16 ^a
KCL3	58.07±0.22 ^c	60.04±0.62 ^f	90.62±0.55 ^{hi}	3.59±0.39 ^d
KCL6	59.79±0.15 ^b	61.02±0.29 ^c	94.25±0.18 ^d	2.89±0.14 ^e
KCL9	60.11±0.37 ^a	61.68±0.18 ^b	97.47±0.06 ^b	2.11±0.09 ^g
KCL12	58.06±0.15 ^c	58.83±0.25 ^j	91.05±0.32 ^h	3.56±0.02 ^k

Means with same superscript letters within the columns are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

4.3.8. Starch digestibility

Crosslinking resulted in reduced RDS, while increasing SDS and RS in the flour samples (Fig. 4.6 a-c). Previously, a direct relation of RS formation with ΔH obtained from DSC was reported (Ge et al., 2022). However, our results were contrary to it. This again suggested a probable issue of insufficient crystallite melting in the DSC-run samples. Or else, it could be suggestive that starch digestibility is governed more by enzyme accessibility and molecular mobility than by crystallinity alone. The formation of covalent phosphate bridges within the predominantly amorphous regions creates a three-dimensional network that reduces chain flexibility. This could have hindered enzyme diffusion and masked glycosidic linkages, thereby slowing down initial hydrolysis and decreasing RDS fraction and increasing SDS.

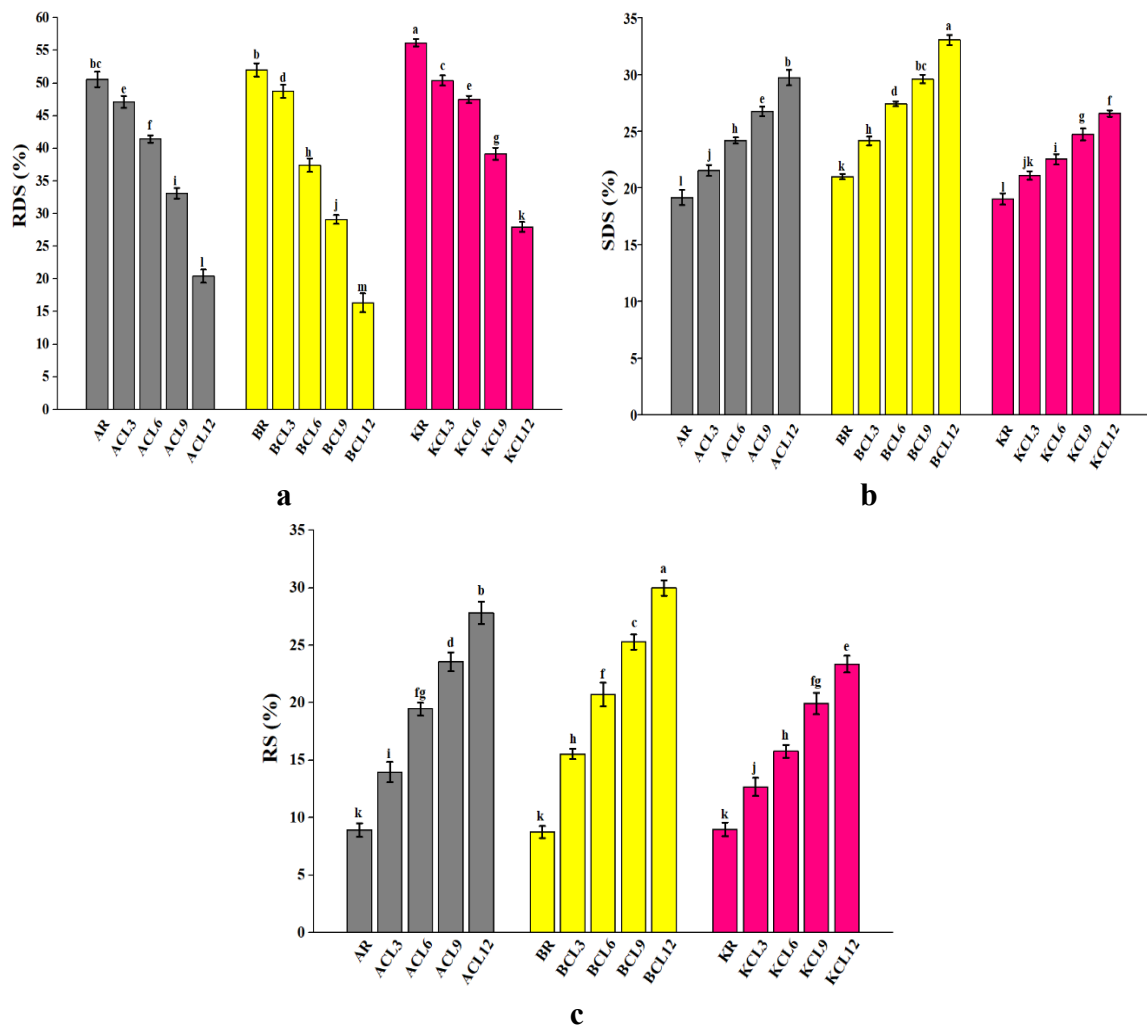


Fig 4.6. (a) Rapidly digestible starch (RDS) (b) Slowly digestible starch (SDS) and (c) Resistant starch (RS) content of the native and crosslinked flours

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within a parameter are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

At 12% crosslinking, the network became denser and more rigid, further limiting enzymatic penetration and promoting the formation of enzyme-resistant RS regions. Thus, starch digestion shifted from rapid to slower and resistant forms (Heo et al., 2017; Malik et al., 2023; Liu et al., 2024). Significant varietal differences ($p < 0.05$) were also observed. Native KR exhibited comparatively higher RDS, followed by BR and AR. Progressive crosslinking (3-12%) resulted in a marked reduction in RDS across all varieties, however, the extent of reduction was more pronounced in *Amona* and *Badal*

CFs than in *Kenkowa* CFs. Concurrently, SDS and RS increased significantly. Among the varieties, *Badal* CFs showed the highest increase in SDS and RS at higher crosslinking levels, *Amona* exhibited a moderate RS formation due to limited availability of linear chains (Kahraman et al., 2015; Kumar et al., 2025). In contrast, despite higher amylose content in *Kenkowa*, its CFs showed a smaller shift toward SDS and RS.

4.3.9. Gel properties

4.3.9.1. Color

Values of L^* , a^* , b^* and ΔE were significantly influenced by the degree of crosslinking. With increasing crosslinking concentration, L^* (lightness) and b^* (yellowness) decreased, whereas a^* (redness) and ΔE increased (Fig. 4.7 a-d). Microstructural densification of the gel matrix could be the major contributor to its reducing L^* . Phosphate-mediated linkages promote a more compact and homogeneous network with fewer microvoids, thereby reducing internal light scattering and resulting in a darker visual appearance (Bagheri et al., 2018). However, the declined b^* could be associated with pigment alterations under the alkaline crosslinking conditions. Flavonoids, which typically contribute to yellow-orange hues, may readily undergo structural modification, leading to reduced yellowness. Anthocyanins are sensitive to pH change and may undergo structural transformations, such as chalcone formation or other pH-dependent rearrangements, altering their chromatic properties and contributing to shifts in color balance (Sharma et al., 2021). The increased a^* suggested a relative intensification of red tones, possibly due to concentration effects within the compact matrix or selective degradation of the non-red pigments. A gradual increase in ΔE with increasing crosslinking indicated pronounced color differences amongst CFs at successive crosslinking levels. This suggested destabilized pigment distribution and increased structural heterogeneity, resulting in less visually uniform samples.

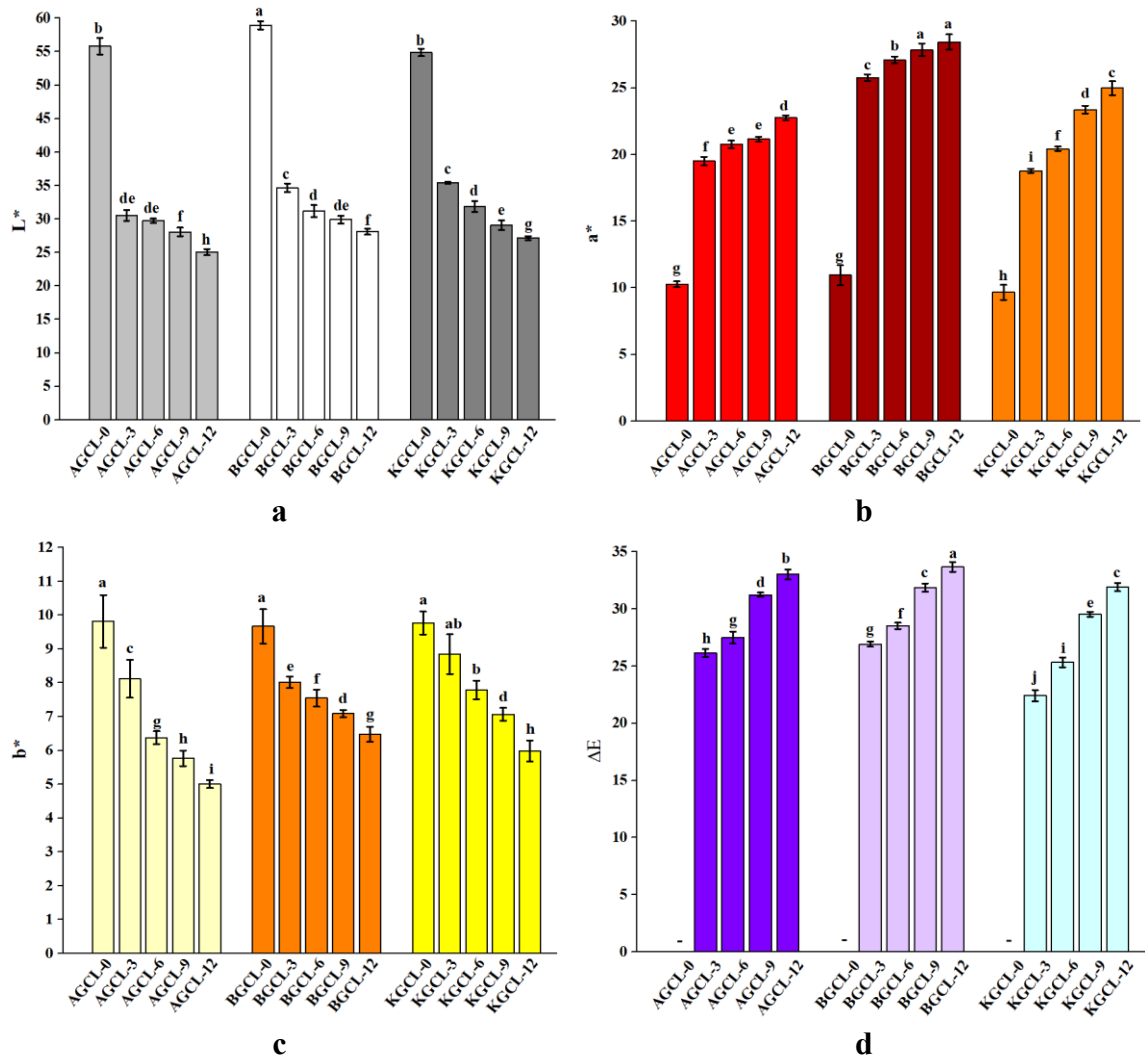


Fig. 4.7. Color values (a) L*, (b) a*, (c) b* and (d) ΔE of the native and crosslinked flour gels

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within a parameter are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test

4.3.9.2. Syneresis

Progressive water expulsion was observed with 7th and 14th day storage in all the samples (Fig. 4.8). AGCL-0, BGCL-0 and KGCL-0 exhibited significantly higher syneresis, reflecting poor water-holding capacity due to structural instability. In contrast, crosslinked samples showed a marked reduction ($p < 0.05$) in syneresis, with the lowest values consistently observed at 9% crosslinking for all the three rice flours.

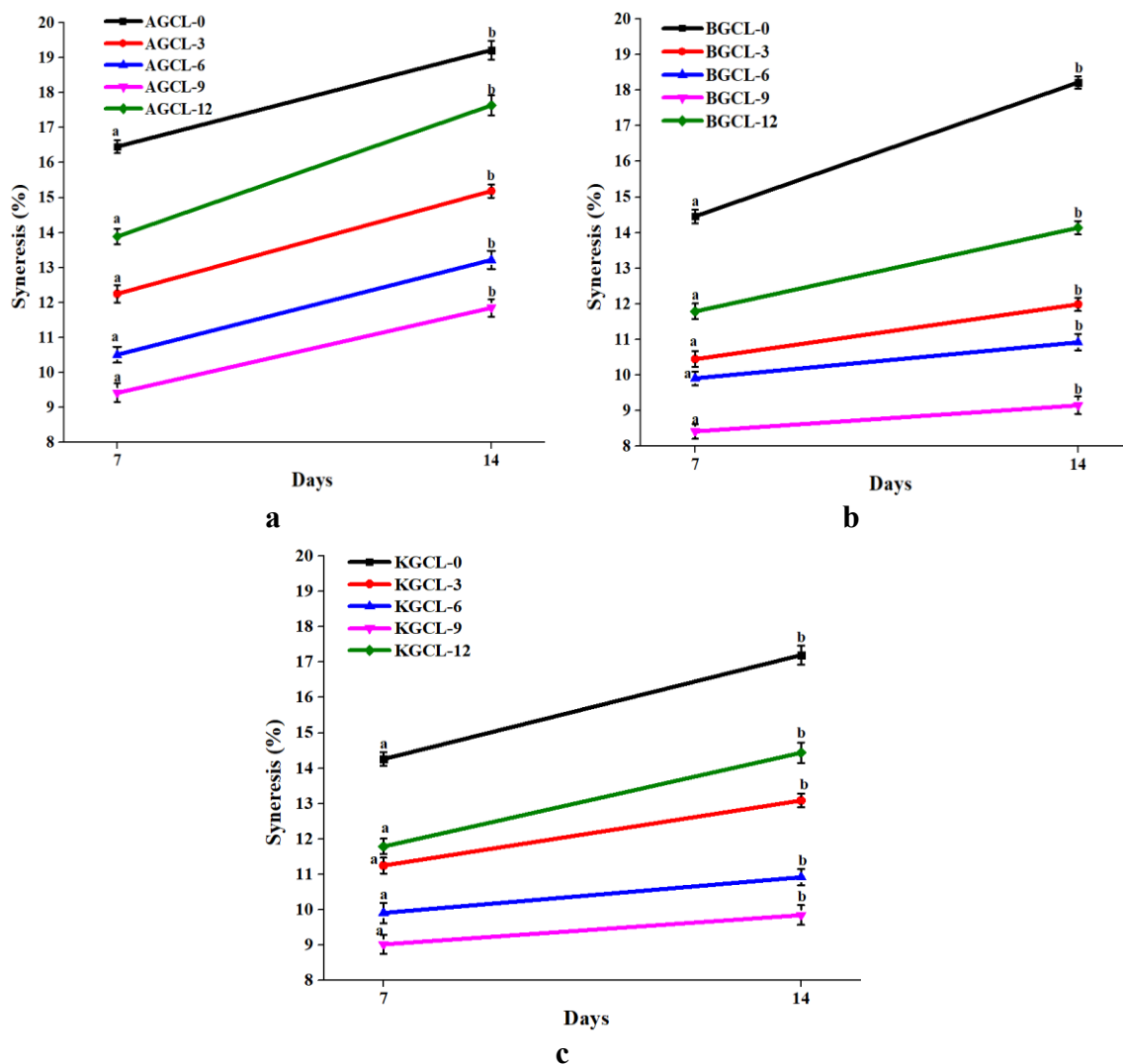


Fig. 4.8. Syneresis (%) of the native and crosslinked flour gels of (a) *Amona bao*, (b) *Badal bao* and (c) *Kenkowa bao*

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters on successive days are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

A progressive decrease in syneresis from 0% to 9% crosslinking suggested the formation of moderate phosphate-mediated intermolecular junctions (P-O-C bridges) between amylose and amylopectin chains, as well as between adjacent granules, as observed under SEM. This reinforced the gel matrix and restricted network collapse during the temperature cycles (Agvaandorj et al., 2025). However, at 12% crosslinking (CL-12), syneresis increased, indicating deterioration in freeze-thaw stability. This behavior

suggested micro-phase separation due developed structural heterogeneity, as also depicted by the previously discussed properties of the CFs. Over-crosslinking may generate highly rigid micro-domains alongside insufficiently crosslinked regions, impairing uniform water entrapment during freezing. Additionally, the alkaline conditions involved in the crosslinking process may promote partial depolymerization or fragmentation of starch chains, further compromising structural cohesion at higher crosslinking levels (Nakkala et al., 2022; Xiaofan et al., 2022).

4.3.9.3. Texture

The TPA values (Table 4.4) were consistent with SB and FV values obtained from RVA. Samples prepared from CFs crosslinked at moderate levels exhibited greater firmness and structural integrity. From 0% to 9%, hardness, cohesiveness, springiness, chewiness and resilience progressively increased ($p < 0.05$), indicating enhanced gel strength and structural integrity. This improvement can be attributed to the formation of phosphate diester linkages in the cooked gels, which increases the density of junction zones within the starch network. The reinforced three-dimensional matrix enables the gels to better resist deformation and recover after compression. In addition, these samples demonstrated reduced syneresis during refrigerated storage, suggesting improved water retention and gel stability due to the formation of a more cohesive starch matrix. A similar trend was reported in purple sweet potato starch following STMP crosslinking (Bodjrenou et al., 2023). At 12% crosslinking, however, these parameters declined, suggesting structural brittleness and reduced flexibility. Excessive crosslink density could have restricted gelatinization and molecular mobility, limiting proper network expansion during heating, as also suggested by the DSC results. Under strongly alkaline conditions, such intense crosslinking may also disrupt uniform chain organization, leading to localized structural irregularities that compromise overall matrix integrity (Sharma et al., 2020). Adhesiveness significantly decreased ($p < 0.05$) from 0 to 9%, reflecting the formation of a stable and cohesive network with minimal surface stickiness. However, at 12%, adhesiveness increased ($p < 0.05$), which may be associated

with structural disruption, minor syneresis or the release of fragmented starch components that contribute to surface tackiness. Among the rices, *Badal* CF gels exhibited the highest hardness, cohesiveness, springiness, chewiness and resilience, along with the lowest adhesiveness, indicating superior structural organization.

Table 4.4. Textural properties of the native and crosslinked flour gels

Samples	Hardness (g)	Adhesiveness (g.s)	Springiness (g)	Cohesiveness (g)	Chewiness (g)	Resilience (g)
AGCL-0	115.76±10.09 ⁱ	-4.12±0.08 ^c	0.250±0.02 ^g	0.08±0.02 ^h	13.19±0.78 ^{hi}	0.064±0.07 ^j
AGCL-3	143.38±5.12 ^{gh}	-2.58±0.12 ^{gh}	0.299±0.05 ^f	0.15±0.05 ^{fg}	19.94±0.45 ^f	0.144±0.05 ^f
AGCL-6	219.94±10.33 ^d	-2.57±0.22 ^{gh}	0.433±0.04 ^d	0.43±0.03 ^b	34.53±0.59 ^c	0.191±0.08 ^c
AGCL-9	322.11±18.02 ^b	-1.09±0.34 ^j	0.674±0.06 ^b	0.68±0.08 ^a	51.40±0.26 ^a	0.238±0.06 ^b
AGCL-12	104.42±9.07 ^j	-3.61±0.29 ^f	0.265±0.02 ^g	0.39±0.05 ^c	12.88±0.63 ⁱ	0.109±0.07 ^h
BGCL-0	162.69±7.05 ^f	-7.33±0.07 ^a	0.218±0.02 ^h	0.17±0.04 ^f	10.88±0.33 ^k	0.040±0.02 ^k
BCGL-3	180.11±5.78 ^{ef}	-4.32±0.19 ^d	0.234±0.06 ^g	0.26±0.02 ^d	12.94±0.19 ^{hi}	0.085±0.09 ^j
BGCL-6	241.12±13.51 ^d	-3.88±0.17 ^c	0.369±0.08 ^c	0.34±0.03 ^c	25.29±0.17 ^c	0.092±0.06 ⁱ
BGCL-9	357.34±11.82 ^a	-2.68±0.09 ^g	0.687±0.07 ^a	0.46±0.04 ^b	57.33±0.11 ^b	0.154±0.06 ^c
BGCL-12	146.92±8.29 ^g	-4.59±0.06 ^b	0.288±0.02 ^f	0.14±0.02 ^g	11.06±1.11 ^j	0.102±0.08 ^h
KGCL-0	133.78±14.45 ^h	-4.55±0.01 ^b	0.356±0.06 ^c	0.07±0.02 ^h	13.87±0.26 ^h	0.056±0.02 ^j
KGCL-3	167.89±12.67 ^e	-3.62±0.11 ^f	0.388±0.03 ^e	0.18±0.03 ^f	25.11±0.45 ^e	0.122±0.06 ^g
KGCL-6	239.67±7.26 ^d	-2.49±0.04 ^h	0.468±0.11 ^c	0.22±0.02 ^c	31.10±0.22 ^d	0.173±0.03 ^d
KGCL-9	313.19±15.08 ^c	-1.77±0.02 ⁱ	0.522±0.02 ^b	0.26±0.01 ^d	43.57±0.02 ^c	0.255±0.03 ^a
KGCL-12	109.45±10.61 ^j	-3.83±0.01 ^c	0.282±0.06 ^f	0.12±0.05 ^{fg}	17.82±1.22 ^g	0.109±0.02 ^h

Means with same superscript letters within the columns are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test

4.3.9.4. Rheological properties

4.3.9.4.1. Steady shear properties

The steady shear response of the STMP-crosslinked *bao* rice flour gels revealed pronounced shear-thinning behavior. This was evident from the progressive increase in γ as τ increases and decrease in η with increasing τ (Fig. 4.9 a-f). All samples exhibited non-Newtonian characteristics ($n < 1$) of structured and shear-sensitive gel systems. The flow data were effectively fitted to the Herschel-Bulkley model. High coefficients of determination ($R^2 > 0.90$) were obtained for all samples, confirming the suitability of the model in describing the rheological behavior of the CF gels (Table 4.5). A systematic increase in η_{50} , K and τ_0 values were observed with increasing crosslinking level up to 9%, while n decreased concurrently, showing a consistent pattern across all three rice flour types.

Table 4.5. Apparent viscosity and Herschel Bulkley model parameters of the crosslinked gels

Samples	Herschel Bulkley model parameters				R^2
	η_{50} (Pa.s)	K (Pa.s)	n	τ_0 (Pa)	
AGCL-0	0.83±0.003 ^o	1.78±1.39 ^k	0.69±0.008 ^a	16.52±1.05 ⁿ	0.992
AGCL-3	2.70±0.001 ^k	14.86±0.56 ⁱ	0.49±0.011 ^e	83.84±4.59 ⁱ	0.972
AGCL-6	4.50±0.005 ^f	22.44±1.01 ^c	0.37±0.002 ^h	179.76±1.88 ^f	0.991
AGCL-9	6.33±0.002 ^c	29.41±2.17 ^c	0.33±0.003 ⁱ	255.58±4.99 ^d	0.959
AGCL-12	2.52±0.006 ^l	13.81±0.56 ^j	0.51±0.007 ^d	62.73±4.62 ^k	0.972
BGCL-0	1.45±0.006 ⁿ	2.11±2.36 ^k	0.66±0.005 ^b	44.52±1.05 ^m	0.991
BGCL-3	3.26±0.003 ⁱ	18.32±0.62 ^f	0.45±0.012 ^f	107.85±2.25 ⁱ	0.959
BGCL-6	5.20±0.001 ^e	26.23±0.75 ^d	0.31±0.021 ^j	211.76±2.19 ^e	0.994
BGCL-9	7.09±0.005 ^b	37.56±2.12 ^b	0.27±0.031 ^k	293.58±3.39 ^e	0.981
BGCL-12	3.11±0.003 ^j	16.72±1.03 ^h	0.49±0.001 ^e	92.74±3.48 ^j	0.951
KGCL-0	1.58±0.004 ^m	2.88±0.56 ^k	0.65±0.005 ^c	49.53±2.60 ^l	0.994
KGCL-3	3.79±0.003 ^g	18.92±0.62 ^f	0.42±0.027 ^g	120.84±5.32 ^g	0.945
KGCL-6	5.86±0.001 ^d	29.47±1.19 ^c	0.30±0.011 ^j	299.76±3.21 ^b	0.992
KGCL-9	7.47±0.005 ^a	40.35±2.36 ^a	0.24±0.003 ^l	312.45±4.72 ^a	0.991
KGCL-12	3.52±0.002 ^h	17.71±2.12 ^g	0.45±0.015 ^f	113.74±6.56 ^h	0.971

Means followed by the same superscript lowercase letters within a column are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test. Here, η_{50} : apparent viscosity, K : consistency index, n : flow behavior index, τ_0 : yield stress

This trend could be attributed to the formation of covalent phosphate linkages introduced during STMP treatment, which reinforced inter and intra-molecular associations within the starch matrix. Such crosslinks limit granular swelling and promote molecular entanglement, thereby increasing resistance to flow and enhancing low-shear viscosity. As shear is applied, these structured networks progressively align and partially disintegrate, resulting in the observed shear-thinning behavior (Kumar et al., 2023). However, at 12% highest crosslinking level, a reduction in η_{50} and K , along with a slight increase in n (from 9%), were seen, indicating the onset of over-crosslinking. The values were comparable to 3% CF gels. Excessive crosslink density may restrict the flexibility of starch chains and hinder effective intermolecular interactions, leading to the formation of a less cohesive and partially disrupted network. This suggests the existence of an optimum crosslinking level beyond which the structural integrity of the gel matrix begins to decline. This phenomenon has been also reported by Qiao et al. (2019), where over-crosslinking reduced hydration capacity and disrupted uniform network formation. Varietal differences were clearly reflected, with *Kenkova* samples exhibiting higher η_{50} , K , and τ_0 values, followed by *Badal* and *Amona*. The stronger shear resistance and enhanced pseudoplasticity observed in the former could be attributed to higher AAC facilitating greater intermolecular association and improved efficiency of crosslinking.

4.3.9.4.2. Dynamic rheology

Viscoelastic properties of the CF gels, expressed in terms of storage modulus (G') and loss modulus (G'') as a function of angular frequency (ω), are presented in Fig. 4.10, 4.11 and 4.12. Across all samples and frequencies, G' remained consistently higher than G'' , with $\tan \delta$ values below unity, indicating the dominance of elastic behavior and confirming the formation of well-developed gel networks. Both G' and G'' increased progressively up to 9% crosslinking level, reflecting the development of a more structured and interconnected internal matrix. The introduction of phosphate crosslinks restricts molecular mobility and enhances the rigidity of the network, thereby improving

its ability to store deformation energy under oscillatory stress. The increase in elastic modulus suggested a higher density of junction zones and stronger intermolecular associations within the gel matrix (Bodjrenou et al., 2023). With increasing angular frequency (ω), both moduli exhibited an upward trend, with G' showing a more pronounced increase over G'' . This behavior indicated that the gels became increasingly elastic at higher deformation rates, demonstrating improved resistance to structural breakdown. The absence of any crossover point between G' and G'' throughout the tested frequency range further confirmed enhanced stability of the gel network and its predominantly solid-like nature. At 12% crosslinked CF gels, a slight reduction in G' were observed, which was consistent with the steady shear results, re-suggesting structural heterogeneity. Nevertheless, the gels retained their elastic dominance, indicating that the overall gel structure remained intact.

Table 4.6. Parameters of power law functions describing storage modulus and loss modulus of the crosslinked gels

Samples	Power law parameters					
	$G' = K'(\omega)^{n'}$			$G'' = K''(\omega)^{n''}$		
	K' (Pa)	n'	R^2	K'' (Pa)	n''	R^2
AGCL-0	3.548±0.014 ^m	0.014±1.01×10 ^{-4a}	0.974	2.154±0.018 ^m	0.014±0.002 ^h	0.984
AGCL-3	4.030±0.015 ^j	0.013±1.12×10 ^{-4b}	0.950	2.360±0.022 ^k	0.022±0.003 ^g	0.951
AGCL-6	4.680±0.003 ^h	0.012±2.15×10 ^{-4c}	0.992	2.601±0.025 ⁱ	0.031±0.003 ^e	0.945
AGCL-9	5.382±0.013 ^e	0.009±9.02×10 ^{-4c}	0.947	3.043±0.025 ^g	0.049±0.001 ^a	0.980
AGCL-12	3.983±0.017 ^k	0.013±3.22×10 ^{-4b}	0.995	2.288±0.024 ^l	0.018±0.003	0.975
BGCL-0	3.804±0.012 ^l	0.014±9.11×10 ^{-4a}	0.947	2.567±0.011 ^j	0.025±0.001 ^{fg}	0.981
BGCL-3	4.415±0.015	0.013±4.15×10 ^{-4b}	0.996	2.741±0.027 ^h	0.030±0.001 ^e	0.972
BGCL-6	5.325±0.013 ^f	0.010±9.03×10 ^{-4d}	0.988	3.118±0.031 ^f	0.041±0.002 ^c	0.954
BGCL-9	6.161±0.015 ^c	0.008±8.60×10 ^{-4f}	0.992	3.994±0.019 ^d	0.048±0.001 ^a	0.996
BGCL-12	4.339±0.016 ⁱ	0.014±6.11×10 ^{-4a}	0.998	2.607±0.025 ⁱ	0.026±0.003 ^f	0.995
KGCL-0	4.696±0.008 ^h	0.013±6.70×10 ^{-4b}	0.977	3.362±0.019 ^c	0.026±0.002 ^f	0.976
KGCL-3	5.384±0.014 ^d	0.009±9.50×10 ^{-4c}	0.992	4.004±0.004 ^d	0.033±0.004 ^d	0.994
KGCL-6	6.272±0.013 ^b	0.008±7.91×10 ^{-4f}	0.976	4.593±0.010 ^c	0.043±0.002 ^e	0.985
KGCL-9	7.213±0.014 ^a	0.007±7.18×10 ^{-4g}	0.992	4.758±0.023 ^b	0.049±0.002 ^a	0.931
KGCL-12	5.314±0.016 ^g	0.010±4.04×10 ^{-4d}	0.927	4.956±0.025 ^a	0.029±0.003 ^e	0.954

Means followed by the same superscript lowercase letters within a column are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test. Here, G' : storage modulus, G'' , loss modulus, K : slope, n : y -intercept

The strengthening of viscoelastic properties with crosslinking could be also linked to increase in complex modulus (G^*) and complex viscosity (η^*) and the corresponding decrease in $\tan \delta$ (Fig. 4.13). The power-law modeling of G' and G'' (Table 4.6) showed positive exponent values across all samples, confirming the formation of stable gel structures. The increase in consistency coefficients (K' and K'') with crosslinking further indicated an increase in three-dimensional network interactions relating to improved gel strength.

Overall, the rheological behavior of the crosslinked gels showed strong agreement with the observed TPA characteristics. The increase in η , K , and τ_0 with increasing crosslinking level corresponded to the development of firmer and more resistant gel structures, as was seen from higher hardness and chewiness values. Similarly, the increase in G' (from 0 to 9%) indicated enhanced elasticity, which contributes to improved cohesiveness. However, at 12% crosslinking, a slight decline in these parameters was observed, which closely paralleled the reduction in textural attributes, suggesting that excessive crosslinking led to a less uniform and partially weakened gel network. The decreasing trend in $\tan \delta$ with increasing crosslinking further supported the transition towards elastic-dominant behavior, which is associated with better shape retention and recovery after deformation. This aligns with the observed increase in springiness and cohesiveness of the 0% to 9% CF gels. Additionally, the decreased gel adhesiveness could be linked to reduced viscous behavior (G'') and increased elastic dominance (G'). As crosslinking strengthens the gel network, less energy is dissipated as flow, leading to lower stickiness and easier detachment. At 12%, the deviation from this trend corresponded with the slight decline in springiness and cohesiveness, reinforcing the impact of over-crosslinking on network integrity. Hence, STMP crosslinking improved gel stability and strength up to 6-9%, but excessive crosslinking (12%) reduced rheological and textural properties.

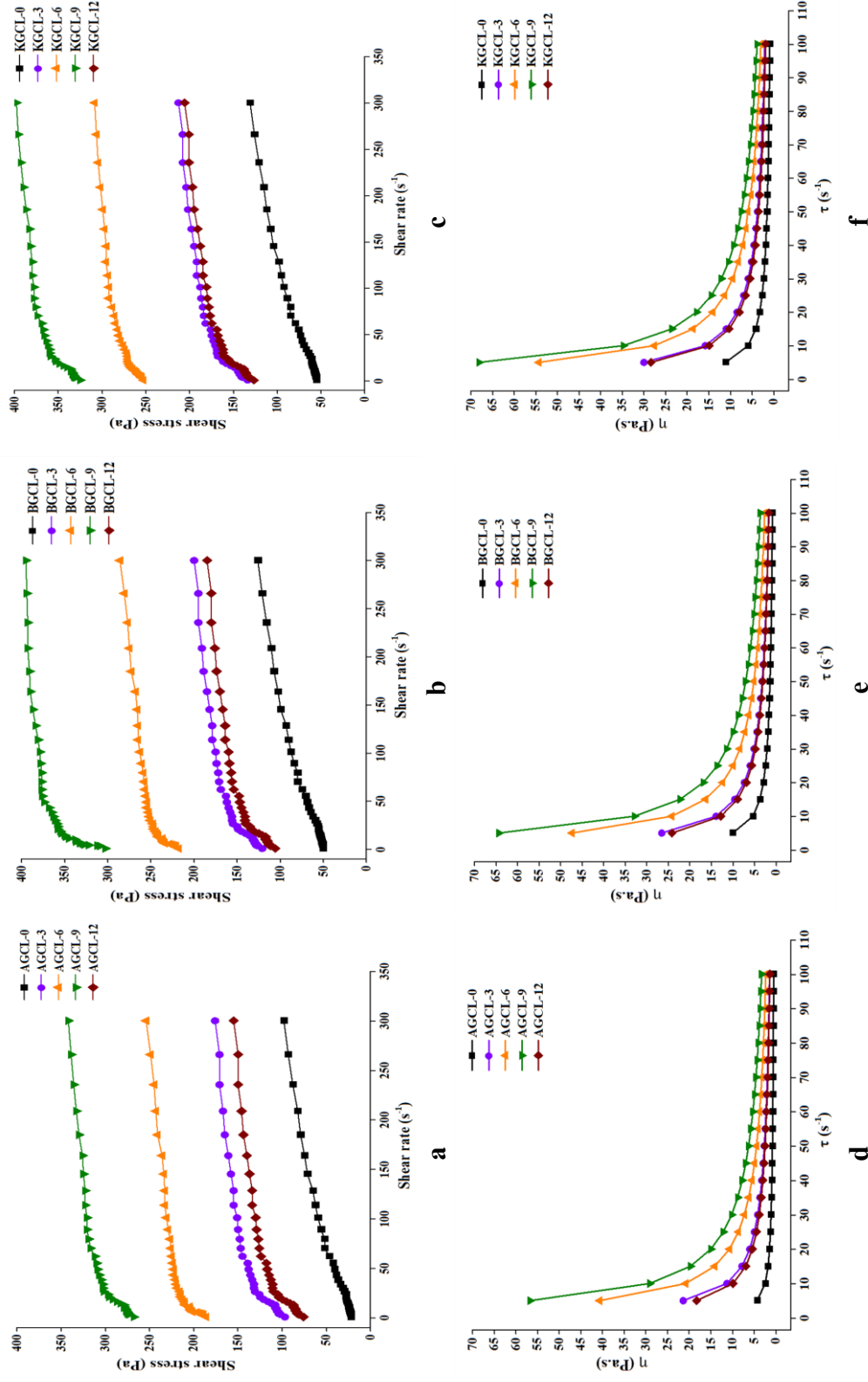


Fig. 4.9. Plots of (a-c) shear rate vs shear stress and (d-f) shear rate vs apparent viscosity of the *Amona*, *Badal* and *Kenkowa bao* crosslinked gels

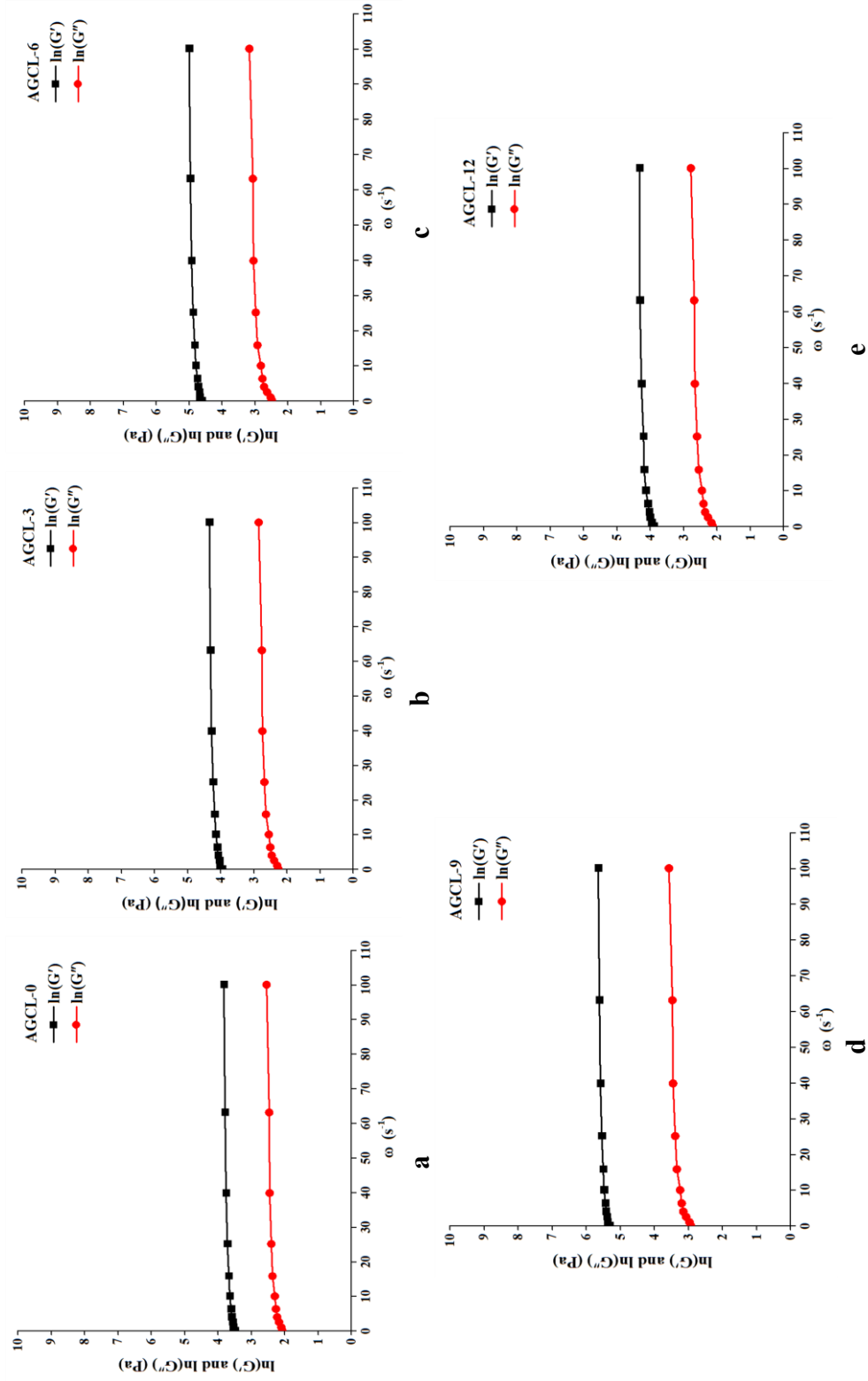


Fig. 4.10. (a-e) Plots of storage (G') and loss (G'') modulus as a function of frequency for the *Amona bao* crosslinked gels developed using 0-12% CF

