

Addition of *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4 to Pressure-cooked Komak (*Lablab purpureus* (L.) Sweet) to Decrease Soaking Time and Water Requirement during Acid Fermentation in Tempe Processing

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Abstract

Background and Objective: Komak beans include high nutritional values, making them promising raw materials for alternative food sources such as tempe. Because the beans are hard, they need soaking in water for 72 h with the water change every 12 h. Soybeans only need soaking for 24 h without changing the water during tempe processing. In this study, pressure cooking was used for Komak beans prior to soaking and a starter culture of *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4 was added to the soaking water to decrease the quantity of water soaking and soaking time.

Material and Methods: Komak beans were pretreated by pressure-cooking for various times and texture and anti-nutritional factors were assessed. The selected pressure-cooked Komak beans were soaked in water and inoculated with *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4. The pH, titratable acidity, soluble protein and minerals were assessed in the soaked water and Komak beans. The soaking water was assessed for viable lactic acid bacteria and anti-nutritional and volatile compounds were assessed in the soaked Komak beans. Mold fermentation was carried out by adding 0.2% (w.w⁻¹) tempe starter culture to the drained Komak beans and incubating for 48 h at room temperature.

Results and Conclusion: Dehulled Komak beans that were pressure-cooked for 15 min included a hardness value of 34.47 N, which was close to the hardness of boiled soybeans in traditional tempe preparing. Pressure-cooking Komak beans significantly decreased anti-nutritional factors. Addition of *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4 during the 24-h soaking step decreased pH of Komak bean from 6.7 to 4.5. Decreases in tannin concentration was observed. Volatile compounds responsible for the beany flavor were not detected in the Komak beans at the end of the soaking. Pressure-cooking and addition of *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4 significantly shortened the soaking time and decreased water needed for Komak Tempe processing. This process provides tempe as an affordable plant-based protein alternative.

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1. Introduction

Komak bean (*Lablab purpureus* (L.) Sweet) is a legume that is widely cultivated in arid areas such as Lombok Island, West Nusa Tenggara, and Indonesia [1]. Commonly

cultivated as a backyard crop, intercrop or monoculture crop, Komak can be harvested four months after planting [2], allowing for year-round use and ensuring supply

availability. Komak beans have traditionally been consumed as vegetables and snacks. These beans are valued for their nutritional composition, characterized by moderate protein, high carbohydrate and low fat contents [3]. Availability and rich nutritional contents of these beans make them promising alternative food sources, especially as plant-based protein options such as tempe. Tempe, a traditional Indonesian fermented food, is processed by fermentation of soybeans with *Rhizopus* spp.

Soybeans are typically used as ingredients in tempe production. However, other beans such as jack beans [4], velvet beans [5] and other common beans [6] can be used. Time needed to prepare tempe varies, especially in the soaking step, depending on characteristics of the beans. Soybeans only need 24 h of soaking to soften the beans in tempe processing [7] but jack beans need 48 h of soaking with water changing every 12 h [4] and velvet beans need a 96-h soaking time with water changing every 12 h [5]. A preliminary study showed that Komak beans could be used as raw materials for Tempe; however, they need 72 h of soaking, with water changing every 12 h. Komak beans are hard with larger bean size, compared to soybeans [7]; however, Komak bean size is smaller than jack [8] and velvet [9] bean sizes. Therefore, Komak Tempe-preparing needs a longer soaking time and consumes six times more water quantity. Tempe production needs a large quantity of water for soaking, changing water and boiling, which hence generates wastewater containing components that can pollute the environment. Therefore, it is necessary to shorten soaking time and decrease water use while creating conditions appropriate for mold fermentation.

Studies have used pressure-cooking methods to speed up softening of beans during soaking. High pressure and uniform heat distribution improved softening of the beans. Time needed for softening can vary depending on size and hardness of the beans [10]. For example, pressure-cooking for 15 min at 110 °C effectively softens common beans [11] and a similar time at 120 °C is appropriate for barlotto beans, chickpeas and kidney beans [12]. Pressure-cooked Komak beans may decrease soaking time and provide a soft texture appropriate for acid fermentation. In soaking, natural acidification occurs due to spontaneous lactic acid bacteria (LAB), decreasing pH of the beans to 4–5, which can inhibit the growth of contaminant microorganisms and pathogenic bacteria [13].

The LAB are addressed for their significant roles in bean soaking of Tempe production. Studies by [14,15] have identified LAB at various stages of soybean tempe production. Moreover, LAB strains isolated at various stages of Tempe processing exhibit antimicrobial activities [16]. The LAB decrease anti-nutritional factors such as phytic acid, tannin [17] and beany flavor [18]. In studies, LAB were added when beans were soaking. These bacteria

include *Lactobacillus plantarum* DSM 20174 in common beans [6] and *Lactobacillus fermentum* HPBD2 in soybeans [19]. This addition could decrease the pH value, shorten the soaking time and inhibit unwanted bacteria such as *Enterobacteriaceae*. The LAB of *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4 isolated from the soaking water of legumes can grow and produce acids in jack bean [15] and black soy [20] milks. Research regarding the combination of pressure-cooking and LAB addition during the soaking stage prior to mold fermentation have not been carried out. Therefore, the major aim of this study was to investigate effects of pressure-cooking time of Komak beans prior to soaking and addition of *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4 starter culture to soaking water in Tempe processing on decrease of soaking-water quantity and soaking time.

2. Materials and Methods

2.1. Materials

Cream-colored Komak jamak putek (*Lablab purpureus* (L.) Sweet) was received from farmers in the North Lombok District of Lombok Island, Indonesia. Samples were collected during a dry season (July–September), 2020. The *L. plantarum* subsp. *plantarum* WGK4 (WGK4) was isolated from water soaked in red lima beans in Tempe production [15], Biotechnology Laboratory, Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia. A commercial Tempe starter (containing *R. oligosporus*) (Raprima, Bandung, Indonesia) was purchased from a local market in Yogyakarta, Indonesia. De Mann, Rogosa and Sharpe (MRS) media were supplied by Merck, Darmstadt, Germany. All chemical reagents were purchased from Sigma-Aldrich, USA.

2.2. Preparation of Starter Culture

The starter culture was prepared as previously described by Yudianti et al. [15]. Culture stock was stored at -20 °C in a sterile solution containing 1:1 ratio of 20% w.v⁻¹ sucrose and 10% w.v⁻¹ skim milk. Working culture was prepared by incubating the culture in MRS broth at 37 °C for 24 h, then inoculating it into MRS deep tube agar. This was incubated at 37 °C for 24 h and stored at 4 °C. Starter culture was activated by inoculation of the working culture into MRS broth and incubation at 37 °C for 24 h twice. Cell pellets were harvested by centrifugation at 2000 rpm for 10 min (Thermo-Fischer Scientific, Germany) and washing twice with sterile saline water. The LAB starter culture was quantified and recorded as CFU.ml⁻¹.

2.3. Preparation of Komak Beans and Pressure-cooking Treatments

Komak beans were peeled using peeler machine (Yamamoto SY 150, Medan, Indonesia). Beans were rinsed



and soaked in distilled water (DW) (1:5 w.v⁻¹) for 6 h at ambient temperature. After discarding the soaking water, beans were washed with DW. Beans were transferred into an Erlenmeyer flask and filled with DW (1:2 w.v⁻¹). Then, flask was subjected to pressure-cooking at 110 °C for 10, 15 and 20 min using portable pressure cooker (All American, Wisconsin, and USA). Raw, soaked dehulled and pressure-cooked soaked beans were assessed for hardness. These samples were freeze-dried (Labconco, Kansas City, USA) and transferred into a freezer at -20 °C until analysis of phytic acid, trypsin inhibitor (TI), tannin and volatile compounds.

