DEVELOPMENT OF SPROUTED SOYBEAN POWDER COMBINED WITH BROWN RICE POWDER AND BLACK SESAME POWDER

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Abstract

The study presents a process for producing a powder from sprouted soybean seeds combined with brown rice and black sesame, with the aim of creating a product of high value and potential applications in the field of functional foods. In this research, sprouted soybean seeds were selected as the primary raw material. The sprouting process facilitates the conversion of oligosaccharides into simple sugars and enhances the content of vitamins and bioactive compounds. The investigation focused on examining factors such as the soaking duration of soybean seeds prior to sprouting, incubation time, as well as drying duration and temperature, in addition to the mixing ratio. Enzyme activity (α-amylase), reducing sugar content, polyphenol content, moisture, and viscosity of the final product were measured. The results indicated that a soaking time of 9 hours, incubation time for 24 hours, drying for 11 hours at 50°C, and a mixing ratio of 70:15:15 for sprouted soybean powder, brown rice powder, and black sesame powder, respectively, produced the most favorable outcome.

Keywords: sprouted soybean seeds, powder, polyphenol, α-amylase, reducing sugar

1. INTRODUCTION

Soybean (scientific name: *Glycine max*), belonging to the Fabaceae family, is widely cultivated for its seeds. Soybean seeds are rich in nutrients, including protein (35-40% dry weight), lipids (18-22% dry weight), numerous essential amino acids, free metals such as K, Fe, Ca, Mg, P, and vitamins B1, B2, B5, B6, E, C, A among others (Jiang et al., 2013). Soybeans are extensively used and are considered a desirable health-promoting ingredient due to their potential in preventing cancer, cardiovascular diseases, osteoporosis, and menopausal symptoms (Adlercreutz and Mazur, 1997, Omoni and Aluko, 2005, Bolanho and Beléia, 2011). However, soybeans contain oligosaccharides that can inhibit trypsin, a crucial digestive enzyme in humans and animals, and also have a high phytate content. Phytate is a digestive inhibitor that forms insoluble complexes with protein and essential metals present in the seeds (Thúy et al., 2018).

Sprouting reduces phytate content, and oligosaccharides that inhibit trypsin are converted into simple sugars. The content of vitamins, carotenoids, folic acid, GABA (gamma-aminobutyric acid), and easily absorbable minerals significantly increases. Sugars and amino acids exist in simple, easily absorbable forms (Zieliński, 2003). Sprouted soybeans contain important compounds in high concentrations, such as polyphenol compounds with antioxidant properties – capable of scavenging free radicals, anti-diabetic, anti-hypertensive, immunomodulatory, anti-cancer, and anti-inflammatory effects (C. Recio et al., 2012, McCue et al., 2005). Specifically, polyphenols prevent cardiovascular diseases by inhibiting the production of Endothelin (a protein that constricts blood vessels, reducing oxygen supply to the heart and causing coronary artery disease) (Reiter et al., 2010). Isoflavones (phytoestrogens) are compounds with antioxidant effects, preventing skin aging, and notably, supplementing hormones in women (Vincent and Fitzpatrick, 2000). Furthermore, sprouted soybeans contribute to anti-aging, improved memory, tissue regeneration, reduced osteoporosis, and enhanced body immunity (Liu, 2004).

Sesame (*Sesamum indicum* L.), a member of the Pedaliaceae family, is a herbaceous plant known for its rich nutritional content and distinctive flavor. It has long been utilized in traditional medicine due to its beneficial health properties (Ma et al., 2022). Sesame seeds are rich in unsaturated fatty acids, primarily linoleic acid, oleic acid, palmitic acid, stearic acid, and small amounts of linolenic acid, along with natural antioxidants and tocopherol analogs (Bedigian and Harlan, 1986, Pathak et al., 2014). Sesame seeds are rich in protein, vitamin

