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The impact of soaking and drying soybeans on soyflour and the gluten-free cookies made from it

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ABSTRACT

This study investigated the effects of drying and soaking conditions on soy flour, as well as their influence on cookie quality. Two drying methods (70°C for 10 hours and 90°C for 6 hours) and three soaking conditions (ambient temperature for 24 hours, 80°C for 2 hours, and 121°C for 10 minutes) were evaluated. High-temperature soaking and drying resulted in darker flour with lower L* values (81.06–89.44) and higher a* values (-0.36–5.25) ($p \leq 0.05$). Scanning electron microscopy showed that the treated flour had rougher surfaces and protein clustering, which made it less foamy, and less emulsifying but better at water absorbing and holding. With heat treatment, the trypsin inhibitor content decreased significantly from 50.21 mg/g to 33.01 mg/g ($p \leq 0.05$). Gluten-free cookies made from soy flour treated at 80°C for 2 hours soaking and 90°C for 6 hours drying showed comparable spread ratios to wheat cookies and lower hardness. This treatment also allowed for higher protein (24.92%) and fiber (22.29%) content in the cookies. Therefore, soaking soybeans at 80°C for 2 hours, followed by drying them at 90°C for 6 hours, is recommended to produce soy flour suitable for developing high-protein, high-fiber, gluten-free cookies.

Contribution/Originality: This study examined how soaking and drying methods affect the functional and physical properties of soy flour, particularly for developing gluten-free cookies aimed at individuals with celiac disease. The findings provide valuable insights for optimizing the process design to produce soy flour that meets the specific requirements of a gluten-free bakery.

1. INTRODUCTION

Soybean (*Glycine max* L.) has been widely used as a food for humans because of its high protein content (40%), fat content (20%), including 19–41% monounsaturated, 46–62% polyunsaturated fatty acids, and 10–15% saturated fatty acids (Szpunar-Krok & Wondołowska-Grabowska, 2022), and mineral content (5%) (Prabakaran et al., 2018). Additionally, it is a good source of dietary fiber (19.02–19.14%), consisting of both soluble fiber (pectin and gums) and insoluble fiber (cellulose, hemicellulose, and lignin) (Li et al., 2021). It also contains essential amino acids (Kudełka, Kowalska, & Popis, 2021) and is confirmed as a possible precursor of bioactive peptides (Ningrum et al., 2022). However, soybeans contain trypsin inhibitors, which affect the digestibility of soybean protein (Qin, Wang, &

Luo, 2022) as well as the impedance of growth and digestive and metabolic diseases. To ensure the accessibility of nutrients in soy beans, reduce the trypsin inhibitor activity during processing (Coscueta et al., 2017).

Soaking is a crucial step in the processing of many soy products, including soymilk, tofu, and tempeh. Soaking can soften the outer layers and affect later operations such as milling, boiling, and soy protein extraction. The efficacy of soaking depended on soaking temperature, soaking water quality, soaking pressure, particle size, soaking method, and soaking time (Guan et al., 2021). Soybeans' soaking time affected the moisture, ash, protein contents, and starch of soy flour (Agume, Njintang, & Mbofung, 2017). Not only soaking but also heating can inactivate trypsin inhibitor activity and chymotrypsin inhibitor activity (CIA), which may be linked to Kunitz and Bowman-Birk structures. Although their structures were different in the amount of disulfide bonds and reactive sites, the inhibitory mechanisms are identical (Takács et al., 2022). Thus, higher cooking temperatures for shorter periods of time had a greater effect on reducing protease-inhibiting activity than lower cooking temperatures for longer periods of time.

Soybeans are dried to reduce moisture content, improve storage stability, and prevent microbial growth. However, drying methods and conditions greatly affected the flavor characteristics of soybeans (Yang, Zhang, Li, Chen, & Liu, 2023). Grinding can transform the dried soybean into soy flour after drying. People have substituted the soy flour in gluten-free bakery products. The demand for gluten-free foods has been increasing due to an increase in the number of people suffering from celiac disease. About 1% of the world's population has suffered from celiac disease, which has become a global health issue (Leonard, Sapone, Catassi, & Fasano, 2017). However, gluten is responsible for the cohesiveness, extensibility, and elasticity of bakery dough. A lack of gluten would pose a challenge to the development of bakery products.

Processing conditions, as mentioned above, influenced the nutritional accessibility and properties of soybeans. Then, this study aimed to determine the impact of soaking and drying on the physical and functional properties of soy flour. Its application in the development of gluten-free cookies was also investigated.

2. MATERIALS AND METHODS

2.1. Materials

Soybean was purchased from Preah Vihear Province in Cambodia and kept in a refrigerator at 8–10°C until it was taken to study the effect of soaking and drying conditions on soybean.