2.4. Acid Fermentation of Komak Beans with Addition of *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4

A starter culture of WGK4 was added to the selected pressure-cooked Komak beans with the soaking water. The primary LAB concentration was 10⁶ CFU.ml⁻¹. Acid fermentation was carried out at room temperature (RT) for 24 h. Soaked pressure-cooked Komak beans without addition of LAB starter culture were used as control. Every 2 h, samples of soaking water and soaked Komak beans were collected and assessed for pH and titratable acidity. Soaking water sample was assessed for viable cells as well. When pH of the Komak beans reached to 5.0 and 4.5, soaking water was analyzed for soluble proteins and minerals: potassium, magnesium, phosphorus, iron and calcium. Soaked beans were freeze-dried and stored at -20 °C until analysis of soluble proteins, minerals, anti-nutritional compounds and volatile compounds.

2.5. Komak Tempe Fermentation

Komak beans were added to a starter culture (WGK4) and soaked to achieve pH appropriate for mold fermentation. Beans were drained and inoculated with a commercial Tempe starter (0.2% w.w⁻¹). Tempe fermentation was carried out for 48 h at RT using perforated plastic bags. Komak Tempe was freeze-dried and stored at -20 °C until analysis of soluble protein, phytic acid, TI and tannin.

2.6. Hardness Assessment

Hardness of the raw, soaked dehulled and pressure-cooked beans was assessed using universal testing machine (Z0.5, Zwick/Roell, and Germany) [9]. Assessment was carried out at a speed of 10 mm.min⁻¹ with pre-load of 0.01 N. Hardness was assessed as the compression force used to deform the beans. Force was assessed in Newtons (N).

2.7. Viable Lactic Acid Bacteria Count

Viable LAB count was assessed based on a procedure described by Yudianti et al. [15]. Viable cells in soaking water were assessed using serial dilution and pour-plate methods as well as MRS agar added with CaCO₃ and incubated at 37 °C for 48 h. After incubation, colonies on plates were calculated and reported as log CFU.mL⁻¹.

2.8. pH and Titratable Acidity

The pH of the soaking water and beans was assessed using pH meter (FiveEasy F20, Mettler-Toledo, Switzerland). Approximately 5 g of the crushed bean sample were mixed with DW (1:1). Titratable acidity analysis was carried out by titration of 5 ml of soaking water or 5 g of crushed bean mixed with DW (1:1) and 0.1 N NaOH using phenolphthalein (PP) as indicator [21].

2.9. Analysis of Minerals Contents

Mineral contents (K, Mg, P, Fe and Ca) were assessed using ICP-OES (Agilent Technologies 700 Series ICP-OES, Santa Clara, CA, USA) and assessed based on AOAC 2011.14 [21]. Nearly 0.5 ml of soaking water and 0.5 g of freeze-dried sample were digested and mineralized by adding 10 ml of concentrated HNO₃, followed by 15 min of heating at 150 °C using microwave oven. Digested sample was set to reach RT and then an internal standard containing 100 mg.l⁻¹ yttrium was added to the sample, followed by addition of DW to reach 50 ml of the final solution. This was filtered before ICP-OES analysis, using filter papers.

2.10. Analysis of Soluble Protein

Soluble protein assessment in soaking water and soaked Komak beans was carried out using Lowry method [22]. Freeze-dried sample (1 g) was added to 10 ml of DW and mixed for 30 min using water bath shaker. After centrifugation at 3000 rpm for 15 min, supernatant (1 ml) and soaking water (1 ml) were mixed with 0.9 ml of Lowry A. Then, mixture was incubated using water bath shaker. Incubation time included 10 min at 50 °C. Cooled mixture was added with 0.1 ml of Lowry B and left for 10 min at RT. Then, 3 ml of Lowry C were added to the mixture and incubated at 50 °C for 10 min using water bath shaker. Mixture was cooled down to RT and absorbance was recorded at 650 nm immediately. Bovine serum albumin was prepared for the standard curve plotting.

2.11. Analysis of Phytic Acid

Phytic acid content was assessed using a method described by Fitriani et al. [23]. Freeze-dried sample (0.1 g) was mixed with 20 ml of 0.5 M HNO₃ and incubated for 4 h at RT using water bath shaker. Then, mixture was filtered and 1 ml of the resulting extract was mixed with 1 ml of 0.005 M FeCl₃.6H₂O and 0.4 ml of DW followed by heating for 20 min in boiling water (100 °C). After cooling down, mixture was mixed with 5 ml of N-amyl alcohol and 0.1 ml of 0.1 M ammonium thiocyanate and centrifuged at 3000 rpm for 10 min. Then, absorbance was assessed at 495 nm. To generate phytic acid standard curve, a mixture of Na-phytate and HNO₃ solution was used.

2.12. Analysis of Tannin

Tannin concentration was assessed using a procedure described by Fitriani et al. [23]. A freeze-dried sample of



0.31 g was mixed with 62.5 ml of DW and boiled at 100 °C for 2 h. After cooling down, solution was filtered through Whatman filter papers no. 1. Then, 0.5 ml of Folin-Ciocalteu reagent and 2 ml of 20% Na₂CO₃ were added to 1 ml solution. Mixture was incubated at RT for 30 min. Absorbance was read at 748 nm.

2.13. Analysis of Trypsin Inhibitor

The TI was analyzed as previously described by Fitriani et al. [23]. The TI analysis was carried out by preparing TI extract, a substrate (BAPNA solution) and trypsin solution. The TI extract was prepared by dissolving 1 g of freeze-dried sample in 50 ml of 0.01 M NaOH. Mixture was agitated for 3 h at RT using water bath shaker, followed by centrifugation at 3500 rpm for 10 min. Substrate was prepared by dissolving 40 mg of BAPNA in 100 ml of 0.05 M tris-buffer (pH 8.2) in 1 ml of DMSO and stored at 37 °C. Substrate was prepared freshly before assessment. Trypsin solution was prepared by dissolving 4 mg of trypsin in 200 ml of 0.001 M HCl and stored at 4 °C. Generally, TI analysis initiated with the preparation of the control and sample solutions. Control solution was prepared by mixing 2 ml of DW, 5 ml of BAPNA and 2 ml of trypsin solution. Sample solution was prepared using a similar procedure but with addition of the extract. Solutions were incubated at 37 °C for 10 min using water bath shaker. Following incubation, 1 ml of 30% acetic acid was added to terminate the reaction and the mixture was centrifuged for 10 min. Absorbance measurement was carried out at 410 nm using UV/visible spectrophotometer. The TI formula was as Eq. 1:

Eq. 1

$$\frac{\text{TIU}}{\text{mg sample}} = \frac{(\text{Absorbance of control} - \text{absorbance of sample}) \times \left(\frac{100}{\text{Dilution factor (ml)}} \right)}{\frac{\text{Weight of sample (mg)}}{\text{Dilution factor (ml)}}}$$

2.14. Analysis of Volatile Compounds

Headspace SPME/GC-MS was used to analyze volatile compounds of freeze-dried samples released in the headspace. Briefly, 5 g of freeze-dried sample were transferred into a headspace vial (22 ml) with polytetrafluoroethylene-silicone septa. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/ CAR/PDMS) fiber was inserted into the vial and incubated at 80 °C for 45 min using water bath. Fiber was immediately injected into the injector port of the GC-MS for 5 min of desorption at 250 °C. Agilent 7890A GC and Agilent 5975C XL EI/CI MS (Santa Clara, CA, USA) were the instruments of analysis. The capillary column included DB-Wax (30 × 250 × 0.25 m). The oven temperature was initially set to 40 °C, increased by 5 °C.min⁻¹ to 120 °C, then increased by 9 °C.min⁻¹ to 240 °C and set for 5 min. Helium was used as carrier gas at a flow rate of 1 ml.min⁻¹. The quadrupole and ion source temperatures were set at 150 and 250 °C, respectively. The volatile compounds were identified based

on their mass spectra and the NIST 14.0 database. The linear index was calculated using retention data from the standard alkane series (C₉-C₃₁). Relative peak area (%) was estimated by comparing the peak area of each compound with the peak area of all compounds [24].

