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Edamame caspian sea soygurt as plant-based yogurt alternatives

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KEYWORDS

Caspian sea soygurt

Edamame

Fermented soybean

Sensory analysis

Yogurt

ABSTRACT

Caspian sea soygurt is made by fermenting edamame milk with *Lactococcus lactis* ssp. *cremoris* and *Acetobacter orientalis* as microbial cultures. To produce good soygurt, edamame milk fermentation requires ideal conditions, such as an optimum carbon source and starter concentration. This study aimed to determine the effect of the proportion of sucrose:skim and concentration of starter on the physical, chemical, microbiological, and sensory characteristics of Caspian sea soygurt. This study used a randomized block design with 2 factors: sucrose:skim proportion (2.5:7.5, 5:5 7.5:2.5) and starter concentration (8, 10, 12% of pasteurized edamame milk) repeated three times. Data were analyzed using ANOVA and continued with the Tukey test. These results was used to select the best treatment using the Zeleny Multiple Attribute Method. The best Caspian soygurt treatment was found in the proportion of sucrose:skim 5:5 (% w/v of pasteurized edamame milk) and starter concentration 12 (% v/v of pasteurized edamame milk), which produced a color (L*) 78.98, (a*) -2.86 (b*) 23.79, viscosity 516.67 cP, protein 3.38%, antioxidant activity 43.12%, pH 4.35, total lactic acid bacteria 9.85 log CFU/mL with a preference level of 3.45 and an acceptance level of 3.55 for the greenness, a preference level of 3.79 and an acceptance level of 3.80 for the brightness, a preference level of 3.15 and an acceptance level 3.21 on the sour aroma, preference level of 3.04 and acceptance level of 3.11for the beany aroma, preference level of 3.04 and acceptance level of 3.18 for the beany flavor, preference level of 3.02 and acceptance level of 3.19 for the sour taste, preference level of 2.98 and acceptance level of 3.09 for sweet taste, preference level of 3.49 and acceptance level of 3.60 for the viscosity.

Introduction

Yogurt is a popular fermented food that is widely consumed because it contains organic acids that are beneficial to health and has a distinctive taste. Yogurt was first discovered in the 18th century, where the bacteria used were *Lactobacillus bulgaricus* and *Streptococcus thermophillus* then developed until there were various types of yogurt(Alam et al., 2016). Caspian sea yogurt is a yogurt that was first traditionally produced in the Caucasus region and then introduced in Japan in 1986 by Dr. Yukio Yamori (Ishida et al., 2002; Kiryu et al., 2009; Uchida et al., 2009). It is classified into yogurt that uses mixed cultures, namely *Lactococcus lactis* ssp. *cremoris* and *Acetobacter orientalis* (Behare et al., 2016; Ishida et al., 2005).

Caspian sea yogurt has the potential to be developed because it has yogurt characteristics that consumers like, namely high viscosity and low acidity (Bayarri et al., 2011; Mantilla et al., 2022). The viscosity of Caspian sea yogurt is 3154 cP, while the viscosity of regular yogurt is around 1500-1600 cP (Niagari, 2023; Alkaisy and Rahi, 2023). *Lactococcus lactis* ssp. *cremoris* produces exopolysaccharides that cause the formation of a characteristic viscosity (Uchida et al., 2009). Exopolysaccharide characterization of *Lactococcus lactis* ssp. *cremoris* can act as a natural stabilizer that improves syneresis and viscosity in low-fat yogurt (Ng et al., 2022). *Acetobacter orientalis* oxidizes lactose in milk and produces lactobionic acid (Kiryu et al., 2009). Lactobionic acid consists of gluconic acid that binds to galactose. Lactobionic acid is a weak acid with an acid solubility degree (pKa) of 3.8 and a sweet taste (Shendurse& Khedkar, 2015).

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Edamame has been cultivated in China for thousands of years as medicine, then became popular since edamame was introduced and produced as an agricultural commodity by Japan and spread to other regions in East Asia and eventually to various regions globally, including Indonesia (Konovsky et al., 2020). Edamame has higher productivity, softer texture, larger seed size, and sweeter taste than soybeans (Carrão-Panizzi et al., 2019; Mozzoni and Chen, 2018). Edamame contains high protein with an amino acid composition that resembles milk protein, fiber, minerals, vitamins, and isoflavones as a source of antioxidants (Capriotti et al., 2015; Chen et al., 2022; Xu et al., 2022; Zeipin et al., 2017).The isoflavones in edamame are glycosides that have low bioavailability. The fermentation process can cause the glycosidic bonds in isoflavone glycosides to be degraded into aglycones, which are easily absorbed and have a higher functional value. (Hasim et al., 2015; Islam et al., 2014; Rafii, 2015). In addition, edamame also has an unpleasant aroma and taste which comes from the activity of the lipoxygenase enzyme. According to Adie (2001) and Barros et al. (2014), when edamame is exposed to oxygen, the lipoxygenase enzyme actively oxidizes polyunsaturated fatty acids and generates hydroperoxidases such as n-hexanal, which results in beany flavor. Deactivating the lipoxygenase enzyme through fermentation is one method used to reduce the unpleasant flavor and odor (Bruzantin et al., 2016). According to Li et al. (2017), increased acidity from soybean fermentation can enhance sensory attributes like taste, texture, aroma, and chemical content.

Processing edamame into Caspian sea soygurt can degrade the structure of the lipoxygenase enzyme, thus decreasing the unfavorable sensory attributes (Yang et al., 2016). The fermentation process also produces organic acids and increase the bioavailability of isoflavones, causing the antioxidants to increase (Lovabyta et al., 2020; Shori, 2013). Bacteria that play a role in fermentation require a carbon source to produce lactic acid. However, carbohydrates in edamame are in the form of oligosaccharidesm which are difficult for bacteria to use to ferment, therefore additional sources of carbon from outside are needed (Handayani & Wulandari, 2016; Mital & Steinkraus, 1975). The carbon sources widely used are sucrose and skim milk (Koswara, 2009). Sucrose is not only easy to obtain but can also reduce the unpleasant aroma and flavor of soybeans (Herlambang & Kusnadi, 2017). Skimmed milk is low-fat milk that contains high protein (FAO, 2018). Skim milk plays a role in improving texture and increasing protein content (Bruzantin et al., 2016). The purpose of this study was to determine the effect of the proportion of sucrose and skim and the concentration of starter on the physical, chemical, microbiological and sensory characteristics of Caspian sea soygurt from edamame milk.

Research and Methods

The study was conducted from November 2022 to April 2023 at the Microbiology Laboratory of Central Laboratory of Life Sciences, Food Processing Technology Laboratory and Laboratory of Sensory and Applied Food Sciences, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia.