2.2. Preparation of Soy Flour by Varying in Soaking and Drying Conditions

The experiment was carried out using a completely randomized design (CRD) to obtain 7 treatments of soaking and drying conditions (T0-T6) for soy flour preparation as follows:

- T0: Soybean without pretreatment (control)
- T1: Soybean was soaked in water at ambient temperature for 24 hours, drained, and dried at 70°C for 10 hours using a hot air oven (Model: SWOF-50, Witeg, Korea).
- T2: Soybean was soaked in water at 80°C for 2 hours using a water bath (Model: WNE14, Memmert, Germany) and dried at 70°C for 10 hours using the hot air oven.
- T3: Soybean was soaked in water at 121°C, 15 psi, for 10 minutes using an autoclave (Model: BKQ-B50II, BIOBASE, China) and dried at 70°C for 10 hours using the hot air oven.
- T4: Soybean was soaked in water at ambient temperature for 24 hours and dried at 90°C for 6 hours using the hot air oven.
- T5: Soybean was soaked in water at 80°C for 2 hours using the water bath and dried at 90°C for 6 hours using the hot air oven.
- T6: Soybean was soaked at 121°C, 15 psi, for 10 minutes using the autoclave and dried at 90°C for 6 hours using the hot air oven.

We ground the soybeans using an electric grinder after soaking and drying them. Then the ground material was sieved using a 60-mesh sieving machine (Model: Test sieve shaker, 1450, London) to obtain soy flour.

2.3. Determination of Soy Flour Quality and Property

Soy flour was taken to analyze water activity at ambient temperature using a water activity meter (Model: LabMaster-aw NGO, Novasina, Switzerland). For bulk density determination, a10 g sample of soy flour was placed in a cylinder to measure its volume, and then, the bulk density was expressed in g/cm^3. We assessed the soy flour sample using a spectrophotometer. (Ultra Scan PRO, Hunter Lab, USA) and color parameters (L*, a*, and b*) were recorded. L* represented lightness/darkness, a* represented greenness/redness, and b* represented blueness/ yellowness.

To determine water absorption capacity**,** a 1 g of soy flour sample was mixed with 10 mL of distilled water and allowed to stand for 30 minutes and then centrifuged at 3000 rpm for 30 minutes (Model: D-78532, Hettich, Germany) (Verem, Dooshima, Ojoutu, Owolabi, & Onigbajumo, 2021). The volume of the supernatant (free water) was measured to determine the water absorption capacity (WAC) using Equation 1 and expressed in mL/g.

$$WAC\ (mL/g)\ = \frac{(Volume\ of\ \text{distilled}\ water - Volume\ of\ supernatant)}{Weight\ of\ sample} \quad (1)$$

To determine water holding capacity, a 1 g of soy flour was mixed with 10 mL of distilled water and incubated at 60 ^{o}C for 30 minutes using a water bath (Model: Maxturdy-30, DAIHAN Scientific). Following incubation, the mixture underwent centrifugation at 4000 rpm for 15 minutes. After removing the supernatant, we measured the weight of the wet sample (Li, Guo, Gao, Wang, & Sun, 2020). Equation 2 estimated the water holding capacity (WHC).

$$WHC\ (g/g)\ = \frac{Weigh\ of\ wet\ sample - Initial\ weigh\ of\ sample}{Initial\ weight\ of\ sample} \quad (2)$$

For foaming capacity and foam stability determination, soy flour sample (2 g) was added to distilled water (100 mL) at 30 ± 2°C in a cylinder. The suspension was mixed and stirred at 8000 rpm for 5 minutes to form foam. The volume of foam at 30 seconds after whipping was expressed as foaming capacity (FC). After that, the foam was poured into a cylinder, and its final foam volume was recorded every 15 minutes, for 90 minutes and the foam stability (FS) was calculated using Equation 3.

$$Foam\ stability\ (\%)\ = \frac{Final\ foam\ volume\ \ x\ 100}{Initial\ foam\ volume} \quad (3)$$

For emulsifying capacity and emulsion stability determination, soy flour sample (1 g) was mixed with 25 mL of deionized water. Then corn oil (10 mL) was added. The mixture was blended in a blender for 5 minutes. After that, the mixture was brought for centrifugation at 5000 rpm for 5 minutes (Model: D-78532, Hettich, Germany). The emulsifying capacity (EC) was calculated using Equation 4.

$$Emulsifying\ capacity\ (\%) = \frac{Height\ of\ emulsion\ \ x\ 100}{Height\ of\ whole\ mixture} \quad (4)$$

After centrifugation, the sample was taken to the water bath (Model: D-78532, Hettich, Germany) at 80^{o}C for 30 minutes, cooled down, and kept at ambient temperature. The emulsion stability (ES) was calculated using Equation 5.

$$Emulsion\ stability\ (\%) = \frac{Height\ of\ emulsion\ after\ heating\ \ x\ 100}{Height\ of\ emulsion\ before\ heating} \quad (5)$$

The structural surface of soy flour was analyzed using a scanning electron microscope (SEM; FEI, FEG Quanta 450, Field Electron and Ion Co., Ltd., Hillsboro, OR, USA) with the gold coating applied using a Quorum SC7620 Sputter Coater (Quorum Technology Co., Ltd., Lewes, UK). The flour samples were coated with gold layers before determination in the microscope chamber at an acceleration voltage of 10.0 kV and a magnification of 1000.

The composition of soy flour was analyzed using the AOAC method (AOAC, 2000). Moisture content was determined by placing the sample in an oven at 105 °C for 4 hours. Ash content was measured by heating 2 g of the sample in a muffle furnace at 600 °C for 3 hours. Nitrogen content was assessed using the semi-micro Kjeldahl method, with crude protein calculated by multiplying total nitrogen by a factor of 6.25. Fat content was extracted using hexane. To find out the percentage of crude fiber, a 5 g sample was mixed with sulfuric acid and sodium hydroxide in a controlled environment. The sample was then extracted, dried, and burned.