B1, and dietary fiber, and they also provide essential minerals such as phosphorus, iron, magnesium, calcium, manganese, copper, and zinc (Pathak et al., 2014). Additionally, sesame seeds contain sesamin and sesamolin, two beneficial lignans that reduce cholesterol in humans, prevent hypertension, and enhance liver detoxification (Cheng et al., 2006, Matsumura et al., 1995). Sesame seeds are a rich source of bioactive compounds including phenolics, phytosterols, and phytates (Pathak et al., 2014). Black sesame has been shown to inhibit myocardial regeneration and protect cardiovascular function (Ha et al., 2017), prevent the onset and progression of atherosclerosis (Narasimhulu et al., 2015), and reduce total cholesterol, low-density lipoprotein, and blood lipids (Namiki, 2007). Moreover, it provides significant protection against chronic liver damage (Kumar et al., 2013), possesses antioxidant, anti-inflammatory (Vittori Gouveia et al., 2016), anti-tumor, anti-cancer (Majdalawieh et al., 2017), and anti-aging properties (Kocher et al., 2015), and has neuroprotective effects (Park et al., 2010). Studies also suggest that black sesame significantly reduces oxidative stress, exhibiting protective effects on the kidneys (Liu et al., 2015a) and preventing osteoporosis (Wanachewin et al., 2012, Srisuthtayanont et al., 2017).

Brown rice retains the germ and bran layers, making it rich in nutrients including vitamins, fiber, and minerals (Zahra and Jabeen, 2020). Brown rice offers numerous health benefits due to its high fiber content, which provides protection against colorectal cancer (Aune et al., 2011) and breast cancer (Goufo and Trindade, 2014). Its abundant B vitamins help prevent beriberi. Beyond B vitamins, brown rice contains a diverse range of nutrients such as dietary fiber, beneficial lipids, essential amino acids, phytosterols, phenolic compounds, flavonoids, anthocyanins, proanthocyanidins, various forms of vitamin E (tocopherols and tocotrienols), minerals, gamma-aminobutyric acid (GABA), and γ-oryzanol (Cho and Lim, 2016, Okarter and Liu, 2010). Simultaneously, it contains a high content of phytic acid (Liu et al., 2015b), which helps lower the glycemic index and prevent type 2 diabetes (Thompson, 2006). A common compound found in brown rice is phenolic acid (Gong et al., 2017), which offers various health benefits including anti-cancer, anti-inflammatory, anti-allergic, and anti-atherosclerotic properties (Tan and Norhaizan, 2017). Brown rice is also a rich source of Vitamin E (Okarter and Liu, 2010), which has antioxidant, immune-supportive, and metabolic functions (Liu, 2007).

The market offers a wide variety of nutritional powders produced from different raw materials such as cereals, barley, green beans, brown rice, lotus seeds, etc., often at relatively high prices, which can deter consumers, especially those with low incomes, from choosing and purchasing these products. Therefore, the research on "Developing a nutritional powder production process from sprouted soybeans combined with brown rice and black sesame" is essential to diversify the nutritional powder products available in the market, creating new products with high nutritional and biological value as a basis for developing functional foods that improve and enhance community health. In terms of nutritional value, all the ingredients positively impact the health of individuals seeking to reduce the risk of cardiovascular disease, prevent aging, manage overweight, and lower cholesterol.

2. MATERIALS AND METHODS

2.1. Raw materials

Soybean seeds were purchased from Hoang Phuong Nutritional Grains store, Tan Binh District, Ho Chi Minh City, Vietnam. Black sesame powder was purchased from Thao Moc Xanh store, Go Vap District, Ho Chi Minh City, Vietnam. Brown rice powder was purchased from Brown Rice store, Tan Binh District, Ho Chi Minh City, Vietnam.

