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Original article

Effects of soybean residue addition on yogurt quality: Physicochemical, functional, and sensory properties

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Abstract. Okara contained bioactive substances, including isoflavones and other natural polyphenols with strong antioxidant activity. We intended to produce better quality yogurt by adding okara; thus, our types of yogurt were prepared: yogurt without additives (control; C), with soy powder (SY), with okara (OY), and with roasted okara (ROY). The yogurt was analyzed for physicochemical (total *Lactobacillus*, titratable acidity, whey separation, and viscosity), functional (isoflavone, total phenols, and antioxidant capacity), and sensory properties. The addition of okara increased the total *Lactobacillu*s count. The OY sample (35.80×10^{12} CFU/g) had a total *Lactobacillus* count that was 18 times higher than that of C (2.80×10^{12} CFU/g). Furthermore, the isoflavone content was the highest in OY at 18.15 mg/kg. The total phenol content and DPPH radical-scavenging capacity were as follows in the order of highest to lowest: SY, ROY, OY, and C. The changes in viscosity during fermentation showed the fastest rate in OY, which seemed to shorten the fermentation time from 8 h to 6 h. No significant difference in the whey separation rate was observed in OY (20.0–20.1%) over 15 days of storage. Additionally, the OY (4.16) and ROY (4.34) samples had better overall acceptability scores than SY (1.78). Okara is an effective additive that can be used to improve the total *Lactobacillus* count and antioxidant capacity of yogurt, and extend its shelf life.

Key words: soybean residue, fermentation, antioxidant capacity, whey separation

Introduction

Yogurt has been recognized as a healthy food option because of the benefits of high amounts of probiotics, protein, and calcium (1). It is a milk product produced from milk fermented by starter cultures, such as *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and lactic acid bacteria (2). Yogurt can be manufactured not only as probiotics, but also as low-fat yogurt and lactose-free yogurt (3), and is considered a healthy food option because it is likely to add benefits that are important aspects of human health, such as dietary fiber (4). The fermentation process in yogurt manufacturing increases the utilization of various nutrients through easy digestion and absorption, and promotes intestinal and immune functions (5). Although yogurt already has health benefits owing to the viable bacteria it contains, several studies have been conducted to increase the health benefits of yogurt, such as by adding dietary fiber (2, 6). These studies focused on improving the nutritional quality and fiber content of yogurt by adding wolfberry fibers (7), soluble fibers from carrots (8), and grapefruit (9). Dietary fibers are prebiotics that induce the growth of beneficial microorganisms, such as lactic acid bacteria, and have health benefits, such as preventing coronary artery disease, hypertension, and diabetes (10). However, the addition of dietary fiber to yogurt can change the texture and taste of the final product through interactions between different ingredients. Recently, yogurt made from the

most commonly consumed plant-based milk, such as soy, almond, and coconut milk, has become very popular among consumers. Soymilk is lactose-free and rich in proteins and other important compounds that are useful in preventing cardiovascular diseases (11). In addition, coconut milk possesses greater antioxidant properties than the milk from goats and cows (12). However, yogurt made from plant-based milk has problems with texture and stability. As such, several types of thickeners, such as pectin and xanthan gum, are added to yogurt to improve stability and adjust viscosity (13).

Soybeans are crop plants that provide protein of the same quality as that from animal sources. Soybean protein is a major component of meat alternatives consumed by individuals who prefer plant-based or low-saturated fat foods (14). Okara is obtained from the processing of tofu or soymilk, and, in Korea, it is consumed as a ground soybean stew (*Kongbiji-jjigae*). Despite its high moisture content leading to most of it being discarded as waste rather than used as food, okara is a valuable source of dietary fiber and proteins (15). It serves as an additive to enhance the nutritional value of products, including noodles (16), cookies (17), cakes (18), fermented condiments (19), and functional drinks (20). Okara has a chewy mouthfeel, a smell reminiscent of beans and grass, and a bland taste (15). Recent studies have highlighted its potential as a prebiotic source that supports probiotic growth (21) and has proven effective in lipid metabolism (22). In this study, okara was used as a supplement in yogurt, aiming to enhance its antioxidant effect and sensory properties through the process of roasting.

