



Thermal processing effects on mung bean juice: Evaluating bioactive stability, viscosity, and metabolite profiles

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ABSTRACT

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Mung bean juice is commonly consumed as a nutritious beverage and contains various bioactive compounds. Thermal processing is needed to ensure microbial safety, but heating may also affect physical properties and bioactive stability. In this study, mung bean juice was pasteurized at 63 °C (T1), 73 °C (T2), and 83 °C (T3) for 30 minutes. The effects of temperature were evaluated by measuring total microbial counts, reducing sugars, cooking loss, total phenolic and flavonoid contents, antioxidant activity (DPPH), viscosity behavior, and metabolite profiles using LC-HRMS. The results showed that thermal conditions reduced microbial levels in all treatments, with total plate counts ranging from 2.81 to 2.99 log cfu/mL. Increasing the processing temperature resulted in higher cooking loss, which increased from 3.51% at 63 °C to 8.85% at 83 °C, while reducing sugar content decreased from 0.358 to 0.216 mg/g. Rheological analysis showed that all samples exhibited shear-thinning behavior, with viscosity increasing with increasing processing temperature. Total phenolic and flavonoid contents did not show significant differences among treatments. However, antioxidant activity increased with temperature, from 75.22% at T1 to 87.36% at T3. LC-HRMS analysis identified 229 metabolites and showed changes related to processing temperature. Several lipid-related compounds, including L- α -palmitin, phospholipids, and citric acid, showed higher peak areas at higher temperatures, while most amino acids remained relatively stable. Overall, pasteurization up to 83 °C increased antioxidant activity and viscosity without causing significant changes in the overall metabolite profile, while microbial safety was maintained.

Contribution/Originality: The paper's primary contribution is that thermal processing conditions affect microbial safety, viscosity, bioactive retention, and metabolite composition of mung bean juice, supporting the selection of thermal conditions for industrial and small-scale beverage processing.

1. INTRODUCTION

Mung beans were appreciated based on their nutritional and health benefits, so they became a beneficial food in the world. Different preparations, in particular mung bean juice, have become popular because of the presence of bioactive compounds and favorable effects on health, which may benefit the overall health outcomes. Mung bean juice

has a nutritional composition of proteins, carbohydrates, vitamins, minerals, and bioactive components such as polyphenols and polysaccharides. The nutrients provide several health benefits, such as antioxidant, liver-protective, and anti-diabetic effects (Hou et al., 2019). Research has shown that mung bean juices have important effects in the management of blood glucose, probably due to the presence of polyphenolic compounds and their ability to increase insulin sensitivity (Tang, Dong, Ren, Li, & He, 2014; Yang et al., 2020; Zheng et al., 2020).

Thermal processing is required to ensure the microbial safety of mung bean juice, both when used as a fermentation medium (Setiyawan et al., 2025; Wu et al., 2015) or consumed directly as a healthy beverage (Andrestian & Hatimah, 2015; Liang et al., 2022). However, heating can also affect the nutritional quality and bioactive properties of the juice. For the traditional culinary process of mung beans, thermal processing significantly affects the nutritional quality (Kabr , Hama-Ba, Sanou , & Savadogo, 2025). Some studies report that higher processing temperatures may reduce antioxidant activity through the degradation of phenolic and flavonoid compounds (Igual, Garc a-Mart nez, Camacho, & Mart nez-Navarrete, 2010) while others indicate that thermal treatment can promote the release of bound phenolics, increasing antioxidant capacity (Liu, Ragaee, Marcone, & Abdel-Aal, 2020). These contrasting results suggest that the impact of thermal processing strongly depends on temperature and conditions, and that responses of mung bean juice in liquid form are not fully characterized.

High temperatures may also reduce heat-sensitive phytochemicals and nutrients and affect the gelatinization behavior and quality of mung bean starch (Piyapattanamongkol, Kongpensook, & Tananuwig, 2022). Although mild heating can enhance certain functional attributes, excessive temperature or prolonged exposure could compromise key bioactive compounds. Despite extensive studies on solid or semi-solid mung bean products and other legume-based beverages, information on how mung bean juice as a liquid system responds to different thermal conditions remains limited. In particular, the combined effects of thermal processing on microbial safety, viscosity, bioactive stability, and metabolite composition have not been fully elucidated.