To determine trypsin inhibitor, soy flour sample (0.5 g) was extracted by 0.01 N NaOH (25 mL) for 3 hours at ambient temperature. In a water bath the sample reactive mixture was pre heated at 37 °C in a 1 mL aliquot. Then, trypsin solution (2 mL), BAPNA solution (5 mL), and acetic acid solution (1 mL) were added and vortexed (Vanga, Wang, & Raghavan, 2020). The mixture was taken to measure the absorbance at 410 nm using a spectrophotometer (Model: UV-1900, Shimadzu). The trypsin inhibitor (TI) was calculated using Equation 6.

$$Trypsin\ inhibitor\ (mg/g) = \left(\frac{Difference\ absorbance}{0.019\ x\ Sample\ weight}\ x\ \frac{Dilution\ factor}{100\ x\ Sample\ volume}\right) \quad (6)$$

2.4. Application of the Treated Soy Flour for Cookie Making

To prepare the wheat and gluten free cookie doughs, 32 g butter and 62 g sugar were mixed, followed by 20 g glucose solution (6%w/v), 10 g water, 100 g flour (wheat flour for wheat cookies and treated soy flours for gluten-free cookies), 1 g baking powder, and 0.75 g salt. We sheeted the doughs to a uniform thickness of 25 mm and then cut them into a cylindrical shape with a diameter of 54 mm. We then baked them in an oven at 180°C for 20 minutes. We then took both wheat and gluten-free cookies to determine their quality.

For textural quality, cookies were brought for hardness measurement using the texture analyzer. The pre-test, test, and post-test speeds were set at 1.0, 1.5, and 10.0 mm/s, respectively. The distance between cookies and the probe was 5 mm (Naseer et al., 2021). Additionally, we measured the diameter and thickness of the cookies using a caliper. The spread ratio was calculated using Equation 7.

$$Spread\ ratio = \frac{Diameter\ (cm)}{Thickness\ (cm)} \quad (7)$$

Color parameters of cookies were determined using a spectrophotometer. The composition analysis of cookies, including moisture, ash, protein, fat, fiber, and carbohydrate content, was conducted using the AOAC methods (AOAC, 2000).

2.5. Statistical Analysis

All experiments were conducted in triplicate, and the data were presented as mean ± standard deviation. We used the analysis of variance (ANOVA) with IBM SPSS Statistics 28.0 software (Thaisoftup Co., Ltd., Bangkok, Thailand) to analyze the variance of data. To determine significant differences among the treatments, Duncan's New Multiple Range Test was employed at a significant level of $p \leq 0.05$. This statistical approach ensured comparison of the experimental results, allowing for interpretation of the effects of different preparation conditions on soy flour characteristics.

3. RESULTS AND DISCUSSION

3.1. Effect Of Soaking and Drying Conditions on the Physical Properties of Soy Flour

According to Table 1, the water activity of all soy flour was in a range of 0.2512–0.3595. The water activity of all soy flour was slightly lower than the reported range of 0.41–0.50 for wheat flour (Carter, Galloway, Morris, Weaver, & Carter, 2015). All treated samples had water activity less than 0.6, suggesting that we could either dry them at 70°C for 10 hours or drying at 90°C for 6 hours to develop shelf-stable flour. In this study, all soy flour contained bulk density in a range of 0.40–0.47 g/cm^3, which was consistent with the previous report at 0.45 g/ cm^3

(Sakare, Jadhav, & John, 2020). and slightly less than the report of Jadhav and Nirval (2019) (0.56–0.74 g/ cm^3). Soaking and drying caused a significant increase in bulk density ($p \leq 0.05$). However, to shorten the soaking time and drying time by increasing the soaking temperature to 121°C and drying temperature to 90°C tended to minimize the bulk density of soy flour ($p \leq 0.05$).

The lightness (L*-value) of soybean and soy flour varied between 81.06 and 89.44. In the case of soaking soybeans at ambient temperature, the increased drying temperature from 70°C to 90°C caused a decrease in L*-value from 89.44 to 83.51. This was because the increased drying temperature could enhance the Maillard browning reaction. The previous study also observed that greater discoloration occurred during the drying process of yam flour (Hsu, Chen, Weng, & Tseng, 2003). In addition, the darkened portions were likely due to water loss during the drying process (Santos et al., 2019). However, soaking soybeans at very high temperature (121°C) yielded the lowest L*-values at 81.06–81.66, presenting the darkness of soy flour regardless of drying temperature.

Considering a*-value, soaking under heating conditions (80°C for 2 h and 121°C for 10 minutes) tended to provide positive a*-value, presenting the redness of soy flour. Increasing drying temperature tended to cause a higher a*-value. Then, the maximum a*-value (5.25) of the flour was observed when the drying temperature was set at 90°C for 6 h. This could be the result of the thermal effect during soaking and drying on the acceleration of the Maillard reaction. For b*-value, all treated soy flour had b*-values ranging from 19.67 to 23.58, indicating the yellowness of soy flour. The variation of b*-values may be due to the difference in fat content of the flour samples. From the previous study Shin, Kim, and Kim (2014) the color of full-fat soy flour presented b*-value of 18.1, while the color of low-fat soy flour had b*-value of 13.2.

