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Optimizing Soaking and Boiling Time in the Development of Tempeh-like Products from Faba Bean (*Vicia faba* L.)

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Abstract: Tempeh is a fermented soybean food with high nutritional value, culinary versatility, and palatability. Its popularity is rising because it aligns with the trend towards sustainable and healthy plant-based diets. However, consumers have exhibited a strong preference for local ingredients over soy. Faba bean is a promising raw material in Scandinavia due to its high protein, dietary fiber, and phytochemical content. In this study, we evaluated the effects of soaking media (vinegar, water and sodium bicarbonate with or without lactic acid bacteria (LAB)) on boiling time and physicochemical properties of faba bean-based tempeh. We demonstrated that sodium bicarbonate, with and without LAB, significantly reduces the boiling time of faba beans (7–8 min), while beans soaked in vinegar and water with and without LAB require longer boiling times for tempeh production (>16 min). Texture analysis has revealed notable variations among the samples, with statistically significant differences ($p < 0.05$) observed across the majority of the measured attributes. Our study has demonstrated that faba beans are suitable for tempeh production and expand the sources of possible raw materials. However, further studies are needed to investigate consumers' preferences and expectations towards faba bean-based tempeh.

Keywords: tempeh; *Vicia faba* L.; plant-based; solid-state fermentation; pre-treatment; LAB; *Rhizopus*

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1. Introduction

In the pursuit of sustainable food production and consumption, the interest toward plant-based diets is increasing, especially in the Nordic countries, where there is a strong focus on reducing dependence on animal-based foods and imported crops [1]. The development of fermented plant-based foods may support this transition [2,3]. Legumes have been part of the human diet for centuries. Their production causes less greenhouse gas emissions compared with other nitrogen-fertilized crops; they serve as carbon sinks, fix nitrogen, promote crop rotation and expend less water than animal-based protein sources [3,4]. Nutritionally, they contain protein, dietary fiber, micronutrients, and phytochemicals, offering health benefits that can improve human health and offer an alternative to animal-based food [5,6].

Faba bean (*Vicia faba* L.) provides substantial nutritional, agronomic, and environmental benefits, making it a promising raw material to address some of the current food system challenges [7]. Faba bean is a good source of protein (26–32% protein), carbohydrates (50–58%), and dietary fiber (8–25%) and contains low amounts of fat (1–4%) [8–10]. Despite the fact that faba bean is already cultivated in Sweden, human consumption is limited due to its undesirable taste, long preparation time, digestive discomfort and low availability in the market [11,12]. Moreover, anti-nutritional factors (ANFs), such as phytic acid,

lectins, raffinose, and stachyose, in addition to vicine and convicine, pose substantial health challenges and make the consumption of faba bean-based products less attractive [13].

Tempeh, originating from Indonesia, is valued for its dense nutritional profile, culinary versatility, and taste. Traditionally made from soybeans fermented with fungal species from Mucoromycetes, such as *Rhizopus microsporus* (formerly, *Rhizopus oligosporus*), that are able to utilize multiple carbon and nitrogen sources, it forms a solid and compact cake-like structure [13,14]. Tempeh is a good source of protein, dietary fiber, vitamins and minerals as well as bioactive compounds with health benefits [15–17]. Tempeh preparation involves cleaning, soaking, dehulling, boiling, pH adjustment and solid-state fermentation (SSF) with food grade inoculum. The soaking step plays a crucial role by affecting processing time and product pH [11,18,19].

Soaking the legumes for 15 min up to 24 h facilitates water absorption, increases internal moisture content, and aids in the removal of water-soluble components such as saponins, oligosaccharides and other ANFs commonly present in legumes [20–22]. Induced fermentation in the soaking process has been widely studied [21,22], for instance the addition of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) enhances the nutritional and sensory characteristics of the legumes [23–25]. In faba bean, *L. plantarum* facilitates the breakdown of phenolic compounds, thereby augmenting antioxidant capacity without elevating phenol concentrations [26–28]. Moreover, it reduces vicine and convicine content by over 91% after several days of fermentation [29–31]. However, the study of the effect of adding *L. plantarum* to the soaking media in the presence of vinegar or sodium bicarbonate on the physicochemical properties of faba beans for subsequent tempeh production has not been investigated.

This study explored faba beans cultivated in Sweden as a novel tempeh ingredient, aiming to optimize the processing line through soaking, boiling, and fermentation to produce tempeh. Specifically, we focused on evaluating different soaking media—vinegar (V), water (W) and sodium bicarbonate (Sb), also in combination with *L. plantarum* (L)—on subsequent tempeh production stages, such as boiling and SSF.