2.2. Chemicals

Chemicals used in the study include: dinitrosalicylic acid (DNS) (98% China), Sodium hydroxide (NaOH) (96% China), Sodium potassium tartrate ($C_4H_4O_6KNa.4H_2O$) (99% China), D-glucose ($C_6H_{12}O_6$) (China), Gallic acid (99% Canada), Folin-Ciocalteu reagent (China), Sodium carbonate (Na_2CO_3) (99.8% China), Ethanol (96% Vietnam), Disodium hydrogen phosphate dodecahydrate (Na_2HPO_4) (99% China), Sodium Dihydrogen Phosphate (NaH_2PO_4) (99% China), Starch (China), Hydrochloric acid (HCl) (36% China), Sodium chloride (NaCl) (99.5% China), Potassium Iodide (KI) (99% China), Iodine (I_2) (China).

2.3. Experimental design

2.3.1. Experiment 1: The effect of soaking time on α-amylase enzyme activity and moisture content of seeds

Soybean seeds were sorted to remove damaged and shriveled seeds. They were then washed and soaked in distilled water at a ratio of 1:5 for different durations (7 hours, 8 hours, 9 hours). The α-amylase enzyme activity and moisture content of the seeds were evaluated to select the appropriate soaking time.

2.3.2. Experiment 2: The effect of incubation time on reducing sugar content

Seeds, after being soaked for the optimal duration selected from Experiment 1, were incubated at room temperature for different durations (1 day, 2 days, 3 days). The reducing sugar content was evaluated to select the appropriate incubation time.

2.3.3. Experiment 3: The effect of drying time on polyphenol content and moisture content of seeds

Seeds, after being soaked and incubated for the optimal durations selected from Experiment 1 and Experiment 2, were dried at 50°C for different durations (10 hours, 11 hours, 12 hours). The polyphenol content and moisture content were evaluated to select the appropriate drying time.

2.3.4. Experiment 4: The effect of drying temperature on polyphenol content and moisture content of seeds

Seeds, after being soaked and incubated for the optimal durations selected from Experiment 1 and Experiment 2, were dried for the optimal duration selected from Experiment 3 at different temperatures (40°C, 50°C, 60°C). The polyphenol content and moisture content were evaluated to select the appropriate drying temperature.

2.3.5. Experiment 5: The effect of mixing ratio on product viscosity

Seeds, after being soaked, incubated, and dried with the optimal parameters selected from Experiments 1, 2, 3, and 4, were finely ground through a sieve (0.15mm pore size). Sprouted soybean powder, brown rice powder, and black sesame powder were then mixed in the ratios of (50:25:25, 60:20:20, and 70:15:15) respectively. The viscosity was evaluated against a control sample to select the appropriate mixing ratio.

2.4. Analytical methods

2.4.1. Determination of moisture content

Moisture content was determined by drying to constant weight using a Sartorius MA 150 infrared moisture analyzer (Germany).

2.4.2. Determination of α-amylase activity

1 ml sample solution was added to 1 ml Sorensen buffer solution, 1 ml starch solution (1%), and 0.5 ml NaCl (3%). The mixture was then incubated at 50°C for 30 minutes. After incubation, 1 ml HCl (1N), 5.5 ml distilled water, and 0.05 ml Lugol's reagent were added.

The absorbance was determined at a wavelength of 620 nm using a UV-Vis spectrophotometer (Nguyễn, 2001).

2.4.3. Determination of reducing sugar content

A volume of 0.5 ml of the sample solution was mixed with 0.5 ml of DNS reagent. The resulting mixture was heated in a boiling water bath at 100°C for 5 minutes, then allowed to cool to room temperature. Subsequently, 4 ml of distilled water was added. The absorbance of the final solution was measured at 540 nm using a UV-Vis spectrophotometer. D-glucose served as the standard, and the concentration of reducing sugars in the extracts was reported as D-glucose equivalents (mg/l) (Miller, 1959).