Table 1. Physical properties of soy flour from various soaking and drying conditions.

Parameters	No treatment (Control)	Soaking at ambient temperature	Soaking at 80°C	Soaking at 121°C	Soaking at ambient temperature	Soaking at 80°C	Soaking at 121°C
		Drying at 70°C for 10 h			Drying at 90°C for 6 h		
Water activity	0.3364±00^c	0.2512±00^e	0.3394±00^c	0.2847±00^d	0.3595±00^a	0.3583±00^a	0.3467±00^b
Bulk density (g/cm^3)	0.43±00^d	0.47±00^a	0.43±00^d	0.44±00^{bc}	0.45±00^b	0.43±00^d	0.40±00^e
L*	88.91±0.89^b	89.44±00^a	87.53±00^c	81.06±00^g	83.51±0.01^e	86.06±00^d	81.66±00^g
a*	-0.43±00^g	-0.36±00^f	0.35±00^e	3.63±0.01^b	5.25±0.01^a	0.65±0.01^d	3.38±0.01^c
b*	19.67±0.36^d	20.04±0.01^c	19.70±0.01^d	20.24±0.01^b	23.58±0.03^a	19.70±0.01^d	20±0.01^c

Note: Data are presented as means ± standard deviation from triplicate analysis. Values followed by different superscripts (a-g) in the same row are significantly different ($p \leq 0.05$).

3.2. Effect of Soaking and Drying Conditions on the Functional Properties of Soy Flour

Soaking and drying caused variations of the functional properties of soy flour, including water absorption capacity (WAC), water holding capacity (WHC), foaming capacity (FC), foam stability (FS), emulsifying capacity (EC), and emulsion stability (ES), as shown in Table 2. The WAC of the soy flour was in the range of 1.54–2.48 ml/g, which was in the same range as the WAC of the roasted soy flour (1.74–1.97ml/g) (Raigar & Mishra, 2018). In this study, soaking under heating conditions (80°C for 2 hours and 121°C for 10 minutes) increased WAC from 1.54–2.02 ml/g to 2.36–2.48 ml/g ($p \leq 0.05$). The variation in WAC was dependent on the presence of hydrophilic carbohydrates to absorb water (Kaushal, Kumar, & Sharma, 2012) and contact time with water (Sakare et al., 2020).

In the current study, reducing soaking time from 2 hours to 10 minutes by increasing soaking temperature from 80 °C to 121°C could still provide a high WAC in the range of 2.36–2.48 mL/g, compared with soaking at ambient temperature for 24 hours. The increased soaking temperature to either 80°C or 121°C possibly caused protein to denature and allowed water to react with the starch easily, resulting in the increased WAC. While soaking at the ambient temperature resulted in a low WAC of soy flour, the WAC could be improved significantly from 1.54 mL/g to 2.02 mL/g by increasing the drying temperature from 70°C to 90°C. The drying temperature's effect on WAC coincided with the previous study on the sprouted soybeans (Agrahar-Murugkar & Jha, 2010).

Table 2. Functional properties of soy flour from various soaking and drying conditions.

Parameters	No treatment (Control)	Soaking at ambient temperature	Soaking at 80°C	Soaking at 121°C	Soaking at ambient temperature	Soaking at 80°C	Soaking at 121°C
		Drying at 70°C for 10 h			Drying at 90°C for 6 h		
WAC (mL/g)	1.94±0.01[c]	1.54±0.02[d]	2.42±0.09[ab]	2.43±00[ab]	2.02±0.01[c]	2.48±0.05[a]	2.36±0.04[b]
WHC (g/g)	0.97±0.08[e]	1.03±0.05[e]	2.19±0.11[b]	2.39±0.05[a]	1.62±0.04[d]	2.02±0.09[c]	2.25±0.06[ab]
FC (%)	161.67±2.88[a]	121.67±2.88[b]	58.33±2.88[d]	60±0.00[d]	95±5.00[c]	56.67±2.88[d]	48.33±2.88[e]
FS (5 min) (%)	76.31±2.18[a]	76.72±2.89[a]	64.49±3.85[b]	65.60±1.92[b]	48.13±4.31[d]	56.96±4.11[c]	58.56±2.56[c]
FS (90 min) (%)	75.64±1.11[a]	74.74±2.90[a]	50.51±0.88[c]	60.13±2.30[b]	46.81±1.72[d]	33.93±1.05[e]	32.89±0.77[e]
EC (%)	33.53±0.64[a]	33.15±0.31[a]	30.44±1.54[b]	24.31±0.87[c]	29.67±0.28[b]	28.81±1.2[b]	18.91±0.76[d]
ES (%)	33.53±0.64[a]	33.15±0.31[a]	26.41±2.17[bc]	18.23±1.67[d]	28.58±1.22[b]	24.45±1.43[c]	13.51±0.88[e]

Note: Data are presented as means ± standard deviation from triplicate analysis. Values followed by different superscripts (a-e) in the same row are significantly different ($p \leq 0.05$).

To determine the ability of flour to retain water, WHC was measured, resulting in a range of 0.97-2.39 g/g. This coincided with the finding of Zhong et al. (2016) reporting WHC of soy flour in a range of 1.69-2.21 g/g. Similar to the WAC, the thermal effect during both soaking and drying could improve the WHC of the soy flour from 1.03-1.62 g/g to 2.02-2.39 g/g ($p \leq 0.05$).