Materials

The starter culture of Caspian sea yogurt contains *L. lactis* ssp. *Cremoris*and *A. orientalis* obtained from Fujicco, Japan. Fresh Edamame was purchased at the modern market, Malang. Commercial pasteurized milk from Greenfields brand. Sugar from Gulaku brand and skim milk from Bonigrasa brand were purchased at the traditional market in Malang, Indonesia. Other material used include distilled water (Hydrobat), MRS agar (Millipore), peptone water (Titan Biotech), DPPH (Sigma-Aldrich), ethanol (Merck), and phosphate buffer (Merck).

Preparation of starter culture

Making Caspian sea yogurt microbial starter culture based on Kiryu et al. (2009). Caspian sea yogurt starter was created by inoculating 0.6% culture starter powder into pasteurized milk at 30±5 °C in the biological safety cabinet, followed by 18 hours of incubation at 27 °C. Microbial starter rejuvenation was carried out using the backsloping method by taking 10% of the previous yogurt starter and inoculating it into pasteurized milk at 30±5°C under sterile conditions, then incubating at 27°C for 18 hours. The starter was rejuvenated three times, and then it can be used.

Preparation of soymilk

Soymilk was made based on a modification of Lovabyta et al. (2020). Edamame (100 g) was peeled, washed with water, and soaked in distilled water (1:1) and $NaHCO_3$ 0.5 g for 9±1 hours. The soaked edamame was blanched with 100 mL distilled water at 90±5 °C for 3 minutes. Then, the blanched edamame was grounded using a food processor by adding distilled water with the ratio of edamame and water 1:3 (w/v) for 3 minutes. The slurry was filtered through a cheese cloth to produce soymilk.

Production of caspian sea soygurt

Caspian sea soygurt production based on a modification of Herlambang and Kusnadi (2017). A 200 mL soymilk was added sucrose:skim milk (2.5:7.5, 5:5, 7.5:2.5% w/v of soymilk) and then pasteurized while stirring until it reached a temperature of 90±5 °C for 5 minutes. Next, soymilk was cooled to room temperature of 30±5 °C, inoculated with Caspian sea yogurt starter (8, 10, 12% v/v of soymilk), and incubated at 27 °C for 18 hours.

Analysis of physic and chemical parameters

The physical analysis included viscosity analysis measured by the method from AOAC(1995) while the color measurements L* , a* and b* were determined as described by Manasika and Widjanarko (2015). The chemical analysis included pH measured by the method from AOAC(1990), protein content was measured by the method from AOAC (2005), and antioxidant activity was determined by measuring the free radical scavenging ability of soygurt extract using DPPH inhibition assay as described by Susilo et al.(2023).

Analysis of total lactic acid bacteria

The total number of lactic acid was calculated using the method of Lee et al. (2015) with some minor modifications. The growth of LABs in the samples was measured using MRS (de Man Rogosa Sharpe) agar media after incubation at 37ºC for 24 h. The samples were serially diluted at 10-7, 10-8, and 10-9 using physiological solutions (NaCl, 0.85% w/v). The presence of LABs was determined by the appearance of a clear zone on the media surrounding the colony due to the reaction of LABs acid with $CaCO_3$ (1% w/v). Observations were conducted after 24 hours of fermentation. Colonies that are able to form clear zones were recorded and counted in terms of colony form unit (CFU)/mL sample (Jannah et al., 2014).

Sensory evaluation

Sensory testing consists of three tests: rating, preference, and acceptance. The rating test measures the intensity of the sample characteristics, the preference test measures the panelists' preferences, and the acceptance test measures the panelists' acceptance of the sample attributes. According to Lawless and Heymann (2010), sensory testing was performed by 104 untrained panelists.

Sensory testing was carried out by presenting 9 samples of Caspian sea Soygurt into 15-20 ml plastic cups and giving a different 3-digit random number code. Panelists were asked to rate the sample attributes, which included greenness, brightness, sour aroma, beany aroma, viscosity, sour taste, sweet taste, and beany flavor. Panelists use five hedonic scales to evaluate sample qualities in the three types of tests. On the test rating scale, 1: not very intense, 2: not intense, 3: moderately intense, 4: intense, 5: very intense. On the preference test scale 1: very dislike, 2: dislike, 3: slightly like, 4: like, 5: very like. On the acceptance test scale 1: not very acceptable, 2: not accepted, 3: slightly acceptable, 4: accepted, 5: very acceptable.

Statistical analysis

Data were expressed as mean±standard deviation (SD) from three independent parallel experiments. The analysis of variance was performed by ANOVA using Minitab 17 and significant differences among the means of samples were analyzed by Tukey's test with a 95 % confidence level. In addition, a correlation analysis was carried out to determine the relationship between the test parameters using Pearson Correlation in Minitab 17. Based on Schober and Schwarte (2018), the Pearson correlation is symbolized by the value (r), which has a scale:

- Scale 0.00–0.10 : negligible correlation
- Scale 0.10–0.39 : weak correlation
- Scale 0.40–0.69 : moderate correlation
- Scale 0.70–0.89 : strong correlation
- Scale 0.90–1.00 : very strong correlation

These results was used to choose the best treatment using the Multiple Attribute Method (Zeleny, 1982), as follows:

1. Determine the optimal value based on expectations, that is, the maximum or the lowest value of a parameter.

Parameters	**Ideal value assumption**
Preference sensory	highest
Acceptance sensory	highest
Physic, chemical, and microbiological	highest

2. Calculate the degree of density (dk) based on the ideal value of each parameter through the following equation:

The highest ideal value, then:

$$dk = \frac{\text{real value that is near to ideal}}{\text{the ideal value of each alternative}} \text{.....................} (1)$$

The lowest ideal value, then:

$$dk = \frac{\text{the ideal value of each alternative}}{\text{real value that is near to ideal}} \text{.....................} (2)$$

3. Calculating the density distance (Lp) assuming that all parameters are important. The density distance (λ) is calculated based on the number of parameters according to the following equations:

$$\lambda = 1 / \sum \text{parameter} \text{ ....} (3)$$
$$\text{L1} = 1 - \sum(\lambda\, 2 \text{ x } (1 - \text{dk})) \text{ ....} (4)$$
$$\text{L2} = \sum(\lambda\, 2 \text{ x } (1 - \text{dk})2) \text{ ....} (5)$$
$$\text{L}\infty = \text{max value } (\lambda \text{ x } (1 - \text{dk})) \text{ ....} (6)$$

4. The treatment with the lowest L1, L2, and L∞ values was chosen as the best.

Results and Discussion

Physical characteristics of caspian sea soygurt

Color (L, a*, b*)*

Caspian sea soygurt has a higher brightness (L*) (76.3-80.3) than edamame milk (73.9±0.46) (Table 1). The brightness of Caspian sea soygurt increased as the sucrose proportion decreased and the skim proportion increased at various starter concentrations. This shows that the brightness (L*) is affected by skim milk. Caspian sea soygurt with a higher proportion of skim (7.5%) than sucrose (2.5%) produced a higher brightness range of 79.9-80.3 compared to Caspian sea soygurt with a lower proportion of skim (2.5-5%) with a brightness range of 77.6-79.0 and the lowest is edamame milk (73.9).Caspian sea soygurt contains skim milk, however edamame does not. Skimmed milk is low or non-fat milk with a maximum fat content of 1.5% m/m (FAO, 2011), and it is whiter in colorthan whole milk, which is yellowish (Sadikin, 2002). Color properties of skimmed milk powder is (L*) 96.94±0.56, (a*) -2.32±0.14, (b*) 11.12±1.32 while color of whole milk is (L*) 81.0±8.1, (a*) -1.5±3.0, (b*) 7.5±4.4 (Milovanovic et al., 2020; Pugliese et al., 2017).

