

**Effect of Fermentation on the Physicochemical, Structural, Textural, Thermal
and Pasting Properties of Adzuki Beans**

Waleed Sultan¹, Muhammad Umair Arshad^{1*}, Ali Imran¹, Farhan Saeed¹

¹Department of Food Science, Faculty of Life Science, Government College University,
Faisalabad, Pakistan

Corresponding author: Muhammad Umair Arshad (umairfood1@gmail.com)

Waleedsultan1@yahoo.com

Effect of Fermentation on the Physicochemical, Structural, Textural, Thermal and Pasting Properties of Adzuki Beans

Abstract:

Fermentation offers innovative opportunities to improve the physicochemical and functional properties of food products. The current research aimed at the impact of Lactic Acid Bacteria (LAB) *Lactobacillus plantarum* mediated fermentation of adzuki bean flours on the physicochemical, structural, thermal and pasting characteristics of non-fermented adzuki bean flour (NFA) and *Lactobacillus plantarum* fermented adzuki bean flour (LPA). Various analytical techniques, including Fourier-Transform Infrared Spectroscopy (FTIR), Rapid Visco Analyzer (RVA), Differential Scanning Calorimetry (DSC) and Scanning Electron Microscopy (SEM) were employed to analyze the compositional and structural modifications. Results indicate that fermentation significantly increased moisture content from 12.10 wt% NFA to 15.10 wt% LPA, enhancing water retention and microbial activity. Protein content rose from 19.90 wt% to 25.31 wt%, while fat content reduced due to enzymatic degradation. Rheological analysis implied improved viscosity and higher thermal stability, with RVA analysis showing a reduction in peak viscosity, suggesting fermentation induced starch modifications. SEM imaging displayed a transition from a smooth to an irregular, fragmented surface, improving hydration properties and structural compactness. FTIR spectra illustrated biochemical transformations in carbohydrates and proteins with characteristic spectral shifts reflecting the breakdown of macromolecules. These outcomes demonstrate that fermentation enhances the nutritional, textural and structural properties of adzuki bean flour, making them a favorable ingredient for functional food applications, particularly in high protein formulations and low carb diets.

Keywords: Fermentation, *Lactobacillus plantarum*, Adzuki Beans, Physicochemical Properties, Rheology, Structural Modification, Functional Foods.

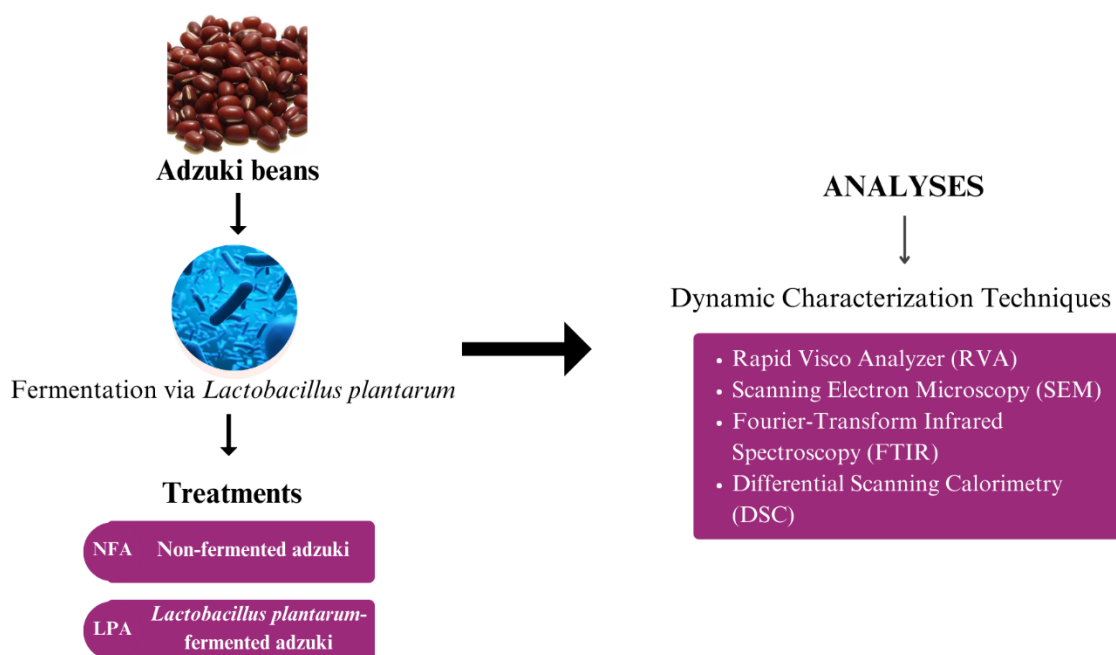


Figure 1. Graphical abstract illustrating the fermentation process of adzuki beans

1. Introduction:

Fermentation presents multiple opportunities to improve the sensory, nutritional and functional characteristics of food products (Yaqoob et al., 2024b). It allows for the development of innovative products with desirable increased viscosity, improved mouthfeel and textures. Foods modified through fermentation exhibit enhanced physicochemical and textural properties, including capacity, gel formation, improved expansion and water holding making them valuable for distinct food applications (Yaqoob et al., 2022). Fermentation also provides economic benefits such as extending shelf life, simplifying production processes and enabling the creation of cost effective products with clean label declarations (Murtaza et al., 2025, Leyeza V.E.B., 2025).

Adzuki beans, a staple legume crop widely cultivated in certain tropical and subtropical areas, hold significant importance as a part of traditional diets in many cultures (Naveed et al., 2024). Rich in nutrients and functional components, adzuki beans have a long history of consumption. They are cultivated globally, particularly in countries like Japan, China, Korea and parts of South Asia, where they are valued for their health benefits and versatility. However, raw adzuki beans contain

50 compounds that can cause irritation and are typically cooked or processed before consumption to
51 ensure edibility and safety (Jia et al., 2021).

52 Recent developments in biotechnology have enabled the modification of legumes through methods
53 like fermentation, enzymolysis and ultrasonication. Among these, fermentation using microbial
54 cultures, such as lactic acid bacteria (*Lactobacillus plantarum*) has gained significant attention for
55 improving the functionality and applicability of food products. Fermentation enhances the extraction
56 of bioactive components, optimizes nutrient profiles and improves the structural and textural
57 properties of food making it a sustainable and eco-friendly processing method (Yaqoob et al., 2024a).

