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Original article

# Effect of the addition of whey protein-basil seed gum on the quality, properties, and antioxidant activities of low-fat mayonnaise

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**Abstract.** In this study, our aim was to develop 50% fat reduced mayonnaise using whey protein isolate (5%) -basil seed gum (BSG) complex, and analyze its physicochemical, storage stability and sensory evaluation. Five different samples were prepared with different concentration of BSG complexes (0 %, 0.2%, 0.4%, 0.6% and 0.8%). The pH and viscosity increased significantly with increasing concentration of basil seed gum complexes($p$<0.001). The *L*-value decreased, while the *a*-value and *b*-value were slightly increased ($p$<0.001). As the viscosity increased, the gel strength, hardness, and cohesiveness also increased ($p$<0.001). The oil separation, emulsion stability and freeze-thaw stability were measured during 16 days. The increase in the total phenol and flavonoid content, and ferric ion reducing antioxidant power (FRAP) with the increase in BSG of complexes increase the antioxidant activities. The sensory evaluation showed no significant difference between the samples except appearance($p$<0.001).

**Keywords:** antioxidant, activity, reduced fat, sensory evaluation, storage stability, mayonnaise

## Introduction

In recent years, the Western pattern of diet, including intake of processed foods with high levels of animal protein and fat has increased due to economic growth and industrialization (1). However, high intake of fat can lead to complications, such as hypertension, cardiovascular disease, diabetes, and obesity (2). Since fat affects the appearance as well as the flavor and texture of food, the development of fat-reduced food using fat substitutes is required rather than reducing the fat content (3). Currently, fat substitutes can be classified into protein-based, carbohydrate-based, fat-based, and synthetic depending on their composition; a combination of two substances might provide the desired properties and texture in food (3). The carbohydrate-based fat substitutes are hydrocolloids that increase the viscosity and form a hard gel, functioning not only as a fat substitute but also as an emulsifier, stabilizer, and foaming agent (1).

Basil seed gum (BSG) is a representative plant-derived hydrocolloid that separates thick mucus membranes by hydrating basil seeds (*4*), which has a high antioxidant activity (5). The BSG has a relatively high molecular weight (2320 kDa) compared to other gums, which imparts viscosity and shearing force to the solution, thus contributing to the maintenance of stable properties (6). In addition, BSG, which is an anionic heteropolysaccharide, contains glucomannan and is used as an emulsifier, foam stabilizer, thickening agent, and gelling agent in food manufacturing and pharmaceuticals (7). The BSG is used as a mixture with proteins and polysaccharides, such as whey protein, galactomannan, and β-lactoglobulin rather than as a sole substance when applied to foods (5). In particular, whey protein contains β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulin, and small amounts of proteins and enzymes (8). Beta-lactoglobulin, which accounts for 70% of the total protein content, imparts properties, such as hydration, gelation, and emulsification (8). Whey proteins are spherical in aqueous solution and form gels at 60°C, and when combined with BSG, gelation and layer separation occur at the same time, resulting in a decrease in the rate of layer separation and rapid formation of a dense gel that imparts the texture (5). Prior studies about using gums as a fat replacer have investigated such as xanthan gum with whey protein mixture (9), Persian gum (10), and tragacanth gum (11).

Mayonnaise is widely used around the world and consists of vinegar, egg yolk, oil, salt and sugar. However, its high fat content of 65-80% not only causes obesity-related illnesses but also easily spoilage during storage due to auto-oxidation of fat (12). To reduce the fat content and oxidation rate, studies were performed using antioxidant materials (13). Previous studies have investigated fat replacement agents in mayonnaise, like whey protein and lecithin complex (14) and guar gum and fibrin complex (15); however, there are no studies using BSG and whey protein isolate.

Therefore, in this study, we developed mayonnaise by adding 50% less oil during the production by using whey protein isolate and BSG as a fat substitute and analyzed the physicochemical properties, quality characteristics, antioxidant activity, and sensory characteristics of mayonnaise to provide an optimal proportion of whey protein isolate- BSG in mayonnaise.

## Materials and methods

### *Materials*

Basil seeds for basil seed gum extraction, which were harvested and dried in India, were purchased from Seohyun Pharmaceutical Farming Association Co., Ltd. (Nonsan, Korea). The whey protein powder (Garunara, Seoul, Korea) and the egg yolk powder (edenownfnb, Co., Ltd, Yeoju, Korea) were purchased through the Internet. Sugar (CJ Cheiljedang Co., Ltd., Seoul, Korea) and salt were purchased at a local market

### *Extraction of basil seed gum*

The extraction of BSG from basil seeds was performed by the method of Hosseini-Parvar et al. (16) with a slight modification. The extraction process was carried out by a juice extractor (NJE-3530, NUC, Daegu, Korea)-assisted extraction as follows: BSG was prepared by soaking under water at 69°C, pH 8, and the total water/seed ratio was 65:1. Before the sample was used in the experiment, BSG was freeze-dried for 96 h, and then, pulverized in a high-speed grinder. Next, BSG solutions (0.2 %, 0.4%, 0.6%, 0.8% were prepared by dissolving the BSG powder in distilled water with 5% whey protein isolate and storing it for 24 h at 4°C and put them 25 °C for 1 h before producing mayonnaise.

## Preparation methods and analyses

### *Preparation of mayonnaise*

The formula of mayonnaise preparation with whey protein isolate and basil seed gum complex is shown in Table 1. The mayonnaise was prepared according to the method of Lee & Song (17) with slight modification. Mixtures used in the preparation of mayonnaise were prepared by adding 5% of whey protein powder to different proportions of basil seed gum (0%, 0.2%, 0.4%, 0.6%, 0.8%) basis on water 35 % used to produce mayonnaise. In the control group, egg yolk powder, vinegar, sugar, salt, and water were put together in a kneader (Chef classic KM400, Kenwood, Havant, England), and then, mixed in five steps every 30 s for a total of 3 min and 30 s. In the complex additive group, egg yolk powder, vinegar, sugar, salt, and water were put together in a kneader and mixed for 1 min at 1 step and 1 min at 3 steps, and then, 10 g of BSG-whey protein mixture was added every 30 s for 5 min. The prepared mayonnaise was stored in a refrigerator at 4°C for 12 h.