Material and methods

Preparation of okara

Okara was produced using the method described by Shin et al. (23). Washed soybeans (*Glycine max* (L.) Merrill, 2016, Ssalnongbu, Geochang-gun, Korea) were soaked in water for 12 h. After draining, the soybeans were mixed with water to obtain soymilk. The soymilk was passed through two layers of cheesecloth for the separation of okara. The okara was then dried in a convection oven (DX9; SJ Science, Seoul, Korea) at 40°C for 8 h. After drying, the okara was milled using a grinder, passed through a 45-mesh sieve (355 μm), sealed with polyethylene bags, and stored at -18°C until analysis. To obtain roasted okara, the dried okara was roasted at 150°C for 2 h in a convection oven (DX9, SJ Science).

Preparation of yogurt

Four types of yogurt samples were prepared with okara: yogurt without additives (control; C), yogurt with 1.5% soy powder (SY), yogurt with 1.5 % okara (OY), and yogurt with 1.5% roasted okara (ROY). To make the yogurt, 5% sugar and 1.5% additives (soy power, okara, or roasted-okara) were added to fresh milk. The mixture was heated to 85 °C for 30 s and cooled down to 40°C. The additives were added to the mixture and inoculated with 0.2% yogurt starter culture (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*). The mixture was transferred to fermentation cups, incubated at 45°C for 8 h, and stored at 4°C overnight before testing. The proximate composition of the yogurt samples was moisture (79–81.2%), total carbohydrate (9.8–11.9%), crude protein (4.2–5.6%), crude fat (2.1–3.4%), and ash (0.7%).

Total lactobacillus count and titratable acidity

The total *Lactobacillus* count in the yogurt samples was determined using the method described by Wang et al. (24). MRS agar (Biokar Diagnostics, France) was used to count viable *Lactobacillus* cells. A 0.1 g of yogurt was mixed with 10 mL of autoclaved distilled water. After mixing, 10-fold serial dilutions were performed for each sample. Thereafter, 0.1 mL of the diluted sample was evenly spread onto an MRS plate with an applicator. The plates inoculated with diluted samples were incubated at 42 °C for 48 h under anaerobic conditions. Enumeration (in colony-forming units per milliliter) was considered as the geometrical mean of at least four plates. The AOAC method (25) was used to measure the titratable acidity of the yogurt samples. Ten grams of the yogurt sample was diluted with 90 mL of distilled water, and 20 g of this solution was used. After phenolphthalein (Sigma-Aldrich, MO, USA) was added

to the solution, 0.1 N NaOH (Sigma-Aldrich, MO, USA) was used to make it appropriate. Titratable acidity was calculated using the following formula: Titratable acidity (%) = (a × f × 0.009/W) × 100,

where a is the volume of 0.1 N NaOH (mL), f is the factor of 0.1 N NaOH (mL), W is the weight of the sample, and 0.009 is the lactic acid amount equivalent to 1 mL of 0.1 N NaOH.

Contents of isoflavone and total phenolics, and DPPH radical-scavenging capacity

The isoflavone content of yogurt was determined at 254 nm using an HPLC system (1525μ binary HPLC pump; UV detector 2487; Waters, Milford, USA) equipped with a YMC-Triart C_{18} column (5 μm, 4.6 mm inner diameter × 150 mm length, YMC, Kyoto, Japan). Daidzein and genistein were used for standard calibrations. The total phenolic content (TPC) of the yogurt samples was determined using a spectrophotometer (Ultrospec 2100 Pro, Amersham Biosciences, Uppsala, Sweden) to measure absorbance values at 725 nm. The TPC was calculated as the gallic acid equivalent (GAE/g), using the standard calibration curve. The 2,2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging capacity was analyzed using a spectrophotometer (Ultrospec 2100 pro, Amersham Biosciences) at 517 nm. Ascorbic acid (Sigma-Aldrich, MO, USA) was used as the standard.

$$\text{DPPH radical-scavenging capacity (\%)} = (1 - Abs_{sample}/Abs_{blank}) \times 100$$