Therefore, this study evaluated the effects of thermal processing at 63 C, 73 C, and 83 C for 30 minutes on total microbial counts, bioactive stability, and metabolite profiles of mung bean juice, to provide scientific support for selecting appropriate thermal conditions in mung bean juice processing.

2. MATERIALS AND METHODS

2.1. Materials of Mung Beans

Mung beans were purchased from a local market and grown in Gunungkidul Regency. Information regarding cultivar and harvest period was not available due to the commercial market source. All beans used in the experiment were purchased in a single procurement and processed under identical laboratory conditions to reduce variability among samples.

2.2. Preparation of Mung Bean Juice (MBJ)

Mung bean juice was prepared using a modified version of the procedure described by Liang et al. (2022) with modifications in the size of the filter. After being cleaned, the mung beans are soaked for 12 hours. After that, it is ground for 3 minutes while water is added, using a 1:10 (v/v) mung bean-to-water ratio. The mung bean juice processing conditions are 63 C for 30 minutes (T1), 73 C for 30 minutes (T2), and 83 C for 30 minutes (T3), followed by filtration through a 30-mesh filter to separate the pulp from the juice.

The production of mung bean juice was carried out in four independent batches for each treatment, which were considered biological replicates. Each batch represented one experimental unit. For each experimental unit, three analytical replicates were performed. Thus, the experiment consisted of 12 experimental units (3 treatments x 4 biological replicates).

2.3. Determination of Total Microbes

Total microbes were evaluated using the total plate count method (Rizqiyati et al., 2023). The media used were Plate Count Agar (Merck) media and physiological NaCl (0.86%). The dilution stage used a 1:9 (v/v) sample-to-sterile diluent ratio. The isolation stage used the pour plate method in duplicate, taking dilution factors of 10^{-2} , 10^{-3} , 10^{-4} , then incubated inverted at 37°C for 24 hours. The microbial colony count analyzed ranged from 30 to 300 cfu/g.

2.4. Determination of Cooking Loss and Reducing Sugar

Cooking loss was analyzed according to the method of Raczyk, Polanowska, Kruszewski, Grygier, and Michałowska (2022). The cooking loss was determined by weighing samples before and after cooking. The initial sample weight was measured, then the sample was pasteurized according to the predetermined method, and the final sample weight was measured after pasteurization. The difference in sample weight indicates the amount of substance lost during pasteurization. Cooking loss is calculated using.

$$\text{Cooking loss} = \frac{\text{initial weight} - \text{weight after pasteurization}}{\text{initial weight}} \times 100\%$$

2.5. Determination of Reducing Sugar

The 3,5-dinitrosalicylic acid (DNS) reagent and reducing sugar were prepared according to the method of Deshavath, Mukherjee, Goud, Veeranki, and Sastri (2020). Briefly, 1 mL of standard or sample was mixed with 3 mL of DNS reagent (Merck), heated in boiling water for 5 min, and the absorbance was measured at 540 nm using a UV-Vis spectrophotometer (Agilent, USA). Distilled water was used as a blank. The DNS assay was applied uniformly across all treatments for comparative evaluation. Potential interference due to heat sensitivity and Maillard reaction products during thermal processing was acknowledged, and similar applications in thermally processed mung beans have been reported by Liang et al. (2021).

2.6. Determination of Total Phenolic Content (TPC)

The TPC method, as described by Hur, Lee, Kim, Choi, and Kim (2014). Briefly, the sample solution (20 μL) and Folin-Ciocalteu (Merck) solution (100 μL) were mixed and incubated in the dark for 5 min. Then, a Na_2CO_3 (Merck) solution (80 μL) was added, and the mixture was reacted for 2 hours. The absorbance was measured at 760 nm using a microplate photometer (Thermo Scientific™ Multiskan™ FC Microplate Photometer). Gallic acid was used as a reference standard, and the results were expressed as milligrams of gallic acid equivalent (GAE) per gram (d.w.).