### *Protein content*

The protein content of mayonnaise was measured by the Bradford method with a slight modification (18). Coomassie blue G-250 (100 mg) was mixed with 50 mL of 95% ethanol and 100 mL of 85% phosphoric acid solution to prepare Coomassie blue reagent. Bovine serum albumin was used as a standard at concentrations of 0, 10, 20, 30, 40, and 50 μg/ml. Mayonnaise sample (1 g) was mixed with 9 mL of distilled water, extracted

**Table 1.** Formula for mayonnaise mixed with whey protein isolate-basil seed gum

| Ingredient | Con[1)] | W-B 0.2% | W-B 0.4 % | W-B 0.6 % | W-B 0.8 % |
|---|---|---|---|---|---|
| Egg yolk | 14 | 14 | 14 | 14 | 14 |
| Soybean oil | 70 | 35 | 35 | 35 | 35 |
| Vinegar | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| Salt | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| sugar | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Gum solution | 0.0 | 35 | 35 | 35 | 35 |
| water | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Total | 100 | 100 | 100 | 100 | 100 |

1) Control = mayonnaise with no addition of whey protein-basil seed gum, W-B 0.2%= mayonnaise with whey protein 5%- Basil seed gum 0.2 %, W-B 0.4%= mayonnaise with whey protein 5%- Basil seed gum 0.4 %, W-B 0.6%= mayonnaise with whey protein 5%- Basil seed gum 0.6 %, W-B 0.8%= mayonnaise with whey protein 5%- Basil seed gum 0.8 %

for 2 h, and filtered twice using Whatman No.1 filter. The absorbance was measured three times at 595 nm using a microplate reader (Infinite 200 PRO, Tecan, Mannedorf, Switzerland), taking 0.05 mL of the sample solution and 1 mL of Coomassie blue reagent in a 1.5 mL tube. The protein content was calculated as a percentage based on the total weight of 100 g.

*Fat content*

To determine the fat content of mayonnaise, 200 g of n-hexane was added to 20 g of the lyophilized ground sample according to the method of Coorey et al. (19) with slight modification. The samples were mixed with 200 mL of n-hexane, and then passed through Whatman No. 1 filter paper. Three replicates were prepared for each sample, and the filtered solution was analyzed after removal of the solvent at 60°C in a rotary evaporator. The fat content was calculated using the following formula:

Fat content (%) = 100 × (weight after extraction – weight before extraction)/initial sample weight

*pH*

To determine the pH of each sample, 10 g of a sample was mixed with 90 mL of distilled water in a 250 mL beaker and homogenized (Unidrive 1000D, CAT M. Zipperer GmbH, Staufen, Germany) for 1 min. The pH of the solution was measured after 15 min.

*Color*

The surface of each sample was evaluated using a colorimeter (CR-400, Konica Minolta, Osaka, Japan) to measure the sample color according to Hunter's color value system. The parameters, $L$ (brightness), $a$ (redness), and $b$ (yellowness) were measured. The National Bureau of Standards (NBS) unit is divided into 6 groups of 0-0.5 (trace), 0.5-1.5 (slight), 1.5-3.0 (noticeable), 3.0-6.0 (appreciable), 6.0-12.0 (much). The NBS unit and the value of ΔE (total color difference) was determined using the following equation.

*Viscosity*

The viscosity of mayonnaise was determined according to the method of Shihata and Shah (20) with some modifications. The samples were analyzed with spindle No. 4 rotation at 100 rpm using a viscometer (LVDV-1 prime, Brookfield Engineering Labs Inc., Middleboro, USA). The readings were recorded during a 5 min measurement period in millipascal seconds (mPa·s). For each sample, we prepared 12 replicates.

$$\Delta E = \sqrt{\left(L_{sample} - L_{standard}\right)^2 + \left(a_{sample} - a_{standard}\right)^2 + (b_{sample} - b_{standard})^2}$$

$$NBS\ unit = \Delta E \times 0.92$$

*Texture*

After cooling for 12 h, the sample was prepared by pouring into the cup which has a uniform size (5 × 5 × 5). Textural properties (hardness, cohesiveness, gel strength) of samples were measured using a rheometer (Sun rheometer Compac-100 II, Sun Scientific Co., Ltd, Tokyo, Japan). The texture profile was set with the two-bite compression test using the following operation conditions: No. 1 Φ25 mm prove; maximum weight, 10 kg; distance, 33%; table speed, 60 mm/min

*Emulsion stability*

The emulsion stability of mayonnaise was determined according to the method of Coorey et al. (21). The 10 g of sample was shaken at 80˚C for 30 min and centrifuged at 4000 g for 20 min using a centrifuge (Universal 32R, Hettich, Tuttlingen, Germany). After removing the supernatant, the weight of the precipitate and the weight of the initial sample were measured three times. The emulsion stability was estimated using the below equation.

Emulsion stability (%) = (precipitate weight after centrifuge / initial weight before centrifuge) × 100

*Freeze-thaw stability*

Freeze-thaw stability of mayonnaise was measured by taking 15 g of each sample into tubes and storing them at -20 ˚C according to the method of Baker et al. (22). The samples were stored at 25 °C for 30 min before the measurement, and then centrifuged at 4000 g for 15 min. The Freeze-thaw stability was measured three times using the following equation below.

Freeze-thaw stability (%) = (weight of the precipitated fraction / initial sample weight) × 100

*Oil separation*

The oil separation of mayonnaise was modified according to the method of Kim et al. (23), and 50 g of the sample was centrifuged at 2000 rpm for 30 min. The amount of oil separated was measured three times and expressed as a percentage.

**Antioxidant activities of mayonnaise**

*Preparation of sample*

Methanol (9 g) was added to 1 g of the mayonnaise sample; the mixture was extracted for 5 h and then centrifuged at 4000 g for 30 min. The extract was cooled in a -80˚C refrigerator for 10 min, and the supernatants were filtered through the Whatman No. 1 filter paper.