58 The current research focuses on examining the impact of fermentation on physicochemical, structural,
59 thermal and pasting characterization of adzuki beans. By leveraging fermentation, this research aims
60 to explore the potential of adzuki beans particularly in the food sector and provide insights into how
61 their functional and nutritional profiles can be optimized for broader applications.

62 **2. Materials and Methods**

63 Adzuki beans were procured from the local market of Lahore, Pakistan. The grains were milled using
64 a laboratory mill (TAISITE, FW100) and subsequently sieved through an 80-mesh screen.
65 *Lactobacillus plantarum* (*L. plantarum*) was cultured on MRS medium at a temperature of 37°C and
66 then stored at 4°C. All other chemicals used were of analytical grade.

67 **2.1 Fermentation process**

68 Isolated strains of *L. plantarum* were cultivated in the liquid MRS medium in an incubator (HZQ-
69 F160) to obtain a bacterial concentration of 10^7 - 10^8 cfu/mL. A 2% of the bacterial suspension was
70 mixed with adzuki bean flour, then incubated at 37°C for 48 hours in the incubator. Dough was
71 prepared by mixing each ingredient thoroughly, then dried by using freeze drying at -60°C for 20
72 hours and stored in zip-lock bags

73

Table 1: Amount of *L. Plantarum* for the fermentation of ABF

Treatments	Volume
NFA	-
LPA	10 mL

Note: This respective value is for 100 g of flour.

Non-fermented Adzuki Bean flour (NFA), *Lactobacillus plantarum*- treated Adzuki bean flour (LPA)

2.3 Physicochemical analysis

2.3.1 Proximate analysis

The proximate parameters of adzuki beans flour were analyzed by the method of AOAC (2023). Moisture percentage was measured by drying the sample in the hot air oven at 105°C until a constant weight was achieved. Crude protein was determined using the Kjeldahl method ($N \times 6.25$) (Shtylla et al., 2024), while crude fat was extracted using the Soxhlet extraction method with n-hexane as a solvent following standard AOAC procedures (Khan et al., 2021). The solvent was completely removed by evaporation. Crude fiber was ascertained by acid-base digestion to remove digestible components. Ash content was determined by incinerating the sample in a muffle furnace at 550°C for 4–6 hours until white ash was obtained. The nitrogen-free extract (NFE) was calculated by difference, using the formula:

$$\text{NFE (\%)} = 100 - (\text{Moisture} + \text{Crude Protein} + \text{Crude Fat} + \text{Crude Fiber} \dots\dots\dots (1)$$

All compositional values presented in the current study are expressed in weight percent (wt%).

2.4 Pasting properties of Adzuki Bean Flour

The pasting properties of adzuki bean samples were assessed using a Rapid Visco Analyzer (RVA 5820, Perten, UK), following the method outlined by Yaqoob et al. (2022). In brief, 3 g of the adzuki bean sample was mixed with 25 mL of water and placed on the RVA cup. The mean values of the breakdown, peak viscosity (PV), trough viscosity (TV), setback, pasting temperature (PT) and final viscosity (FV) were calculated for respective samples.

97 **2.5 Micromorphological analysis**

98 The micromorphological characteristics of the samples ABF were analyzed using a Scanning Electron
99 Microscope (G2-Phenon™, Pakistan) microscope at 1000× magnification as described by Yaqoob et
100 al. (2019a). Before the imaging, the flour samples were mounted on specimen holders using double-
101 sided adhesive tape, sputter-coated with a gold layer and scanned under vacuum at an accelerating
102 voltage of 5 kV potential difference.

103 **2.6 Fourier-transformed infrared (FTIR) spectra**

104 The FTIR spectra of the adzuki bean flour samples were obtained following the method outlined by
105 Yaqoob et al. (2019b). The spectra were recorded in the infrared range of 400–4000 cm⁻¹. The
106 molecular structural changes in the adzuki bean samples were analyzed by scanning the pellet using
107 FTIR (Bruker, Vertex-70, Massachusetts, USA). A mixture of 100 mg potassium bromide and 1 mg
108 of the adzuki bean sample was placed under a hydraulic press to form a tablet, which was then
109 analyzed in the FTIR to obtain the spectral peaks.

110 **2.7 Textural profile of adzuki bean flour**

111 The dough was prepared by adding 50 g of adzuki flour to 50 g of water and stirring and leaving it
112 for 60 minutes at room temperature by following the method of (Yaqoob et al., 2022). To evaluate
113 textural properties of the dough (hardness, cohesiveness, springiness, gumminess and resilience),
114 (TA.XT Plus) texture analyzer was used. Hardness was defined as the maximum force achieved
115 during analysis. Analysis conditions were as follows: P/0.5 probe and with a test distance of 10 mm
116 at a speed of 0.5 mms⁻¹.

117 **2.8 Thermal characterization of adzuki bean flour**

118 Differential scanning calorimetry (DSC, TA Q 2100) was used to evaluate the thermal properties of
119 flour samples, following the method outlined by Yaqoob et al. (2022). An empty aluminum pan was
120 used as the reference standard for the samples. A 3 mg adzuki bean flour sample was mixed with 6

121 μL of H_2O and placed in the DSC pan. The analysis was carried out under a nitrogen atmosphere to
122 ensure an inert environment. The optimum temperature range was set from 30 to 90°C, with a constant
123 heating rate of 10 °C min⁻¹. The respective parameters onset (T_o), peak (T_p), conclusion (T_c),
124 gelatinization temperature range (T_c-T_o) and ΔH were determined using Universal Analysis 2000
125 software.

126 **2.9 Statistical analysis**

127 The statistical analysis of the collected data for all parameters was conducted using Origin Pro 8.5
128 software. The t-test was used to measure the statistical difference between samples at the significance
129 level (95%) due to the comparison between two treatments in which one is the dependent and the
130 other is the independent variable. Each analysis was performed three times to ensure statistical
131 significance.

