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Shelf-Life Assessment of Bread Partially Substituted with Soy Protein Isolate

Yu-Han Chang [1], Cheng-Ming Chang [1,2] and Pei-Ting Chuang [1,*]

[1] Institute of Food Safety and Risk Management, National Taiwan Ocean University, Keelung City 202301, Taiwan
[2] Department of Food Science, National Taiwan Ocean University, Keelung City 202301, Taiwan
* Correspondence: ptchuang@mail.ntou.edu.tw; Tel.: +886-2-24622192 (ext. 5173)

Abstract: Partial substitution of flour with soy protein isolate in bread making increases the protein content and nutritional value of baked goods as it contains more lysine than wheat flour. However, changes in the bread recipe alter the pH and amino acid content of the baked good, and product assessment is required to determine whether the product is a non-time/temperature control for safety food. This study examines the effects of substituting high-gluten flour with 2–8% soy protein isolate on bread quality and on the shelf life of the bread using the microbiological challenge test. The results indicate that increased soy protein isolate substitution reduces the volume and specific volume of bread. Six percent soy protein isolate-fortified bread also had a poorer taste, and, therefore, the optimal substitution amount is 4%. Based on the yeast and mold growth during the storage period, the 4% soy protein isolate-fortified bread has a shelf life of four days, while the 2% soy protein isolate-fortified bread has a shelf life of five days. The microbiological challenge test results revealed that the substitution of flour with soy protein isolate is conducive to *Staphylococcus aureus* growth within the bread. To summarize, the optimal soy protein isolate substitution in bread is 4%, which offers a four-day shelf life at room temperature.

Keywords: bread; soy protein isolate; product assessment; microbiological challenge test; shelf life

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1. Introduction

In food processing, the addition of protein to the initial ingredients improves the nutritional value of the final food product, allowing consumers to increase protein intake. These products are particularly beneficial to the elderly as enhancing daily protein consumption promotes the maintenance of dietary habits and quickly compensates for any protein deficiencies. Protein-enriched bread is among the high-protein foods that elderly people would gladly purchase and consume [1–7]. Furthermore, some people choose to adopt a gluten-free diet. Gluten-free products are often described as tasteless, flavorless, and having poor texture, which amplifies the importance of the choice of gluten substitutes used in food manufacturing [8,9]. White bread comprises wheat flour and has a protein content of 8–9%, and protein-enriched bread is made by adding soy protein to the raw ingredients, which increases the protein content to 13–14%. The addition of soy protein not only improves the protein content of the bread products, but it also increases lysine concentrations, which occur in low levels in wheat [10,11]. Additionally, soy protein retains the water content of bread during baking, thus prolonging the freshness of the bread while also improving dough handling and crust color [12,13]. To examine the effects of soy protein on the product quality of dough and flour, previous studies were often conducted by removing the soy protein isolate that does not contain non-protein constituents [14]. SPI is a product rich in protein that does not contain certain anti nutritional factors, including the trypsin inhibitor, lipoxygenase, and it does not have a beany flavor. Numerous studies have examined the effects of SPI on bread quality by adding SPI to bread recipes or by substituting a portion of the wheat dough with SPI [11,12,15–18]. SPI does not contain

gluten and forms net-like structures in the dough similar to those formed by gluten. The addition of SPI in gluten-free bread production improves the quality and texture of the bread [18–21].

A time/temperature control for safety food (TCS food) is a food in which pathogenic microorganisms grow if it is placed under an unsafe temperature for some time. The Food Code has explicit temperature control requirements for the processing and storage environments of TCS foods [22]. In bread making, baking limits the growth of pathogens within the bread, and the low water activity (a_w) prevents the growth of pathogens on the surface of the bread. Hence, baked breads can be stored safely at room temperature. The traditional uses of bread in human activity and its history as a food provide a basis for its effective and safe storage at room temperature, and, therefore, bread could be regarded as a non-TCS food. However, because breads differ by recipe, pH, a_w, and means of storage and shipping, the external and internal factors affecting microorganism growth in bread vary, and different types of bread should be assessed separately in determining TCS food status [23]. The interaction between pH and a_w may be a factor that determines whether a food product requires specific time and temperature controls, and the microbiological challenge test for product assessment is utilized to ascertain whether a food product requires time and temperature controls [23,24].

The goal of the microbiological challenge test is to verify whether a food product remains safe and stable after it is accidentally contaminated with pathogenic or spoilage microorganisms [25]. The National Advisory Committee on Microbiological Criteria for Foods suggests that the selection of pathogenic bacteria should be based on its association with the food, its probability of survival, and the expected use of the product [24]. The pH and a_w conditions of bread potentially support the growth of *Staphylococcus aureus* (*S. aureus*). Feeherry et al. [26] studied the growth kinetics of *S. aureus* in bread and reported that an a_w <0.83 and >0.83 restricts and promotes its growth, respectively. Therefore, changes in bread recipes can be based on microbiological challenge test results conducted using *S. aureus* as the test strain. Moreover, food spoilage and shelf life are often associated with the microbial count. Therefore, the National Advisory Committee on Microbiological Criteria for Foods recommends that the shelf life of bread can be determined based on the growth of typical spoilage microorganisms, such as mold and yeast [24].

The popularity of the "clean label" trend has resulted in continuous demands for redesigning recipes; consumer preferences and demands for healthier, more natural foods have driven manufacturers to develop new products and modify the recipes of old products [27]. For instance, reduced salt content in processed foods, including baked foods and meat, is an issue of concern for the British Food Standards Agency [28]. Another recent trend is the substitution of sugar with sugar alternatives, despite knowing this approach may promote increased microorganism growth during storage [25]. Thus, the quality of new products and old products with modified recipes may differ, and it is crucial to validate the safety and stability of the final products in question.

Numerous studies have examined how the quality of bread is affected by adding more protein or substituting a portion of wheat dough with protein. However, no study to date has performed an in-depth assessment of the effect of substituting wheat flour with protein on the shelf life of baked goods. Microbiological shelf-life testing is a common means of ensuring food safety [29,30]. However, no study thus far has evaluated the microbiological testing of SPI-substituted flour in bread making. This study aims to examine the effect of SPI on bread quality by substituting a portion of high-gluten flour with SPI and to assess the shelf life of this bread based on the growth of spoilage microorganisms and microbiological challenge test results. The results of this study are expected to function as a reference for food manufacturers in developing techniques for incorporating soy protein into baked goods.

2. Materials and Methods

2.1. Raw Material

The high-gluten flour (protein content = 13.7%) was purchased from Yifeng Food Co., Ltd. (New Taipei City, Taiwan). SPI powder (protein content = 90.84%) was purchased from Wellsun Health International Co., Ltd. (New Taipei City, Taiwan). Granulated sugar was purchased from Taiwan Sugar Corp. (Tainan City, Taiwan). Unsalted butter was purchased from Fonterra Ltd. (Auckland, New Zealand). Salt was purchased from Taiyen Biotech Co., Ltd. (Tainan City, Taiwan). Yeast was purchased from Guangxi Danbaoli Yeast Co., Ltd. (Guangxi, China).