2.15. Statistical Analysis

A completely randomized experimental design with three replications was used in this study. Data were analyzed using one-way ANOVA followed by Duncan's multiple range test when significant differences were present ($p < 0.05$). Software used for the analysis was SPSS software v.25 (SPSS, Chicago, USA). Results are presented as mean and standard deviation (SD).

3. Results and Discussion

3.1. Pressure cooking treatment

Komak beans were pressure cooked for various quantity of time and the hardness and anti-nutritional contents were assessed. This study demonstrated that pressure-cooking treatment significantly affected hardness ($p < 0.05$). Findings showed that raw Komak beans included the highest hardness (341.93 ± 0.86 N). Dehulling followed by soaking for 6 h (RBPS) significantly decreased the hardness to 84.29 N ± 0.73 (Table 1). In soaking, dehulled beans absorbed water, decreasing hardness and increasing swelling of the beans, which might decrease the cooking time [10].

Pressure-cooking of the beans further decreased their hardness. The longer the pressure-cooking time, the softer the Komak beans. This softening was associated to structural changes in the beans. Breakdown of cell walls in the beans resulted in the release of pectin and protein middle lamella, which contributed to binding and firmness of the beans [11]. Dehulled Komak beans that were pressure-cooked for 15 min included a hardness value of 34.47 N ± 0.51, which was similar to the value of the hardness of boiled soybeans from traditional Tempe (34 N).

Phytic acid, trypsin inhibitor and tannin content in raw Komak beans were 11.77 mg.g⁻¹ ± 0.97, 2.14 TIU.mg⁻¹ ± 0.02 and 11.94 mg.g⁻¹ ± 0.23, respectively (Table 2). Dehulling and soaking of komak beans significantly decreased the trypsin inhibitor and tannin content ($p < 0.05$) but did not significantly decrease the phytic acid content ($p > 0.05$). Pressure-cooking treatment significantly decreased the anti-nutrient factors in this study. Phytic acid concentration of the pressure-cooked dehulled komak beans for 10, 15 and 20 min decreased by 47.1-51%, compared to dehulled beans (RBPS). Phytic acid could bind to minerals such as calcium, copper, zinc and iron, delaying their absorption.



Table 1. Hardness of the raw, soaked and pressure-cooked Komak beans

Treatment	Hardness (N)
RB	341.93 ± 0.86 ^e
RBPS	84.29 ± 0.73 ^d
RBPS10	46.38 ± 0.89 ^c
RBPS15	34.47 ± 0.51 ^b
RBPS20	19.33 ± 0.17 ^a

RB: raw bean, RBPS: Dehulled bean soaked for 6 h, RBPS10: RBPS followed by pressure cooking for 10 min, RBPS15: RBPS followed by pressure cooking for 15 min, RBPS20: RBPS followed by pressure cooking for 20 min. Values are represented as mean ± SD (n = 3). The means values in the same column with different superscripts indicate significant differences (p < 0.05).

It has been reported that soaking and pressure-cooking of soaked kidney beans decreased phytic acid contents by 19 and 62%, respectively [25]. The higher phytic acid decrease in kidney beans could be attributed to longer soaking time and higher temperature during pressure-cooking. Soaking time for kidney beans was 12 h and pressure-cooking temperature and time were 121 °C and 30 min, respectively [25]. Trypsin inhibitor content of Komak beans was 2.14 TIU.mg⁻¹ ± 0.02 and trypsin inhibitor loss during soaking was 3.7%. However, a high decrease rate in trypsin inhibitor was detected in the pressure-cooking treatment (91.1-93.9%). High temperature and pressure could disrupt covalent and non-covalent bonds of proteins, resulting in inactivation of trypsin inhibitor activity [26]. Compared to raw beans, dehulled soaked beans decreased the tannin content by 7.8%, whereas pressure-cooked beans decreased the tannin content by 41.6-64.8% for 10-20 min. Decreases in tannin concentration during the soaking process might be caused by leaking of its compounds into the soaking water, while the pressure-cooking process might decrease the tannin content by thermal degradation of these compounds [27]. Decreases in tannin concentration due to pressure cooking of presoaked and soaked kidney beans and cowpeas have been reported as well [25,27]. This suggests that pressure-cooking Komak beans for 15 min can decrease the

hardness similar to that of soaked soybeans and significantly decrease phytic acid, trypsin inhibitor and tannin contents. The pressure-cooking method can be used to soften beans and decrease anti-nutrient substances prior to the soaking step.

3.2. Acid Fermentation of Komak Beans with Addition of *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4

3.2.1. Viable cells, pH and Total Titratable Acid of Soaking Water and Komak Beans

Acid fermentation of Komak beans by pressure-cooking treatment for 15 min was carried out by the addition of WGK4 starter culture, followed by 24 h of incubation at RT. The soaking water used included water from the pressure-cooking step. Acid fermentation without starter culture addition was used as control (Figure 1A). No viable LAB were present during the soaking time. A possible explanation for this might be that natural LAB from the raw materials and soaking water were killed by heating during the pressure-cooking. Thus, titratable acidity of the soaking water and Komak beans in the control did not increase and pH of soaking water and Komak beans was approximately similar during soaking.

Acid fermentation supplemented with WGK4 starter culture (Figure 1B) showed increased LAB growth from 6.2 to 9.5 log CFU.ml⁻¹ by the end of the soaking time. Viable LAB count increased significantly for 12 h and then was relatively constant until the completion of soaking. The LAB needed carbon and nitrogen sources, vitamins and minerals for their growth and metabolic activities [28]. Fermentable sugars in soaked beans and soaking water were used by LAB and metabolized to compounds, especially lactic acid, increasing titratable acidity and decreasing pH of the soaked beans and soaking water. Sucrose was the most common sugar in Komak beans.

Table 2. Phytic acid, trypsin inhibitor, and tannin of raw, soaked, and pressure-cooked Komak beans

Treatment	Phytic acid (mg.g ⁻¹)	Trypsin Inhibitor (TIU.mg ⁻¹)	Tannin (mg.g ⁻¹)
RB	11.77 ± 0.97 ^b	2.14 ± 0.02 ^d	11.94 ± 0.23 ^d
RBPS	11.21 ± 0.47 ^b	2.06 ± 0.03 ^c	11.00 ± 0.34 ^c
RBPS10	5.93 ± 0.50 ^a	0.19 ± 0.00 ^b	6.97 ± 0.30 ^b
RBPS15	5.78 ± 0.20 ^a	0.16 ± 0.00 ^{ab}	4.57 ± 0.53 ^a
RBPS20	5.49 ± 0.41 ^a	0.13 ± 0.01 ^a	4.20 ± 0.24 ^a

RB: raw bean, RBPS: dehulled bean soaked for 6 h, RBPS10: RBPS followed by pressure cooking for 10 min, RBPS15: RBPS followed by pressure cooking for 15 min, RBPS20: RBPS followed by pressure cooking for 20 min. Values are represented as mean ± SD (n = 3). The means values in the same column with different superscripts indicate significant differences (p < 0.05).