132

133 3. Results and Discussion

134 3.1 Compositional Analysis of Adzuki Bean Flour (ABF)

135 The proximate analysis of Non-Fermented Adzuki (NFA) and *Lactobacillus plantarum*-Fermented
136 Adzuki (LPA) revealed notable differences as a result of the fermentation process Figure 2. The
137 moisture content significantly increased from 12.10 ± 0.24 % in NFA to 15.10 ± 0.30 % in LPA. This
138 increase can be attributed to the fermentation process, where microbial activity leads to cell wall
139 loosening and an increase in hydrophilic compounds, which enhances the water-binding capacity of
140 the substrate as mentioned in the study of SarojMalik and Kaur (2025). Additionally,
141 exopolysaccharides produced by *L. plantarum* during fermentation may have contributed to greater
142 moisture retention (XiongLiu and Huang, 2023). LPA exhibited an increase in protein content
143 25.31 ± 0.51 % as compared to NFA 19.90 ± 0.40 %. This rise could be attributed to the biosynthesis
144 of microbial proteins and enhanced solubilization of native proteins due to proteolytic activity by *L.*
145 *plantarum* during fermentation. Furthermore, fermentation is known to reduce antinutritional factors
146 such as trypsin inhibitors and tannins thereby increasing protein digestibility and apparent content
147 (Christensen et al., 2022). Fermentation reduces antinutritional factors in adzuki beans through
148 microbial enzymatic activity. *Lactobacillus plantarum* produces proteases enzymes that degrade
149 trypsin inhibitors so improving the protein digestibility. Tannins are broken down by tannase and
150 phenolic acid decarboxylases, reducing their interference with protein and mineral absorption.
151 Fermentation also alters pH which enhances the breakdown of complex antinutrients. These processes
152 increase the availability and extractability of proteins thus leading to improved nutritional quality and
153 an apparent rise in protein content in fermented adzuki bean flour. The crude fat content showed a
154 slight reduction in LPA 0.44 ± 0.01 % compared to NFA 0.50 ± 0.01 %. This marginal decline may be
155 due to the utilization of lipids by fermentative microbes as an energy source or the enzymatic
156 degradation of fat molecules during fermentation. Although the difference is minor but it indicates a
157 potential shift in lipid metabolism, consistent with similar findings of Tomás-Pejó et al. (2023). The
158 fiber content in both treatments was slightly different, with 6.00 ± 0.12 % in NFA and 6.01 ± 0.11 % in

159 LPA. The lack of significant variation is consistent with prior literature suggesting that dietary fiber,
160 being structurally robust and non-digestible by most fermentative microbes, remains largely
161 unaffected during lactic acid fermentation. This retention is advantageous for maintaining the
162 functional benefits of dietary fiber (DeehanMocanu and Madsen, 2024).

163 Ash content showed negligible variation between NFA $3.49 \pm 0.07\%$ and LPA $3.52 \pm 0.07\%$,
164 indicating that the fermentation has no effect on mineral profile similar observation in previous
165 studies reported by Guillen-Guerrero and de la Rosa-Millan (2025) and N'zi et al. (2021), where
166 mineral elements were unaffected due to their non-volatile and chemically inert nature during
167 microbial fermentation. Nitrogen free extract (NFE) decreased markedly in LPA $49.62 \pm 0.99\%$
168 compared to NFA $58.01 \pm 1.16\%$, reflecting microbial utilization of fermentable carbohydrates.
169 *Lactobacillus plantarum* metabolizes available sugars to produce organic acids, leading to a reduction
170 in the overall carbohydrate fraction (Tejedor-Sanz et al., 2022). The fermentation of ABF using
171 *Lactobacillus plantarum* significantly influenced their proximate composition. The increase in
172 moisture and protein content, along with a reduction in NFE suggests an enhanced nutritional profile.
173 These changes are consistent with previous research so underscoring the role of microbial
174 fermentation in enhancing the quality of legumes.

175

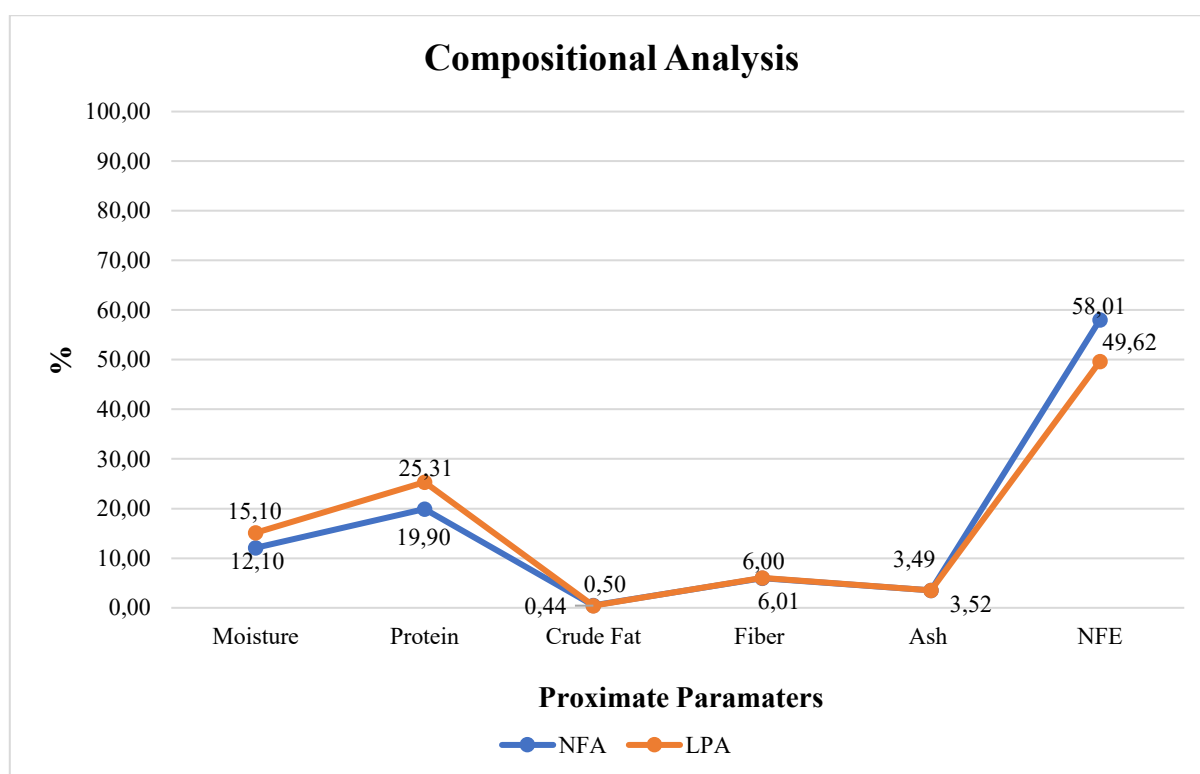


Figure 2: Compositional analysis of ABF
NFA (non-fermented adzuki), LPA (*Lactobacillus plantarum*-fermented adzuki)