*Total phenol content*

The total polyphenol content was analyzed using the method of Folin-Denis with a slight modification (24). A solution containing 0.9 M Folin-Ciocalteu reagent (Junsei Chemistry, Tokyo, Japan) and 20% (w/v) sodium carbonate solution (Merck kGaA, Darmstadt, Germany) was prepared and 10 μL of samples were vortexed with 790 μL of distilled water for 1 min. A total of 50 μL of 0.9 N Folin-Ciocalteu reagent (Junsei Chemistry, Tokyo, Japan) was added to 150 μL of 20% sodium carbonate solution (Merck kGaA, Darmstadt, Germany), and the reaction mixture was incubated for 30 min at room temperature in the dark. The absorbance of each sample was measured at 750 nm using a microplate reader. Gallic acid (Merck kGaA, Darmstadt, Germany) was used as a standard and the absorbance of each sample was converted to gallic acid equivalent (GAE).

*Total flavonoid content*

The flavonoid content was measured as previously described (25). A total of 1 mL sample was vortexed with 150 μL of 5% sodium nitrite (Junsei Chemistry, Tokyo, Japan) for 6 min in the dark (25°C), followed by the addition of 300 μL of 10% aluminum chloride (Junsei Chemistry, Tokyo, Japan) and incubation in the dark (25°C) for 5 min. The solution was reacted with 1 mL of 1 N sodium hydroxide solution (Daejung Chemicals & Metals, Gyeonggi, Korea). Each sample was measured at 520 nm. Absorbance calibration curves were prepared using quercetin (Sigma-Aldrich Co., Ltd, Mo, USA) as a standard, and the total flavonoid content of each sample was converted to quercetin equivalent.

*FRAP*

Ferric-reducing antioxidant power was measured by the method of Thaipong et al. (26) with some modification. The reagents used included 0.2 M phosphate buffer (pH 6.6), 1% potassium ferricyanide (Merck kGaA), 10% trichloroacetic acid (Sigma-Aldrich Co., Ltd, Mo, USA), and 0.1% ferric chloride ($FeCl_3$) (Junsei Chemistry, Tokyo, Japan). A mixture of 250 μL of the sample, 250 μL of 0.2 M phosphate buffer, and 1% potassium ferricyanide were allowed to react for 30 min at 50°C, and then, treated with 250 μL of 10% trichloroacetic acid. A total of 0.5 mL of this mixture was treated with 0.5 mL of distilled water and 0.1% $FeCl_3$. The absorbance of each solution was measured at 700 nm.

*Sensory evaluation*

A panel of 30 individuals (20-30 years of age) participated in this test and analyzed the appearance, oily odor, odor amplitude, mouthfeel, oily taste, and overall acceptability of samples using the 9-point scale method (strong dislike = 1 and strong like = 9).

*Statistical analysis*

All data were expressed as the mean ± standard deviation of triplicate experiments and evaluated by one-way analysis of variance (ANOVA) using SPSS ver. 23.0 (SPSS InC., Chicago, IL, USA). The significance between the means of measured experimental values was analyzed by the Duncan's multiple range test ($p$<0.05).

## Results and discussion

*Protein and fat content*

The protein and fat contents of mayonnaise were analyzed and showed in Table 2. The protein content of the control and BSG complex added group was 14.96 % and 14.97 %, respectively ($p$<0.001). The fat content was significantly different, with 74.34 % in the control group and 35.79 % - 36.61 % in the BSG complex added group ($p$<0.001).

*pH*

Razavi et al. (27) had reported that the pH of BSG has a great effect on the viscosity of an emulsion that the lower pH decrease the viscosity. However, in terms of structure, alkaline condition decreases the particle size of the emulsion, and the addition of BSG increases the stability of the emulsion and the gel formation time (16). The pH of the mayonnaise containing the BSG complex is shown in Table 2. The control group showed the highest pH value (5.02), and W-B 0.2 % group had the lowest value (4.39) ($p$<0.001). This result is different to results previously obtained with additon of polysaccharide gum (28) that the pH decreases as the amount of polysaccharide gum increases. In this study, it was due to the difference in the extraction solvent. The major factors affecting the pH are the difference in each material.

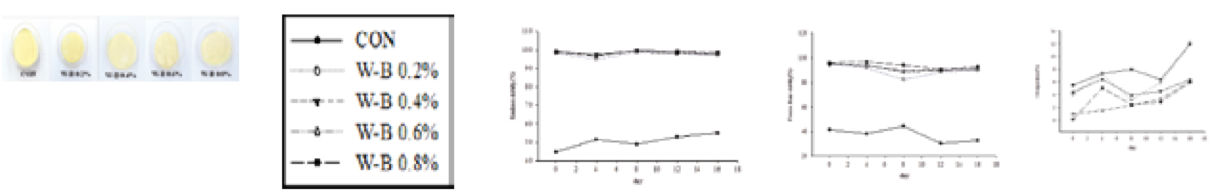
*Color*

The color of the BSG complex added to mayonnaise is shown in Table 2 and Fig 1. The $L$-value of the control was the highest at 81.47 and decreased significantly as the BSG content of the BSG complex increased ($p$<0.001). The $a$-value of the control group was the highest at 0.25 and the W-B 0.2 % was lowest at -0.75 also BSG complex added group were increased as the concentration of