2.4.4. Determination of polyphenol content

1 ml sample solution was added to 5 ml Folin-Ciocalteu reagent (10%) and mixed thoroughly. After 5 minutes, 4 ml Na_2CO_3 (7.5%) was added. After 1 hour, absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm. Gallic acid was used as the standard, and the total phenolic content of the extracts was expressed as gallic acid equivalents (mgGAE/g) (Mishra et al., 2014).

2.4.5. Viscosity

Viscosity was measured using an automatic Brookfield viscometer (USA).

2.5. Data processing method

All experiments were conducted in triplicate, and the results are presented as the mean ± Standard Deviation of three independent replicates. Statistical differences among treatments were evaluated using analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test. Data processing and analysis were performed using Microsoft Excel and R version 4.3.3. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Effect of soaking time on α-amylase activity and moisture content of seeds

Figure 1 illustrates the change in α-amylase enzyme activity with soaking time. At soaking times of 7 hours, 8 hours, and 9 hours, the α-amylase enzyme activities were 0.482 (UI/g), 0.566 (UI/g), and 0.71 (UI/g), respectively. This indicates that increasing the soaking time leads to stronger α-amylase enzyme activity.

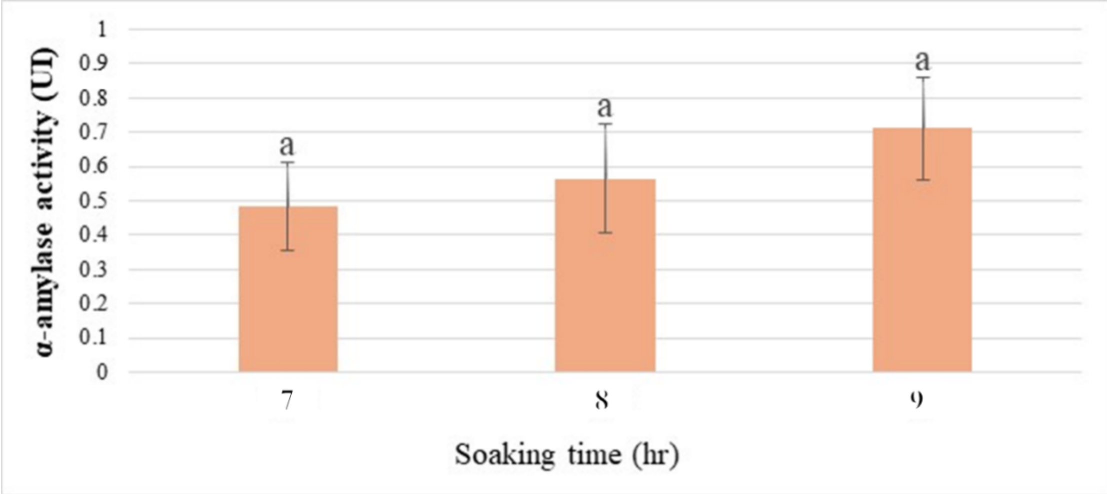

Figure 1. The effect of soaking time on α-amylase activity (Different lowercase letters indicate a statistically significant difference ($p<0.05$))

This result is consistent with the study by Jamil et al., where soaking black gram for 12 hours and 18 hours increased α-amylase enzyme activity (Jamil et al., 2000). According to Lu et al., quinoa seeds soaked for 4 and 6 hours showed higher enzyme activity than those soaked for 2 hours (Lu et al., 2024). In 2007, Hotz and Gibson reported that α-amylase activity increased during cereal grain soaking. Although increasing soaking time led to an increase in α-amylase enzyme activity, there was no significant difference ($p > 0.05$) between the soaking times. The increase in α-amylase activity during soaking is attributed to respiratory activity and metabolic exchange under the influence of oxygen and water absorbed by the seeds. Water facilitates the mobilization and activation of water-soluble phytohormones like gibberellins, which are naturally present in seeds. It also enables the diffusion of enzymes from the aleurone layer into the endosperm. These phytohormones, in turn, stimulate the production of hydrolytic enzymes such as amylases, nucleases, and proteases that are essential for degrading stored nutrients, promoting cell division, and supporting cell elongation. Consequently, extending the soaking duration enhances water uptake by the seeds, which leads to an increase in α-amylase activity (Klang et al., 2021). Among the three soaking durations investigated, the results showed that α-amylase enzyme activity reached its highest when soaked for 9 hours. Hence, we selected a soaking time of 9 hours for subsequent investigations.