3.2 Textural analysis of adzuki flour

The textural properties of adzuki bean samples as shown in Table 2 were considerably modified by *Lactobacillus plantarum* fermentation. Non-fermented adzuki (NFA) exhibited a hardness value of 5.22 ± 0.20 , which decreased to 4.78 ± 0.09 after fermentation (LPA), indicating substantial softening. This reduction in hardness aligns with previous studies demonstrating that microbial fermentation degrades cell wall polysaccharides, particularly pectins and hemicelluloses in leguminous matrices as reported in the study of MiaoLi and Wu (2022). Springiness declined from 0.92 ± 0.03 in NFA to 0.89 ± 0.02 in LPA, suggesting a reduction in elastic recovery. The slight decline in cohesiveness from 0.81 ± 0.02 to 0.79 ± 0.02 may be attributed to protein aggregation or exopolysaccharide formation during fermentation as expressed by Zhang et al. (2023). The resilience of fermented adzuki LPA: 0.48 ± 0.01 was lower than that of the NFA 0.51 ± 0.02 , indicating reduced structural recovery after deformation. This effect likely results from proteolytic activity disrupting the protein network, as observed in other fermented legume

products (Liaquat et al., 2025). In addition to this, the gumminess reduces in NFA from 4.87 ± 0.11 to 0.48 ± 0.01 in LPA, which suggests that chewiness is enhanced, possibly due to the modified protein-water interactions, as studied by Zhang et al. (2022). These outcomes demonstrate that *L. plantarum* fermentation induces complex changes in adzuki bean texture, including softening, reduced elasticity and altered protein matrix behavior. The current study illustrates that the fermented flour creates a less sticky and softer textural commodities having better mouthfeel. Because of this, LPA flour is suitable for making soft baked items like gluten-free cakes, muffins, cookies and snack bars. These products benefit from a tender texture and moist feel. A key challenge in scaling up fermented adzuki bean flour-based products is ensuring the consistent fermentation as the metabolic activity of *Lactobacillus spp.* is sensitive to variables like temperature, pH, inoculum level and fermentation duration. Minor fluctuations in these parameters can significantly affect product texture and sensory attributes (Guiné, 2022).

Table 2: Effect of *L. Plantarum* on the textural properties of adzuki flour dough

<i>Treatment</i>	<i>Hardness</i>	<i>Springiness</i>	<i>Cohesiveness</i>	<i>Gumminess</i>	<i>Resilience</i>
<i>NFA</i>	5.22 ± 0.20^a	0.92 ± 0.03^a	0.81 ± 0.02^a	4.87 ± 0.11^a	0.51 ± 0.02^a
<i>LPA</i>	4.78 ± 0.09^b	0.89 ± 0.02^b	0.79 ± 0.02^b	4.16 ± 0.06^b	0.48 ± 0.01^b
<i>p-values</i>	0.250	0.221	0.282	0.006	0.081

NFA (non-fermented adzuki), **LPA** (*Lactobacillus plantarum*-fermented adzuki)

Means in the same columns with different letters were significantly different ($p < 0.05$) from each other.

3.3 Thermal characteristics of flour

The differential scanning calorimetry (DSC) analysis revealed certain differences in the thermal properties of flours having different treatments, as shown in Table 3. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures observed higher in the fermented flours in comparison with non-fermented ones, highlighting the enhanced thermal stability of starch granules due to fermentation. However, the highest T_o : 71.05 ± 0.27 and T_p : 78.64 ± 0.48 values were observed in LPA treatment, respectively. These results are in line with the outcomes of Dou et al. (2023), who demonstrated that

216 fermentation strengthens crystalline order of starch, thereby increasing its resistance to thermal
 217 breakdown. The gelatinization temperature range (T_c - T_o) was broader for the non-fermented sample
 218 NFA: 15.63 ± 0.15 , indicating a more heterogeneous starch structure. While in non-fermented sample
 219 the ΔH values were lowered NFA: 2.82 ± 0.05 , indicative of less stable starch granules.

220 This can be due to the fermentation action of *Lactobacillus plantarum*, that has been reported to
 221 improve the structural and functional properties of flours Valsalan et al. (2024). The improved
 222 thermal properties of these treatments are linked with the better baking performance because higher
 223 thermal stability and narrower gelatinization ranges contribute to enhanced crumb structure, moisture
 224 retention and texture of the product. These outcomes highlight the transformative role of fermentation
 225 in modifying the thermal properties of legume flours, making them more suitable for high-quality
 226 bakery applications. The results are consistent with previous studies by Shiferaw Terefe and Augustin
 227 (2020), which demonstrated that fermentation-induced changes in starch properties improve the
 228 functional and nutritional quality of food products. Nevertheless, the enhanced thermal behavior of
 229 fermented flours in treatments underlines their potential for use in superior product, offering both
 230 functional and sensory benefits.

231 **Table 3:** Effect of *L. Plantarum* on the Thermal characterization of adzuki dough

Treatment	$T_o(^{\circ}\text{C})$	$T_p(^{\circ}\text{C})$	$T_c(^{\circ}\text{C})$	$T_c-T_o(^{\circ}\text{C})$	$\Delta H (\text{J g}^{-1})$
NFA	67.82 ± 0.05^b	72.89 ± 0.15^b	83.45 ± 1.05^b	15.63 ± 0.15^a	2.82 ± 0.05^a
LPA	71.05 ± 0.27^a	78.64 ± 0.48^a	85.89 ± 0.98^a	14.84 ± 0.02^b	5.56 ± 0.02^a
p-values	0.003	0.004	0.421	0.008	0.005

235 NFA: non-fermented adzuki, LPA: *Lactobacillus plantarum*-fermented adzuki
 236 Means in the same columns with different letters were significantly different ($p < 0.05$) from each
 237 other.