**Table 2.** Physiochemical properties of mayonnaise prepared with whey protein-basil seed gum complex

| properties | CON[2)] | W-B 0.2% | W-B 0.4 % | W-B 0.6 % | W-B 0.8 % | F-value ($p$-value) |
|---|---|---|---|---|---|---|
| Protein content (%) | 14.96±0.00$^{b}$ | 14.97±0.00$^{a}$ | 14.97±0.00$^{a}$ | 14.97±0.00$^{a}$ | 14.97±0.00$^{a}$ | 169.756[1)] (0.000) |
| Fat content (%) | 74.24±1.05$^{a}$ | 35.97±0.63$^{b}$ | 36.61±1.12$^{b}$ | 36.09±1.20$^{b}$ | 36.27±1.62$^{b}$ | 637.198$^{***}$ (0.000) |
| pH | 5.02±0.01$^{a}$ | 4.39±0.01$^{e}$ | 4.45±0.01$^{d}$ | 4.56±0.01$^{c}$ | 4.73±0.01$^{b}$ | 4134.143$^{***}$ (0.000) |
| viscosity (cP) | 5353.33±109.64$^{b}$ | 1464.00±60.00$^{e}$ | 4923.00±24.98$^{d}$ | 5227.00±42.14$^{c}$ | 6395.00±24.00$^{a}$ | 2842.341$^{***}$ (0.000) |
| *L* | 81.47±0.32$^{a}$ | 80.78±0.03$^{b}$ | 80.34±0.09$^{c}$ | 79.66±0.39$^{d}$ | 79.74±0.12$^{d}$ | 30.657$^{***}$ (0.000) |
| *a* | 0.25±0.03$^{a}$ | -0.75±0.04$^{d}$ | -0.47±0.04$^{c}$ | -0.19±0.01$^{b}$ | -0.18±0.01$^{b}$ | 872.965$^{***}$ (0.000) |
| *b* | 27.71±0.07$^{a}$ | 22.36±0.02$^{d}$ | 22.87±0.08$^{c}$ | 23.23±0.02$^{b}$ | 23.26±0.02$^{b}$ | 5834.149$^{***}$ (0.000) |
| $\Delta E$ | 0.07±0.06$^{d}$ | 30.14±0.17$^{a}$ | 25.23±0.69$^{b}$ | 23.69±1351$^{c}$ | 23.03±0.49$^{c}$ | 679.197$^{***}$ (0.000) |
| NBS unit | 0.07±0.06$^{d}$ | 27.73±0.16$^{a}$ | 23.21±0.63$^{b}$ | 21.79±1.39$^{c}$ | 21.18±0.45$^{d}$ | 679.197$^{***}$ (0.000) |
| Hardness (g/cm$^2$) | 0.49±0.00$^{a}$ | 0.39±0.00$^{b}$ | 0.39±0.00$^{b}$ | 0.49±0.00$^{a}$ | 0.52±0.06$^{a}$ | 17.500$^{***}$ (0.000) |
| Cohesieveness (%) | 127.95±6.27$^{b}$ | 117.86±0.94$^{b}$ | 123.05±8.12$^{b}$ | 172.61±7.76$^{a}$ | 186.86±12.27$^{a}$ | 47.584$^{***}$ (0.000) |
| Gel strength (g·Cm) | 38.20±3.56$^{b}$ | 24.74±0.51$^{c}$ | 27.85±2.29$^{c}$ | 40.23±3.55$^{b}$ | 46.67±1.30$^{a}$ | 37.950$^{***}$ (0.000) |

[1)] Values are expressed as mean ± SD of triplicate observations
$^{a\text{-}d}$Different superscripts indicate significant differences between values in the same row according to Duncan's range test ($p$<0.05)
$^{***}p$ < 0.001
[2)] Control = mayonnaise with no addition of whey protein-basil seed gum, W-B 0.2%= mayonnaise with whey protein 5%- Basil seed gum 0.2 %, W-B 0.4%= mayonnaise with whey protein 5%- Basil seed gum 0.4 %, W-B 0.6%= mayonnaise with whey protein 5%- Basil seed gum 0.6 %, W-B 0.8%= mayonnaise with whey protein 5%- Basil seed gum 0.8 %




CON W-B 0.2% W-B 0.4% W-B 0.6% W-B 0.8%

**Figure 1.** Mayonnaise made with whey protein-basil seed gum complex

BSG increased. The *b*-value was the highest in the control group and lowest in the W-B 0.2 % at 27.71 and 22.36, respectively, but the value increased with increasing BSG concentration in the BSG complex added group ($p$<0.001). In the case of ΔE and NBS unit, the difference between the samples decreased as the amount of BSG increased. The W-B 0.6%-W-B 0.8% showed the least difference from the control. Lee et al. (29) reported that as the concentration of xanthan gum increased, the *L*-value and *b*-value decreased whereas the *a*-value increased that slightly different decrease in xanthan gum content results in a color value similar to the control. On the other hand, Su et al. (28) showed that the *L*-value of the group supplemented with guar gum was higher than that of the control group. The difference in the color of mayonnaise is thought to be caused by the difference in the color between oil and the BSG complex.

**Viscosity**

Viscosity is a measure of the resistance to friction within the fluid, which means that as the viscosity increases, the texture of the aqueous solution becomes dense (19). The viscosity of the mayonnaise with BSG complex shown in Table 2. In the case of viscosities, the W-B 0.8 % showed the highest value of 6395 cP, and the control group showed a similar value, 5353 cP. In the addition group, the viscosity increased as the BSG content of the complex increased, indicating a significant difference from the control ($p$<0.001). Razavi et al. (27) reported that the increase in the BSG concentration induces the molecular interaction and the self-aggregation of the molecular chains to increase the viscosity. In addition, the use of higher than W-B 0.3 % increases the viscosity of the aqueous solution due to the non-absorbed polysaccharide, and reduces the efficiency of fragmentation during homogenization, thereby changing the physical properties of the aqueous solution (4). In the study by Hashemi et al. (30), the viscosity was similar to that of the present study, and the viscosity increased as the content of gum arabic increased. Lee et al. (29) also found that the viscosity increased with increasing xanthan gum content of the composites, consistent with the results of this study. Contratsingly, mayonnaise supplemented with 0.5% guar gum and 0.375% cellulose (Golchoobi et al., 2016) showed no significant difference from the control group viscosity. The results were slightly different as the viscosity was higher compared to this sudy. Basil seeds contain a high content of mucus and consist of a solid core in the interior and a pectinous fibrillar outer membrane that forms a hydration seed in water (31). The polysaccharides extracted from the basil seeds are composed of glucosamine (43%) with an acid-stable glucose and mannose ratio of 10: 2 and acidic side chains C-2 and C-3 of the xylosyl residue (32), which is composed of xylenes (24.29%) and glucan (2.31%). The molecular weight of PER-BSG (5980 kDa) and that of SUPER-BSG (1045 kDa) are divided and the ratio of these two parts varies depending on the extraction method and greatly affects the viscosity (33). In a study by Razavi et al. (27), the viscosity of BSG decreased as the shear rate increased, suggesting that the BSG consists of two or more different polysaccharides. In addition, the viscosity of BSG is affected by pH, temperature, presence of salt and sugar, and thawing and freezing (33). In this study, the viscosity of the mayonnaise increased as the BSG of the complex increased, the movement of oil particles of mayonnaise decreased due to BSG trapped the oil particles and makes more compact structure to the mayonnaise.