2.7. Determination of Total Flavonoid Content (TFC)

TFC was analyzed according to the method of Supasatyanukul, Saisriyoot, Klinkesorn, Rattanaporn, and Sae-Tan (2022). Briefly, 10 μL of the sample was added to each well of the 96-well plate solution, followed by 60 μL of methanol (Merck), 10 μL of 50 g/L NaNO_2 (Merck), and 120 μL of distilled water. After a 5-minute incubation, 10 μL of 100 g/L AlCl_3 (Merck) was added to the mixture. The plate was shaken for 30 minutes in the plate reader before measuring absorbance at 415 nm using a microplate photometer (Thermo Scientific™ Multiskan™ FC Microplate Photometer). Quercetin was used as a reference standard, and the results were expressed as milligrams of quercetin equivalent (QE) per gram (d.w.).

2.8. Determination of Radical DPPH Scavenging Activity

Determination of radical DPPH scavenging activity according to Zheng et al. (2020). 100 μL of the sample and 100 μL of 0.2 mM DPPH (Merck) in ethanol (Merck) were briefly mixed in a 96-well plate. The plate was incubated in the dark for 30 minutes before absorbance was measured at 517 nm using a microplate photometer (Thermo Scientific Multiskan FC Microplate Photometer).

2.9. Viscosity Behavior Analysis

Determination of viscosity behavior using MCR 302 Rheometer Anton Paar, with plate 25 mm, gap 1 mm, temperature setting in 25°C, and shear rate: $d(\gamma)/dt = 0,1 \dots 500$ 1/s linear. The time settings are 25 data points and no point duration setting.

2.10. Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS) Analysis

LC-HRMS was performed according to Indrianingsih et al. (2024) for analyzing mung bean juice in thermal processing. Orbitrap high-resolution mass spectrometry and Thermo Scientific™ Vanquish™ UHPLC Binary Pump (Thermo Scientific, USA) were used. Methanol was used to dissolve the materials, and a mixture of methanol and water containing 0.1% formic acid was used to elute the solvent. At a flow rate of 0.3 mL/min, a 3 µL injection volume was used. The ion source ran in both positive and negative modes at 3.30 kV. LC-HRMS analysis was performed in full-scan mode over a mass range 66.7 to 1000 m/z with a mass accuracy within ±5 ppm. Quality control (QC) samples were prepared by pooling aliquots of all samples and injected periodically to monitor instrument stability. Metabolite identification was performed based on accurate mass matching and database comparison using mzCloud and ChemSpider. Identification confidence was assigned according to the Metabolomics Standards Initiative (MSI) guidelines (Sumner et al., 2007) corresponding mainly to MSI level 2 (putatively annotated compounds).

2.11. Data Analysis

The experiments were conducted in four replicates, resulting in 12 experimental units, each including three analysis replicates. A one-way ANOVA analyzed the data. Post hoc tests, including Duncan's Multiple Range Test (DMRT), were applied if results were statistically significant ($p < 0.05$). Data processing used CoSTAT Version 6.400. The number of units was appropriate for evaluating processing effects.

Raw LC-HRMS data were processed and analyzed using MetaboAnalyst (version 6.0). Data preprocessing included peak filtering, normalization, and scaling before multivariate analysis. Principal component analysis (PCA) and partial least squares–discriminant analysis (PLS–DA) were used to explore metabolic differences among thermal processing treatments.

3. RESULTS AND DISCUSSION

3.1. Characteristics of Mung Bean Juice During Thermal Conditions

The total number of microbes is critical to evaluate, as it is significant for food products that will be consumed. Mung bean juice is used as a healthy beverage (Pravitasari, Hariyadi, & Mulyanita, 2020), a fermentation beverage Liang et al. (2022) or as a substitute for milk in yoghurt or kefir medium (Setiyawan et al., 2025; Yi, Li, Zhu, Liu, & Quan, 2021). For Table 1, the total number of microbes in mung bean juice after processing was not significantly different across the thermal parameters. The values for total microbes were T1 (2.811 ± 0.07 log cfu/mL), T2 (2.823 ± 0.02 log cfu/mL), and T3 (2.987 ± 0.38 log cfu/mL). In Indonesia, the National Agency of Drug and Food Control established a limit of microbial contamination of less than 5 log cfu/mL as a plate count determination limit. It shows that the processing temperatures between 63 °C and 83°C can be used.

Table 1. Determination of total microbes, reducing sugars, and cooking loss after processes.