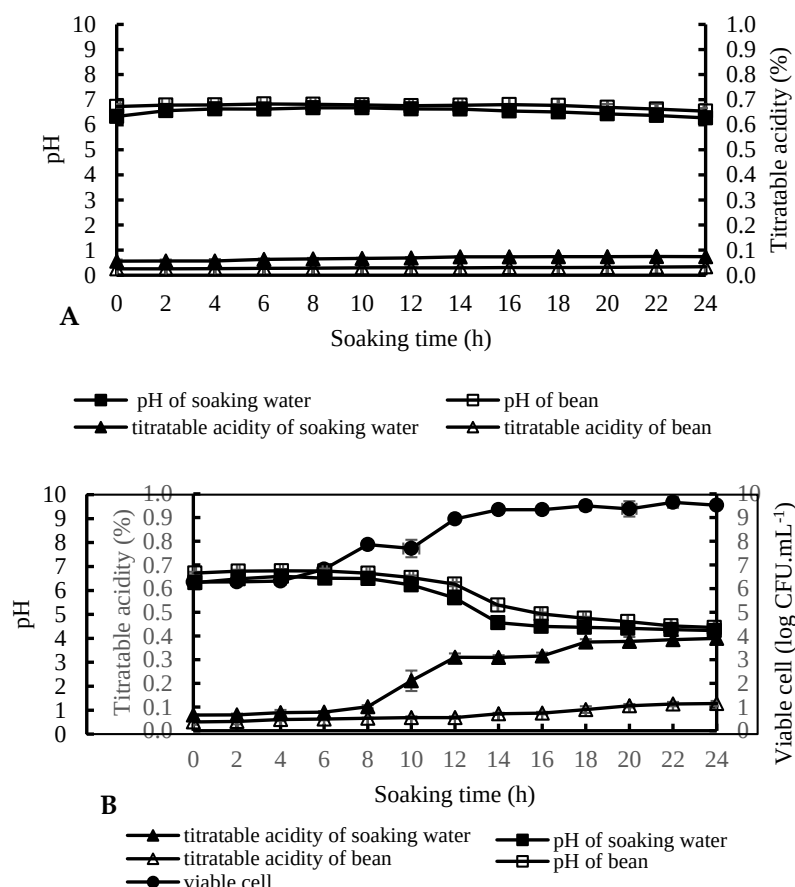


Figure 1. pH, acid production, and growth of WGK4 during soaking of Komak bean (A) without *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4, (B) with 10^6 CFU.mL⁻¹ of *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4 starter culture

The beans also contained stachyose (2.77%) and raffinose (0.45%) [29]. The WGK4 has been reported to use fructose, glucose, sucrose and raffinose for growth [15, 20]. According to [30], *L. plantarum* C6 used stachyose and raffinose to grow through the production of α -galactosidase.

In this study, WGK4 showed a good growth rate during fermentation of the soaked Komak bean. After 24 h of fermentation, growth of WGK4 reached to $9.5 \log \text{CFU.mL}^{-1}$. In addition, black soymilk fermentation using WGK4 at 37°C increased the cell count from 6.4 to $9.1 \log \text{CFU.mL}^{-1}$ [20]. This indicated that WGK4 could use nutrients in black soymilk for its growth. Kitum et al. [17] detected that addition of *L. plantarum* BFE 5092 to soaked kidney beans increased the cell count by 2 log cycles from 6.5 to $8.5 \log \text{CFU.mL}^{-1}$ following fermentation for 24 h. The WGK4 growth during acid fermentation of Komak beans increased by more than 3 log cycles. It is possible that during pressure-cooking prior to soaking, nutrients from the Komak beans needed for the growth of WGK4 were released into the soaking water. Tables 3 and 4 show increases in soluble protein and minerals in the soaking water after pressure-cooking.

Table 3. Soluble protein of soaking water and Komak beans in *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4 soaking

Treatment	Soluble protein (%)	
	Soaking water	Komak beans
Pressure cooking for 15 min ¹	0.28 ± 0.03^a	4.16 ± 0.05^d
Soaking for 24 h without WGK4	0.48 ± 0.05^b	2.09 ± 0.16^c
Soaking for 16 h with WGK4	0.42 ± 0.01^b	1.10 ± 0.05^b
Soaking for 22 h with WGK4	0.37 ± 0.12^{ab}	0.87 ± 0.02^a

Values are represented as mean $\pm$ SD (n = 3). The means values in the same column with different superscripts indicate significant differences ($p < 0.05$). ¹After pressure cooking for 15 min, water and Komak beans were measured for soluble protein. Soaking means soaking after pressure cooking for 15 min without changing the water.

3.2.2. Soluble Protein and Mineral of Soaking Water and Komak Beans

After pressure-cooking treatment for 15 min, protein contents in water and Komak beans were $0.28\% \pm 0.03$ and $4.16\% \pm 0.05$, respectively (Table 3). Pressure-cooking increased the soluble protein of Komak beans, compared to raw beans. Total and soluble s of the Komak beans were 26 and 1.67%, respectively. Water used for the pressure-cooking was used for acid fermentation without changes or addition of fresh water.



Table 4. Minerals of Komak beans and soaking water in *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4 soaking

Sample	Treatment			
	Pressure cooking for 15 min ¹	Soaking for 24 h without WGK4	Soaking for 16 h with WGK4	Soaking for 22 h with WGK4
Beans (mg.100 g ⁻¹)				
K	490.98 ± 0.33 ^d	360.35 ± 1.08 ^c	240.94 ± 0.09 ^b	232.96 ± 0.14 ^a
Mg	89.63 ± 0.37 ^d	59.52 ± 0.09 ^c	52.31 ± 0.02 ^b	50.08 ± 0.09 ^a
P	235.45 ± 0.83 ^d	233.45 ± 0.21 ^c	231.04 ± 0.50 ^b	205.66 ± 0.63 ^a
Fe	2.05 ± 0.05 ^d	1.88 ± 0.06 ^c	1.77 ± 0.00 ^b	1.35 ± 0.01 ^a
Ca	43.07 ± 0.32 ^d	33.43 ± 0.67 ^c	31.83 ± 0.70 ^b	26.17 ± 0.11 ^a
Soaking water (mg.100 mL ⁻¹)				
K	40.67 ± 0.41 ^a	142.99 ± 0.24 ^d	78.48 ± 0.74 ^c	73.48 ± 0.73 ^b
Mg	4.65 ± 0.04 ^a	15.07 ± 0.00 ^d	5.37 ± 0.02 ^c	5.01 ± 0.10 ^b
P	6.73 ± 0.12 ^a	24.28 ± 0.03 ^d	12.88 ± 0.07 ^c	10.19 ± 0.10 ^b
Fe	0.67 ± 0.00 ^c	0.89 ± 0.03 ^d	0.48 ± 0.01 ^b	0.42 ± 0.00 ^a
Ca	0.21 ± 0.00 ^a	5.75 ± 0.04 ^d	5.48 ± 0.15 ^c	3.44 ± 0.05 ^b

Values are represented as mean ± SD (n = 2). The means values in the same row with different superscripts indicate significant differences (p < 0.05). ¹After pressure cooking for 15 min, water and Komak beans were measured for minerals. Soaking means soaking after pressure cooking for 15 min without changing the water.

During soaking, soluble protein in the Komak beans decreased, while soluble protein in the soaked water, either soaking without or with the addition of starter culture WGK4, increased. Decreases in soluble protein in the Komak beans without LAB addition could be due to the protein leaking from the beans to the soaking water. Addition of WGK4 lowered the soluble protein in soaking water and beans. During soaking, LAB used nutrients, including soluble proteins, for their growth and metabolic activity. These bacteria could produce proteolytic enzymes such as proteases and peptidases, which provided nitrogen sources for their growth and metabolic activity. Meng et al. [31] showed that soluble proteins in bean milk (soy, peanut and chickpea) decreased after fermentation with *Lactobacillus fermentum* GD01. The WGK4 grew well in black soymilk containing amino acids such as leucine, valine, glutamic acid, cysteine, arginine, methionine and histidine [20]. Raw Komak beans contain 18 various amino acids, including arginine, glutamic acid, leucine, valine and cysteine. These essential amino acids (EAAs) are needed by LAB, especially *L. plantarum* [32]. Soluble proteins in soaking water and Komak beans might contain these EAAs.

Minerals are micronutrient compounds detected in legumes. Komak beans are rich in potassium, magnesium, phosphorus, iron and calcium [2]. Raw beans contain 1686.95 mg of potassium, 150.26 mg of magnesium, 358.05 mg of phosphorus, 4.71 mg of iron and 123.07 mg of calcium per 100 g. After pressure-cooking, mineral content of the Komak beans decreased. Potassium, magnesium, phosphorus, iron and calcium concentrations in pressure-cooked Komak beans were nearly one-third to two-thirds lower than those in raw beans. The pressure-cooking process, which uses water as a medium, could cause minerals to leak into cooking water [33]. Mineral contents in Komak beans and soaking water after pressure-cooking soaked without and with WGK4 are shown in Table 4. The mineral mostly lost in the Komak beans was potassium.