Edamame milk and Caspian sea soygurt have a negative chromaticity index (a*), indicating a green color(Lara et al., 2019). According to Table 1, the green color of edamame milk is higher (-3.6±0.10) than Caspian sea soygurt ((-2.7)-(-3.1)). The decrease in the green color (a*) of Caspian sea soygurt occurred along with the decrease in the proportion of sucrose and the increase in the proportion of skim at various starter concentrations. This shows that the decrease in green color (a*) occurs as the white color (L*) increases due to skim milk. Caspian sea soygurt with a higher proportion of skim (7.5%) than sucrose (2.5%) (G1S1, G1S2, G1S3) produced a greenish range of -2.7-(-2.8) lower than Caspian sea soygurt with a lower proportion of skim (2.5- 5%) with a greenish range of -2.8-(-3.1) and the highest is edamame milk (-3.6). The green color in edamame milk and Caspian sea yogurt is caused by chlorophyll contained in the chloroplasts of edamame. According to Hasibuan and Arini (2011), chlorophyll is a green pigment found in plants that comes in two varieties: chlorophyll-a, which is dark green, and chlorophyll-b, which is light green. The intensity of the greenish color can be changed by the heating process and the addition of other ingredients (i.e., skim milk). Processing of edamame milk into Caspian sea soygurt requires a pasteurization process where high temperatures will cause a decrease in the concentration of green color. This occurs by chlorophyll breakdown at 60-70°C, which is characterized by the release of chlorophyll molecules up to 80°C (Lipova et al., 2010).

Edamame milk and Caspian sea soygurt produce a positive chromaticity index (b*), which means they have a degree of yellowish color (Lara et al., 2019). Edamame milk has the same yellowish color (b*) (24.5±0.2) as Caspian sea soygurt (23.1-24.2). Furthermore, there was no variation in the quantities of the Caspian sea soygurt treatment in yellowish color (b*), as shown in Table 1. This means that the yellowish color is caused only by edamame chlorophyll pigment and is unaffected by sucrose or skim milk. This is because sucrose is a food additive in the form of colorless crystals (Plaza-Diaz and Gil, 2016) while skim milk is white in color due to dispersed fat globules, casein, and calcium phosphate (Prasetyaji, 2018) and so has no impact. According to Puspita et al. (2021), the light green color in edamame is due to chlorophyll b, which absorbs light blue and reddish orange wavelengths to produce a yellowish-green color.

Table 1. Physico-chemical and microbiological characteristics of Caspian sea soygurt edamame

Sample	Color			Viscosity (cP)	Protein (%)	Antioxidant (%)	pH	Total LAB (log cfu/ml)
	L*	a*	b*					
EM	73.9±0.46	-3.6±0.10	24.5±0.26	70±20.00	1.53±0.12	38.63±2.87	-	-
G1S1	79.9±0.35^{ab}	-2.8±0.29^{ab}	23.9±0.73^{a}	556.67±28.43^{bc}	3.10±0.33^{a}	49.18±0.78^{ab}	4.47±0.04^{c}	9.67±0.65^{d}
G1S2	80.0±0.32^{a}	-2.8±0.35^{ab}	24.0±0.79^{a}	751.67±112.40^{a}	3.37±0.06^{a}	51.28±0.95^{a}	4.52±0.03^{b}	9.73±0.69^{bc}
G1S3	80.3±0.12^{a}	-2.7±0.36^{a}	23.6±0.60^{a}	611.67±67.52^{b}	3.36±0.12^{a}	46.89±7.63^{abc}	4.51±0.03^{b}	9.73±0.62^{bc}
G2S1	78.9±0.03^{c}	-2.8±0.39^{ab}	24.2±0.55^{a}	451.67±45.52^{de}	2.75±0.36^{ab}	44.38±0.71^{abcd}	4.34±0.05^{e}	9.70±0.62^{cd}
G2S2	78.6±0.41^{cd}	-2.9±0.41^{bc}	24.2±0.49^{a}	491.67±68.25^{cd}	3.04±0.08^{a}	45.10±0.84^{abc}	4.40±0.03^{d}	9.89±0.61^{a}
G2S3	79.0±0.51^{bc}	-2.9±0.27^{abc}	23.8±0.55^{a}	516.67±50.08^{cd}	3.38±0.05^{a}	43.12±1.51^{bcd}	4.35±0.02^{e}	9.85±0.65^{a}
G3S1	76.3±0.46^{f}	-3.1±0.29^{c}	24.0±0.38^{a}	381.67±37.53^{ef}	2.90±0.04^{ab}	45.01±1.16^{abc}	4.20±0.03^{g}	9.72±0.67^{cd}
G3S2	77.6±0.67^{e}	-2.9±0.37^{bc}	24.1±0.48^{a}	395±20.00^{ef}	2.82±0.04^{ab}	36.62±1.61^{d}	4.30±0.02^{f}	9.74±1.01^{bc}
G3S3	77.6±0.12^{de}	-2.9±0.28^{bc}	23.1±0.34^{a}	353.33±35.12^{f}	2.32±0.17^{b}	41.08±0.14^{cd}	4.56±0.03^{a}	9.78±0.60^{b}

Notes: Values are means ± standard deviation (n = 3). Different letter in the same column mean significant different at α = 5% ($p < 0.05$). EM=Edamame Milk, G1S1=sucrose:skim milk 2.5:7.5% starter 8%, G1S2=sucrose:skim milk 2.5:7.5% starter 10%, G1S3= sucrose:skim milk 2.5:7.5% starter 12%, G2S1=sucrose:skim milk 5:5% starter 8%, G2S2=sucrose:skim milk 5:5% starter 10%, G2S3=sucrose:skim milk 5:5% starter 12%, G3S1= sucrose:skim milk 7.5:2.5% starter 8%, G3S2=sucrose:skim milk 7.5:2.5% starter 10%, and G3S3=sucrose:skim milk 7.5:2.5% starter 12%.