238 3.4 Pasting properties of flour

239 The pasting characteristics of unfermented and LAB fermented flour samples shown in Table 4
 240 determined by Rapid Visco Analyzer (RVA) revealed that certain changes in viscosity profile of LAB

241 fermented samples consistently reduced key parameters including peak viscosity, final viscosity,
242 pasting temperature, setback and breakdown values. These alterations can be attributed to the
243 structural degradation of starch macromolecules during fermentation. The findings are in line with
244 previous findings of Yan et al. (2024), who demonstrated that acidification during fermentation
245 increases starch granule fragility. Luo et al. (2022) observed similar reductions in breakdown and
246 setback values in fermented rice flour due to modifications in starch crystallinity. The decreased PV
247 particularly reflects changes in amylopectin polymerization state and chain length. Fermentation of
248 ABF with *Lactobacillus plantarum* led to a significant pH reduction due to the production of organic
249 acid. This acidification altered starch functionality. The lower pH likely promoted partial hydrolysis
250 of amylopectin and weakened starch granule integrity, reducing swelling and retrogradation. As a
251 result, starch granules exhibit impaired structural integrity, evidenced by decreased swelling power
252 and solubility. Furthermore, the increased acidity disrupts the crystalline regions of starch granules
253 so weakening the intermolecular hydrogen bonding. This promotes gelatinization at lower
254 temperatures and reduces the tendency for retrogradation during storage. For instance, CappelliOliva
255 and Cini (2020) reported similar reductions in peak and final viscosities in *Lactobacillus*-fermented
256 chickpea flour attributing to enzymatic degradation of starch molecules and partial hydrolysis of
257 complex carbohydrates during fermentation. The viscoelastic properties appear to be governed by
258 three principal components e.g., proteins, lipids and amylose content. While amylopectin mediates
259 starch swelling, amylose maintains granule integrity while restricting excessive swelling Varghese et
260 al. (2022), explaining the inverse correlation between amylose content and viscosity parameters.
261 During cooling, leached amylose molecules reassociate to form junction zones that contribute to
262 setback and final viscosity development (Woodbury and Mauer, 2023). The decreased setback and
263 final viscosity in LPA suggest improved stability and lower staling tendency making it suitable for
264 baked products with extended softness. These changes are also supported by (EmirYildiz and Sumnu,
265 2023), who demonstrated that fermentation of lentils reduces the tendency of starch to recrystallize
266 thereby improving the shelf life and consumer acceptability in gluten-free bakery products. These

changes collectively demonstrate that the pasting properties of fermented flour are predominantly influenced by the extent of polymer leaching along with the degree of starch damage and internal granule morphology, all of which are capable to modify molecular interactions with water and determine the functional characteristics.

Table 4: Effect of *L. Plantarum* on the RVA characterization of adzuki flour dough

Samples	Peak Viscosity (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Pasting Temperature (°C)	Trough Viscosity (cP)
NFA	200.3±7.61 ^a	41.2±1.57 ^a	155.2±5.79 ^a	110.2±4.19 ^a	66.2±2.49 ^a	298±11.32 ^a
LPA	159.4±6.06 ^b	24.5±0.76 ^b	134.6±4.17 ^b	65.3±2.02 ^b	61.3±2.33 ^b	175±6.65 ^b
p-values	0.001	0.007	0.005	0.009	0.674	0.008

NFA: non-fermented adzuki, LPA: *Lactobacillus plantarum*-fermented adzuki

Means in the same columns with different letters were significantly different ($p < 0.05$) from each other.

3.5 FTIR Spectra of Adzuki Bean Flour

The FTIR spectra of different samples of adzuki bean reflect distinct molecular modifications that align with previous studies on fermentation-induced changes in legume-based flours. The NFA sample showed typical absorption peaks for hydroxyl 3264 cm^{-1} and aliphatic 2920 cm^{-1} and 2842 cm^{-1} groups as illustrated in Figure 3, which are characteristic of non-fermented legume flours (Neji et al., 2022). These findings are consistent with previous reports, where the molecular structure of raw legumes is dominated by carbohydrate and protein components, as indicated by peaks at 1239 cm^{-1} and 1029 cm^{-1} for carbohydrate-related C–O stretching (Perera et al., 2023). The LPA sample exhibited more pronounced changes, particularly in the fingerprint region, with significant shifts at 1587 cm^{-1} and 1401 cm^{-1} , corresponding to aromatic and carboxylate groups. These changes reflect the production of organic acids and the transformation of phenolic compounds during *Lactobacillus plantarum* fermentation. The peaks noted at 1587 cm^{-1} in the FTIR spectrum of *Lactobacillus plantarum*-fermented adzuki bean flour reflects enhanced aromatic C=C as compared to non-fermented flour. This change in the peak arises from *L. plantarum*'s proteolytic activity which

290 catabolizes aromatic amino acids (e.g., phenylalanine & tyrosine) and from enzymatic degradation
 291 and microbial transformation of bound phenolic acids such as ferulic and chlorogenic acids originally
 292 immobilized in the adzuki bean matrix. These metabolic processes increase free aromatic structures
 293 in the flour thereby intensifying conjugated vibrational signatures. Similar findings were reported by
 294 EmkaniOliete and Saurel (2022), who noted that *Lactobacillus plantarum* fermentation in legumes
 295 leads to greater breakdown of phenolic and acidic compounds, altering their molecular structure.
 296 Additionally, the appearance of peaks at 1234 cm^{-1} and 839 cm^{-1} in the LPA spectrum highlights
 297 modifications in polysaccharides, which is consistent with the effects of *Lactobacillus plantarum* on
 298 the depolymerization of complex carbohydrates which is previously reported by Landete et al. (2021).

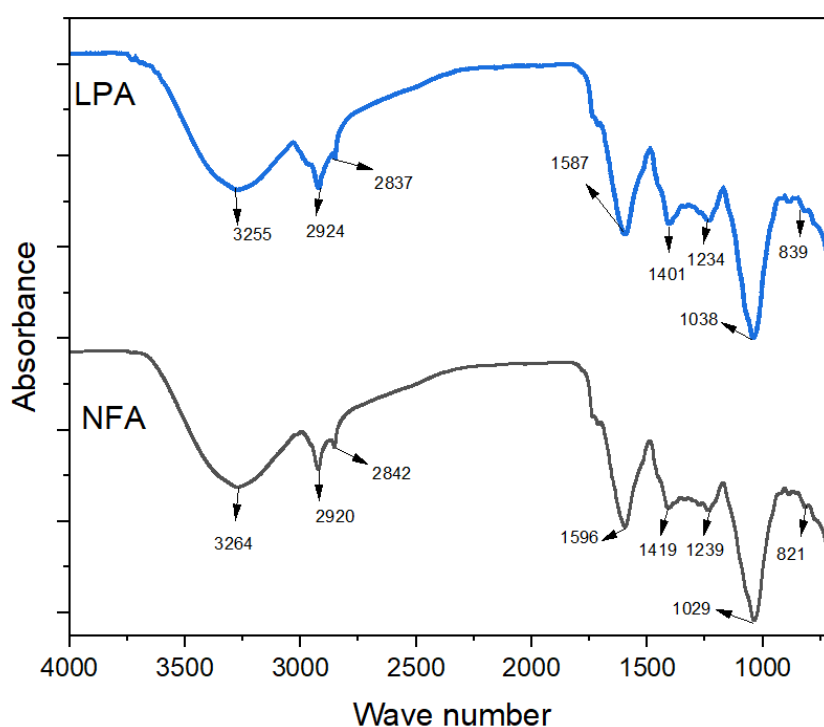


Figure 3: Structural characterization of different treatments through FTIR.