FC of soy flour was in a range of 48.33-161.67%, while FS was in a range of 48.13-76.27% at 5 minutes and 32.89-75.64% at 90 minutes. Proteins generated an interfacial coating that kept air bubbles suspended and reduced the rate of coalescence, primarily determining the the FC and FS. The proteins and other components, such as carbohydrates, presented in flours may have influenced the foaming properties (Sreerama, Sashikala, Pratape, & Singh, 2012). In this study, raw soybean (T0) had the highest FC at 161.67%. Soaking and drying significantly reduced the FC to 48.33-121.67%. Using heat during soaking caused a significant reduction in FC due to the thermal effect on protein denaturation. Even though soaking was carried out at ambient temperature, the FC was still reduced due to the high temperature during drying. Drying at 90°C caused a lower FC than drying at 70°C, although drying time was shortened.

Similar to FC, soaking at high temperatures reduced FS at both 5 and 90 minutes. In fact, FS at 5 minutes did not differ significantly from FS at 90 minutes. Similarly, earlier researchers Mao et al. (2024) discovered that soaking led to a decline in FC and FS. The researchers also discovered that soaking led to the deterioration of FC and FS. Drying at 70°C did not cause a significant reduction in FS when soybeans were soaked at ambient temperature. However, increasing the drying temperature to 90°C reduced FS significantly, regardless of soaking conditions.

The soy flour's EC and ES were in the range of 18.91–33.53% and 13.51–33.53%, respectively. Without treatment, soybeans had an EC and ES of 33.53%, the highest among all soy flours. To ensure the EC and ES of the treated soy flour remain comparable to those of the untreated soy flour, soybeans should be soaked at ambient temperature and then dry them at 70°C for 10 h. However, this process required a long soaking time (24 hours), compared with soaking at high temperatures (10–120 minutes). The protein's emulsifying property in soy flour was dependent on its initial solubility. The better solubility would allow proteins to stay at the interface between the oil phase and the continuous phase, resulting in an improvement in emulsifying properties (Pednekar, Das, Rajalakshmi, & Sharma, 2010). An increase in soaking temperatures to both 80°C and 121°C resulted a significant reduction in EC and ES. The least EC and ES were observed when drying at 90°C and soaking at 121°C were used for the preparation of soy flour. Similarly, drying conditions affected the EC of mung bean (*Vigna radiata*) protein isolate powder, as found by Brishti et al. (2020).

According to scanning electron microscope images (Figure 1), the protein bodies of raw soybean and the treated soy flour (soaking and drying) were different. In the case of soybeans without any treatment, the protein bodies were spherical in shape and had a smooth surface, which set them apart from the material that made up cell walls. Soaking and drying the soybeans resulted in a rough and irregular surface structure of the soy flour. Protein aggregation and folding were detected. Similar to the previous study by Khotchai, Therdthai, and Soontrunnarudrungsri (2023) a higher degree of protein aggregation, clustering, and roughness of surface in soymilk powder was observed after the temperature of soymilk was increased to 67–91°C during microwave heating. In this study, the high temperature during soaking (80–121 °C) induced protein bodies to combine, become less distinct, and cluster. This made it more densely structured (Politiek et al., 2023). Consequently, the structure of the treated soy flour became less separated larger, and less soluble.