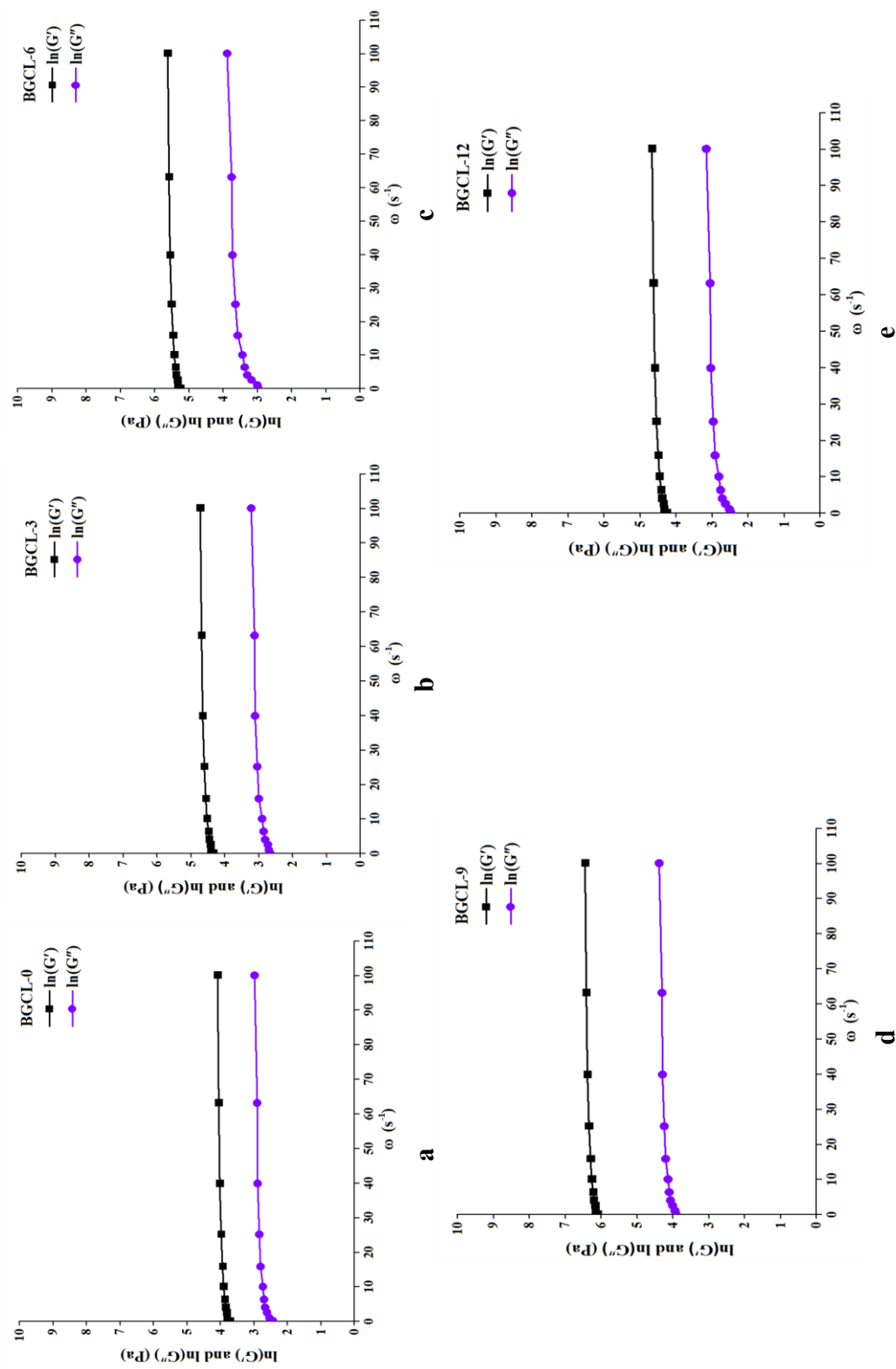


Fig. 4.11. (a-e) Plots of storage (G') and loss (G'') modulus as a function of frequency for the *Badal bao* crosslinked gels developed using 0-12% CF

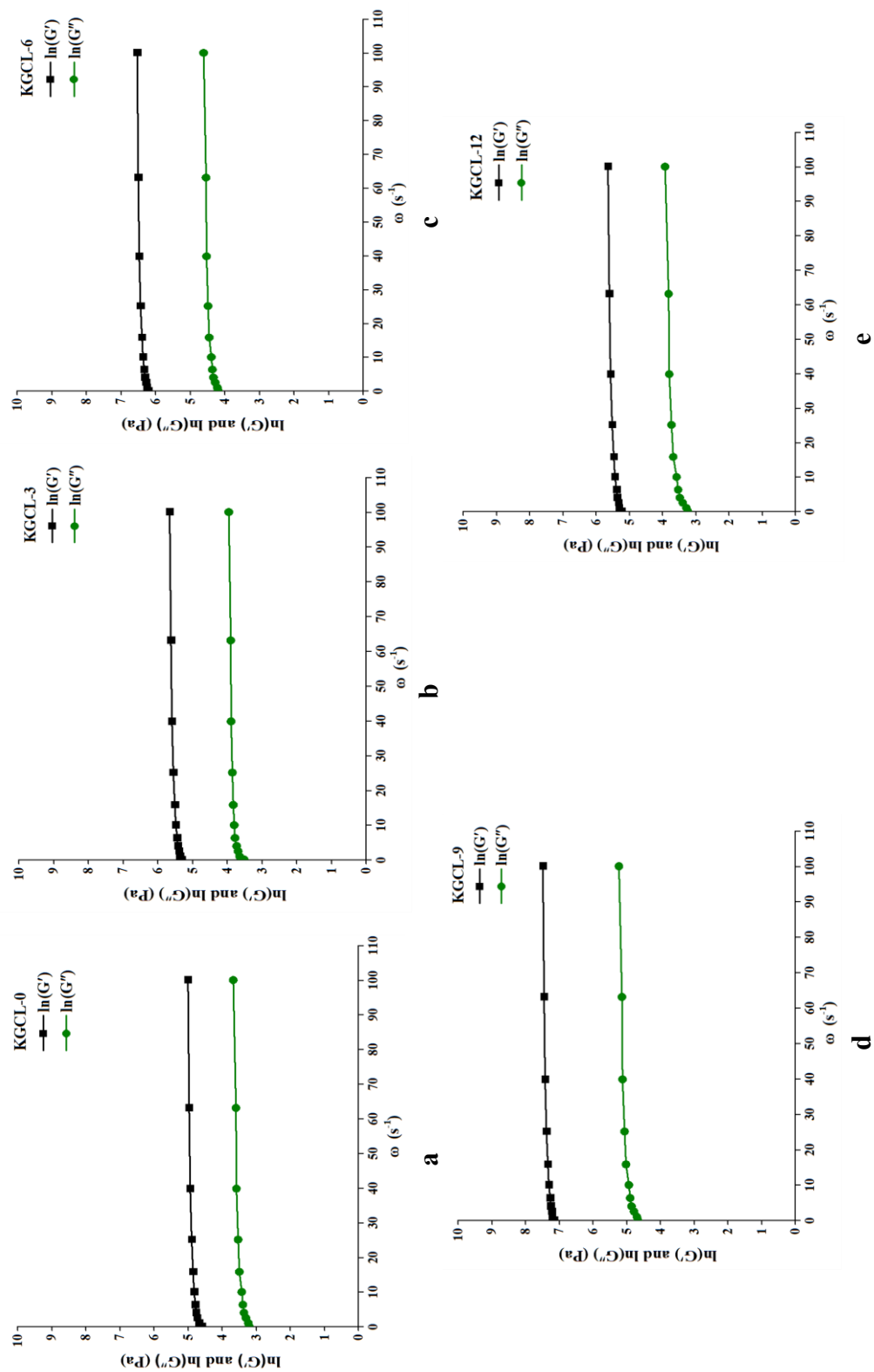
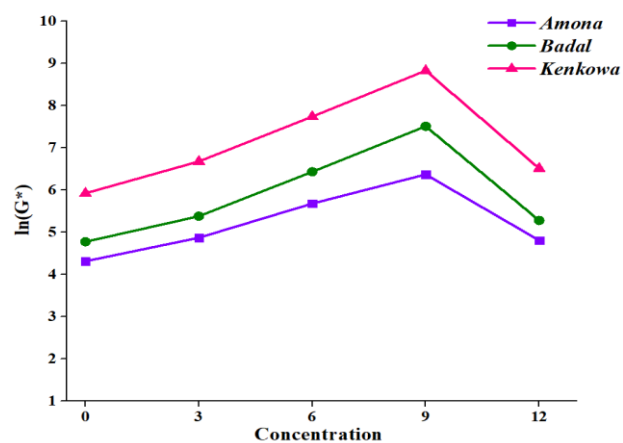
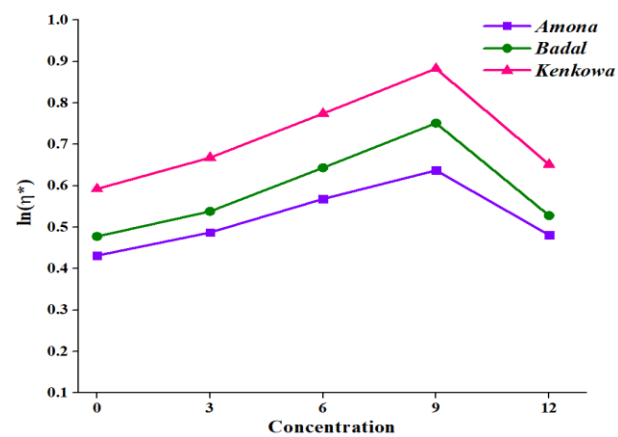


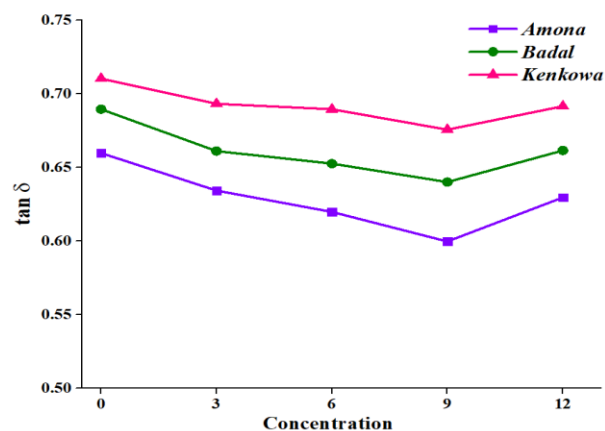
Fig. 4.12. (a-e) Plots of storage (G') and loss (G'') modulus as a function of frequency for the *Kenkoba biao* crosslinked gels developed using 0-12% CF



a



b



c

Fig. 4.13. Plots showing variation in (a) complex modulus (G^*), (b) complex viscosity (η^*) and (c) loss tangent ($\tan \delta$) as a function of % CF substitution for crosslinked gels of *Amona*, *Badal* and *Kenkowa* bao

4.3.9.5. Bioactive properties

Crosslinking caused a drastic decline ($p < 0.05$) in TPC, TFC, DRSA and FRAP (Fig. 4.14 a-d). The reduction may be attributed to either and all of thermal exposure during processing, alkaline-induced degradation and decreased extractability in solvent due to starch-polyphenol interactions.

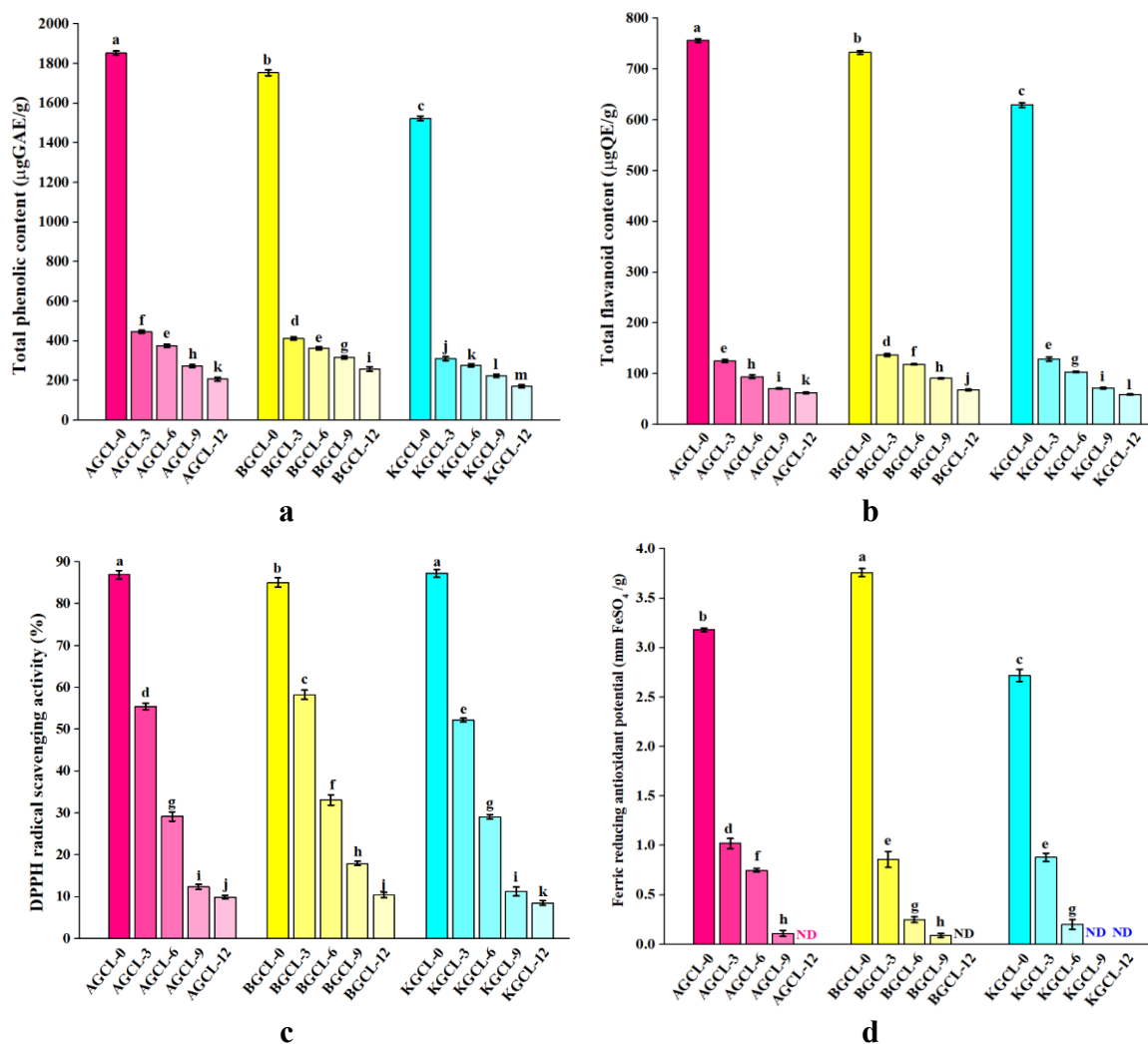


Fig. 4.14. (a) Total phenolic content, (b) Total flavanoid content, (c) DPPH radical scavenging activity and (d) Ferric reducing antioxidant potential of the native and crosslinked flours

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within a parameter are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test

Under high pH conditions, anthocyanins are susceptible to structural transformations such as ring opening to chalcone forms and subsequent oxidative degradation, leading to loss of antioxidant functionality (Gu et al., 2024). In addition, the formation of a crosslinked polymer matrix may entrap phenolic acids within the starch network, limiting their release during extraction. Interaction between the phosphate groups of STMP and reactive hydroxyl groups of anthocyanins may further reduce the availability of active sites responsible for radical scavenging activity. Similar observations were reported in by Khadsai et al. (2024) in crosslinked cassava starch and Yildiz et al. (2022) in faba bean starch. Among the varieties, the reduction in TPC, TFC and DRSA was most pronounced in *Kenkowa*, followed by *Amona* and *Badal*. In contrast, the decline in FRAP values was highest in *Kenkowa*, followed by *Badal* and *Amona*. FRAP was not detected in KGCL-9, KGCL-12, AGCL-12 and BGCL-12, indicating greater susceptibility of its antioxidant constituents to modification or entrapment during crosslinking.

4.3.10. Crosslinking levels and prospective CF usage

Understanding the functional implications of different crosslinking levels is important for identifying appropriate applications of the modified flours in food systems. At the lowest modification level (3% STMP), the starch structure remained only partially stabilized. Although crosslink formation began to restrict granule swelling, the network formed was relatively weak and insufficient to produce a highly stable starch matrix. Functional analyses showed relatively higher syneresis (Fig. 4.8) and moderate solubility (Fig 4.4b) compared with more extensively crosslinked samples. Textural properties (Table 4.4) also indicated reduced hardness and cohesiveness, suggesting that the starch matrix lacked sufficient structural reinforcement. Thermal analysis (Fig. 4.5, Table 4.3) supported these observations, as the gelatinization enthalpy remained relatively high, indicating that the native crystalline structure was only partially disrupted. Consistently, rheological parameters (low η , K , τ_0 and G' with higher n and $\tan \delta$) reflected a weak and poorly organized gel network with limited elasticity.

Consequently, 3% CFs may be more suitable for applications where moderate viscosity and partial swelling are desirable, such as thickening agents in soups, gravies, or light sauces where excessive rigidity is not required.

Increasing the crosslinking concentration to 6% resulted in noticeable improvements in structural stability and functional performance. Syneresis decreased compared with the 3% samples, while swelling (Fig 4.4a) reduced and paste stability improved (Table 4.2) due to stronger intermolecular interactions within the starch granules. Solubility also decreased, indicating reduced amylose leaching during heating. Textural parameters showed improved firmness and cohesiveness, suggesting the formation of a more stable gel network than the 3% CFs. Thermal transitions obtained from DSC also indicated partial modification of the native starch structure, with reduced gelatinization enthalpy compared with the native and 3% CFs. This was supported by rheological enhancement, where higher η , K , τ_0 and G' along with lower n and $\tan \delta$ indicated a stronger, more elastic gel network. These characteristics suggested that 6% CFs may be appropriate for food products requiring moderate structural stability, such as sauces, fillings and certain processed cereal products that are necessarily rigid but melt in mouth, where controlled viscosity and stability during heating are required.

Further, upon increasing the crosslinking level to 9%, the starch matrix achieved an optimal crosslink density that allowed controlled swelling while maintaining strong structural integrity. Syneresis reached its lowest level, indicating improved water retention and enhanced network stability. Swelling power and solubility were also minimized, reflecting limited amylose leaching and improved matrix cohesion. Textural analysis revealed the highest values of hardness, cohesiveness, springiness and resilience, indicating the formation of a dense and stable gel network. Thermal analysis further supported these findings, as the lowest gelatinization enthalpy suggested effective disruption of native crystalline regions and improved molecular rearrangement during gel formation. FTIR analysis (Fig. 4.2) also indicated the formation of well-defined phosphate crosslinks without excessive structural rigidification. Rheologically,

peak η , K , τ_0 and G' with minimum n and $\tan \delta$ confirmed the formation of a dense, highly elastic, and stable gel network. Collectively, these results demonstrate that 9% crosslinking provides the most favorable balance between granule stability, hydration capacity and network formation. Such functional characteristics make this level particularly suitable for applications requiring firm, cohesive and stable gel structures.

Increasing the crosslinking concentration to 12% resulted in a decline in several functional properties, indicating excessive modification of the starch matrix. At this level, the high density of phosphate crosslinks enhanced granule hydration and increased swelling capacity. As a result, swelling power increased and the ability of starch granules to form a cohesive gel network was compromised. Syneresis increased again, suggesting that excessive crosslinking produced a heterogeneous structure incapable of effectively retaining water during storage. Textural parameters also decreased, indicating a brittle and less cohesive matrix. Thermal analysis showed an increase in gelatinization temperatures, which may be attributed to the increased rigidity of the crosslinked starch network that requires greater thermal energy to initiate gelatinization. FTIR spectra further indicated intensified phosphate-related bands, suggesting excessive crosslink formation that may have disrupted the balance between rigidity and flexibility required for optimal gel formation. In agreement, reduced η , K , τ_0 and G' with increased n and $\tan \delta$ indicated structural heterogeneity and weakened viscoelastic integrity (Fig. 4.9-4.13). Therefore, although highly crosslinked starches may provide strong resistance to thermal and shear stresses, excessive crosslinking can negatively affect swelling behavior and structural cohesiveness, making the 12% CFs unsuitable for use as functional food ingredient.

4.3.11. Probable effects on non-starch constituents

The present study discussed the effects of STMP crosslinking primarily in relation to starch. However, it is important to recognize that its influence on the non-starch minor constituents would be also a matter of future research. This is important because with the

abundance of hydroxyl groups, similar reactions are also expected to occur with other non-starch polysaccharides present in rice flour, contributing to the formation of a reinforced composite network. Direct evidence of STMP interaction has been established for hemicellulose, where the treatment resulted in enhancing its mechanical strength and barrier properties (Zhao et al., 2021). Also, rice proteins, predominantly glutelin, along with prolamin, albumin, and globulin fractions could play a significant role in modulating the system. Although these proteins do not undergo substantial covalent crosslinking with STMP, they interact strongly with phosphorylated starch chains through hydrogen bonding and physical entanglement. Such protein-starch interactions form a continuous matrix surrounding starch granules, restricting their swelling and enhancing gel firmness (Alavarse et al., 2022). Lipids, due to the absence of sufficient reactive hydroxyl groups, do not directly participate in STMP-mediated reactions, but indirectly influence the system by stabilizing amylose-lipid inclusion complexes within the crosslinked matrix, thereby further restricting swelling and solubility. Therefore, while covalent modification is primarily confined to starch and other hydroxyl-rich polysaccharides, the overall physicochemical and functional behavior of the system is governed by synergistic interactions among all flour components, resulting in the formation of a highly integrated composite biopolymer network.

4.4. Conclusion

The three pigmented *bao* rice whole flours, when crosslinked with increasing concentrations of STMP (0, 3, 6, 9 and 12%), exhibited distinct variations in physicochemical, structural, thermal and functional properties. These variations reflected progressive changes in starch molecular interactions and granule integrity with increasing crosslink density. Lightly crosslinked starches (3%) may function as moderate thickening agents in liquid-based foods, whereas moderately crosslinked starches (6%) could provide improved stability in semi-structured systems such as sauces and fillings. More extensively crosslinked starches (9%) exhibited optimal

balance between swelling, water retention and structural strength, making them suitable for structured starch-based food systems that require strong and cohesive gel networks. However, excessively crosslinked starches (12%) exhibited restricted swelling and reduced structural flexibility, limiting their applicability in products requiring cohesive gel formation.

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CHAPTER 5

Development of a dessert gel from pigmented *bao* rices and characterization of its techno-functional, antioxidant and nutritional properties

5.1. Introduction

Growing consumers' demand for health-oriented convenient food products has intensified efforts to diversify functional food ingredients. Starch-based hydrogels with tunable viscoelasticity and structural integrity have attracted considerable interest for use as thickening, stabilizing, and gelling agents (Obadi and Xu, 2021). Their soft yet stable texture, mouthfeel, and ability to serve as carriers of functional ingredients have made them a class of most extensively researched hydrogels (Hirao et al., 2021). Besides food formulations such as puddings, sauces, dairy analogues, and ready-to-eat (RTE) foods such as noodles, instant formulations among others, their notable non-food applications range to pharmaceutical (as controlled-release drug carriers), biomedical (as wound dressings, scaffolds and hydrogels for tissue engineering), cosmetic (as thickeners or stabilizers in emulsions) and packaging (as film-forming agents) industries (Bangar et al., 2024).

The fundamental mechanism of starch gelation involves gelatinization upon heating and retrogradation upon cooling, forming a semi-solid matrix that entraps water and other ingredients. However, the textural and storage properties of starch-based gels are highly dependent on the botanical source and the molecular composition and structure of the starch (Li et al., 2020). Among various botanical sources, high-amylose starches have been promising candidates for hydrogel applications due to their extended linear glucan chains, elevated gelation temperature, and superior molecular alignment during retrogradation, promoting the formation of denser, more cohesive gel matrices characterized by higher elasticity and firmness (Gong et al., 2024; Zhang et al., 2022a).

Moreover, high-amylose starches exhibit slower retrogradation kinetics and greater resistance to thermal, shear, and acid-induced breakdown, rendering them especially suitable for applications that demand prolonged structural stability, such as heat-processed or refrigerated food systems. Considering the fact that native starches exhibit several limitations in acquirement of desired functionality, the concept of starch modification emerged. Stable starch gel matrices have been developed through modification strategies such as dual thermal cycling, enzymatic restructuring, physical blending with hydrocolloids or chemical modifications (Obadi and Xu, 2021).

Chemical modification by starch crosslinking stands out for its ability to enhance paste viscosity, reduce syneresis, and increase resistance of a starch gel to shear and acid breakdown (Kou et al., 2022). Crosslinking, particularly with food-grade agents like sodium trimetaphosphate (STMP), introduces phosphate bridges between hydroxyl groups of adjacent starch chains, resulting in a denser and more stable gel network (Bangar et al., 2024; Bodjrenou et al., 2023). STMP preferentially reacts with the linear amylose component, making high-amylose starches better suitable for this modification. Crosslinked starches exhibit enhanced storage modulus (G'), lower breakdown during thermal processing, improved freeze–thaw stability, and reduced digestibility, which are highly valued attributes in dessert gels, frozen products, and slow-energy-release foods (Adamczyk et al., 2022; Butt et al., 2021). Recent studies have reported successful use of STMP-crosslinking on rice, waxy wheat, waxy maize starches in hydrogels and RTE food matrices (Obadi and Xu, 2021; Gong et al., 2024). However, excessive STMP can increase residual phosphorus beyond safe limits. Regulatory bodies like Codex and Joint FAO/WHO Expert Committee on Food Additives (JECFA) have set a maximum limit of 0.4% (w/w, as P) for food-grade crosslinked starches, making it essential to balance functional benefits with regulatory compliance. While crosslinking enhances textural stability, it may also have unintended effects on nutritional and functional components (Khadsai et al., 2024; Gu et al., 2024; Yildiz et al., 2022). With most studies reported on crosslinking of isolated starch and their food applications, research on its effects on

whole flour have been limited (Bangar et al., 2024, Gong et al., 2024, Sharma et al., 2021).

Latterly, pigmented rices have emerged as highly nutritious grain crops, delivering substantial amounts of phenolics, flavonoids and anthocyanins (Gogoi et al., 2025). When used in their whole-flour form, they offer not only gelling properties but also added value through antioxidant capacity, color enhancement, and dietary fiber. The dehulled rice grains contain about 60-70% starch, that predominantly control their processed functional characteristics. When milled to flour, the bioactive compounds can co-contribute to antioxidant functionality and color stability in products including gels. However, this hypothesis is contradicted by previous reports of Khadsai et al (2024) and Yildiz et al (2022) mentioning decline in antioxidant potential of cassava starch and faba bean flour following crosslinking with citric acid. This was attributed to covalent masking or transformation of phenolic hydroxyl groups by carboxyl groups of citric acid. Notably, no study to date has reported the formulation of firm and stable gel-based desserts using crosslinked pigmented rice flours.

Red-pigmented *bao* rice landraces taken up for this research, namely *Kenkowa*, *Amona*, and *Badal* are not only nutritionally rich but are also ecologically unique (Mudoi and Das, 2019). The characteristic red color of pigmented rice is primarily due to high levels of proanthocyanidins, which contribute to multiple health benefits including strong antioxidant, anti-inflammatory, and potential anti-diabetic effects (Sahewalla et al., 2023; Thakur et al, 2025). Although these varieties reportedly demonstrate high phenolic content (up to 1357 mg catechol/100 g), antioxidant activity (> 80% 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity) and cooking stability, they have remained underutilized in modern food systems (Mudoi and Das, 2019; Mudoi and Das, 2020). There is a complete lack of scientific data on their starch functionality, modification potential, or suitability in RTE product formulations, revealing a critical research gap that this work aimed to address.

Primarily, we hypothesized that whole *bao* rice flour can serve as an equally effective substrate for crosslinking, owing to the naturally high starch content which get exposed to reagents, especially after milling and grinding. It would nullify the cost and time associated with starch isolation. It was further hypothesized that STMP-induced crosslinking of whole pigmented *bao* rice flours would enhance techno-functional properties while retaining a substantial fraction of antioxidant activity, thus making it a promising ingredient for functional food design. Hence, this study focused on the development of an innovative dessert gel using STMP-crosslinked whole grain flours of *Amona*, *Badal* and *Kenkowa bao* rice. In this context, physical, physicochemical, antioxidant, rheological, and sensorial properties of the developed dessert gels were investigated. As an innovative approach to eliminate sensorial bias and human error in sample tasting, fuzzy logic was implemented on the sensorial data to obtain the best performing gels. The final dessert gel samples were evaluated for nutritional status and starch digestibility. Finally, a shelf-stability study over refrigerated storage was carried out based on microbiological safety, texture, color retention and overall sensory acceptability of the gels.

5.2. Materials and methods

5.2.1. Materials

Freshly harvested pure line paddy samples of *Amona*, *Badal* and *Kenkowa bao* paddy were dehusked using a laboratory-scale huller and milled to flour with a grinder (Phillips HL7707/00, India, particle size=100-150 μm). The flour moisture was adjusted at $12\pm 1\%$ and stored in airtight containers until further use. All analytical grade chemicals used in this study were purchased from SRL chemicals, Lobachemie, Himedia and Sigma-Aldrich.

5.2.2. Crosslinking

Crosslinking was conducted using sodium trimetaphosphate (STMP) as per Kumar et al. (2022) with slight modifications. Rice flour (50 g) was dispersed in distilled water (DW,

100 ml) and stirred continuously at 45 °C. Maintaining the temperature and stirring, 4.5 g STMP corresponding to 9% of flour weight and 1 g sodium sulfate as catalyst (2% w/w) were added. The mixture pH was adjusted to 11.0 with 0.5 mol.l⁻¹ sodium hydroxide. The reaction was maintained for 3 h. Following this, pH was brought down to 6.5 using 1.0 mol.l⁻¹ hydrochloric acid. The suspension was centrifuged (2325 g, 5 min) to separate the modified flour as precipitate, washed six times with DW to remove any residual reagent, and dried at 40 °C till 12% moisture content to obtain the crosslinked flours (CF) of the respective varieties.

Based on our previous study detailed in Chapter 4 (Table 4.2), where we estimated the phosphorus content of different concentrations of STMP, the residual phosphorus content in the CFs remained below the Codex Alimentarius and JECFA safety threshold of 0.4% (w/w, as retained phosphorus), confirming their suitability for food applications.

5.2.3. Preparation of dessert gel

Dessert gels were prepared from the raw and partially CF-substituted (0-50%) flours of the respective varieties, as detailed in the next paragraph. All flour and gel samples were coded based on rice variety (*Amona* = A, *Badal* = B, *Kenkova* = K), CF substitution level, and product type (flour or gel). For example, AF-40 referred to *Amona* flour with 40% CF substitution, and AG-40 refers to its corresponding dessert gel. Native flours and gels (0% CF) were coded as AF-0, BF-0, KF-0 and AG-0, BG-0, KG-0, respectively (Table 5.1). Fig 1 shows the native flours and their respective CFs.

For preparation, 10 g of each flour mixture was added to 100 ml DW (1:20, w/v) taken in a beaker and cooked at 85±5 °C for 5 min with mixing to allow complete and uniform starch gelatinization. Confirmation of complete gelatinization was ensured by the viscosity values of the samples (Table 5.2). Sucrose (10%, w/w) was added as sweetener at the 5th min of cooking. The resulting gels were transferred into molding tray, cooled to room temperature, and then refrigerated (4±2 °C) for 12 h before analyses. Dessert gel cubes with dimensions of 30×30×10 mm (length × width × height) were obtained.

5.2.3.1. Confirmation of complete gelatinization at chosen cooking temperature

Apparent viscosity values between 1.740 and 3.016 Pa.s confirmed complete gelatinization of starch in all dessert gel samples (Table 5.2). Literature indicates that fully gelatinized starch systems typically exceed 1.600-1.800 Pa.s (Zhou et al., 2021). The chosen cooking condition (85±5°C for 5 min, 1:20 w/v) provided sufficient heat and water, as supported by viscosity data and previous studies. Higher viscosities reflect improved matrix integrity (Hoover & Ratnayake, 2002; Kaur et al., 2019).

Table 5.1. Coding of samples

Rice variety	CF substitution level (%)	Flour sample code	Dessert gel sample code
<i>Amona bao</i>	0	AF-0	AG-0
	10	AF-10	AG-10
	20	AF-20	AG-20
	30	AF-30	AG-30
	40	AF-40	AG-40
	50	AF-50	AG-50
	100	AF-100	-
<i>Badal bao</i>	0	BF-0	BG-0
	10	BF-10	BG-10
	20	BF-20	BG-20
	30	BF-30	BG-30
	40	BF-40	BG-40
	50	BF-50	BG-50
	100	BF-100	-
<i>Kenkowa bao</i>	0	KF-0	KG-0
	10	KF-10	KG-10
	20	KF-20	KG-20
	30	KF-30	KG-30
	40	KF-40	KG-40
	50	KF-50	KG-50
	100	KF-100	-



Fig. 5.1. Native flours of *Amona* (AF-0), *Badal* (BF-0) and *Kenkowa* (KF-0) and 9% crosslinked flour of *Amona* (AF-100), *Badal* (BF-100) and *Kenkowa* (KF-100)

Table 5.2. Apparent viscosity of the slurries of substituted flours as measured by a Brookfield viscometer (1:20 w/v ratio, 85±5 °C for 5 min, measured at 40 °C, spindle #4, 20 rpm)

Rice variety	CF substitution level (%)	Apparent viscosity (Pa.s)
<i>Amona bao</i>	0	1.740±0.048 ^{f12}
	10	1.830±0.052 ^{e11}
	20	2.120±0.038 ^{d9}
	30	2.395±0.029 ^{c7}
	40	2.675±0.066 ^{b5}
	50	2.890±0.032 ^{a2}
<i>Badal bao</i>	0	1.865±0.033 ^{f11}
	10	1.940±0.062 ^{e10}
	20	2.235±0.046 ^{d8}
	30	2.510±0.070 ^{c6}
	40	2.795±0.039 ^{b4}
	50	2.980±0.027 ^{a1}
<i>Kenkowa bao</i>	0	1.892±0.055 ^{f11}
	10	1.985±0.042 ^{e10}
	20	2.280±0.062 ^{d8}
	30	2.555±0.053 ^{c6}
	40	2.835±0.036 ^{b3}
	50	3.016±0.069 ^{a1}

Means followed by the same superscript lowercase letter within a column for a given variety, and the same superscript number within a column across varieties, are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

5.2.4. Apparent amylose content

Apparent amylose contents (AAC) of the substituted flour samples were quantified using an iodometric assay described by Govindaraju et al. (2022). Briefly, 0.1 g flour was treated with 1 ml of 95% ethanol and 9 ml of 1 N sodium hydroxide, followed by incubation in a boiling water bath for 10 min and further cooling to ambient temperature. The final volume was adjusted to 100 ml with distilled water. From this, a 5 ml aliquot was transferred to a test tube and 1 ml glacial acetic acid and 2 ml iodine solution (0.2% I₂ in 2% KI) were added. The mixture was then diluted to a final volume of 100 ml using distilled water. After incubation at room temperature for 10 min, absorbance was measured at 620 nm. AAC was determined by comparing the absorbance values with a standard calibration curve of purified potato amylose.

5.2.5. Flour hydration properties

Solubility and swelling power (SP) of the flour samples were determined following the combination method of Bora et al. (2023). Briefly, 1 g flour was vortexed well with 10 ml DW and incubated at 50 °C for 60 min with gentle stirring. After centrifugation (1308 g, 15 min), supernatant was collected and dried at 60 °C for 24 h before weighing. The sediment was also weighed. Values of SP and solubility were calculated using the following equations (Eq. 5.1 and 5.2)

$$\text{Solubility (\%)} = \frac{\text{Weight of dried supernatant}}{\text{Dry weight of flour taken}} \times 100 \quad \text{Eq. 5.1}$$

$$\text{SP(g/g)} = \frac{\text{Weight of sediment}}{\text{Dry weight of flour taken} - (100\% - \% \text{ of dried supernatant})} \times 100 \quad \text{Eq. 5.2}$$

5.2.6. Physicochemical properties of dessert gels

5.2.6.1. Color values

The CIE color values, namely L* (lightness or darkness), a* [(greenness (-) to redness (+)) and b* [(blueness (-) to yellowness (+))] of the gel samples were obtained using a Color Quest spectrophotometer (Hunter Associates Laboratory, USA) calibrated with standard white and dark tiles. The total color difference (ΔE) was calculated using the following equation (Eq. 5.3)

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \text{Eq. 5.3}$$

5.2.6.2. Syneresis

Freshly prepared dessert gel sample (20 g) was poured in a pre-weighed test tube, sealed, cooled to ambient temperature, and stored at 4±2 °C. After each 24 h interval, the tube was brought to ambient temperature for 4 h and centrifuged (6300 g, 5 min). The separated aqueous layer was carefully decanted and weighed to calculate syneresis (Eq. 5.4) (Park et al., 2024).

$$\% \text{ Syneresis} = \left(\frac{\text{Weight of decanted liquid (g)}}{\text{Total gel weight before centrifugation (g)}} \right) 100 \quad \text{Eq. 5.4}$$

5.2.6.3. *Texture*

Texture profile analysis (TPA) of the dessert gels was conducted in a TA.XT. Plus texture analyzer system (Stable Microsystems, UK). Double compression test at the center of the gel cube was performed using a 20 mm cylindrical probe fitted on a 10 kg load cell with 3 mm.s⁻¹ pretest speed, 1mm.s⁻¹ test speed and 1mm.s⁻¹ post-test speed to a compression distance of 2 mm. A penetration depth of 5 mm into the center of each sample was achieved using a probe with a trigger force set at 5 g. Hardness, adhesiveness, cohesiveness, springiness and chewiness values were determined from the generated graphs using the inbuilt software (Exponent Connect).

5.2.7. *Antioxidant activity*

5.2.7.1. *Sample extraction*

Lyophilized (-40 °C for 24 h followed by primary drying at -35 °C under 100 mTorr vacuum for 24 h) gel samples were extracted using absolute ethanol (1:10 w/v, sample to solvent) at 40 °C for 24 h under constant agitation. After centrifuging (845 g, 60 min), supernatant was collected.

5.2.7.2. *DPPH radical scavenging activity (DRSA) and ferric ion reducing antioxidant potential (FRAP)*

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (DRSA) and ferric ion reducing antioxidant potential (FRAP) were determined as per Pradipta et al. (2020) and Zhang et al. (2022b), respectively. DRSA was evaluated (Eq. 5.5) and expressed in percentage, while FRAP expressed as milimole of ferrous equivalent Fe(II) per g (mM/g) of sample.

$$\% \text{ DRSA} = \frac{(A_0 - A_s)}{A_0} \times 100 \quad \text{Eq. 5.5}$$

where, A_0 is the absorbance of the blank, A_s is the absorbance of the sample extract

5.2.8. Rheological properties

Rheological characterization of the dessert gels was carried out using a Physica MCR 101 rheometer (Anton Paar, Austria) fitted with a cone-and-plate measuring system (50 mm diameter, 1° cone angle, 0.1 mm gap). The steady shear, time-dependent, and dynamic viscoelastic properties were assessed at 4 °C (Bora et al., 2023). Each gel sample was allowed to rest for 2 minutes after loading to facilitate structural relaxation and thermal stabilization before running the tests.

5.2.8.1. Steady shear

For steady shear analysis, the samples were subjected to a forward shear rate sweep from 0 to 300 s⁻¹ over 2 min, followed by a reverse sweep back from 300 s⁻¹ to 0 s⁻¹ over the next 2 min. Apparent viscosity was measured across the shear ranges, with a specific focus on the value at 50 s⁻¹ (η_{50}), which is considered physiologically relevant to oral processing. The flow characteristics were interpreted using the Herschel-Bulkley model, which defines the relationships between shear stress and shear rate in terms of yield stress, consistency coefficient and flow behavior index (Eq. 5.6).

$$\tau = \tau_0 + K\dot{\gamma}^n \quad \text{Eq. 5.6}$$

where, τ is shear stress (Pa), τ_0 is yield stress (Pa), K is the consistency coefficient (Pa.s ^{n}), $\dot{\gamma}$ is shear rate (s⁻¹) and n is the flow behavior index (dimensionless).

5.2.8.2. Dynamic shear

For dynamic rheology, frequency sweep tests were conducted at 4 °C over a range of 0.1 to 100 rad·s⁻¹ at a fixed strain of 1%. Storage modulus (G'), representing elastic behavior, and the loss modulus (G''), representing viscous behavior, were recorded as functions of angular frequency (ω). The loss tangent ($\tan \delta$), indicative of the relative dominance of viscous or elastic behavior, was calculated (Eq. 5.7).

$$\tan\delta = \frac{G''}{G'} \quad \text{Eq. 5.7}$$

Further, the overall viscoelastic behavior of the samples under oscillatory strain was examined by calculating the complex modulus (G^*) and complex viscosity (η^*) values (Eq. 5.8 and 5.9).

$$G^* = \sqrt{(G')^2 + (G'')^2} \quad \text{Eq. 5.8}$$

$$\eta^* = \frac{G^*}{\omega} \quad \text{Eq. 5.9}$$

where, ω represents angular frequency

The relationship between ω , G' and G'' was examined through nonlinear regression analysis, allowing for the determination of intercepts (K' and K''), slopes (n' and n''), and corresponding coefficients of determination (R^2), based on the power-law model (Eq. 5.10 and 5.11)

$$G' = K'(\omega)^{n'} \quad \text{Eq. 5.10}$$

$$G'' = K''(\omega)^{n''} \quad \text{Eq. 5.11}$$

5.2.9. Sensory evaluation using fuzzy logic

The sensory analytical procedures involving human subjects were approved by the Human Ethical Committee of Mizoram University (Reference No. MZU/HEC/2024/013, dated 17/06/2024). A pre-screening of 20 panelists (10 females and 10 males) out of 35 individuals (15 males and 20 females), aged between 22 and 38 years was done based on a consent and survey conducted. Individuals with health issues, pregnant women and habitual tobacco consumers were excluded. Two orientation sessions were conducted to standardize the evaluation protocols and familiarize the 20-member panel with the assessment procedures.