3.2. Effect of incubation time on reducing sugar content

As shown in Figure 2, incubation time had a significant impact on the reducing sugar content of soybeans, with a notable difference ($p < 0.05$) observed between incubated and nonincubated samples. The reducing sugar content increased to 16.125%, 15.57%, and 13.764% after 24, 48, and 72 hours of incubation, respectively, in contrast to 6.542% in the untreated control.

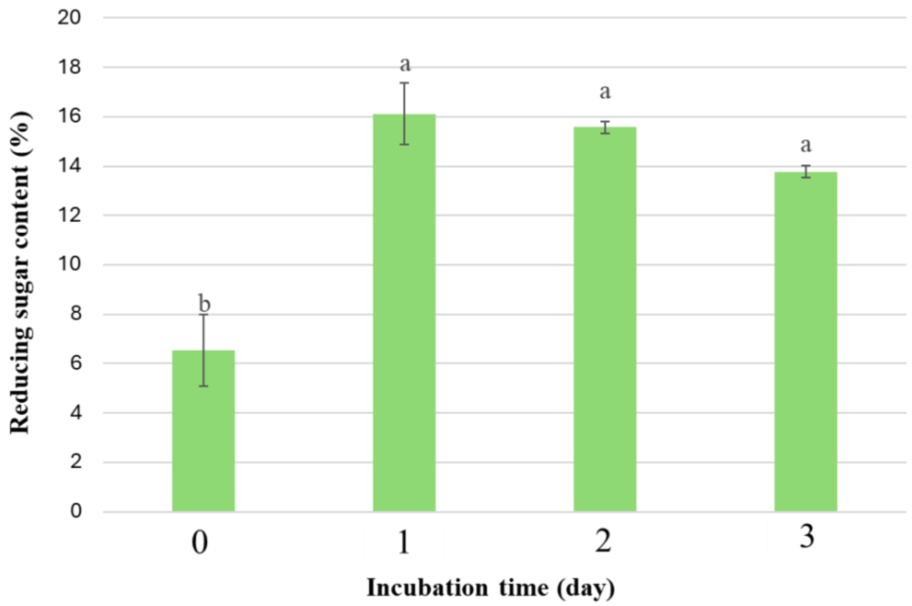

Figure 2. The effect of incubation time on reducing sugar content (Different lowercase letters indicate a statistically significant difference ($p<0.05$))

According to Ohtsubo et al. (2005), the sprouting process significantly increases the reducing sugar content due to starch hydrolysis by α-amylase, leading to several times the initial sugar content (Ohtsubo et al., 2005, El-Safy et al., 2013). However, increasing the incubation time showed a decreasing trend in reducing sugar content. This might be because, in the early stages of sprouting, seeds utilize simple and easily absorbable sugars for development, which aligns with the study by Shi et al., where sugar content decreased from 19.9% to 14% during 7 days of sprouting (Shi et al., 2010). The decrease in reducing sugar content with increased incubation time was not statistically significant ($p > 0.05$). After sprouting, the reducing sugar in the seeds tends to decrease due to the respiratory activity of the seeds and consumption for seedling nourishment, thus reducing the reducing sugar content (Thúy et al., 2018, Quyên et al., 2015). The results show that the reducing sugar content of sprouted soybean seeds reached its highest value when incubated for 1 day. Therefore, we chose an incubation time of 1 day for subsequent experiments.