2.2. Bacterial Strain and Chemicals

Staphylococcus aureus (BCRC 10780) was purchased from the Bioresource Collection and Research Center (Hsinchu City, Taiwan). pH 4.01 buffer solution and pH 7.00 buffer solution were purchased from GOnDO Electronic Co., Ltd. (Taipei City, Taiwan). Glycerol (100%) was purchased from Macron Fine Chemicals (Radnor, PA, USA). Tryptic soy broth (TSB), peptone (from casein), and dichloran glycerol chloramphenicol agar (DG 18) were purchased from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate (KH_2PO_4, 99%) and sodium bicarbonate ($NaHCO_3$, 99.5%) were purchased from Showa Chemical Industry Co., Ltd. (Tokyo, Japan). Sodium hydroxide (NaOH, 98%) was purchased from Honeywell Research Chemicals (Morris Plains, NJ, USA). *S. aureus* test plates (3M Petrifilm™ Staph Express Count Plate and 3M Petrifilm™ Staph Express Disk) were purchased from 3M (St. Paul, MN, USA).

2.3. Bread Making

The bread recipe (Table 1) in this study was based on Mondal and Datta (2008), CNS 3899, and FDA definitions of baked goods as well as the white pan bread recipe in the revised Taiwanese Technician for Baking Food examination [31–33]. An automatic bread maker (CBK-100, Cuisinart, East Windsor, NJ, USA) was used to make the bread sample. Bread-making experiments were carried out in a laboratory at room temperature (27 °C). The baking conditions were as follows: loaf size of 900 g; crust color set as "light"; and bread type set as "basic/white". The baking temperature was set to 205 °C, and the bread was baked for 30 to 35 min until the top of the loaf was brown in color.

Table 1. Recipes and costs of the breads in this study.

Material	Ratio *				
High-gluten flour	100	98	96	94	92
Soy protein isolate powder	0	2	4	6	8
Water	50	50	50	50	50
Granulated sugar	8	8	8	8	8
Unsalted butter	8	8	8	8	8
Salt	2	2	2	2	2
Yeast	1.5	1.5	1.5	1.5	1.5
Cost (NTD)	23.27	23.83	24.39	24.96	25.52
Group	Control	2%	4%	6%	8%

* Total of flour + SPI is 100, the other ingredients were calculated in terms of weight percentage.

2.4. Specific Volume (SV)

The bread weight was measured using methodologies devised by Ribotta et al. and Masure et al. [11,34]. The baked loaf of bread was removed from the pan entirely, cooled for two hours at room temperature, and weighed using an electronic balance. The bread volume was measured using the AACC 10-05.01 rapeseed displacement method [35]. The first step was to fill a container with rapeseed, smooth the surface, and then measure the total volume of the container (V1). Then, the bottom of the container was filled with

rapeseed, and the cooled loaf of bread was placed into the container, followed by rapeseed. The surface was smoothed, and the total volume of the rapeseed (V2) was measured. The difference between V1 and V2 is the volume (in mL) of the bread measured by the container. The specific volume (SV) of the bread was obtained according to Shin et al. [36] methodologies, in which the volume of the bread is divided by its weight.

$$\text{Specific volume} = \text{bread volume (mL)}/\text{bread weight (g)}$$

2.5. Moisture Content, a_w, and pH

The moisture content of the bread was measured using AACC Method 44-01.01 [37]. The a_w of the bread was measured using a water activity meter (HP23-a_w-A, Rotronic AG, Bassersdorf, Switzerland). A 3 g sample was placed smoothly and uniformly in the container, with its height not exceeding the container. The test was initiated by pressing the switch, and the a_w of the sample was taken when the sample was in equilibrium inside the container [38]. To measure the pH of the bread, a ten-fold dilution of 10 g of the sample using pure water was prepared, and the solution was homogenously mixed using a stomacher blender (Stomacher 400, Seward Ltd., West Sussex, UK). The pH of the sample was measured using a pH meter that was calibrated using pH 7.0 and 4.0 standard solutions [39].

2.6. Sensory Evaluation

After the bread was made, a $5 \times 5 \times 2$ cm^3 sample was cut from the center of the loaf, and the 9-point hedonic test was performed to assess consumer preferences based on the appearance, taste, hardness, texture, and overall acceptance of soy protein breads with different ratios of SPI [36]. The measures were 1 = extremely dislike, 3 = moderately dislike, 5 = neutral, 7 = moderately like, and 9 = extremely like. A total of 58 consumers were randomly recruited on the street to participate in the sensory evaluation.

2.7. Total Yeast and Mold Count

The total yeast and mold count was performed in accordance with the Taiwan Food and Drug Administration Methods of Test for Food Microbiology—Test of Mold and Yeast Count [40]. An amount of 10 g of the bread center was placed into a sterile homogeneous bag and 90 mL of 0.1% peptone diluent was added. The bag was placed into a stomacher blender for two minutes to produce a ten-fold diluted liquid sample, 1 mL of which was added into a 9 mL diluent to produce 100-fold and 1000-fold diluted samples. Using the spread plate method, 0.1 mL of the samples were plated into DG18 agar and spread evenly using a sterile glass rod. The dishes were incubated for five days in the dark at 25 °C, after which yeast and mold counts were taken.

2.8. Microbiological Challenge Test

The following method was used to activate and quantify *S. aureus* in cryovials: After the bread loaf cooled, a $5 \times 5 \times 2$ cm^3 sample was cut from the center for *S. aureus* inoculation [41]. The sample was stored inside an incubator with a constant temperature of 25 °C and a constant relative humidity of 75%. The initial *S. aureus* plate count was taken after two hours. The plate count was taken daily from the second day until the fifth day.

2.8.1. Strain Activation and Storage

S. aureus type strain (BCRC 10780) was activated in TSB at 37 °C inside a shaking incubator. Glycerol (30%) was added to the activated culture, and the mixture was transferred into cryovials and stored at −80 °C until analysis [42].

2.8.2. Preparation of Diluent

Preparation of Butterfield's phosphate-buffered dilution water (BPBW): 34 g of KH_2PO_4 was dissolved in 500 mL distilled water, and the pH was adjusted to 7.2 using 1 N NaOH. A

1000 mL stock solution was prepared by adding more distilled water to the solution, which was sterilized for 15 min at 121 °C and subsequently refrigerated. During analysis, 1.25 mL of the stock solution was taken and diluted with 1000 mL distilled water, transferred to dilution bottles, and sterilized for 15 min at 121 °C [43].

2.8.3. Quantification of *S. aureus*

S. aureus culture (20 µL) was transferred from a cryovial to 10 mL TSB and incubated at 45 °C for six hours at 130 rpm inside a shaking incubator. Two mL of the inoculum was collected and centrifuged at 7000× *g* rpm (4 °C, 15 min). The supernatant was removed, and the precipitate was rinsed three times with BPBW. The inoculum on the surface of the precipitate was removed. The precipitate was mixed with 1 mL BPBW and shaken, and the *S. aureus* inoculum was prepared through serial dilutions [44].

2.8.4. *S. aureus* Inoculation

The inoculation method must not alter the original properties of the food sample [24]. The preliminary test results indicate that inoculating 0.5 wt. % of *S. aureus* (Colony count: 4.97 log CFU g^{-1}) onto the surface of our bread sample has no significant effects on the pH and water activity of the sample. The initial *S. aureus* count was determined two hours after inoculation.