NFA: non-fermented adzuki, LPA: *Lactobacillus plantarum*-fermented adzuki

306 SEM Analysis of Adzuki Bean Samples

303 Scanning electron microscopy (SEM) analysis revealed significant morphological differences
 304 between native and bacterially fermented adzuki flour (Figure 3). Native flour maintained intact
 305 starch granules characterized by smooth surfaces and preserved structural integrity, while fermented
 306 samples exhibited extensive surface modifications including granule fragmentation, increased surface

307 porosity, irregular edges and surface corrosion patterns. These structural transformations can be
 308 attributed to bacterial enzymatic activity, particularly through α -amylase-mediated endo-hydrolysis
 309 of glycosidic bonds and glucoamylase-induced surface corrosion of granule surfaces compounded by
 310 microbial acid production that induces phase transitions in starch crystallinity. The resultant increase
 311 in surface area-to-volume ratio enhances water absorption kinetics but reduces water binding capacity
 312 due to disruption of hydroxyl group networks in damaged starch, loss of crystalline domains in
 313 amylopectin clusters and amylose leaching from compromised granule matrices. As reported in
 314 previous studies by (Chang et al., 2021, Ma et al., 2022) confirm these morphological alterations
 315 represent a consistent pattern in fermented cereal matrices, with the extent of modification dependent
 316 on bacterial strain specificity, fermentation duration, substrate availability and environmental
 317 parameters (pH, temperature and water activity). The observed structural degradation pathways
 318 suggest a complex interplay of enzymatic and physicochemical mechanisms driving starch granule
 319 modification during bacterial fermentation.

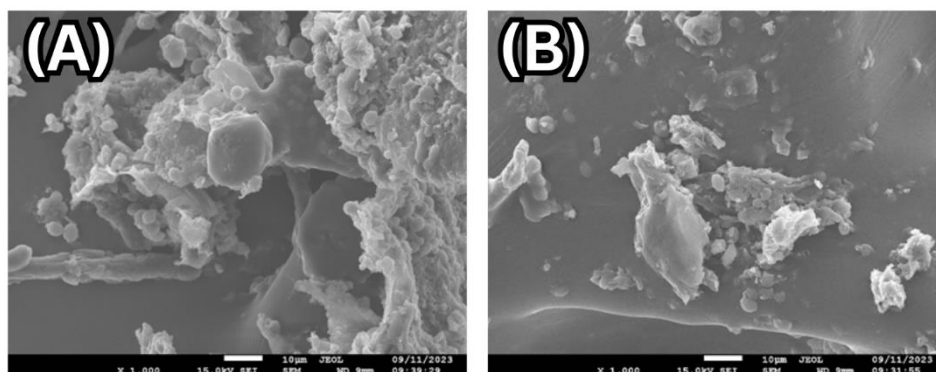


Figure 3: SEM photomicrographs of different treatments.

Micromorphology of each sample was captured at a magnification of x 1000.

A: NFA (non-fermented adzuki), **B:** LPA (*Lactobacillus plantarum*-fermented adzuki)

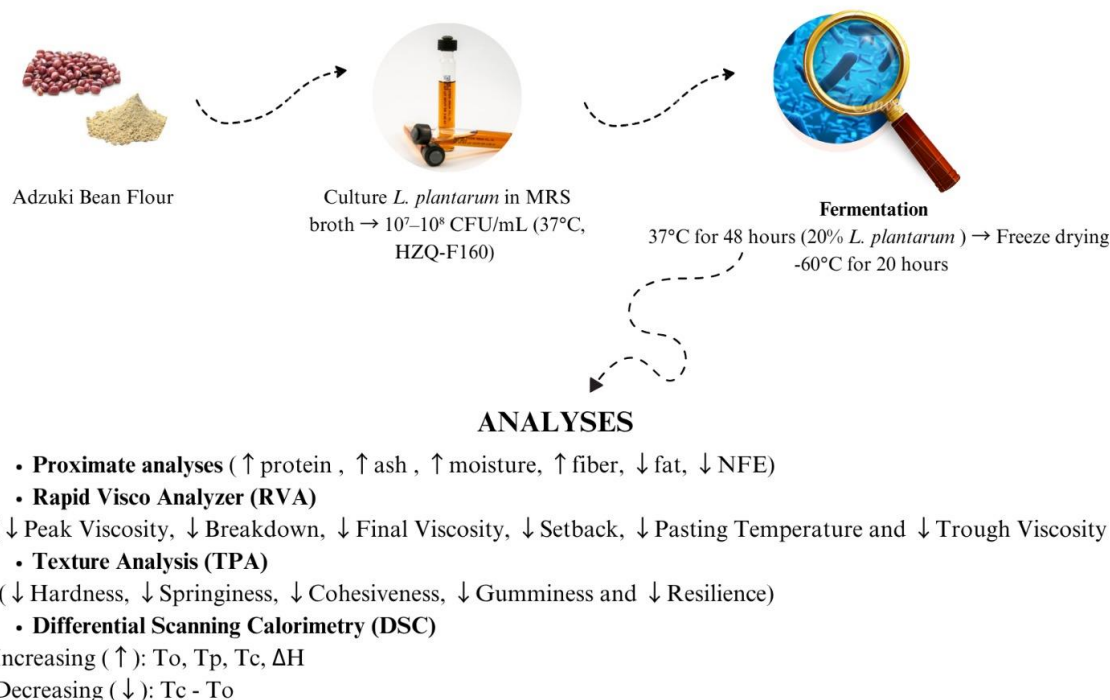


Figure 4: Integrated Fermentation and Multi-Parameter Evaluation of *Lactobacillus plantarum*-Treated Adzuki Bean Flour

4 Conclusion

This study demonstrated that *Lactobacillus plantarum*-mediated fermentation significantly enhances the physicochemical, structural, rheological and thermal properties of adzuki beans, making them a valuable ingredient for functional food applications. Fermentation led to an increase in protein content (from 19.90 to 25.31%), improved mineral retention and enhanced hydration properties, while reducing fat and fiber content. Structural analysis revealed that fermentation disrupted starch-protein matrices, leading to a more porous, hydrated structure, as evidenced by SEM imaging. Rheological analysis confirmed higher viscosity and improved stability, with RVA results indicating a decrease