3.3. Effect of drying time on polyphenol content and moisture content

Figure 3 indicates a directly proportional relationship between polyphenol content and drying time. Polyphenol content decreased to 2.175 (mgGAE/g), 2.015 (mgGAE/g), and 1.547 (mgGAE/g) as drying time increased from 10 to 12 hours, respectively.

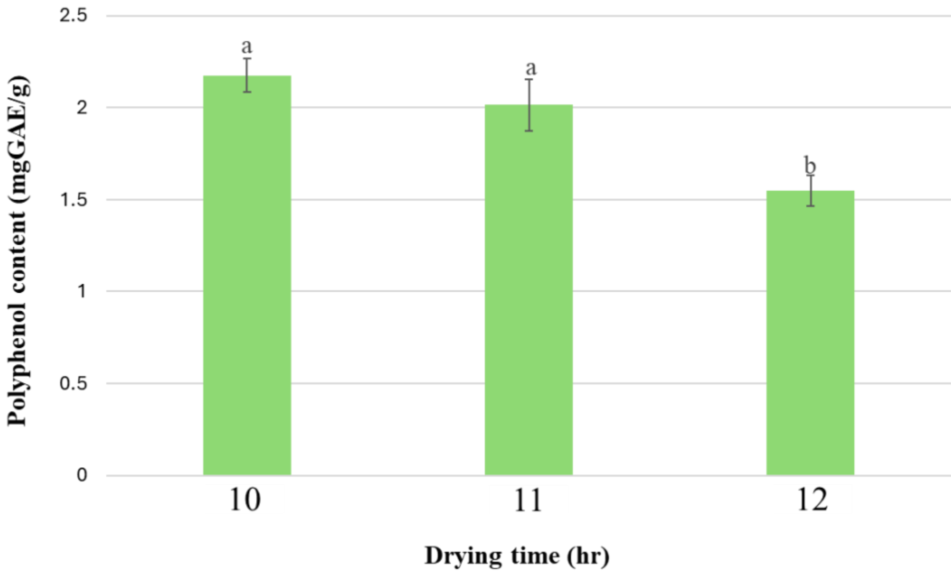

Figure 3. The effect of drying time on polyphenol content (Different lowercase letters indicate a statistically significant difference (p<0.05))

This result is consistent with the research by Kyi et al. (2005) on the degradation and reduction of polyphenol content over time during the drying process (Kyi et al., 2005). The survey results show a decreasing trend in polyphenol content with increasing drying time because prolonged drying time leads to faster oxidation of polyphenol compounds by air, thus increasing polyphenol loss. Increasing drying time also means increasing the heat exposure time on polyphenols, leading to a decrease in polyphenol content (Dwiyanti et al., 2018). Figure 3 shows that the polyphenol content of sprouted soybeans dried for 10 hours was better than the other two durations. However, drying at 10 hours resulted in a moisture content of 5.29%, which exceeds the permissible standard (<5%) for powdered products (according to QCVN 5-2:2010/BYT). When dried for 11 hours, the moisture content was 4.77%, which is within the permissible range, and the polyphenol content was also relatively high. Finally, while drying for 12 hours achieved the lowest moisture content at 4.47%, the polyphenol content significantly decreased compared to 10 hours and 11 hours. Therefore, we chose a drying time of 11 hours for subsequent experiments.