2.8.5. Detection of *S. aureus*

This study used a revised method developed by Silbernagel et al. [45] during the *S. aureus* detection experiments. A 5 g bread sample was placed into a sterile homogeneous bag; 45 mL of BPBW diluent was added to the bag; and the pH of the sample was adjusted to 6~8 using 1 N NaOH. The sample was placed in the stomacher blender for one minute, and 1 mL of the diluent was taken and plated into the center of a test plate with its top film removed. The top film was repositioned carefully to prevent bubbles from forming, and the test plate was gently clamped for one minute to allow gel solidification. The culture was incubated at 37 ± 1 °C for 24 h. *S. aureus* was noted as present only when purple-red colonies are formed. Otherwise, *S. aureus* was identified using a confirmation disc. The disc was placed onto the test plate with its top film removed, which was repositioned carefully to prevent bubbles from forming. The culture was incubated at 37 ± 1 °C for 1–3 h, and *S. aureus* was noted as present when pink halos formed around the colonies.

2.8.6. Calculating the Generation Time and Reproductive Rate of *S. aureus*

Based on a daily count of *S. aureus* for 2–5 days, the generation time and reproductive rate were calculated [46].

$$\text{Generation time (G)} = (t1 - t0)/n$$

$$\text{Reproductive rate} = (\text{G on the } s\text{-th day} - \text{G on the } s + 1\text{-th day})/(\text{G on the } s\text{-th day}) \times 100$$

where,

t1 = 24 × t (t is the number of days);
t0 = 0;
n = 3.3 × (log y − log x); y = Colony count on Day t, x = Colony count on Day 0.

2.8.7. Statistical Analysis

Triplicate bread samples were made using three automatic bread makers. The data were expressed as the mean ± standard deviation. A one-way analysis of variance (ANOVA) was performed using Statistical Products and Services Solution (SPSS) software, and the statistical significance between the mean of each group was assessed through Duncan's multiple range test ($p < 0.05$). The statistical results between the groups are expressed in English letters, and different letters indicate the presence of significant differences ($p < 0.05$).

3. Results and Discussion

3.1. Volume, Weight, and SV

The post-cooling constant weight and subsequent specific volume of the entire loaf of bread were measured on an electronic balance using the rapeseed displacement method. The results are presented in Table 2. The bread volume significantly decreased ($p < 0.05$) with increasing SPI use, and the SV significantly decreased ($p < 0.05$) with increasing weight. The SVs of the bread in all groups were within the standard range of 3.5–6 mL g^{-1}. The external appearances of the breads in all groups are shown in Figure 1. Based on the external appearance, irregular shapes were formed on the bread surfaces when SPI use exceeded 6%. This indicates that different proteins were not completely homogenized during dough fermentation.

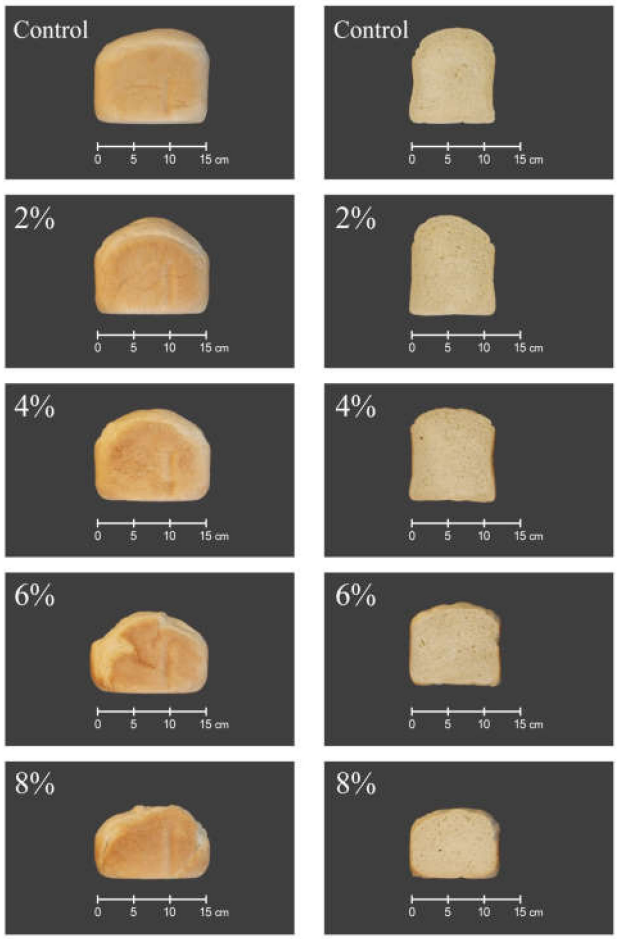

Figure 1. The effects of partially substituting high-gluten flour with soy protein isolate on the external appearance and cross-section of bread. (**A**) External appearance. (**B**) Cross section.

Table 2. Effects of partially substituting high-gluten flour with soy protein isolate on the volume, weight, and specific volume of bread.

Group	Volume (mL)	Weight (g)	Specific Volume (mL g^{-1})
Control	3226.00 ± 64.09 ^{a}	617.83 ± 1.57 ^{a}	5.22 ± 0.10 ^{a}
2%	3104.67 ± 38.28 ^{b}	621.53 ± 2.95 ^{ab}	5.00 ± 0.04 ^{b}
4%	2860.67 ± 10.02 ^{c}	621.43 ± 5.83 ^{ab}	4.60 ± 0.03 ^{c}
6%	2773.67 ± 46.36 ^{d}	625.20 ± 2.70 ^{b}	4.44 ± 0.09 ^{d}
8%	2390.00 ± 39.74 ^{e}	625.97 ± 2.73 ^{b}	3.82 ± 0.08 ^{e}

$^{a–e}$: Means within the same column values with different superscript letters are significantly different ($p < 0.05$).

SPI affects the structure and specific volume of bread because soy protein and wheat protein form covalent, disulfide bonds. These bonds affect the arrangements of gluten proteins and other macromolecules within the dough, forming a dilution effect that causes the dough to lose its gas-retaining ability [17,47]. Additionally, the substitution of soy protein reduces the size of the bread, which could be due to the variation in the denaturation temperature, foaming properties, and soy protein composition or properties. Zhou et al. [48] highlighted that gluten proteins are primary gluten networks formed by prolamin and glutenin. Since wheat protein has a lower denaturation temperature, it stabilizes the dough structure during the early phases of baking. Soy protein consists of β-conglycinin and glycinin, which have higher denaturation temperatures. Hence, the presence of soy protein affects the form and stability of the dough structure by weakening it. Meanwhile, Horstmann et al. [49] proposed that, because of its higher foaming capacity and its influence on the stability of multiphase food systems, SPI affects the porosity of bread structure.

The SPI-fortified bread was heavier, probably because of the increased water retention rate of soy protein. The β-conglycinin and glycinin in soy protein are spherical, and soy protein is more hydrophilic than wheat gluten because of its amino acid composition [50]. Additionally, soy protein retains water molecules and prevents them from evaporating during the baking process [51]. Thus, adding an equal ratio of water to the raw ingredients increases the weight of the SPI-fortified bread, which also has a lower baking loss. Baking loss refers to the weight lost during the baking and cooling processes; while it is primarily associated with water evaporation, it also promotes the loss of organic substances, such as the fermented carbohydrates released as CO_2. Baking loss is directly correlated with the surface volume of bread [49]. These results indicate that the baking loss had reduced significantly in the SPI-fortified bread. The control bread had a greater surface volume for evaporation compared to the SPI-fortified bread, which subsequently affected baking loss.