Evaluations were conducted in four sessions over two days. Initial three sessions involved five samples and the last session involved two samples. Two-hour intervals were maintained between successive sessions. Panelists were instructed to abstain from food and beverages, except water, for at least 30 minutes prior to each session. The panelists independently evaluated the anonymized and randomized dessert gel samples using a five-point hedonic scale: NS (Not Satisfactory), F (Fair), M (Medium), G (Good), and E (Excellent), focusing on the key sensory attributes, namely color, taste, texture, and mouthfeel. Following evaluation of each sample, participants rinsed their mouths thoroughly with water and observed a two-minute rest before proceeding to the next sample. No discomfort or allergic reactions were reported by any of the panelists during or after the sensory evaluation sessions.

The five-point rating scale was: NS (Not Satisfactory), F (Fair), M (Medium), G (Good), and E (Excellent), as tabulated in Tables 5.8, 5.9 and 5.10, focusing on the key sensory attributes, namely color, taste, texture, and mouthfeel. In addition to hedonic scoring, relative importance of individual sensory attributes was assessed using a five-point importance scale: NI (Not at all Important), SI (Somewhat Important), I (Important), HI (Highly Important), and EI (Extremely Important) as tabulated in Table 5.11. This involved a stepwise fuzzy-logic evaluation, performed in Microsoft Excel by inserting the respective equations. Step 1: The responses of the 20-member sensory panel were first converted into triangular fuzzy triplets for each sample and each quality attribute (color, taste, texture, and mouthfeel). Triplets obtained from the hedonic scale formed the sample-associated fuzzy numbers (SC, STa, STex, SM), while triplets derived from the importance scale generated attribute-associated fuzzy numbers (QC, QTa, QTex, QM). Step 2: Relative weightages of the sensory attributes were calculated from the attribute triplets (QC_{rel} , QTa_{rel} , $QTex_{rel}$, QM_{rel}). Step 3: These weightages were then applied to their corresponding sample triplets to compute the overall sensory triplet (SO_x) for each gel sample. Step 4: The overall membership values (B0-B5) were subsequently obtained from SO_x through the fuzzy membership rules. Step 5: Membership functions for each attribute were computed in the same manner from their

triplet values and denoted as BC, BTa, BTex and BM. Step 6 : Similarity values comparing each sample (S0-S5) with the standard fuzzy scale “not satisfactory (F1), fair (F2), satisfactory (F3), good (F4), very good (F5) and excellent (F6)” in case of the dessert gels and as “not at all necessary (F1), somewhat necessary (F2), necessary (F3), important(F4), highly important (F5) and extremely important (F6)” in case of the quality attributes were calculated to find the relative sensory standing and rank of each sample (Deka et al., 2024).

The highest-ranked sample in terms of the highly ranked parameters from each rice variety was identified as the optimized formulation. The samples were then further evaluated for their static and dynamic rheological properties and instrumental textural properties. This was used to validate the fuzzy logic-based ranking and confirm the selected samples as the optimized formulations suitable for further physicochemical, functional, and shelf-life studies.

5.2.10. Proximate composition

Moisture, protein, fat and ash contents (% w.b.) of the dessert gel samples were assessed by AOAC (2019) protocols as detailed in section 3.2.5 (Chapter 3). Total carbohydrate content was calculated by difference. Energy content of the samples was calculated using Atwater general factors (4 kcal/g for protein, 4 kcal/g for carbohydrates, and 9 kcal/g for fat), following the method described by Merrill and Watt (1973).

5.2.11. Starch digestibility

The *in vitro* protocol described by Roy et al. (2019) was adapted with minor modifications to quantify digestibility of starch in the dessert gels. First, total starch (TS) content of each sample was determined by an AOAC (2019) protocol. For digestion, the lyophilized gel flour samples (100 mg) were initially subjected to deproteinization (pH 1.5, 1ml pepsin in HCl-KCl buffer, 37 °C, 1 h). Following this, pH was adjusted to 5.4 using 7 ml of acetate buffer. To this, 3 ml of an enzymatic mixture (220 U/ml invertase,

3000 U/mL pancreatic α -amylase, 15 U/ml amyloglucosidase) was added and incubated at 37 °C to allow digestion. Aliquots were taken out at the 20 and 120 minutes and analyzed for glucose content. Rapidly (RDS) and slowly (SDS) digestible starches were calculated from these values respectively by multiplying with a factor of 0.9 and expressed as percentage ratio to that of total starch (TS) (Eq. 5.12 and 5.13). The undigested starch remaining after 120 minutes was considered as resistant starch (RS) (Eq. 5.14).

$$RDS(\%) = \left[\frac{G_{20} \times 0.9}{TS} \right] \times 100 \quad \text{Eq. 5.12}$$

$$SDS(\%) = \left[\frac{(G_{120} - G_{20}) \times 0.9}{TS} \right] \times 100 \quad \text{Eq. 5.13}$$

$$RS(\%) = [TS - (RDS + SDS)] \times 100 \quad \text{Eq. 5.14}$$

5.2.12. Storage stability

Freshly prepared dessert gel cubes were individually packed in airtight polypropylene containers and stored in refrigerator (4±2 °C) for analyzing stability during storage.

5.2.12.1. Microbial analysis

Microbial stability was evaluated at 2-day intervals. Plate Count Agar (PCA) was used for total aerobic plate count (TAPC) and Potato Dextrose Agar (PDA) acidified with 10% tartaric acid was employed for yeast and mold count (YMC). On each sampling day, three independent sample packages were randomly selected, a 10 g portion aseptically weighed and transferred into 100 ml of sterile physiological saline (0.85% NaCl) taken in a sterile bag. The mixture was homogenized using a laboratory blender to ensure even microbial dispersion. The resulting homogenate was then subjected to serial dilution using 0.1% sterile peptone water to obtain appropriate dilution levels for plating. Aliquots from suitable dilutions were surface plated onto the PCA and PDA media and incubated at 37 °C for 48 h and at 25 °C for 5 days, respectively. Post

incubation period, colonies were counted and the results were expressed as colony-forming units per gram (cfu g⁻¹) of the dessert gel (Larrea-Wachtendorff et al. 2022).

The storage period was selected based on the duration over which the samples remained microbiologically safe, in line with regulatory standards for ready-to-eat (RTE) food products (FSSAI, 2020). Samples maintaining TAPC below 5 log cfu g⁻¹ and YMC under 2-3 log cfu g⁻¹ were deemed acceptable. This microbiologically safe duration was then adopted for subsequent quality assessments.

5.2.12.2. Color, syneresis, texture and overall sensory acceptability

During this defined microbiologically safe period, samples were systematically evaluated for CIE color attributes, syneresis and texture profile using standardized methodologies as described in previous sections. Furthermore, the sensory overall sensory acceptability (OAA) was evaluated over the storage period using a 9-point rating scale ranging from 1 (dislike extremely) to 9 (like extremely) by the panelists based on their preferences.

5.2.13. Statistical analysis

All the analyses of this study were performed in triplicates, with results reported as mean $\pm$ standard deviation. Significance of differences among the results were assessed using one-way ANOVA using Duncan's multiple range test at a 95% significance level ($p \leq 0.05$) using IBM SPSS 21 (IBM Corporation, USA). Graphs and model fitting were performed using Origin 9.0 (OriginLab Corporation, USA). Fuzzy logic analysis was performed in Microsoft Excel 2021 (Microsoft, WA, USA).

5.3. Results and Discussions

5.3.1. Apparent amylose content

A significant AAC reduction ($p \geq 0.05$) of approximately 25% was recorded from AF-0 to AF-50. Decline of about 30% was recorded for samples BF-0 to BF-50 and KF-0 to KF-50 exhibited (Table 5.3). The phosphate diester bonds between hydroxyl groups introduced due to STMP crosslinking, formed a denser starch matrix, restricting dispersion and solubility of external amylose during the iodine-binding assays, thereby lowering the quantifiable AAC (Bangar et al., 2024). Steric hindrance imposed by phosphate cross-bridges may also shield the internal amylose chains from interacting with iodine (Xiaofan et al., 2022). Further, amylose-amylopectin associations induced by crosslinking and enhanced molecular rigidity could have additionally contributed to reductions in measured AAC (Sukhija et al., 2019). This combined effect in CF combined with progressive compositional dilution in the substituted flours resulted in the overall lowering of AAC.

5.3.2. Flour hydration properties

5.3.2.1. Swelling power

High-amylose starches typically exhibit lower swelling due to the presence of extended linear chains, which promote strong internal bonding and entanglement, thereby restricting granule expansion (Wang et al., 2024). The present data support this trend, with KF-0 showing a lower SP than AF-0 and BF-0 (Table 1). SP decreased consistently ($p \geq 0.05$) with increasing CF concentration. Limited starch chain mobility preventing granular expansion imparted by crosslinking could be attributed to it. Similar observations were reported by Sharma et al. (2020) in crosslinked faba bean starches. Notably, the decline in SP was evident even at lower substitution levels, suggesting that even minimal CF incorporation was sufficient to restrict granule hydration behavior.

Similar reductions in SP were reported in phosphorylated corn and epichlorohydrin crosslinked sorghum starches (Ma et al., 2025; Sandhu et al., 2021)

Table 5.3. AAC, SP and Solubility of the substituted flours

Samples	Apparent amylose content (%)	Swelling power	Solubility (%)
AF-0	25.87±0.14 ^{a7}	4.75±0.03 ^{a1}	2.44±0.06 ^{a1}
AF-10	24.19±0.12 ^{b8}	4.61±0.05 ^{b2}	2.29±0.03 ^{b2}
AF-20	22.95±0.22 ^{c9,10}	4.54±0.04 ^{c3}	2.18±0.07 ^{c3,4}
AF-30	22.07±0.15 ^{d12}	4.45±0.02 ^{d4}	2.06±0.04 ^{d5}
AF-40	21.12±0.33 ^{e14}	4.31±0.07 ^{e6}	1.95±0.02 ^{e6,7}
AF-50	19.84±0.12 ^{f16}	4.22±0.04 ^{f7}	1.84±0.01 ^{f8}
AF-100	16.58±0.22 ^{h17}	-	-
BF-0	29.14±0.15 ^{a2}	4.39±0.03 ^{a5}	2.20±0.02 ^{a3}
BF-10	27.73±0.19 ^{b4}	4.18±0.05 ^{b7}	1.98±0.05 ^{b6}
BF-20	26.05±0.11 ^{c6}	4.07±0.02 ^{c8}	1.83±0.02 ^{c8}
BF-30	24.17±0.41 ^{d8}	3.89±0.04 ^{d9}	1.72±0.01 ^{d10}
BF-40	22.89±0.22 ^{e10}	3.72±0.01 ^{e11}	1.62±0.03 ^{e11}
BF-50	21.72±0.12 ^{f13}	3.60±0.02 ^{f12}	1.53±0.04 ^{f12}
BF-100	19.66±0.36 ^{f16}	-	-
KF-0	30.20±0.19 ^{a1}	4.22±0.08 ^{a7}	2.14±0.06 ^{a4}
KF-10	28.88±0.43 ^{b3}	4.09±0.05 ^{b8}	1.93±0.01 ^{b7}
KF-20	27.02±0.15 ^{c5}	3.91±0.01 ^{c9}	1.78±0.03 ^{c9}
KF-30	25.64±0.25 ^{d7}	3.79±0.04 ^{d10}	1.65±0.04 ^{d11}
KF-40	23.12±0.17 ^{e9}	3.58±0.02 ^{e12}	1.57±0.05 ^{e12}
KF-50	22.40±0.21 ^{f11}	3.46±0.05 ^{f13}	1.49±0.03 ^{f13}
KF-100	20.32±0.27 ^{g15}	-	-

Means followed by the same superscript lowercase letter within a column for a given variety, and the same superscript number within a column across varieties, are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

5.3.2.2. Solubility

Attributed to the reduced amylose leaching behavior of CF in the composite flour samples, solubility also declined. It was highly prominent in BF-50 and KF-50, likely due to the combined effects of high AAC and chemical crosslinking, which together produce a compact and reinforced granular structure resistant to thermal and solvent-induced disruption. (Table 5.3). Comparable findings were previously reported for crosslinked purple sweet potato starch (Bodjrenou et al., 2023) and cassava starch (Khadsai et al., 2024) and barnyard millet starch (Bhatt et al., 2025). This was attributed to the formation of hydrophobic ester linkages and a decrease in the availability of hydrophilic hydroxyl groups, together inhibiting chain disentanglement and reduced

starch-water interactions. The synergistic effect of high amylose content and crosslinking thus yielded a structurally rigid starch matrix characterized by both low swelling and low solubility. This trend was also evident in native samples, where KF-0 consistently showed lower solubility than AF-0 and BF-0, which was in line with their amylose contents.

5.3.4. Gel color

A progressive decrease in L^* values was observed with increasing levels of CF substitution across all varieties ($p \geq 0.05$) (Table 2). Clear drop in L^* and highly significant increase in ΔE values to more 7.0 in the three 50% substituted flours supported the visible color differences, that were well above the perceptibility threshold value of 3.0 (Francis and Clydesdale, 1975; Aghajanzadeh et al., 2023). This darkening was due to enhanced anthocyanin retention, facilitated by covalent phosphate bridges formed due to starch crosslinking. Crosslinking preserves pigment integrity and enhance light absorption and scattering through increased matrix density and granular rigidity (Bagheri et al., 2021). The reinforced starch matrices reduced pigment leaching and thermal degradation by encapsulating anthocyanins within a more rigid structure. Positive redness (a^*) values increased with CF substitution. Conversely, yellowness (b^*) decreased in each rice flour type. These indicated a perceptible color shift toward from yellowish-red to more reddish-brown tones and deeper red hues. This pattern was consistent with anthocyanin stabilization. This chromatic transition may also be influenced by the partial breakdown and polymerization of flavonoids during thermal processing. Furthermore, phenolic-starch interaction, particularly with starch-lipid complexes may also enhance matrix density and opacity, contributing to reduced L^* values in retrograded gels (Sandhu et al., 2022; Sharma et al., 2021). The presence of added sugar (10%) in the gel formulation could have likely contributed to pigment stabilization by creating a protective microenvironment that shields pigments from oxidative and thermal degradation (El hosry et al., 2025).

5.3.5. Syneresis

STMP crosslinking establishes covalent phosphate diester bridges, which reinforce the internal starch granule structure, reducing vulnerability to ice-induced damage and phase separation during freeze-thaw cycles (Nakkala et al., 2022). Syneresis values decreased significantly from non-substituted flour gels (AG-0, BG-0, KG-0) to those with 50% CF substitution (AG-50, BG-50, KG-50) (Table 5.4). Kenkowa rice gels exhibited the lowest syneresis, consistent with its higher amylose content, which enhances gel strength and water retention. Bagheri et al. (2018) demonstrated that high-amylose starches undergo greater retrogradation upon cooling, forming double-helical crystalline regions that effectively trap water and limit syneresis. In refrigerated conditions, amylose molecules reassociate to form a thermally stable, semi-crystalline network. The incorporation of CF further strengthened this network through restricted granule swelling and increased structural rigidity, resulting in a synergistic barrier to water release. Notably, at higher substitution levels, BG and KG formulations demonstrated superior freeze-thaw resistance, with syneresis consistently below 5%. The introduction of phosphate crosslinks generated steric hindrance and densified the starch matrix, effectively restricting water mobility and enhancing system stability under freeze-thaw conditions (Xiaofan et al., 2024; Agvaandorj et al., 2025). Additionally, the consistent inclusion of 10% sucrose also uniformly functioned as cryoprotectant by lowering the freezing point and stabilizing the starch matrix by forming hydrogen bonding with starch chains. This mitigated ice crystal formation and prevented network disruption (Arunyanart and Charoenrein, 2008; Guo et al., 2025).

Table 5.4. Color and syneresis values of the developed dessert gels

Samples	Color values				Syneresis (%)
	L*	a*	b*	ΔE	
AG-0	55.81±0.22 ^{a6}	10.26±0.12 ^{f13}	9.82±0.09 ^{a1}	-	7.83±0.16 ^{a1}
AG-10	54.40±0.39 ^{b7}	10.58±0.11 ^{e10,11}	9.60±0.11 ^{b3}	1.49±0.23 ^{e6}	7.56±0.11 ^{b2}
AG-20	53.10±0.33 ^{c9}	10.84±0.04 ^{d9}	9.31±0.09 ^{c5}	2.91±0.13 ^{d5}	6.92±0.21 ^{c3}
AG-30	51.76±0.88 ^{d10}	11.12±0.01 ^{c7}	9.04±0.02 ^{d7}	4.33±0.15 ^{c6}	5.87±0.19 ^{d5}
AG-40	50.40±0.17 ^{e11}	11.38±0.05 ^{b5}	8.92±0.07 ^{e8}	5.76±0.22 ^{b5}	5.09±0.08 ^{e6}
AG-50	49.26±0.24 ^{f12}	11.66±0.02 ^{a3}	8.56±0.06 ^{f10}	7.10±0.10 ^{a3}	4.27±0.12 ^{f8}
BG-0	58.95±0.12 ^{a2}	10.85±0.05 ^{f9}	9.67±0.10 ^{a2}	-	6.79±0.09 ^{a3}
BG-10	57.40±0.27 ^{b4}	10.99±0.11 ^{e8}	9.42±0.07 ^{b4}	1.57±0.08 ^{e6}	6.52±0.07 ^{b4}
BG-20	54.07±0.53 ^{c7}	11.29±0.06 ^{d6}	9.21±0.04 ^{c6}	2.94±0.17 ^{d5}	5.77±0.12 ^{c5}
BG-30	50.44±0.19 ^{d11}	11.48±0.04 ^{c4}	8.97±0.07 ^{d8}	4.68±0.34 ^{c6}	4.69±0.15 ^{d7}
BG-40	48.55±0.21 ^{e13}	11.72±0.01 ^{b2}	8.78±0.05 ^{e9}	6.39±0.26 ^{b4}	4.12±0.08 ^{e9}
BG-50	46.76±0.13 ^{f14}	11.88±0.03 ^{a1}	8.51±0.02 ^{f10}	7.49±0.19 ^{a1}	3.20±0.14 ^{f10}
KG-0	60.89±0.55 ^{a1}	9.66±0.06 ^{f15}	9.62±0.14 ^{a3}	-	6.65±0.17 ^{a4}
KG-10	58.76±0.12 ^{b3}	9.87±0.09 ^{e14}	9.39±0.11 ^{b4,5}	1.21±0.22 ^{e7}	6.49±0.13 ^{b4}
KG-20	56.32±0.32 ^{c5}	10.26±0.07 ^{d13}	9.18±0.06 ^{c6}	2.71±0.44 ^{d5}	5.71±0.07 ^{c5}
KG-30	55.89±0.45 ^{d6}	10.38±0.08 ^{c12}	9.07±0.08 ^{d7}	4.87±0.29 ^{c6}	4.60±0.16 ^{d7}
KG-40	53.62±0.10 ^{e8}	10.46±0.12 ^{b11}	8.94±0.05 ^{e8}	6.36±0.09 ^{b4}	4.09±0.11 ^{e9}
KG-50	51.80±0.11 ^{f10}	10.69±0.17 ^{a10}	8.79±0.07 ^{f9}	7.21±0.17 ^{a2}	3.02±0.09 ^{f10}

Means followed by the same superscript lowercase letter within a column for a given variety, and the same superscript number within a column across varieties, are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

5.3.6. Texture

With increasing levels of CF substitution, hardness, adhesiveness, cohesiveness, springiness and chewiness of gels significantly increased. This reflected the overall elasticity and energy-retaining capacity of the crosslinked networks in both amorphous and crystalline starch regions stabilized by phosphate ester bridges, reducing fragmentation during cooling and storage (Table 5.5). Hardness values increased from 2.10 N to 2.95N with increasing CF substitution in *Amona*, 2.45N to 3.22N in *Badal* and 2.52 to 3.23N in *Kenkova* gels respectively. Adhesiveness increased by 50%, cohesiveness by 20% and chewiness by 65% in all the varieties. Amylose retrogradation during refrigerated storage creates crystalline zones that act like elastic springs, allowing the gel to recover shape after deformation (Chakraborty et al., 2023). Crosslinked starch chains add resilience, promoting a bouncier texture. This behavior aligns with the dynamic mechanical analyses of storage-stable starch-based gels, where springiness

positively correlates with gel network reinforcement (Bangar et al., 2024, Bodjrenou et al., 2023). Cohesiveness was significantly improved owing to denser and more elastic networks. Added sugar can additionally intensify the gel network strength by promoting interactions between starch chains and sucrose, reducing water availability and enhancing the elastic modulus (Lopez-Sanchez et al., 2018). High cohesiveness is desirable in ready-to-eat gels, contributing to better mouthfeel and minimal crumbling.

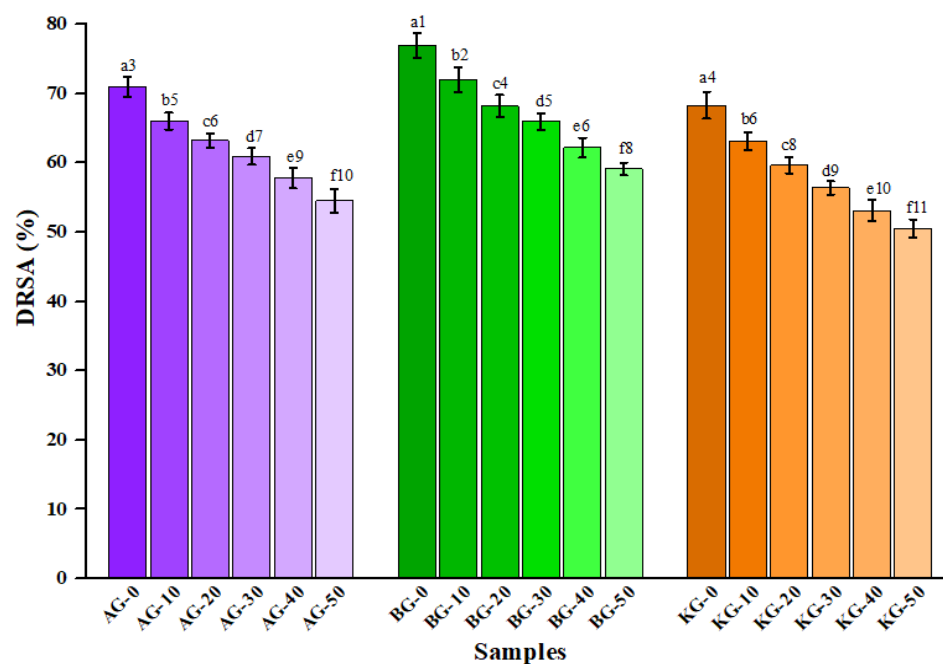
Table 5.5. Textural properties of the developed dessert gels

Samples	Hardness (N)	Adhesiveness (N-s)	Cohesiveness	Springiness	Chewiness (N-mm)
AG-0	2.10±0.008 ^{fl4}	-0.35±0.009 ^{a1}	0.57±0.011 ^{fl2}	0.81±0.003 ^{fl1}	1.14±0.007 ^{fl8}
AG-10	2.31±0.010 ^{e13}	-0.41±0.017 ^{b3}	0.61±0.013 ^{e11}	0.85±0.012 ^{e10}	1.29±0.012 ^{e17}
AG-20	2.54±0.006 ^{d11}	-0.43±0.012 ^{c4}	0.63±0.004 ^{d9}	0.90±0.010 ^{d8,9}	1.38±0.006 ^{d16}
AG-30	2.72±0.014 ^{e9}	-0.47±0.004 ^{d7}	0.65±0.003 ^{c7}	0.92±0.009 ^{c7}	1.54±0.005 ^{c13}
AG-40	2.89±0.015 ^{b7}	-0.51±0.006 ^{e8}	0.68±0.007 ^{b6}	0.93±0.007 ^{b6}	1.67±0.004 ^{b10}
AG-50	2.95±0.010 ^{a6}	-0.56±0.010 ^{fl0}	0.73±0.004 ^{a5}	0.95±0.004 ^{a4}	1.81±0.009 ^{a8}
BG-0	2.45±0.018 ^{fl2}	-0.39±0.010 ^{a2}	0.62±0.005 ^{fl0}	0.89±0.013 ^{fl9}	1.42±0.013 ^{fl5}
BG-10	2.64±0.013 ^{e10}	-0.42±0.005 ^{b3,4}	0.65±0.007 ^{e7}	0.91±0.011 ^{e7,8}	1.63±0.004 ^{e12}
BG-20	2.85±0.005 ^{d8}	-0.45±0.004 ^{c5}	0.68±0.006 ^{d6}	0.94±0.006 ^{d5}	1.79±0.005 ^{d9}
BG-30	3.09±0.017 ^{c5}	-0.46±0.007 ^{d6}	0.72±0.007 ^{e5}	0.96±0.004 ^{c3}	1.99±0.010 ^{c6}
BG-40	3.17±0.016 ^{b4}	-0.53±0.003 ^{e9}	0.74±0.014 ^{b4}	0.97±0.007 ^{b2}	2.10±0.003 ^{b4}
BG-50	3.22±0.017 ^{a2}	-0.58±0.018 ^{fl1}	0.78±0.003 ^{a2}	0.98±0.003 ^{a1}	2.22±0.005 ^{a2}
KG-0	2.52±0.011 ^{fl1}	-0.42±0.012 ^{a3,4}	0.64±0.005 ^{fl8}	0.90±0.006 ^{fl8}	1.47±0.018 ^{fl4}
KG-10	2.72±0.013 ^{e10}	-0.46±0.007 ^{b6}	0.66±0.012 ^{e7}	0.91±0.013 ^{e7}	1.66±0.006 ^{e11}
KG-20	2.96±0.010 ^{d6}	-0.50±0.016 ^{c8}	0.68±0.005 ^{d6}	0.93±0.004 ^{d6}	1.89±0.005 ^{d7}
KG-30	3.10±0.006 ^{c5}	-0.55±0.005 ^{d10}	0.73±0.010 ^{c4}	0.94±0.003 ^{c5}	2.07±0.007 ^{c5}
KG-40	3.19±0.006 ^{b3}	-0.58±0.004 ^{e11}	0.75±0.008 ^{b3}	0.95±0.002 ^{b4}	2.15±0.009 ^{b3}
KG-50	3.25±0.007 ^{a1}	-0.62±0.010 ^{fl2}	0.80±0.009 ^{a1}	0.97±0.003 ^{a2}	2.29±0.003 ^{a1}

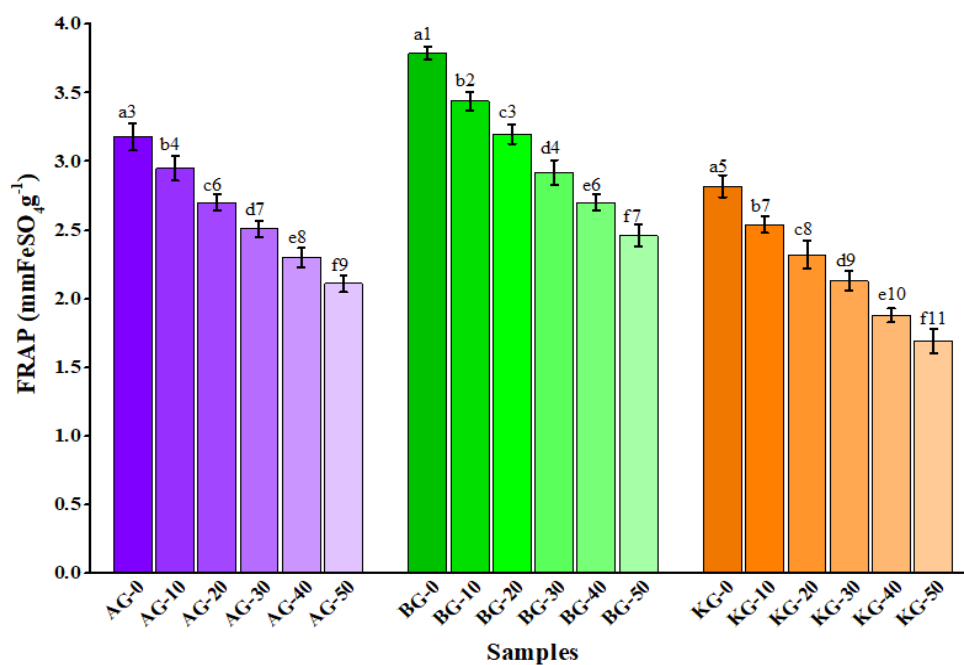
Means followed by the same superscript lowercase letter within a column for a given variety, and the same superscript number within a column across varieties, are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

5.3.7. Antioxidant activity

Progressive decline in DRSA and FRAP were recorded in the gels of each *bae* rice (Fig 2). Thermal processes and pH changes during processing causes degradation of bioactive anthocyanins, often also leading to color changes by forming inactive chalcone or polymerized forms (Zhang et al., 2023a). Processes involving high moisture, like STMP modification, also causes anthocyanin leaching promoting oxidation. Up to 20% decrease in DRSA was found in CF substituted samples. Decrease in FRAP amounting up to 25% in *Amona* gels, 30% in *Badal* gels and 35% in *Kenkova* gels were observed.



a



b

Fig. 5.2. (a) DRSA and (b) FRAP of the dessert gels

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within variety and same numbers across varieties are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

Apart from esterification of hydroxyl moieties present in rice starch granules, STMP-mediated phosphate crosslinking under alkaline conditions also esterifies anthocyanins and other phenolic compounds, masking of their active sites. This reduces the number of free functional groups available for radical scavenging (Xue et al., 2024). Moreover, structurally dense modified starch-phosphate network matrix can encapsulate polyphenolic compounds, reducing their extractability for antioxidant assays (Yildiz et al., 2022; Khadsai et al., 2024). Apart from esterification of hydroxyl moieties present in rice starch granules, STMP-mediated phosphate crosslinking under alkaline conditions also esterifies anthocyanins and other phenolic compounds, masking of their active sites. This reduces the number of free functional groups available for radical scavenging (Xue et al., 2024). Moreover, structurally dense modified starch-phosphate network matrix can encapsulate polyphenolic compounds, reducing their extractability for antioxidant assays (Yildiz et al., 2022; Khadsai et al., 2024). This change is more prominent in high amylose processed starches forming semi-crystalline zones that can entrap phenolic compounds more effectively. While this enhances structural integrity and gel firmness, it indirectly limits the diffusion and accessibility of antioxidants during DRSA and FRAP assays due to increased matrix rigidity as evidenced by higher reduction in FRAP values in *Kenkowa* and *Badal* gels compared to *Amona* gels which also showed lower syneresis and better textural profile. Yildiz et al. (2022) attributed the loss in antioxidant potential of curcumin in citrate-crosslinked starch films to interactions between carboxyl groups of citric acid and the active sites of curcumin, as well as the hydroxyl and amino groups of chitosan, leading to masking of the antioxidant-active sites. Khadsai et al. (2024) emphasized that increased hydrogen bonding between citric acid and cassava starch and participation of curcumin's active sites in covalent bonding with citric acid diminished the antioxidant effectiveness. Although STMP crosslinking resulted in a 20-35% reduction in antioxidant activity, the dessert gels still retained appreciable levels of antioxidant functionality, with DRSA remaining at ~50-60% and FRAP values between 2.0 and 2.9 mmol FeSO₄/g. These values fall within the range typically reported for antioxidant-rich pigmented rice

products, indicating that the gels continue to provide meaningful dietary antioxidant activity despite the expected losses associated with chemical modification (Cañizares et al., 2024; Das et al., 2023). Such reductions are commonly observed in modified cereal matrices and largely reflect a decrease in extractable phenolic fractions rather than a complete loss of functionality. Antioxidant retention can be further improved through simple formulation refinements such as addition of antioxidant rich cereal flours like millets and controlled processing adjustments.

5.3.8. Rheological properties

5.3.8.1. Steady shear

The steady shear properties of the CF-substituted dessert gels exhibited a distinct shear-thinning behavior, indicative of pronounced pseudoplasticity, with all samples showing reductions in apparent viscosity as shear rate increased (Fig. 5.3). All formulations showed non-Newtonian flow behavior ($n < 1$). The Herschel-Bulkley model adequately described the flow behavior, with $R^2 > 0.97$. η_{50} , K and τ_0 increased for the samples with greater levels of CF substitution, while n decreased progressively showing similar trend for each rice (Table 5.6). This could be attributed to the formation of phosphate crosslinks introduced by STMP, that reduced granule swelling by enhancing molecular entanglements, resulting in elevated low-shear viscosity. These structured networks gradually break down upon prolonged shearing, exhibiting pseudoplastic flow and shear thinning behavior. Zhang et al. (2023b) reported parallel observations for physically modified heat-moisture treated rice starch-hydrocolloid complexes. Kumar et al. (2023) demonstrated that crosslinking buckwheat starch with citric acid and STMP resulted in gels characterized by shear-thinning flow viscoelastic dominance. Gels formulated with *Kenkwa* and *Badal* rice flours gave higher values of η_{50} , K and τ_0 , confirming a stronger shear-thinning response than the *Amona* rice samples. The concurrent increase in τ_0 , a critical parameter influencing gel quality and sensory perception, suggested the formation of firmer, more cohesive, and stable gel structures with increasing CF substitution.

Table 5.6. Apparent viscosity and Herschel Bulkley model parameters of the dessert gels

Samples	Herschel Bulkley model parameters				
	η_{50} (Pa.s)	K (Pa.s)	n	C_0 (Pa)	R ²
AG-0	0.83±0.002 ^{a18}	7.64±1.39 ^{a15}	0.72±0.001 ^{f1}	16.52±4.25 ^{a17}	0.99
AG-10	3.84±0.002 ^{b15}	16.09±0.56 ^{b12}	0.64±0.00 ^{e4}	110.31±2.15 ^{b14}	0.991
AG-20	5.23±0.005 ^{c12}	28.65±1.01 ^{c10}	0.56±0.001 ^{d7}	212.53±3.39 ^{c11}	0.991
AG-30	6.74±0.006 ^{d9}	36.42±2.17 ^{d8}	0.48±0.003 ^{c8}	313.32±2.09 ^{d8}	0.988
AG-40	9.59±0.004 ^{e6}	44.81±0.79 ^{e7}	0.39±0.00 ^{b11}	366.64±14.35 ^{e7}	0.996
AG-50	10.91±0.005 ^{f4}	50.09±0.39 ^{f5}	0.27±0.002 ^{a15}	433.66±5.70 ^{f4}	0.992
BG-0	1.11±0.004 ^{a17}	12.11±2.36 ^{a13}	0.67±0.001 ^{f3}	30.76±1.05 ^{a16}	0.991
BG-10	4.58±0.002 ^{b14}	27.32±0.62 ^{b11}	0.55±0.002 ^{e6}	146.41±2.25 ^{b13}	0.992
BG-20	6.29±0.003 ^{c11}	36.53±0.73 ^{c8}	0.46±0.001 ^{d9}	265.46±2.19 ^{c10}	0.994
BG-30	8.56±0.005 ^{d8}	47.23±2.05 ^{d6}	0.37±0.001 ^{c12}	404.64±3.39 ^{d6}	0.992
BG-40	10.72±0.006 ^{e5}	52.77±1.03 ^{e4}	0.29±0.001 ^{b14}	424.86±10.56 ^{e4}	0.991
BG-50	12.58±0.003 ^{f2}	64.23±1.28 ^{f2}	0.20±0.001 ^{a17}	518.74±5.82 ^{f2}	0.993
KG-0	1.26±0.005 ^{a16}	11.88±0.56 ^{a14}	0.68±0.002 ^{f2}	38.85±2.90 ^{a15}	0.994
KG-10	4.82±0.003 ^{b13}	32.72±1.23 ^{b9}	0.56±0.001 ^{e5}	157.34±3.34 ^{b12}	0.991
KG-20	6.48±0.003 ^{c10}	45.71±0.79 ^{c6,7}	0.48±0.001 ^{d8}	274.56±3.28 ^{c9}	0.992
KG-30	8.92±0.006 ^{d7}	51.30±2.36 ^{d4,5}	0.42±0.003 ^{c10}	422.58±5.70 ^{d5}	0.991
KG-40	11.85±0.004 ^{e3}	62.49±2.17 ^{e3}	0.33±0.001 ^{b13}	477.85±9.56 ^{e3}	0.995
KG-50	13.43±0.004 ^{f1}	65.70±0.55 ^{f1}	0.25±0.001 ^{a16}	556.55±3.28 ^{f1}	0.991

Means followed by the same superscript lowercase letter within a column for a given variety, and the same superscript number within a column across varieties, are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

Here, η_{50} : apparent viscosity, K: consistency index, n: flow behavior index, C_0 : yield stress

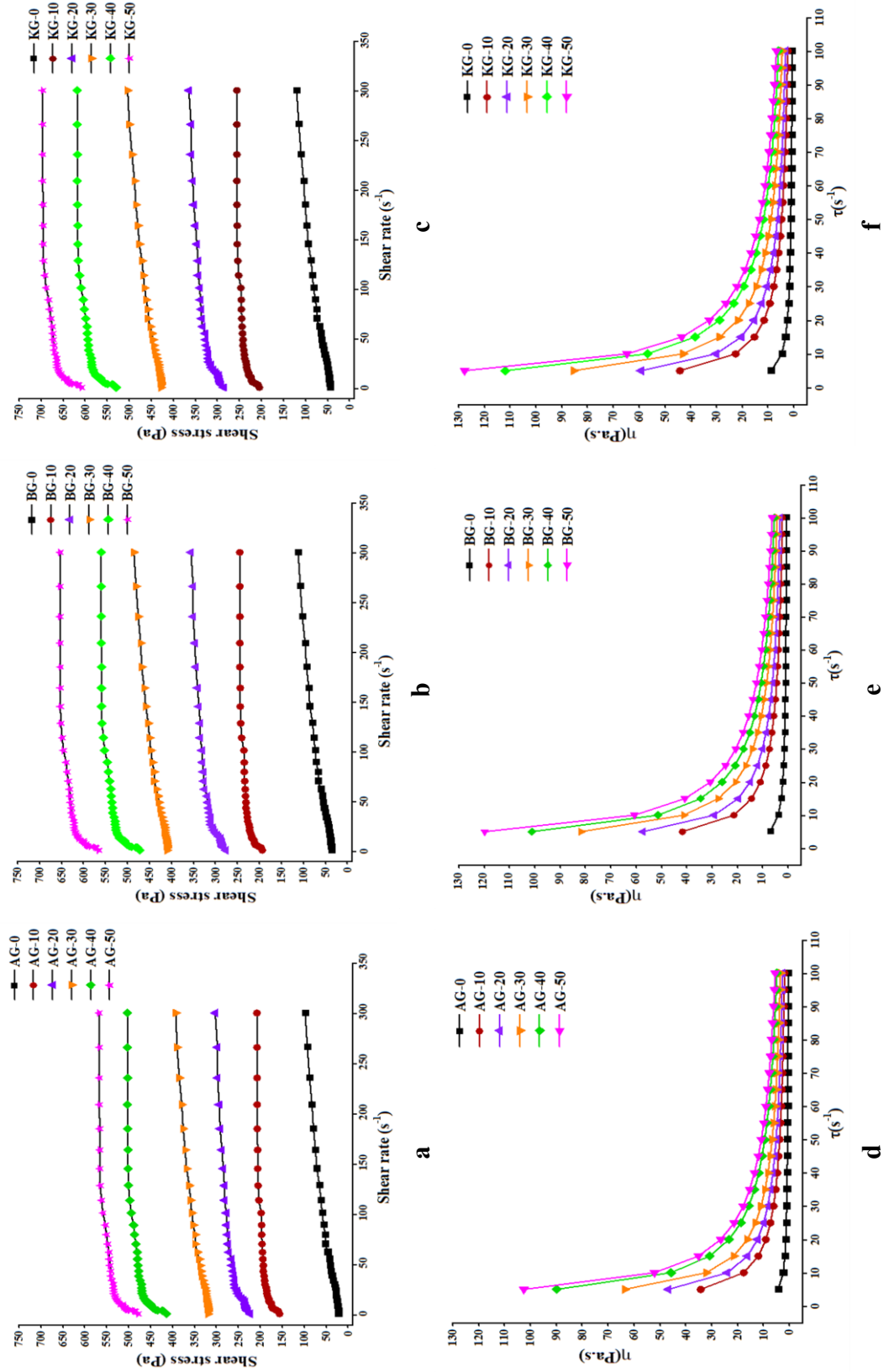


Fig. 5.3. Plots of (a-c) shear rate vs shear stress and (d-f) shear rate vs apparent viscosity of the *Amona*, *Badal* and *Kenkoba bao* dessert gels

5.3.8.2. *Dynamic shear*

The dynamic rheological properties of the gels, namely storage modulus (G') and loss modulus (G''), as a function of frequency (ω), are presented in Fig 5.4, 5.5 and 5.6. Throughout the frequency range across all samples, G' values consistently exceeding G'' and $\tan \delta < 1$ indicated dominant elastic behavior and confirmed the formation of a true gel network (Fig. 5.7). Both moduli increased with rising CF substitution, suggestive of a more structured and cohesive internal matrix development. Rigid phosphate crosslinks between starch chains restricts molecular slippage and enhances network strength, thereby improving energy storage capacity under oscillatory stress (Bodjrenou et al., 2023). With increasing frequency, G' showed a steeper rise than G'' , indicating that more of the applied energy was stored in the gel's elastic structure rather than lost as viscous flow. This showed greater resistance to deformation and improved structural stability. Temperature ramp tests further supported these findings by showing an upward shift in the G'/G'' crossover temperature, implying enhanced thermal stability. STMP crosslinking delayed the crossover point by stabilizing amylose chains against thermal breakdown. No crossover point between G' and G'' was observed in any sample across the tested frequency range, reaffirming the predominance of elastic behavior irrespective of CF substitution levels.