Viscosity

Edamame milk has a lower viscosity (70±20.00 cP) than Caspian sea soygurt (353.33-751.67 cP). The viscosity of edamame milk is affected by the denaturation of edamame protein caused by the blanching process. The viscosity of edamame milk in Cornelia and Lessy (2018) was lower (65.46 ± 4.20 cP) compared to the results in this study. This is due to changes in pasteurization temperature, which was utilized in this study at 90±5°C, whereas Cornelia and Lessy (2018) used a temperature of 70°C. The higher the heating temperature, the more the protein structure is modified. According to Liu and Chang (2007), the structure of β-conglycinin (7S) is denatured at 70°C while glycinin (11S) is denatured at 90°C. The greater the heating temperature, the more the subunits and polypeptides separate from the two proteins, causing aggregation with other components and polymerization to a lesser extent. This produces a rise in total dissolved solids, followed by an increase in viscosity (Shin et al., 2014).

A higher proportion of skim than sucrose at various starter concentrations leads to a higher increase in viscosity of Caspian sea soygurt. Table 1 shows that the Caspian sea soygurt with the highest viscosity was in the proportion of sucrose:skim 2.5:7.5 at various starter concentrations (556.67-751.67 cP) while the lowest was in the proportion of sucrose:skim 7.5:2.5 at various starter concentrations (353.33-395 cP). This indicates that raising the amount of skim milk protects probiotic cells from extracellular mechanisms, whereas calcium cations in skim milk can enhance the stability of cellular structures(Fu et al., 2018). According to research byBruzantin et al. (2016) and Marafon et al. (2011), powdered skimmed milk is frequently used to prevent texture damage due to syneresis by maintaining solid levels.

Microorganism activity during fermentation generates a restructuring of the protein structure, resulting in agglomeration and increased viscosity (Tamime and Robinson, 2007). As a result of the lactic acid generated by microbial activity, the pH drops below the isoelectric point (pH 4.6), causing the casein micelles to become unstable and coagulated(Lesme et al., 2020; Sinaga et al., 2016). Coagulation occurs by disulfide bonds created between k-casein and β-lactoglobulin, which are denatured by temperature and heating duration, protein content, and pH (Mahomud et al., 2017). Additionally, *Lactococcus lactis* ssp. *cremori*s as lactic acid bacteria produces exopolysaccharide filaments that bind mucosal bacteria with a protein matrix, which causes an increase in viscosity (Guzel-Seydim et al., 2005). Yogurt manufactured with polysaccharide-producing cultures has a higher viscosity than yogurt made without polysaccharide-producing cultures (Amatayakul et al., 2006; Patel et al., 2012).

Chemical characteristics of caspian sea soygurt

Protein

The protein content in edamame milk (1.53%) is lower than in Caspian sea soygurt (2.32-3.38%). However, the protein content of edamame milk in this study was higher than that of edamame milk in the study by Amtiran et al. (2018), which was 0.86%. This difference is caused by the heating time and temperature during the edamame blanching process, which in this study used a temperature of 90 ± 5°C for 15 minutes. In contrast,Amtiran et al. (2018) used a temperature of 85-100°C for 20 minutes. According to Putri et al. (2021), the longer the heating process on soybeans, the more protein content will decrease by 0.06% per minute. This occurs by protein denaturation, which reduces protein solubility and activity (Kristiningrum and Susanto, 2015).

Table 1 shows that the increase in protein content of Caspian sea soygurt was found with and increasing proportion of skim at each starter concentration. The percentage of sucrose:skim 2.5:7.5 with various starting concentrations had the highest protein content (3.10-3.37%), while the proportion of sucrose:skim 7.5:2.5 with various starter concentrations had the lowest (2.32-2.90%). This is due to the use of skim as a carbon source, which contains 33% protein(Fu et al., 2018). According to Tamime and Robinson (2007), increasing the amount of skim in yogurt causes an increase in protein content followed by an increase in total solids and viscosity.

Antioxidant

The antioxidant activity of edamame milk is relatively lower (38.63%) than that of Caspian sea soygurt, which was 44.74% on average. At each starter concentration, antioxidant activity in Caspian sea soygurt increased as the proportion of skim increased, with the proportion of sucrose:skim 2.5:7.5 producing antioxidant activity 46.89-51.28% higher than the proportion of sucrose:skim 5:5 (43.12-45.10%) and the lowest on sucrose:skim 7.5:2.5 (36.62-45.01%). The components in edamame milk that have antioxidant activity are polyamines (putrescine, spermidine, and spermine) and isoflavones (Ha et al., 1998; Fujisawa and Kadoma, 2005). The fermentation process in Caspian sea soygurt causes the breakdown of protein in edamame into amino acids, which results in higher antioxidant activity. Protein structural changes to peptides result in more active amino acid R groups that are more open, resulting in higher antioxidant activity compared to complete proteins (Chen et al., 1998).According to Matoba (2002), conglycinin and glycinin exhibit an increase in antioxidant capacity up to 3-5 times. Lovabyta et al. (2020) discovered that genistein and daidzein, which are isoflavones in the form of aglycones, resulted in a 13.56 mg/mL increase in IC50 antioxidant activity above genistin and daidzin, which are isoflavones in the form of glycosides.

pH

Caspian sea soygurt has a pH range of 4.20 to 4.56. The resulting pH range is less than 4.6, indicating the acidification process by lactic acid bacteria (Lee and Lucey, 2010). The increase in acidity indicated by Caspian sea soygurt's low pH value was demonstrated by increasing the amount of sucrose in each starter concentration (G3S1 = 4.20, G3S2 = 4.30, and G3S3 = 4.56). This study showed the same results as Herlambang and Kusnadi (2017) study, which reported decreases in pH from 4.37 and 4.28 to 4.12 and 4.10 in Caspian sea soygurt made from yellow and black soybeans with a rise in sucrose and a decrease in skim (75:25). This is because one of the Caspian sea yogurt bacteria, *Acetobacter orientalis*, has higher specificity in oxidizing d-glucose (97%) than other monosaccharides such as lactose, maltose, and cellobiose due to the presence of a membrane-bound glucose dehydrogenase enzyme with low lactose oxidation activity, i.e., 0.04% (Kiryu et al., 2009). *Lactococcus lactis* ssp. *cremoris*, another Caspian sea yogurt bacteria, can also break down sucrose and lactose via hexokinase and phospho-beta-galactosidase enzymes (Mills et al., 2011). Furthermore, the proportion of sucrose:skim 7.5:2.5 with a starter concentration of 8% generated the lowest pH, 4.20, compared to the concentrations of 10 and 12% at the same sucrose and skim proportions. Lactic acid bacteria have limits in their ability to convert glucose into lactic acid during fermentation (Syahputra et al., 2015). These constraints include the availability of important components such as Fe^{2+} and Ca^{2+}, as well as the availability of various carbon sources required by microorganisms to create organic acids, which might result in a pH reduction (Hayek and Ibrahim, 2013).