3.4. Effect of drying temperature on polyphenol content and moisture content

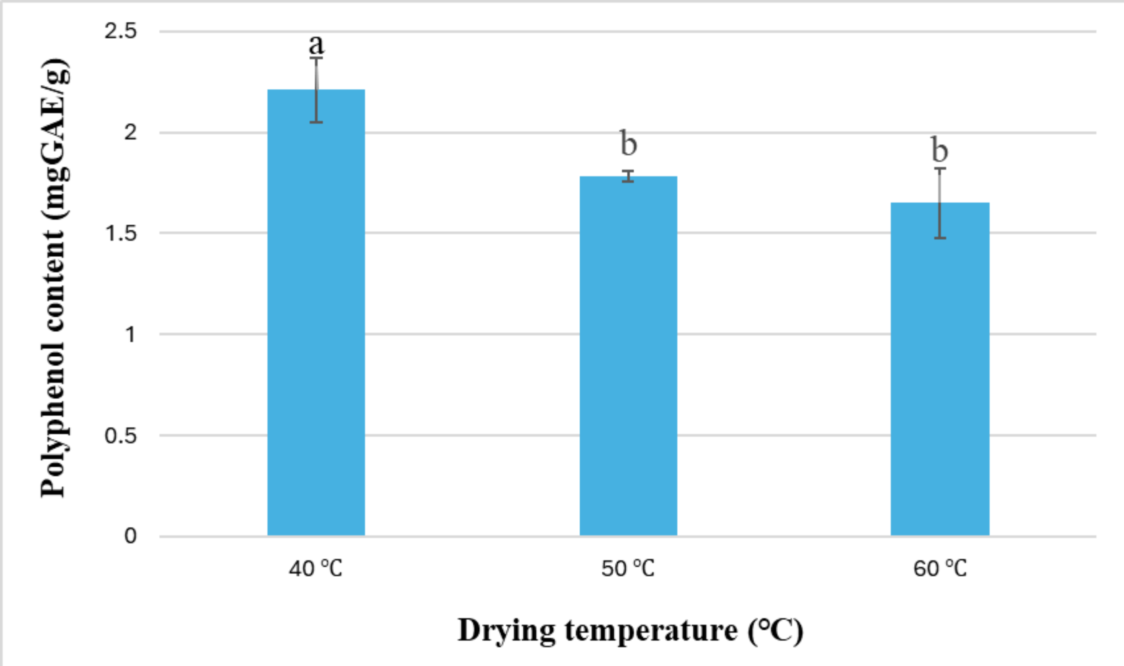

Figure 4. The effect of drying temperature on polyphenol content (Different lowercase letters indicate a statistically significant difference ($p<0.05$))

Figure 4 shows that when dried at 40°C, the polyphenol content was the highest at 2.211 (mgGAE/g), at 50°C it was 1.786 (mgGAE/g), and at 60°C, the polyphenol concentration was the lowest at 1.65 (mgGAE/g). According to Kyi et al. (2005), this is due to enzymatic oxidation of polyphenols (Kyi et al., 2005). Another study by Cheng et al. (2013) reported that drying at low temperatures does not destroy polyphenol oxidase enzymes, while high-temperature drying destroys these enzymes (Cheng et al., 2013). The survey results indicate a decreasing trend in polyphenol content with increasing drying temperature, as high temperatures during drying lead to the degradation of heat-sensitive phenolic compounds (Izli et al., 2018). According to Alean et al. (2016), if the temperature increases, the polyphenol content will decrease (Alean et al., 2016). Many authors have researched the effect of drying temperature on polyphenol content. However, all studies concluded that polyphenol content decreases with increasing drying temperature (Kyi et al., 2005, Stephenus et al., 2023, López et al., 2010). Figure 4 shows that the polyphenol content of sprouted soybeans dried at 40°C was better than the other two temperatures. However, drying at 40°C resulted in a moisture content of 8.94%, which exceeds the permissible standard (<5%) for powdered products (according to QCVN 5-2:2010/BYT). Subsequently, drying at 50°C yielded a moisture content of 4.47%, which is within the permissible range, and the polyphenol content was also relatively high. Finally, while drying at 60°C achieved the lowest moisture content at 3.79%, the polyphenol content significantly decreased compared to 40°C and 50°C. Therefore, we chose a drying temperature of 50°C for subsequent experiments.