Whey separation and viscosity

The yogurt sample (5 g) was centrifuged at 3000 rpm for 10 min, and the supernatant was removed and precipitated using the method described by Wang et al. (24). The viscosity of the sample was determined using a digital viscometer (DV1 Digital Viscometer, ANETEK Brookfield Inc., MA, USA) with a spindle (No. 21) at 10 rpm for 4 min at 25°C.

$$\text{Whey separation (\%)} = (\text{weight of separated whey (g)} \div \text{weight of yogurt (g)}) \times 100$$

Sensory evaluation

Sensory evaluation of the yogurt was conducted using a consumer preference test with a 7-point hedonic scale (1 = very weak, 7 = very strong). Yogurt samples were served to 32 panelists (22 women and 10 men between the ages of 20 and 25 years), and assessed for flavor (beany), texture (cohesiveness), taste (sourness and sweetness), and overall acceptability. Yogurt samples were stored at 4°C for 10 h before sensory evaluation, and served in an odorless and colorless disposable cup at room temperature (18±2°C) immediately after being removed from refrigeration. Drinking water was provided to the panelists to rinse their mouths during the evaluations.

Statistical analysis

All experimental data were expressed as the mean ± standard deviation of triplicate experiments. Data analysis was performed using ANOVA. Duncan's multiple range test was also used to identify significant differences between means at a significance level of $p<0.05$, using SPSS (version 24.0; SPSS Inc., Chicago, IL, USA).

Results and discussion

Total lactobacillus count and titratable acidity of yogurt

The total *Lactobacillus* count and titratable acidity of yogurt are presented in Table 1. Among the treatments, C (2.80×10^{12} CFU/g) showed the lowest number of *Lactobacillus*. Samples SY, OY, and ROY showed similar values from 35.80×10^{12} CFU/g to 36.80×10^{12} CFU/g. Regarding titratable acidity, C was 106.20°T; ROY was 107.20 °T; and SY and OY had high titratable acidity values of 113.50°T and 112.70°T, respectively. The increase in *Lactobacillus* was related to the role of okara or soy as a fermentation source and prebiotic (7). These results are similar to those of Fernandez-Garcia and McGregor (26), which showed that the levels of acetic acid and propionic acid were significantly higher in fiber-fortified products. Furthermore, a study by Yu et al. (27) found

Table 1. Total *Lactobacillus* count and titratable acidity of yogurt.

Sample	C	SY	OY	ROY
Total Lactobacillus count (10^{12} CFU/g)	2.80±0.45[b]	36.40±3.21[a]	35.80±2.59[a]	36.80±2.59[a]
Titratable acidity (°T)	106.20±1.48[b]	113.50±1.50[a]	112.70±0.58[a]	107.20±2.75[b]

Abbreviations: C: yogurt without additives; SY: yogurt with 1.5 % soy powder; OY: yogurt with 1.5 % okara; ROY: yogurt with 1.5 % roasted okara. [ab] Different superscripts within the same row indicate significant differences according to Duncan's multiple range test (p<0.05).

Table 2. The contents of isoflavone and total phenolics, as well as the DPPH radical-scavenging capacity of yogurt.

Sample	C	SY	OY	ROY
Isoflavone (mg/kg)	11.48±0.20[d]	14.42±0.51[c]	18.15±0.28[a]	17.22±0.24[b]
Total phenols (mg GAE/g)	0.58±0.01[d]	1.48±0.05[a]	0.97±0.04[c]	1.14±0.01[b]
DPPH radical scavenging capacity (%)	6.47±0.43[d]	18.73±0.32[a]	12.26±0.52[c]	15.01±0.66[b]

Abbreviations: C: yogurt without additives; SY: yogurt with 1.5 % soy powder; OY: yogurt with 1.5 % okara; ROY: yogurt with 1.5 % roasted okara. [a–d] Different superscripts within the same row indicate significant differences according to Duncan's multiple range test (p<0.05).