Microbiological characteristics of caspian sea soygurt

Total lactic acid bacteria

Total Caspian sea soygurt lactic acid bacteria ranged from 9.67-9.89 log cfu/ml. This range indicates that Caspian sea soygurt has fulfilled the yogurt quality standard's minimal requirement for total lactic acid bacteria, which is 10^7 colonies/g or 7 log CFU/mL (BSN, 2009). Table 1 demonstrates that the same quantities of sucrose and skim produce higher total lactic acid bacteria at each starter concentration, with concentrations of 10 and 12% yielding more total lactic acid bacteria (9.89 and 9.85 log CFU/mL) than concentration 8% (9.70 log CFU/mL). The findings of this study are consistent with the results of Larasati et al. (2016), who discovered that a high concentration of starter established at the start of Caspian sea yogurt fermentation resulted in a higher total lactic acid bacteria at the end of the fermentation process. Carbohydrates, as a source of microbial carbon, are another component that plays a role. Tamime and Robinson (2007) discovered that adding 4-5% sucrose to yogurt resulted in optimum development of lactic acid bacteria. Due to variations in osmotic pressure, the addition of too much sucrose inhibits the development of lactic acid bacteria (Koswara, 2009). Moreover, a high proportion of skim milk in the probiotic drink reduced total lactic acid bacteria (Hakiki et al., 2022). This is because excessively concentrated skim will produce hypertonic conditions in which the bacteria will lead to plasmolysis (Waluyo, 2005). The amount of skim that can be added to yogurt fermentation ranges between 1-6% (Tamime and Robinson, 2007).

Sensory characteristics of Caspian sea soygurt

Greenness and brightness

Figures 1a and 1b show that the interactions of sucrose:skim and starter concentrations were not significantly different in greenness and brightness color characteristics of. Color is an obvious visual component that impacts the views of panelists (Lestari and Susilawati, 2015). However, the study's findings revealed that variations in the proportions of sucrose:skim and starter concentrations created no significant differences in the greenness intensity and brightness of Caspian sea soygurt. The level of preference and acceptance also did not show significant differences between treatments. Herlambang and Kusnadi (2017) research found the same phenomenon, with panelists responding to the color of Caspian sea soygurt with the treatment of sugar and skim concentrations.

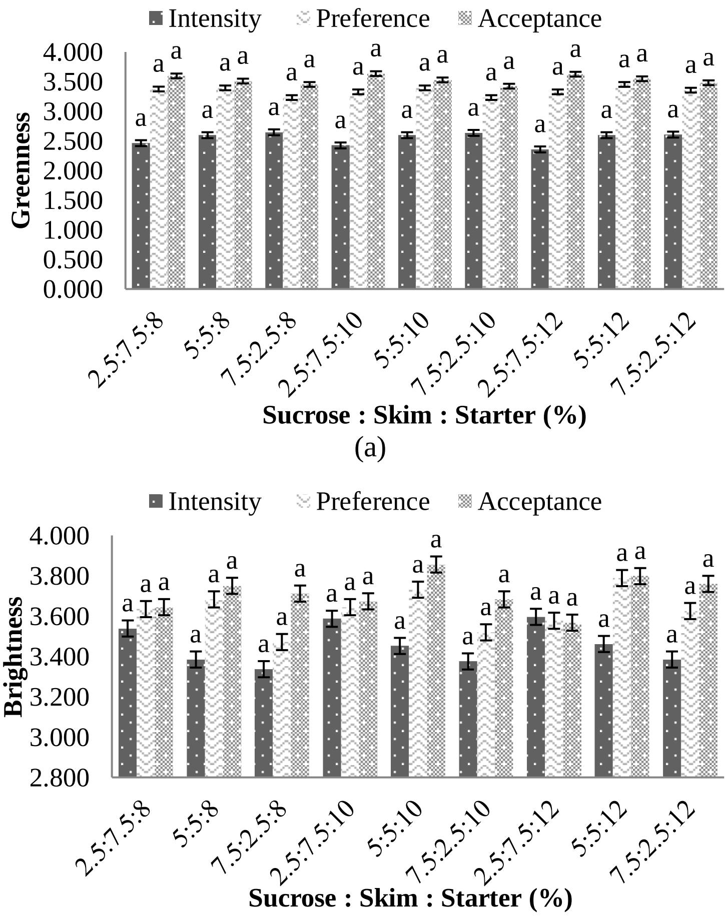


Figure 1. The effect of the proportion of sucrose:skim on the greenness (a) and brightness (b) attribute at various starter concentrations. Error bars represent standard deviation from three measurements. Different letter means significant different at α = 5% ($p < 0.05$).

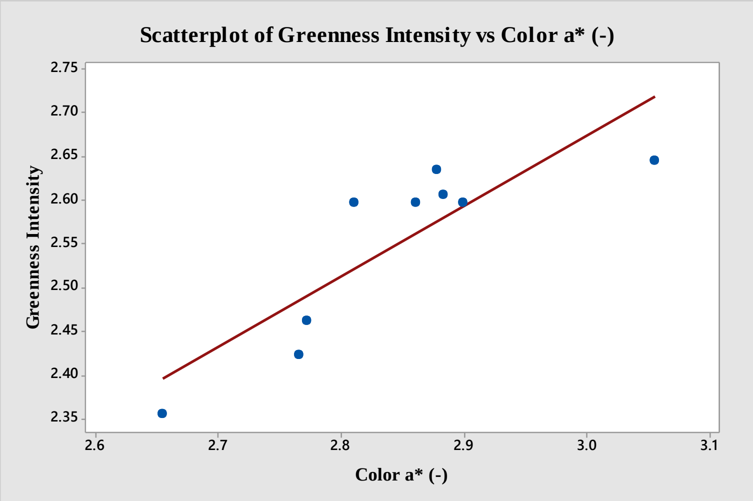


Figure 2. Correlation of greenness intensity with color a*(-) Caspian sea soygurt

The results of testing the level of greenness intensity and brightness by the panelists are in accordance with the results of the color tests (L* and a*) by the color reader. The relationship between greenery and color attributes (a*) shows a strong correlation with a value of r=0.859 (Figure 2). Likewise, the correlation between the brightness attribute and color (L*) has a value of r=0.914. The factor that affects the green intensity and brightness of Caspian Sea Soygurt is skim milk. Caspian sea soygurt with the proportion of sucrose:skim 7.5:2.5% starter concentration of 8% produced the highest greenness intensity and the lowest brightness. While, the proportion of sucrose:skim 2.5:7.5% starter concentration of 12% produced the lowest greenness intensity and the highest brightness. According to Sadikin (2002), skim milk is non-fat milk that is whiter in color than whole milk and, when added to food items, increases the white color index or brightness.