The observed increase in viscoelastic strength could be attributed to enhanced water-binding and holding capacity of the crosslinked starch matrix. As CF substitution increased, so did molecular entanglement and internal bonding density, improving the gel's ability to resist structural breakdown under dynamic shear. These observations were further supported by increases in η^* , and G^* , and decreases in $\tan \delta$ values, collectively representing a transformation from viscous-dominant form to a more solid-like gel system with enhancing substitution levels (Fig. 5.7). The decreasing trend in $\tan \delta$ values for higher CF levels and the corresponding increase in G^* reflected gradual increases in the rigid structure and improved gel performance under stress. This was likely due to the reinforcement of the gel network through phosphate crosslinking, which

restricts molecular mobility and increases the energy storage capacity (G') relative to energy dissipation (G'') (Fig 5.4, 5.5, 5.6). In a similar study, Siroha et al. (2019) reported $\tan \delta$ values below 1 in acetylated pearl millet starch, suggesting a predominantly elastic (solid-like) behavior of the gel matrix. Similar observations for buckwheat starch crosslinked with citric acid and STMP was also reported (Kumar et al., 2023)

The relationship between G' and G'' was modeled using the power law. The results are summarized in Table 5.7. Positive values of the power-law exponent (n) across all formulations confirmed the characteristics of strong gel. Furthermore, the observed increase in the consistency coefficients K' and K'' with CF substitution suggested a rise in both the number and strength of the three-dimensional network bonds formed within the gel matrix.

Table 5.7. Parameters of power law functions describing storage modulus and loss modulus of the dessert gels

Samples	Power law parameters					
	$G'=K'(\omega)^{n'}$			$G''=K''(\omega)^{n''}$		
	K' (Pa)	n'	R^2	K'' (Pa)	n''	R^2
AG-0	3.601±0.003 ^{f18}	0.013±3.85×10 ⁻⁴ a1	0.991	2.230±0.005 ^{f18}	0.029±9.11×10 ⁻⁴ a1	0.990
AG-10	4.272±0.003 ^{e16}	0.011±3.27×10 ⁻⁴ b3	0.992	2.623±0.005 ^{e16}	0.024±7.32×10 ⁻⁴ b2	0.990
AG-20	4.924±0.003 ^{d13}	0.009±2.86×10 ⁻⁴ c5	0.992	2.925±0.005 ^{d14}	0.021±6.27×10 ⁻⁴ c3	0.995
AG-30	5.409±0.003 ^{c12}	0.009±2.62×10 ⁻⁴ d5	0.997	3.203±0.005 ^{c11}	0.019±6.14×10 ⁻⁴ d5	0.993
AG-40	5.973±0.003 ^{b9}	0.008±2.37×10 ⁻⁴ e6	0.995	3.506±0.004 ^{b10}	0.018±5.60×10 ⁻⁴ e6	0.997
AG-50	6.769±0.003 ^{a6}	0.007±2.12×10 ⁻⁴ f7	0.991	3.902±0.006 ^{a7}	0.017±5.26×10 ⁻⁴ f7	0.991
BG-0	3.864±0.003 ^{f17}	0.012±2.73×10 ⁻⁴ a2	0.996	2.668±0.003 ^{f17}	0.024±4.89×10 ⁻⁴ a2	0.995
BG-10	4.598±0.003 ^{e15}	0.010±2.69×10 ⁻⁴ b4	0.995	3.128±0.003 ^{e15}	0.020±4.87×10 ⁻⁴ b4	0.993
BG-20	5.435±0.003 ^{d11}	0.008±2.60×10 ⁻⁴ c6	0.997	3.440±0.003 ^{d12}	0.018±4.01×10 ⁻⁴ c6	0.997
BG-30	6.265±0.003 ^{c8}	0.007±2.27×10 ⁻⁴ d7	0.992	3.908±0.003 ^{c9}	0.016±3.63×10 ⁻⁴ d8	0.996
BG-40	7.136±0.003 ^{b4}	0.006±2.00×10 ⁻⁴ e8	0.998	4.361±0.005 ^{b6}	0.015±3.99×10 ⁻⁴ e9	0.998
BG-50	7.547±0.003 ^{a2}	0.006±1.89×10 ⁻⁴ e8	0.992	4.661±0.008 ^{a4}	0.014±4.99×10 ⁻⁴ f10	0.997
KG-0	4.781±0.003 ^{f14}	0.009±2.45×10 ⁻⁴ a5	0.992	3.362±0.004 ^{f13}	0.019±4.66×10 ⁻⁴ a5	0.992
KG-10	5.697±0.003 ^{e10}	0.008±2.34×10 ⁻⁴ b6	0.991	4.004±0.004 ^{e8}	0.016±4.52×10 ⁻⁴ b8	0.993
KG-20	6.390±0.003 ^{d7}	0.007±2.23×10 ⁻⁴ c7	0.996	4.593±0.004 ^{d5}	0.015±4.11×10 ⁻⁴ c9	0.992
KG-30	6.796±0.003 ^{c5}	0.006±2.10×10 ⁻⁴ d8	0.994	4.758±0.004 ^{c3}	0.014±3.50×10 ⁻⁴ d10	0.995
KG-40	7.347±0.003 ^{b3}	0.006±1.94×10 ⁻⁴ d8	0.992	4.956±0.004 ^{b2}	0.013±3.40×10 ⁻⁴ e 11	0.991
KG-50	7.847±0.003 ^{a1}	0.005±1.79×10 ⁻⁴ e9	0.994	5.332±0.005 ^{a1}	0.012±4.07×10 ⁻⁴ f12	0.996

Means followed by the same superscript lowercase letter within a column for a given variety, and the same superscript number within a column across varieties, are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

Here, G' : storage modulus, G'' : loss modulus, K : slope, n : y -intercept

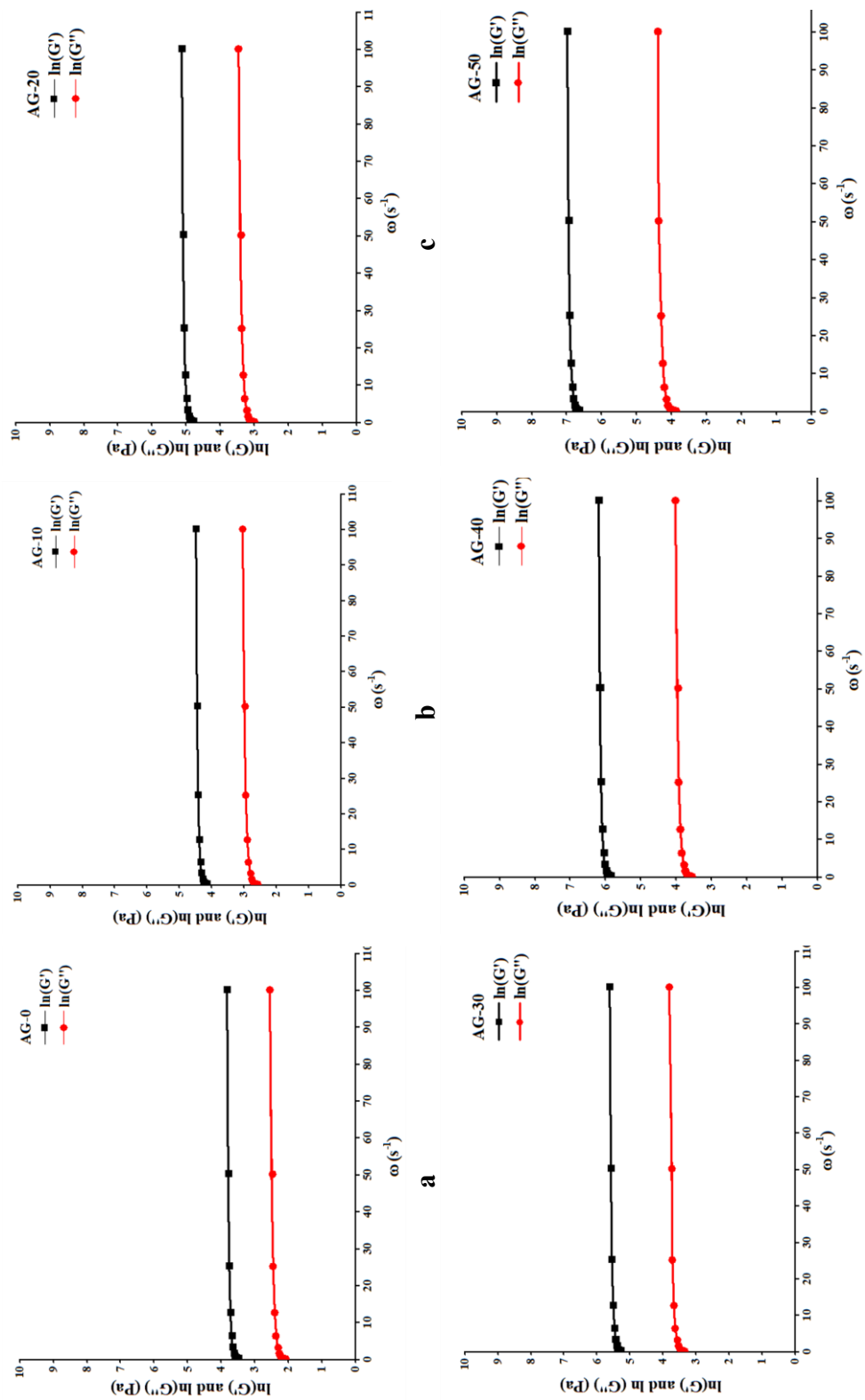


Fig. 5.4. Plots of (a-f) storage (G') and loss (G'') modulus as a function of frequency for the *Amona bao* dessert gels developed using 0-50% CF

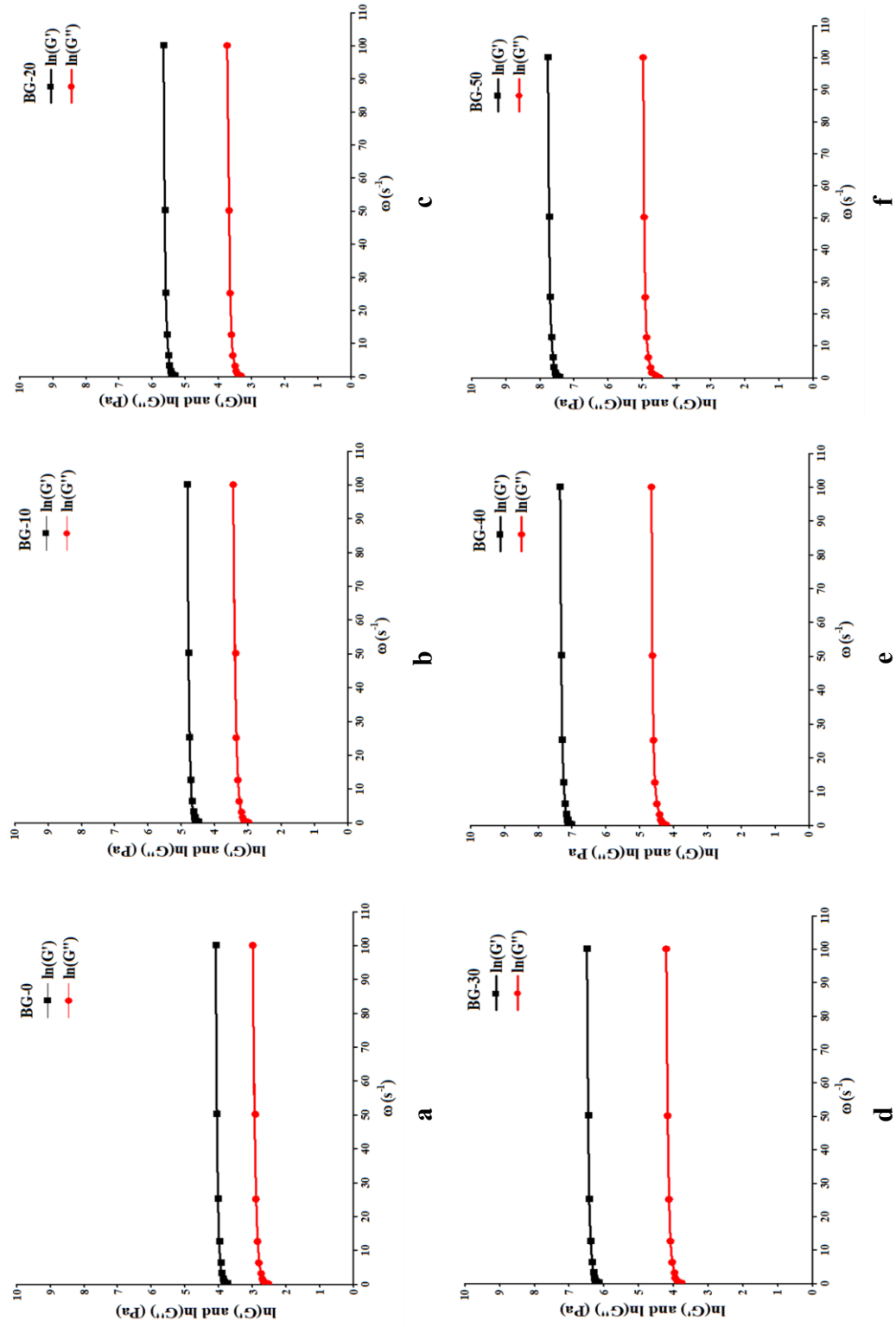


Fig. 5.5. Plots of (a-f) storage (G') and loss (G'') modulus as a function of frequency for the *Badao bao* dessert gels developed using 0-50% CF

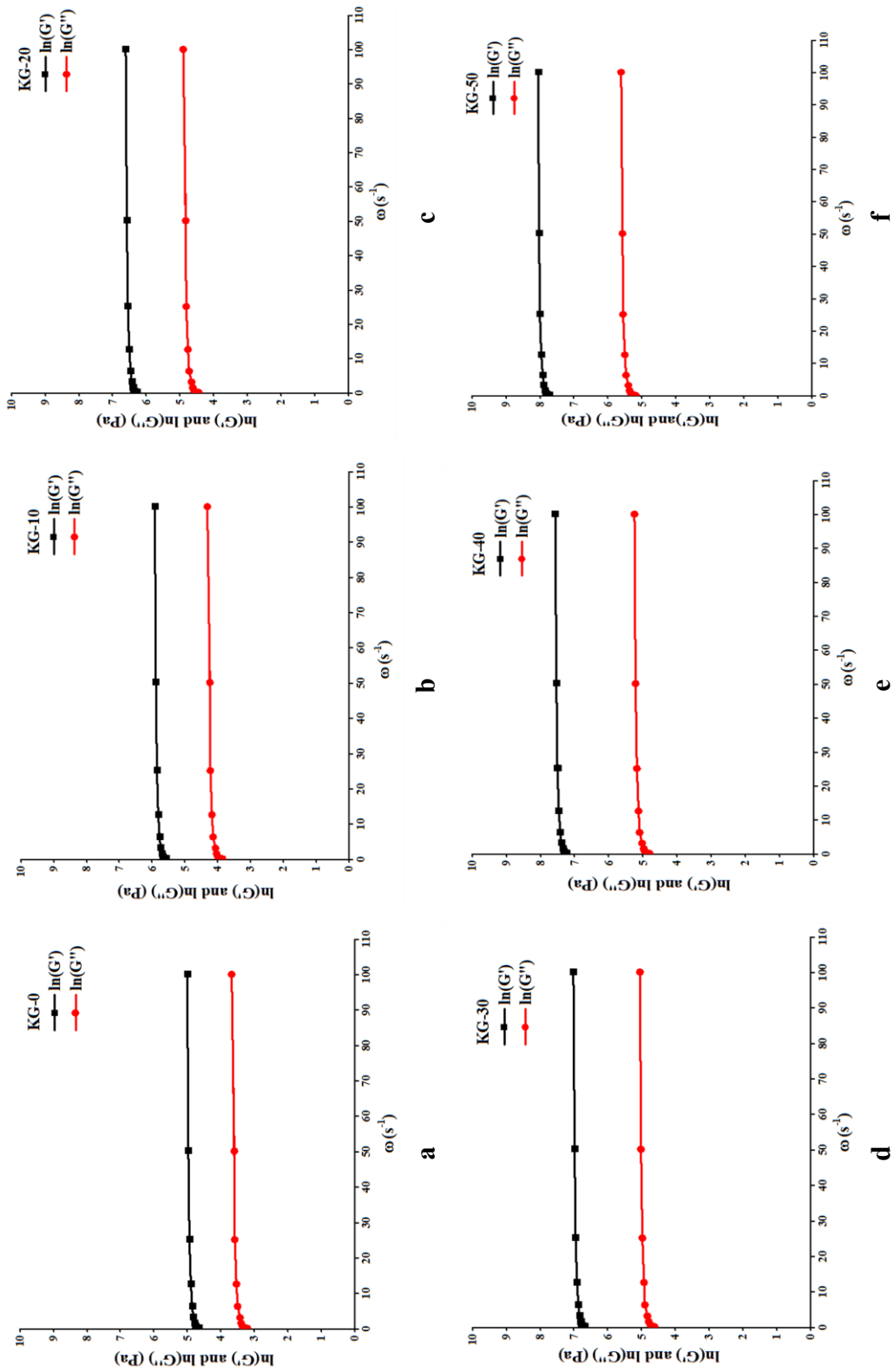


Fig. 5.6. Plots of (a-f) storage (G') and loss (G'') modulus as a function of frequency for the *Kenkowa bao* dessert gels developed using 0-50% CF

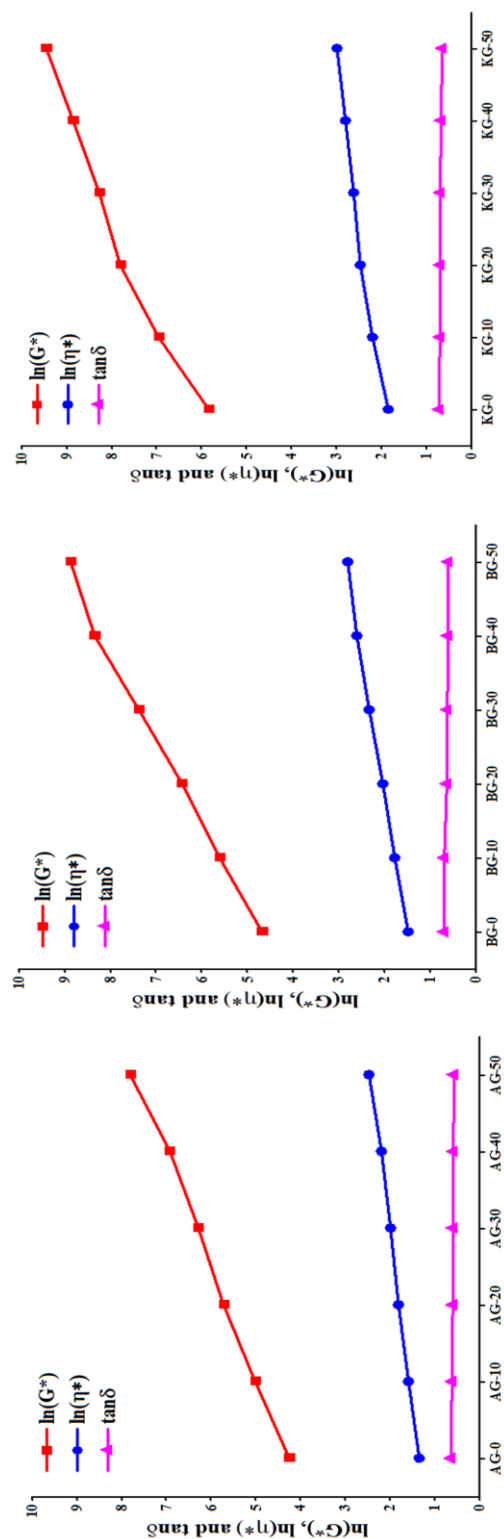


Fig. 5.7. Plots showing variation in complex modulus (G^*), complex viscosity (η^*) and loss tangent ($\tan\delta$) as a function of substitution % of CF for (a) *Amona*, (b) *Badal* and (c) *Kenkoba baos* dessert gels

Both the steady shear and dynamic rheology results showed patterns that were consistent with the textural properties of the gels. In steady-state measurements, the increases in η_{50} , K and τ_0 with higher CF substitution indicated a more structured and shear-resistant system. Gels with higher apparent viscosity and yield stress require greater force to deform, which directly corresponded to the increased hardness and chewiness observed in the texture profile analysis (Kumar et al., 2023). Dynamic rheology further supported these observations. The rise in G' and G'' at higher substitution levels reflected the development of a stronger, more elastic gel network. A higher G' explained the increase in hardness, while the steeper $G'-G''$ gap at elevated substitution levels corresponded with the improved cohesiveness of the gels. Importantly, $\tan \delta$ values decreased with increasing CF incorporation, indicating a shift toward elastic-dominant behavior. This aligned well with the higher springiness and cohesiveness measured in TPA, as gels with lower $\tan \delta$ recover more efficiently after deformation. The increase in G'' also matched the rise in adhesiveness, since gels with higher viscous dissipation tend to show greater stickiness during probe withdrawal.

Overall, the combined rheological data demonstrated that CF substitution and phosphate crosslinking enhanced the internal structural stability of the gels. The strengthening of the elastic and viscous components (higher G' , G'' , τ_0 , η_{50} and lower $\tan \delta$) was directly reflected in the improved hardness, adhesiveness, springiness, cohesiveness and chewiness of the dessert gels.

5.3.9. Sensory analysis of the gels using fuzzy logic approach

Based on the responses provided by the 20-member sensory panel, triplets associated with the tested samples and their quality parameters were calculated (Eq. 5.15). These triplets were designated as SC, STa, STex and SM (as can be seen in Table 5.8, 5.9 and 5.10). Similarly, the triplets associated with the quality parameters, namely color, taste, texture and mouthfeel were QC, QTa, QTex and QM (as in Table 5.11).

$$S_x a / Qa = \frac{n_1(0 \ 0 \ 25) + n_2(25 \ 25 \ 25) + n_3(50 \ 25 \ 25) + n_4(75 \ 25 \ 25) + n_5(100 \ 25 \ 0)}{n_1 + n_2 + n_3 + n_4 + n_5} \quad \text{Eq. 5.15}$$

where, S_{xa} : triplets associated with sensory score; x : sample number; a : quality attributes; Qa : triplets associated with different quality attributes in general; n_1 - n_5 : number of judges providing each response.

The overall relative weightages of the sensory parameters (Q_{rel}) were then calculated using Eq 5.16. These values were used to calculate the overall sensory scores (SO_x) of the gels (Eq 5.17). The overall membership function was then calculated from SO_x (Eq. 5.18, Table 5.12) and denoted between B0-B5. Likewise, the membership functions of the quality parameters were calculated from their triplet values and denoted as BC, BTa, BTex and BM.

$$Qa_{rel} = \frac{Qa}{Q_{sum}} \quad \text{Eq. 5.16}$$

where, Qa_{rel} : triplets associated with the relative weightage for each quality attributes; Qa : triplet associated with different quality attributes; Q_{sum} : the sum of the first digit of the triplets of the quality attributes.

$$SO_x = S_xC \times QC_{rel} + S_xTa \times QTa_{rel} + S_xTex \times QTex_{rel} + S_xM \times QM_{rel} \quad \text{Eq. 5.17}$$

where, SO_x : overall sensory scores for each sample; S_xC : triplets of the dessert gels for color; S_xTa : for taste; S_xTex : for texture; S_xM : for mouthfeel; QC_{rel} : for the relative weightage of color; QTa_{rel} : for the relative weightage of taste; $QTex_{rel}$: for the relative weightage of texture; QM_{rel} : for the relative weightage of mouthfeel

Table 5.8. Triplet sensory scores of preferences for different quality attributes of the *Amona* dessert gels

Attributes	Not Satisfactory	Fair	Medium	Good	Excellent	Codes	Triplets of the sensory scores		
Color									
S0	7	6	5	2	0	S0C	27.50	16.25	25
S1	4	7	5	4	0	S1C	36.25	20	25
S2	3	5	8	4	0	S2C	41.25	21.25	25
S3	0	4	5	7	4	S3C	63.75	25	20
S4	0	2	3	8	7	S4C	75	25	16.25
S5	0	3	6	8	3	S5C	63.75	25	21.25
Taste									
S0	8	7	5	0	0	S0Ta	21.25	15	25
S1	3	10	6	1	0	S1Ta	31.25	21.25	25
S2	0	4	5	7	4	S2Ta	63.75	25	20
S3	0	0	2	10	8	S3Ta	82.5	25	15
S4	0	0	2	6	12	S4Ta	87.5	25	10
S5	2	4	7	6	1	S5Ta	50	22.5	23.75
Texture									
S0	8	8	4	0	0	S0Tex	20	15	25
S1	5	7	5	3	0	S1Tex	32.50	18.75	25
S2	0	2	3	8	7	S2Tex	75	25	16.25
S3	0	2	5	8	5	S3Tex	70	25	18.75
S4	0	0	7	7	6	S4Tex	73.75	25	17.5
S5	1	2	5	8	4	S5Tex	65	23.75	20
Mouthfeel									
S0	8	9	3	0	0	S0M	18.75	15	25
S1	5	8	5	2	0	S1M	30	18.75	25
S2	0	3	6	8	3	S2M	63.75	25	21.25
S3	0	1	6	8	5	S3M	71.25	25	18.75
S4	0	0	6	7	7	S4M	76.25	25	16.25
S5	1	2	4	9	4	S5M	62.25	23.75	20

Here, S0-S5: AG-0-AG-50; S0C-S5C: triplets associated with the quality attribute of color for samples; S0Ta-S5Ta: triplets associated with the quality attribute of taste for samples; S0Tex-S5Tex: triplets associated with the quality attribute of texture of samples; S0M-S5M: triplets associated with the quality attribute of mouthfeel for samples

Table 5.9. Triplet sensory scores of preferences for different quality attributes of the *Badal* dessert gels

Attributes	Not Satisfactory	Fair	Medium	Good	Excellent	Codes	Triplets of the sensory scores
Color							
S0	6	7	6	1	0	S0C	27.5 17.5 25
S1	3	8	5	4	0	S1C	37.5 21.25 25
S2	2	5	8	5	0	S2C	45 22.5 25
S3	0	3	6	7	4	S3C	65 25 30
S4	0	1	2	9	8	S4C	80 25 15
S5	0	3	5	7	5	S5C	67.5 25 18.75
Taste							
S0	6	9	4	1	0	S0Ta	25 17.5 25
S1	3	8	8	1	0	S1Ta	33.75 21.25 25
S2	0	5	8	5	2	S2Ta	55 25 22.5
S3	0	0	1	10	9	S3Ta	85 25 13.75
S4	0	0	1	7	12	S4Ta	88.75 25 10
S5	1	2	7	7	3	S5Ta	61.25 23.75 21.25
Texture							
S0	5	7	5	3	0	S0Tex	32.5 18.75 25
S1	3	5	9	3	0	S1Tex	40 21.25 25
S2	0	2	7	7	4	S2Tex	66.25 25 20
S3	0	0	3	7	10	S3Tex	83.75 25 12.5
S4	0	0	2	4	14	S4Tex	90 25 7.5
S5	0	3	4	8	5	S5Tex	68.75 25 18.75
Mouthfeel							
S0	4	7	6	3	0	S0M	35 20 25
S1	3	5	8	4	0	S1M	41.25 21.25 25
S2	0	2	7	6	5	S2M	67.5 25 18.75
S3	0	0	2	7	11	S3M	86.25 25 11.25
S4	0	0	1	6	13	S4M	90 25 8.75
S5	0	2	4	9	5	S5M	71.25 25 18.75

Here, S0-S5: BG-0-BG-50; S0C-S5C: triplets associated with the quality attribute of color for samples; S0Ta-S5Ta: triplets associated with the quality attribute of taste for samples; S0Tex-S5Tex: triplets associated with the quality attribute of texture of samples; S0M-S5M: triplets associated with the quality attribute of mouthfeel for samples

Table 5.10. Triplet sensory scores of preferences for different quality attributes of the *Kenkwa* dessert gels

Attributes	Not Satisfactory	Fair	Medium	Good	Excellent	Codes	Triplets of the sensory scores
Color							
S0	8	6	5	1	0	S0C	27.75 15 25
S1	5	8	5	2	0	S1C	30 18.75 25
S2	4	5	7	4	0	S2C	38.75 20 25
S3	0	4	7	6	3	S3C	60 25 21.5
S4	0	2	3	9	6	S4C	73.75 25 17.5
S5	0	3	5	8	4	S5C	66.25 25 20
Taste							
S0	8	9	3	0	0	S0Ta	18.75 15 25
S1	3	9	6	2	0	S1Ta	33.75 21.25 25
S2	0	7	8	5	0	S2Ta	47.5 25 25
S3	0	0	3	9	8	S3Ta	81.25 25 15
S4	0	0	3	6	11	S4Ta	85 25 11.25
S5	1	4	7	7	1	S5Ta	53.75 23.75 23.75
Texture							
S0	5	8	4	3	0	S0Tex	31.25 18.75 25
S1	3	6	9	2	0	S1Tex	37.5 21.25 25
S2	0	2	8	6	4	S2Tex	65 25 20
S3	0	0	4	8	8	S3Tex	80 25 15
S4	0	0	2	5	13	S4Tex	88.75 25 8.75
S5	0	2	5	8	5	S5Tex	70 25 18.75
Mouthfeel							
S0	4	8	5	3	0	S0M	33.75 20 25
S1	3	6	8	3	0	S1M	38.75 21.25 25
S2	0	2	8	5	5	S2M	66.25 25 18.75
S3	0	0	4	7	9	S3M	81.25 25 13.75
S4	0	0	1	7	12	S4M	88.75 25 10
S5	0	2	4	9	5	S5M	71.25 25 18.75

Here, S0-S5: KG-0-KG-50; S0C-S5C: triplets associated with the quality attribute of color for samples; S0Ta-S5Ta: triplets associated with the quality attribute of taste for samples; S0Tex-S5Tex: triplets associated with the quality attribute of texture of samples; S0M-S5M: triplets associated with the quality attribute of mouthfeel for samples

The similarity values of quality attributes and dessert gel samples calculated using Eq. 5.19 are mentioned in Tables 5.13 and 5.14 respectively. The defuzzified sensory data were then categorized under linguistic adjectives, namely “not satisfactory, fair, medium, good, very good and excellent” in case of the dessert gels and as “not at all necessary, somewhat necessary, necessary, important, highly important and extremely important” in case of the quality attributes.

$$\begin{aligned}
 B_y &= \frac{y - (a - b)}{b}, \text{ for } (a - b) < y < a \\
 &= \frac{(a + c) - y}{c}, \text{ for } a < y < (a + c) \\
 &= 0, \text{ for all other values}
 \end{aligned}
 \tag{Eq.5.18}$$

where, B_y : value of the membership function of different samples; a, b, c: triplets associated with the overall sensory scores; y: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100

$$S_m(F_j B_y) = \frac{F_j \times B'_y}{\text{maximum of } (F_j \times F'_j \text{ and } B_y \times B'_y)}
 \tag{Eq. 5.19}$$

$$F_1 = (1 \ 0.5 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0)$$

$$F_2 = (0.5 \ 1 \ 1 \ 0.5 \ 0 \ 0 \ 0 \ 0 \ 0)$$

$$F_3 = (0 \ 0 \ 0.5 \ 1 \ 1 \ 0.5 \ 0 \ 0 \ 0)$$

$$F_4 = (0 \ 0 \ 0 \ 0 \ 0.5 \ 1 \ 1 \ 0.5 \ 0)$$

$$F_5 = (0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0.5 \ 1 \ 1)$$

$$F_6 = (0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0.5 \ 1)$$

where, S_m : similarity values of the samples and their quality attributes; F_j : $F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8, F_9, F_{10}, F_{11}, F_{12}, F_{13}$ of standard 6-point fuzzy scale; B_x : overall

membership functions for the samples and the quality attributes (Chakraborty et al., 2025).

Similarity values of the quality attributes revealed that mouthfeel was the most critical parameter influencing consumer preference for the dessert gel samples, followed by texture, taste and color. Mouthfeel, texture and taste were rated under the “highly important” category. While mouthfeel received the highest weight among the three, color was categorized as “important” (Table 5.13). Based on these similarity values, the sample S4 (40% CF substitution) was ranked highest (Rank 1), followed by S3 and S2.

Dessert gel prepared with 40% CF substitution of *Amona* flour (AG-40) was categorized as “very good.” In contrast, for the *Badal* and *Kenkowa* varieties, dessert gels prepared with both 40% and 30% substitution (BG-40, BG-30, KG-40, and KG-30) were also placed under the “very good” category. Of these, BG-40 and KG-40 scored highest, indicating superior sensory acceptability. AG-30 and AG-50 were “good,” while AG-20 was ranked “satisfactory.” Both 30% and 50% substituted gels of *Badal* and *Kenkowa* were “good,” with the BG-30 demonstrating higher similarity values. No significant difference was observed in overall sensory perception between the highest-scoring BG-40 and KG-40 gels, suggesting similar consumer experience. However, AG-40, the highest-ranked *Amona* sample, exhibited statistically lower similarity value than BG-40 and KG-40 (Table 5.14). This difference could be attributed to the inherently higher AAC of *Badal* and *Kenkowa* rices. As previously discussed, their gels also demonstrated lower syneresis and superior textural properties, that likely contributed to their superior sensory performances. Irrespective of rice type, the dessert gels with 40% substitution consistently received higher preference scores than 50% substitution. The firmer, rigid texture of the later could be less appealing to the panelists. Instead, the 40% substitution level appeared to offer an optimal balance between structure and palatability (Gupta et al., 2021). This trend was also reflected in its rheological properties. While moderate viscosity is associated with desirable mouthfeel, excessive viscosity at 50% substitution might have negatively impacted the ease of oral manipulation and overall acceptability.

Table 5.11. Total number of judges with different preferences, triplets associated with scores and the relative weightage for the quality attributes of the dessert gels

Quality attributes	Not important	Somewhat important	Important	Highly important	Extremely important	Triplets for sensory scores			Triplets for relative weightage				
Color	0	2	9	9	0	QC	58.75	25	20.25	QC _{rel}	0.18007	0.076628	0.07662
Taste	0	0	0	8	12	QTa	90	25	10	QTa _{rel}	0.27586	0.076628	0.03065
Texture	0	0	1	6	13	QTex	90	25	8.75	QTex _{rel}	0.27586	0.076628	0.02681
Mouthfeel	0	0	1	8	11	QM	87.5	25	11.25	QM _{rel}	0.26819	0.076628	0.03448

QC: triplet for the sensory score of color; QTa: triplet for the sensory score of taste; QTex: triplet for the sensory score of texture; QM: triplet for the sensory score of mouthfeel; QC_{rel}: triplet for relative weightage of color; QTa_{rel}: triplet for relative weightage of taste; QTex_{rel}: triplet for relative weightage of texture; QM_{rel}: triplet for relative weightage of mouthfeel

Table 5.12. Values of overall membership functions of the dessert gels and their quality attributes

	B0	B1	B2	B3	B4	B5	BC	BTa	Btex	BM
Amona	0.481983	0.252021	0	0	0	0	0	0	0	0
	0.937978	0.589577	0.004677	0	0	0.03377	0	0	0	0
	1	0.927094	0.237220	0.092973	0.010371	0.269595	0	0	0	0
	0.701473	1	0.469762	0.305600	0.214677	0.505421	0.25	0	0	0
	0.355949	0.744139	0.702305	0.518288	0.418982	0.741246	0.65	0	0	0
	0.010425	0.417787	0.934848	0.730855	0.623287	0.9770726	1	0	0	0
	0	0.091435	1	0.943482	0.827592	1	0.95	0.2	0.2	0.3
	0	0	0.756226	1	1	0.715256	0.55	0.6	0.6	0.7
	0	0	0.417572	0.417572	0.944053	0.399848	0.15	1	1	1
	0	0	0.078917	0.078917	0.585721	0.084441	0	1	1	0.7777
	B0	B1	B2	B3	B4	B5	BC	Bta	Btex	BM
Badal	0.270990	0.144976	0	0	0	0	0	0	0	0
	0.631870	0.448597	0.066095	0	0	0	0	0	0	0
	0.992741	0.752217	0.301601	0	0	0.179508	0	0	0	0
	1	1	0.537108	0.164218	0.074820	0.400507	0.25	0	0	0
	0.672849	0.941437	0.772614	0.366152	0.268153	0.621507	0.65	0	0	0
	0.338983	0.622998	1	0.568086	0.461487	0.842506	1	0	0	0
	0.005117	0.304560	0.988888	0.770021	0.654820	1	0.95	0.2	0.2	0.3
	0	0	0.660947	0.9719554	0.848153	0.906673	0.55	0.6	0.6	0.7
	0	0	0.333006	1	1	0.581894	0.15	1	1	1
	0	0	0.005065	0.677080	0.911485	0.257116	0	1	1	0.7777
	B0	B1	B2	B3	B4	B5	BC	Bta	Btex	BM
Kenkowa	0.331526	0.192769	0	0	0	0	0	0	0	0
	0.721952	0.509950	0.122297	0	0	0	0	0	0	0
	1	0.827131	0.367598	0.022863	0	0.212585	0	0	0	0
	0.902085	1	0.612899	0.230416	0.113301	0.436428	0.25	0	0	0
	0.561909	0.851678	0.858200	0.437972	0.310282	0.660270	0.65	0	0	0
	0.221734	0.525683	1	0.6455269	0.507264	0.884112	1	0	0	0
	0	0.199688	0.861194	0.853081	0.704245	1	0.95	0.2	0.2	0.3
	0	0	0.532219	1	0.901226	0.846470	0.55	0.6	0.6	0.7
	0	0	0.203245	0.895008	1	0.528129	0.15	1	1	1
	0	0	0	0.535628	0.800956	0.209788	0	1	1	0.7777

Table 5.13. Similarity values of the quality attributes of the dessert gels and their rankings

Scale factors	Color	Taste	Texture	Mouthfeel
Not at all necessary, F1	0	0	0	0
Somewhat necessary, F2	0.046082949	0	0	0
Necessary, F3	0.516129032	0	0	0
Important, F4	0.940092166	0.2	0.2	0.26
Highly important, F5	0.433179724	0.8800019	0.8800366	0.895556
Extremely important, F6	0.02764977	0.625	0.625	0.586137
Rank	IV	III	II	I

Highlighted numbers indicate the highest scores of the similarity values of each quality parameter

Table 5.14. Similarity values of the dessert gels and their rankings

Similarity values of the <i>Amona</i> dessert gels and their rankings						
Scale factors	S0	S1	S2	S3	S4	S5
Not satisfactory, F1	0.348216	0.181827	0.000689	0	0	0.004808
Fair, F2	0.926299	0.7125	0.140369	0.082599	0.033351	0.158336
Satisfactory, F3	0.572188	0.803586	0.517604	0.415306	0.269303	0.532462
Good, F4	0.068986	0.293056	0.784343	0.81783	0.6121	0.77031
Very good, F5	0	0.015202	0.504401	0.648218	0.751028	0.471905
Excellent, F6	0	0	0.084703	0.096691	0.299693	0.08097
Rank	VI	V	III	II	I	IV

Here, S0-S5: AG-0-AG-50

Similarity values of the *Badal* dessert gels and their rankings

Scale factors	S0	S1	S2	S3	S4	S5
Not satisfactory, F1	0.193912	0.117034	0.009425	0	0	0
Fair, F2	0.746901	0.562013	0.181453	0.023596	0.011442	0.108026
Satisfactory, F3	0.772810	0.833223	0.559124	0.234039	0.175479	0.436077
Good, F4	0.224802	0.443156	0.771633	0.5768	0.512156	0.741462
Very good, F5	0.000810	0.048262	0.4252	0.77461	0.80482	0.602229
Excellent, F6	0	0	0.04893	0.338258	0.431721	0.1559
Rank	VI	V	III	II	I	IV

Here, S0-S5: BG-0-BG-50

Similarity values of the *Kenkova* dessert gels and their rankings

Scale factors	S0	S1	S2	S3	S4	S5
Not satisfactory, F1	0.24646	0.148116	0.018372	0	0	0
Fair, F2	0.665273	0.6396	0.239254	0.039701	0.017083	0.123342
Satisfactory, F3	0.590207	0.836304	0.647416	0.288279	0.204219	0.470994
Good, F4	0.142993	0.380826	0.768044	0.637638	0.548007	0.755138
Very good, F5	0	0.033029	0.350331	0.744535	0.800278	0.566749
Excellent, F6	0	0	0.030531	0.282686	0.392311	0.135669
Rank	VI	V	III	II	I	IV

Here, S0-S5: KG-0-KG-50

Highlighted numbers indicate the highest scores of the similarity values of each sample

5.3.10. Identification of the best performing samples

Dessert gel is a product primarily consumed for its pleasurable eating experience. Hence, the final selection of the best formulations was necessarily made on the basis of overall sensorial appeal. The fuzzy logic-based approach minimized bias, enhancing the robustness of the outcome. The effectiveness of the fuzzy logic approach was further supported by the strong alignment between sensory similarity scores and instrumental color, texture and rheological behavior across substitution levels. Instrumental measurements showed that 10-20% gels were the lightest (high L^* , low a^*) and mechanically weakest, with the lowest hardness (~ 2.1 - 2.5 N), low cohesiveness and low viscoelastic strength (lower G' , higher $\tan \delta$), which matched their lower sensory rankings in texture and mouthfeel. At 30% substitution, color deepened (low L^* , slightly higher a^*) and both hardness and cohesiveness increased, supported by higher η_{50} and moderate increases in G' and G'' ; this corresponded well with their “good” sensory ratings in fuzzy, indicating that panelists perceived them as more stable than 20% and 10% gels. Although 50% gels showed the highest instrumental firmness (>3.2 N), greatest viscosity and yield stress (η_{50} , τ_0) and the strongest elastic network (highest G' , lowest $\tan \delta$), their excessive rigidity and stickiness reduced sensory acceptance and the panelists found them harder and less pleasant in mouthfeel, resulting in only “good” to “very good” similarity values. In contrast, the 40% gels consistently matched the highest sensory preference because they achieved sufficient hardness (~ 2.9 - 3.2 N) and cohesiveness to form a stable structure, strong elastic dominance (high G' but not excessively low $\tan \delta$) and moderate viscosity that supported smooth oral processing (Gupta et al., 2021). Their deeper red color (low L^* , high a^*) also aligned with the highest sensory color scores. This relation where instrumental data identified 40% gels as structurally optimal and sensory fuzzy logic independently ranked them as highest. AG-40, BG-40 and KG-40 were identified as the top-performing samples and are depicted in Fig. 5.8. These samples demonstrated superior similarity values across the key sensory attributes including mouthfeel, texture, taste and color. These samples were considered for further comprehensive analyses of nutritional quality and shelf-life

performances, including proximate composition, starch digestibility characteristics, and storage stability under refrigerated conditions.