1.9×10^9 CFU/g and 3.6×10^9 CFU/g of *Lactobacillus* when comparing the numbers in plain yogurt and peanut sprout-supplemented yogurt, respectively. It was confirmed that vegetable additives improved the viability or growth of probiotics in yogurt. Factors affecting the survival of *Lactobacillus* in yogurt include the strain of probiotic bacteria, pH, hydrogen peroxide, storage atmosphere, concentration of metabolites such as lactic acid and acetic acids, dissolved oxygen, and buffers such as whey proteins (28). In particular, the concentration of pH and hydrogen peroxide had a notable effect on the lactic acid bacteria content of the yogurt. The production rate of lactic acid bacteria in soybean drinks was faster than that of milk, and the growth of lactic acid bacteria was also greater in soymilk fermented drinks than in fermented milk (29). *Lactobacillus* growth during fermentation affected the pH and titratable acidity of the yogurt. During fermentation, the yogurt's pH showed a decreasing trend, whereas titratable acidity showed an increasing trend. This is due to the rapid fermentation of lactose to produce lactic acid (30). When the pH of yogurt reached 4.6, accumulated lactic acid was supplied to lactic acid again, resulting in the inhibition of bacterial activity, and the slowing of changes in pH and titratable acidity (31). Moreover, Seo et al. (32) confirmed that when nanopowdered chitosan and commercially powdered chitosan were added, the pH decreased and the titratable acidity increased. The pH of commercial yogurt products ranges from 4.0 to 4.4 (33), and the titratable acidity of yogurt tends to increase as the storage period increases. The increase in titratable acidity during fermentation improves the water-binding capacity of the protein and the stability of the yogurt (34).

Content of isoflavone and total phenolics, and the DPPH Radical scavenging capacity of yogurt

The isoflavone, total phenolic contents, and the DPPH radical-scavenging capacity of yogurt are presented in Table 2. The isoflavone content of yogurt was the highest with OY (18.15 mg/kg), followed by ROY (17.22 mg/kg), SY (14.42 mg/kg), and C (11.48 mg/kg). For the total phenolic content, the SY showed the highest at 1.48 mg GAE/g, and C had the lowest at 0.58 mg GAE/g. The DPPH radical-scavenging capacity was the highest in SY at 18.73%; meanwhile in ROY, OY, and C it was 15.01%, 12.26%, and 6.47%, respectively. In particular, ROY exhibited high antioxidant activity among the additive groups. Similarly, there was an increase in antioxidant activity when hickory-black bean (27) and mulberry fruit juice (35) were added to yogurt. Soybean has high antioxidant activity owing to its isoflavone and polyphenol contents (36). Xu and Chang (37) reported that heat treatment methods increased the polyphenol compounds

in soybeans. Shin et al. (38) reported that the antioxidant capacity of heat-treated soy powder is higher than that of non-heat-treated soy powder. In particular, roasted soy powder showed higher antioxidant activity than steamed soy powder. In addition, organic acids produced by the metabolic activity of microorganisms during fermentation affect their antioxidant activities (35). Moreover, the antioxidant activity of lactic acid bacteria increased when soybean was added to yogurt (39), which coincided with the total *Lactobacillus* count (Table 1).

Viscosity changes in yogurt

The viscosity changes in yogurt during the fermentation period are shown in Figure 1A. The