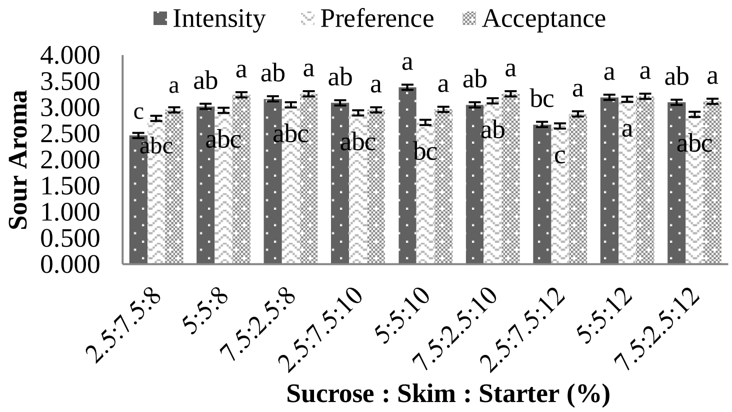
Sour aroma attribute

Aroma is an attribute that can provide a quick assessment of whether a food product is liked or disliked. Figure 3a shows that the interaction between sucrose:skim and starter concentration had a significant effect ($p<0.05$) on the level of intensity and preference for sour aroma.The proportion of sucrose:skim 2.5:7.5% and starter 8% produced a Caspian sea soygurt with the lowest intensity of sour aroma and distinct from the other treatments. This demonstrates that decreasing the proportion of sucrose as a carbon source and decreasing the number of lactic acid-producing bacteria affect the quantity of lactic acid generated, resulting in a low intensity of the sour aroma. Sucrose is the primary carbon source for lactic acid bacteria, which produces lactic acid and acetaldehyde, which contribute to the odor and flavor of yogurt (Panagiotidis and Tzia, 2007; Pineli et al., 2016).

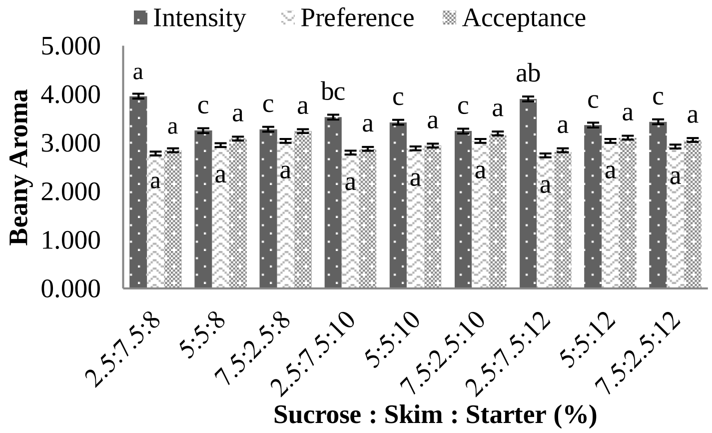
The highest preference for sour aroma was found in the proportion of sucrose:skim 5:5% starter 12%, while the lowest preference level was in the proportion sucrose:skim 2.5:7.5% starter 12%, but both had the same level of acceptance. This shows that Caspian sea soygurt with the highest intensity of sour aroma is preferable than lower intensity of sour aroma but still acceptable to the panelists. Panelists' preference for sour aromas with higher intensity is due to high sour aromas can mask or reduce unfavorable sensory characteristics such as the aroma of beany and the taste of soybeans. This is supported by the correlation between sour aroma with beany aroma and soybean flavor, which shows a strong and opposite correlation (r=-0.814 and r=-0.686) (Figure 4).

Beany aroma attribute

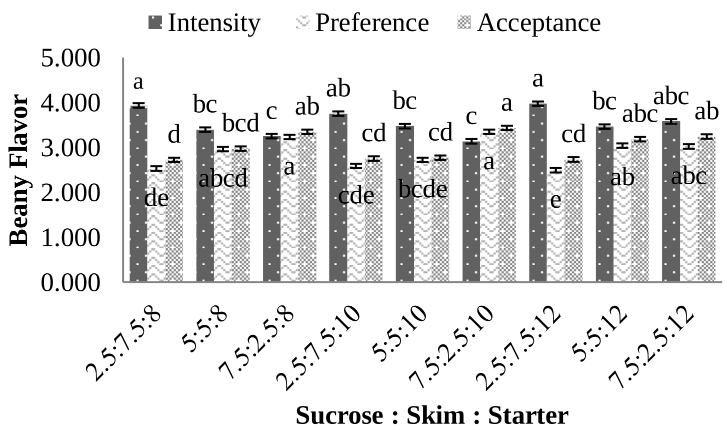
The beany aroma is a distinctive aroma that comes from soybeans and their processed products. Figure 3b shows that the interaction between sucrose:skim and starter concentration had a significant effect ($p<0.05$) on the intensity of the beany aroma. The highest intensity level of beany aroma is found in the proportion of sucrose:skim 2.5:7.5% with 8% starter. This shows that the high intensity of beany aroma is influenced by the proportion of sucrose and low starter concentration. Snyder and Wilson (2003) stated that sucrose is a natural additive that plays a role in disguising the aroma and taste of soybeans.



(a)



(b)



(c)

Figure 3. The effect of the proportion of sucrose:skim on the sour aroma (a), beany aroma (b), and beany flavour (c) attribute at various starter concentrations. Error bars represent standard deviation from three measurements. Different letter means significant different at α = 5% ($p < 0.05$).

Figure 3b shows that the proportion of sucrose:skim and starter concentration did not significantly affect the liking and acceptance level of beany aroma indicated by the same notation. This is assumed to be due to the difficulty in detecting the beany aroma because, according to Wilkens et al. (1970), the aroma of beany is derived from the volatile molecule hexanal, which has the lowest detection threshold.

Beany flavor attribute

Flavor is a combination of all the perceptions of the senses of smell, feeling, and sight that determine consumer preferences. Figure 3c shows that a low proportion of sucrose at various starter concentrations results in a high intensity of soybean flavor but a low level of preference and acceptance. This aligns with the findings of this study on the beany aroma attributes.Decreasing the proportion of sucrose results in an increase in the intensity of the soybean flavor accompanied by an increase in the intensity of the beany aroma. The correlation between the two attributes occurs in the same direction and is very strong, with a value of r = 0.934 (Figure 5). Caspian sea soygurt with the highest level of preference and acceptance of the beany flavor attribute was in the proportion of sucrose:skim 7.5:2.5% and 10% starter concentration which had the lowest beany flavor intensity. Sucrose is a carbon source for microbes to produce lactic acid. Therefore, an increase in sucrose will increase lactic acid, which may increase the sour aroma and decrease the beany aroma and beany flavor (see Figure 5). The taste and aroma of soybeans are unwanted sensory attributes formed due to the oxidation of unsaturated fatty acids by lipoxygenase enzymes in soybeans (Yang et al., 2016).