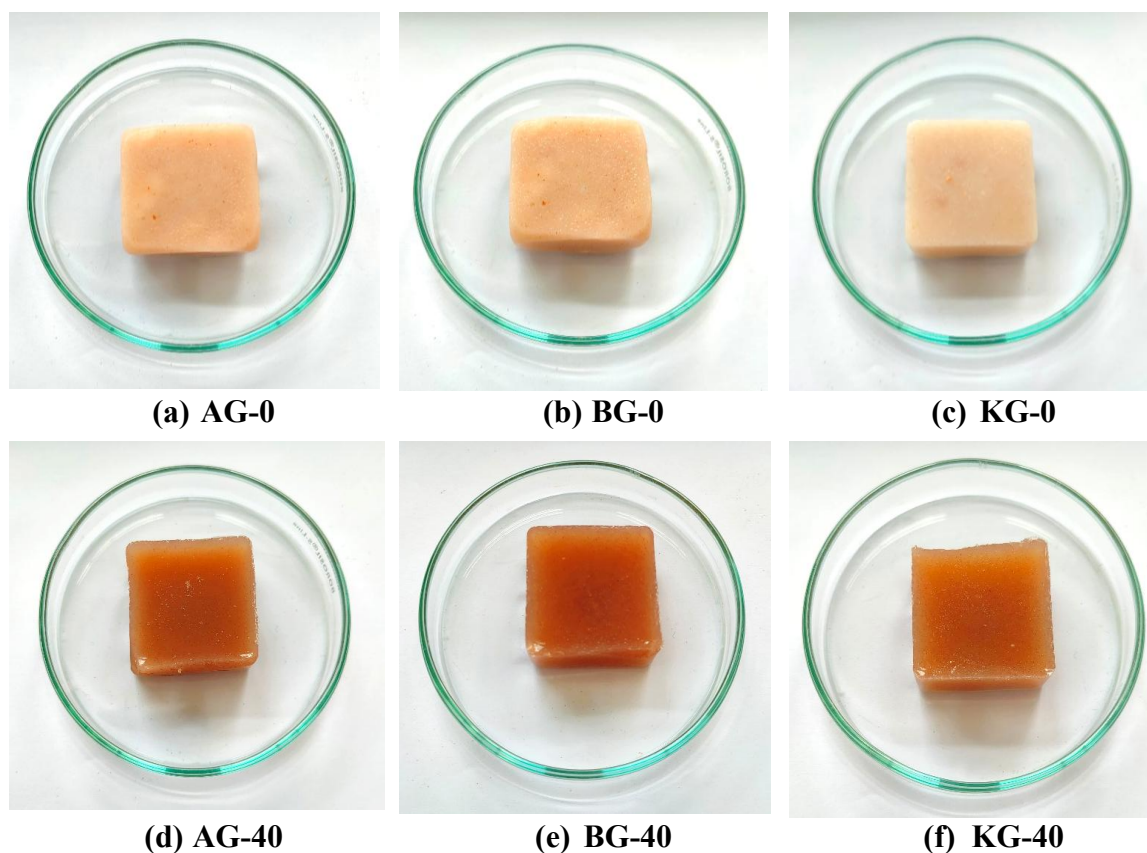


Fig. 5.8. Dessert gels prepared from 0% CF substituted flours (a) AG-0, (b) BG-0 and (c) KG-0 and 40% CF substituted flours of (d) AG-40, (e) BG-40 and (f) KG-40

5.3.11. Proximate composition

The proximate composition of the dessert gel samples showed minor variations, depending on the rice variety and formulation (Table 5.15). Moisture content ranged between 58.12-64.44%. BG-40 and KG-40 exhibited slightly lower moisture content, attributable to their more compact matrices as confirmed from previous textural and rheological analyses. Higher protein content in BG-40 was potentially due to the inherently higher flour protein. Fat, ash and carbohydrate content did not show any statistically significant variations. Energy values of the optimized samples ranged from

142.40 to 157.21 kcal/100 g and were relatively low across all formulations. This reduced caloric density, macronutrient levels, suggests that the samples may potentially exhibit a lower glycemic response.

Table 5.15. Proximate composition properties of the optimized dessert gels

Samples	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Carbohydrate (%)	Energy (kcal/100g)
A40	64.44±0.93 ^c	1.25±0.02 ^a	4.67±0.12 ^a	0.78±0.05 ^b	32.18±1.67 ^a	142.40±2.39 ^b
B40	58.12±0.88 ^a	1.36±0.04 ^c	5.12±0.22 ^c	0.80±0.01 ^a	32.67±2.04 ^a	155.18±2.12 ^a
K40	60.09±1.58 ^b	1.33±0.06 ^b	4.85±0.09 ^b	0.80±0.03 ^b	32.74±1.44 ^a	157.21±3.15 ^a

Means followed by the same superscript lowercase letters within a column are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

5.3.12. Starch digestibility

Presence of both retrograded starch (RS type 3) and crosslinked starch (RS type 4) can resist amylolysis. Agvaandorj et al. (2025) also found that STMP crosslinking of mung bean starch significantly increased RS content by converting amorphous RDS into RS type 4. Among the optimized dessert gels, BG-40 showed the lowest RDS (38.78%) and highest SDS (24.1%) and RS (23.72%), indicative of a slower glucose release profile (Table 5.16). Comparatively, AG-40 and KG-40 exhibited significantly higher ($p \geq 0.05$) RDS (42.63 and 46.19%, respectively) and lower ($p \geq 0.05$) RS values (21.01 and 19.78%), suggesting more extensive starch breakdown and proneness to enzymatic hydrolysis. A lesser dense starch matrix in these gels could have been also a factor in promoting rapid enzymatic degradation. Lower AAC generally correlates with a more open, amorphous starch structure, making the starch chains more accessible to α -amylase (Wang et al., 2024).

Table 5.16. Starch digestibility properties of the optimized dessert gels

Samples	RDS (%)	SDS (%)	RS (%)
A40	42.63±1.23 ^b	21.32±1.02 ^b	21.01±0.16 ^b
B40	38.78±1.19 ^a	24.71±0.71 ^c	23.72±0.43 ^c
K40	46.19±1.52 ^c	20.06±0.55 ^a	19.78±0.22 ^a

Means followed by the same superscript lowercase letters within a column are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test

Higher AAC (29.14%) in *Badal* rice, effective crosslinking and rich phenolic composition, may have also contributed to a tightly bound starch-polyphenol network, reducing enzymatic accessibility and thus increasing RS fractions. However, KG-40 with highest AAC (30.20%), showed intermediate RDS and RS values. A similar observation by Liu et al. (2021) could explain this. Although higher AAC generally promotes formation of enzyme-resistant crystalline lattices upon retrogradation, for crosslinked starches this reorganization may not always translate into higher RS due to changes in moisture dynamics (Bodjrenou et al., 2023).

5.3.13. Storage stability

5.3.13.1. Microbial analysis

STMP crosslinking definitely contributed to lowering water mobility and porosity in the dessert gels, reducing the available niche for microbial colonization. During refrigerated storage (4 ± 2 °C), increases in the values of TAPC and YMC were observed with each succeeding day till 14th day (Fig. 5.9). However, they remained well within acceptable microbiological standards for chilled, ready-to-eat products (FSSAI, 2011). All samples initially exhibited low microbial loads ($\text{TAPC} < 2.0 \log \text{cfu g}^{-1}$ and $\text{YMC} < 1.5 \log \text{cfu g}^{-1}$). Gradual increases were observed, with AG-40 and KG-40 reaching borderline spoilage thresholds ($\sim 5.0 \log \text{cfu g}^{-1}$ TPC and $\sim 4.0 \log \text{cfu g}^{-1}$ YMC) by day 10, indicating onset of microbial deterioration. BG-40 maintained significantly lower microbial loads till day 12 with $\text{TPC} < 5.0$ and $\text{YMC} < 3.0 \log \text{cfu g}^{-1}$. This could be attributed to a synergistic effect of more compact gel matrix and higher phenolic

concentration in *Badal* flour conferring mild antimicrobial activity, thus inhibiting microbial proliferation (Kawee-Ai, 2025). Based on these observations, the storage stability analysis for further physicochemical and sensorial evaluations was conducted up to Day 8, during which all samples remained microbiologically safe and well within regulatory limits.

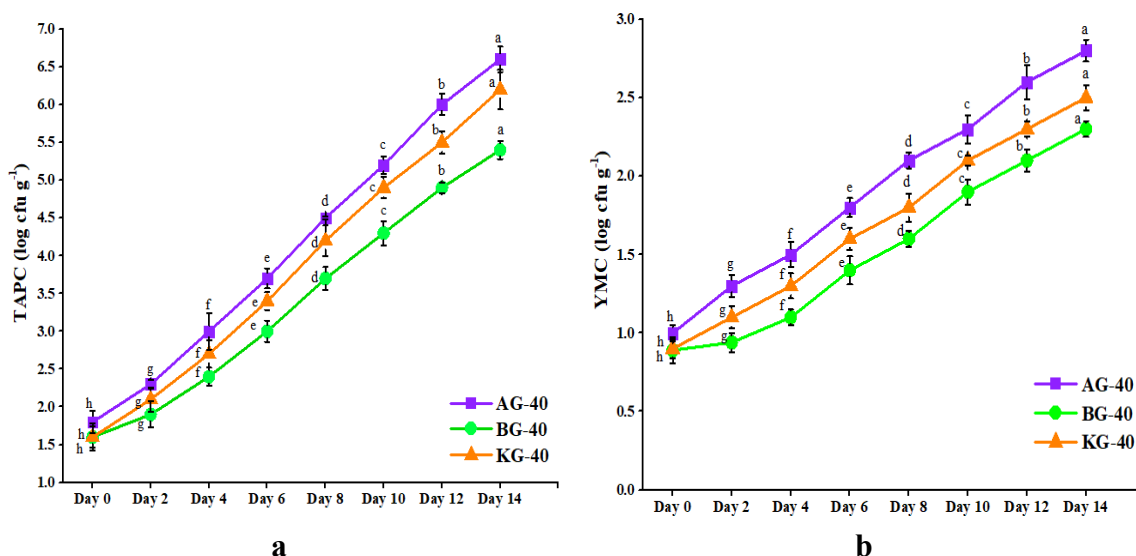


Fig. 5.9. (a) TAPC and (b) YMC of the optimized dessert gels over storage

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within each storage day are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

5.3.13.2. Syneresis

The syneresis profiles of the three optimized dessert gel formulations during refrigerated storage (4 ± 1 °C) over 8 days are presented in Fig. 5.10a. All samples exhibited gradual yet significant increases in syneresis over time, indicative of progressive water release due to structural relaxation and starch retrogradation. BG-40 showed a modest increase from 4.01% on Day 0 to 4.61% on Day 8. In contrast, AG-40 exhibited the highest syneresis values, rising significantly from 5.02% to 6.31% over the same period. KG-40 showed an intermediate behavior (4.03% to 5.53%). The superior water-holding and minimal syneresis in BG-40 can be attributed to its higher AAC making it more

effective for STMP-induced crosslinking to form denser gel network that resists phase separation during storage (Gong et al., 2024). Conversely, AG-40, with lowest AAC, formed a comparatively weaker gel matrix prone to retrogradation, leading to greater water expulsion. Although KG-40 had the highest amylose content (30.20%), its intermediate syneresis suggested that amylose level alone does not govern gel stability, which may also depend on the extent of crosslinking and internal moisture distribution (Nie et al., 2026). These findings corroborate the textural and digestibility trends previously discussed and support the selection of BG-40 as the most storage-stable formulation.

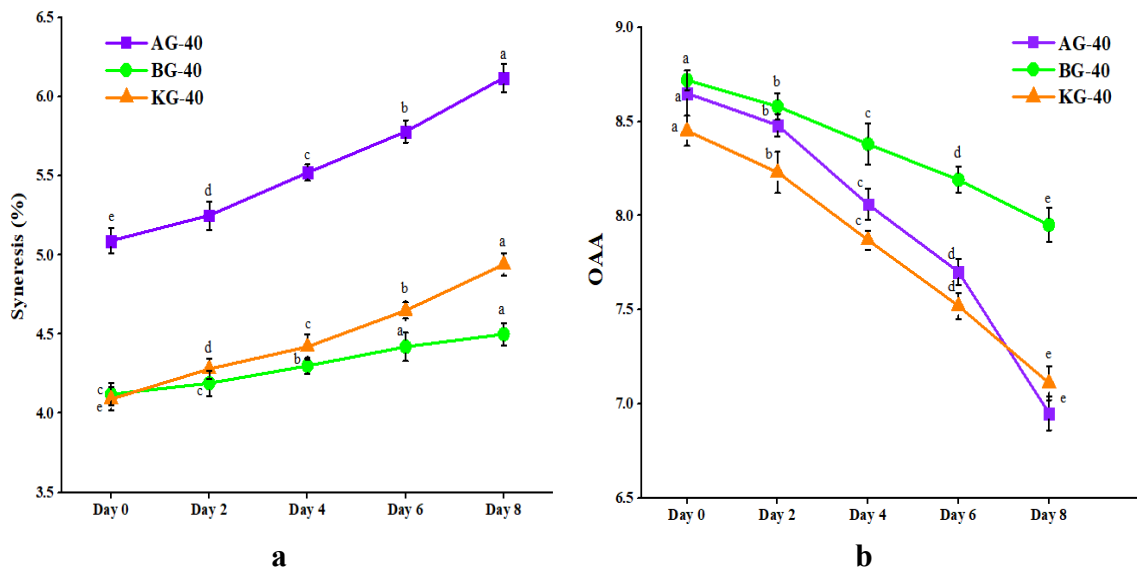


Fig. 5.10. (a) Syneresis and (b) OAA of the optimized dessert gels over storage
Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within each storage day are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

5.3.13.3. Color

The color values of dessert gels till 8th day of refrigerated storage are presented in Fig. 5.11. Across all formulations, gradual decrease in L^* , b^* and increased a^* and ΔE were observed with significant differences ($p \geq 0.05$) found each succeeding day. This suggested progressive pigment

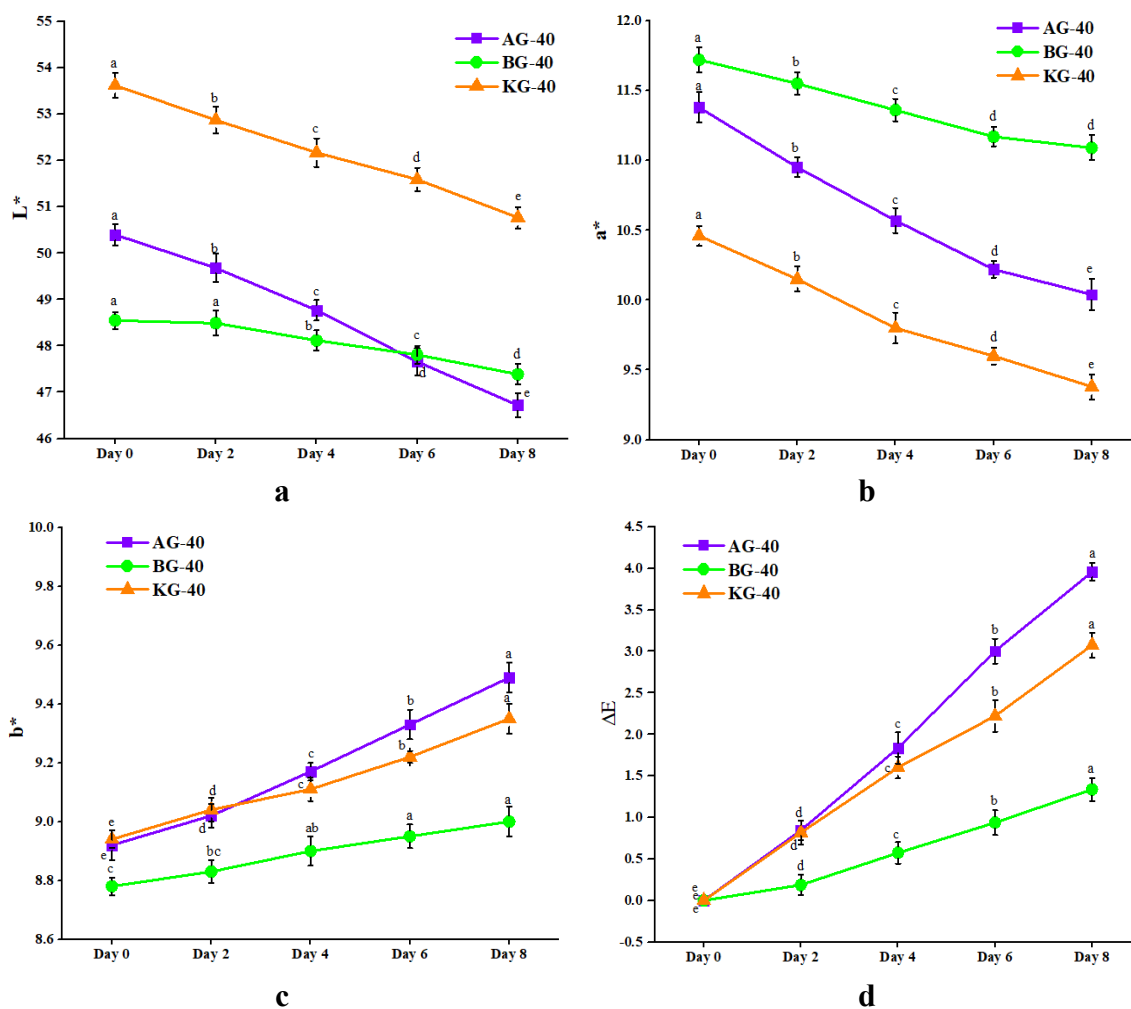


Fig. 5.11. Color values (a) L^* , (b) a^* , (c) b^* and (d) ΔE of the optimized dessert gels over storage

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within each storage day are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

fading and structural alterations of anthocyanins and phenolic compounds. Progressive syneresis could be identified as another factor (Bodjrenou et al., 2023). Retrogradation during refrigerated storage can also disrupt chromophore entrapment and increase light scattering, thereby reducing perceived color intensity (Das et al., 2023). AG-40 and KG-40 exhibited the highest ΔE values of 4.03 and 3.91 on day 8. Possibly weaker phenolic-starch interactions in them contributed to lower chromatic retention. In contrast, BG-40 maintained the most stable color profile throughout the storage period, with a $\Delta E=1$ on day 8, remaining well within the visually imperceptible threshold ($\Delta E < 3$).

5.3.13.4. Texture

Changes in the texture profiles of refrigeration stored optimized dessert gels are plotted in Fig. 5.12. AG-40, followed by KG-40 and BG-40, exhibited increases in hardness amounting approximately 20%, 6% and 3%, respectively. This was due to progressive starch retrogradation and matrix firming. The elevated syneresis observed in AG-40 may have contributed to increased gel rigidity through matrix dehydration and tighter molecular packing. A statistically significant difference ($p \leq 0.05$) in textural parameters was observed on each successive day for AG-40 and KG-40, reflecting ongoing structural reorganization. BG-40 demonstrated the most consistent texture stability, with only a marginal increase in hardness over the 8-day period and minimal changes ($p \leq 0.05$) in cohesiveness and springiness. This suggested stability of BG-40 gel network during retrogradation, potentially due to more effective STMP-induced crosslinking (Wang et al., 2024). The stable springiness and adhesiveness values of BG-40 indicated a resilient and elastic gel matrix capable of retaining moisture, consistent with its superior freeze-thaw stability. KG-40, though initially formed a strong network due to its high AAC, exhibited a marked decline in cohesiveness over time ($p \leq 0.05$), indicating a gradual loss of internal structural integrity. Its initially higher chewiness also decreased sharply, indicating enhanced amylose recrystallization and restricted polymer mobility (Villas-Boas et al., 2020). Overall, BG-40 emerged as the most texturally stable formulation, likely due to its balanced starch architecture and phenolic-mediated interactions, which can attenuate starch retrogradation and maintain gel elasticity over time. The observed increases in hardness and adhesiveness, along with decreased cohesiveness, springiness and chewiness were consistent with starch retrogradation behavior and has been associated with the average chain length of amylopectin. Specifically, a higher proportion of longer amylopectin

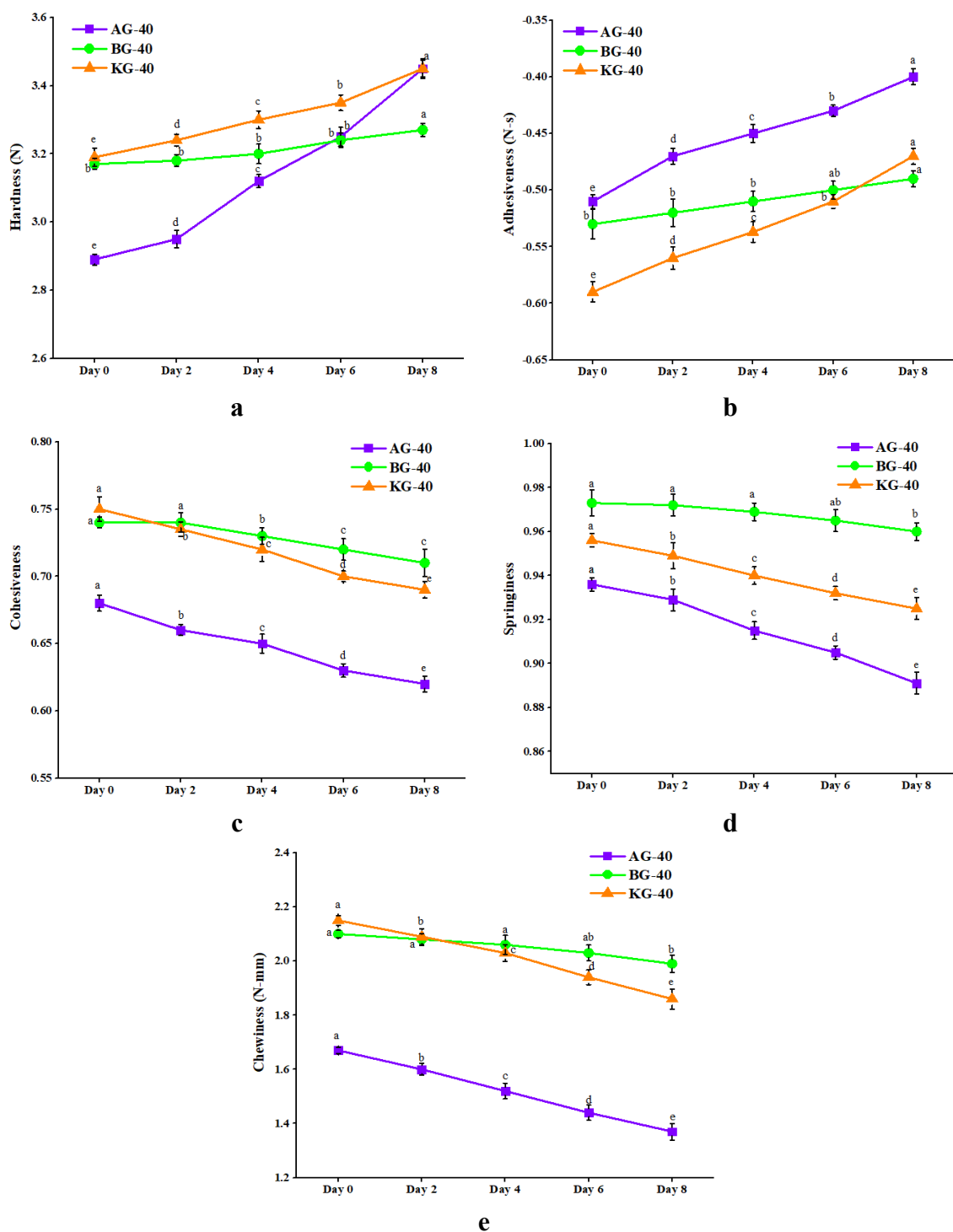


Fig. 5.12. Textural properties (a) Hardness, (b) Adhesiveness, (c) Cohesiveness, (d) Springiness and (e) Chewiness of the optimized dessert gels over storage

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within each storage day are not significantly different at $p \leq 0.05$ as indicated through Duncan's multiple range test.

chains can accelerate retrogradation and further influence these texture-related parameters (Agvaandorj et al., 2025). The improved microbial and textural stability of BG-40 could be explained by two complementary factors, its higher phenolic content and effective STMP-induced network formation. *Badal* flour showed highest antioxidant activity due to the presence of higher level of polyphenols which exert mild antimicrobial effects and can delay microbial proliferation during refrigerated storage. This explains why BG-40 maintained lower TAPC and YMC values up to day 12, while AG-40 and KG-40 approached spoilage thresholds earlier. In addition, the starch composition of *Badal* flour favored a more compact and cohesive crosslinked structure, resulting in lower syneresis and minimal firmness increase over the 8-day storage period. A denser gel matrix limits available free water, reduces micro pore spaces and restricts moisture migration which contribute to slower microbial growth and enhanced textural retention (Bodjrenou et al., 2023; Gu et al., 2024). Thus, BG-40 performed better due to the synergistic effects of higher phenolic content and a more cohesive crosslinked matrix.

5.3.13.5. Sensory overall acceptability

Results of sensory evaluation of the optimized dessert gels over 8 days of refrigerated storage using a 9-point hedonic scale are presented in Fig 5.10b. On day 0, all samples scored highly (>8.0), indicating favorable sensory attributes. However, a steady decline ($p \leq 0.05$) was noted over storage duration, particularly in AG-40 and KG-40 dropping to 6.91 and 7.22 respectively by day 8. This decline correlated with increased syneresis, firmness, and loss of color. These factors negatively influence consumer perception of gel-based products (Wang et al., 2023). BG-40 consistently maintained higher acceptability scores throughout the storage period, ending at 7.3 on day 8, suggesting a more stable sensory profile. The reduced syneresis, sustained textural elasticity, and better chromatic stability (high a^* value) of BG-40 likely contributed to its superior mouthfeel, appearance, and flavor retention. The presence of polyphenolic compounds in BG40 might also have imparted subtle but favorable flavor nuances, masking

potential retrogradation-associated off-notes (Fabela-Morón, 2024). The alignment between sensory scores and instrumental quality measures namely microbial load, syneresis, textural properties and color further validates BG-40 as a functionally superior formulation.

5.4. Conclusion

Unlike most prior studies that focus on chemical modification of isolated starches, this study valorized the entire nutrient-rich pigmented rice flour. Incorporation of STMP-crosslinked pigmented rice flours into raw flours to develop dessert gels significantly modulated gel structure and improved functional performances. Desirable improvements in the ready-to-eat refrigerated desserts' gel elasticity, cohesiveness and firmness were evidenced. Substitution led to significant reduction in syneresis, improved freeze-thaw stability and better color retention, all of which are critical for maintaining product quality during storage. A moderate decline in antioxidant activity occurred due to cross-linking. However, the functional benefits outweighed this limitation. Our fuzzy logic-based evaluation revealed that 40% CF substitution resulted in the most acceptable dessert gels from flours of the three rice. This highlights the importance of controlled modification for balancing texture to meet consumers' expectations of the gel product. Increased SDS and RS in the substituted gels suggested potential nutritional benefits of the developed gels and possibility of projecting the CF flour as a functional food ingredient. The results demonstrated that amylose content, effectiveness of starch crosslinking and native antioxidant profiles collectively played a pivotal role in sustaining consumer-relevant quality parameters of the refrigerated product. Microbiological stability up to a minimum of 8 days under refrigerated conditions, along with consistently high sensory scores, confirmed the product's shelf stability and consumer acceptability. Besides demonstrating the functional potential of crosslinked pigmented rice flours in dessert gel development, the results of this study also back the practical significance of pigmented *baio* rice, an underutilized crop widely cultivated by marginalized communities in flood-prone regions of Assam and its transformation into

value-added products. This would offer potential economic upliftment and support germplasm conservation by creating new market pathways for these landraces. Future researches may explore alternative modification techniques (physical, dual-modification, enzymatic, heat-moisture treatment) and tailoring the base gel for diverse applications such as hydrogels, binders, spreads and nutraceutical matrices. Advanced shelf-life studies and evaluation of bioavailability of phenolics would further strengthen the functional food potential of the developed products.

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CHAPTER 6

Optimization of hydrothermally processed pigmented *bao* rice for gluten-free pasta with improved functional and nutritional properties

6.1. Introduction

Pasta is one of the most widely consumed cereal-based foods worldwide due to its affordability, ease of preparation, long shelf life and sensory versatility (Gupta and Sharma, 2023). Conventionally, pasta is produced from durum wheat semolina, in which gluten plays a central role in determining cooking quality, firmness, low cooking loss and resistance to overcooking. During processing and cooking, gluten proteins form a continuous viscoelastic network that entraps swollen starch granules, thereby stabilizing the pasta matrix and governing its textural properties (Wang et al., 2024).

Despite its technological advantages, wheat-based pasta exhibits nutritional limitations. They are rich in digestible carbohydrates but relatively low in dietary fiber, essential amino acids and bioactive compounds (Zingale et al., 2023). In addition, wheat cultivation is restricted in many tropical and subtropical regions, contributing to higher production costs and import dependency (Parenti et al., 2025). More critically, gluten consumption has been found to trigger adverse health effects in individuals affected by gluten-related disorders, including celiac disease, wheat allergy and non-celiac gluten sensitivity. Global prevalence of gluten intolerance has increased in recent years (Capriles et al., 2023).

Consequently, the demand for gluten-free (GF) foods, including pasta, has expanded beyond clinical populations to health-conscious consumers seeking improved digestibility and perceived wellness benefits. However, commercially available cereal based GF products are frequently nutritionally imbalanced, characterized by low fiber content, high glycemic index and inferior protein quality, when compared with their gluten-containing counterparts (Capriles et al., 2021; Scarton and Clerici, 2022). These

limitations have prompted extensive research into reformulation strategies aimed at improving both nutritional and technological qualities of GF pasta. Studies have reported the use of millets and pseudocereals as GF raw material for pasta development (Cervini et al., 2021). However, among GF cereals, rice (*Oryza sativa* L.) is the most extensively used raw material for pasta production owing to its bland flavor, hypoallergenic nature, high digestibility, light color and consumer acceptance. Rice-based pasta currently represents a substantial share of the global GF pasta market (Park and Kim., 2023). From a structural standpoint, rice starch is the dominant component controlling pasta processability and quality. The amylose-to-amylopectin ratio strongly influences starch gelatinization, swelling behavior, retrogradation and enzymatic digestibility. High-amylose rice varieties have been shown to yield pasta and noodle products with greater firmness, reduced stickiness, increased resistant starch content and lower postprandial glycemic response due to restricted starch swelling and slower hydrolysis (Ramos et al., 2023).

The food structure of pasta is largely dictated by processing induced transformations of starch and protein. Thermal and mechanical treatments during pasta manufacture promote starch gelatinization and protein aggregation, leading to the formation of a compact matrix that improves cooking tolerance and texture. In gluten-free rice pasta, absence of a gluten network renders the starch matrix more susceptible to leaching, structural breakdown and excessive cooking loss (Ramos et al., 2023). To address these challenges, technological interventions such as hydrothermal pretreatments and starch modification have been widely explored.

Hydrothermal treatments, particularly parboiling and pre-gelatinization, are effective in modifying rice starch functionality without the use of synthetic additives. Parboiling induces partial starch gelatinization followed by retrogradation, promotes amylose-lipid complex formation and enhances protein aggregation. These structural rearrangements reduce starch swelling and amylose leaching during cooking, resulting in increased firmness, reduced stickiness and improved cooking quality. Moreover, parboiling

facilitates the migration of vitamins and minerals into the endosperm and increases resistant starch content, thereby enhancing nutritional value (Taddei et al., 2021). The combined effects of heat, pressure and shear in extrusion cooking disrupt the native crystalline structures of starch and promote starch-protein interactions, enabling the formation of cohesive matrices in the absence of gluten. Processing parameters such as feed moisture, barrel temperature and screw speed critically influence gelatinization extent, molecular organization and microstructure, ultimately determining cooking loss, firmness and textural stability of extruded rice pasta (Zhao et al., 2024).

Beyond processing strategies, formulation approaches including protein and fiber enrichment have been investigated to enhance the nutritional quality of rice pasta (Demir and Bilgiçli, 2021; Park and Kim, 2023). Incorporation of legume proteins, egg proteins, fish, pseudocereals and rice bran fractions increases protein and fiber content but may also interfere with starch continuity if not properly optimized, leading to higher cooking loss and compromised texture (Aínsa et al., 2021; Messina et al., 2021; Mostafa et al., 2025).

While most commercial rice pasta products utilize polished white rice flour, increasing attention is being directed toward whole and pigmented rice varieties due to their superior nutritional profiles. Pigmented rice is rich in dietary fiber, phenolic compounds, anthocyanins, vitamins and minerals, which confer antioxidant and potential glycemic-modulating effects (Tiozon et al., 2021). However, pigmented rice remains underutilized in pasta applications due to challenges related to pigment stability, starch functionality and processing induced quality changes.

Of particular interest are the underutilized deepwater, flood-resistant pigmented *bao* rice landraces cultivated in the flood-prone regions of Assam, India (Mudoï and Das, 2020; Sahewalla et al., 2023). Despite their agronomic resilience and nutritional richness, *bao* rice varieties are largely confined to traditional consumption and have received limited scientific attention for value-added applications, such as pasta. Therefore, in this study, the development of gluten-free pasta from pigmented, underutilized, flood-resistant *bao*

rice was carried out as a sustainable approach to diversify, enhance their nutritional quality and add value to the climate-resilient indigenous rice germplasm. With emphasis on optimization and standardization of hydrothermal pretreatment, structural modifications on the pasta was done. The textural attributes, nutritional implications and storability of the developed pasta were also analyzed.

6.2. Materials and Methods

6.2.1. Materials

Freshly harvested, *Kenkowa*, *Amona* and *Badal bao* paddy were procured. Analytical grade chemicals were purchased from SRL chemicals, Lobachemie, Himedia, Merck and Sigma-Aldrich.

6.2.2. Pretreatment process of paddy and preparation of pregelatinized flour

Pigmented bao rice paddy was cleaned to remove any adhering extraneous matter and subjected to steam parboiling under atmospheric pressure (0 psig) at 100 °C for durations ranging from 15-30 min. The parboiled paddy was dried at room temperature (27±2 °C) and dehusked in a laboratory dehuller (A-Grain, India). The obtained rice was milled into flour and sieved to obtain flour particles withing a size range of 150-100 µm, followed by oven-drying at 40 °C to constant moisture content of 8±2% and refrigerated storage in airtight containers for 24 h until further processing. This dried flour was subjected to pressure steaming (121 °C, 15 psig) for 15-30 min, cooled to room temperature, redried (8±2%) and stored in airtight containers. The resulting pregelatinized flour was designated as pretreated rice flour.

6.2.3. Processing of pasta

Pasta dough was prepared by mixing the pretreated rice flour with potable water maintained at 60±5 °C, along with 1% (w/w) salt and 2% (w/w) edible oil, based on flour weight. The flour-to-water ratio was maintained at 1:2 (w/v), and mixing was

carried out until a homogeneous, cohesive dough was obtained. The prepared dough was immediately processed using a laboratory-scale pasta maker (Philips Viva Collection Electric Pasta Machine HR2345/27, China) fitted with a fettuccine die (≈ 6 mm width, ≈ 1 mm thickness) at room temperature. The extruded pasta strands were manually cut and dried in a hot-air oven (60 ± 2 °C) until a stable moisture content of $8 \pm 2\%$ was attained. The dried pasta samples were cooled to room temperature, packaged in sealed polypropylene bags and stored under ambient conditions.

6.2.4. Pretreatment process optimization

Optimization of the pretreatment process was performed using response surface methodology (RSM). A central composite face-centered (CCFC) design was employed using Design-Expert software (version 12). Steam parboiling time (X_1) and pressure steaming time (X_2) were selected as independent variables and the response variables evaluated were cooking time (min), cooking loss (%), rehydration ratio, and textural properties including hardness (g), cohesiveness (g.s) and chewiness (g.s) of the cooked pasta. Experimental data were fitted to a second-order polynomial model as expressed in the following equation (Eq. 6.1).

$$Y = a_0 + a_1X_1 + a_2X_2 + a_{11}X_1^2 + a_{22}X_2^2 + a_{12}X_1X_2 \quad \text{Eq. 6.1}$$

where, Y represents the predicted response for the dependent variables, a_0 is the intercept, a_1 and a_2 are the linear coefficients, a_{11} and a_{22} are quadratic coefficients and a_{12} is the interaction coefficient. X_1 and X_2 are the independent variables. Model adequacy and significance were evaluated by analysis of variance (ANOVA) at 95% confidence level ($p \leq 0.05$) for significance.