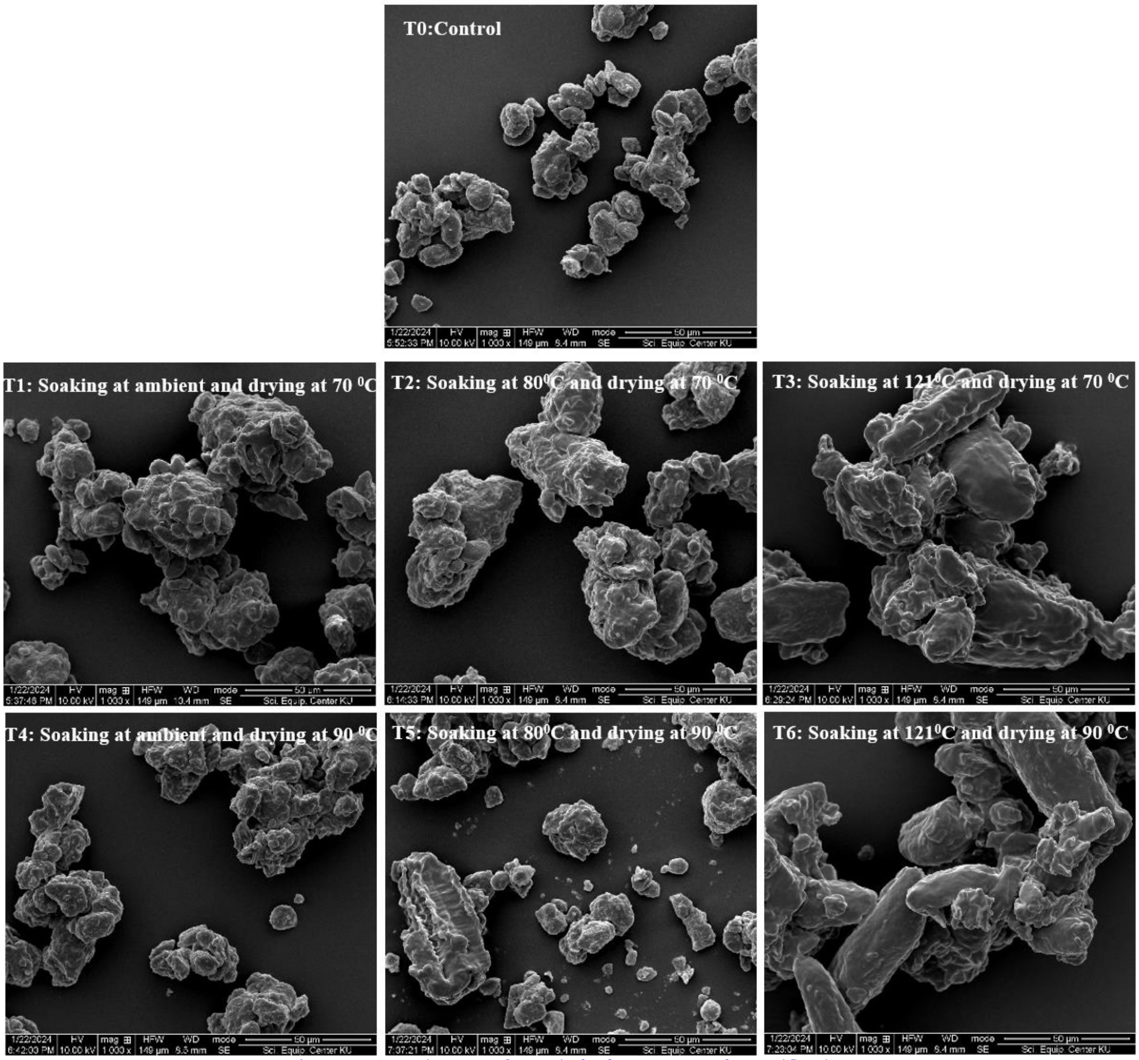

Figure 1. SEM images of protein body at 1000x of magnification.

3.3. Effect of Soaking and Drying Conditions on Composition and Trypsin Inhibitor in Soy Flour

The moisture content was 6.65%. The soaking and drying processes reduced the moisture content of the treated soy flour to a range of 2.28–3.36%. The flour should then be shelf-stable, as shown in Table 3. Ash content was generally a useful indicator of the total mineral content of food. In this study, the ash content of the soy flour was in the range of 2.48–4.88%, which was similar to the previous study by Koriyama, Teranaka, Tsuchida, and Kasai (2022) reporting the ash content of soybeans from various cultivars with different seed coat colors in the range of 4.0–4.5%. The current study discovered that soaking caused the reduction in ash content, particularly soaking at higher temperatures (80°C and 121°C). This was because higher temperatures promoted the breakdown of mineral compounds in soybeans. Then, soaking water could led to the loss of some mineral compounds, resulting in a reduction in the soy flour's mineral content and decrease in its ash content. This coincided with the previous study Agume et al. (2017) that observed a reduced ash content of 3.5% after soaking and roasting.

Table 3. Nutritional composition and trypsin inhibitor in soy flour.

Parameters	No treatment (Control)	Soaking at ambient temperature	Soaking at 80°C	Soaking at 121°C	Soaking at ambient temperature	Soaking at 80°C	Soaking at 121°C
		Drying at 70°C for 10 h			Drying at 90°C for 6 h		
Moisture (%)	6.65±0.26^{a}	3.16±0.04^{bc}	3.36±0.39^{b}	2.88±0.19^{cd}	2.96±0.06^{cd}	2.60±0.13^{de}	2.28±0.17^{e}
Ash (%)	4.88±0.09^{a}	3.78±0.24^{c}	2.90±0.02^{d}	2.48±0.28^{e}	4.26±0.02^{b}	2.91±0.02^{d}	2.66±0.03^{de}
Fat (%)	25.67±0.55^{bc}	23.18±0.85^{d}	26.24±0.88^{ab}	27.36±1.17^{a}	24.55±0.64^{cd}	25.88±0.61^{bc}	27.41±0.61^{a}
Fiber (%)	23.43±0.39^{a}	22.34±0.41^{c}	23.02±0.76^{ab}	23.15±0.56^{ab}	18.79±0.38^{d}	22.81±0.48^{ab}	22.24±0.28^{c}
Protein (%)	45.60±1.47^{a}	44.17±0.75^{b}	43.46±0.24^{b}	44.08±0.60^{b}	42.94±0.37^{c}	43.42±0.82^{b}	41.93±0.41^{c}
TI (mg/g)	50.21±0.71^{a}	42.94±0.94^{b}	39.88±0.94^{c}	38.38±0.85^{c}	48.69±2.83^{a}	33.01±1.28^{d}	33.52±1.72^{d}

Note: Data are presented as means ± standard deviation from triplicate analysis. Values followed by different superscripts (a-e) in the same row are significantly different ($p \leq 0.05$).