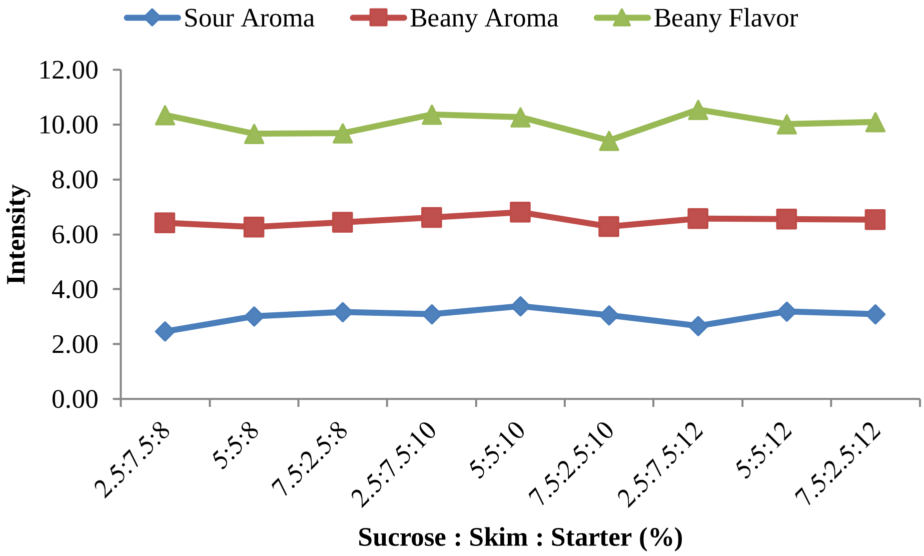


Figure 4. Correlation of the intensity of sour aroma with beany aroma and beany flavor of Caspian sea soygurt

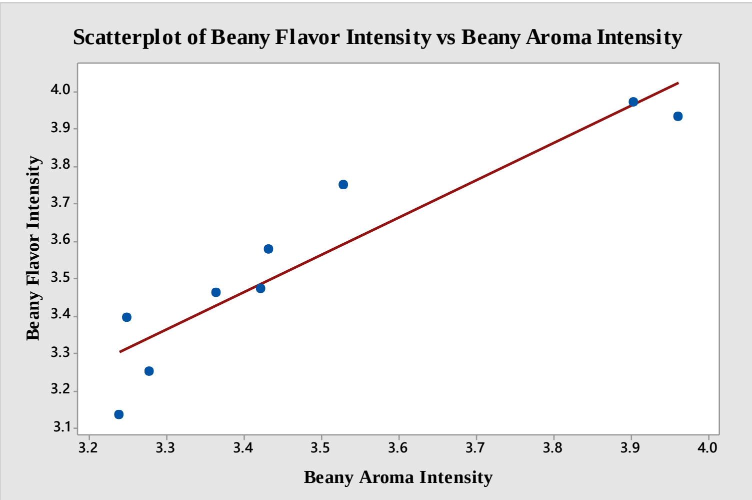
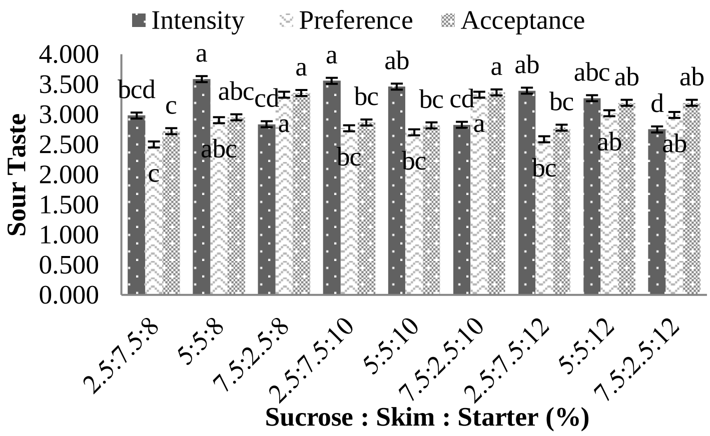
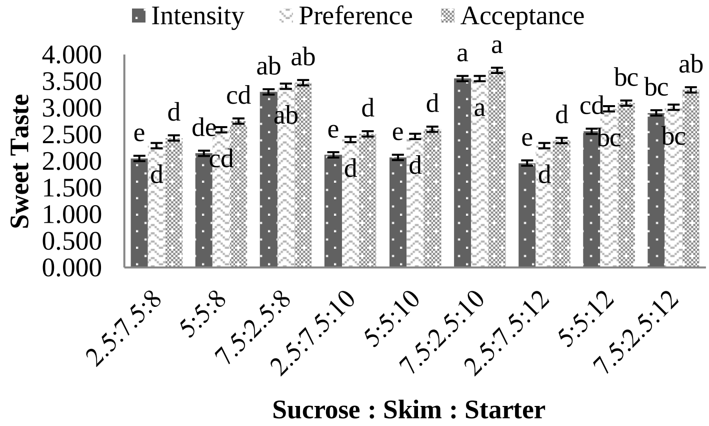


Figure 5. Correlation of the intensity of beany flavor with beany aroma of Caspian sea soygurt



(a)



(b)

Figure 6. The effect of the proportion of sucrose:skim on the sour taste (a) and sweet taste (b) attribute at various starter concentrations. Error bars represent standard deviation from three measurements. Different letter means significant different at $\alpha = 5\%$ ($p < 0.05$).

Sour taste attribute

Taste is a sensation formed from the combination of the ingredients and the composition of a food product captured by the senses of taste (Yansyah et al., 2016). Figure 6a shows that high sucrose and low skim proportion at various starter concentrations produce a low sour taste intensity but have a high degree of preference and acceptance. According to research Baldwin et al. (2008) and Peris (2016), sucrose has a sweet taste and can reduce the sour taste generated by lactic acid bacteria fermentation. As stated by Bayarri et al. (2011), fermented products with a low level of sour flavor are favored by customers.

Sweetness attribute

Sweet taste sensory testing involves the activation of taste receptor cells distributed throughout the surface of the oral cavity, which then results in transmission and is sent to parts of the brain involved in taste processing (Low et al., 2014). Figure 6b shows that the proportion of high sucrose and low skim at various starter concentrations results in a high level of sweet taste intensity accompanied by increased liking and acceptance. According to Tamime and Robinson (2007), sucrose provides a sweet flavor while suppressing the acidity of yogurt. Herlambang and Kusnadi (2017) also reported that increasing sucrose in Caspian sea soygurt from yellow and black soybeans could increase panelist preferences. Wan et al. (2021) confirm this by stating that adding sugar can mask an unpleasant flavor and improve the acceptability of a product's taste.