**Texture**

The texture of the mayonnaise with BSG was measured in terms of hardness, cohesiveness, and gel strength shown in Table 2. In the case of hardness, 0.52 g / $cm^2$ was the highest value in W-B 0.8 % and the value was similar to the W-B 0.6 % at 0.49 g / $cm^2$. In the BSG complex added group, the hardness increased with the increase in BSG content of BSG complex($p$<0.001). Similar to hardness, the cohesiveness values of W-B 0.4 % was similar to those of the control group at 127 %, and the value increased with the content of BSG, which was the highest, 186 % in the W-B 0.8 % group. There was a significant difference between the samples ($p$<0.001). Basil seed gum is close to a black fibrillar structure so the size of the aggregate is reduced by adding BSG to an aqueous solution due to a connection between the spherical protein by a thin fibril of BSG then create a binary continuous microstructure (34). In the study by Rafe et al. (34), the structural change forms a space capable of retaining moisture and forms a suitable gel. As the content of BSG increases, the structure becomes dense and the space inside becomes smaller, increasing the hardness and gel strength. In addition, in the study by Javidi et al. (35), the addition of BSG increased the hardness and cohesiveness of the sample and was consistent with the results of this study. Gel strength was the lowest, 24.74 in the W-B 0.2 % and the value increased as the content of BSG increased; it was the highest, 46.67 g · Cm in the W-B 0.8 % group. The gel strength of the control group and W-B 0.6 % was 38.20 g · Cm. Lee and Chin (36) reported that the gel strength increased by the addition of a combination of carbohydrate and protein; similarly, the gel strength of the sample increased with the addition of BSG. Golchoobi et al. (15) reported that hardness and cohesiveness increase with the decreasing emulsion particle size. In this study, addition of BSG decreased the particle size of the aqueous solution, and increased the binding of protein and carbohydrate, thus, increasing the coagulation degree of emulsion, and cohesiveness.

**Emulsion stability**

The emulsion stability of mayonnaise is influenced by the oil content, yolk content, relative ratio of oil and water, preparation method, temperature, viscosity, size of lipid, emulsifier type, concentration, and distribution ratio (29). The emulsion stability of mayonnaise with BSG complex was measured at 4°C for 16 days, as shown in Fig 2. The stability of the emulsion was not correlated with the storage period; the lowest value was observed in the control group throughout the storage period, and the stability also increased as the BSG content of the BSG complex increased, showing the highest stability in the W-B 0.8 % group. Basil seed gum contains a pectin layer composed of non-esterified galacturonic acid with high hydration ability (37). In the study by Osano et al. (4), a small amount of protein impurities and the high hydration ability of BSG stabilized the dispersion state of O/W emulsion. Addition of 0.3 % (w/w) of BSG resulted in the precipitation and aggregation of O/W and helped maintain a stable state. In addition, Osano et al. (4) reported that as the ratio of SUPER-BSG in BSG increases, the emulsifying power increases due to the increase in the uronic acid content and the flexibility of SUPER-BSG. In addition, the increase in SUPER-BSG and the hydrophobic part of the BSG leads to an increase in the emulsifying power of the aqueous solution and the increase in the PER-BSG part of the BSG increases the emulsion stability.

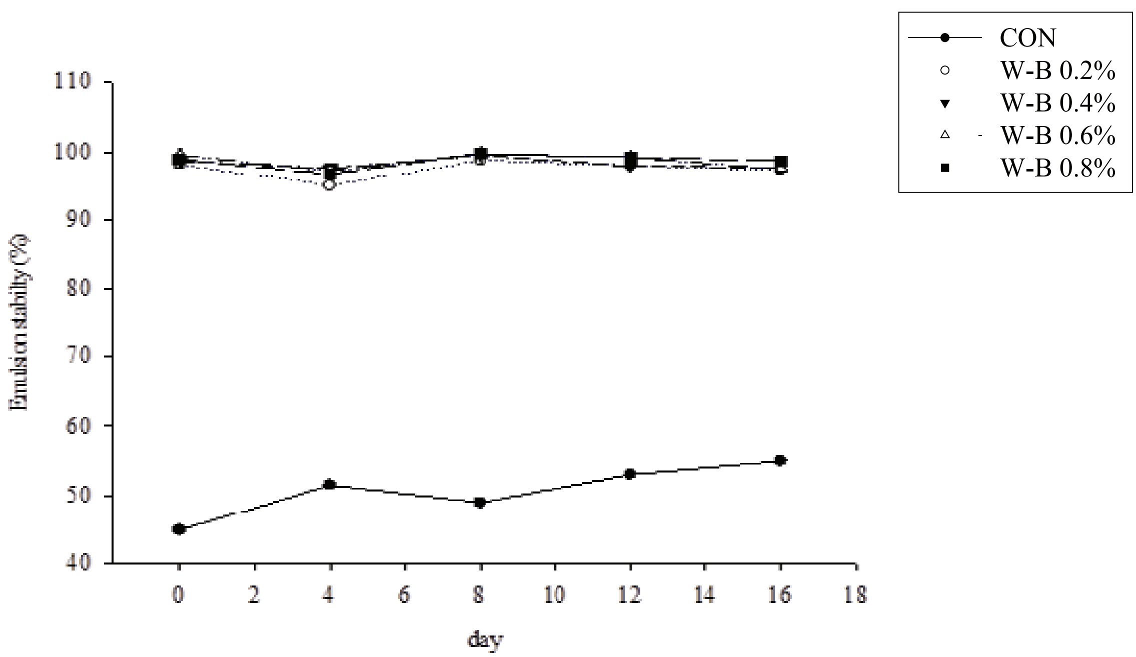



**Figure 2.** Mayonnaise made with whey protein-basil seed gum complex

In the study by Hashemi et al. (30), the addition of lysozyme and arabic gum combination to mayonnaise showed that protein and polysaccharide interact with oil and water to promote emulsification and increase emulsion stability. Lee et al. (29) also reported that the emulsion stability of mayonnaise increased as the content of xanthan gum increased. These results suggest that the addition of BSG to the mixture of mayonnaise stabilizes the dispersed state of the emulsion and that the emulsion stability is increased by forming the tissue more tightly than the control.