According to Damodaran et al. [34], potassium is present in foods as a free ion. Martinez-Pineda et al. [35] reported that pressure-cooking soaked chickpeas decreased potassium and phosphorus contents by 88.9 and 67.2% with final values of 95 and 104.6 mg per 100 g, respectively. Pressure-cooking was carried out at 118 °C for 40 min. The pressure-cooking process led to the loss of the mineral concentration of the beans. After pressure-cooking, Komak beans were soaked without and with addition of WGK4.

The current study reported that soaking Komak beans decreased the mineral content. Soaking with addition of WGK4 decreased the potassium content by more than 50%. Decreases in minerals of the Komak beans resulted in increases in minerals in the soaking water. The mineral loss during soaking might be due to the release of minerals into the soaking water [36]. They reported that soaking white faba beans for 24 h decreased iron and phosphorus by 28 and 16%, respectively. Iron content of the cowpeas decreased by 14% after soaking for 24 h [37]. However, concentration of the minerals in soaking water with the addition of WGK4 was much lower than that without the addition of WGK4 (Table 4). Increasing the soaking time with WGK4 significantly decreased minerals in Komak beans and soaking water. Technically, LAB need minerals in their growth media because they are unable to synthesize these essential nutrients. Therefore, media containing minerals must be provided. Soaking water provides minerals used by LAB for growth and metabolic activity. Examples of the minerals needed by LAB are Fe, Mg, Mn and Zn and their quantities vary depending on the microbial strain [38]. As reported by Leksono et al. [20], WGK4 LAB grew well in black soymilk containing minerals including iron, zinc, magnesium and manganese. Minerals were used to grow *L. acidophilus* KLDS 1.0738. During 14 h of fermentation, *L. acidophilus* KLDS 1.0738 utilized iron, magnesium and potassium at rates of 83.5, 27.2 and 9.6%,



respectively [28]. This finding indicated that nutrients in the media could support growth of WGK4.

3.2.3. Anti-nutrient Factors of Komak Beans

Raw Komak beans contained $11.77 \text{ mg.g}^{-1} \pm 0.97$ phytic acid, $2.14 \text{ mg.g}^{-1} \pm 0.02$ trypsin inhibitor and $11.94 \text{ mg.g}^{-1} \pm 0.23$ tannin (Table 2). Pressure-cooking for 15 min decreased more than 50% of these anti-nutrient factors to $5.77 \text{ mg.g}^{-1} \pm 0.34$, $0.16 \text{ mg.g}^{-1} \pm 0.03$ and $4.56 \text{ mg.g}^{-1} \pm 0.58$ for phytic acid, trypsin inhibitor and tannin, respectively. Soaking the Komak beans without WGK4 addition did not significantly decrease contents of the anti-nutrient factors, except for tannin which decreased by more than 50% after 24 h of soaking (Table 5). Soaked Komak beans with the addition of WGK4 significantly decreased the anti-nutrient factors, compared to pressure-cooked beans. Decreases in phytic acid contents throughout fermentation might be associated to the phytase enzyme activity of beans and microbial fermentation [17]. Previous studies have shown that LAB fermentation can decrease the phytic acid contents of materials [17, 39]. For example, fermentation of kidney beans and faba bean flour for 24 h, using *L. plantarum* BFE5092 and *L. plantarum* E-78076, resulted in 28 (5.02 to 3.61 mg.g^{-1}) and 14% (9.7 to 8.9 mg.g^{-1}) decreases in phytic acid, respectively. Pressure-cooking followed by soaking with addition of WGK4 significantly eliminated phytic acid in the Komak beans. Although phytic acid is an anti-nutritional component, low levels of phytic acid are beneficial for health such as prevention of diabetes [40]. Consumption of phytic acid is safe and can be consumed up to 4500 mg per day [41].

As shown in Table 5, trypsin inhibitor content was unaffected by addition of LAB to Komak beans. It showed relatively consistent values ranging 0.13 - 0.15 TIU.mg^{-1} . Chandra-Hioe et al. [42] reported no significant differences in the content of trypsin inhibitor of chickpeas and faba bean flour fermented by LAB for 16 h. Addition of LAB significantly decreased tannin content of the Komak beans, with tannin content ranging 1.16 - 1.45 mg.g^{-1} . Tannin content in soaked kidney beans fermented with *L. plantarum* BFE 5092 for 24 h decreased by 7% (3.07 to 2.83 mg.g^{-1}) [17]. Decreases in tannin in Komak beans fermented with

WGK4 could be linked to the ability of LAB to hydrolyze the tannin complex. Tannin content in Komak beans could be decreased by pressure-cooking followed by the addition of WGK4 during soaking. Tannin is safe to consume and consumption less than 1.5 - 2.5 g per day shows no side effects [43].

3.2.4. Volatile Compounds of Komak Beans

Volatile compounds in raw Komak beans were identified as aldehydes, alcohols, phenols, hydrocarbons, ketones, pyrazines, acids, esters, terpenes, furans and naphthalene (Table 6). After pressure-cooking for 15 min, concentrations of the volatile compounds, except aldehydes, mostly decreased. Further decreases in volatile compound concentrations were detected when Komak beans were soaked with or without addition of WGK4. Soaking Komak beans for 16 h with addition of WGK4 were selected since it achieved an appropriate pH value for mold fermentation. Similar to other legumes, Komak beans include a distinctive aroma that can affect consumer perception and hence limit their use. This distinct aroma is associated with an unpleasant odor, also called beany flavor. Volatile compounds contributing to the beany flavor have been described as beany, green and earthy [44, 45], derived from volatile aldehydes such as hexanal, nonanal and (E, E)-2, 4-nonadienal; alcohols such as 1-hexanol and 1-octen-3-ol; ketones such as 3-octen-2-one; and furan such as 2-pentyl furan [18, 44, 46]. In Komak beans, volatile compounds of the aldehyde group associated with beany flavor include nonanal and (E, E)-2, 4-nonadienal. Nonanal concentration of the beans soaked with addition of WGK4 was significantly lower than that of the pressure-cooking treatment. Nonanal and (E, E)-2, 4-nonadienal was detected at the beginning of soaking because of pressure-cooking. Increases in aldehyde compounds in yellow and grey peas (*Pisum sativum*) were reported due to enzymatic and non-enzymatic oxidation processes during heating [47]. Moreover, (E, E)-2, 4-nonadienal was undetected in Komak beans fermented with WGK4. According to Pei et al. [46], pea flour fermentation with *L. rhamnosus* L08 for 6 h decreased this compound to undetectable levels.

Table 5. Phytic acid, trypsin inhibitor, and tannin of Komak beans in *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4 soaking

Treatment	Phytic acid (mg.g^{-1})	Trypsin Inhibitor (TIU.mg^{-1})	Tannin (mg.g^{-1})
Pressure cooking for 15 min [§]	5.77 ± 0.34^b	0.16 ± 0.03^b	4.56 ± 0.58^c
Soaking for 24 h without WGK4	5.39 ± 0.21^{ab}	0.15 ± 0.01^{ab}	2.10 ± 0.38^b
Soaking for 16 h with WGK4	4.96 ± 0.21^a	0.13 ± 0.01^a	1.45 ± 0.16^a
Soaking for 22 h with WGK4	4.95 ± 0.43^a	0.13 ± 0.01^a	1.16 ± 0.37^a

Values are represented as mean $\pm$ SD (n = 3). The means values in the same column with different superscripts indicate significant differences (p < 0.05).

[§]After pressure cooking for 15 min, Komak beans were measured for phytic acid, trypsin inhibitor, and tannin. Soaking means soaking after pressure cooking for 15 min without changing the water.