2. Materials and Methods

2.1. Raw Materials and Microorganisms

The faba beans (*Vicia faba* L. var. *Gloria*), both raw and prepared for tempeh production, were obtained from the Research Institutes of Sweden (RISE). Cultivated in central Sweden, these beans were harvested in 2020 and dehulled in 2021 (Hi-Tech Machinery Manufacturing Co., Ltd., Ningbo, China). Sodium bicarbonate and white apple cider vinegar 5% were acquired from a supermarket in Uppsala, Sweden. For the faba bean pre-fermentation stage in the soaking process, *L. plantarum* (Harvest LB-1, pure culture freeze-dried DVS provided by Chr Hansen, Hørsholm, Denmark) was used with a total cell count of $>10^{11}$ CFU/g inoculum as stated by the manufacturer. This strain was directly added to three different soaking media at an equal concentration of 0.1 g of freeze-dried bacteria per 100 g of dry faba beans, mirroring the method typically employed in the industrial production of plant-based fermented beverages and food by incorporating this microbial agent directly into the processing line, as the culture is ready for inoculation (Chr Hansen, Hørsholm, Denmark).

R. microsporus, in powder form, was purchased from the commercial brand TopCultures (Zoersel, Belgium). A small amount of the powder was evenly distributed on MEA agar plates (Oxoid, Basingstoke, UK) and incubated at 30 °C (Binder BF series convection, class 3.1, Tuttlingen, Germany). After 5–6 days, spores were collected in 50 mL Falcon tubes and subjected to five wash cycles in aqueous 9 g/L NaCl. The suspensions were centrifuged (Thermo Scientific Sorvall LYNX 4000, Waltham, MA, USA) at 5000× *g* for 10 min, after which 50% of the supernatant was removed. Following resuspension to homogenize the spore suspension, the concentration was determined using a Bürker counting chamber (Hirschmann EM, Eberstadt, Germany). The prepared suspension was stored at 2 °C (Beko

model B5RCNA365HW, Istanbul, Turkey) prior to the use in tempeh production for a maximum of 2 days.

2.2. Soaking and pH Measurement

Dehulled faba beans (100 g) were rinsed with tap water and subsequently soaked in 250 mL of tap water within a 600 mL beaker. The beaker was placed in an incubator room maintained at a temperature of 25 °C for a duration of up to 12 h. The pH values were initially measured with a pH meter (PHM92, Radiometer Analytical A/S, Copenhagen, Denmark) at time zero and subsequently every 2 h up to 12 h.

Six different soaking media were evaluated: one consisting of 250 mL of tap water with 1 mL vinegar, a second comprising 250 mL of tap water, and a third containing 250 mL of tap water with 1 g sodium bicarbonate. All three treatments were administered with or without 0.1 g *L. plantarum* (Table 1). After the soaking period, the faba beans were drained, the soaking water was discarded, and the beans were heated following the procedure described subsequently.

Table 1. Soaking media description.

Media Code	Media	Composition	Initial pH
V	Vinegar	250 mL of tap water + 1 mL vinegar	5.0 ± 0.02
VL	Vinegar + *L. plantarum*	250 mL of tap water + 1 mL vinegar + 0.1 g *L. plantarum*	5.2 ± 0.01
W	Water	250 mL of tap water	6.7 ± 0.03
WL	Water + *L. plantarum*	250 mL of tap water + 0.1 g *L. plantarum*	6.6 ± 0.03
Sb	Sodium bicarbonate	250 mL of tap water + 1 g Sodium bicarbonate	7.6 ± 0.03
SbL	Sodium bicarbonate + *L. plantarum*	250 mL of tap water + 1 g Sodium bicarbonate + 0.1 g *L. plantarum*	7.5 ± 0.03

All procedures were undertaken in triplicate.

2.3. Boiling Time

Regular boiling under atmospheric pressure of the faba beans in boiling tap water was conducted. Dry weight of faba beans and tap water was used in a ratio of 1:4. The water was boiling vigorously when the faba beans were submerged and they were boiled for 8 min and 16 min. These two timepoints were chosen based on preliminary experiments, yielding faba beans with sufficient softness yet maintaining a full round shape which is needed for the subsequent SSF to produce tempeh. The process was repeated until three replicates were obtained.

2.4. Preparation of Tempeh

Dehulled faba beans were utilized for tempeh production. The tempeh was prepared according to the method described by Rumah Tempe Indonesia (RTI) [32] with modifications (Figure 1). In brief, the raw faba beans were weighed, rinsed, and soaked as described above. Following the soaking process, the media was discarded and the beans were rinsed again, boiled, drained and rinsed with cold water. Excess water was removed with paper towels. The beans were then allowed to cool to room temperature before being inoculated with 1 mL of spore suspension (approximately 108 CFU/g per inoculum) per 100 g of wet beans. Subsequently, 150 g of the inoculated faba beans were tightly packaged in perforated food grade zip-lock plastic bag to facilitate oxygen exchange while maintaining faba bean arrangement; the package size was 100 mm× 150 mm × 0.05 mm LDPE food grade material (VWR, Europe). The beans were incubated for 40 h at 30 °C. Visual inspections of the tempeh were periodically conducted from above and below the package. All tempeh samples were produced in November 2023. Samples were stored at −18 °C (Beko, Turkey)

prior to analysis, considering that around 50% of soybean tempeh sold in Sweden is a frozen product.