Parameter	T1	T2	T3
Total microbes (log cfu/ml)	2.811 ± 0.07^{ns}	2.823 ± 0.02^{ns}	2.987 ± 0.38^{ns}
Reducing sugars (mg/g)	0.358 ± 0.03^b	0.230 ± 0.02^a	0.216 ± 0.03^a
Cooking loss (%)	3.512 ± 0.79^a	5.901 ± 2.21^b	8.845 ± 0.44^c

Note: ^{ns}) not a significant difference; ^{a,b,c}) significant difference between the treatments. T1 : 63°C for 30 minutes; T2 : 73°C for 30 minutes; T3: 83°C for 30 minutes.

The thermal process reduced the sugar content of the mung bean juice. The research showed that increasing the temperature to 83°C for 30 minutes resulted in a decrease in the sugars. It is because the gelatinization process reduces sugar content during thermal treatment. Research on sweet potatoes has shown that the gelatinization process occurs when starch is heated, and this process converts starch into reducing sugars to some degree. It also describes that long-term heating decreases sugar content by reaction caramelization or additional degradation at the higher temperatures and thus decreases the supply of sugar (Sawai, Nakai, Hashimoto, & Shimizu, 2004; Sawai, Nakai, & Shimizu, 2009). The temperature of the cooking loss is directly proportional to the percentage of cooking loss. Losses in cooking occur at a lower temperature of 63°C. Protein denaturation should be minimized at this temperature, which assists in preserving the water content in the protein matrix (Diao, Tao, Chen, Zhang, & Wang, 2022). However, increasing the cooking temperature will cause protein denaturation. The starch-protein interaction is disrupted, and the starch is released, and the water retention is reduced (Huang, Cheng, Chan, Lu, & Li, 2024).

3.2. Viscosity Analysis of Mung Bean Juice in Different Thermal Processing Conditions

Figure 1 shows the viscosity of mung bean juice in different thermal processing conditions. Shear-thinning behaviour was observed in all treatments, and viscosity decreased with increasing shear rate. The rheological behaviour is that of a non-Newtonian pseudoplastic fluid, meaning the viscosity of the sample increases with increasing shear rate, which simplifies movement (Setiyawan et al., 2025). At low shear rates (25–100 s⁻¹), the viscosity in all treatments was relatively high, indicating the presence of protein-fat interactions resistant to deformation. These structures disintegrated as the shear rate increased, leading to a significant reduction in viscosity until reaching a constant value at high shear rates. Among these treatments, T3 (83°C) showed the highest viscosity value, followed by T2 (73°C) and T1 (63°C). The increase in viscosity with increasing processing temperature is likely related to protein denaturation. The increase in viscosity was also linearly related to increasing cooking losses (Table 1), which increased significantly ($p < 0.05$) from $3.51 \pm 0.79\%$ (T1) to $8.85 \pm 0.44\%$ (T3).

According to this trend, despite increased temperature (83°C), increasing internal structural rigidity, indicated by increased viscosity, also increases moisture and soluble compound loss, hence decreasing the water-holding capacity. Also, increased temperatures during pasteurisation may cause the gelatinisation of starches (Sharma, Singh, Hussain, & Sharma, 2017) and reduced protein stability, worsening the loss of moisture during processing (Zhang et al., 2022). By contrast, the treatment with the lowest temperature (T1) had a higher water content, and it had a lower viscosity and cooking loss (Table 1), and therefore suggested a less aggregated protein matrix that was more balanced.