3.2. Moisture Content, a_w, and pH Value

The variations in the moisture content, a_w, and pH are shown in Figure 2. Moisture loss is one of the factors that cause dough staling, while moisture retention promotes chemical or microbial interactions. Therefore, the moisture content is a factor affecting bread quality. In Figure 2A, the moisture content of the control bread on the first day of storage was 37.85 ± 0.17%, while the moisture contents of the 2%, 4%, 6%, and 8% SPI-fortified breads were 37.87 ± 0.08%, 37.67 ± 0.15%, 37.62 ± 0.08%, and 37.50 ± 0.36%, respectively. On the fifth day of storage, the moisture content of the control had decreased to 36.88 ± 0.26%, while the moisture contents of the 2%, 4%, 6%, and 8% SPI-fortified bread had decreased to 37.17 ± 0.63%, 36.98 ± 0.81%, 37.32 ± 0.18%, and 37.30 ± 0.48%, respectively. There were no significant changes in the water content of the central bread structure, mainly because, as the dough stales, the water migrates closer to the crust. Thus, there were no significant changes in the moisture content in the center of the bread. This finding is consistent with that of Besbes et al. [52]. However, during the sixth day of storage, the moisture content of the control (36.67 ± 1.01%) was significantly lower ($p < 0.05$) than those of the SPI-fortified breads (37.83 ± 0.29~37.30 ± 0.36%). During the seventh day of storage, the moisture content of the control (36.37 ± 0.75%) was also significantly lower ($p < 0.05$) than those of the SPI-fortified breads (37.70 ± 0.01~37.43 ± 0.43%). This is because soy protein is more hydrophilic than wheat protein, due to β-conglycinin and glycinin, which have higher water-binding capacities. Furthermore, during the preparation of SPI, many proteins had partially denatured or expanded, which increased their water retention capacities. The use of SPI promotes the water retention capacity of bread, which was observed from the sixth day of storage onwards.

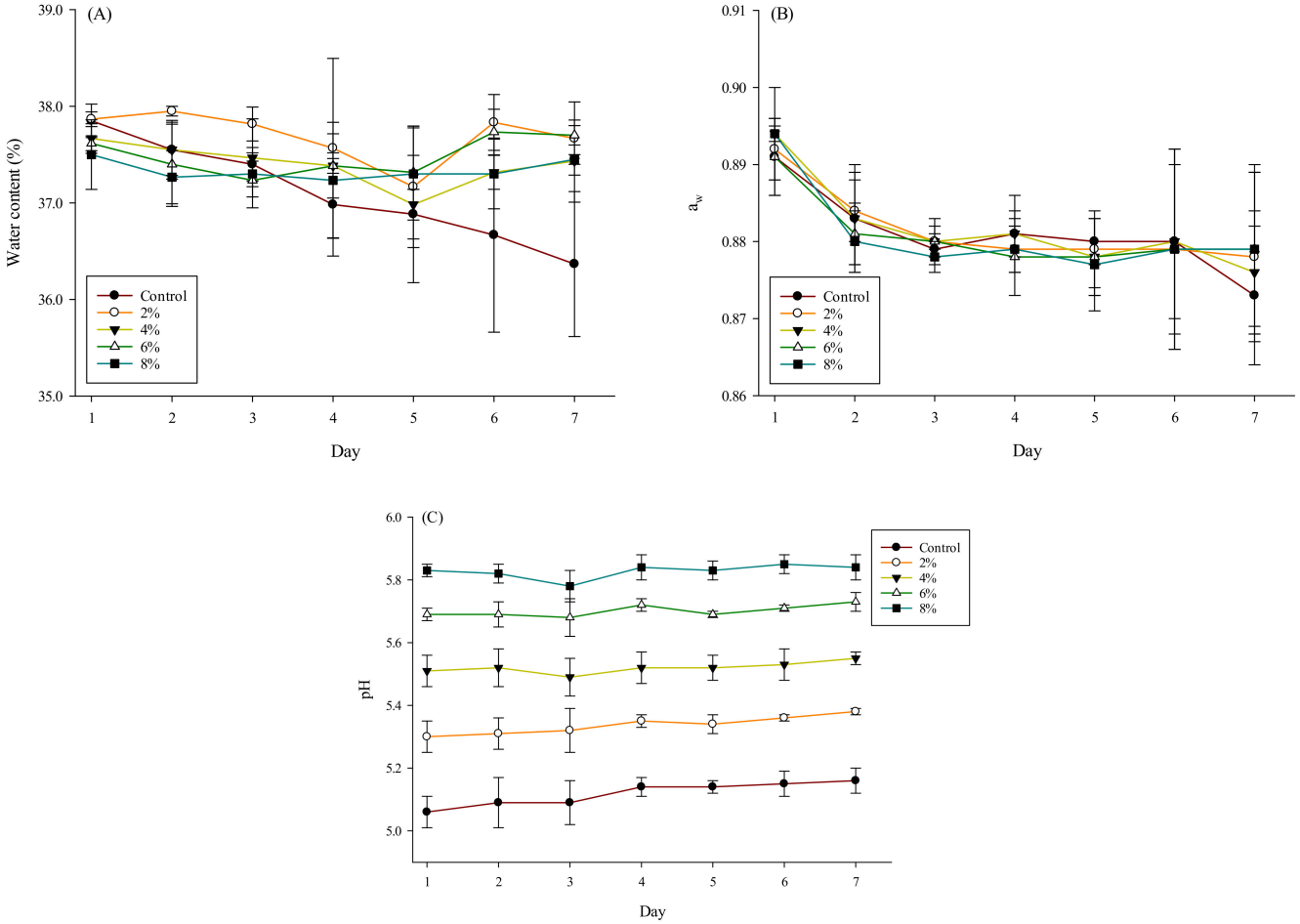

Figure 2. Effects of partially substituting high-gluten flour with soy protein isolate on the (**A**) moisture content, (**B**) a_w, and (**C**) pH of bread.

Water activity is closely associated with microbial growth. Most foods become moldy and spoil when the a_w exceeds 0.80. In Figure 2B, except for the control in which the a_w had decreased to 0.87 on the seventh day, no significant differences were observed in the a_w of the control and SPI-fortified breads during the storage period, which were all greater than 0.88. The a_w of the control and SPI-fortified breads had decreased on the second day because the rearrangement of water molecules within the bread had altered the bound water/free water ratio when the water migrated from the internal bread structure to the crust. This finding is consistent with that of Czuchajowska and Pomeranz [53].

The variation in bread pH during the storage period is shown in Figure 2C. The pHs of all the breads significantly increased with increasing SPI incorporation ($p < 0.05$), likely because of the higher pH of the SPI raw ingredient (8.05), but were not significantly different over time. On the first day, the mean pH values of the control, 2%, 4%, 6%, and 8% SPI-fortified breads were 5.12, 5.34, 5.52, 5.70, and 5.83, respectively.

3.3. Sensory Evaluation

The sensory evaluation was performed with respect to the appearance, taste, softness and hardness, texture, and overall acceptance of soy protein breads. The consumers' acceptance of the sensory properties of breads fortified with different SPI ratios was evaluated on a 9-point hedonic scale, in which 1 = extremely dislike, 3 = moderately dislike, 5 = neutral, 7 = moderately like, and 9 = extremely like. A total of 58 questionnaires were administered, 51 of which were valid. Male and female evaluators accounted for 54.90% and 45.10% of the participants, respectively. Individuals under 20 years of age comprised 3.92%, 21~30-year-olds accounted for 41.80%, 31~40-year-olds comprised 25.49%, and 41~50-year-

olds accounted for 25.49% of the participants. Participants above 50 years of age accounted for 3.92% of the total.