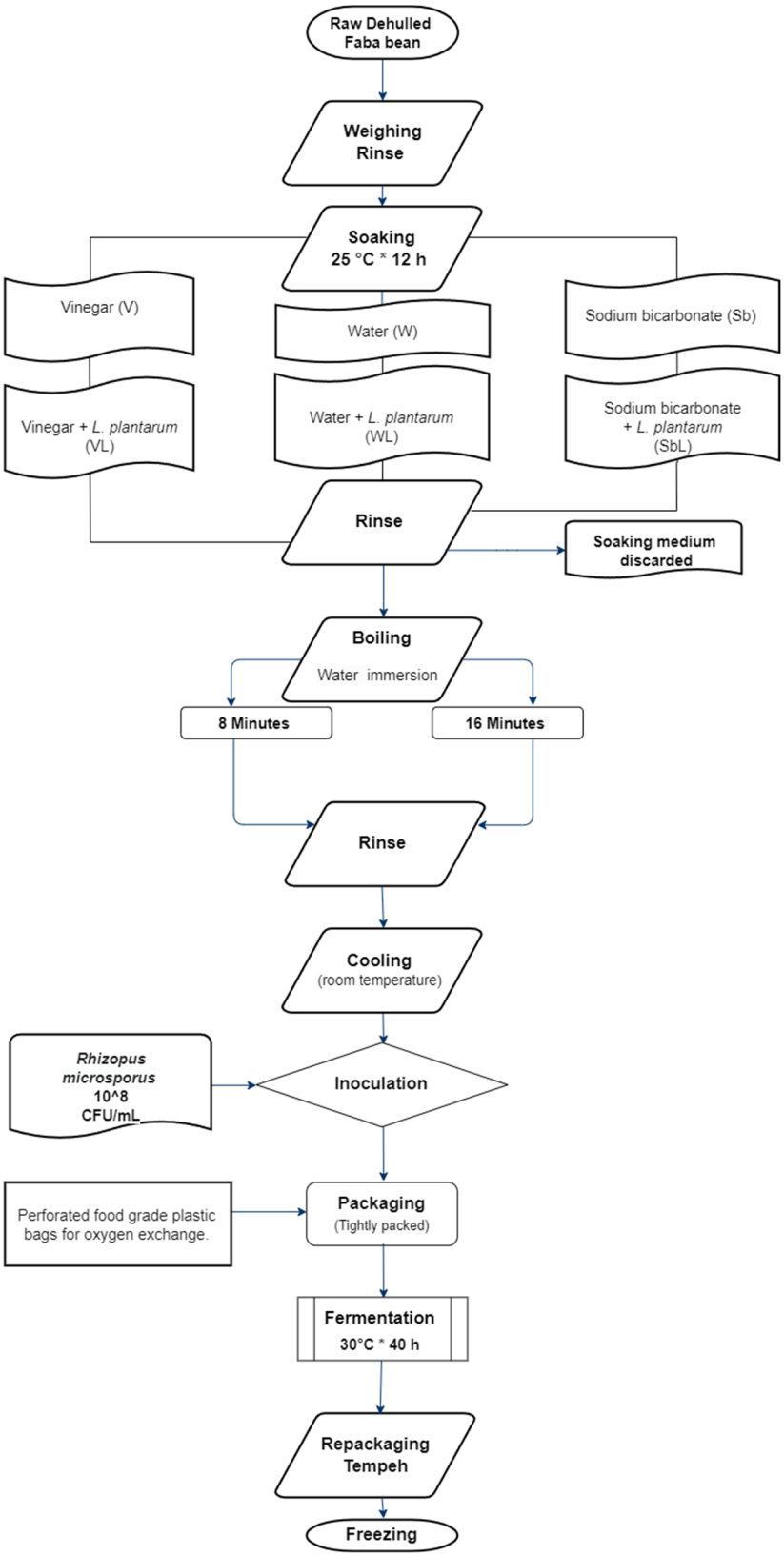

Figure 1. The developed lab-scale faba bean tempeh production processing line. * = for.

2.5. pH Measurement in Tempeh

Tempeh products (20 g) were thawed at room temperature for 2 h and homogenized by stirring by hand for 3 min in a 1:10 (g/L) dilution with deionized water. The pH measurement process was undertaken using a pH meter (PHM92, Radiometer Analytical A/S, Denmark).

2.6. Colour

The colour of the thawed faba bean tempeh was assessed in ten replicates in different spots of the surface, at room temperature using a Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Osaka, Japan). Samples were taken from the tempeh surface where mycelial growth was observed, characterizing mostly the mycelium color formed since tempeh is, by definition, a compact cake with a whitish or grey appearance on its surface. On each sample, the CIE L* (lightness), a* (redness), and b* (yellowness) were calculated (CIE, 1986).