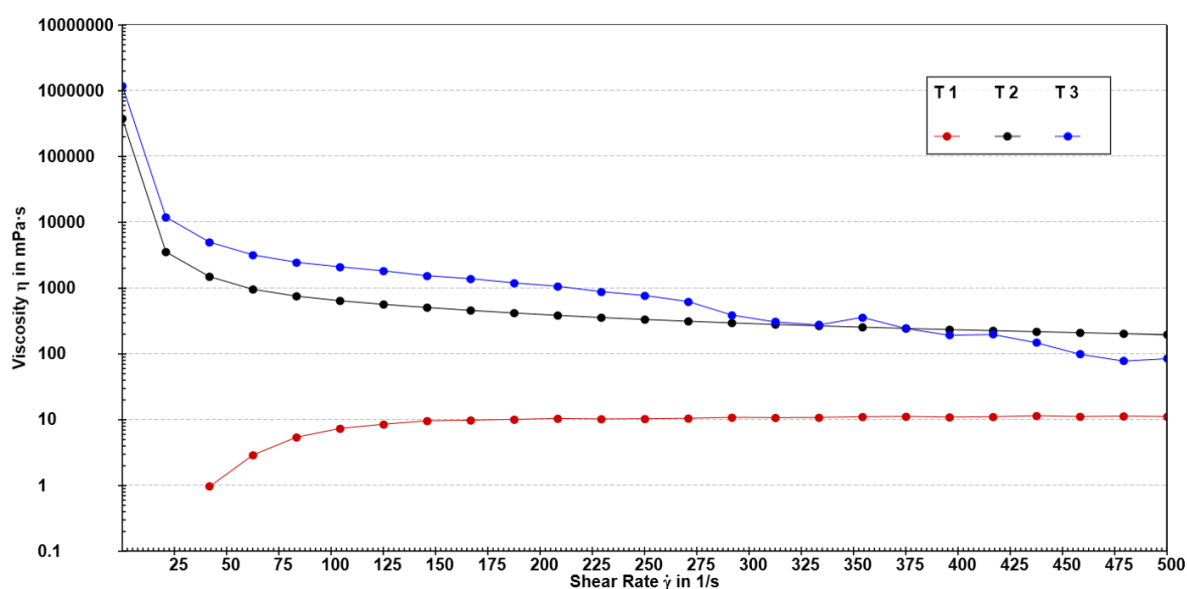


Figure 1. Viscosity depends on the shear rate of mung bean juice in different thermal processing conditions.

3.3. Determination of Bioactive Stability (Phenolic, Flavonoid, and DPPH inhibitors) of Mung Bean Juice

Determination of bioactive compound stability in mung bean juice is shown in Table 2. The temperature increase could not be significantly different from that of phenolic and flavonoid compounds. The heating process changes the structure of mung bean extract, causing gelatinization. Figure 1 shows that the viscosity of T3 is higher than that of the others. This chemical modification also results in the creation of a globulin-polyphenol complex, thus enhancing protein stability and preserving some of the bioactive components (Ma et al., 2024; Zhang et al., 2022).

Antioxidant activity increased significantly with increasing temperature from 75.216% to 87.359% (Table 2). Such improvement is an indicator that the heating process will possibly promote the release of bound antioxidant compounds or even the production of new antioxidant compounds that will raise antioxidant capacity. The study of the effects of various cooking methods on black beans found that high-pressure heat treatment can lead to the formation of Maillard reaction products, which exhibit significant antioxidant activity. It shows that the modifications in phenolic compounds of cooking increase their capacity to counter free radicals (Xu & Chang, 2008). In line with this study, the cooking procedure focuses on the concentration of the phenolic compounds in the broth and the overall antioxidant capability of the black beans in the post-cooking phase (Huber, Brigide, Bretas, & Canniatti-Brazaca, 2016). The increase in antioxidant activity at 83°C may be attributed to the increased release of antioxidant-related metabolites and the formation of thermally produced compounds. LC–HRMS analysis in Table 3 shows higher peak areas for several metabolites associated with antioxidant activity, such as lipid moieties, in samples processed at 83°C. This suggests that increasing the processing temperature does not damage the bioactive compound but may enhance the antioxidant properties of mung bean juice.

Table 2. Determination of bioactive stability of mung bean juice in different thermal conditions.

Parameter	T1	T2	T3
Phenolic total (mg GAE/g)	0.068±0.02 ^{ns}	0.069±0.02 ^{ns}	0.076±0.02 ^{ns}
Flavonoid (mg QE/g)	0.008±0.01 ^{ns}	0.007±0.01 ^{ns}	0.007±0.01 ^{ns}
DPPH inhibitor (%)	75.216±7.24 ^a	83.428±2.21 ^b	87.359±0.44 ^b

Note: ^{ns}) not a significant difference; ^{a,b,c}) significant difference between the treatments. T1: 63°C for 30 minutes; T2: 73°C for 30 minutes; T3: 83°C for 30 minutes.