The optimized pasta samples were compared with two control formulations prepared from each rice variety. Pasta made from the three untreated raw *bao* rice flours served as the respective negative controls (NC), while pasta prepared from the respective raw flours with 0.5% xanthan gum served as the positive controls (PC). Samples were coded

as A, B, and K for *Amona*, *Badal* and *Kenkowa*, respectively. Thus, A(NC), B(NC), and K(NC) represented negative controls, whereas A(PC), B(PC), and K(PC) represented positive controls. The optimized samples were designated as AO, BO, and KO for the corresponding varieties.

6.2.5. Cooking Time

The optimal cooking time (OCT) was determined according to Ramos et al. (2023) with minor modifications. Dried pasta strands were first cut into ~0.1 g portions. A total of 10 g was boiled in 250 ml distilled water. At 30 s intervals, 3-4 strands were removed and compressed between two glass slides. The OCT was recorded as the time at which the central opaque core completely disappeared upon pressing.

6.2.6. Cooking loss

Cooking loss was determined according to Zhao et al. (2024) using the same sample size and cooking conditions as described for optimal cooking time. Pasta strands were cut into uniform 0.1 g portions, and 10 g of dried gluten-free pasta was cooked in 250 ml of boiling distilled water. After cooking, the pasta and cooking water were allowed to cool to room temperature. The cooking water (pasta cooking liquor) was then transferred to a 500 ml volumetric flask and homogenized. An aliquot of 100 ml of the homogenized cooking water was transferred into a pre-weighed 250 ml beaker and dried in a hot air oven at 105 °C until constant weight. Cooking loss was calculated as the percentage of solid residue relative to the initial dry weight of pasta using the formula given below (Eq. 6.2).

$$CL\% = \frac{5M}{G \times (100 - W)} \times 100 \quad \text{Eq. 6.2}$$

where CL is the cooking loss (%), G is the weight of pasta sample before cooking (g), M is the dry matter mass of 100 ml of pasta soup (g), and W is the moisture content of pasta (%).

6.2.7. Rehydration ratio

Rehydration ratio (RR) was subsequently determined using the cooked pasta obtained after cooking loss analysis. After cooking, the pasta samples were drained for 2 min and gently blotted with filter paper to remove excess surface water. The cooked pasta was then weighed. Rehydration ratio was calculated as the ratio of the weight of cooked pasta to the initial dry weight of pasta (Eq. 6.3), following the method described by Ramos et al. (2023).

$$RR = \frac{\text{Weight of cooked pasta (g)}}{\text{Weight of dry pasta (g)}} \quad \text{Eq. 6.3}$$

6.2.8. Texture

Textural analysis was conducted following the methodology described in a previous study by Duan et al. (2024). Briefly, the pasta samples were boiled to the optimal cooking time and then taken out to soak in water at room temperature for 30 s, drained and measured with a texture analyser (TA. XT. Plus, Stable Microsystems, U.K). The parameters were set as follows. TPA probe: P/36R; pre-test, test and post-test speed: 3, 1, and 3 mm s⁻¹ respectively, strain, 50%; trigger force, 5.0 g.

6.2.9. Digestive properties

Rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) fractions in the pasta samples were determined using a modified method of Roy et al. (2019). Samples were deproteinized by incubation with pepsin in HCl-KCl buffer (pH 1.5) at 37 °C for 1 h. The pH was subsequently adjusted to 5.4 using 7 ml acetate buffer, followed by the addition of 3 ml of an enzyme mixture containing invertase (220 U/ml), pancreatic α -amylase (3000 U/ml), and amyloglucosidase (15 U/ml). Enzymatic hydrolysis was conducted at 37 °C, and aliquots were withdrawn at 20 and 120 min for glucose determination. Glucose released at 20 min (G_{20}) was used to calculate RDS (Eq. 6.4), while glucose released between 20 and 120 min ($G_{120} - G_{20}$) was used to calculate

SDS (Eq. 6.5). Glucose values were converted to starch equivalents using a conversion factor of 0.9 and expressed as a percentage of total starch (TS) content (AOAC, 2019). The starch fraction remaining undigested after 120 min was defined as RS (Eq. 6.6).

$$RDS(\%) = \left[\frac{G_{20} \times 0.9}{TS} \right] \times 100 \quad \text{Eq. 6.4}$$

$$SDS(\%) = \left[\frac{(G_{120} - G_{20}) \times 0.9}{TS} \right] \times 100 \quad \text{Eq. 6.5}$$

$$RS(\%) = [TS - (RDS + SDS)] \times 100 \quad \text{Eq. 6.6}$$

6.2.10. Bioactive properties

The pasta sample was powdered to 100-212 µm particle size. One-gram powder was then extracted in 10ml ethanol at 40 °C for 4 h under constant agitation. After centrifuging (845 g, 60 min), supernatant was collected.

6.2.10.1. Total phenolic content

Total phenolic content (TPC) of the samples was estimated using the Folin-Ciocalteu Reagent (FCR) method. Initially, 500 µl of the ethanolic extract was mixed with 2.5 ml of 10% (w/v) FCR. Within 8 minutes, 2ml of 7.5% sodium carbonate was added and vortexed before incubating in dark at 30 °C for 30 min. Absolute ethanol served as blank. Absorbance was measured at 765nm using an UV-Visible spectrophotometer (Orion AquaMate 8100, Thermo Fisher Scientific, Germany) and compared with a standard curve prepared with gallic acid. Value of TPC was expressed as gallic acid equivalents per gram (µg GAE/g) of sample (Kalpan et al., 2021)

6.2.10.2. Total flavonoid content

Total flavonoid content (TFC) was measured by a method described by Deka et al. (2024). Briefly, 0.5 ml ethanolic extract was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled

water. After vortexing, the mixture was allowed to stand for 5 min and 1 ml of 1 M sodium hydroxide was added. Absorbance was measured at 510 nm and plotted against a standard curve prepared using standard quercetin. TFC values were expressed as quercetin equivalent per hundred grams ($\mu\text{g QE}/100 \text{ g}$) of sample.

6.2.10.3. DPPH radical scavenging activity

DPPH radical scavenging activity (DRSA) was determined by the method of Kalpan et al. (2021) with slight modifications. A blank solution was prepared by mixing 200 μl ethanol with 2.8 ml of 0.1 mM DPPH. Simultaneously, 200 μl ethanolic sample extract was mixed with 2.8 ml of the DPPH solution (0.1 mM) and incubated in dark for 30 min. Absorbance was measured at 517 nm and result expressed as percentage DRSA using the following equation (Eq. 6.7).

$$\% \text{ DRSA} = \frac{(A_0 - A_s)}{A_0} \times 100 \quad \text{Eq. 6.7}$$

where, A_0 is the absorbance of the blank, A_s is the absorbance of the sample extract.

6.2.10.4 Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) was determined using a modified method of Zhang et al. (2022). FRAP solution was freshly prepared by mixing 5 ml of 10 mM 2,4,6-TPTZ [2,4,6-tri(2-pyridyl)-1,3,5-triazine] solution, 40mM hydrochloric acid, 5ml of 20mM ferric chloride and 50 ml of 0.3 M acetate buffer (pH 3.6). Hundred microliters aliquot of the sample extract was thoroughly mixed with 3ml of the FRAP solution and incubated at 37 °C for 4 min. Absorbance was determined at 593 nm taking distilled water as blank. A calibration curve was prepared using a concentration range of 0.1 to 1 mM aqueous ferrous sulphate. FRAP values were expressed as millimoles of ferrous equivalent Fe(II) per g (mmol/g) of sample.

6.2.11. Color

The CIE color parameters including L* (lightness), a* (greenness [−] to redness [+]), and b* (blueness [−] to yellowness [+]) of the dry pasta samples were measured using a ColorQuest spectrophotometer (Hunter Associates Laboratory, USA). The instrument was calibrated prior to measurement using white and black calibration tiles.

6.2.12. Sensory overall acceptability

A sensory evaluation study was conducted with approval by the institutional ethics committee (Clearance No. MZU/HEC/2024/013, dated 17/06/2024). A sensory panel of 30 members (aged 20-45 years, healthy, non-smoking) with prior experience of sensory evaluation was selected. Informed consent was obtained from each participant prior to evaluation. Two training sessions were conducted to familiarize panelists with the evaluation protocol. Sensory assessment was carried out in three sequential cycles, with four samples per cycle and a 1-hour interval between consecutive cycles. To minimize sensory fatigue and external influences, the panelists were restricted from consuming food and beverages, except for drinking water for 30 minutes prior to each evaluation cycle. Cooked and anonymized pasta samples (pasta-to-water ratio of 1:25) were presented. Assessments were done independently using a 9-point hedonic scale on overall acceptability (OAA). The hedonic scale ranged from 1 (extremely disliked) to 9 (extremely liked) for rating.

6.2.13. Proximate composition

Moisture, protein, fat and ash content of the samples were assessed by standard AOAC (2019) protocols as detailed in section 3.2.5 (Chapter 3). Total carbohydrate content was calculated through difference method. Energy values were calculated using the Atwater factors (4 kcal/g for protein, 4 kcal/g for carbohydrates, and 9 kcal/g for fat (Merrill and Watt, 1973)).

6.2.14. Scanning electron microscopy

The microstructural characteristics of the raw pasta samples were analyzed using scanning electron microscopy (SEM, JSM 6390LV, JEOL Ltd., Japan). Prior to imaging, the samples were vacuum dried to eliminate residual moisture. Small fragments were mounted on aluminum stubs using double-sided carbon adhesive tape and subsequently sputter-coated with a thin layer of gold (~20 nm thickness) to enhance surface conductivity. To visualize the overall morphology and integrity of the entire cut piece, both surface and cross-sectional views were initially examined at low magnification (30-33X). This allowed assessment of macrostructural features such as surface uniformity, fissures, and overall matrix continuity. For detailed evaluation of internal structural organization, including starch fusion, pore distribution, and matrix compactness, cross-sectional micrographs were further captured at higher magnification (500X).

6.2.15. Storage study

The optimized pasta samples (AO, BO and KO) were subjected to accelerated storage studies to evaluate physicochemical, microbiological and textural stability. Samples were packed in laminated polyethylene pouches and stored under controlled accelerated conditions at 45 ± 2 °C and 75% relative humidity (RH) for a period of 35 days. Analyses were performed at 7 day intervals (0, 7, 14, 21, 28 and 35 days).

Moisture content and water activity (a_w) were determined to assess hygroscopic behavior and microbial susceptibility. Microbiological stability was evaluated through total aerobic plate count (TAPC) and yeast and mold count (YMC), expressed as log cfu g⁻¹. Lipid oxidation was monitored by determining free fatty acid content (FFA, % oleic acid), peroxide value (PV, meq O₂/kg) and thiobarbituric acid reactive substances (TBARS, mg MDA/kg). Textural properties (hardness, cohesiveness and chewiness) were measured on cooked pasta samples using a texture analyzer. Cooking loss and overall acceptability (OAA) were also evaluated during storage.

Quality acceptability thresholds were predefined as follows: moisture content <13%, a_w <0.6, TAPC <4 log cfu g⁻¹, YMC <2.5 log cfu g⁻¹, FFA <1.5%, PV <10 meq O₂/kg, TBARS <1.0 mg MDA/kg, cooking loss <6%, hardness >500 g, cohesiveness >0.8, chewiness >200 g and OAA >7. These criteria were used to determine product acceptability during storage.

Shelf-life prediction was carried out using the Q₁₀ accelerated model (Hutami et al., 2025), where the shelf life at ambient temperature (25 °C) was calculated using the following equation (Eq. 6.8).

$$\text{Shelf life at ambient} = \text{Shelf life at accelerated} \times Q_{10}^{\frac{T_2 - T_1}{10}} \quad \text{Eq. 6.8}$$

A Q₁₀ value of 2.5 was assumed, indicating that the rate of deterioration increases 2.5 fold for every 10 °C rise in temperature.

6.2.15.1. Moisture content

Moisture content of the stored pasta samples was done using oven drying method (105 °C) to constant weight (AOAC, 2019).

6.2.15.2. Water activity (a_w)

Water activity of the stored pasta samples was done using a calibrated water activity meter (METER Aqualab PRE, USA).

6.2.15.3. Microbiological analysis

Microbiological stability of the pasta samples was assessed during accelerated storage at 45°C over 35 days, with analyses conducted at two-day intervals. At each sampling point, three independent packages were randomly selected and handled aseptically. A 10 g portion of pasta was weighed and transferred into 100 ml of sterile physiological saline solution (0.85% NaCl) in a sterile bag and homogenized to obtain a uniform microbial suspension. The homogenate was serially diluted using 0.1% sterile peptone water.

Appropriate dilutions were surface plated on Plate Count Agar (PCA) for total aerobic plate count (TAPC) and on Potato Dextrose Agar (PDA) acidified with 10% tartaric acid for yeast and mold count (YMC). PCA plates were incubated at 37 °C for 48 h, while PDA plates were incubated at 25°C for 5 days. Colonies were enumerated after incubation and results were expressed as log colony forming units per gram (log cfu g⁻¹) (Larrea-Wachtendorff et al. 2022).

6.2.15.4. Free fatty acid (FFA) value

2 g flour sample was dissolved in a neutral solvent composed of petroleum ether and ethanol (1:1 v/v) and titrated with 0.1 N potassium hydroxide using phenolphthalein as indicator (AOAC, 2023). Acid value and FFA content (expressed as oleic acid) were calculated using the following equations respectively (Eq. 6.9 and 6.10)

$$\text{Acid value (mg KOH/g)} = \frac{TV \times N \times 56.1}{W} \quad \text{Eq. 6.9}$$

where, TV: titer value, N: normality of KOH, W: weight of the sample

$$\text{FFA (\%)} = \text{Acid value} \times 0.5 \quad \text{Eq. 6.10}$$

6.2.15.5. Peroxide value (PV)

5 g sample was dissolved in a 10 ml mixture of acetic acid and chloroform (3:2 v/v). To it, 0.5 ml of saturated potassium iodide (KI) solution was introduced, and the mixture was dark incubated for 1 min. Then, 30 ml distilled water was added and the solution was titrated with 0.01 N sodium thiosulfate using a 1% starch solution as indicator. A stable blue color indicated reaction endpoint. PV was calculated using the following formula (AOAC, 2019) (Eq. 6.11).

$$\text{PV (meq/kg)} = \frac{TV \times N \times 1000}{W} \quad \text{Eq. 6.11}$$

where, TV: titer value, N: normality of Na₂S₂O₃, W: weight of the sample

6.2.15.6. Thiobarbituric acid reactive substances (TBARS)

TBARS of the stored pasta samples were assessed following the protocol described by Houketchang Ndomou et al. (2023), with minor modifications. Briefly, 0.25 g sample was combined with aqueous 0.1% trichloroacetic acid (TCA) solution and vortexed. Subsequently, 1 ml each of 0.375% thiobarbituric acid, 15% TCA, and 0.25 N HCl were sequentially added. After vortexing, the mixture was incubated at 95 °C for 30 min and cooled down to room temperature. Absorbance of the aliquot was recorded at 532 nm against a blank containing all the reagents but not the sample. TBARS was calculated using the following formula (Eq. 6.12).

$$TBARS (mg\ MDA/kg) = \frac{Abs \times VTCA \times 2 \times M \times 10^{-2}}{1.56 \times W} \quad \text{Eq. 6.12}$$

where, Abs: absorbance, VTCA: volume of TCA solution, M: molar mass of malonaldehyde (72 g/mol), W: weight of the sample

6.2.16. Statistical analysis

Optimization of processing parameters was conducted using Design Expert 12.0 (Stat-Ease, USA). All the analyses were performed in triplicates, with results reported as mean±standard deviation. Significance of differences among the results were assessed using Duncan's multiple range test at a 95% confidence level ($p \leq 0.05$) using SPSS 21 (IBM, USA).

6.3. Results and Discussions

6.3.1. Modelling and fit summary

Response surface methodology was employed to model the influence of parboiling time (X_1) and pressure steaming time (X_2) on the cooking and textural properties of pasta. Experimental layouts of independent and response variables from the CCFC design are reported in Table 6.1, 6.2 and 6.3 for *Amona Badal* and *Kenkowa bao* respectively. The experimental data were fitted into second order polynomial models and analysis of

variance indicated that the models were statistically significant with high coefficient of determination ($p \leq 0.05$, $R^2 > 0.9$) explaining strong explanatory power and minimum unexplained variability. Quadratic model was the most suitable for describing relationships between the independent variables and responses (Table 6.4, 6.5 and 6.6). The non-significant lack of fit ($p > 0.05$) further confirmed the adequacy of the models in predicting the response behavior within the experimental domain. Significant linear, quadratic and interaction terms revealed that hydrothermal intensity and its synergistic effects governed structural transitions in starch systems. The relatively low prediction error between experimental and model-estimated values indicated robust predictive capability, validating the suitability of RSM for describing the nonlinear behavior of the hydrothermally modified gluten-free pasta.

6.3.2. Impact of independent variables on responses

For all the three rice varieties, cooking time and cooking loss exhibited similar response surface trends, characterized by negative linear coefficients for parboiling time (X_1) and pressure steaming time (X_2), along with positive quadratic coefficients (X_1^2 and X_2^2) (Eq. 6.13, 6.14, 6.19, 6.20, 6.25, 6.26). This indicated that moderate hydrothermal treatment reduced cooking time and cooking losses, whereas excessive treatment reversed this effect. In contrast, rehydration ratio showed positive linear and negative quadratic coefficients for both X_1 and X_2 (Eq. 6.15, 6.21, 6.27), suggesting that water absorption improved with increasing treatment intensity up to an optimum level, followed by a decline. The textural parameters followed comparable quadratic behavior. Hardness and chewiness displayed positive linear and negative quadratic relationships with X_1 and X_2 (Eq. 6.16, 6.18, 6.22, 6.24, 6.28, 6.30), indicating progressive strengthening of the matrix with treatment severity up to an optimal point, followed by slight softening under the extreme processing conditions. Cohesiveness also demonstrated positive linear and negative quadratic effects, with minor positive interaction coefficients (Eq. 6.17, 6.23, 6.29), reflecting synergistic contributions of the

two processing variables. Contour plots (Fig. 6.1, 6.2 and 6.3) depict the combined and interactive effects of the independent variables on the responses.

Parboiling and pressure steaming significantly influenced cooking loss, cooking time, rehydration ratio and textural properties through modulated structural transformations of starch. Increasing the parboiling time initiated partial gelatinization by disrupting the amylopectin crystalline regions and swelling amorphous domains, enhancing amylose mobility. Subsequent pressure steaming intensified granule disintegration and facilitated amylose leaching. At moderate levels, this sequential hydrothermal treatment promoted controlled amylose redistribution and strengthened intermolecular hydrogen bonding during drying. This resulted in the formation of compact retrograded network that reduced cooking loss by limiting solid diffusion into the cooking medium. However, excessive steaming caused over gelatinization, structural heterogeneity and microvoid formation, leading to marginal increases in cooking loss (Yang et al., 2020). Cooking time decreased under moderate treatments due to improved water penetration into partially gelatinized matrices but increased slightly under prolonged steaming in the *Kenkowa* samples due to their higher amylose resulting in enhanced amylose reassociation and matrix densification that restricted hydration kinetics (Deka et al., 2026). Rehydration ratio reflected internal structural organization. Controlled gelatinization could have created uniform porosity that facilitated water absorption whereas excessive retrogradation reduced polymer mobility and swelling capacity. Textural attributes were predominantly governed by amylose content and retrogradation behavior. Hardness and chewiness increased with hydrothermal severity due to formation of a dense amylose network. *Kenkowa* samples exhibited the highest firmness because of greater linear chain availability for double helix aggregation, while *Badal* demonstrated optimal cohesiveness attributable to balanced amylose-amylopectin interactions preventing brittleness (Zainab et al., 2025). The significant interaction between parboiling and pressure steaming confirmed that sequential thermal moisture exposure dictates crystalline melting, amylose migration and subsequent network stabilization.

Table 6.1. Experimental layout of the independent variables and their responses as given by the CCFC model for *Amona bao*

Run	Parboiling time (min)	Pressure steaming time (min)	Cooking time (min)	Cooking loss (%)	Rehydration ratio	Hardness (g)	Cohesiveness (g.s)	Chewiness (g.s)
1	25	30	8.71	4.71	1.92	575.21	0.75	255.12
2	17.5	22.5	8.55	4.5	2.10	590.09	0.95	280.09
3	10	30	8.86	4.86	1.69	560.06	0.65	240.06
4	17.5	30	8.56	4.56	1.95	581.12	0.85	274.97
5	17.5	22.5	8.35	4.40	2.15	590.02	0.91	269.82
6	17.5	15	8.78	4.78	1.79	571.15	0.72	264.59
7	17.5	22.5	8.30	4.38	2.25	595.36	0.99	268.22
8	25	15	8.93	4.93	1.72	560.17	0.65	241.26
9	25	22.5	8.65	4.65	1.98	582.15	0.81	260.11
10	10	22.5	8.80	4.80	1.71	568.42	0.70	257.67
11	10	15	9.18	5.08	1.41	545.32	0.56	229.39
12	17.5	22.5	8.444	4.35	2.30	585.15	0.85	275.18
13	17.5	22.5	8.20	4.30	2.35	580.06	0.80	285.32
			R²	0.9597	0.9737	0.9254	0.9706	0.9587
			Adjusted R²	0.9423	0.9521	0.9672	0.9638	0.9472
			Predicted R²	0.9189	0.9031	0.9426	0.9374	0.8942

Table 6.2. Experimental layout of the independent variables and their responses as given by the CCFC model for *Badal bao*

Run	Parboiling time (min)	Pressure steaming time (min)	Cooking time (min)	Cooking loss (%)	Rehydration ratio	Hardness (g)	Cohesiveness (g.s)	Chewiness (g.s)
1	17.5	30	8.3	4.61	1.98	584.1	0.87	274.54
2	10	22.5	8.8	4.85	1.74	570.19	0.72	259.23
3	10	30	8.91	4.9	1.71	565.08	0.67	252
4	17.5	22.5	8.35	4.44	2.15	595.07	0.97	282.54
5	17.5	22.5	8.4	4.4	2.2	590.12	0.92	271.44
6	25	15	8.97	4.98	1.75	565.12	0.67	246.24
7	17.5	22.5	8.3	4.55	2.35	600.29	1.01	281.12
8	17.5	15	8.82	4.82	1.81	574.35	0.74	257.45
9	17.5	22.5	8.25	4.22	2.3	587.23	0.87	270.67
10	25	30	8.75	4.74	1.95	577.12	0.77	259.12
11	25	22.5	8.69	4.7	2.02	583.22	0.83	264.12
12	17.5	22.5	8.49	4.3	2.25	581.34	0.82	276.45
13	10	15	9.23	5.12	1.45	554.52	0.58	231.35
			R²	0.9697	0.9723	0.9754	0.9816	0.9522
			Adjusted R²	0.9024	0.9211	0.9672	0.9671	0.8972
			Predicted R²	0.9452	0.9031	0.9526	0.9406	0.9042

Table 6.3. Experimental layout of the independent variables and their responses as given by the CCFC model for *Kenkowa bao*

Run	Parboiling time (min)	Pressure steaming time (min)	Cooking time (min)	Cooking loss (%)	Rehydration ratio	Hardness (g)	Cohesiveness (g.s)	Chewiness (g.s)
1	22.5	12.5	7.36	3.5	2.07	585.03	0.99	278.01
2	15	5	8.05	4.08	1.46	531.45	0.5	218.96
3	22.5	12.5	7.46	3.35	2.14	581.01	0.89	270.14
4	15	20	7.82	3.86	1.66	540.12	0.59	230.02
5	30	20	7.64	3.71	1.88	575.05	0.69	244.69
6	30	12.5	7.58	3.65	1.95	586.02	0.74	250.07
7	22.5	5	7.74	3.78	1.76	564.78	0.66	255.16
8	15	12.5	7.76	3.8	1.74	557.98	0.64	248.17
9	22.5	20	7.51	3.56	1.92	564.92	0.79	247.89
10	22.5	12.5	7.26	3.25	2.25	570.44	0.95	290.12
11	30	5	7.9	3.93	1.69	570.22	0.59	232.44
12	22.5	12.5	7.3	3.4	2.3	575.12	0.9	280.31
13	22.5	12.5	7.56	3.3	2.2	590.43	0.92	294.78
R²								
			0.9395	0.9456	0.9654	0.9706	0.9782	0.9987
Adjusted R²								
			0.9423	0.9421	0.9672	0.9638	0.9671	0.9272
Predicted R²								
			0.9189	0.9031	0.9533	0.9374	0.9378	0.9642

Table 6.4. Analysis of variance showing the quadratic interaction and lack of fit of the response variable for *Amona bao*

Source of variation	df	Response variable									
		Cooking time (min)		Cooking loss (%)		Rehydration ratio		Hardness (g)		Cohesiveness (g.s)	
		Sum of squares	F value	Sum of squares	F value	Sum of squares	F value	Sum of squares	F value	Sum of squares	F value
Regression	5	0.8410	14.53*	0.6963	31.56*	0.8593	16.54*	2329.65	23.06*	0.1674	9.90*
Residual error	7	0.0810		0.0309		0.0727		141.43		0.0237	
Lack of fit	3	0.0096	0.1784^{ns}	0.0090	0.6772^{ns}	0.0297	0.9215^{ns}	11.43	0.1172^{ns}	0.0006	0.3336^{ns}
Pure error	4	0.0715		0.0219		0.0430		130.00		0.0231	
Cor total	12	0.9221		0.7272		0.9320		2471.08		0.1910	

* significant at $p \leq 0.05$; ^{ns} not significant

Table 6.5. Analysis of variance showing the quadratic interaction and lack of fit of the response variable for *Badal bao*

Source of variation	df	Response variable									
		Cooking time (min)		Cooking loss (%)		Rehydration ratio		Hardness (g)		Cohesiveness (g.s)	
		Sum of squares	F value	Sum of square	F value	Sum of squares	F value	Sum of squares	F value	Sum of squares	F value
Regression	5	1.09	26.03*	0.8093	13.23*	0.8336	22.82*	1827.79	12.02*	0.1678	9.94*
Residual error	7	0.0587		0.0857		0.0511		212.83		0.0236	
Lack of fit	3	0.0244	0.9487^{ns}	0.0208	0.4269^{ns}	0.0261	1.39^{ns}	1.83	0.0116^{ns}	0.0006	0.0323^{ns}
Pure error	4	0.0343		0.0649		0.0250		210.99		0.0231	
Cor total	12	1.15		0.8949		0.8847		2040.61		0.1915	

* significant at $p \leq 0.05$; ^{ns} not significant

Table 6.6. Analysis of variance showing the quadratic interaction and lack of fit of the response variable for *Kenkwa bao*

Source of variation	df	Response variable									
		Cooking time (min)		Cooking loss (%)		Rehydration ratio		Hardness (g)		Cohesiveness (g.s)	
		Sum of squares	F value	Sum of squares	F value	Sum of squares	F value	Sum of squares	F value	Sum of squares	F value
Regression	5	0.6172	13.96*	0.7595	21.05*	0.7395	18.698	3346.05	15.08*	0.2920	27.28*
Residual error	7	0.0619		0.0505		0.0554		310.72		0.0150	
Lack of fit	3	0.0022	0.0491^{ns}	0.0135	0.4871^{ns}	0.0227	0.9263^{ns}	60.72	0.3238^{ns}	0.0084	1.69^{ns}
Pure error	4	0.0597		0.0370		0.0327		250.00		0.0066	
Cor total	12	0.6791		0.8100		0.7949		3656.77		0.3070	

* significant at $p \leq 0.05$; ^{ns} not significant

Regression equations for *Amona bao*

Cooking time

$$Y = 8.38 - 0.0917 X_1 - 0.1267 X_2 + 0.3053 X_1^2 + 0.2503 X_2^2 + 0.0250 X_1 X_2 \quad \text{Eq. 6.13}$$

Cooking loss

$$Y = 4.40 - 0.0750 X_1 - 0.1100 X_2 + 0.2840 X_1^2 + 0.2290 X_2^2 + 0.0020 X_1 X_2 \quad \text{Eq. 6.14}$$

Rehydration ratio

$$Y = 2.20 + 0.1350 X_1 + 0.1067 X_2 - 0.2884 X_1^2 - 0.2634 X_2^2 - 0.0200 X_1 X_2 \quad \text{Eq. 6.15}$$

Hardness

$$Y = 588.28 + 7.33 X_1 + 6.67 X_2 - 13.97 X_1^2 - 13.97 X_2^2 - 0.00120 X_1 X_2 \quad \text{Eq. 6.16}$$

Cohesiveness

$$Y = 0.8959 + 0.0483 X_1 + 0.0533 X_2 - 0.1405 X_1^2 - 0.1055 X_2^2 + 0.0025 X_1 X_2 \quad \text{Eq. 6.17}$$

Chewiness

$$Y = 278.59 + 2.87 X_1 + 5.94 X_2 - 24.20 X_1^2 - 13.39 X_2^2 + 1.10 X_1 X_2 \quad \text{Eq. 6.18}$$

Regression equations of *Badal bao*

Cooking time

$$Y = 8.36 - 0.0833 X_1 - 0.1767 X_2 + 0.3957 X_1^2 + 0.2107 X_2^2 + 0.0250 X_1 X_2 \quad \text{Eq. 6.19}$$

Cooking loss

$$Y = 4.41 - 0.0750 X_1 - 0.1117 X_2 + 0.3095 X_1^2 + 0.2495 X_2^2 - 0.0050 X_1 X_2 \quad \text{Eq. 6.20}$$

Rehydration ratio

$$Y = 2.22 + 0.1367 X_1 + 0.1050 X_2 - 0.2783 X_1^2 - 0.2633 X_2^2 - 0.0150 X_1 X_2 \quad \text{Eq. 6.21}$$

Hardness

$$Y = 590.76 + 5.94 X_1 + 5.38 X_2 - 13.94 X_1^2 - 11.42 X_2^2 + 0.3600 X_1 X_2 \quad \text{Eq. 6.22}$$

Cohesiveness

$$Y = 0.9166 + 0.0500 X_1 + 0.0533 X_2 - 0.1379 X_1^2 - 0.1079 X_2^2 + 0.0025 X_1 X_2 \quad \text{Eq. 6.23}$$

Chewiness

$$Y = 277.00 + 4.48 X_1 + 8.44 X_2 - 16.72 X_1^2 - 12.40 X_2^2 - 1.94 X_1 X_2 \quad \text{Eq. 6.24}$$

Regression Equations for *Kenkwa bao*

Cooking time

$$Y = 7.40 - 0.0850 X_1 - 0.1250 X_2 + 0.2557 X_1^2 + 0.2107 X_2^2 - 0.0075 X_1 X_2 \quad \text{Eq. 6.25}$$

Cooking loss

$$Y = 3.38 - 0.0750 X_1 - 0.1100 X_2 + 0.2974 X_1^2 + 0.2424 X_2^2 + 0.0010 X_1 X_2 \quad \text{Eq. 6.26}$$

Rehydration ratio

$$Y = 2.17 + 0.1100 X_1 + 0.0917 X_2 - 0.2653 X_1^2 - 0.2603 X_2^2 - 0.0025 X_1 X_2 \quad \text{Eq. 6.27}$$

Hardness

$$Y = 580.38 + 17.00 X_1 + 2.50 X_2 - 9.83 X_1^2 - 16.33 X_2^2 - 1.25 X_1 X_2 \quad \text{Eq. 6.28}$$

Cohesiveness

$$Y = 0.9152 + 0.0483 X_1 + 0.0533 X_2 - 0.1881 X_1^2 - 0.1531 X_2^2 + 0.0025 X_1 X_2 \quad \text{Eq. 6.29}$$

Chewiness

$$Y = 277.93 + 4.83 X_1 + 2.67 X_2 - 25.26 X_1^2 - 22.76 X_2^2 + 0.7500 X_1 X_2 \quad \text{Eq. 6.30}$$

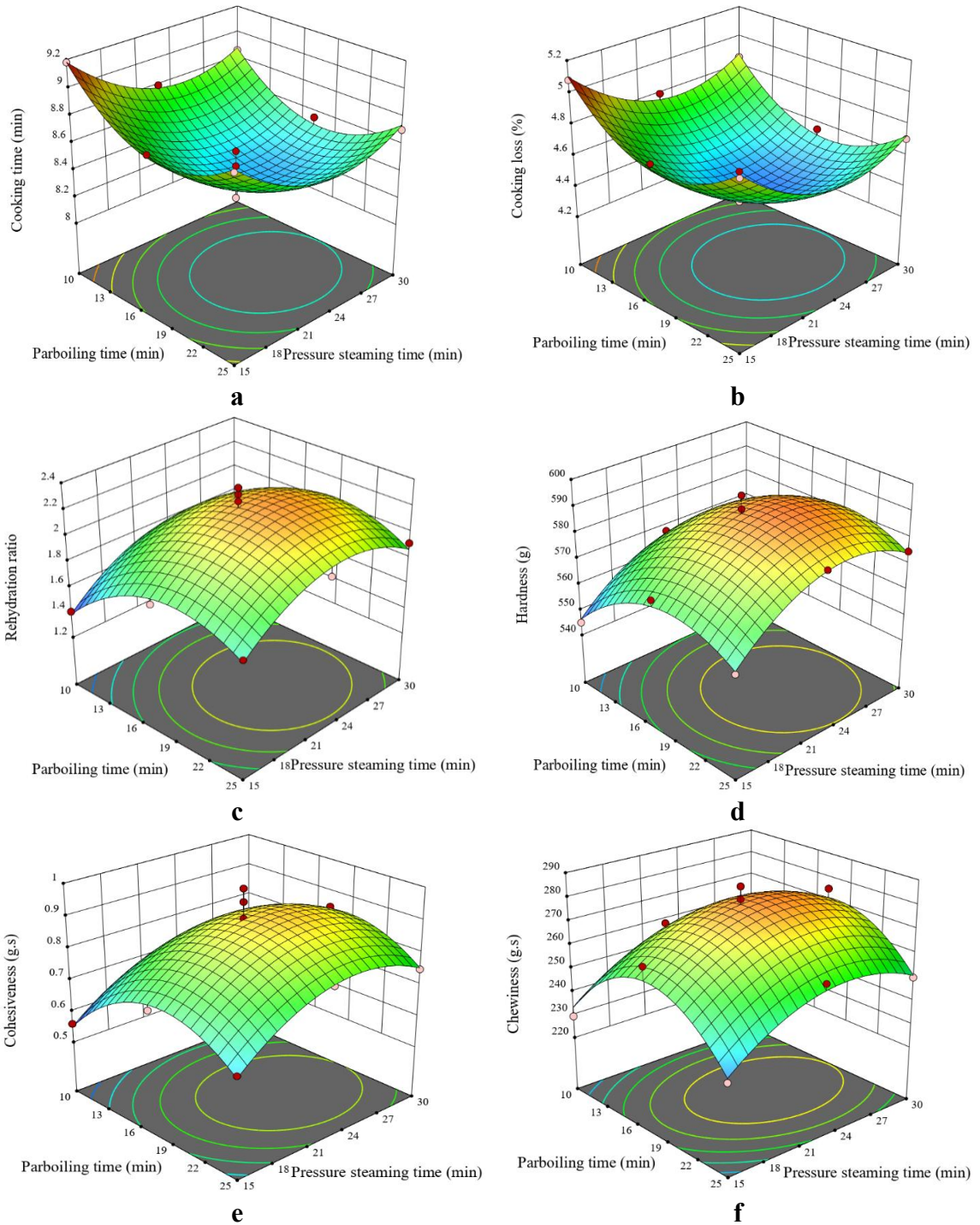


Fig. 6.1. Variation of response variables (a) Cooking time, (b) Cooking loss, (c) Rehydration ratio, (d) Hardness, (e) Cohesiveness and (f) Chewiness due to the interaction between the independent variables rice parboiling time and pressure steaming time in *Amona bao*

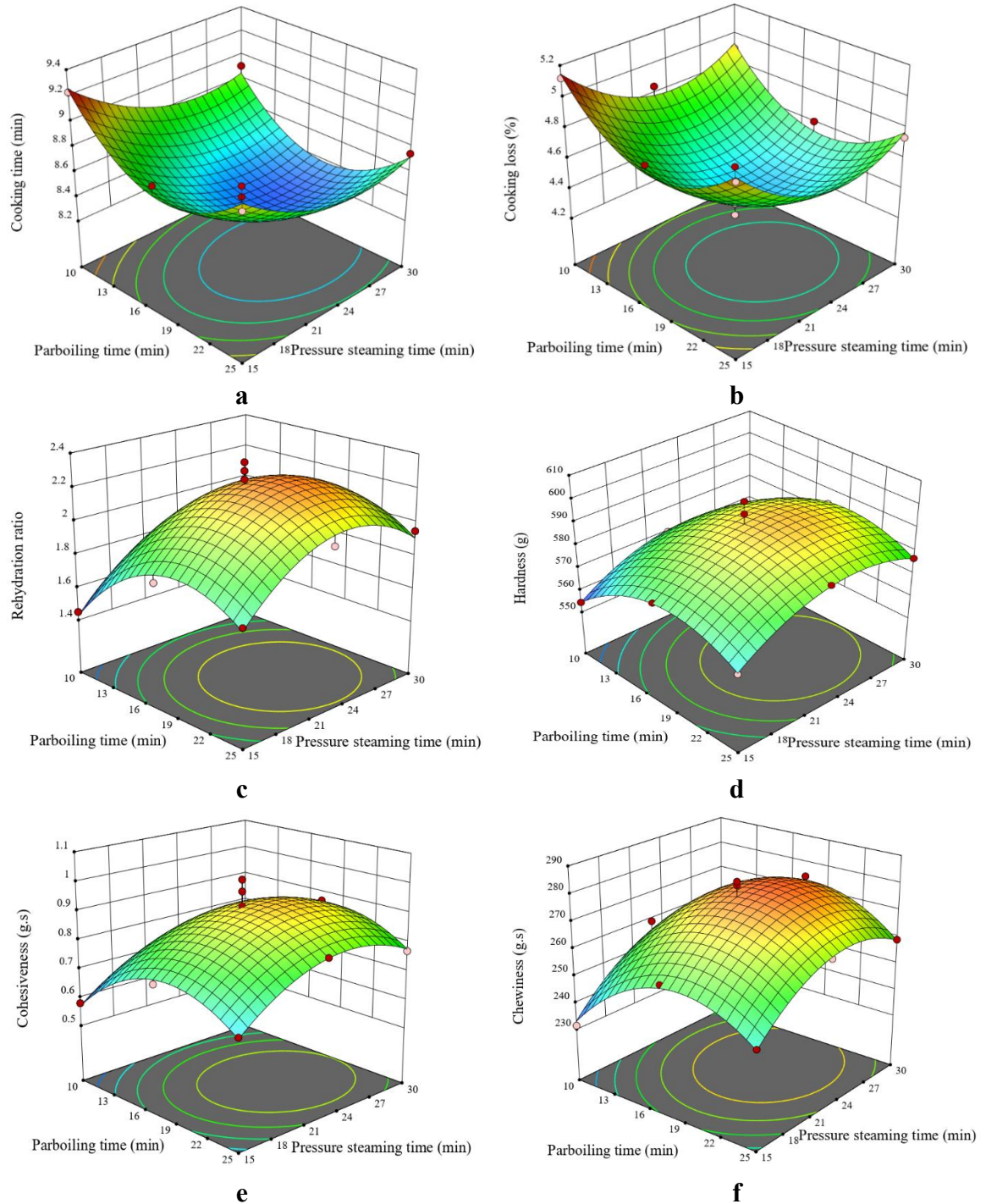


Fig. 6.2. Variation of response variables (a) Cooking time, (b) Cooking loss, (c) Rehydration ratio, (d) Hardness, (e) Cohesiveness and (f) Chewiness due to the interaction between the independent variables rice parboiling time and pressure steaming time in *Badal bao*

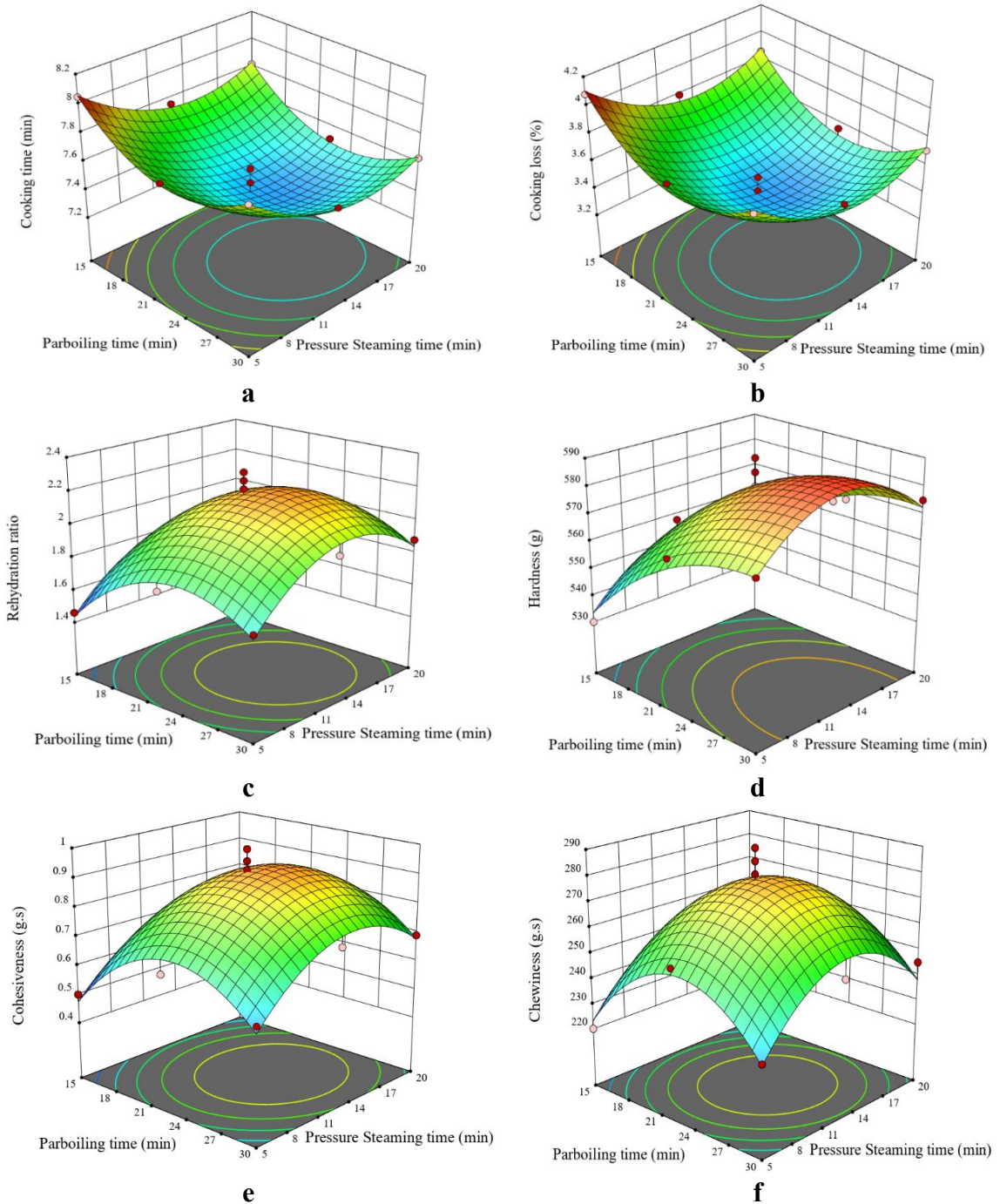


Fig. 6.3. Variation of response variables (a) Cooking time, (b) Cooking loss, (c) Rehydration ratio, (d) Hardness, (e) Cohesiveness and (f) Chewiness due to the interaction between the independent variables rice parboiling time and pressure steaming time in *Kenkowa bao*

6.3.3. Optimization

Numerical optimization using a desirability function approach targeted simultaneous minimization of cooking loss and cooking time while maximizing hardness, chewiness and acceptable cohesiveness. The optimized region corresponded to moderate parboiling combined with intermediate to high pressure steaming, where controlled gelatinization and regulated amylose retrogradation produced a structurally cohesive yet sufficiently porous matrix. Under these conditions, starch molecular rearrangement was optimized to balance hydration kinetics with mechanical strength, resulting in minimal solid leaching and enhanced textural integrity. Variety wise differences were governed primarily by amylose content. *Kenkowa* required slightly controlled steaming to prevent excessive densification, whereas *Amona* benefited from comparatively higher treatment intensity to compensate for lower amylose driven reinforcement.