2.7. Texture Analysis

Texture characterization of faba bean tempeh was performed using a Texture Analyzer TA-XTplus (model TA-HDi; Stable Micro Systems Ltd., Surrey, UK) by adopting the method from Erkan et al. [19] with modifications. For texture profile analysis (TPA), the double compression model was employed. This analytical method mimics the act of chewing by sequentially compressing a food sample twice in a directional motion. Frozen tempeh samples were thawed at room temperature for 2 h and cut into cubes 20 mm on each side. The samples underwent a compression to 50% of their initial height, facilitated by an acrylic cylindrical probe 20 mm in diameter. The parameter setting for the pre-test was 2.0 mm/s and was 5.0 mm/s for the actual test and for the post-test, across a 5 s interval, and employing a load cell with a 50 kg capacity. The key textural attributes, in brief, include hardness as the peak force in the first compression in (N); adhesiveness as the area under the curve for the first negative peak in (N*s); cohesiveness from the ratio of the area under the second compression curve to that under the first compression curve; springiness as ratio of percentage of the product to recover to its initial height; and chewiness as the product from hardness, cohesiveness, springiness in (N) [33]. These parameters were quantitatively determined for each cubic sample, with calculations performed with the software associated to the Stable Micro Systems standard for TPA macros, software version TA.XT plus C (Stable Micro Systems, TA-HDi, Surrey, UK). The measurements were performed six times on three different batches of faba bean tempeh production.

2.8. Moisture Content

The moisture content of all faba bean tempeh samples were determined in triplicate as a difference between weights before and after overnight oven drying at 105 °C (Model 2000655, J:P: Selecta, Barcelona, Spain) based on AOAC official method 934.01.

2.9. Statistical Analyses

Statistical analyses were performed using RStudio version 4.3.2 (RStudio Inc., Boston, MA, USA). Homogeneity of variance and normality of the data set was evaluated using Levene's test and the Shapiro–Wilk test. Differences in pH, color, textural profile parameters, and moisture content in relation to the soaking and boiling process were estimated using a one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test. Values are presented as least square means (LSmeans) and standard errors (SE).

3. Results and Discussion

3.1. Soaking and pH Measurement

The initial pH varied significantly across media (Figure 2). However, adding *L. plantarum* resulted in a drastic pH decrease after 12 h of soaking (Figure 2). In the SbL groups, sodium bicarbonate buffering capacity moderated this effect, leading to only a slight decrease in pH. Similarly, in group Sb, there was also a slight reduction in pH due to

spontaneous fermentation from the endogenous microbiota present in faba bean, which has been shown to contain various strains of LAB [34,35].

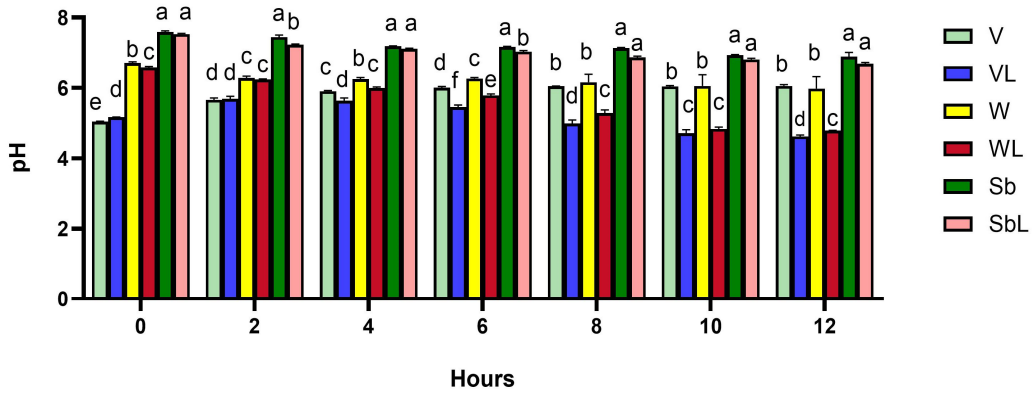

Figure 2. pH values in the soaking media used as pre-treatment for faba bean, V: vinegar, W: water, Sb: sodium bicarbonate with and without L: *L. plantarum*. Different letters indicate statistically significant differences per time group treated separately ($p < 0.05$).

Soaking beans in water softens their structure, allowing easier soluble penetration into starch granules and proteins. The addition of salt and sodium bicarbonate in the soaking media reduces the boiling time of the legumes and improves nutritional quality by lowering levels of tannins and trypsin inhibitors and increasing nutrient bioaccessibility [36–38].

The observed behavior of media Sb may be explained by the dissociation of sodium bicarbonate into sodium (Na^+) ions and bicarbonate (HCO_3^-) ions. The bicarbonate ion acts as a weak base, producing hydroxide ions that increase the pH, thus making it more alkaline [37,39]. The buffering capacity appears to be strong against the organic acids especially lactic acid formed by LAB [26,30,39]. Moreover, there were no statistically significant differences between the treatments with Sb alone and those combined with LAB in the pH measurements ($p < 0.05$).

The effects of alkaline pH in the soaking media of beans and its combined effect on boiling have previously been studied. In white and red kidney beans, lentils, chickpeas, black velvet beans, and mung beans, a 6-h soak with Sb reduced boiling time by 45 to 60%. An alkaline soak decreased starch digestibility, as well as the concentrations of tannins, phytic acid, and oligosaccharides [21,38]. Moreover, soaking mung bean in Sb increased the chemically extractable phenolic compounds, bioaccessible phenolics, total phenolic content and antioxidant activity, as well as the extractability of protein [37].