Table 6. Volatile compounds of raw beans, soaked beans without and with *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4

Volatile compound	Detected in sample (% Area)				Odor description
	Raw beans	Pressure cooking for 15 min ¹	Soaking for 24 h without WGK4	Soaking for 16 h with WGK4	
<i>Aldehyde</i>					
Pentanal	nd	0.38 ± 0.02 ^b	0.25 ± 0.04 ^a	nd	fermented bready fruity nutty berry
Nonanal	nd	0.56 ± 0.03 ^b	0.50 ± 0.08 ^{ab}	0.44 ± 0.05 ^a	waxy aldehydic rose fresh orris orange peel fatty peely
Benzaldehyde	0.68 ± 0.08 ^a	0.70 ± 0.29 ^a	0.54 ± 0.24 ^a	0.69 ± 0.24 ^a	strong sharp sweet bitter almond cherry
Furfural	1.18 ± 0.24 ^b	0.28 ± 0.07 ^a	0.36 ± 0.00 ^a	nd	sweet woody almond fragrant baked bread
(E, E)-2,4-Heptadienal	nd	0.48 ± 0.13 ^a	0.45 ± 0.03 ^a	nd	fatty green oily aldehydic vegetable cake cinnamon
(E, E)-2,4-Nonadienal	nd	0.05 ± 0.04 ^a	0.04 ± 0.02 ^a	nd	fatty melon waxy green, violet leaf cucumber tropical fruit chicken fat
(E)- 2-Tridecenal	0.13 ± 0.02	nd	nd	nd	waxy citrus rind tangerine fatty creamy soapy watery cilantro
(E)-Cinnamaldehyde	0.07 ± 0.01	nd	nd	nd	sweet spice candy cinnamon red hots warm
<i>Total</i>	2.06	2.45	2.14	1.13	
<i>Alcohol</i>					
Ethanol	nd	nd	nd	1.36 ± 0.70	alcoholic ethereal medical
1-Octen-3-ol	1.15 ± 0.03 ^c	1.03 ± 0.07 ^b	0.93 ± 0.06 ^a	nd	mushroom earthy green oily fungal raw chicken
cis-Linalool oxide	0.61 ± 0.03 ^a	1.25 ± 0.30 ^b	1.16 ± 0.13 ^b	nd	earthy floral sweet woody
2-Methylthioethanol	0.04 ± 0.04 ^a	1.76 ± 0.71 ^b	1.93 ± 0.55 ^b	nd	sulfurous meaty
6-methyl-5-Hepten-2-ol	0.14 ± 0.25	nd	nd	nd	sweet oily green coriander
(E)- 2-Nonen-1-ol	0.16 ± 0.01	nd	nd	nd	waxy green violet melon
1-Nonanol	0.64 ± 0.06 ^c	0.16 ± 0.07 ^a	0.34 ± 0.05 ^b	nd	fresh clean fatty floral rose orange dusty wet oily
Phenylethyl Alcohol	0.55 ± 0.06 ^c	0.04 ± 0.02 ^a	0.13 ± 0.03 ^b	0.05 ± 0.01 ^a	floral rose dried rose flower rose water
Benzyl alcohol	2.00 ± 0.24 ^b	nd	nd	0.26 ± 0.09 ^a	floral rose phenolic balsamic
<i>Total</i>	5.29	4.24	4.49	1.67	
<i>Phenol</i>					
2-Formylphenol	0.64 ± 0.06 ^b	0.10 ± 0.03 ^a	0.15 ± 0.01 ^a	nd	medical spicy cinnamon wintergreen cooling
o-Guaiacol	0.78 ± 0.03 ^b	0.06 ± 0.04 ^a	0.08 ± 0.01 ^a	0.10 ± 0.06 ^a	phenolic smoke spice vanilla woody
p-Cresol	nd	0.23 ± 0.03 ^a	0.53 ± 0.09 ^b	nd	phenolic
2-methoxy-4-methylphenol	0.04 ± 0.01	nd	nd	nd	spicy clove vanilla phenolic medicinal leathery woody
Phenol	0.17 ± 0.04 ^b	0.04 ± 0.02 ^a	nd	nd	phenolic plastic rubber
m-Xylenol	0.04 ± 0.01	nd	nd	nd	weak smoky roasted dark
m-Cresol	0.03 ± 0.00 ^{ab}	0.02 ± 0.01 ^a	0.08 ± 0.02 ^c	nd	medicinal woody leather phenolic
2,6-Dimethoxyphenol	0.03 ± 0.00 ^a	0.02 ± 0.01 ^a	0.05 ± 0.02 ^a	nd	smoky phenolic balsamic bacon powdery woody
m-Eugenol	0.74 ± 0.11 ^b	0.02 ± 0.00 ^a	0.07 ± 0.02 ^a	nd	spicy carnation
4-Vinylguaiacol	2.72 ± 0.35 ^b	0.04 ± 0.01 ^a	0.17 ± 0.06 ^a	nd	sweet spicy clove carnation phenolic peppery smoky woody powdery
2,4-Di-tert-butylphenol	1.72 ± 0.44 ^b	0.13 ± 0.02 ^a	0.32 ± 0.05 ^a	nd	phenolic
4-tert-Butylphenol	0.06 ± 0.05	nd	nd	nd	oakmoss leather
Butylated hydroxytoluene	0.20 ± 0.03	nd	nd	nd	mild phenolic camphor
<i>Total</i>	7.17	0.68	1.45	0.10	
<i>Hydrocarbon</i>					
Dodecane	2.27 ± 0.29 ^b	nd	nd	0.22 ± 0.20 ^a	alkane
Tetradecane	1.65 ± 0.19 ^b	nd	nd	0.14 ± 0.01 ^a	mild waxy
Pentadecane	1.63 ± 0.18 ^b	0.91 ± 0.17 ^a	0.73 ± 0.15 ^a	nd	waxy
Nonadecane	0.24 ± 0.03 ^a	1.33 ± 0.32 ^c	0.21 ± 0.04 ^a	0.96 ± 0.05 ^b	bland
Quinoline	0.03 ± 0.01	nd	nd	nd	medical musty tobacco rubber earthy
2-Methoxyanisole	1.71 ± 0.12 ^b	0.05 ± 0.02 ^a	0.08 ± 0.04 ^a	0.18 ± 0.04 ^a	sweet creamy vanilla phenolic musty
Methyl eugenol	0.04 ± 0.01 ^a	0.03 ± 0.02 ^a	nd	0.02 ± 0.02 ^a	sweet fresh warm spicy clove carnation cinnamon
<i>Total</i>	7.57	2.32	1.02	1.52	
<i>Ketone</i>					
2-Nonanone	nd	0.12 ± 0.02 ^a	0.14 ± 0.02 ^a	0.18 ± 0.06 ^a	fresh sweet green weedy earthy herbal
3,5-Octadien-2-one	nd	0.18 ± 0.02 ^b	0.17 ± 0.05 ^b	0.10 ± 0.06 ^a	fruity fatty mushroom
Isophorone	0.40 ± 0.04 ^b	0.03 ± 0.01 ^a	0.03 ± 0.01 ^a	nd	cooling woody sweet green camphor fruity musty cedarwood tobacco leather
2-Undecanone	nd	Nd	0.07 ± 0.01 ^a	0.17 ± 0.04 ^b	waxy fruity creamy fatty orris floral
Geranyl acetone	nd	0.03 ± 0.01 ^a	0.05 ± 0.02 ^a	0.05 ± 0.01 ^a	fresh green fruity waxy rose woody magnolia tropical