Design Expert 12 predicted parboiling time and pressure steaming time as 18.75 min and 24.25 min respectively for *Amona bao*, 19.32 min and 20.99 min respectively for *Badal bao* and 22.64 min and 14.64 min for *Kenkowa bao* respectively as optimum process variables to develop pasta (Table 6.7, 6.8, 6.9). The desirability values were 0.998, 0.985 and 0.992 respectively. The cooking loss, cooking time, rehydration ratio and textural parameters namely hardness, cohesiveness and chewiness of the cooked pasta obtained from the optimized process variables for each variety strongly correlated with the predicted values of responses (Table 6.10, 6.11, 6.12). The optimized pasta samples, along with the corresponding positive and negative controls are illustrated in Fig. 6.4.

Table 6.7. Estimated optimal conditions of the independent variables for *Amona bao*

Factor	Low level	High level	Optimal level
Parboiling time (min)	10	25	18.75
Pressuresteaming time (min)	15	30	24.25

Table 6.8. Estimated optimal conditions of the independent variables for *Badal bao*

Factor	Low level	High level	Optimal level
Parboiling time (min)	10	25	19.32
Pressure steaming time (min)	15	30	20.99

Table 6.9. Estimated optimal conditions of the independent variables for *Kenkowa bao*

Factor	Low level	High level	Optimal level
Parboiling time (min)	15	30	22.60
Pressure steaming time (min)	5	20	14.64

Table 6.10. The predicted and experimental values of the responses for *Amona bao*

Responses	Predicted value	Experimental value
Cooking time (min)	8.35	8.26
Cooking loss (%)	4.38	4.35
Rehydration ratio	2.227	2.234
Hardness (g)	589.90	589.84
Cohesiveness (g.s)	0.907	0.905
Chewiness (g.s)	278.17	277.88

Table 6.11. The predicted and experimental values of the responses for *Badal bao*

Responses	Predicted value	Experimental value
Cooking time (min)	8.40	8.20
Cooking loss (%)	4.43	4.40
Rehydration ratio	2.20	2.18
Hardness (g)	589.82	590.12
Cohesiveness (g.s)	0.905	0.903
Chewiness (g.s)	275.05	272.92

Table 6.12. The predicted and experimental values of the responses for *Kenkowa bao*

Responses	Predicted value	Experimental value
Cooking time (min)	7.37	7.39
Cooking loss (%)	3.36	3.32
Rehydration ratio	2.17	2.18
Hardness (g)	580.11	582.04
Cohesiveness (g.s)	0.913	0.911
Chewiness (g.s)	279.58	277.52



(a) A(NC)



(b) B(NC)



(c) K(NC)



(d) A(PC)



(e) B(PC)



(f) K(PC)



(g) A(O)



(h) B(O)



(i) K(O)

Fig. 6.4. Pasta developed from raw flour (NC), raw flour with 0.5% xanthan gum (PC) and optimized pretreatment conditions (O)

6.3.4. Cooking time

The optimized pasta samples exhibited significantly lower cooking time compared to the negative controls across all three varieties, while remaining statistically comparable to the positive control (Fig. 6.5a). Reduction in cooking time of the optimized samples could be attributed to hydrothermal pretreatment induced partial gelatinization, which disrupted the native semicrystalline starch architecture and enhanced water accessibility during subsequent cooking. Parboiling and pressure steaming likely induced melting of amylopectin crystalline lamellae and swelling of amorphous domains, thereby reducing the energy barrier required for complete gelatinization during cooking (Kraithong et al., 2019). In contrast, the negative control retained intact crystalline regions that required longer hydration and thermal input to achieve core softening. The similarity between optimized samples and positive control pasta suggests that hydrothermal restructuring effectively modified hydration kinetics without compromising structural integrity. Among the varieties, *Kenkowa* exhibited slightly higher cooking time relative to *Amona* and *Badal*, which may be associated with increased amylose reassociation and matrix densification that moderately restricted water diffusion.

6.3.5. Cooking loss

Cooking loss was significantly reduced in optimized samples compared to the negative control (Fig. 6.5b), indicating enhanced structural integrity and resistance to solid leaching during cooking. The higher cooking loss observed in negative control pasta is attributable to weak inter granular bonding and rapid solubilization of starch components, particularly amylose, into the cooking medium. In contrast, hydrothermal optimization promoted controlled amylose exudation followed by retrogradation during drying, leading to formation of double-helical aggregates and strengthened intermolecular hydrogen bonding. This reorganized network acted as a barrier matrix, restricting diffusion of soluble solids and thereby minimizing cooking loss (Yan et al., 2025). The optimized samples demonstrated cooking loss values statistically comparable

to the positive control, suggesting that retrograded starch network formation effectively mimicked the stabilizing and viscosity enhancing role of xanthan gum. The lowest cooking loss was observed in *Kenkowa*, likely due to its higher amylose content facilitating stronger network reinforcement through enhanced retrogradation (Wei et al., 2022).

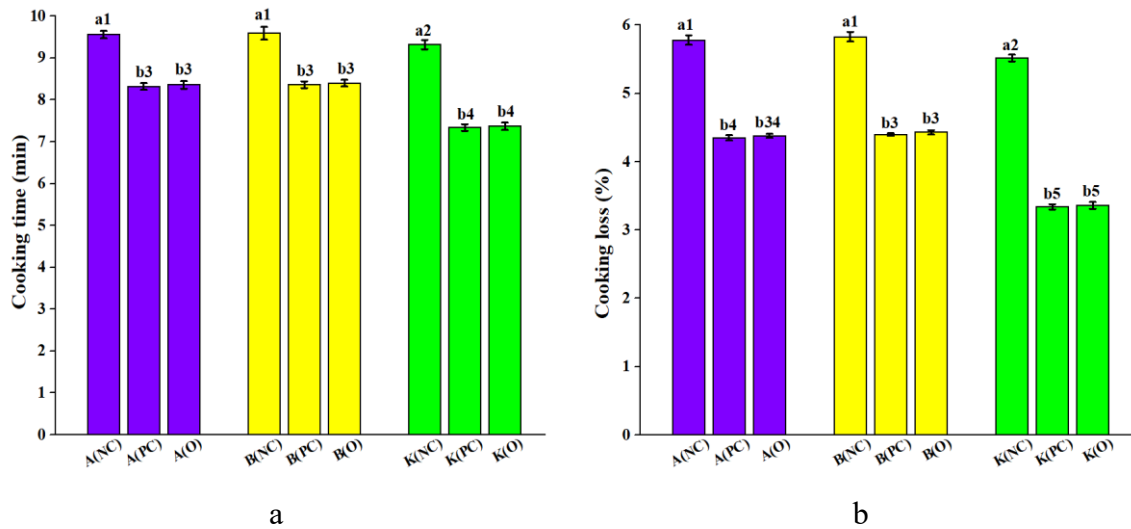


Fig. 6.5. (a) Cooking time and (b) Cooking loss of the developed pasta

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within variety and same numbers across varieties are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

6.3.6. Texture

Optimized samples exhibited significantly higher hardness than the negative control and were statistically comparable to the positive control (Fig. 6.6a). The increased hardness was attributed to the formation of a dense retrograded amylose network generated during hydrothermal processing and subsequent drying. Partial gelatinization allowed amylose chains to leach and redistribute within the matrix and upon cooling, these chains reassociated into ordered double-helical structures, increasing matrix rigidity. The negative control lacked this pre-structured network, resulting in a weaker and softer texture. *Kenkowa* demonstrated the highest hardness among varieties, which is

consistent with its higher amylose content and greater tendency for crystallite formation and structurally reinforced network development.

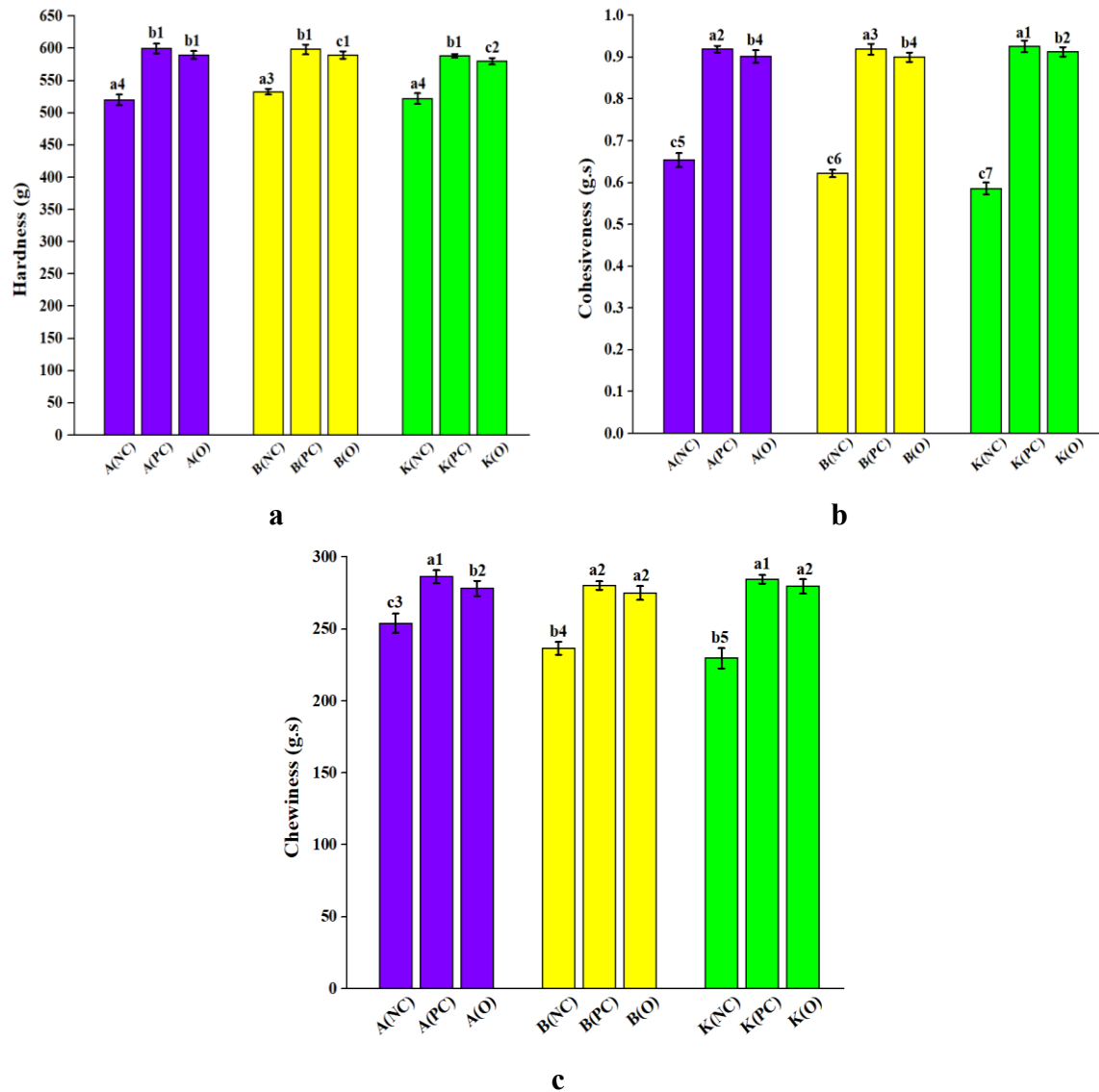


Fig. 6.6. Textural properties (a) Hardness, (b) Cohesiveness and (c) Chewiness of the developed pasta

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within variety and same numbers across varieties are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

Cohesiveness significantly improved in optimized samples compared to raw flour pasta (Fig. 6.6b), indicating enhanced internal bonding and resistance to structural breakdown

under compressive force. Hydrothermal processing likely promoted improved intermolecular hydrogen bonding within the starch matrix, resulting in greater structural continuity. The negative control exhibited lower cohesiveness due to poor internal matrix organization and limited polymer reassociation. Interestingly, *Badal* showed relatively balanced cohesiveness, suggesting that intermediate amylose content allows sufficient retrogradation for network formation without inducing excessive brittleness that may compromise structural recovery (Wei et al., 2022).

Chewiness was significantly higher in the optimized samples than in the negative controls and closely resembled that of the positive control pasta (Fig. 6.6c). The increase in chewiness reflects the formation of a compact and elastic starch network capable of sustaining deformation energy before breakdown. Hydrothermal restructuring enhanced molecular alignment and subsequent recrystallization of amylose chains, contributing to greater resistance to mastication (Gao et al., 2023). *Kenkowa* exhibited the highest chewiness values, correlating with its enhanced retrogradation potential and stronger three-dimensional network formation. The similarity between optimized and positive control pasta confirms that hydrothermal modification successfully imparted structural reinforcement typically achieved through hydrocolloid incorporation.

6.3.7. Digestive properties

The optimized pasta samples exhibited significantly lower RDS values compared to the negative control across all three rice varieties (Fig. 6.7a), indicating reduced susceptibility to rapid enzymatic hydrolysis. The high RDS observed in raw flour pasta can be attributed to intact but weakly organized starch matrices that undergo rapid gelatinization during cooking, leading to extensive granule swelling and exposure of amorphous regions to α -amylase attack. In contrast, hydrothermal optimization promoted controlled gelatinization followed by amylose reassociation and retrogradation during drying, resulting in formation of ordered double-helical structures and compact domains that limited enzyme accessibility. This restructured matrix reduced the

proportion of readily hydrolyzable starch fractions (Oliveira et al., 2026). Compared to the positive control, optimized samples showed comparable or slightly reduced RDS values, suggesting that hydrothermal restructuring of starch can effectively mimic the diffusion-barrier effect of hydrocolloids by physically restricting enzymatic penetration. Among the varieties, *Kenkowa* exhibited the lowest RDS, likely due to its higher amylose content facilitating greater retrogradation and formation of enzyme-resistant domains.

SDS content was significantly higher in optimized samples compared to the negative control (Fig. 6.7b), indicating a shift from rapid to gradual glucose release kinetics. The increase in SDS can be attributed to partial gelatinization followed by controlled retrogradation, which generated semi-ordered regions that were not completely resistant but hydrolyzed at a slower rate. These semi-crystalline structures likely formed through amylose-amylopectin interactions and chain realignment during cooling. In the negative control, absence of pre-structured organization resulted in rapid granule disintegration during cooking, favoring rapid digestion and limiting formation of intermediate digestion fractions. The optimized samples demonstrated SDS levels comparable to or higher than positive control pasta, suggesting that hydrothermal processing enhanced internal matrix density and modulated enzymatic diffusion pathways. *Kenkowa* and *Badal* showed greater SDS, reflecting increased potential for forming compact but partially accessible starch domains.

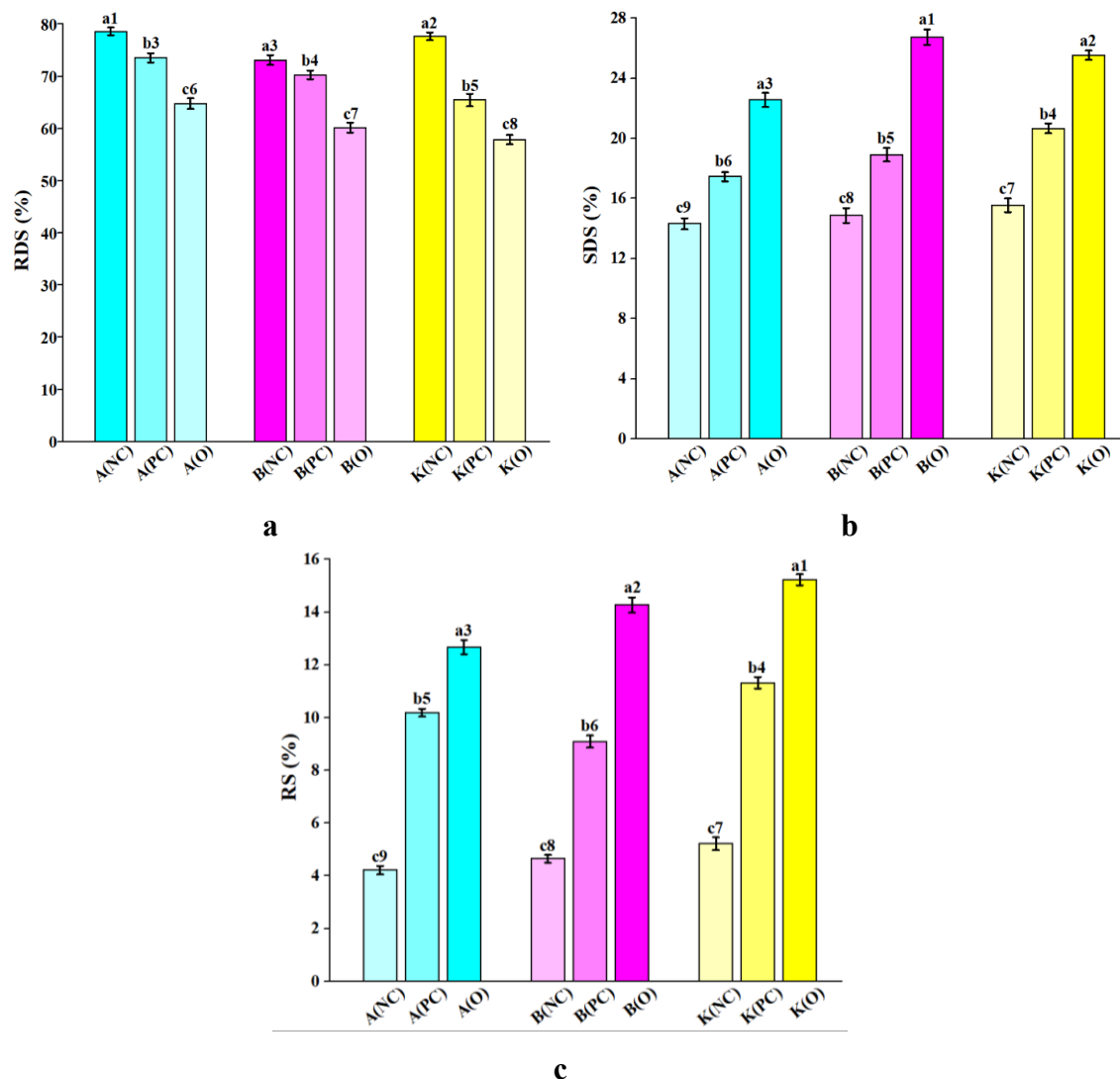


Fig. 6.7. Digestive properties (a) RDS, (b) SDS and (c) RS of the developed pasta
 Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within variety and same numbers across varieties are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

RS content was markedly increased in optimized samples relative to the negative control (Fig. 6.7c), demonstrating the effectiveness of hydrothermal processing in promoting retrogradation induced enzyme resistance. The elevated RS fraction can be attributed primarily to formation of retrograded amylose (RS type III), generated during cooling and drying after hydrothermal treatment. Amylose chains reassociated into tightly packed double helices and crystalline aggregates stabilized by extensive intermolecular

hydrogen bonding, which restrict enzymatic hydrolysis. The negative control exhibited the lowest RS due to lack of prior gelatinization and insufficient molecular mobility required for retrogradation. While xanthan gum may reduce enzyme diffusion through viscosity effects, it does not directly promote retrograded crystallite formation. Thus, the optimized samples showed comparable or higher RS levels than the positive control. *Kenkowa* exhibited the highest RS content, consistent with its higher amylose concentration and greater retrogradation potential.

6.3.8. Bioactive properties

The bioactive properties of the developed pasta, including total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity (DRSA) and ferric reducing antioxidant power (FRAP), are presented in Fig. 6.8. The optimized samples showed a slight but significant reduction in TPC and TFC compared to the negative control across all three rice varieties. This decrease can be attributed primarily to thermal degradation and leaching of phenolic compounds during parboiling and pressure steaming. Hydrothermal processing may also induce partial oxidation or structural transformation of bound phenolics, thereby reducing extractable phenolic fractions (Kanan et al., 2024). However, despite this reduction, the optimized samples retained substantial phenolic content, indicating that pigmented rice matrices exhibit considerable thermal stability of bioactive compounds. The positive control did not demonstrate significant improvement in TPC or TFC compared to the negative control, confirming that hydrocolloid incorporation does not directly influence intrinsic phenolic concentration. The antioxidant activities measured through DRSA and FRAP followed trends consistent with phenolic content. Optimized samples exhibited slightly lower DRSA and FRAP compared to negative control, likely due to partial degradation of thermolabile antioxidant compounds during hydrothermal processing (Kabira et al., 2025). Nevertheless, antioxidant activity remained relatively high, suggesting preservation of a substantial fraction of polyphenolic constituents. The reduction in FRAP values in optimized samples may also be associated with structural modification

of phenolic hydroxyl groups responsible for electron donation capacity (Muchlisiyah et al., 2023). Importantly, antioxidant activity in optimized pasta was slightly lower than the positive control. Varietal differences were evident, with *Amona* and *Badal* exhibiting higher TPC, TFC and DRSA than *Kenkowa* however in case of FRAP *Badal* showed the highest potential followed by *Amona* and *Kenkowa*, reflecting intrinsic differences in pigment concentration and phenolic composition among the rice varieties.

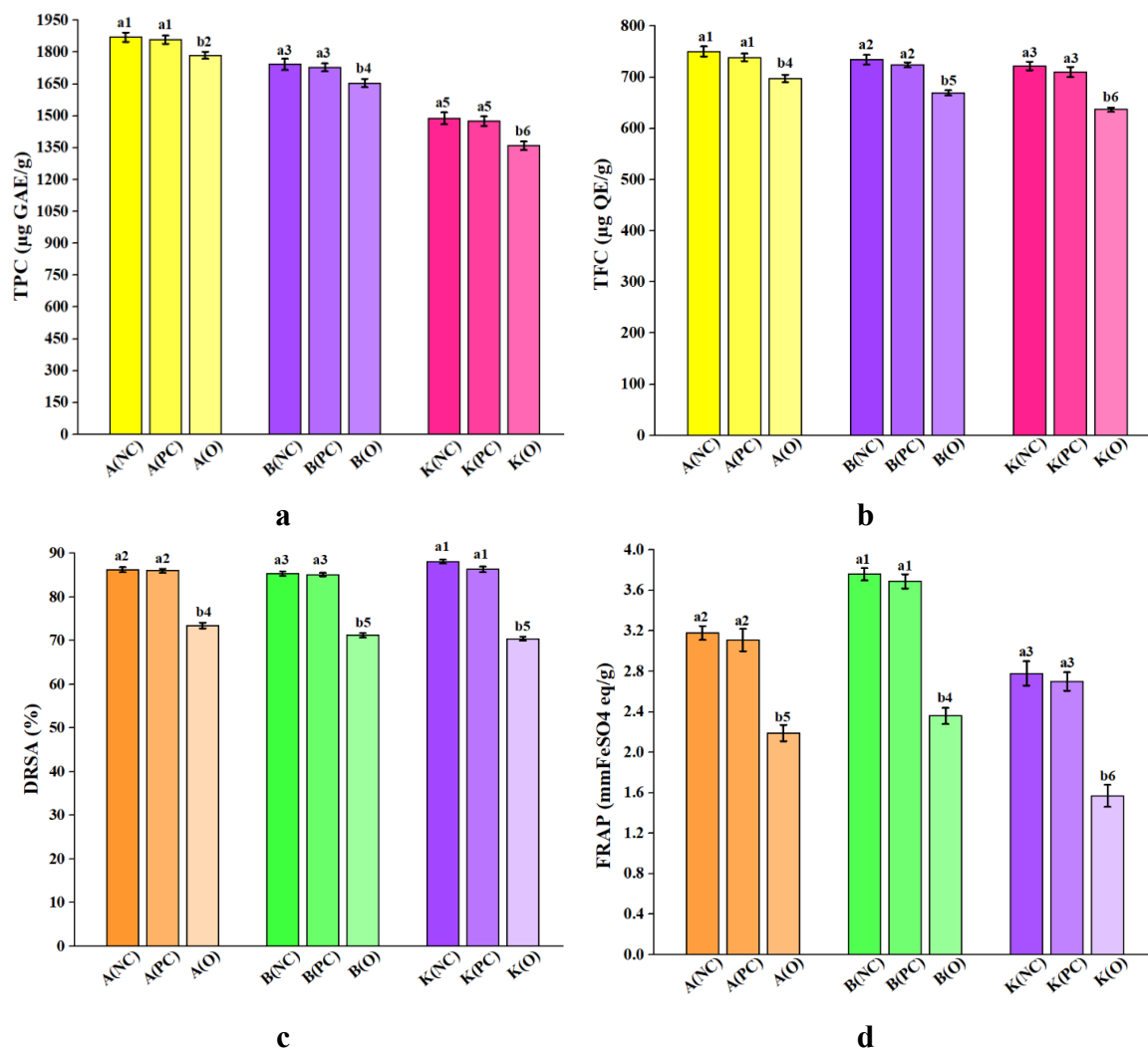


Fig. 6.8. Bioactive properties (a) TPC, (b) TFC, (c) DRSA and (d) FRAP of the developed pasta

Values are expressed as mean $\pm$ SD. Bars sharing the same lowercase letters within variety and same numbers across varieties are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

6.3.9. Color values

The color attributes of the developed pasta are presented in Table 6.13. Significant differences ($p \leq 0.05$) were observed among the NC, PC and O samples within each rice variety. The optimized samples exhibited significantly lower L^* values compared to both NC and PC, indicating darker appearance. This reduction in lightness can be attributed to intensified pigment concentration and possible thermal induced browning reactions during hydrothermal processing.

Table 6.13. Color values and sensory OAA of the developed pasta

Samples	L^*	a^*	b^*	OAA
A(NC)	52.12±2.12 ^{a2}	10.63±0.10 ^{b4,5}	8.47±6.72 ^{a3}	6.45±0.23 ^{c7}
A(PC)	53.92±1.34 ^{a2}	10.71±0.09 ^{b4}	8.44±4.56 ^{a3}	8.33±0.17 ^{b4}
AO	48.90±2.12 ^{b3}	11.80±0.05 ^{a2}	8.16±5.32 ^{b5}	8.69±0.12 ^{a1}
B(NC)	55.75±1.56 ^{a1}	10.88±0.05 ^{b3}	8.56±4.55 ^{a2}	6.49±0.19 ^{c7}
B(PC)	55.43±1.65 ^{a1}	10.95±0.07 ^{b3}	8.59±2.92 ^{a2}	8.26±0.12 ^{b5}
B(O)	43.82±1.92 ^{c4}	11.98±0.09 ^{a1}	8.05±6.72 ^{b6}	8.58±0.22 ^{a2}
K(NC)	52.12±1.44 ^{a2}	10.59±0.07 ^{b5}	9.08±7.12 ^{a1}	6.29±0.25 ^{c7}
K(PC)	53.46±2.08 ^{a2}	10.72±0.07 ^{b4}	9.15±3.05 ^{a1}	8.19±0.17 ^{b6}
KO	48.04±1.79 ^{a3}	11.85±0.08 ^{a2}	8.88±5.66 ^{b4}	8.44±0.15 ^{a3}

Means followed by the same superscript lowercase letter within a column for a given variety, and the same superscript number within a column across varieties, are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test.

Parboiling and pressure steaming may have enhanced pigment diffusion from bran layers into the starch matrix, resulting in intensified color development. The redness parameter (a^*) significantly increased in optimized samples across all varieties. The higher a^* values suggest enhanced expression or stabilization of anthocyanins and other pigmented phenolics during hydrothermal treatment. Moreover, maillard reactions and pigment oxidation could also occur during processing (Jafari et al., 2017). Structural modification of the starch matrix may have improved pigment retention within the pasta

network, thereby intensifying red tonal characteristics. In contrast, the NC and PC samples showed comparatively lower a^* values, reflecting less pigment integration into the matrix. The yellowness parameter (b^*) showed slight variation among treatments, with optimized samples generally exhibiting marginally lower b^* values compared to controls. This shift may reflect pigment structural changes or dilution effects associated with matrix reorganization during hydrothermal processing. Similar trends have been reported in high-temperature short-time treated rice noodles (Yan et al., 2025). Overall, the optimized pasta demonstrated a darker and more intense red hue, which may contribute positively to consumer perception.

6.3.10. Sensory overall acceptability

Sensory evaluation revealed significant differences in overall acceptability among treatments (Table 6.13). The optimized samples recorded the highest OAA scores across all three rice varieties, followed by the positive control, while the negative control received the lowest scores. The lower OAA of NC samples can be attributed to softer structure and higher cooking loss, reduced cohesiveness and chewiness. Incorporation of xanthan gum in the positive control improved structural integrity and sensory perception. However, the optimized samples surpassed PC in acceptability, indicating that hydrothermal restructuring provided superior textural balance and mouthfeel. The improved OAA of optimized pasta may be associated with its enhanced firmness, balanced chewiness, reduced cooking loss and intensified color characteristics, which collectively contribute to better visual appeal and eating quality. The darker and more vibrant red tone of optimized samples likely reinforced the perception of whole grain authenticity and functional quality. Among varieties, slight differences in OAA were observed, potentially linked to intrinsic variations in amylose content and pigment composition influencing both texture and flavor perception.

6.3.11. Proximate composition

The proximate composition and calculated energy values of the optimized pasta samples are presented in Table 6.14. Moisture content ranged narrowly among AO, BO and KO, with no significant differences ($p \leq 0.05$), indicating uniform drying efficiency and comparable storage stability across varieties. The fat content remained low (~ 2.1 - 2.3%), reflecting the intrinsic lipid content of red rice flour and suggesting minimal alteration during hydrothermal processing. Protein content was also comparable among samples (~ 8.1 - 8.3%), demonstrating that hydrothermal optimization primarily modified starch structure without significantly affecting protein composition. Similarly, ash content showed minor variation, representing stable mineral retention from the pigmented rice varieties. Carbohydrate content constituted the major macronutrient fraction (~ 78 - 80%), consistent with cereal-based pasta products. Slight varietal differences in carbohydrate percentage may be attributed to intrinsic compositional variation among *Amona*, *Badal* and *Kenkowa*. The calculated energy values ranged approximately between 338 and 349 kcal/100 g, with AO exhibiting slightly higher energy due to marginally elevated carbohydrate levels. However, overall caloric differences were minimal and nutritionally comparable across varieties.

Table 6.14. Proximate composition and energy value of the optimized pasta

Samples	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Carbohydrate (%)	Energy (kcal/100g)
AO	8.99 $\pm$ 1.58 ^a	2.13 $\pm$ 0.06 ^c	8.18 $\pm$ 0.09 ^b	1.60 $\pm$ 0.03 ^b	79.74 $\pm$ 0.54 ^a	349.21 $\pm$ 1.15 ^a
BO	8.95 $\pm$ 1.09 ^a	2.21 $\pm$ 0.09 ^b	8.21 $\pm$ 0.15 ^a	1.69 $\pm$ 0.06 ^a	78.94 $\pm$ 0.67 ^a	343.77 $\pm$ 2.12 ^b
KO	8.90 $\pm$ 1.16 ^a	2.30 $\pm$ 0.12 ^a	8.24 $\pm$ 0.08 ^a	1.71 $\pm$ 0.08 ^a	79.06 $\pm$ 0.29 ^a	338.92 $\pm$ 1.49 ^c

Means followed by the same superscript lowercase letters within a column are not significantly different ($p \leq 0.05$) as indicated through Duncan's multiple range test

6.3.12. Scanning electron microscopy

Scanning electron micrographs of surface morphology, cross-sectional structure and 500X magnification revealed pronounced microstructural differences among the NC, PC and O pasta samples across all three rice varieties (Fig. 6.9). The NC samples exhibited rough, irregular surfaces with visible cracks and discontinuities, indicating weak intergranular bonding and absence of starch gelatinization prior to drying. The presence of discrete granule remnants and structural heterogeneity suggests insufficient molecular integration and minimal intermolecular interactions, resulting in a porous internal architecture. Such discontinuities facilitate rapid water penetration and enzymatic accessibility during cooking, explaining the higher cooking loss, lower hardness and chewiness and elevated RDS values observed in NC samples. The PC samples showed comparatively smoother and more compact surfaces due to xanthan gum incorporation, which likely formed a viscous external network that filled void spaces and enhanced matrix continuity (Kraithog and Rawdkuen, 2020). However, minor micro cracks were still evident, indicating that reinforcement was primarily due to hydrocolloid thickening rather than intrinsic starch restructuring.

In contrast, the optimized samples displayed a markedly smoother, denser and more continuous surface with reduced fissures and minimal void spaces in cross-section. Hydrothermal treatment likely induced the formation of a cohesive three-dimensional network stabilized by intermolecular hydrogen bonding. At 500X magnification of the cross-section samples, optimized samples exhibited fused starch domains with minimal granular remnants and reduced porosity, reflecting effective gelatinization and molecular reassociation. This compact and homogeneous architecture restricted solid leaching and enzyme diffusion (Yan et al., 2025). Overall, SEM analysis confirms that hydrothermal optimization produced a dense matrix comparable to or superior to xanthan gum stabilization and directly supporting the observed improvements in cooking quality, texture and digestibility.

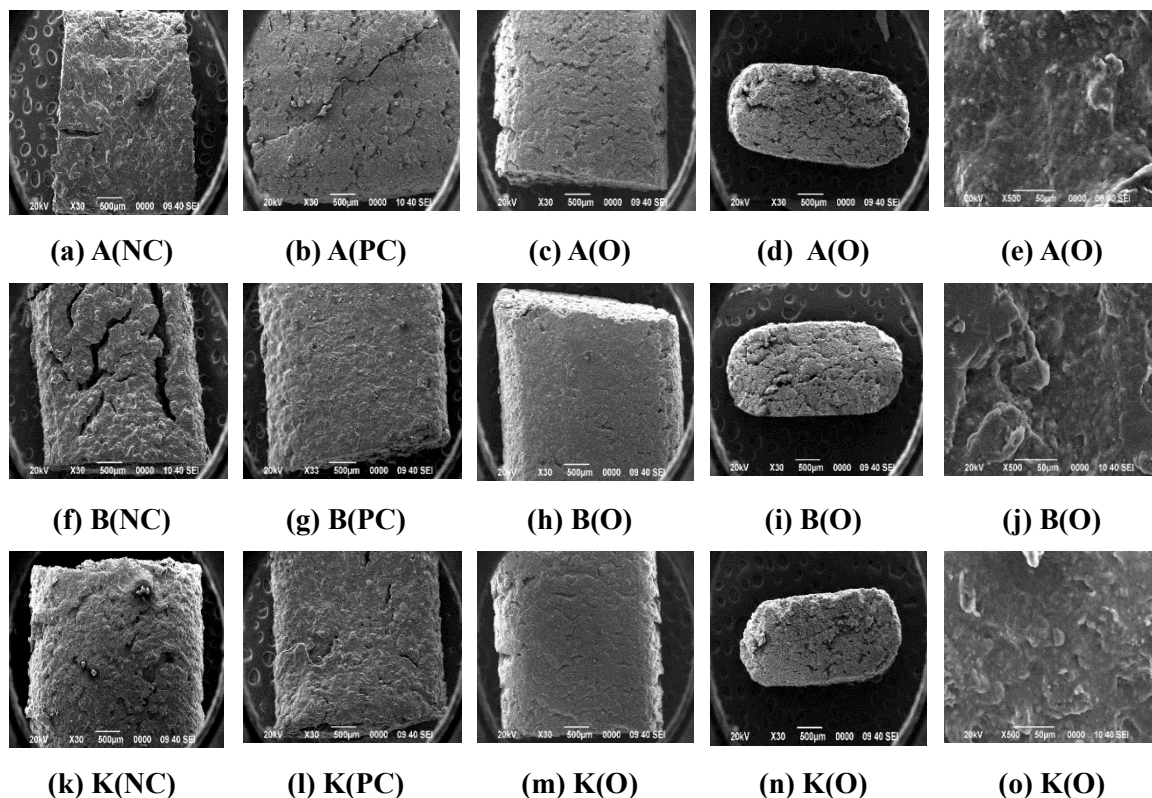


Fig. 6.9. SEM micrographs of (a-c, f-h, k-m) surface, (d, e, i) cross-section and (e, j, o) 500X magnification (of the cross-section) of the pasta samples

6.3.13. Storage study

Moisture content increased significantly ($p \leq 0.05$) with storage time in all optimized samples (AO, BO and KO), rising from approximately 9-10% at day 0 to about 11-12% by day 35 (Fig. 6.10a). A similar increasing trend was observed for water activity (a_w), which increased significantly ($p \leq 0.05$) across successive storage intervals (Fig. 6.10b). The progressive rise in moisture and a_w can be attributed to moisture absorption under high relative humidity (75% RH). Although significant differences were observed across storage days, a_w values remained below 0.60 throughout the study, indicating limited susceptibility to rapid microbial proliferation. Among samples, KO exhibited significantly lower moisture uptake at later stages ($p \leq 0.05$), possibly due to its denser retrograded starch matrix restricting moisture diffusion.