To the best of our knowledge, the combination of Sb and LAB (SbL) in soaking faba beans has not been previously reported. In the present study, the use of Sb in soaking faba bean, prevented acidification by *L. plantarum* by stabilizing the pH of the media. Although *L. plantarum* can adapt to alkaline media by alkalizing its cytoplasm, it operates optimally at a neutral pH; here, the initial pH with Sb was 7.6 ($p < 0.05$). In fact, *L. plantarum* is considered a highly-alkali-tolerant species, which is able to grow up to maximum pH between 8.5 and 8.9. Therefore, this pH value in the media permits the growth of *L. plantarum*, enabling it to bioconvert faba bean substrates with its own enzymes, such as α-amylase, esterase, lipase, and α-glucosidase [40]. These processes might lead to changes in nutritional composition and ANF content, which requires further studies.

In the groups of V and VL, initial pH levels were low at 5 and 5.1, respectively ($p < 0.05$) compared to the other media, due to the acetic acid from the vinegar solution. During the 12 h, a pH increase to 6 was observed in group V ($p < 0.05$) (Figure 2), likely from organic alkaline compound solubilisation, sugar degradations and protein breakdown into peptides, stabilizing the pH. Acid media disrupts protein ionic interactions inducing protein degradation and formation of free amino acid that could tend to increase the media pH due to their buffering capacity [10,16,41]. As expected, group VL experienced a pH drop to 4.6 at 12 h of soaking, attributed to LAB activity during fermentation, from the

ensuing organic acid production [20,29,42,43]. Vinegar in tempeh production lowers pH during soaking to prevent spoilage and alter taste and smell. However, vinegar combined with *L. plantarum* may restrict the LAB activity due to the initial pH of around 5 in the soaking media [44].

In the samples using tap water W and WL as a soaking media, initial pH was 6.7 and 6.6 respectively then the final pH dropped significantly to 6 in W and 4.8 in WL, where the action of *L. plantarum* was noticeable due the production of organic acids and acidification of the media in WL. Therefore, the induced fermentation (WL) had significant higher effect than the spontaneous fermentation in the soaking treatment with W. The soaking process in tempeh production reduces bean boiling time and adjusts pH for SSF, enhancing *R. microsporus* growth and reducing the proliferation of spoilage microorganisms [18,44]. Even with tap water soaking, pH dropped to 5.9 due to endogenous and spontaneous action of LAB coming from faba bean (Figure 2).

In Figure 3 the physical appearance of the faba bean could be observed from different soaking media and boiling times that boiling in condition SbL for 7 to 8 min was the optimal boiling time for faba bean; longer boiling led to the seed coat breaking at around 17 min, which is visualized from 9 to 10 min of boiling the faba bean from SbL media, similar results for all the media were obtained from soaking without *L. plantarum*. This is in agreement with previous results that excessive boiling causes protein denaturation, starch gelatinization and leakage of the internal seed contents [35].

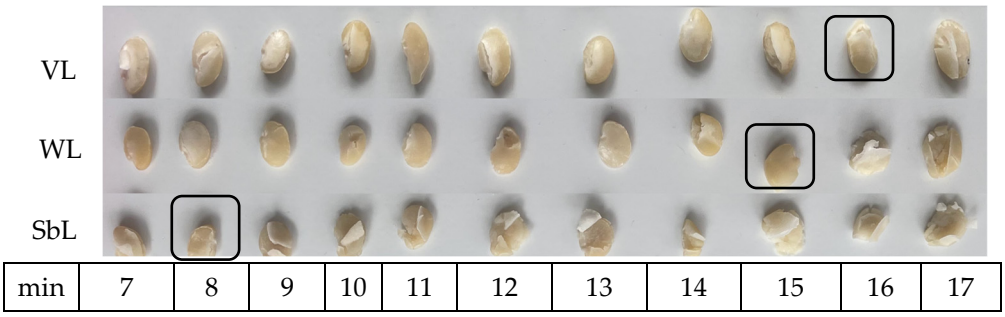

Figure 3. Visual comparative analysis of faba bean texture post-boiling from different pre-treatment soaking media (SbL, VL, and WL). The softness and full shape of the beans are critical to ensure the SSF with *R. microsporus* species to produce tempeh; optimal times are marked with a square figure over the specimen.

Soaking in V, VL, W and WL extended the boiling time needed to achieve the proper texture comparing with Sb and SbL soaking media. In tempeh production, boiling time is crucial as it affects *R. microsporus* mycelial growth and the success of SSF. Soaking in Sb, with or without LAB, decreased the boiling time needed to around 50% from 16 min (soaking with V, VL, W or WL) to 8 min. The use of sodium bicarbonate in the soaking process has previously shown the same effect as shortening the boiling time needed to obtain soft, boiled common beans (*P. vulgaris* L., carioca group) by Schoeninger et al. (2014) [38] and common beans (*P. vulgaris* L., 9 different varieties) by Kinyanjui et al. (2015) [35].