Volatile compound	Detected in sample (% Area)				Odor description
	Raw beans	Pressure cooking for 15 min ¹	Soaking for 24 h without WGK4	Soaking for 16 h with WGK4	
cis-Geranylacetone	0.22 ± 0.00 ^a	0.19 ± 0.11 ^a	0.22 ± 0.13 ^a	nd	fatty metallic
2-Tridecanone	0.16 ± 0.02 ^c	0.02 ± 0.01 ^a	0.03 ± 0.01 ^a	0.10 ± 0.06 ^b	fatty waxy dairy milky coconut nutty herbal earthy
cis-Jasmone	0.05 ± 0.01	nd	nd	nd	woody herbal floral spicy jasmin celery
γ-Caprolactone	0.20 ± 0.02	nd	nd	nd	herbal coconut sweet coumarin tobacco
γ-Nonalactone	0.32 ± 0.04	nd	nd	nd	coconut creamy waxy sweet buttery oily
Maltol	0.07 ± 0.01 ^b	0.03 ± 0.04 ^a	nd	nd	sweet caramel cotton candy jam fruity baked bread
Perhydrofarnesyl acetone	0.15 ± 0.04 ^{ab}	0.08 ± 0.03 ^a	0.18 ± 0.09 ^{ab}	nd	oily herbal jasmin celery woody
Farnesyl acetone	0.06 ± 0.04	Nd	nd	nd	flower ether
<i>Total</i>	1.63	0.70	0.97	0.60	
<i>Pyrazine</i>					
2-Methoxy-3-(2-methylpropyl)Pyrazine	0.12 ± 0.10 ^a	0.57 ± 0.27 ^b	nd	nd	green pea green bell pepper green pea galbanum
<i>Total</i>	0.12	0.57	-	-	
<i>Acid</i>					
Acetic acid	nd	nd	nd	0.24 ± 0.05	sharp pungent sour vinegar
Caprylic acid	0.04 ± 0.01 ^a	0.02 ± 0.01 ^a	0.04 ± 0.02 ^a	nd	fatty waxy rancid oily vegetable cheesy
Lauric acid	0.05 ± 0.01	nd	nd	nd	mild fatty coconut bay oil
Hexadecanoic acid	0.12 ± 0.00	nd	nd	nd	slightly waxy fatty
<i>Total</i>	0.21	0.02	0.04	0.24	
<i>Ester</i>					
Ethyl salicylate	nd	0.20 ± 0.08 ^a	0.25 ± 0.02 ^a	nd	sweet wintergreen mint floral spicy balsam
Butyl butyrate	nd	0.06 ± 0.02 ^a	0.10 ± 0.02 ^b	nd	fruity banana pineapple green cherry tropical fruit
Methyl salicylate	0.12 ± 0.01 ^c	0.02 ± 0.01 ^a	0.04 ± 0.03 ^{ab}	0.07 ± 0.01 ^b	ripe fruit juicy fruit
Methyl palmitate	0.12 ± 0.02	nd	nd	nd	sweet wintergreen mint
<i>Total</i>	0.24	0.28	0.39	0.07	oily waxy fatty orris
<i>Terpene</i>					
α-Copaene	nd	0.38 ± 0.08 ^a	0.36 ± 0.07 ^a	nd	woody spicy honey
β-Linalool	0.80 ± 0.05 ^b	0.52 ± 0.15 ^a	0.59 ± 0.07 ^{ab}	0.43 ± 0.10 ^a	citrus floral sweet bois de rose woody green
Linalyl acetate	nd	0.05 ± 0.03 ^a	0.10 ± 0.10 ^a	nd	blueberry
Geraniol	nd	0.06 ± 0.05 ^a	0.05 ± 0.01 ^a	nd	sweet green citrus bergamot lavender woody
β-Ionone	nd	0.04 ± 0.02 ^a	0.12 ± 0.04 ^b	nd	sweet floral fruity rose waxy citrus
3-Carene	nd	nd	nd	0.32 ± 0.04	floral woody sweet fruity berry tropical beeswax
D-Limonene	nd	nd	nd	0.94 ± 0.12	citrus
cis-β-Farnesene	nd	nd	nd	0.04 ± 0.02	citrus
<i>Total</i>	0.80	1.05	1.22	1.73	citrus green
<i>Furan</i>					
2-Pentyl-furan	nd	0.48 ± 0.12 ^a	0.38 ± 0.06 ^a	nd	fruity green earthy beany vegetable metallic
<i>Total</i>	-	0.48	0.38	-	
<i>Naphthalene</i>					
Naphthalene	1.65 ± 0.53 ^b	0.05 ± 0.00 ^a	0.09 ± 0.05 ^a	nd	pungent dry tarry
Dehydro-ar-ionene	0.21 ± 0.02 ^b	0.03 ± 0.01 ^a	0.07 ± 0.04 ^a	nd	licorice
<i>Total</i>	1.86	0.08	0.16	-	
<i>Others</i>					
sec-Butylamine	0.39 ± 0.19 ^a	0.62 ± 0.03 ^b	nd	nd	fishy ammonia
Benzothiazole	nd	0.22 ± 0.16 ^b	0.44 ± 0.17 ^{bc}	0.04 ± 0.02 ^a	Sulfurous rubbery vegetable cooked nutty coffee
Methyl nerolate	0.02 ± 0.03	nd	nd	nd	meaty
<i>Total</i>	0.41	0.86	0.44	0.04	floral herbal citrus fruity green geranium

nd = not detected. Values are represented as mean ± SD (n = 3). The means values in the same row with different superscripts indicate significant differences (p < 0.05). **The Good Scents Company Information System <http://www.thegoodscentscompany.com/search2.html>. ¹After pressure cooking for 15 min, Komak beans were measured for volatile compound. Soaking means soaking after pressure cooking for 15 min without changing the water.

After soaking Komak beans with addition of WGK4 for 16 h, 1-octen-3-ol, an alcohol compound and 2-pentyl-furan, contributing to the beany flavor, were not detected. It might be linked to the enzymatic activity of these LAB. Significant decreases in 1-octen-3-ol were observed in *L.*

plantarum X7021 soymilk fermentation. These decreases might be attributed to the strong oxidoreductase system of these LAB [48]. This result is similar to that of Saadoun et al. [49] in okara fermentation using *L. acidophilus* 8151, *P. acidilactici* 3992 and *L. rhamnosus* 1473. Furthermore,



decreases in 2-pentyl furan by LAB fermentation were demonstrated by Saadoun et al. [49]. In addition to disappearance of volatile compounds contributing to the beany flavor, several pleasant odors were detected in Komak beans during 16-h soaking with addition of WGK4. These volatile compounds included 3-carene (citrus), D-limonene (citrus) and cis- β -farnesene (citrus green). This indicated that addition of WGK4 in the soaking step not only decreased pH of the Komak beans to desirable levels, but also decreased the beany flavor and tannin levels and produced volatile compounds responsible for the pleasant flavors.

3.3. Komak Tempe with Soaking in *Lactiplantibacillus plantarum* subsp. *plantarum* WGK4

Pressure-cooking followed by soaking Komak beans for 16 h with addition of WGK4 shortened the soaking time and decreased water requirements to one-sixth, compared to traditional Komak Tempe production. Tempe production needs a large quantity of water for soaking, water changes and boiling, which subsequently generates wastewater containing components that can pollute the environment. In the pre-mold fermentation process of jack bean Tempe production, beans with a hard texture were soaked for 24 h, boiled for 30 min and then soaked for 24 h. Then, beans were peeled and sliced into 4–6 pieces and soaked for 48 h. Soaking water was changed every 12 h. Sliced beans were boiled for 30 min. This process needed a large quantity of water and a soaking time of 96 h [4]. In this study, the soaking time was 16 h, which included a faster method. Studies by [5] on velvet beans revealed that the beans were soaked for 24 h, boiled for 30 min, peeled, sliced, soaked for 96 h with water changes every 12 h and boiled again for 30 min. This process needed eight times more water and included a longer time. Therefore, method used in this study was shorter and involved a fewer steps than that other processing methods did.

A 48-h fungal fermentation of the Komak beans formed compact mycelia, white cake-form products (data not shown), containing 6.23% $\pm$ 0.55 soluble protein and anti-nutrient factors of 3.46 mg.g⁻¹ $\pm$ 0.21 phytic acid, 0.13 TIU.mg⁻¹ $\pm$ 0.03 trypsin inhibitor and 6.69 mg.g⁻¹ $\pm$ 0.26 tannin. Mold fermentation increased the soluble protein of the Komak Tempe by 59.8%. Raw Komak beans included a protein solubility value of 1.67%. Increases in soluble protein during Tempe fermentation were caused by the presence of protease enzymes produced by the molds

throughout the fermentation [50]. Proteolytic enzyme activity is considered as the major factor in protein hydrolysis during Tempe fermentation, causing releases of peptides and free amino acids, thereby increasing dissolved nitrogen. Protein solubility in soybean, chickpea, pea and horse bean Tempe respectively increased by 66.4, 62.7, 62.3 and 60.7%, compared to raw beans [51]. Increases in soluble protein during Tempe fermentation could include positive effects on the nutritional quality of Tempe. As the protein in beans is broken down into simpler forms during fermentation, protein solubility increases, making it easier for the body to absorb the protein.