The fat content of all soy flour was in the range of 23.18–27.41%, which was similar to the previous report at 25.7% (Agume et al., 2017). An increase in soaking temperature from ambient to 80°C and 120°C tended to increase the fat content in the soy flour. In contrast, raising the drying temperature from 70°C to 90°C did not have a significant effect on the fat content. Similarly, the previous study by Correia, Leitão, and Beirão-da-Costa (2009) found no significant effect of drying temperature on the total fat content in chestnut flour. However, the increased drying temperature could reduce the fiber content of soy flour from 22.34–23.15% to 18.79–22.81%. Specifically, we observed the lowest fiber content when a long soaking time (24 hours) at ambient temperature before drying at 90°C.

Soybeans primarily contained 45.6% protein. The soaking and drying process significantly reduced the protein content in flour to 41.93–44.17%. the soaking water could lose the water-soluble proteins during soaking (Agrahar-Murugkar & Jha, 2010). The additional loss of protein was observed when a higher temperature (90°C) was used during drying.

Considering the bio-accessibility of proteins, the soybean contained a significantly higher trypsin inhibitor (50.21 mg/g) than the soaked and dried soy flour (33.01–48.69 mg/g). In the previous studies, the trypsin inhibitor of soybean was in the range of 18.6 to 74.8 mg/g (Kumar, Rani, Mittal, & Shuaib, 2019), while the trypsin inhibitor of soy flour was in the range of 50 to 75 mg/g (Kumar, Rani, Shuaib, & Mittal, 2018). In this study, soaking soybeans at ambient temperature did not reduce trypsin inhibitors as much as soaking at high temperatures (80°C and 121°C). Similarly, Egounlety and Aworh (2003) founded that soaking soybeans at ambient temperature did not significantly affect the trypsin inhibitor content, even when they extended the soaking time to 12-14 hours. The previous study also reported that the heating under high pressure in the autoclave was the most effective in reducing trypsin inhibitor activity (Agrawal, Panigrahi, & Eri, 2024). The current study found that soaking at either 80°C or 121°C, followed by a high drying temperature of 90°C for 6 hours, yielded the lowest trypsin inhibitor. This was because of the combined thermal effect of both soaking and drying on protein structure. Temperatures in the range of 80–110 °C could significantly reduce trypsin inhibitor activity (Vagadia, Vanga, Singh, Gariepy, & Raghavan, 2018).

3.4. Quality of Gluten-Free Cookies Made from Treated Soy Flour

All gluten-free dough from the treated soy flour and wheat dough was baked to develop cookies. Table 4 presents the nutritional composition of the cookies. The nutritional composition of wheat cookies included 1.55% moisture, 6.19% protein, 13.65% fat, 19.24% fiber, 0.88% ash, and 77.71% carbohydrate. This was similar to other wheat cookies containing 15.2% protein and 20.2% fat (Olagunju & Ifesan, 2013). The addition of treated soy flour to the cookie formula increased the nutritional contents such as protein, fat, fiber, and ash to ranges of 23.18–25.92%, 24.1–29.31%, 20.05–22.29%, and 1.96–2.74%, respectively, while decreasing the carbohydrate content to 41.98–45.71%. This was because soy flour contained more protein, fat, fiber, and ash than wheat flour. Similar to the report from the previous study by Ghoshal and Kaushik (2020), the protein content in cookies was increased from 14.3 to 16.7% when soy flour was used to replace wheat flour by 15–25%. Compared with other gluten-free cookies, such as okara cookies, which contain 11.6% protein, soy cookies contain much higher protein (Park, Choi, & Kim, 2015). Therefore, the gluten-free cookies made from the treated soy flour in this study could be a beneficial source of protein. In addition, the carbohydrate content was lower, while the fiber content of the gluten-free cookies was higher than that of the wheat cookie. Using treated soy flour, low-carb and high-fiber cookies can be made.

Table 4. Nutritional composition of cookies made from wheat flour and soy flour.

Parameters	Wheat flour	Soaking at ambient temperature	Soaking at 80°C	Soaking at 121°C	Soaking at ambient temperature	Soaking at 80°C	Soaking at 121°C
		Drying at 70°C for 10 h			Drying at 90°C for 6 h		
Moisture (%)	1.55±0.02c	4.37±0.14a	1.58±0.14c	0.55±0.08e	3.06±0.02b	2.98±00b	0.88±0.05d
Ash (%)	0.88±0.12f	2.60±0.05b	2.16±0.02d	2.19±0.09d	2.74±0.02a	1.96±0.02e	2.37±0.01c
Fat (%)	13.65±0.16e	24.14±0.15d	27.71±0.16bc	29.32±0.32a	27.96±0.74b	27.15±0.55c	27.46±0.16bc
Fiber (%)	19.24±0.73b	22.05±1.38a	20.99±0.74ab	20.05±1.31ab	20.30±1.26ab	22.29±1.11a	21.13±1.44ab
Protein (%)	6.19±0.11c	23.18±0.24b	25.07±0.42a	25.52±0.08a	23.78±0.63b	25.92±0.44a	25.50±0.88a
Carbohydrate (%)	77.72±0.38a	45.71±0.44b	43.48±0.54c	42.42±0.43d	42.46±0.13d	41.98±0.21d	43.80±0.97c

Note: Data are presented as means ± standard deviation from triplicate analysis. Values followed by different superscripts (a-f) in the same raw are significantly different ($p \leq 0.05$).