3.2. pH Measurement in Tempeh

The pH of tempeh at the start time 0 and after 40 h of SSF were measured, assessing the combined effect of the soaking media and boiling duration. The results in Figure 4 suggest that shorter boiling times result in a lower final pH in tempeh, possibly because the seed coat remains more intact compared to longer boiling times, preventing protein release which could raise the pH towards neutrality [19].

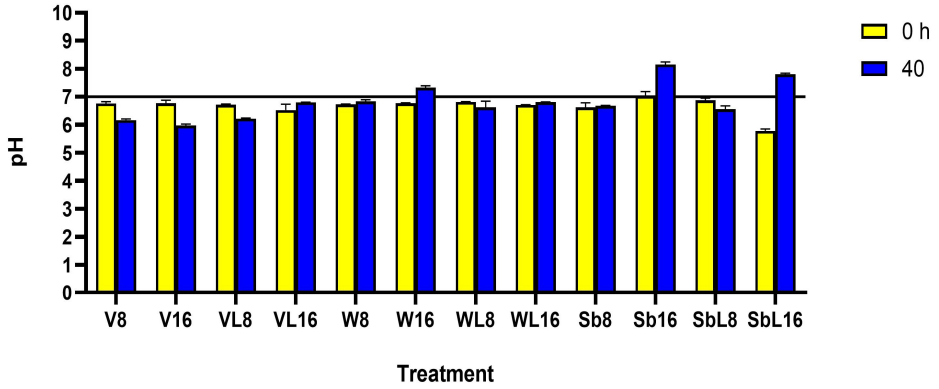

Figure 4. pH in Tempeh at time 0 and 40 h after SSF with *Rhizopus microsporus*, a comparative analysis of the effect from soaking media and boiling times in combination on faba bean (n = 3).

On the other hand, excessive boiling for 16 min of beans soaked in Sb and SbL also led to suboptimal conditions for SSF (Figure 5). The final tempeh had dark colour, high pH value of 8.2 for Sb and 7.8 for SbL, bad odours and it was not edible. This could be due to oxidation of the substrate.

(**a**) Tempeh from VL 8 min (undercooked beans)

(**b**) Tempeh from SbL 8 min (cooked beans)

(**c**) Tempeh from SbL 16 min (overcooked beans)

Figure 5. Tempeh visual appearance from different pre-treatment soaking and boiling in combination. (**a**) VL beans undercooked at 8 min. (**b**) SbL tempeh with cooked beans at 8 min and (**c**) SbL beans overcooked at 16 min.

Of the edible samples, optimal tempeh was found using V, VL, W, and WL, heated for 16 min, and with Sb and SbL, heated for 8 min. Their pH after 40 h of SSF, ranged from 6.0 to 7.3 on V16 and W16 respectively ($p < 0.05$) (Figure 4). Despite the higher initial pH when soaking with Sb8 and SbL8 (Figure 2, time 0), the final tempeh from the beans boiled for 8 min had pH level of 6.7 and 6.6 respectively.

Erkan et al. [19] studied the physicochemical properties of tempeh made from several beans including soybean, which is traditionally used for tempeh production, as well as faba bean. The faba beans underwent a 2 h soaking in tap water, boiling for 10 min and then grape vinegar was added at the same time of the inoculation with *R. microsporus*. Fermentation lasted for 32 h at 30–34 °C. The pH of tempeh varied from 6.48 to 7.56 for different beans, with faba bean tempeh having pH 6.87 [19]. This pH aligns with tempeh produced in our study for WL16 and VL16 with an average pH of 6.8, and SbL8 at pH 6.6.

Other studies have reported soybean tempeh pH after 48 h of SSF ranging from 7.2 to 7.6, which is slightly high compared to faba bean tempeh and probably at a higher risk of spoilage [16,44].

The findings suggested that W, V, and Sb combined with LAB produces tempeh with an optimal pH, moreover, the addition of LAB in W or Sb soaking media eliminates the use of vinegar in any part of the process. Also, LAB may enhance the final product sensory appeal and improve nutrient profiles while mitigating ANFs [32,45–47].

3.3. Colour

Colourimetric analysis on different tempeh surface locations aimed to evaluate its visual characteristics without visualizing the inner product since there is high variability between the bean colour and mycelium. Table 2 shows L*, a*, and b* values, this approach ensures an intact assessment of faba bean tempeh colour. The L* value ranges from 60.7 to 85.5. The a* value ranges from −11.5 to −4.86, and for b*, from 33.7 to 41.3 (Table 2).

Table 2. Colour measurement data of tempeh produced from faba bean from different pre-treatment in combination of soaking and boiling.