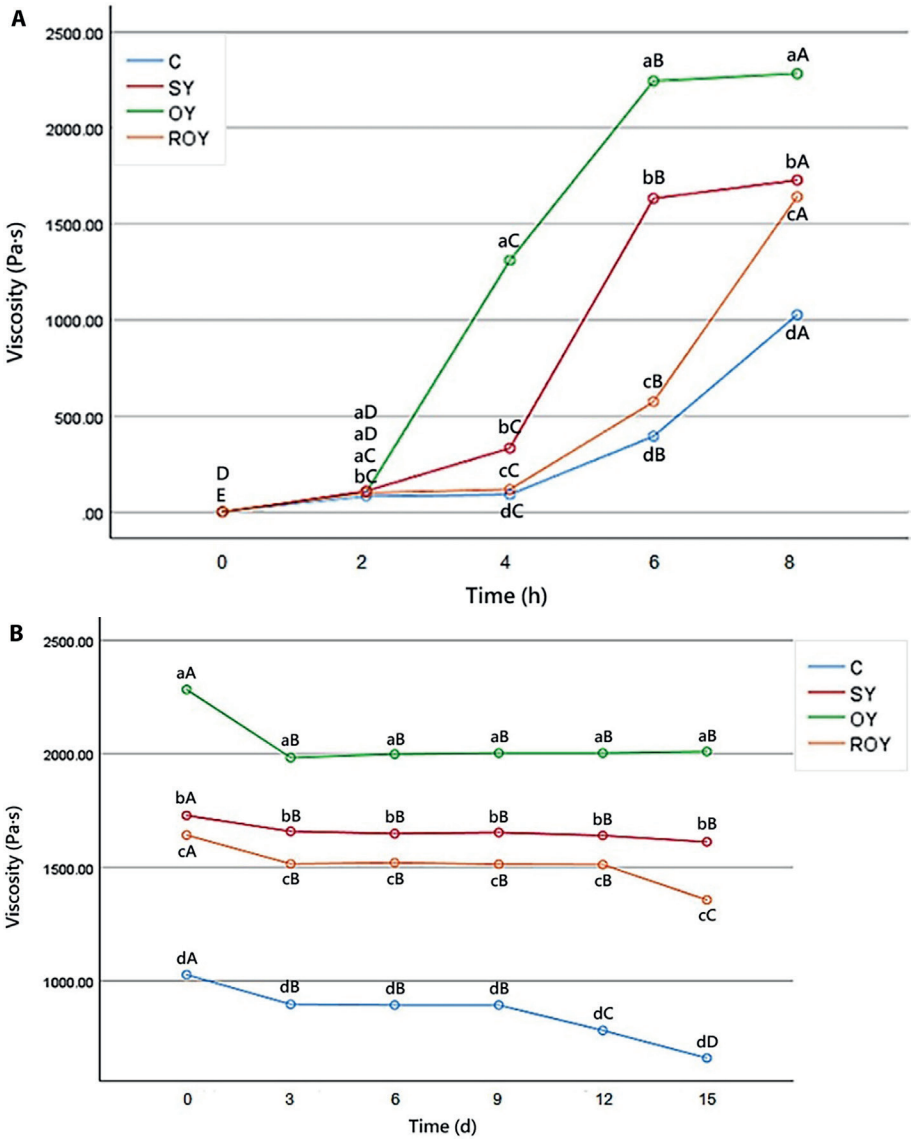

Figure 1. Viscosity changes in yogurt during the fermentation period (A) and 15-day storage period (B). Abbreviations: C: yogurt without additives; SY: yogurt with 1.5 % soy powder; OY: yogurt with 1.5 % okara; ROY: yogurt with 1.5 % roasted okara. $^{A–E}$ Different superscripts within the same samples indicate significant differences according to Duncan's multiple range test (p<0.05). $^{a–d}$ Different superscripts within the same fermentation or storage period indicate significant differences according to Duncan's multiple range test (p<0.05).

viscosity of yogurt is associated with the aggregation of casein micelles and gel formation, which is attributed to biochemical and physicochemical changes during milk fermentation (9). Overall, the viscosity of the yogurt in all samples showed a tendency to increase. The viscosities of SY and OY increased rapidly, and those of C and ROY showed a similar pattern, with a gradual increase. This finding is consistent with that of a previous study showing that the addition of fiber increases the apparent viscosity of yogurt owing to an increased acidification rate (7). The increased viscosity has been attributed to the interaction between exogenous hydrocolloids and dairy proteins (40). Fibers also have a water-holding capacity that influences their viscosity (9). Okara contains more than 50% dietary fiber, while soybean contains approximately 25% dietary fiber (15), which suggests that OY has a higher viscosity than SY. The viscosity of ROY was lower than that of SY. After 8 h, OY had the highest viscosity at 2283 Pa·s, followed by that of SY (1728 Pa·s), ROY (1642 Pa·s), and C (1027 Pa·s).