3.4. LC-HRMS Analysis

Based on LC-HRMS analysis, the compounds resulting from thermal processing effects on mung bean juice are shown in Table 3. The 229 metabolites were identified by non-target LC-HRMS in positive and negative ionization modes. Metabolite identification using the mzCloud and ChemSpider databases selected the top ten antioxidant compounds and two original antioxidant compounds in mung bean juice with a similarity level of more than 80%.

Table 3. Metabolite compounds of thermal processing effects on mung bean juice from LC-HRMS analysis.

No.	Name	Formula	Calc. MW	RT (Min)	Area		
					T1	T2	T3
1	L-alpha-PALMITIN	C ₁₉ H ₃₈ O ₄	330.276	17.620	3999688650	4972417706	5306558058
2	Choline	C ₅ H ₁₃ NO	103.099	0.801	1948557665	2268830451	2144936133
3	gamma-Glutamyl-S-methylcysteine	C ₉ H ₁₆ N ₂ O ₅ S	264.078	1.247	1623087794	1798559879	1395090432
4	L-Phenylalanine	C ₉ H ₁₁ NO ₂	165.079	1.886	743441849	778517222	695381557
5	1-palmitoyl-2-linolenoyl-sn-glycero-3-phosphocholine	C ₄₂ H ₇₈ NO ₈ P	755.546	18.781	660375310	649559099	665476395
6	L-Dilinoylecithin	C ₄₄ H ₈₀ NO ₈ P	781.561	19.118	89901962	390304575	322194397
7	Citric acid	C ₆ H ₈ O ₇	192.026	0.965	370299142	329917463	429271911
8	L-Isoleucine	C ₆ H ₁₃ NO ₂	131.095	1.230	268506843	318056161	256894294
9	Valine	C ₅ H ₁₁ NO ₂	117.079	0.847	136264382	165937554	168488268
10	Pheophytin alpha	C ₅₅ H ₇₄ N ₄ O ₅	870.565	19.404	134467080	127127926	139605120
11	Nicotinic acid	C ₆ H ₅ NO ₂	123.032	1.063	37329446	25436666	13911697
12	Glycitein	C ₁₆ H ₁₂ O ₅	284.068	13.547	299181	238458	295515

Note: T1: 63°C for 30 minutes; T2: 73°C for 30 minutes; T3: 83°C for 30 minutes.

LC-HRMS analysis showed that the metabolite groups in mung bean juice changed during heating. Despite the changes, the metabolite composition remained stable. As shown in Table 2, there are no significant differences in total phenols and flavonoids between treatments. In the lipid group, particularly L-alpha-palmitine and phospholipids, the peak area tended to increase with increasing processing temperature. It suggests that heating facilitates the release of more lipid components from mung bean juice. These components are known to have antioxidant properties (Ali, Ahsan, Zia, Siddiqui, & Khan, 2020; Iuchi et al., 2016). It confirms Table 3, which shows that increasing processing temperature increases the antioxidant capacity of mung bean juice.

The increased antioxidant activity is likely due to the higher in citric acid in treatment three (T3), as shown in Table 3. According to Johnson, Tian, Roman, Decker, and Goddard (2015) and Mozuraityte, Kristinova, Rustad, and Storror (2016) citric acid is likely able to bind metal ions such as Fe^{2+} and Cu^{2+} , thus preventing oxidation reactions. However, high temperatures decrease non-heat-stable metabolites, such as γ -glutamyl-S-methylcysteine and vitamin B3 (nicotinic acid). This is because sulfur compounds and vitamins are easily oxidized or degraded when exposed to heat (Macdonald et al., 2011; Zhang, Walk, Sorbara, Cowieson, & Stamatopoulos, 2022). Table 3 shows that amino acids tended to remain unchanged in the temperature range of 63°C to 83°C. Phenylalanine, isoleucine, and valine experienced relatively small changes during the thermal process.