Samples	L*	a*	b*
V8	85.2 ± 1.63^{bc}	-11.2 ± 0.45^{e}	37.9 ± 0.09^{cde}
VL8	68.4 ± 1.32^{h}	-5.64 ± 1.31^{ab}	40.2 ± 0.70^{ab}
V16	74.7 ± 1.34^{ab}	-7.67 ± 0.49^{c}	41.3 ± 1.90^{a}
VL16	65.0 ± 2.51^{cd}	-4.86 ± 1.42^{a}	39.7 ± 1.69^{abcd}
W8	84.0 ± 3.60^{ef}	-10.7 ± 0.96^{e}	38.2 ± 0.75^{bcde}
WL8	72.6 ± 1.40^{g}	-7.80 ± 0.58^{cd}	37.8 ± 0.60^{cde}
W16	81.1 ± 1.80^{de}	-9.76 ± 0.75^{de}	37.7 ± 0.75^{e}
WL16	72.3 ± 3.38^{fg}	-7.58 ± 1.60^{bc}	39.3 ± 1.65^{abcde}
Sb8	85.5 ± 1.60^{a}	-11.5 ± 0.64^{e}	37.7 ± 0.66^{abc}
SbL8	60.7 ± 1.31^{a}	-4.91 ± 0.70^{a}	33.7 ± 0.31^{f}
Sb16	78.6 ± 4.03^{a}	-8.26 ± 1.38^{cd}	39.9 ± 1.05^{abc}
SbL16	NA	NA	NA

Values represent LSmeans and SE. Different letters indicate statistically significant differences ($p < 0.05$). L* implies a light, nearly white color; negative a* suggests greenish colour, and positive b* indicates yellowness. NA: Non-applicable.

In SSF of faba bean, *R. microsporus* showed effective growth on the beans soaked, with or without *L. plantarum.* However, data analysis indicates that incorporating *L. plantarum* into the soaking media leads to reduced L* values, signifying a departure from white towards a darker colour, which may derive from the inherent colour of faba beans.

Erkan et al. [19] reported colour values for faba bean tempeh of L* 66.94, which aligns with the colour values of the edible tempeh samples in the present study, such as VL16, V16, and SbL8. However, the values for a* and b* do not seem to be similar to those in their experiment, considering the different SSF times [19]. On the other hand, some L* values (Table 2) from faba bean tempeh are similar to the soybean tempeh of 79.2 from Handoyo and Morita [46] and 72.32 from Erkan et al. [19]. This can be considered a positive finding, considering the similarity in appearance of faba bean-based tempeh to traditional tempeh, and may facilitate a smoother transition for consumers from soybean-based to faba bean-based tempeh [16,44].

For the SbL16 faba bean samples, overboiling led to the complete leakage of seed contents, preventing the reporting of colour values or texture property profiles. This hindered mycelial growth and caused the SSF process to fail, rendering the samples inedible.

3.4. Texture Analysis

The texture properties of tempeh were assessed according to various attributes such as hardness, adhesiveness, cohesiveness, springiness and chewiness (Table 3). The results indicated a clear influence of soaking media (W, V and Sb with and without LAB) and boiling duration (8 and 16 min) on the textural properties of the tempeh. Overall, the samples from the soaked pre-treatment with W, V and Sb had slightly higher values on hardness compared to the beans soaked in WL, VL, and SbL ($p < 0.05$).

Table 3. Moisture content and texture analysis profile in the tempeh (n = 3) from different soaking media (V, W, Sb with and without *L. plantarum*) in combination with boiling time at 8 min and 16 min.

Sample	Moisture (%)	Hardness (N)	Adhesiveness (N.s)	Cohesiveness	Springiness (%)	Chewiness (N)
V8 *	64.6 ± 0.94^{ab}	21.4 ± 1.91^{ab}	-0.08 ± 0.01^{ab}	0.26 ± 0.03^{e}	48.8 ± 1.24^{a}	2.76 ± 0.41^{abcd}
VL8 *	62.1 ± 1.81^{b}	15.4 ± 1.85^{bcd}	-0.20 ± 0.05^{bc}	0.33 ± 0.02^{abcd}	39.9 ± 1.06^{b}	2.03 ± 0.16^{bcd}
V16	69.5 ± 0.39^{a}	8.5 ± 1.48^{d}	-0.05 ± 0.03^{a}	0.31 ± 0.00^{de}	50.3 ± 2.35^{a}	1.29 ± 0.21^{d}
VL16	64.4 ± 2.98^{ab}	27.6 ± 1.20^{a}	-0.16 ± 0.08^{abc}	0.38 ± 0.00^{ab}	43.0 ± 1.42^{ab}	4.61 ± 0.10^{cd}
W8 *	63.5 ± 3.00^{ab}	26.3 ± 2.38^{a}	-0.14 ± 0.06^{abc}	0.30 ± 0.03^{de}	49.2 ± 3.09^{a}	3.96 ± 0.39^{ab}
WL8 *	63.3 ± 2.94^{b}	24.7 ± 5.36^{ab}	-0.16 ± 0.03^{abc}	0.39 ± 0.02^{a}	42.4 ± 2.69^{ab}	4.19 ± 1.21^{ab}
W16	66.9 ± 0.85^{ab}	23.8 ± 4.94^{ab}	-0.09 ± 0.04^{ab}	0.33 ± 0.02^{bcd}	45.7 ± 2.00^{ab}	3.60 ± 1.01^{abc}
WL16	67.4 ± 1.38^{ab}	22.3 ± 5.92^{ab}	-0.19 ± 0.04^{bc}	0.38 ± 0.02^{abc}	43.8 ± 2.51^{ab}	3.82 ± 1.32^{abc}
Sb8	67.1 ± 3.68^{ab}	27.8 ± 3.77^{a}	-0.09 ± 0.06^{ab}	0.32 ± 0.03^{de}	46.1 ± 2.63^{ab}	4.11 ± 1.13^{ab}
SbL8	63.9 ± 1.09^{ab}	18.8 ± 2.32^{abc}	-0.23 ± 0.04^{c}	0.34 ± 0.01^{abcd}	43.7 ± 2.54^{ab}	2.87 ± 0.25^{abcd}
Sb16 *	67.9 ± 1.26^{ab}	10.3 ± 1.83^{cd}	-0.1 ± 0.01^{abc}	0.30 ± 0.01^{cd}	50.3 ± 6.89^{a}	1.72 ± 0.338^{cd}
SbL16 *	65.9 ± 0.15^{ab}	NA	NA	NA	NA	NA