342 in peak viscosity (200.3 to 159.4), suggesting better gelatinization properties. FTIR and DSC analyses
343 further highlighted molecular transformations, improving the thermal resilience of the fermented
344 beans. These findings underscore the potential of fermentation as a sustainable processing method for
345 improving nutritional value, texture and industrial functionality of legume-based ingredients. The
346 enhanced properties of fermented adzuki beans make them particularly suitable for use in high-protein
347 formulations, gluten-free alternatives and low-carb dietary products. Future research should focus on
348 improving the consumer acceptance for fermented products specially made up of indigenous legume
349 flours like adzuki bean. Such products can help to overcome malnutrition and enhance food security
350 by providing affordable and protein rich alternatives in vulnerable areas. Fermentation can improve
351 taste, texture and nutrient bioavailability so making these products more appealing. Utilizing locally
352 available legumes in product development supports sustainable nutrition and strengthens local food
353 systems while offering a practical solution to dietary deficiencies and dependence on imported foods.

354

- AOAC (2023). Official Methods of Analysis (22nd ed., Vol. II). Association of Official Analytical Chemists, Gaithersburg, MD, USA. Official Methods 925.09, 923.03, 979.09, 962.09, 4.5.01, and 923.05.
- CAPPELLI, A., OLIVA, N. & CINI, E. 2020. A systematic review of gluten-free dough and bread: Dough rheology, bread characteristics, and improvement strategies. *Applied Sciences*, 10, 6559.
- CHANG, D., MA, Z., LI, X. & HU, X. 2021. Structural modification and dynamic in vitro fermentation profiles of precooked pea starch as affected by different drying methods. *Food & Function*, 12, 12706-12723.
- CHRISTENSEN, L. F., GARCÍA-BÉJAR, B., BANG-BERTHELTSEN, C. H. & HANSEN, E. B. 2022. Extracellular microbial proteases with specificity for plant proteins in food fermentation. *International Journal of Food Microbiology*, 381, 109889.
- DEEHAN, E. C., MOCANU, V. & MADSEN, K. L. 2024. Effects of dietary fibre on metabolic health and obesity. *Nature reviews Gastroenterology & hepatology*, 21, 301-318.
- DOU, X., REN, X., ZHENG, Q., HE, Y., LV, M., LIU, L., YANG, P., HAO, Y., CHEN, F. & TANG, X. 2023. Effects of lactic acid bacteria fermentation on the physicochemical properties of rice flour and rice starch and on the anti-staling of rice bread. *Foods*, 12, 3818.
- EMIR, A. A., YILDIZ, E. & SUMNU, G. 2023. Utilization of Lentils in Different Food Products. *Lentils: Production, Processing Technologies, Products, and Nutritional Profile*, 237-259.
- EMKANI, M., OLIVETE, B. & SAUREL, R. 2022. Effect of lactic acid fermentation on legume protein properties, a review. *Fermentation*, 8, 244.
- GUILLEN-GUERRERO, K. M. & DE LA ROSA-MILLAN, J. 2025. Effects of Fermentation Temperature on the Physicochemical Properties, Bioactive Compounds, and In Vitro Digestive Profile of Cacao (*Theobroma cacao*) Seeds. *Fermentation*, 11, 167.
- GUINÉ, R. P. 2022. Textural properties of bakery products: A review of instrumental and sensory evaluation studies. *Applied Sciences*, 12, 8628.
- JIA, R., GE, S., REN, S., LUO, Y., XIU, L., SANABIL, LIU, H. & CAI, D. 2021. Antibacterial mechanism of adzuki bean seed coat polyphenols and their potential application in preservation of fresh raw beef. *International Journal of Food Science & Technology*, 56, 5025-5039.
- LANDETE, J. M., RODRÍGUEZ, H., CURIEL, J. A., DE LAS RIVAS, B., DE FELIPE, F. L. & MUÑOZ, R. 2021. Degradation of phenolic compounds found in olive products by *Lactobacillus plantarum* strains. *Olives and Olive Oil in Health and Disease Prevention*. Elsevier.
- LEYEZA V.E.B., L.-A. R. C. M., FLANDEZ L.E.L. AND CASTILLO-ISRAEL K.A.T., 2025. Enhancing the Physicochemical Characteristics and Biological Activities of Phenolic-rich 'Bignay' (*Antidesma bunius* (L.) Spreng.) Fruit Beverage by Two-stage Fermentation. *Chiang Mai Journal of Science*, 52(2): e2025011.
- LIAQUAT, A., ASHRAF, H., AHSAN, M., UL-HAQ, I.-., MUGABI, R., ALSULAMI, T. & NAYIK, G. A. 2025. Enzymatic influence on dough rheology and cookie quality: protease and lipase as functional modifiers. *International Journal of Food Properties*, 28, 2489490.
- LUO, S., ZHOU, B., CHENG, L., HUANG, J., ZOU, P., ZENG, Y., HUANG, S., CHEN, T., LIU, C. & WU, J. 2022. Pre-fermentation of rice flour for improving the cooking quality of extruded instant rice. *Food Chemistry*, 386, 132757.
- MA, S., WANG, Z., TIAN, X., SUN, B., HUANG, J., YAN, J., BAO, Q. & WANG, X. 2022. Effect of synergistic fermentation of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* on thermal properties of wheat bran dietary fiber-wheat starch system. *Food Chemistry*, 373, 131417.
- MIAO, W., LI, N. & WU, J.-L. 2022. Food-polysaccharide utilization via in vitro fermentation: Microbiota, structure, and function. *Current Opinion in Food Science*, 48, 100911.
- MURTAZA, M. S., YAQOOB, S., MUBEEN, B., SAMEEN, A., MURTAZA, M. A., REHMAN, A., ALSULAMI, T., KORMA, S. A., KHALIFA, I. & MA, Y. K. 2025. Investigating the triple-frequency ultrasound-assisted fermented rice lees: Impact on physicochemical, structural, morphological, and metabolic properties. *Ultrasonics Sonochemistry*, 112, 107176.
- N'ZI, K. P., ADINGRA, K. M.-D., N'GUESSAN, K. F., ATTACHELOUWA, C. K. & TANO, K. 2021. Effect of spontaneous fermentation time on physicochemical, nutrient, anti-nutrient and microbiological composition of Lima Bean (*Phaseolus lunatus*) flour. *Journal of Applied Biosciences*, 162, 16707-16725.