3.5. Principal Component Analysis (PCA)

The PCA analysis in Figure 2 shows that heating at 63°C, 73°C, and 83°C resulted in clustering of mung bean extract samples. This indicates that the metabolite compound profile shifts with increasing temperature. At 63°C, treatment at this temperature tends to retain heat-sensitive compounds, such as aromatic amino acids, sulfur-containing peptides, and phospholipids. In other words, at lower temperatures, many components of the mung bean juice were still preserved from heat damage. In the meantime, at 73°C, the sample position was 63°C to 83°C, and some changes were also observed, but not to degrade seriously. At 83°C, the PCA position of the samples shifts more distinctly, indicating a considerable change in metabolite composition. The association with compounds such as citric acid and lipid-related metabolites suggests that higher temperatures may promote the transformation or breakdown of more complex biomolecules. Overall, these results indicate that increasing temperature accelerates changes in metabolite composition, reflecting temperature-dependent thermal effects on mung bean extract. Figure 2 principal component analysis (PCA) (A) and partial least squares–discriminant analysis (PLS-DA) (B) of mung bean juice under different thermal processing conditions. Note: T1: 63°C for 30 min; T2: 73°C for 30 min; T3: 83°C for 30 min.

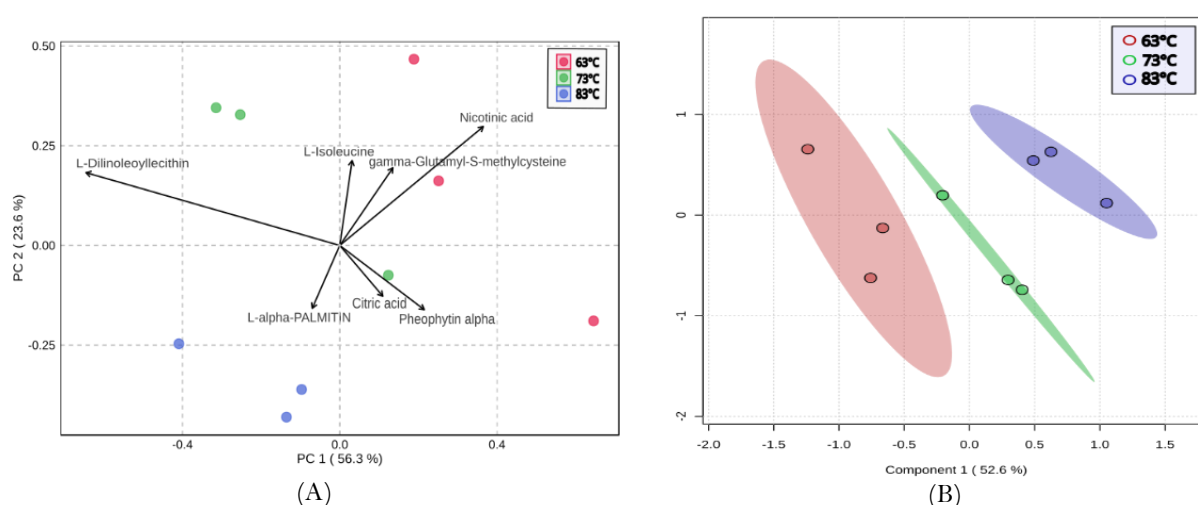


Figure 2. PCA analysis (A) and Partial least squares–discriminant analysis (B) of mung bean juice in different thermal processing conditions.

Further PLS-DA analysis, a supervised pattern recognition technique, produced a score plot similar to that obtained with PCA (Figure 2), confirming the PCA results. Overall, the PLS-DA plots showed that the three temperature treatments also differed, demonstrating that each temperature level also induces distinctive changes in metabolites. This pattern suggests that at lower temperatures, more natural compounds are well preserved, while at intermediate temperatures, changes are already visible, although not as drastic. The greatest changes occur at high temperatures (83°C), and these changes are usually related to the development of metabolites due to heating or degradation.

4. CONCLUSION

Thermal processing of mung bean juice at 63–83 °C affected microbial levels, physicochemical properties, and selected metabolites. Heating up to 83 °C maintained total microbial counts below acceptable limits and increased antioxidant activity from 75.22% to 87.36%. Cooking loss and viscosity increased with temperature, and all samples exhibited non-Newtonian shear-thinning behavior, while total phenolic and flavonoid contents were not significantly different among treatments. LC–HRMS analysis indicated temperature-dependent changes in several lipid-associated metabolites, whereas most amino acids remained relatively stable. These findings suggest that thermal processing primarily modifies physical properties and selected lipid metabolites of mung bean juice, while the overall bioactive composition remains stable across the evaluated temperature range. However, further studies incorporating targeted metabolomics, bioavailability assessment, and scalability evaluation are required to support industrial applications and policy development.

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