The preferential evaluation results are displayed in Table 3. In terms of appearance, the control had the highest score (6.37), which was not significantly different from that of the 2% SPI-fortified bread (5.65). The mean scores of the 4%, 6%, and 8% SPI-fortified breads were 5.43, 5.25, and 4.33, respectively, all of which were significantly lower than the control. The evaluation results indicate that the appearance scores decreased with increased SPI use.

Table 3. Consumer preferences toward breads made by partially substituting high-gluten flour with soy protein isolate.

Group	Appearance	Taste	Softness/Hardness	Texture	Overall Acceptance
Control	6.37 ^{a}	6.53 ^{a}	6.84 ^{a}	6.63 ^{a}	7.02 ^{a}
2%	5.65 ^{ab}	5.84 ^{ab}	6.14 ^{ab}	6.02 ^{a}	6.10 ^{b}
4%	5.43 ^{b}	5.59 ^{b}	5.65 ^{bc}	5.84 ^{a}	5.92 ^{b}
6%	5.25 ^{b}	5.18 ^{b}	4.94 ^{c}	4.88 ^{b}	5.25 ^{b}
8%	4.33 ^{c}	4.31 ^{c}	3.82 ^{d}	4.22 ^{b}	4.16 ^{c}

$^{a–d}$: Means within the same column values with different superscript letters are significantly different ($p < 0.05$).

In terms of taste, the score of the control (6.53) was not significantly different from the score of the 2% SPI-fortified bread (5.84). The mean taste score decreased significantly ($p < 0.05$) when the SPI use exceeded 4%. The mean scores of the 4%, 6%, and 8% SPI-fortified breads were 5.59, 5.18, and 4.31, respectively. A possible reason is that SPI contains polyunsaturated fatty acids, which produce volatile compounds during oxygenation, resulting in beany or grassy flavors. Moreover, polyphenolic compounds, such as isoflavones, saponin, and phenolic acids, create an astringent taste in the bread [54]. As such, the consumers felt that the tastes of the ≥4% SPI-fortified breads were significantly different from that of the control.

In terms of softness/hardness, the mean score of the control was 6.84 while those of the 2%, 4%, 6%, and 8% SPI-fortified breads were 6.14, 5.56, 4.94, and 3.82, respectively. Consumer preference decreased with increasing SPI substitution, which was significant in the ≥4% SPI-fortified breads.

In terms of texture, the mean score of the control was 6.63 while those of the 2%, 4%, 6%, and 8% SPI-fortified breads were 6.02, 5.84, 4.88, and 4.22, respectively. The score of the control was not significantly different with those of the 2% and 4% SPI-fortified breads, but it was significantly different from the 6% and 8% SPI-fortified breads. A possible reason for this is that the SV of bread decreases with increasing SPI use and creates a denser structure. The consumers' preferences for 6% and 8% SPI-fortified breads were significantly lower.

3.4. Detection of Yeast and Mold

Mold growth affects the shelf life of baked goods and is also the main cause of spoilage [55–57]. The breads were sliced and stored at room temperature, and the yeast and mold growth are shown in Figure 3. No yeast or mold was detected in any groups from the first to the fourth day of storage. The yeast and mold counts of the 4–8% SPI-fortified breads on the fifth day were 4.06 log CFU g^{-1}, 4.23 log CFU g^{-1}, and 3.57 log CFU g^{-1}, respectively. A possible reason could be the pH values of the 4–8% SPI-fortified breads (Figure 2C), which were 5.52, 5.69, and 5.83, respectively. Comparatively, the control (pH = 5.14) and the 2% SPI-fortified bread (pH = 5.34) were slightly more acidic and more unfavorable for mold growth. On the sixth day of storage, the yeast and mold counts of the control and the 2% SPI-fortified bread were 4.69 log CFU g^{-1} and 4.29 log CFU g^{-1}, respectively. From the sixth day onwards, mycelia and dark patches, discernible with the naked eye, were observed on the sliced surfaces of the breads. The yeast and mold counts of the 4–8% SPI-fortified breads were 5.43 log CFU g^{-1}, 4.90 log CFU g^{-1}, and 4.57 log CFU g^{-1}, respectively. The yeast and mold colonies continued to grow on the seventh

day, on which the mold counts of the control, 2%, 4%, 6%, and 8% SPI-fortified breads were 5.58 log CFU g^{-1}, 5.44 log CFU g^{-1}, 6.95 log CFU g^{-1}, 6.15 log CFU g^{-1}, and 5.77 log CFU g^{-1}, respectively. Additionally, soy protein has a higher water retention capacity, as exemplified on the sixth and seventh days, when the moisture contents of the SPI-fortified breads were significantly higher than that of the control (Figure 2A). This could be a reason why the yeast and mold counts of SPI-fortified breads were higher than those of the control.

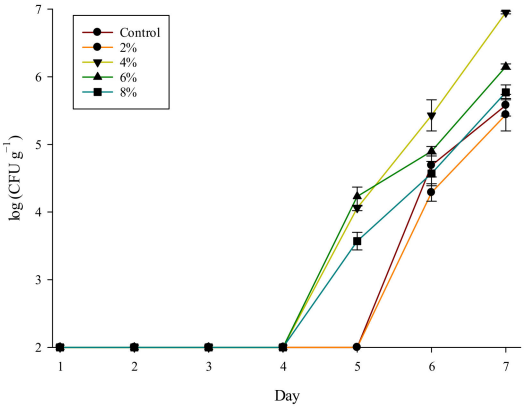

Figure 3. Effects of partially substituting high-gluten flour with soy protein isolate on the mold and yeast growth in bread.

Baking inhibits the growth of mold and mold spores in bread. Mold growth is caused by recontamination after baking, such as during the cooling, slicing, packaging, and storage processes. Bread is contaminated when exposed to mold spores in the air, and the surface of sliced bread is an ideal environment for mold growth. Therefore, the experimental environment in this study may have affected mold growth on the bread. However, under the same conditions, yeast and mold were only detected in the 4% SPI-fortified bread from the fourth day of storage onwards, while they were only detected in the control and 2% SPI-fortified breads on the fifth day. Thus, SPI use exceeding 4% in bread promotes faster yeast and mold growth.

3.5. S. aureus Challenge Test

Lower inoculant can be used to precisely indicate the capacity of a product to support microbial growth. In this study, a 5 × 5 × 2 cm^3 sample was cut from the center of the loaf after it had cooled, and the challenge test was performed by inoculating 0.5 wt. % culture with 2–3 log CFU g^{-1} *S. aureus*. Because *S. aureus* produces enterotoxins at levels above 6 log CFU g^{-1} [23], the outcome of the challenge test is based on a threshold of 6 log CFU g^{-1}. The results are shown in Figure 4. Because discernible mold had appeared on the bread from the sixth day onwards, the observation period ended on the fifth day. The initial *S. aureus* counts inoculated on all groups of bread on the first day were within 2.62–2.70 log CFU g^{-1}, and no significant differences were observed on the second day of *S. aureus* inoculation as this was the lag phase. The *S. aureus* counts of the control, 2%, 4%, 6%, and 8% SPI-fortified breads were 3.28 log CFU g^{-1}, 3.62 log CFU g^{-1}, 3.55 log CFU g^{-1}, 3.44 log CFU g^{-1}, and 3.68 log CFU g^{-1}, respectively. On the third day, the *S. aureus* count of the control (3.90 log CFU g^{-1}) was slightly lower than those of the 2%, 4%, 6%, and 8% SPI-fortified breads (4.50 log CFU g^{-1}, 4.44 log CFU g^{-1}, 4.21 log CFU g^{-1}, and 4.45 log CFU g^{-1}, respectively.). On the fourth day, the *S. aureus* counts of the control, 2%, 4%, 6%, and 8% SPI-fortified breads were 4.38 log CFU g^{-1}, 5.44 log CFU g^{-1}, 5.44 log CFU g^{-1}, 5.25 log CFU g^{-1}, and 5.37 log CFU g^{-1}, respectively. This shows that the SPI-fortified breads had a significantly higher *S. aureus* count than the control ($p < 0.05$). On the fifth day, the *S. aureus* counts of the control, 2%, 4%, and 6% SPI-fortified breads were 5.12 log CFU g^{-1}, 5.79 log CFU g^{-1}, 5.81 log CFU g^{-1}, and 5.63 log CFU g^{-1}, respectively, while that of the 8% SPI-fortified bread had risen to 6.58 log CFU g^{-1}.