NAVEED, H., SULTAN, W., AWAN, K. A., IMTIAZ, A., YAQOOB, S., AL-ASMARI, F., FARAZ, A., QIAN, J.-Y., SHARMA, A. & MUGABI, R. 2024. Glycemic impact of cereal and legume-based bakery products: Implications for chronic disease management. *Food Chemistry: X*, 101959.

NEJI, C., SEMWAL, J., KAMANI, M. H., MÁTHÉ, E. & SIPOS, P. 2022. Legume protein extracts: The relevance of physical processing in the context of structural, techno-functional and nutritional aspects of food development. *Processes*, 10, 2586.

PERERA, D., DEVKOTA, L., GARNIER, G., PANOZZO, J. & DHITAL, S. 2023. Hard-to-cook phenomenon in common legumes: Chemistry, mechanisms and utilisation. *Food chemistry*, 415, 135743.

SAROJ, R., MALIK, M. A. & KAUR, D. 2025. Effect of solid-state yeast fermentation on the physicochemical properties, antioxidant and anti-nutritional activity of wheat bran. *Cereal Research Communications*, 1-17.

Shtylla B., Pikuli K., Morava K., Karapanci N., Zhidro N. (2024), Determination of proteins by the Kjeldahl method in cereals of the markets in Korça city, *JASRD Journal of Agriculture and Sustainable Rural Development*, 2(3-4), 26–33.

SHIFERAW TEREFE, N. & AUGUSTIN, M. A. 2020. Fermentation for tailoring the technological and health related functionality of food products. *Critical reviews in food science and nutrition*, 60, 2887-2913.

Khan A., Talpur F.N., Bhanger M.I., Musharraf S.G., Afridi H.I. (2021), Extraction of fat and fatty acid composition from slaughterhouse waste by evaluating conventional analytical methods, *American Journal of Analytical Chemistry*, 12(5), 202–225.

TEJEDOR-SANZ, S., STEVENS, E. T., LI, S., FINNEGAN, P., NELSON, J., KNOESEN, A., LIGHT, S. H., AJO-FRANKLIN, C. M. & MARCO, M. L. 2022. Extracellular electron transfer increases fermentation in lactic acid bacteria via a hybrid metabolism. *Elife*, 11, e70684.

TOMÁS-PEJÓ, E., GONZÁLEZ-FERNÁNDEZ, C., GRESES, S., KENNES, C., OTERO-LOGILDE, N., VEIGA, M. C., BOLZONELLA, D., MÜLLER, B. & PASSOTH, V. 2023. Production of short-chain fatty acids (SCFAs) as chemicals or substrates for microbes to obtain biochemicals. *Biotechnology for Biofuels and Bioproducts*, 16, 96.

VALSALAN, A., KOKSEL, F., ROSELL, C. M. & MALALGODA, M. 2024. Ingredient technologies and process modifications for increasing the use of ancient grains in bakery applications. *Food Reviews International*, 40, 2873-2892.

VARGHESE, S., AWANA, M., MONDAL, D., RUBIYA, M., MELETHIL, K., SINGH, A., KRISHNAN, V. & THOMAS, B. 2022. Amylose–amylopectin ratio: Comprehensive understanding of structure, physicochemical attributes, and applications of starch. *Handbook of biopolymers*. Springer.

WOODBURY, T. J. & MAUER, L. J. 2023. Oligosaccharide, sucrose, and allulose effects on the pasting and retrogradation behaviors of wheat starch. *Food Research International*, 171, 113002.

XIONG, J., LIU, D.-M. & HUANG, Y.-Y. 2023. Exopolysaccharides from *Lactiplantibacillus plantarum*: isolation, purification, structure–function relationship, and application. *European Food Research and Technology*, 249, 1431-1448.

YAN, X., MCCLEMENTS, D. J., LUO, S., YE, J. & LIU, C. 2024. A review of the effects of fermentation on the structure, properties, and application of cereal starch in foods. *Critical Reviews in Food Science and Nutrition*, 1-20.

YAQOOB, S., CAI, D., LIU, M., ZHENG, M., ZHAO, C.-B. & LIU, J.-S. 2019a. Characterization of microstructure, physicochemical and functional properties of corn varieties using different analytical techniques. *International Journal of Food Properties*, 22, 572-582.

YAQOOB, S., GOUDA, M. M., MUBEEN, B., IMTIAZ, A., MURTAZA, M. S., AWAN, K. A., REHMAN, A., ALSULAMI, T., KHALIFA, I. & MA, Y. 2024a. Synergistic Enhancement in the Mulberry Juice's Antioxidant Activity by Using Lactic Acid Bacteria Co-culture Fermentation.

YAQOOB, S., IMTIAZ, A., AWAN, K. A., MURTAZA, M. S., MUBEEN, B., YINKA, A. A., BOASIAKO, T. A., ALSULAMI, T., REHMAN, A. & KHALIFA, I. 2024b. Impact of fermentation through synergistic effect of different lactic acid bacteria (mono and co-cultures) on metabolic and sensorial profile of mulberry juice. *Journal of Food Measurement and Characterization*, 1-21.

YAQOOB, S., LIU, H., LIU, M., ZHENG, M., AWAN, K. A., CAI, D. & LIU, J. 2022. The effect of lactic acid bacteria and co-culture on structural, rheological, and textural profile of corn dough. *Food Science & Nutrition*, 10, 264-271.

- 465 YAQOOB, S., LIU, H., ZHAO, C., LIU, M., CAI, D. & LIU, J. 2019b. Influence of multiple
466 freezing/thawing cycles on a structural, rheological, and textural profile of fermented and
467 unfermented corn dough. *Food Science & Nutrition*, 7, 3471-3479.
- 468 ZHANG, H., ZHAO, X., CHEN, X. & XU, X. 2022. Thoroughly review the recent progresses in improving
469 O/W interfacial properties of proteins through various strategies. *Frontiers in Nutrition*, 9, 1043809.
- 470 ZHANG, X., LAPOINTE, G., LIU, Y., WANG, X., XIAO, L., ZHAO, X. & LI, W. 2023. Comparative
471 analysis of exopolysaccharide-producing *Lactiplantibacillus plantarum* with ropy and non-ropy
472 phenotypes on the gel properties and protein conformation of fermented milk. *Food Chemistry*, 420,
473 136117.

474