The wheat dough expanded with a spread ratio of 5.68 during baking, taking into account its physical quality. To obtain the non-significant difference in the spread ratio (5.52–5.64) compared to the wheat cookies, the gluten-free cookies could be prepared from the soy flour treated by soaking at ambient temperature or at 80°C and drying at 70–90°C (Table 5). Too high soaking temperature of 121°C affected the protein structure in flour, reduced the dough consistency, and then increased the spread ratio of cookies. The spread ratio was an important quality factor that directly affected the size, shape, and texture of cookies (Hamdani, Wani, & Bhat, 2020). For texture, the wheat cookies had a hardness of 56.68 N. The use of soy flour treated by soaking at ambient temperature and drying at 70°C for 10 h could yield the cookies with a non-significantly different hardness (53.30 N) compared with the wheat cookies. Increasing the soaking and drying temperatures caused the increased water absorption capacity in soy flour and the increased spread ratio, yielding a wider and thinner shape in cookies. The reduction in hardness to 23.87–42.51 N led to crispy texture in the cookies, a desirable quality.

Table 5. Quality of gluten-free cookies from the treated soy flour.

Parameters	Wheat flour	Soaking at ambient temperature	Soaking at 80°C	Soaking at 121°C	Soaking at ambient temperature	Soaking at 80°C	Soaking at 121°C
		Drying at 70°C for 10 h			Drying at 90°C for 6 h		
Spread ratio	5.68±0.04bc	5.64±0.14bc	5.60±0.37bc	6.57±0.22a	5.52±0.12c	5.52±0.04c	5.87±0.03b
Hardness (N)	56.68±3.62a	53.30±2.72a	34.33±4.28c	23.87±1d	33.36±1.81c	42.51±1.05b	27.80±0.62d
L*	77.78±0.32a	68.53±0.41d	71.86±0.77b	69.54±0.58c	65.58±0.96e	71.34±0.63b	71.21±0.77b
a*	3.38±0.37e	10.65±0.35b	7.23±0.83d	8.43±0.32c	12.52±0.71a	7.61±0.42d	7.13±0.51d
b*	28.81±0.60f	39.12±0.38a	32.69±0.70c	31.42±0.40d	34.98±0.65b	34.83±0.56b	30.24±0.62e

Note: Data presented as means ± standard deviation from triplicate analysis. Values followed by different superscripts (a-f) in the same raw are significantly different ($p \leq 0.05$).

In terms of color of cookies (Figure 2), the wheat biscuits had lightness (L*) of 77.78, redness (a*) of 3.38, and yellowness (b*) of 28.81. Cookies became darker by replacing wheat flour with soy flour, with a significant decrease in lightness (65.58–71.86) and an increase in redness (7.13–12.52) and yellowness (30.24–39.12). This was because the treated soy flour contained a higher protein content than wheat flour, especially lysine, which likely caused the darker color (Pokharel et al., 2023). In addition, the higher protein content also enhanced the Maillard reaction between amino acids and reducing sugar during the baking process (Sulieman et al., 2019). The previous study Park, Kim, Na, Oh, and Cho (2021) found that a 25-100% substitution of soy protein isolate in cokkies resulted in lower lightness and higher saturation.

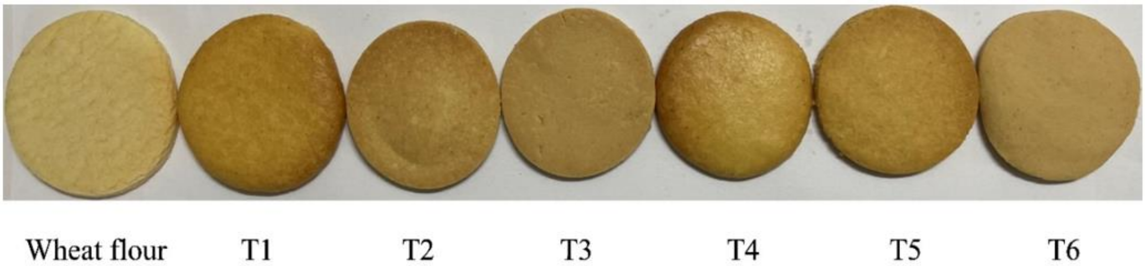

Figure 2. Gluten-free cookies made from wheat flour and treated soy flour (T1–T6).

4. CONCLUSION

All soaking and drying conditions caused a change in protein bodies and could reduce the trypsin inhibitor from 50.21 mg/g to 33.01–48.69 mg/g. This could improve the availability of nutrients in soy products. By soaking at high temperatures, either 80°C for 2 hours or 121°C for 10 minutes, WAC and WHC were improved, but FC, FS, EC, and ES were decreased. In addition, soaking at 121°C caused the soy flour to be darker with the decreased lightness and increased redness. Similarly, the increased drying temperature from 70 to 90°C caused the darker flour color. The gluten-free cookies made from treated soy flour were not exactly the same as the wheat cookies because of a lack of gluten. To obtain the non-significant difference in the spread ratio, similar color, and lower hardness than the wheat cookies and the minimized trypsin inhibitor, soybeans should be soaked at 80°C for 2 hours prior to drying at 90°C for 6 hours to prepare soy flour for the gluten-free cookies. Due to the better nutritional values of the soy flour, the gluten-free cookies contained significantly more protein and fiber than the wheat cookies. Therefore, with proper soaking and drying operations, the soy flour could be an alternative material for the development of gluten-free, high-protein, low-carb, and high-fiber products.

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