The viscosity changes in yogurt over a storage period of 15 days are presented in Figure 1B. During the 15-day storage period, the addition of okara maintained the high viscosity of yogurt. The OY sample (2283–1983 Pa·s) had the highest viscosity during the storage period, followed by SY (1728–1612 Pa·s), ROY (1641–1512 Pa·s), and C (1027–600 Pa·s). The viscosities of all the samples tended to decrease gradually as the storage period increased. The OY and SY samples presented no significant decrease after the third day of storage. Meanwhile, the viscosity of ROY decreased significantly after 12 days of storage, and that of the control yogurt decreased after 9 days of storage. The water retention capacity of roasted okara decreases upon thermal degradation during heating, thereby reducing its effect on viscosity (38). The addition of okara appears to extend the shelf life of yogurt by improving its viscosity.

Whey separation changes in yogurt over a 15-day storage period

The changes in whey separation during storage are shown in Figure 2. The addition of okara reduced the whey separation rate in the yogurt. During the storage

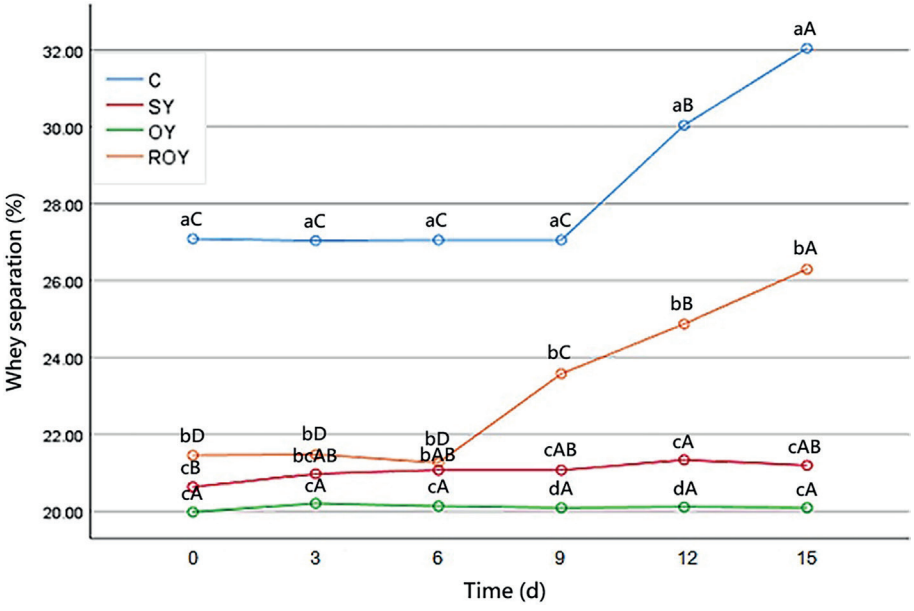

Figure 2. Whey separation changes in yogurt during storage period of 15 days. Abbreviations: C: yogurt without additives; SY: yogurt with 1.5 % soy powder; OY: yogurt with 1.5 % okara; ROY: yogurt with 1.5 % roasted okara. $^{A–D}$ Different superscripts within the same samples indicate significant differences according to Duncan's multiple range test (p<0.05). $^{a–d}$ Different superscripts within the same fermentation or storage period indicate significant differences according to Duncan's multiple range test (p<0.05).

period, OY (20.0–20.1%) showed no significant difference in whey separation, whereas a significant increase in the whey separation rate was observed in the control (27.0–32.0%) and ROY (21.5–26.3%). The whey separation was regarded as the removal of whey from the continuous yogurt network (8). Whey separation decreased as the water-holding capacity increased. The fiber in okara has an increased water-holding capacity, which, when released by the gel structure, results in reduced whey separation (9). Yoshida and Prudencio (41) reported that okara dietary fiber modified with a carbohydrate mixture improved water absorption and water-holding capacity. In a study on steamed rice cake (18), okara-supplemented batter showed a 3–6 fold increase in water-holding capacity. Okara contains very high dietary fiber (40–65%) and protein (25%) contents; therefore, it is considered to increase the water-holding capacity, which reduces whey separation (8). In the case of roasted okara-supplemented yogurt, there was no significant change in the whey separation rate until 6 days of storage. However, a significant ($p < 0.05$) increase in whey separation rate was observed after the 6 days (ROY: 21.5–26.3%). Water-holding capacity is associated with protein; therefore, more protein particles lead to an increased water-holding capacity (24). This is why SY showed the second less reduction in whey separation. In addition, the water-holding capacity was high when protein particles were combined with polysaccharides, which reduced whey separation (42). Whey separation also affects the viscoelastic network of the yogurt, and creates liquid on the surface of the yogurt, yielding undesirable sensory mouthfeel properties to consumers (43). The whey separation resulted in undesirable textural and flavor characteristics, and the loss of water-soluble nutrients reduced the nutritional value of the product (44). The improved whey separation from adding okara is expected to improve the quality and stability of yogurt.