Viscosity attribute

Viscosity is a textural attribute where the test is the outcome of a tactile sense reaction to a kind of physical stimulation when the components in the mouth cavity come into contact with food (Tarwendah, 2017).

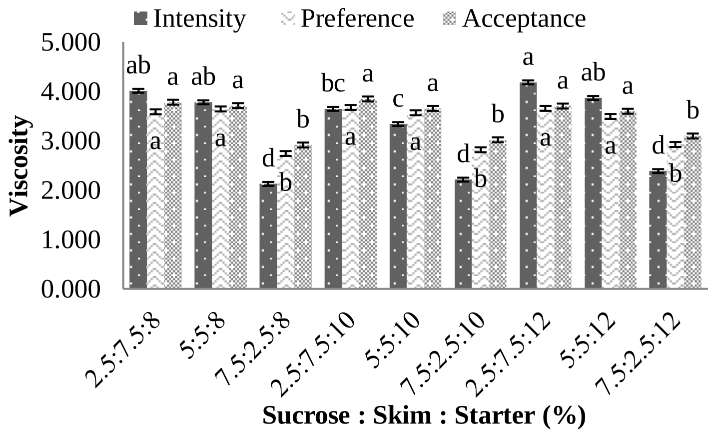


Figure 7. The effect of the proportion of sucrose:skim on the viscosity attribute at various starter concentrations. Error bars represent standard deviation from three measurements. Different letter means significant different at $\alpha = 5\%$ ($p < 0.05$).

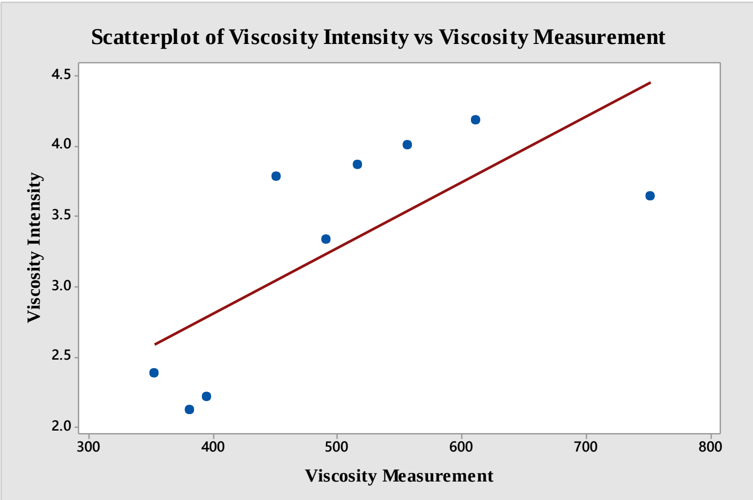


Figure 8. Correlation of the intensity of viscosity assessed by panelists with viscosity measured by viscometer of Caspian sea soygurt

Figure 7 shows that the proportion of low sucrose and high skim at various starter concentrations results in a high level of viscosity intensity accompanied by an increase in liking and acceptance. Factors that influence the increase in viscosity are skim, which has a high protein content and the role of microbial starter. According to Lesme et al. (2020) and Mahomud et al. (2017), the lactic acid produced during fermentation will lower the pH to an isoelectric point where k-casein and β-lactoglobulin will denature and agglomerate to form disulfide bonds. In addition, the increase in starter also affects the viscosity. According to Guzel-Seydim et al. (2005), the exopolysaccharide filaments produced by *Lactococcus lactis* ssp. *cremoris* as Caspian sea yogurt cultures play a role in binding the mucosal bacteria with the protein matrix so that the viscosity increases. The level of viscosity intensity assessed by the panelists correlated strongly with the results of viscosity measurements using a viscometer. This correlation value was r = 0.723, as shown in Figure 8. In addition, panelists who prefer and accept Caspian sea soygurt with high viscosity intensity are in accordance with research by Mantilla et al. (2022), which states that consumers prefer yogurt with thick texture, soft, and can be spooned.

The best treatment

Determining the best treatment for Caspian sea soygurt was carried out based on the Zeleny method. The parameters used as determinants were the level of preference, acceptance and physical, chemical, and microbiological characteristics. The selection of the best treatment quantitatively by looking at the highest and lowest averages for each parameter according to the expected value, then choosing the smallest sum of L1, L2 and Lmax.

Caspian sea soygurt made with a sucrose:skim ratio of 5:5 and a 12% starter concentration was selected as the best treatment. The result is color (L*) 78.98, (a*) -2.86 (b*) 23.79, viscosity 516.67 cP, protein 3.38%, antioxidant activity 43.12%, pH 4.35, total lactic acid bacteria 9.85 log CFU/mL. Previous research found a difference between this result and Caspian sea yogurt as a control, with color (L*) 86.7, (a*) -1.2, (b*) 13.7, viscosity 1249 cP, pH 4.58, and total lactic acid bacteria 8.75 log CFU/mL (Niagari, 2023).

The best treatment of Caspian sea soygurt resulted in a preference level of 3.45 and an acceptance level of 3.55 for the green attribute, a preference level of 3.79 and an acceptance level of 3.80 for the brightness attribute, a preference level of 3.15 and an acceptance level 3.21 on the sour aroma attribute, 3.04 preference level and 3.11 acceptance level for the beany aroma attribute, 3.04 preference level and 3.18 acceptance level for the soybean flavor attribute, 3.02 preference level and 3.19 acceptance level for the sour taste attribute, 2.98 preference level and 3.09 acceptance level for attribute of sweet taste, level of preference 3.49 and level of acceptance of 3.60 on the attribute of viscosity.There were variations observed in the outcomes between the best treatment and Caspian sea yogurt used as a control in earlier research. In that study, the hedonic ratings were 4.29 for color, 3.55 for aroma, 4.21 for taste, and 3.95 for texture(Herlambang and Kusnadi, 2017).

Conclusions

Interaction of sucrose:skim proportions and starter concentration in the manufacturing of Caspian sea soygurt showed a significant ($p<0.05$) effect on color (L*, a*), viscosity, protein, antioxidant activity, pH, total lactic acid bacteria, intensity of greenness and brightness, beany aroma, intensity and preference of sour aroma, intensity, preference and acceptance of beany flavor, sour taste, sweet taste, and viscosity. The strong correlation between experimental data and sensory data was found in the color parameters of brightness, greenness, and viscosity. The proportion of sucrose:skim 5:5 and 12% starter concentration was the best treatment for Caspian sea soygurt.

Declarations

Conflict of interests The authors declare no competing interests.

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