Total aerobic plate count (TAPC) and yeast and mold count (YMC) increased significantly ($p \leq 0.05$) during storage for all samples (Fig. 6.10c, 6.10d). The increase was gradual and correlated with rising moisture content and a_w values. However, microbial counts remained within acceptable safety limits ($<4 \log \text{ cfu g}^{-1}$ for TAPC and $<2.5 \log \text{ cfu g}^{-1}$ for YMC) throughout the 35-day accelerated storage period. Significant differences were observed between initial and final storage intervals ($p \leq 0.05$), but differences among varieties were comparatively minor. KO showed significantly lower microbial counts at storage intervals ($p \leq 0.05$), which may be associated with its comparatively lower a_w and compact microstructure limiting microbial accessibility. Free fatty acid (FFA), peroxide value (PV), and TBARS increased significantly ($p \leq 0.05$) throughout storage (Fig. 6.10e, f and Fig. 6.11a), indicating progressive lipid hydrolysis and oxidation under accelerated conditions. The rise in PV and TBARS between storage intervals reflected the formation of primary and secondary lipid oxidation products, respectively. Significant varietal differences ($p \leq 0.05$) were also observed, likely due to differences in lipid composition and endogenous antioxidant content among the rice varieties. Despite these increases, FFA ($<1.5\%$), PV ($<10 \text{ meq O}_2/\text{kg}$), and TBARS ($<1.0 \text{ mg MDA/kg}$) values remained within acceptable quality limits throughout the 35-day storage period. Hardness, cohesiveness and chewiness decreased significantly ($p \leq 0.05$) with storage time (Fig. 6.11). The decline in hardness was statistically significant after 14 days of storage, likely due to moisture induced plasticization of the retrograded starch network. Increased moisture disrupts intermolecular hydrogen bonding and reduces crystalline stability within reassociated amylose domains, resulting in matrix softening. Cohesiveness and chewiness showed similar significant reductions ($p \leq 0.05$), indicating progressive weakening of internal structural integrity. Differences among varieties were significant at certain storage intervals ($p \leq 0.05$), with KO generally retaining higher structural stability.

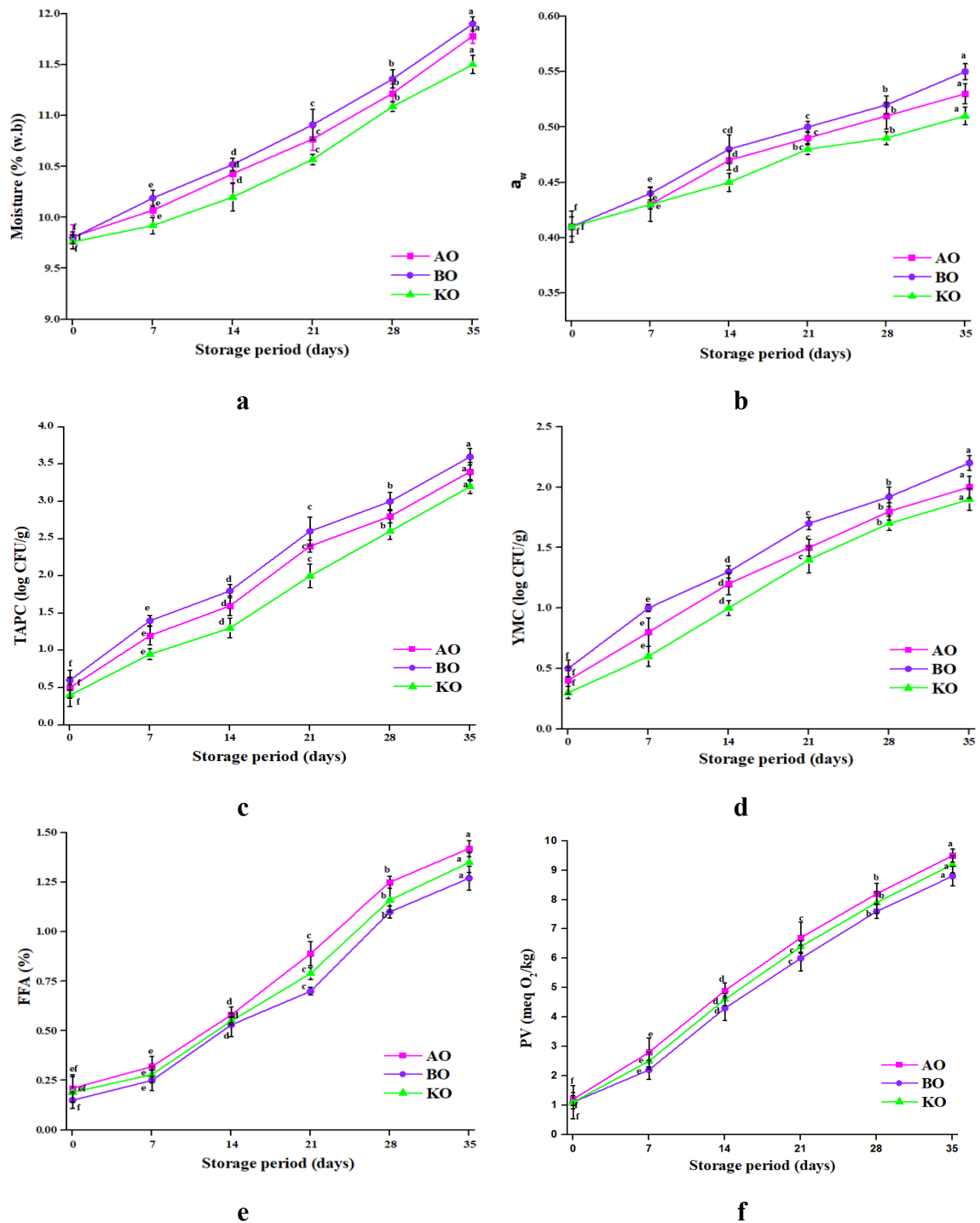


Fig. 6.10. Changes in (a) Moisture content, (b) Water activity (a_w), (c) Total aerobic plate count (TAPC), (d) Yeast and mold count (YMC), (e) Free fatty acid value (FFA) and (f) Peroxide value (PV) of the optimized pasta samples during storage period of 35 days
 Values are expressed as mean $\pm$ SD. Different lowercase letters on successive days indicate significant differences ($p \leq 0.05$) as indicated through Duncan's multiple range test

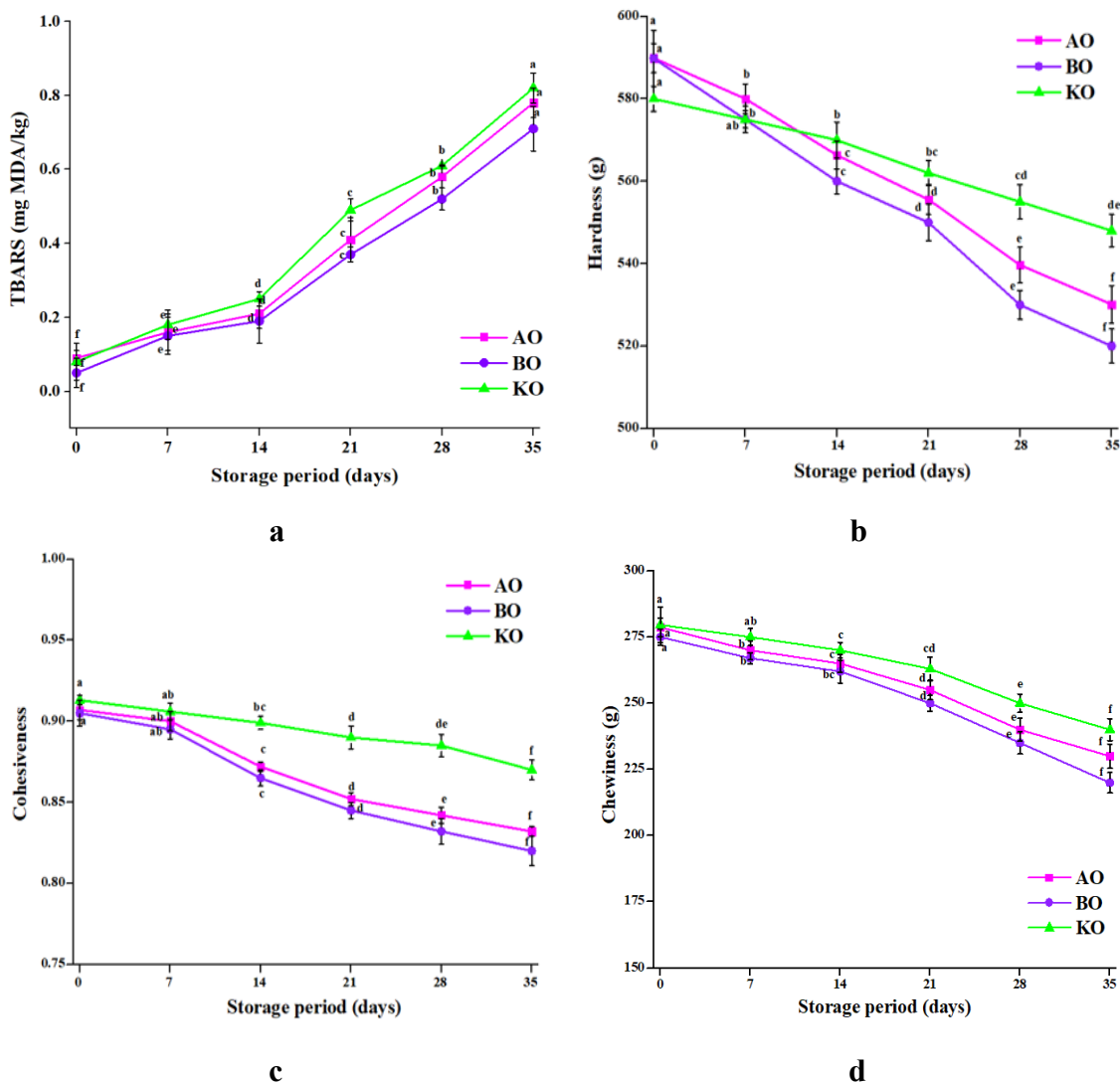


Fig. 6.11. Changes in (a) TBARS, (b) Hardness (cooked), (c) Cohesiveness (cooked) and (d) Chewiness (d) of the optimized pasta samples during storage period of 35 days

Values are expressed as mean $\pm$ SD. Different lowercase letters on successive days indicate significant differences ($p \leq 0.05$) as indicated through Duncan's multiple range test

Cooking loss increased significantly ($p \leq 0.05$) over storage time (Fig. 6.12a), which may be attributed to structural relaxation and partial weakening of the retrograded starch network caused by moisture absorption. The increase became more pronounced after 21 days of storage. Correspondingly, overall acceptability (OAA) decreased significantly

($p \leq 0.05$) during storage (Fig. 6.12b), primarily due to texture softening leading to minor oxidative changes (Naseer et al., 2021). Despite significant declines at later stages, OAA scores remained above the acceptable sensory threshold (>7) up to 28 days under accelerated conditions

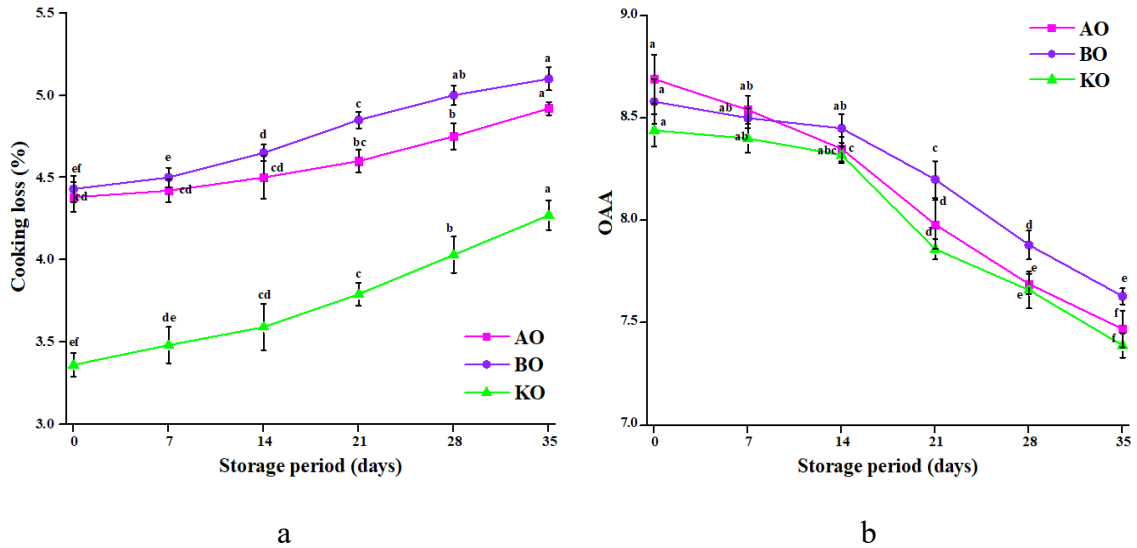


Fig. 6.12. Changes in (a) Cooking time and (b) Overall acceptability (OAA) of the optimized pasta samples during storage period of 35 days

Values are expressed as mean $\pm$ SD. Different lowercase letters on successive days indicate significant differences ($p \leq 0.05$) as indicated through Duncan's multiple range test

6.3.13.1. Shelf-life prediction

Based on the Q_{10} model ($Q_{10} = 2.5$), the predicted ambient shelf life at 25 °C was calculated as follows:

$$\text{Shelf life} = 35 \times 2.5^2 = 35 \times 6.25 \approx 218 \text{ days } (\sim 6\text{-}7 \text{ months})$$

Considering that all quality parameters remained within predefined acceptable limits during accelerated storage, the optimized pasta is projected to maintain acceptable quality for approximately 6-7 months under ambient conditions.

6.4. Conclusion

The present study successfully demonstrated the development and optimization of gluten-free pasta from underutilized pigmented *bao* rice landraces (*Amona*, *Badal* and *Kenkowa*) through controlled dual hydrothermal pretreatment. Application of response surface methodology effectively established the combined influence of parboiling and pressure steaming on cooking, textural and functional attributes of pasta, with statistically robust models confirming strong predictive capability. Hydrothermal modification played a crucial role in restructuring starch architecture through partial gelatinization followed by amylose reassociation and retrogradation, resulting in formation of a compact and cohesive matrix. This significantly improved cooking quality by reducing cooking loss and cooking time, while enhancing rehydration behavior and textural properties. The optimized samples exhibited performance comparable to xanthan gum-based controls, indicating that hydrothermal processing can effectively replace hydrocolloid dependency in gluten-free pasta systems. Nutritionally, the optimized pasta exhibited improved starch digestibility, suggesting potential for lower glycemic response. Although slight reductions in bioactive compounds occurred due to thermal processing, substantial functional components were retained. Microstructural analysis confirmed a dense and homogeneous network with reduced porosity, correlating with improved cooking stability and enzymatic resistance. Sensory evaluation showed higher overall acceptability of optimized samples compared to controls, attributed to improved texture, lower cooking loss and enhanced color. Accelerated storage studies further confirmed physicochemical, microbiological and oxidative stability within acceptable limits, supporting shelf-life suitability. Therefore, hydrothermally processed pigmented *bao* rice offers a promising clean-label approach for developing nutritionally enhanced gluten-free pasta while promoting the utilization of underexploited indigenous rice varieties.

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CHAPTER 7

Summary and conclusion

The present study was undertaken with the central objective of exploring the structural, functional, nutritional and application potential of underutilized, submergence-tolerant pigmented *bao* rice landraces, namely *Amona*, *Badal* and *Kenkowa*. These indigenous rice varieties, traditionally cultivated in flood-prone regions of Assam, represent a valuable yet underexploited genetic resource with inherent nutritional richness and adaptability to climatic stress. Through a systematic and integrated approach encompassing hydrothermal processing, chemical modification and product development, the study successfully demonstrated the feasibility of transforming these landraces into multiple value-added functional food products, including instant porridge mix, modified flour systems, dessert gel and gluten-free pasta. The research established clear structure-function relationships, besides highlighting broader implications of utilizing the climate-resilient crops for innovating sustainable food system.

Initially, development of the instant porridge mix highlighted the effectiveness of hydrothermal processing in enhancing techno-functional properties of the *bao* rice flours. The optimization of process variables, namely rice concentration and cooking time resulted in significant improvements in techno-functional parameters, including increased water absorption index and swelling power, along with reduced solubility and bulk density. These changes were indicative of enhanced starch gelatinization and improved water retention capacity, that are essential for instant food applications. Structural analyses confirmed that hydrothermal treatment led to gelatinization-induced modifications in the native crystalline architecture of *bao* rice starch, leading to crystallinity loss, reduced gelatinization enthalpy and granule deformation. These transformations translated improved consistency and cohesiveness in the developed porridge. However, a notable trade-off observed across all hydrothermally treated systems was the reduction in bioactive compounds, including phenolics and flavonoids, primarily

due to thermal degradation and leaching. Despite this, increased resistant starch content and improved digestibility profile provided a compensatory nutritional advantage, indicating the potential of these products as functional foods with lower glycemic response. The extended storage stability of the porridge mix, up to 12 months under ambient conditions, further reinforced its industrial applicability and commercial viability. Among the varieties, *Kenkowa* followed by *Badal* demonstrated superior performance, indicating their suitability for the instant porridge applications.

Then, the study further advanced into the modification of the *bae* rice whole flours using sodium trimetaphosphate (STMP) crosslinking, which enabled precise control over starch functionality. Crosslinking introduced controlled structural reinforcement within the starch matrices. The STMP-crosslinked rice flours exhibited progressive structural changes with increasing crosslinking levels. This resulted in reduced swelling power and solubility owing to restricted granule hydration. Among the levels evaluated, 9% crosslinking emerged as optimal, providing a desirable balance between structural rigidity and functional performance. At this level, the modified flour demonstrated enhanced gel strength, reduced syneresis and improved rheological properties, characterized by their visco-elastic behavior. Additionally, crosslinking shifted starch digestibility toward increased slowly digestible and resistant starch fractions, thereby suggesting improved nutritional properties. However, excessive crosslinking (12%) resulted in structural heterogeneity and reduced flexibility, limiting its applicability. A decline in antioxidant properties was also observed, which was consistent with structural alterations and possible phenolic interactions. Furthermore, functional implications of different crosslinking levels important for identifying appropriate applications of the modified flours in food systems could be addressed from the results obtained.

Building from this perspective, the modified flour was further explored for the development of a pigmented *bae* rice dessert gel, which served as a model system to evaluate gel-based food applications. Incorporation of 9% crosslinked flour into native flour at varying substitution levels resulted in significant improvements in textural

properties, including hardness, cohesiveness, adhesiveness, springiness and chewiness. The optimized formulations, containing 40% crosslinked flour substitution, produced the most desirable gel structure with enhanced firmness and stability. The developed dessert gel also exhibited improved freeze-thaw stability with significantly reduced syneresis, indicating effective water retention within the matrix. Rheological analysis confirmed the formation of a strong and stable viscoelastic network. Although a moderate reduction in antioxidant activity was observed due to processing-induced degradation, the functional and structural advantages outweighed this limitation. The dessert gel maintained acceptable microbial stability for up to 8 days under refrigerated conditions, with high sensory acceptability scores. Among the varieties, *Badal bao* produced the most premium dessert gel.

Finally, pasta was developed from dual stage hydrothermally pretreated flours of the pigmented *bao* rices, addressing a critical gap in gluten-free product formulation. The study successfully demonstrated that hydrothermal pretreatment could effectively compensate for the absence of gluten by restructuring the starch matrix. The optimized hydrothermal treatment effectively modified starch structure through partial gelatinization followed by controlled retrogradation, leading to the formation of a dense and cohesive matrix capable of mimicking the structural role of gluten. This structural transformation led to significant improvements in cooking quality, including reduced cooking time and cooking loss, coupled with enhanced rehydration capacity. The textural properties of the optimized pasta, particularly hardness, cohesiveness and chewiness, were found to be comparable to those of synthetic hydrocolloid-based systems, thereby eliminating the need for synthetic additives such as xanthan gum. From a nutritional perspective, the increase in slowly digestible and resistant starch fractions indicated a favorable shift toward lower glycemic response, enhancing the health-promoting potential of the product. Sensory evaluation further confirmed the acceptability of the optimized pasta, with scores clearly suggesting improved texture and visual appeal. Storage studies demonstrated that the product maintained acceptable physicochemical and microbiological stability over extended periods, reinforcing its potential for commercialization. Notably, all three rice

varieties produced equally acceptable pasta, demonstrating their versatility in this application.

Overall, this study successfully established that pigmented *bao* rice, when subjected to controlled structural modification, can be transformed into a versatile and high-value raw material for the development of functional food products. The findings contributed significantly to the scientific understanding of starch modification in pigmented cereals and provided a strong foundation for the development of safe, gluten-free and nutritionally enhanced food systems.

Across all experimental chapters, the critical role of starch structural modification in determining product functionality and performance were elucidated. Both hydrothermal processing and chemical crosslinking contributed to the reorganization of starch molecular architecture, influencing hydration behavior, textural attributes, digestibility and storage stability. While these modifications led to substantial improvements in functional and technological properties, they were invariably accompanied by a reduction in antioxidant activity, highlighting an inherent challenge in processing pigmented cereal systems. Nevertheless, the retention of a significant proportion of bioactive compounds, combined with enhanced resistant starch content, ensured that the developed products maintained their functional food relevance.

Varietal differences also played a significant role in determining product suitability. *Kenkowa* demonstrated superior performance in instant applications due to higher amylose content and enhanced retrogradation behavior. This contributed to improved structural integrity. *Badal* exhibited optimal characteristics for gel-based systems, reflecting a balanced composition that favored both elasticity and cohesiveness. *Amona*, while slightly lower in certain functional parameters, still contributed effectively across all applications. The comparable performance of all three varieties in pasta development further emphasized their adaptability to diverse processing conditions and product requirements.

Based on the comprehensive findings of the present investigation, the following specific recommendations are proposed to guide research and technological development.

1. Hydrothermal processing parameters (temperature, time and moisture content) should be optimized in combination, as their interactive effects govern starch gelatinization behavior, hydration properties and structural transformations rather than acting independently.
2. Moderate processing intensity is recommended. Excessive thermal treatment, although improves functional properties, leads to significant degradation of phenolic and flavonoid compounds.
3. Chemical crosslinking levels should be maintained within an optimal range, as lower and excessive crosslinking results in increased structural heterogeneity and limited applicability in diverse food systems.
4. Modified flour systems when used in combination with native counterparts as partial incorporation rather than complete substitution, provides a better balance between structural strength, functional performance and sensory acceptability.
5. Selection of raw material (rice variety) should be aligned with functional requirements, since variations in amylose content and composition significantly influence retrogradation behavior, gel formation and hydration characteristics.
6. Processing strategies should be designed to balance structural enhancement with bioactive retention, as all modification techniques demonstrated a trade-off between improved techno-functional properties and reduction in antioxidant activity.
7. Physical modification approaches should be prioritized over excessive reliance on synthetic additives, supporting clean-label product development.
8. Combined treatment strategies should be encouraged, since it is shown to improve overall performance compared to single-treatment systems.
9. Storage stability considerations should be integrated during product design, as moisture migration, starch retrogradation and microbial growth significantly influence shelf life and quality.

Future research prospects

The present study opens several promising avenues for further research and technological advancement in the field of functional food development using pigmented *bao* rice.

Future research could focus on exploring alternative and complementary starch modification techniques, such as heat-moisture treatment, annealing, enzymatic modification, and dual modification approaches, which may offer improved retention of bioactive compounds while maintaining desirable functional properties. Comparative studies between chemical and physical modification techniques would provide deeper insights into optimizing processing strategies for different product applications.

In terms of product diversification, there is significant scope for extending the application of pigmented *bao* rice into a wider range of food products, including extruded puffed snacks, breakfast cereals, bakery items and ready-to-eat meals. The incorporation of these modified flours into composite formulations with protein-rich ingredients such as pulses and millets could enhance the overall nutritional profile, particularly in terms of protein quality and micronutrient content. Such developments would further strengthen the role of pigmented rice in addressing nutritional security.

The current study has extensively evaluated *in vitro* starch digestibility. Future investigations should focus on *in vivo* glycemic index studies to validate the physiological impact of increased slowly digestible and resistant starch fractions. Additionally, studies on the bioavailability and metabolic fate of phenolic compounds after processing are essential to better understand their functional contribution in the human body.

Shelf-life studies conducted under accelerated conditions should be complemented with real-time storage studies to obtain more accurate predictions of product stability under commercial conditions. Mathematical modelling of shelf life and quality deterioration kinetics would also be beneficial for industrial applications.

Consumer acceptance and market feasibility studies represent another important area for future research. Large-scale sensory evaluation across diverse demographic groups, along with consumer preference mapping, would provide valuable insights for product optimization and commercialization strategies. Techno-economic analysis and life cycle assessment should be also undertaken to evaluate the sustainability and economic viability of large-scale production.

Finally, the broader impact of promoting pigmented *bao* rice cultivation should be explored in terms of agricultural sustainability, climate resilience and rural livelihood development. Integration of these underutilized crops into mainstream food systems has the potential to contribute significantly to food security, biodiversity conservation and economic growth in flood-prone regions.

In conclusion, the present research demonstrated that strategic structural modification of pigmented *bao* rice can effectively bridge the gap between traditional underutilized crops and modern functional food applications. By combining scientific innovation with indigenous knowledge, this study provided a comprehensive framework for the valorization of the climate-resilient *bao* rice varieties, paving the way for sustainable and nutritionally enriched food systems.

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ANNEXURE-I

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Name & Institutional Address of the Supervisor/Joint Supervisor	Dr. Himjyoti Dutta, Mizoram University, Aizawl	
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DR. HIMJYOTI DUTTA
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Ph.D. Course Work : SGPA of 9.29

Present Occupation Details

Organization : Not Applicable

Rank : Not Applicable

Job Profile : Not Applicable

LIST OF PUBLICATIONS AND CONFERENCES ATTENDED

Journal Publications

1. **Deka, G.**, Singha, S., Yadav, D.K., Chakraborty, I., Mazumder, N., Mal, S.S., & Dutta H. (2026). Hydrothermal process optimization, product characterization and storage stability evaluation of red *bao* rice instant porridge mix. *Journal of Food Science and Technology*. 1-14. <https://doi.org/10.1007/s13197-026-06618-7> (ISSN No. 0975-8402)
2. **Deka, G.**, Chakraborty, S., Singha, S., Yadav, D. K., Chowdhury, D., & Dutta, H. (2026). Development of pigmented bao rice dessert gel and characterization of its techno-functional, antioxidant and nutritional properties. *Journal of Food Measurement & Characterization*. 1-27. <https://doi.org/10.1007/s11694-026-04235-8> (ISSN No. 2193-4134)

Conferences attended

1. **Poster presentation** on “Effect of crosslinking on gelation, functional properties and textural attributes of red *bao* rice starch gels” in **CARBO-XXXVIII**, 2024, at Gauhati University, Assam, India during 4-6th December 2024.
2. **Oral presentation** on “Standardization of process parameters for the development of RTE porridge mix using three underutilized red pigmented rice of Assam” in the International Conference on “Recent Advances and future Prospects in Formatting a Healthier Food System” at **7th AMIFOST 2024**, Amity University, Noida during 19th-20th March 2024.



Hydrothermal process optimization, product characterization and storage stability evaluation of red *bao* rice instant porridge mix

Gunjana Deka¹ · Siddhartha Singha² · Dev Kumar Yadav³ · Ishita Chakraborty⁴ · Nirmal Mazumder⁴ · Sib Sankar Mal⁵ · Himjyoti Dutta¹ 

Received: 21 March 2025 / Revised: 13 October 2025 / Accepted: 31 December 2025
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Abstract

Dry instant porridge mix was developed by optimizing hydrothermal processing using Response Surface Methodology for three red pigmented *bao* rice namely *Kenkowa*, *Amona* and *Badal*. The optimized rice concentration and cooking time were 18.68 g, 10 min for *Kenkowa* and 22.5 g, 12.5 min for *Amona* and *Badal*, resulting in high WAI and SP and low WSI and p_b . Post optimization, the technofunctional properties improved enabling instant properties. X-Ray diffractometry revealed loss of crystallinity, Differential scanning calorimetry showed reduced enthalpy, Fourier transform infrared spectroscopy confirmed molecular modifications and Scanning electron microscopy indicated granule deformation all owing to gelatinization. Resistant starch content increased by ~35% across varieties. Consistency improved by ~30% and cohesiveness by ~75%. L^* decreased while a^* values increased ($p \leq 0.05$). Bioactive compounds decreased ($p \leq 0.05$), with total phenolic content reducing by ~65–75%, total flavonoid content by ~40–65%, DPPH radical scavenging activity by ~40–45% and ferric reducing antioxidant potential by ~55%. Principal component analysis (96.55%) depicted highest overall acceptability (OAA) in the optimized *Kenkowa* mix (8.10), followed by *Badal* (8.05) and *Amona* (7.95). Storage elevated free fatty acid, peroxide value and thiobarbituric acid reactive substances, while reducing consistency, taste and OAA. *Kenkowa* and *Badal* varieties were found to be more promising for instant food applications.

Keywords Pigmented · Rehydration · Instant · Hydrothermal · Optimization

Introduction

The global demand for ready-to-eat (RTE) and rehydratable food products continues to rise, driven by urbanization and the increasing need for convenient meal solutions. A wide range of RTE products, including instant noodles, rice, porridge, kheer, phirni and soup mixes, have gained prominence in modern markets (Mottan et al. 2024; Naseer et al. 2021; Washmin et al. 2022). These products utilize diverse ingredients, catering to evolving consumer preferences.

Cereals are widely used in RTE and rehydratable porridge industry due to their high starch content, which contribute to the desired thick consistency. Hydrothermal processes involving heat treatments under added moisture improves hydration and modify crystallinity, thermal, pasting properties, digestibility and content of antinutritional factors of starchy cereal flours, (Deka et al. 2023). Rheology and sensorial properties of rehydrated porridge depend largely on starch granule behavior. Pregelatinization accompanied by severe leaching of simpler glycosidic components

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Development of pigmented *bao* rice dessert gel and characterization of its techno-functional, antioxidant and nutritional properties

Gunjana Deka¹ · Sayantan Chakraborty¹ · Siddhartha Singha² · Dev Kumar Yadav³ · Dhiren Chowdhury⁴ · Himjyoti Dutta¹

Received: 16 July 2025 / Accepted: 24 February 2026

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Abstract

This study is a novel attempt to develop a structurally firm and stable dessert gel using flours of three underutilized, submergence-tolerant pigmented *bao* rice landraces - *Amona*, *Badal* and *Kenkowa*. To impart desired gel structure, the starchy flours were crosslinked (9%, w/w) with sodium trimetaphosphate and substituted with respective raw flours at levels of 0–50%. Crosslinking reduced amylose content (25–30%), swelling power (10–15%) and solubility (20–30%). Prominently decreased lightness, yellowness and increased redness were recorded. Freeze-thaw stability improved with syneresis substantially reducing by 45–55%. Textural parameters, namely hardness, adhesiveness, cohesiveness, springiness and chewiness changed desirably. Rheometry exhibited higher storage and loss moduli, with loss tangent values lower than 1, indicating improved gel strength. 2,2-diphenyl-1-picrylhydrazyl scavenging and ferric reducing antioxidant capacities were reduced by up to 20% and 25–30%, respectively. This was attributable to pH-driven anthocyanins degradation, oxidative losses, partial phenolic esterification and encapsulation within the dense crosslinked network. Here, fuzzy logic based sensory evaluation was employed as an innovation to minimize commonly occurring panelist bias. The study identified each 40% substituted gel as better than the rest. Increased slowly digestible and resistant starches suggested their improved glycemic potentials. The gels maintained microbial counts within standard safety limits for 8 days at 4 ± 2 °C, with overall sensory scores above 7 in a 9-point hedonic scale. 40% crosslinked flour substituted *Badal bao* produced the best dessert gel. Overall, the study demonstrated the feasibility of producing value-added functional dessert gel from pigmented *bao* rice using controlled starch crosslinking.

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using three underutilized pigmented rice of Assam
presented in Technical session... *Novel Foods & Novel Ingredients*
during 7th International Conference on “Recent Advances and Future
Prospects in Formatting a Healthier Food System”
on 19th-20th March 2024 at Amity University Uttar Pradesh, Noida.

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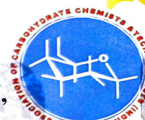
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organized by Department of Bioengineering and Technology, Gauhati University and the Association of
Carbohydrate Chemists and Technologists India (ACCTI), held from 4-6 December 2024.

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LIST OF AWARDS AND FELLOWSHIPS

List of Awards

1. **Awarded first position** in oral presentation at the 7th AMIFOST “International Conference on “Recent Advances and future Prospects in Formatting a Healthier Food System” at Amity University, Uttar Pradesh, India during 19th-20th March 2024.

List of fellowships

1. **CSIR Direct SRF** from 1st August 2025.
2. **SRF** under DRDO sponsored project titled “**Development of Pigmented Rice Based Antioxidant Rich Traditional and Innovative Food Products as Adaptogens for Reducing Oxidative Stress**”. From 16th September, 2024 to 30th July 2025.
3. **JRF** under DRDO sponsored project titled “**Development of Pigmented Rice Based Antioxidant Rich Traditional and Innovative Food Products as Adaptogens for Reducing Oxidative Stress**”. From 15th September, 2022 to 15th September 2024.

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DEGREE : Ph.D.
DEPARTMENT : FOOD TECHNOLOGY
TITLE OF THE THESIS : PROCESS STANDARDIZATION,
CHARACTERIZATION AND SHELF-
LIFE ANALYSIS OF READY-TO-EAT
PRODUCTS MADE FROM
PIGMENTED *BAO* RICE OF ASSAM
DATE OF ADMISSION : 06.09.2021
APPROVAL OF RESEARCH
PROPOSAL
1. DRC : 30.03.2022
2. BOS : 25.05.2022
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ABSTRACT

**PROCESS STANDARDIZATION, CHARACTERIZATION AND
SHELF-LIFE ANALYSIS OF READY-TO-EAT PRODUCTS MADE
FROM PIGMENTED *BAO* RICE OF ASSAM**

**AN ABSTRACT SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

GUNJANA DEKA

MZU REGN NO. 2107558

Ph.D. REGN NO. MZU/Ph.D./1797 of 06.09.2021



**DEPARTMENT OF FOOD TECHNOLOGY
SCHOOL OF ENGINEERING AND TECHNOLOGY
JUNE 2026**

**PROCESS STANDARDIZATION, CHARACTERIZATION AND
SHELF-LIFE ANALYSIS OF READY-TO-EAT PRODUCTS MADE
FROM PIGMENTED *BAO* RICE OF ASSAM**

**BY
GUNJANA DEKA
DEPARTMENT OF FOOD TECHNOLOGY**

Name of Supervisor: DR. HIMJYOTI DUTTA

Submitted

**In partial fulfilment of the Degree of Doctor of Philosophy in Food
Technology of Mizoram University, Aizawl**

Prominent pigmented *bao* rice landraces of Assam, namely *Amona*, *Badal* and *Kenkowa*, represent an underutilized group of submergence-tolerant, climate-resilient rice varieties possessing high nutritional value, bioactive compounds and distinctive functional properties. Yet, their systematic utilization in value-added food applications remain largely unexplored. The present investigation was therefore undertaken to comprehensively evaluate the structural, functional, nutritional and application potential of these indigenous pigmented rice varieties through an integrated approach involving processing, modification, product development, product characterization, sensory assessment and shelf-life analysis. The specific objectives of the study were:

1. To standardize processes for three *bao* red rice-based products, namely porridge mix, rice gel and pasta
2. To analyze the physicochemical, digestibility and phytochemical status of the developed products
3. To study the storability and shelf-life of the developed products

The study demonstrated that hydrothermal processing significantly enhanced the functional properties of pigmented *bao* rice flour, particularly water absorption index, swelling power and structural integrity, that are critical for instant food applications such as porridge mix. The optimized porridge mix exhibited high hygroscopicity superior reconstitution behavior, improved textural characteristics and extended storage stability under ambient conditions. However, hydrothermal processing resulted in reductions of phenolic and flavonoid content, attributable to degradation and leaching, although this was partially offset by an increase in resistant starch content, suggesting a shift toward lower glycemic response.

Sodium trimetaphosphate-induced crosslinking of the whole flour of *bao* rice effectively modified the starch matrix through the formation of phosphate linkages, leading to enhanced structural stability, restricted granule swelling, improved rheological behavior and increased resistance to thermal and mechanical stresses. Among the different levels,

moderate crosslinking was identified as optimal, providing a desirable balance between structural reinforcement and functional performance. However, lower levels and over crosslinking depicted lower structural stability. Crosslinking also influenced starch digestibility by increasing slowly digestible and resistant starch fractions, while a concomitant decline in antioxidant activity was observed. This was likely due to interactions between phenolic compounds and the modified starch network. The dessert gel developed from crosslinked *bao* rice flour substituted with native flour exhibited marked improvements in textural attributes, including hardness, cohesiveness, springiness and chewiness, along with enhanced freeze-thaw stability and reduced syneresis. The formulation containing moderate crosslinked flour substitution demonstrated the most favorable performance, yielding a firm yet elastic gel structure with high sensory acceptability. The product maintained microbiological stability for up to 8 days under refrigerated storage. Although a significant reduction in antioxidant activity was observed, the gel retained significant functional and nutritional relevance.

Dual hydrothermal pretreatment facilitated successful development of gluten-free pasta from pigmented *bao* rice by restructuring the starch matrix to compensate for the absence of gluten. The optimized pasta exhibited reduced cooking loss, improved rehydration capacity and desirable textural properties comparable to hydrocolloid-based systems, thereby eliminating the need for synthetic additives. From a nutritional perspective, the increased resistant starch content indicated potential reduction in glycemic response and enhanced functional quality.

Overall, the study established pigmented *bao* rice landraces as promising raw materials for the development of functional, gluten-free and ready-to-eat food systems. The findings provided a comprehensive scientific basis for their value addition through targeted processing and modification strategies, supporting the sustainable utilization of underexploited climate-resilient crops, while contributing to nutritional security and diversification of modern food systems.

The present thesis comprises seven chapters, systematically arranged to address the objectives of the study.

Chapter 1 establishes the conceptual framework of the study by highlighting the global importance of rice and the nutritional limitations of polished white rice. It positions pigmented rice as a superior alternative due to its rich bioactive composition. Particular emphasis is given to the underutilized pigmented *bao* rice landraces of Assam (*Amona*, *Badal* and *Kenkowa*), characterized by high amylose content, nutritional richness and submergence tolerance. The chapter identifies the lack of systematic research on their processing behavior and value addition, thereby justifying the need for their scientific exploration in functional food applications. The chapter also outlines the objectives of the study for development, characterization and shelf life analysis of products from *bao* rice landraces.

Chapter 2 presents a critical review of literature on pigmented rice, covering its classification, genetic regulation of pigmentation and bioactive composition. It examines physicochemical and functional properties, with emphasis on starch structure, gelatinization and digestibility. Processing and modification techniques are discussed in relation to their impact on functionality and nutritional quality. The chapter concludes by identifying key research gaps, particularly the limited work on processing and product development from the pigmented *bao* rices.

Chapter 3 focuses on the development and optimization of hydrothermally processed instant porridge mix from pigmented *bao* rice. The effects of processing variables on hydration properties and bulk density were evaluated. Hydrothermal treatment-induced structural changes in starch are correlated with improved reconstitution and textural characteristics. Nutritional composition, starch digestibility and bioactive retention were also assessed, highlighting improved functional performances alongside partial loss of phenolics. The developed product was further evaluated for sensory quality and storage stability.

Chapter 4 investigates the effect of sodium trimetaphosphate-induced crosslinking on the structural, physicochemical and rheological properties of pigmented *bao* rice whole flours. The study identified an optimal level of modification that balances functional performance with structural integrity. The impact of crosslinking on starch digestibility and antioxidant properties was also examined, linking molecular modification with techno-functional and nutritional outcomes.

Chapter 5 describes the development of a gel-based dessert using crosslinked pigmented *bao* rice flour. The influence of flour substitution levels on gel structure, textural and rheological properties was systematically evaluated. Changes in antioxidant activity and nutritional composition were assessed, and a sensory evaluation using fuzzy logic identified the most acceptable product. Storage studies confirmed its microbiological safety and quality retention under refrigerated conditions.

Chapter 6 presents the development and optimization of gluten-free pasta from dual stage hydrothermally pretreated pigmented *bao* rice. Hydrothermal pretreatment was shown to restructure starch, enabling formation of a cohesive matrix that could compensate for the absence of gluten. Optimization of processing conditions improved cooking quality, reduced cooking loss and enhanced textural attributes. Nutritional evaluation indicated increased resistant starch content, suggesting a lower glycemic response and improved functional quality.

Chapter 7 integrates the findings of the study, demonstrating the successful transformation of underutilized pigmented *bao* rice into value-added products including instant porridge mix, modified flour systems, dessert gel and gluten-free pasta. The chapter highlights the potential of these landraces for functional food development and sustainable utilization, while outlining future research directions focused on scale-up, process optimization and product diversification.