**Freeze-thaw stability**

The Freeze-thaw stability is to measure the separation of water and oil in the emulsion during thawing and freezing. The stability of the mayonnaise with BSG was measured at -20°C for 16 days and the result is shown in Fig 3. In the case of freeze-thaw stability, the stability was the lowest in the control group (41 %) at day 0 and increased with the addition BSG of BSG complex. Freeze-thaw stability decreased slightly with the increase in storage period, but did not show any significant correlation with the storage period. All of them showed a similar tendency on day 0, and the control group showed the lowest stability. Puligundla et al. (38) reported that in mayonnaise added with starch gel, the stability of thawing increased as the amount of gel added increased. However, as the storage period increased, the stability decreased significantly. On the other hand, Coorey et al. (19) reported that as the content of xanthan gum and gelatin increased, the stability increased because of the water-binding capacity. In the study of Zameni et al. (39), the BSG was shown to be stable in the thawing and freezing process, while the BSG was not frozen below 0°C. The unfrozen portion has a very large viscosity and the polymer concentration increases from ice to water and it is reported that it maintains a stable aqueous solution even after freezing by forming a chain aggregate. In addition, when the BSG hydrated gel is frozen, the gel strength is increased and a dense tissue structure is formed (39). In the present study, the freeze-thaw stability of the mayonnaise supplemented with BSG was higher

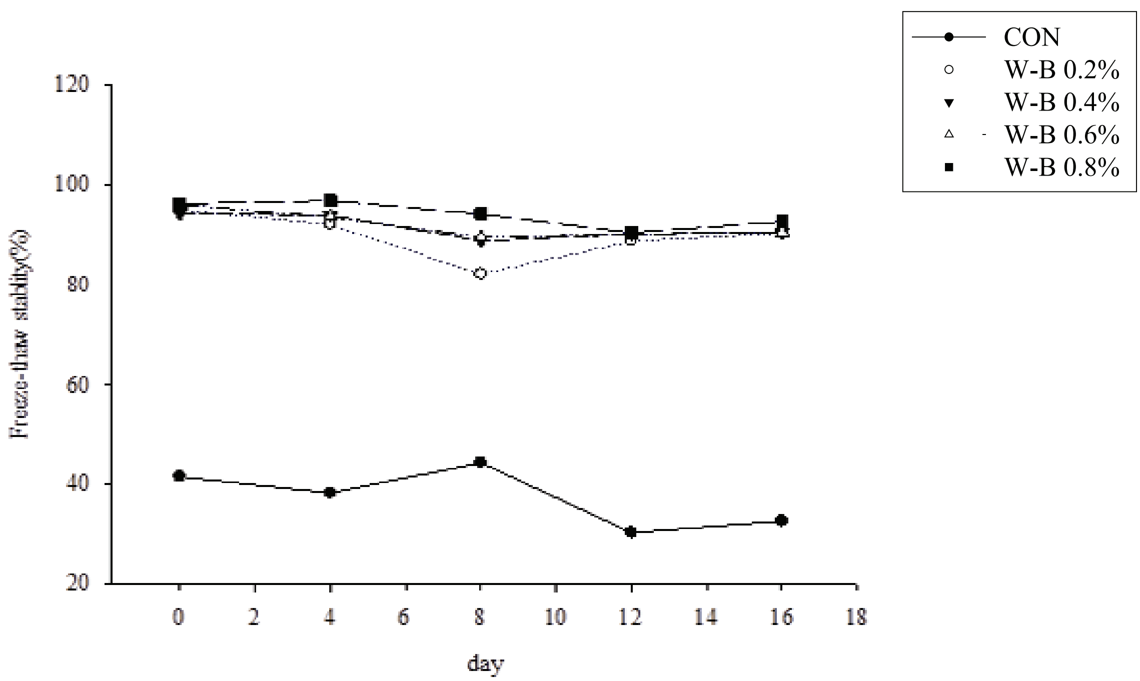



**Figure 3.** Freeze-thaw stability (%) mayonnaise made with whey protein-basil seed gum complex

than that of the control because the addition of BSG increased the stability of the emulsion, decreased the separation of water and oil due to high water binding capacity and disturbance of oil particle movement.

## Oil separation

The oil separation of the mayonnaise with the BSG compound was measured for 16 days at 4˚C and the result is shown in Fig 4. The oil level of the control group was the highest at 5.5 % storage at day 0. The W-B 0.2 %, W-B 0.4 %, and W-B 0.6 % were 1.04 %, 1.01 %, and 0.14 %, respectively. On the 4th day after storage, the highest value was observed in the control group (7.34 %), but the W-B 0.2 % and W-B 0.4 %, increased the oil separation 7.22 % and 6.44 %, respectively. The W-B 0.6 % and W-B 0.8 % decreases the separation to 5.41 % and 5.06 %, respectively. On the 8th day of storage, the degree of separation was similar to that of the control group as on day 0 and 4th day. In the group with BSG, however, the degree of separation decreased from day 0 to the 4th day to 2.12 %. On the 12th day of storage, the control group showed the highest oil separation with a similar tendency to the previous storage days, and decreased with increasing BSG content of complex. On the 16th day of storage, a relatively high oil separation was observed in all samples. The highest value was 12.17 % and 11.93 % for W-B 0.2 % and the control group, 6.37% for W-B 0.4% and 6.13% for W-B 0.6%. The W-B 0.8 % showed the lowest level of 5.98%. In a study by Johary et al. (40), oil particles were trapped and stopped by BSG so that forming a sustainable network and increasing emulsification and reducing oil separation. In the study of mayonnaise prepared by the addition of gum, such as guar gum and xanthan gum by Lee and Song (17), there were no differences in oil separation, but the overall addition of the gum decreased the oil separation of mayonnaise, which is consistent with the results of this study. It is considered that the addition of gum reduces the degree of separation of oil because the oil binding ability and water binding ability of the gum is high, and the stability of the emulsion is increased and the separation degree is reduced.