Values represent LSmeans and SE. Different letters indicate statistically significant differences ($p < 0.05$). * Tempeh not edible. NA: Non-applicable.

Analysis of the TPA data reveals notable variations among the samples, with statistically significant differences ($p < 0.05$) observed across the majority of measured attributes. The hardness values vary from 8.5 to 27.8 N, for V16 and Sb8, respectively including edible and not edible tempeh samples. Erkan et al. [19] found that faba bean tempeh had a hardness of 27.8 N ($p < 0.05$), which is comparable to the texture of the Sb8 samples of 27.8 N in this experiment ($p < 0.05$). Additionally, they observed that control tempeh made from soybeans had a hardness of 16.99 N, closely aligning with the 18.8 N hardness of the tempeh from the SbL8 treatment in this study.

For all the samples, edible and not edible, the adhesiveness varied from −0.23 to −0.05 N.s for SbL8 and V16 respectively. Cohesiveness was the lowest in V8 (0.26), reflecting less internal binding, and the highest in WL8 (0.39), indicating stronger internal cohesion, as higher the cohesiveness is the higher the number of chews is required to break the food down. The VL8 sample had the minimum springiness at 39.9%, compared to the Sb16 sample, which showed the greatest springiness at 50.3%. The chewiness parameter showed the lowest value of 1.29 N for V16 and the highest at 4.61 N for VL16. Compared to the 1.66 N reported for faba bean tempeh by Erkan et al. [19], these values are similar to those observed in the Sb16 samples of 1.72 N. However, the Sb16 samples were inedible due to the lack of mycelium formation and overcooked beans.

In a comparison of only edible samples V16, VL16, W16, WL16, Sb8, and SbL8; specifically V16 values for hardness and springiness were statistically significantly different compared to all the others ($p < 0.05$). Notably, when examining V16 and VL16, significant differences are found in all the parameters. These differences highlight the substantial impact of pre-treating with *L. plantarum*, particularly when used in combination with vinegar in the soaking medium.

Tempeh is a product of SSF from *R. microsporus*, and is supposed to have a solid, chewable, and compact texture. In the present study, over-boiling hinders SSF (Figure 5c) due to bean seed coat breakdown and content leakage, while under-boiling results in a raw texture (Figure 5a). In further studies, textural attributes should be complemented with sensory evaluation by panellists and hedonic consumer testing to assess consumer's preferences and expectations [48].

3.5. Moisture Content

The moisture content ranged from 62.1 to 69.5% (Table 3). Comparing only the edible tempeh samples, V16 had the highest moisture content, and it was statistically different ($p < 0.05$) from most other samples. On the other hand, VL8 had the lowest moisture

content, however VL8 treatment produced inedible tempeh due to raw beans, resulting in a very hard and dry product.

The large variation in the moisture content found in these tempeh samples could be determined by the initial physical structure post soaking and boiling of the faba beans. Shorter boiling time yields harder, nearly raw beans, leading to lower moisture tempeh. Conversely, longer boiling softens the seed coat, increasing moisture content in the tempeh. The moisture content around 64% in edible faba bean tempeh, observed in SbL8 and VL16, aligns closely with findings reported by Erkan et al. [19]. Nevertheless, the CODEX Alimentarius standard 313R-2013 [49] for soybean tempeh or "tempe" specifies a moisture content of maximum 63% w/w.

This data underscores the variability in moisture content across different tempeh samples depending on the pre-treatments. However, it should be noted that Sb16 treatment failed in the SSF process and exhibited the highest moisture content and pH levels over 8, due to over boiling, whereas SbL8 treatment shows similar moisture content as Erkan et al. [19], a positive outcome of the combination of Sb and LAB.

4. Conclusions

The adoption of the SbL8 pre-treatment protocol at laboratory scale resulted in physical attributes such as colour, texture, pH, and moisture content similar to tempeh by the traditional definition, as well as reduced boiling time of the beans. Therefore, the suggested protocol could be used to optimize the faba bean tempeh manufacturing processes, facilitating the development of tempeh with textures that appeal to consumer preferences and compare well with traditional soybean tempeh.

Future research should be focused on how soaking in *L. plantarum* solutions and the process of SSF impact nutritional quality, ANFs, and sensory properties of faba bean tempeh.

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