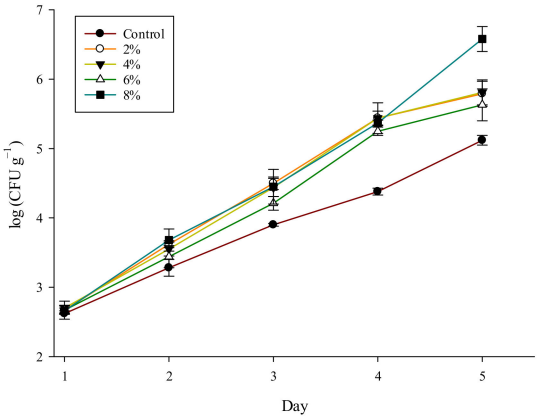

Figure 4. Microbiological challenge test results using *Staphylococcus aureus*.

According to the challenge test results, the *S. aureus* count was below 6 log CFU g^{-1} within the first four days of storage and had successfully passed the challenge test. The control bread passed the test within the first five days of storage. A possible reason for this difference is the amino acid composition of SPI in the SPI-fortified and control breads, as well as the difference between their pH values.

Comparing SPI and flour protein composition, SPI contains 6.14% lysine and 2.47% methionine and cysteine, while flour protein contains 1.99% lysine and 4.52% methionine and cysteine [58,59]. Hence, a more balanced intake of essential amino acids can be achieved by eating bread made from these two different sources of protein. Leucine, isoleucine, and valine are branched-chain amino acids that provide environments that are conducive to *S. aureus* protein and branched-chain fatty acid formation. Grispoldi et al. [60] found a positive correlation between *S. aureus* growth and leucine and isoleucine levels in composite milk. The branched-chain amino acids in SPI account for 16.08% of all amino acids, while flour protein contains 13.69% branched-chain amino acids [58,59]. This could be a reason why SPI-fortified bread is more conducive to *S. aureus* growth than the control.

As shown in Figure 2C, the bread pH increases with SPI use. Feeherry et al. [26] reported that lowering the dough pH prolongs the lag phase of *S. aureus* growth and shortens the microbial growth rate. Tango et al. [61] studied the effects of pH on the biofilm formation of *S. aureus* and found a positive correlation between pH and *S. aureus* growth. The *S. aureus* count exceeded 5 log CFU g^{-1} on the fourth day.

Table 4 shows the effect of SPI on the generation time and the reproductive rate of *S. aureus*. The generation time of *S. aureus* in the control on the second day of growth was 21.83 h, while those of the 2%, 4%, 6%, and 8% SPI-fortified breads were 15.30, 17.06, 18.96, and 14.33 h, respectively. The use of SPI reduced the generation time of *S. aureus*. Additionally, the generation times of the control from the third to fifth days had decreased, and the reproductive rate of *S. aureus* in the 4% and 6% SPI-fortified breads was fastest on the third day. On the fifth day of storage, the reproductive rate of *S. aureus* in the 2–6% SPI-fortified breads had reduced, due to mold and yeast growth. In general, SPI-fortified bread has a shorter shelf life and *S. aureus* generation time because it provides a favorable environment for *S. aureus* growth. We recommend a shelf life of four days for SPI-fortified bread.

Table 4. Effects of partially substituting high-gluten flour with soy protein isolate on the growth count, generation time, and reproductive rate of *Staphylococcus aureus*.

Group		Day 0	Day 2	Day 3	Day 4	Day 5
Control	Growth count (log CFU g^{-1})	2.62	3.28	3.90	4.38	5.12
	Generation time (hr)		21.83	17.07	16.50	14.52
	Reproductive rate (%)			21.84	3.33	11.99
2%	Growth count (log CFU g^{-1})	2.67	3.62	4.50	5.44	5.79
	Generation time (hr)		15.30	11.94	10.52	11.67
	Reproductive rate (%)			21.92	11.94	−10.94
4%	Growth count (log CFU g^{-1})	2.70	3.55	4.44	5.44	5.81
	Generation time (hr)		17.06	12.55	10.63	11.70
	Reproductive rate (%)			26.44	15.31	−10.08
6%	Growth count (log CFU g^{-1})	2.67	3.44	4.21	5.25	5.63
	Generation time (hr)		18.96	14.20	11.30	12.31
	Reproductive rate (%)			25.08	20.43	−8.90
8%	Growth count (log CFU g^{-1})	2.66	3.68	4.45	5.37	6.58
	Generation time (hr)		14.33	12.22	10.75	9.28
	Reproductive rate (%)			14.67	12.02	13.71

4. Conclusions

In this study, bread was made by partially substituting flour with 2–8% SPI. The bread volume and SV decreased with increased SPI use, while irregularities formed on the bread surface when 6% SPI was used, which all affect the appearance of the bread. Based on consumers' preferential evaluations, 6% SPI use significantly affected the texture of the bread, and hence, the optimal amount should be 4% SPI. Based on the mold and yeast growth during the storage period, ≥4% SPI-fortified breads have a shelf life of four days, while 2% SPI-fortified bread has a shelf life of five days. SPI use affects the pH of bread by increasing the amount of branched-chain amino acids, thus providing a favorable environment for *S. aureus* growth and shortening the *S. aureus* generation time. We recommend a shelf life of four days for 2–8% SPI-fortified breads. Even though partially substituting bread flour with SPI increases the cost of raw ingredients, it compensates for the limiting amino acid (lysine) in wheat flour and is applicable in various baked goods. For manufacturers who wish to create and commercialize a wider range of baked goods, the partial substitution of bread flour with soy protein in baked goods offers promising business opportunities.