Sensory properties of yogurt

The sensory properties of yogurt are presented in Table 3. The flavor and rheology of okara yogurt are important for consumer acceptance. For beanyness, C scored the lowest at 1.50, while SY obtained the highest value of 5.00. The ROY group showed low scores (4.45) compared with those in the additive groups. For cohesiveness, OY and C obtained high scores of 5.36 and 5.20, respectively. Furthermore, the cohesiveness of OY (5.36) was significantly ($p < 0.05$) higher than that of the control (3.00), SY (3.91), and ROY (3.73). These results were similar to what was observed in the viscosity levels of the yogurt samples (Fig. 1B). Sourness and sweetness values exhibited opposite trends in the samples. The sourness of OY (5.09) was significantly higher than that of the other yogurt samples (3.00 to 3.50), while OY (3.82) was significantly ($p < 0.05$) lower in sweetness values than the control (5.10), SY (4.91), and ROY (4.82). Okara-supplemented yogurt (OY: 4.16, ROY: 4.34) showed higher overall acceptability than SY (1.78), which was lower than that of C (5.90). In most studies, when additives were added to yogurt, they scored lower than the control in taste, flavor, and overall acceptability owing to the unique aroma and taste of the additives. Nevertheless, in some cases, the taste score was high when the additives hid the sour taste of the yogurt (42). In particular, with additives that underwent heat

Table 3. Sensory properties of yogurt.

Sample	C	SY	OY	ROY
Beanyness	1.50±0.70^{b}	5.00±1.34^{a}	4.55±0.93^{a}	4.45±1.21^{a}
Cohesiveness	5.20±0.91^{a}	3.91±1.04^{b}	5.36±0.67^{a}	3.73±1.01^{b}
Sourness	3.50±1.08^{b}	3.00±1.41^{b}	5.09±0.83^{a}	3.18±1.17^{b}
Sweetness	5.10±0.87^{a}	4.91±1.58^{a}	3.82±0.60^{b}	4.82±0.75^{a}
Overall acceptability	5.90±1.02^{a}	1.78±0.97^{c}	4.16±1.48^{b}	4.34±1.45^{b}

Abbreviations: C: yogurt without additives; SY: yogurt with 1.5 % soy powder; OY: yogurt with 1.5 % okara; ROY: yogurt with 1.5 % roasted okara.
$^{a–c}$ Different superscripts within the same row indicate significant differences according to Duncan's multiple range test ($p<0.05$).

treatment, such as roasting, the flavor of the yogurt was improved, and a good score was obtained in sensory evaluation (38). This study also found that yogurt supplemented with roasted okara had less sourness than that with raw okara, along with particularly high scores for sweetness and overall acceptability.

Conclusion

This study was conducted to develop nutritious and high-quality yogurt by adding okara. The results showed that okara increased the number of *Lactobacillus* in yogurt, showed high antioxidant effects and significantly reduced the whey separation of yogurt. Specifically, it was the high water-holding capacity of okara that had an effect on reducing whey separation, which resulted in the preferred viscosity of yogurt. It is believed that this will have the effect of extending the shelf life by improving the yogurt's stability. In particular, roasted okara increased the antioxidant effects and improved the sensory properties; therefore, the addition of roasted okara improved the quality of the yogurt.

Conflict of Interest Statement: The authors declare that there is no conflict of interest

Author Contributions: YZ conducted the analysis and drafted the original manuscript. JK developed the methodology. KY revised the manuscript. YK reviewed, edited, and approved the final manuscript.

Data Availability: The data presented in this study are available on request from the corresponding author.

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