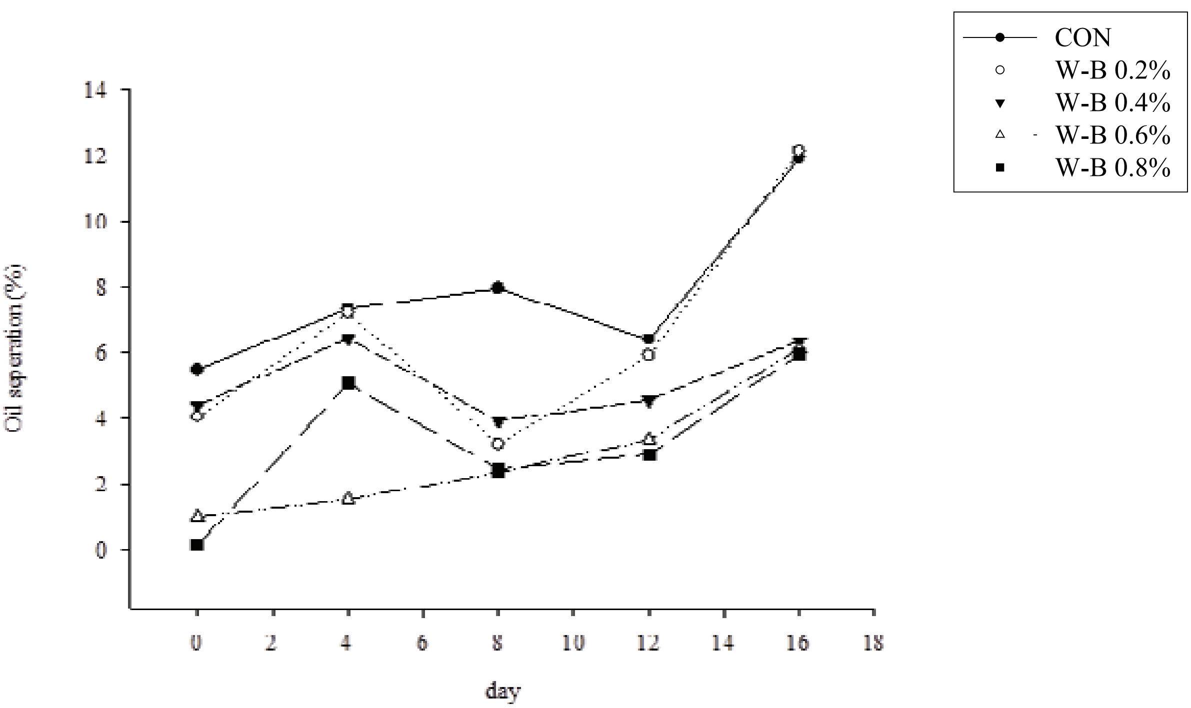



**Figure 4.** Oil separation of mayonnaise made with different concentration of whey protein-basil seed gum complex during storage (16 day)

### Total phenol content, flavonoid content, and FRAP

Antioxidants are substances that have the ability to inhibit or delay the oxidation of lipids by inhibiting the early or progressive stages of the oxidation reaction (41). The most representative components of antioxidants are polyphenols, which are included in plants, vegetables, and fruits and the secondary metabolites of plants have defense systems against pathogens and ultraviolet rays (42). Polyphenols affect the bitter taste, flavor, and oxidative stability of foods and reduce the risk of various diseases in humans, such as cancer, heart disease, obesity, osteoporosis, and neurodegenerative diseases (43). Flavonoids are a subgroup of polyphenols that impart color to plants and fruits, and include six subgroups of flavonols, flavones, flavanones, flavanols, anthocyanins, and isoflavones (42). Polyphenols play a role as antioxidants through redox reactions, depending on their location (44). Table 3 shows the results of analysis of total polyphenol and flavonoid content of mayonnaise containing BSG complex. The total polyphenol and flavonoid contents increased with increasing BSG content of the complex, and the highest value was 52.88 μg GAE / mg and 22.42 μg QE / mg in the W-B 0.8%, showing a significant difference ($p<0.001$). The polyphenol content of basil seeds is smaller than that of basil leaves but also contains phenolic compounds, such as rosmarinic acid, chicoric acid, p-hydroxybenxoic acid, and p-coumaric acid (45). The FRAP has the ability to reduce ferric ion to ferrous ion, changing the reactant color from yellow to green to blue, and measuring the absorbance of the reactant at 700 nm (46). In the case of FRAP, the antioxidant activity tended to increase as the total polyphenol and flavonoid content increased with the content of total polyphenol and flavonoid contents. The value of FRAP in the control group was the lowest at 0.41 and the value of FRAP was increased as the BSG content of the composite increased. The ability to chelate metal ions is important because it reduces the ability to catalyze the transfer of metals during lipid peroxidation (47). Gülçin et al. (47) reported that FRAP was increased as the content of basil and phenolic compound increased. The results of this study suggest that FRAP is higher than that of the control group because the content of phenolic compounds increases as the content of BSG of the complex increases.