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References

1. Bauer, J.; Biolo, G.; Cederholm, T.; Cesari, M.; Cruz-Jentoft, A.J.; Morley, J.E.; Phillips, S.; Sieber, C.; Stehle, P.; Teta, D.; et al. Evidence-based recommendations for optimal dietary protein intake in older people: A position paper from the PROT-AGE Study Group. *J. Am. Med. Dir. Assoc.* **2013**, *14*, 542–559. [CrossRef] [PubMed]
2. Boirie, Y. Physiopathological mechanism of sarcopenia. *J. Nutr. Health Aging* **2009**, *13*, 717–723. [CrossRef]
3. Costa, A.I.A.; Jongen, W.M.F. Designing new meals for an ageing population. *Crit. Rev. Food Sci.* **2010**, *50*, 489–502. [CrossRef]

4. Deutz, N.E.P.; Bauer, J.M.; Barazzoni, R.; Biolo, G.; Boirie, Y.; Bosy-Westphal, A.; Cederholm, T.; Cruz-Jentoft, A.; Krznariç, Z.; Nair, K.S.; et al. Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. *Clin. Nutr.* **2014**, *33*, 929–936. [CrossRef] [PubMed]
5. Nieuwenhuizen, W.F.; Weenen, H.; Rigby, P.; Hetherington, M.M. Older adults and patients in need of nutritional support: Review of current treatment options and factors influencing nutritional intake. *Clin. Nutr.* **2010**, *29*, 160–169. [CrossRef]
6. Wolfe, R.R.; Miller, S.L.; Miller, K.B. Optimal protein intake in the elderly. *Clin. Nutr.* **2008**, *27*, 675–684. [CrossRef]
7. Van der Zanden, L.D.T.; van Kleef, E.; de Wijk, R.A.; van Trijp, H.C.M. Knowledge, perceptions and preferences of elderly regarding protein-enriched functional food. *Appetite* **2014**, *80*, 16–22. [CrossRef] [PubMed]
8. Kreutz, J.M.; Heynen, L.; Arayess, L.; Vreugdenhil, A.C.E. Celiac disease and the gluten free diet during the COVID-19 pandemic: Experiences of children and parents. *Medicina* **2023**, *59*, 425. [CrossRef]
9. Bian, X.; Xing, T.L.; Yang, Y.; Fan, J.; Ma, C.M.; Liu, X.F.; Wang, Y.; He, Y.Y.; Wang, L.D.; Wang, B.; et al. Effect of soy protein isolate on physical properties of quinoa dough and gluten-free bread quality characteristics. *J. Sci. Food Agric.* **2023**, *103*, 118–124. [CrossRef] [PubMed]
10. Boyacioglu, M.H. Soy ingredients in baking. In *Soy Applications in Food*, 1st ed.; Riaz, M.N., Ed.; CRC Taylor and Francis: Boca Raton, FL, USA, 2006; pp. 63–81.
11. Ribotta, P.D.; Arnulphi, S.A.; León, A.E.; Añón, M.C. Effect of soybean addition on the rheological properties and breadmaking quality of wheat flour. *J. Sci. Food Agric.* **2005**, *85*, 1889–1896. [CrossRef]
12. Crockett, R.; Ie, P.; Vodovotz, Y. Effects of soy protein isolate and egg white solids on the physicochemical properties of gluten-free bread. *Food Chem.* **2011**, *129*, 84–91. [CrossRef]
13. Endres, J.G. Uses in food systems. In *Soy Protein Products: Characteristics, Nutritional Aspects, and Utilization*, 1st ed.; Endres, J.G., Ed.; AOCS Press: Champaign, IL, USA, 2001; pp. 31–34.
14. Zhang, Y.; Guo, X.; Xiong, H.; Zhu, T. Effect of modified soy protein isolate on dough rheological properties and noodle qualities. *J. Food Process. Preserv.* **2022**, *46*, e16371. [CrossRef]
15. Fang, Y.; Zhang, B.; Wei, Y. Effects of the specific mechanical energy on the physicochemical properties of texturized soy protein during high-moisture extrusion cooking. *J. Food Eng.* **2014**, *121*, 32–38. [CrossRef]
16. Kristiawan, M.; Micard, V.; Maladira, P.; Alchamieh, C.; Maigret, J.-E.; Réguerre, A.-L.; Emin, M.A.; Della Valle, G. Multi-scale structural changes of starch and proteins during pea flour extrusion. *Food Res. Int.* **2018**, *108*, 203–215. [CrossRef]
17. Pérez, G.T.; Ribotta, P.D.; Steffolani, M.E.; León, A.E. Effect of soybean proteins on gluten depolymerization during mixing and resting. *J. Sci. Food Agric.* **2008**, *88*, 455–463. [CrossRef]
18. Zhang, Y.; Guo, X.; Shi, C.; Ren, C. Effect of soy proteins on characteristics of dough and gluten. *Food Chem.* **2020**, *318*, 126494. [CrossRef] [PubMed]
19. Zheng, L.; Regenstein, J.M.; Zhou, L.; Wang, Z. Soy protein isolates: A review of their composition, aggregation, and gelation. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 1940–1957. [CrossRef]
20. Sahagún, M.; Benavent-Gil, Y.; Rosell, C.M.; Gómez, M. Modulation of in vitro digestibility and physical characteristics of protein enriched gluten free breads by defining hydration. *LWT–Food Sci. Technol.* **2019**, *117*, 108642. [CrossRef]
21. Srikanlaya, C.; Therdthai, N.; Ritthiruangdej, P.; Zhou, W. Effect of hydroxypropyl methylcellulose, whey protein concentrate and soy protein isolate enrichment on characteristics of gluten-free rice dough and bread. *Int. J. Food Sci. Technol.* **2018**, *53*, 1760–1770. [CrossRef]
22. FDA. Food Code 2017. US Food and Drug Administration. 2017. Available online: https://www.fda.gov/media/110822/download (accessed on 12 January 2023).
23. FDA. Evaluation and definition of potentially hazardous foods. *Compr. Rev. Food Sci. Food Saf.* **2003**, *2*, 6–50. Available online: https://www.fda.gov/files/food/published/Evaluation-and-Definition-of-Potentially-Hazardous-Foods.pdf (accessed on 12 January 2023).
24. National Advisory Committee on Microbiological Criteria for Foods. Parameters for determining inoculated pack/challenge study protocols. *J. Food Protect.* **2010**, *73*, 140–202. [CrossRef]
25. Komitopoulou, E. Microbiological challenge testing of foods. In *Food and Beverage Stability and Shelf Life*, 1st ed.; Kilcast, D., Subramaniam, P., Eds.; Woodhead Publishing: Cambridge, UK, 2011; pp. 507–523.
26. Feeherry, F.E.; Doona, C.J.; Taub, I.A. Effect of water activity on the growth kinetics of *Staphylococcus aureus* in ground bread crumb. *J. Food Sci.* **2003**, *68*, 982–987. [CrossRef]
27. Asioli, D.; Aschemann-Witzel, J.; Caputo, V.; Vecchio, R.; Annunziata, A.; Næs, T.; Varela, P. Making sense of the "clean label" trends: A review of consumer food choice behavior and discussion of industry implications. *Food Res. Int.* **2017**, *99*, 58–71. [CrossRef] [PubMed]
28. MacGregor, G.A.; He, F.J.; Pombo-Rodrigues, S. Food and the responsibility deal: How the salt reduction strategy was derailed. *Brit. Med. J.* **2015**, *350*, 20–22. [CrossRef]
29. Coombs, C.E.; Holman, B.W.; Friend, M.A.; Hopkins, D.L. Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Sci.* **2017**, *125*, 84–94. [CrossRef]
30. Barcenilla, C.; Álvarez-Ordóñez, A.; López, M.; Alvseike, O.; Prieto, M. Microbiological safety and shelf-life of low-salt meat products—A review. *Foods* **2022**, *11*, 2331. [CrossRef]
31. Mondal, A.; Datta, A.K. Bread baking-A review. *J. Food Eng.* **2008**, *86*, 465–474. [CrossRef]