The anti-nutritional components of Komak Tempe resulting from the addition of WGK4 during soaking are present in Table 7. Phytic acid in Komak Tempe decreased from 4.96 to 3.46 mg.g⁻¹ compared to beans prior to Tempe fermentation (beans soaked with WGK4) (Tables 5 and7). Fermentation of *Rhizopus oligosporus* generates phytase enzymes that hydrolyze phytic acid present in beans into organic phosphate and inositol [52]. Decreases in phytic acid were reported in soybean Tempe from 32.30 to 28.13 mg.g⁻¹ and kidney bean Tempe from 3.90 to 3.53 mg.g⁻¹, compared to cooked beans from dehulled soaked beans [53]. Phytic acid content observed in the Komak Tempe was lower than that in soybean and kidney bean Tempe.

Trypsin inhibitor in the fermented Komak Tempe did not decrease. Trypsin inhibitor in the Komak Tempe was 0.13 TIU.mg⁻¹. According to [6], thermal use (cooking and autoclaving) is more effective in eliminating trypsin inhibitors than the activity of microorganisms alone. For tannin contents, Tempe fermentation increased tannin contents of Komak Tempe, compared to the beans prior to Tempe fermentation (Tables 5 and 7). Tannin content of the Komak Tempe was 6.69 mg.g⁻¹. This increase might be due to the enzymatic hydrolysis of condensed tannins [52]. Increases in tannin content of the velvet bean Tempe have been reported by Ezegbe et al. [54]. Tannin content of soybean Tempe was 7.6 mg.g⁻¹ [52]. Although soybean Tempe contains anti-nutritional components such as phytic acid and tannin, it is widely consumed by Indonesian people, especially the Javanese. Therefore, Komak Tempe can safely be consumed as an alternative to soybean Tempe. Based on the findings of this study, addition of WGK4 during acid fermentation of softened Komak beans can be used for the Tempe production, resulting in Komak Tempes that are safe for consumption.

Table 7. The soluble protein, phytic acid, trypsin inhibitor, and tannin of Komak Tempe

Treatment	Soluble protein (%)	Phytic acid (mg.g ⁻¹)	Trypsin Inhibitor (TIU.mg ⁻¹)	Tannin (mg.g ⁻¹)
Soaking for 16 h with WGK4	6.23 $\pm$ 0.55	3.46 $\pm$ 0.21	0.13 $\pm$ 0.03	6.69 $\pm$ 0.26

Values are represented as mean $\pm$ SD (n = 3).



4. Conclusion

Dehulled Komak beans pressure cooked for 15 min included a hardness value of 34.47 N. This value was close to the hardness value of boiled soybeans from traditional Tempe preparing. Pressure-cooking of Komak beans significantly decreased their anti-nutritional components, including phytic acid, trypsin inhibitor and tannin. Addition of *L. plantarum* subsp. *plantarum* WGK4 during the soaking step for 24 h decreased pH of the beans from 6.7 to 4.5.

These LAB use nutrients in the media such as soluble proteins and mineral elements for growth and metabolism. At the end of the soaking period, tannin level significantly decreased and the volatile compounds associated with the beany flavor were no longer available in the Komak beans. Therefore, *L. plantarum* subsp. *plantarum* WGK4 can potentially be used as a starter culture for acid fermentation in Tempe processing. Pressure-cooking and addition of these LAB significantly decreased the soaking time and water needed for Komak Tempe processing. These findings can highly help Tempe processing in resource-rich regions, as they shorten the processing time and save water. However, it is necessary to provide LAB culture starters in further applicable powdered forms.

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6. Conflict of Interest

The authors report no conflicts of interest.

7. Authors Contributions

Conceptualization, T.U. and E.S.R.; methodology, W.W.; software, W.W.; validation, T.U., E.S.R. and W.S.; formal analysis, W.W.; investigation, W.W.; resources, T.U.; data curation, T.U., E.S.R. and W.S.; writing-original draft, W.W.; writing-review and editing, T.U., E.S.R. and W.S.; visualization, W.W.; supervision, T.U.; project administration, W.W.; funding acquisition, T.U.

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افزودن لاکتی پلانتهی باسیلوس پلانتاروم زیر گونه پلانتاروم WGK4 به کوماک پخته شده تحت فشار (بلب پریپروس شیرین) برای کاهش زمان خیساندن و نیاز به آب در طی تخمیر اسیدی در فرایند تمپه

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چکیده

سابقه و هدف: لوبیا کومک دارای ارزش غذایی بالایی است و ماده خام امیدوارکننده‌ای به عنوان جایگزین منابع غذایی مانند تمپه است. از آنجایی که حبوبات سفت هستند، نیاز است ۷۲ ساعت در آب خیسانده و هر ۱۲ ساعت آب آن عوض شود. در مورد دانه‌های سویا فقط نیاز است به مدت ۲۴ ساعت بدون تعویض آب خیسانده شود. در این مطالعه از پخت تحت فشار برای لوبیای کماک قبل از خیساندن، کشت آغازگر لاکتی پلانتهی باسیلوس پلانتاروم زیر گونه پلانتاروم WGK4 به آب مرحله خیساندن اضافه شد تا میزان آب و زمان خیساندن کاهش یابد.

مواد و روش ها: لوبیای کومک در زمان‌های گوناگون تحت فشار پخت، پیش تیمار شد و پارامترهای بافتی و ضدتغذیه‌ای مورد ارزیابی قرار گرفت. لوبیای کومک تحت فشار پخت انتخابی در آب خیسانده و بالاکتی پلانتهی باسیلوس پلانتاروم زیر گونه پلانتاروم WGK4 تلقیح شدند. pH، اسیدیته قابل تیتراسیون، پروتئین محلول و مواد معدنی در آب خیسانده شده و لوبیای کومک ارزیابی شد. آب خیسانده برای باکتری‌های لاکتیک اسید زنده و ترکیبات ضدتغذیه‌ای و فرار در لوبیای کماک خیسانده ارزیابی شد. تخمیر کپک با افزودن ۰/۲٪ (وزنی/وزنی) کشت آغازگر تمپه به لوبیای کومک آبکش شده و گرمخانه‌گذاری به مدت ۴۸ ساعت در دمای اتاق انجام شد.

یافته‌ها و نتیجه گیری: لوبیای کومک بدون پوست که به مدت ۱۵ دقیقه تحت فشار پخته شد، دارای مقدار سختی ۳۴/۴۷ نیوتن بود که نزدیک به سختی سویای پخته شده در تهیه تمپه سنتی بود. در لوبیا کومک پخته شده تحت فشار به طور قابل توجهی عوامل ضد تغذیه‌ای کاهش یافته بود. افزودن لاکتی پلانتهی باسیلوس پلانتاروم زیر گونه پلانتاروم WGK4 طی مرحله خیساندن ۲۴ ساعته، pH لوبیا کومک را از ۶/۷ به ۴/۵ کاهش داد. کاهش غلظت تانن مشاهده شد. ترکیبات فرار مسئول طعم لوبیا در لوبیا کومک در پایان خیساندن شناسایی نشد. پخت و پز تحت فشار و افزودن لاکتی پلانتهی باسیلوس پلانتاروم زیر گونه پلانتاروم WGK4 به طور قابل توجهی زمان خیساندن را کوتاه کرد و آب مورد نیاز برای فرایند کومک تمپه را کاهش داد. این فرایند تمپه، آن را به عنوان یک جایگزین پروتئین گیاهی مقرون به صرفه مطرح می‌کند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافی مرتبط با انتشار این مقاله ندارند.