**Table 3.** Antioxidant activities of mayonnaise prepared with whey protein-basil seed gum complex

| **Sample** | **CON**[2)] | **W-B 0.**2% | **W-B 0.**4 % | **W-B 0.**6 % | **W-B 0.**8 % | **F-value (*p*-value)** |
|---|---|---|---|---|---|---|
| Total phenol content (μgGAE/mg) | 17.01±0.95$^{e}$ | 40.45±0.21$^{d}$ | 46.42±0.36$^{c}$ | 49.29±2.18$^{b}$ | 52.88±1.56$^{a}$ | 369.192$^{***}$[1)] (0.000) |
| Flavonoid content (μg QE/mg) | 14.13±0.28$^{d}$ | 15.86±0.11$^{c}$ | 18.09±0.93$^{b}$ | 21.99±0.21$^{a}$ | 22.42±0.49$^{a}$ | 161.046$^{***}$ (0.000) |
| FRAP | 0.41±0.01$^{d}$ | 0.56±0.01$^{c}$ | 0.59±0.00$^{b}$ | 0.60±0.00$^{a}$ | 0.60±0.00$^{a}$ | 814.319$^{***}$ (0.000) |

1) Values are expressed as mean ± SD of triplicate observations

a-e Different superscripts indicate significant differences between values in the same row according to Duncan's range test ($p<0.05$)

$^{***}p < 0.001$

2) Control = mayonnaise with no addition of whey protein-basil seed gum, W-B 0.2%= mayonnaise with whey protein 5%- Basil seed gum 0.2 %, W-B 0.4%= mayonnaise with whey protein 5%- Basil seed gum 0.4 %, W-B 0.6%= mayonnaise with whey protein 5%- Basil seed gum 0.6 %, W-B 0.8%= mayonnaise with whey protein 5%- Basil seed gum 0.8 %.

*Sensory evaluation*

The sensory evaluation of mayonnaise with BSG complex was conducted for 6 catogory and evaluated as follows: appearance, oily odor, oil amplitude, mouthfeel, oily taste and overall acceptability. In the case of appearance, the value increased as the BSG content of the complex increased, resulting in the highest value 6.14 in the W-B 0.8 % and 3.88 in the W-B 0.2% but it was in the same category as the control group in the W-B 0.4 %-W-B 0.6 %. However, as the BSG content of the complex increased, the value also tended to increase ($p$<0.001). These results were consistent with the results of the study of Hashemi et al. (30) because the significance of the mayonnaise samples was not verified. In the study of Javidi et al. (35), the addition of BSG improved the texture which was similar to that of the present study. On the other hand, Golchoobi et al. (15) reported that as the content of guar gum increased, the sensory evaluation of mayonnaise was adversely affected.

## Conclusion

In this study, the oil content of mayonnaise was reduced by 50 % by using a mixture of whey protein and BSG as a fat substitute, and the stability, quality characteristics, antioxidant activity, and sensory evaluation during storage were investigated. The pH was the highest in the control group and increased as the BSG content of the mixture increased in the addition group, which was significantly different from the control group ($p$<0.001). The hardness, cohesiveness, gel strength, and viscosity were similar to each other, and the value also increased with increasing BSG content of the complex. The BSG complex addition group showed a similar texture to the control group.

**Table 4.** Sensory evaluation of mayonnaise made with whey protein-basil seed gum complex

| Sample | CON[3)] | W-B 0.2% | W-B 0.4 % | W-B 0.6 % | W-B 0.8 % | F-value ($p$-value) |
|---|---|---|---|---|---|---|
| Appearance | 5.88±1.54[ab1)] | 3.88±5.08[c] | 5.08±1.63[b] | 5.29±1.43[ab] | 6.14±1.70[a] | 7.346*** (0.000) |
| Oily odor | 5.58±1.44 | 5.12±1.79 | 5.16±1.52 | 5.75±1.54 | 5.64±2.22 | 0.696[NS2)] (0.596) |
| Odor amplitude | 5.50±1.67 | 4.96±1.79 | 4.92±1.66 | 5.67±1.49 | 5.36±2.08 | 0.881 [NS] (0.478) |
| Mouthfeel | 5.50±1.87 | 3.96±1.74 | 4.72±1.62 | 5.50±1.56 | 5.24±2.30 | 3.172 [NS] (0.016) |
| Oily taste | 5.25±1.59 | 4.52±1.56 | 4.92±1.58 | 5.367±1.81 | 4.82±2.46 | 1.416 [NS] (0.233) |
| Overall acceptability | 5.75±1.59 | 4.04±1.74 | 4.72±1.77 | 5.38±1.61 | 5.23±2.33 | 3.254 [NS] (0.014) |

1) Values are expressed as mean ± SD of triplicate observations
[a-b]Different superscripts indicate significant differences between values in the same row according to Duncan's range test ($p$<0.05)
***$p$ < 0.001
2) NS : No significant difference
3) Control = mayonnaise with no addition of whey protein-basil seed gum, W-B 0.2%= mayonnaise with whey protein 5%- Basil seed gum 0.2 %, W-B 0.4%= mayonnaise with whey protein 5%- Basil seed gum 0.4 %, W-B 0.6%= mayonnaise with whey protein 5%- Basil seed gum 0.6 %, W-B 0.8%= mayonnaise with whey protein 5%- Basil seed gum 0.8 %.

The *L*-value of color was the highest in the control group and significantly decreased as the BSG content of the complex increased ($p<0.001$). Contrastingly, the values of *a*- value and *b*-value showed a tendency to increase as the BSG content of the complex increased in the additive group; the value of the control group was the highest and the value of the W-B 0.2% was the lowest. Freeze-thawing stability and emulsion stability during 16 days of storage were not correlated with storage days, but the stability increased with increasing BSG content in all storage days. Total polyphenol, flavonoid, and FRAP showed an increase in the total polyphenol and flavonoid content as the BSG content of the complex increased, and FRAP also increased as the total polyphenol and flavonoid content increased, showing the highest value in the W-B 0.8 %. Sensory evaluation results showed that there was no significant difference in all category except for appearance; therefore, it seems that the substitution of mayonnaise will not have a big influence on the sensory quality of consumers. The results of this study suggested that BSG as a lipid system can be used as a basic data for various low-fat and non-fat product development.

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