32. *CNS 3899*; Breads. National Standards of the Republic of China. Ministry of Economic Affairs: Taipei City, Taiwan, 2012.
33. FDA. US Department of Health and Human Services. CFR-Code of Federal Regulations Title 21, Part 136 Bakery Products. 2019. Available online: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=136&showFR=1 (accessed on 12 January 2023).
34. Masure, H.G.; Wouters, A.G.B.; Fierens, E.; Delcour, J.A. Impact of egg white and soy proteins on structure formation and crumb firming in gluten-free breads. *Food Hydrocolloid* **2019**, *95*, 406–417. [CrossRef]
35. AACC International. AACC Method 10-05.01 Guidelines for Measurement of Volume by Rapeseed Displacement. In *Approved Methods of the American Association of Cereal Chemists*, 11th ed.; American Association of Cereal Chemists: St. Paul, MN, USA, 2010.
36. Shin, M.; Gang, D.O.; Song, J.Y. Effects of protein and transglutaminase on the preparation of gluten-free rice bread. *Food Sci. Biotechnol.* **2010**, *19*, 951–956. [CrossRef]
37. AACC International. AACC International. AACC Method 44-01.01 Calculation of Percent Moisture. In *Approved Methods of the American Association of Cereal Chemists*, 11th ed.; American Association of Cereal Chemists: St. Paul, MN, USA, 2000.
38. Sanmartin, C.; Taglieri, I.; Venturi, F.; Macaluso, M.; Zinnai, A.; Tavarini, S.; Botto, A.; Serra, A.; Conte, G.; Flamini, G.; et al. Flaxseed cake as a tool for the improvement of nutraceutical and sensorial features of sourdough bread. *Foods* **2020**, *9*, 204. [CrossRef]
39. Brewer, M.S.; Potter, S.M.; Sprouls, G.; Reinhard, M. Effect of soy protein isolate and soy fiber on color, physical and sensory characteristics of baked products. *J. Food Qual.* **1992**, *15*, 245–262. [CrossRef]
40. TFDA. *Methods of Test for Food Microorganisms-Test of Mold and Yeast Count*; Taiwan Food and Drug Administration: Taipei City, Taiwan, 2013.
41. Aguirre, J.F.; Osella, C.A.; Carrara, C.R.; Sánchez, H.D.; Buera, M.D.P. Effect of storage temperature on starch retrogradation of bread staling. *Starch–Stärke* **2011**, *63*, 587–593. [CrossRef]
42. Yoo, M.H.; Huh, M.D.; Kim, E.H.; Lee, H.H.; Jeong, H.D. Characterization of chloramphenicol acetyltransferase gene by multiplex polymerase chain reaction in multidrug-resistant strains isolated from aquatic environments. *Aquaculture* **2003**, *217*, 11–21. [CrossRef]
43. TFDA. *Methods of Test for Food Microorganisms-Test of Coliform Bacteria*; Taiwan Food and Drug Administration: Taipei City, Taiwan, 2013.
44. Osés, S.M.; Diez, A.M.; Gómez, E.M.; Wilches-Pérez, D.; Luning, P.A.; Jaime, I.; Rovira, J. Control of *Escherichia coli* and *Listeria monocytogenes* in suckling-lamb meat evaluated using microbial challenge tests. *Meat Sci.* **2015**, *110*, 262–269. [CrossRef]
45. Silbernagel, K.M.; Jechorek, R.P.; Carver, C.N.; Horter, B.L.; Lindberg, K.G. 3M Petrifilm Staph Express count plate method for the enumeration of *Staphylococcus aureus* in selected types of processed and prepared foods: Collaborative study. *J. AOAC Int.* **2003**, *86*, 954–962. [CrossRef]
46. Monod, J. The growth of bacterial cultures. *Annu. Rev. Microbiol.* **1949**, *3*, 371–394. [CrossRef]
47. Lorimer, N.L.; Zabik, M.E.; Harte, J.B.; Stachiw, N.C.; Uebersax, M.A. Effect of navy bean protein flour and navy bean globulins on composite flour rheology, chemical bonding, and microstructure. *Cereal Chem.* **1991**, *68*, 213–220.
48. Zhou, J.; Liu, J.; Tang, X. Effects of whey and soy protein addition on bread rheological property of wheat flour. *J. Texture Stud.* **2018**, *49*, 38–46. [CrossRef]
49. Horstmann, S.W.; Foschia, M.; Arendt, E.K. Correlation analysis of protein quality characteristics with gluten-free bread properties. *Food Funct.* **2017**, *8*, 2465–2474. [CrossRef]
50. Kinsella, J.E. Functional properties of soy proteins. *J. Am. Oil Chem. Soc.* **1979**, *56*, 242–258. [CrossRef]
51. Otegbayo, B.O.; Adebiyi, O.M.; Bolaji, O.A.; Olunlade, B.A. Effect of soy enrichment on bread quality. *Int. Food Res. J.* **2018**, *25*, 1120–1125.
52. Besbes, E.; Jury, V.; Monteau, J.-Y.; Le Bail, A. Effect of baking conditions and storage with crust on the moisture profile, local textural properties and staling kinetics of pan bread. *LWT–Food Sci. Technol.* **2014**, *58*, 658–666. [CrossRef]
53. Czuchajowska, Z.; Pomeranz, Y. Different scanning calorimetry, water activity, and moisture contents in crumb center and near-crust zones of bread during storage. *Cereal Chem.* **1989**, *66*, 305–309.
54. Damodaran, S.; Arora, A. Off-flavor precursors in soy protein isolate and novel strategies for their removal. *Annu. Rev. Food Sci. Technol.* **2013**, *4*, 327–346. [CrossRef]
55. Corsetti, A.; Gobbetti, M.; Rossi, J.; Damiani, P. Antimould activity of sourdough lactic acid bacteria: Identification of a mixture of organic acids produced by *Lactobacillus sanfrancisco* CB1. *Appl. Microbiol. Biot.* **1998**, *50*, 253–256. [CrossRef] [PubMed]
56. Guynot, M.E.; Ramos, A.J.; Sanchis, V.; Marın, S. Study of benzoate, propionate, and sorbate salts as mould spoilage inhibitors on intermediate moisture bakery products of low pH (4.5–5.5). *Int. J. Food Microbiol.* **2005**, *101*, 161–168. [CrossRef]
57. Legan, J.D. Mould spoilage of bread: The problem and some solutions. *Int. Biodeter. Biodegr.* **1993**, *32*, 33–53. [CrossRef]
58. Tamaru, S.; Kurayama, T.; Sakono, M.; Fukuda, N.; Nakamori, T.; Furuta, H.; Nagata, Y.; Tanaka, K. Dietary soybean peptides containing a low-molecular fraction can lower serum and liver triglyceride levels in rats. *J. Nutr. Sci. Vitaminol.* **2014**, *60*, 436–442. [CrossRef]
59. Abou-Zaid, A.; Elbandy Mohamed, A.S. Production and quality evaluation of nutritious high quality biscuits and potato puree tablets supplemented with crayfish ProcombarusClarkia protein products. *J. Appl. Sci. Res.* **2014**, *10*, 43–53.

60. Grispoldi, L.; Karama, M.; Ianni, F.; La Mantia, A.; Pucciarini, L.; Camaioni, E.; Sardella, R.; Sechi, P.; Natalini, B.; Cenci-Goga, B.T. The relationship between *S. aureus* and branched-chain amino acids content in composite cow milk. *Animals* **2019**, *9*, 981. [CrossRef]
61. Tango, C.N.; Akkermans, S.; Hussain, M.S.; Khan, I.; Van Impe, J.; Jin, Y.-G.; Oh, D.H. Modeling the effect of pH, water activity, and ethanol concentration on biofilm formation of *Staphylococcus aureus*. *Food Microbiol.* **2018**, *76*, 287–295. [CrossRef] [PubMed]