3.5. Effect of mixing ratio on viscosity

In this study, we formulated various powders (sprouted soybean, brown rice, and black sesame) to develop a nutritional powder product with high sensory value, consumer preference, and a viscosity similar to products currently available in the market.

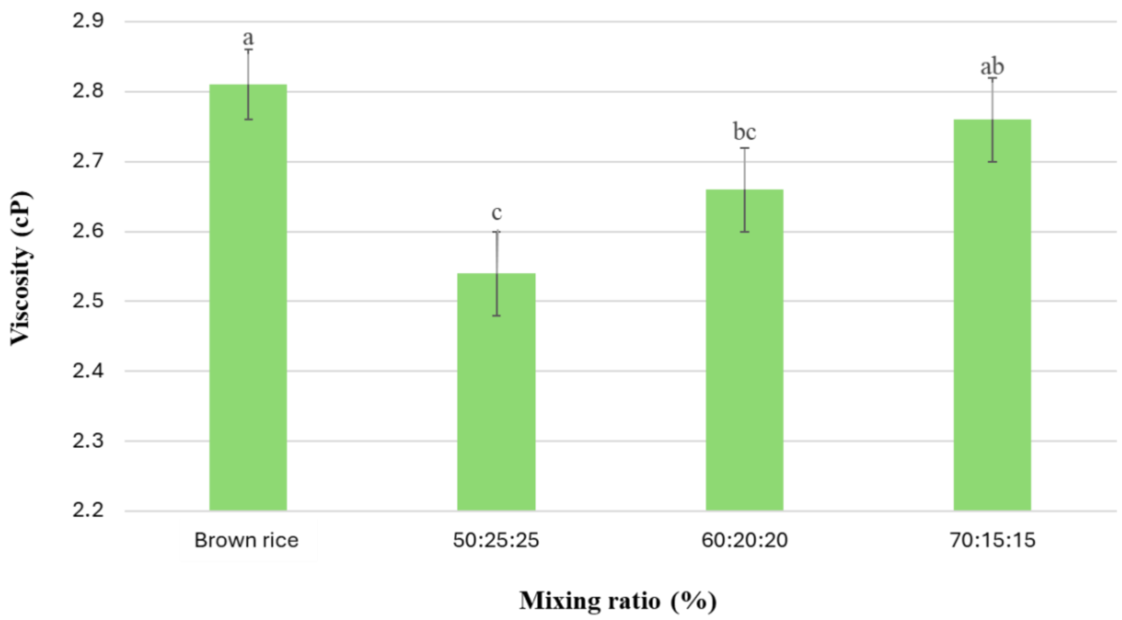

Figure 5. The effect of mixing ratio on viscosity (Different lowercase letters indicate a statistically significant difference ($p<0.05$))

The results in Figure 5 show that when sprouted soybean powder, brown rice powder, and black sesame powder were mixed in the ratios of 50:25:25, the viscosity was 2.54 cP; for the 60:20:20 ratio, the viscosity was 2.66 cP; and finally, for the 70:15:15 ratio, the viscosity was 2.76 cP. It can be observed that increasing the proportion of sprouted soybean powder while reducing brown rice and black sesame powders increased product viscosity. This increase in viscosity is attributed to the high protein content in sprouted soybean powder, which has strong water-binding and gel-forming capabilities, contributing to increased viscosity when dissolved. Figure 5 demonstrates that the product achieved a viscosity comparable to market products when using the 70:15:15 mixing ratio. Therefore, we selected the mixing ratio of 70:15:15 for sprouted soybean powder, brown rice powder, and black sesame powder.

4. CONCLUSION

In this study, soybean seeds were soaked for 9 hours, then incubated for 1 day at room temperature to promote germination. Subsequently, the sprouted soybean seeds were dried for 11 hours at 50°C. Finally, the sprouted soybean powder was mixed with brown rice powder and black sesame powder at a ratio of 70:15:15. The product developed in this research holds significant potential for practical application in creating high-quality products that meet consumer demands.

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