Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 9, No. 8, 965-975
2025
Publisher: Learning Gate
DOI: 10.55214/2576-8484.v9i8.9514

The influence of the composition of "Alcosoy" milk and variations in lactose levels on fermentation by lactic acid bacteria regarding the formation of lactic acid, the growth of lactic acid bacteria, viscosity, antioxidant activity, and Vitamin B_1

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Abstract: Almond milk yogurt, corn, and soy (Alcosoy) is a probiotic drink that has a relatively complete nutritional value, with the ingredients reinforcing each other to support health. This study has been conducted with experimental treatment factors including the composition of alcosoy almond milk: corn: soy (1:1:1; 2:1:1; 1:2:1; and 1:1:2) and lactose concentrations of 4%; 5%; and 6%. The results of this study show a significant difference in response to the levels of lactic acid, the growth of BAL microorganisms, viscosity, and the content of vitamin B1 in alcosoy yogurt. Meanwhile, the response to antioxidant activity showed no significant difference. The composition 1:2:1 with a lactose concentration of 6% shows a lactic acid level of 2.28% and a growth of lactic acid bacteria (LAB) at 4.03E7 cfu/ml. The response of viscosity composition 2:1:1 and a lactose content of 4% showed the highest viscosity of 0.0614 cm^2/s. The response to antioxidant activity overall exhibited strong activity (50 – 100 ppm), vitamin B1 content composition 2:1:1 and a lactose concentration of 4% showed the highest level at 0.31 mg/100 ml.

Keywords: *Alcosoy, Almond, Corn, Fermentation, Soybean, Yogurt.*

1. Introduction

Yogurt is known to play a vital role in health, including benefits for those with lactose intolerance, a symptom of lactose malabsorption that is common among the population, particularly children. These various benefits are primarily due to the bacteria used in the yogurt fermentation process [1]. Consuming yogurt offers numerous benefits to the body, including regulating the digestive tract, antidiarrheal, anticancer, promoting growth, helping those with lactose intolerance and regulating blood cholesterol levels, reducing tumors and cancer in the digestive tract, combating the growth of pathogenic bacteria in the digestive tract, stimulating bowel movements, and containing calcium, which is excellent for bone health and can prevent osteoporosis [2].

Yogurt is a coagulated product of milk resulting from the fermentation process of lactose into lactic acid by lactic acid bacteria (LAB) as starters, namely L. *bulgaricus* and S. *thermophilus* [3]. LAB act as probiotics, namely live bacteria that can provide health effects to their hosts when consumed in sufficient quantities [4]. Probiotics are included in functional foods, this is because probiotics can maintain the balance of gut microbiota, improve the body's immunity, and provide other health benefits [5]. Another use of LAB can also be to enhance the texture and flavour of yogurt, for example, in the cheese-making process. In this research, the substrate used is not cow's milk, but a plant-based substrate formulated from almond milk, corn milk and soy milk. (*Alcosoy* Milk).

History: Received: 13 June 2025; Revised: 1 August 2025; Accepted: 5 August 2025; Published: 18 August 2025
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Vegetable juice is a juice made from plants. Generally, the varieties most often processed into vegetable juice are those derived from cereals and legumes. Each variety has distinct superior characteristics [6]. These vegetable juices can be combined and made into yogurt, which can result in the characteristics of a vegetable juice yogurt made from a single type of vegetable juice. In this study, vegetable extract or vegetable milk was used as a substitute for animal milk.

Plant-based milk is a beverage produced or made from plants, particularly nuts and cereals. Plant-based milk is an alternative dairy product for those allergic to lactose from cow's milk (animal milk). One of the ingredients in plant-based milk is fiber. Fiber is difficult to obtain from animal milk, such as cow's milk. This fiber has excellent benefits for the body, including improving digestion [7]. Plant-based milk is also rich in vitamins and minerals such as vitamin E, vitamin B, antioxidants, phosphorus, and isoflavones. Currently, there are not many types of processed beverages made from plant extracts or plant-based milk on the market. The most popular or well-known types are soy milk, mung bean milk, and almond milk [8].

Almond is known as one of the alternative raw materials in the production of plant-based products because it has a low risk of causing allergies in individuals with specific conditions. The antioxidant compounds *flavonol* and flavone-3-ols contained in almonds have many benefits to the digestive tract and can be antiviral, anti-inflammatory, antiallergenic, anti *mutagene*, anticancer and also anti-cholesterol [9]. These antioxidants can suppress the occurrence of increased oxidative stress in individuals with Autism Spectrum Disorder (ASD) conditions [10]. Almonds contain high nutrients, per 100 grams of total fat (nutrition) of 49.9 g, food *fiber* 12.2 g, vitamin B (B1, B2, B3, B6) 4.7 mg, vitamin E 25.63 mg, and high Ca, K, and P respectively 269.481, and 733 mg [11]. In addition, the *fiber* content found in almonds also has prebiotic potential, making it suitable as a raw material for the production of plant-based yogurt. One of the uses of almonds is to make milk (almond milk), in this research, almond milk is used as the raw material for the production of "*alcosoy* yogurt".

Corn is a cereal crop that has the potential to be developed as a functional food product because corn contains essential amino acids, minerals, dietary fiber, essential fatty acids, and other things that the body needs [12]. In addition, corn is also a source of raw materials for the industrial sector, including the food industry. Corn contains many functional food components such as dietary fiber needed by the body, essential fatty acids, isoflavones, minerals (Ca, Mg, K, Na, P, Ca, and Fe). Anthocyanins, beta-carotene (provitamin A), and others [13]. The chemical composition of corn kernels generally consists of carbohydrates, proteins, fats, vitamins, minerals, and other organic materials. Carbohydrates in corn kernels contain reducing sugars (glucose and fructose), sucrose, polysaccharides, and starch, amounting to 10-11% [14]. Corn carbohydrates consist of starch, sugars, dietary fiber, and pentosans. Corn starch consists of amylose and amylopectin, while sugar is in the form of sucrose. The advantages of corn juice compared to cow's milk are that it is lactose-free and contains more fiber, making it suitable for those on a diet and those who are lactose intolerant [15]. Corn contains essential dietary fiber and has a relatively low glycemic index (GI) compared to rice. In this research, the production of corn milk has been carried out as a raw material for the production of plant-based yogurt. (*alcosoy* yogurt).

Soybeans are a source of plant-based protein that is widely utilized as a food source for the world's population in several countries, especially in developing nations. Soy milk is a processed product extracted from soybeans. Soy protein has a similar amino acid structure to cow's milk, making it a popular substitute for cow's milk. Processed soybean products mostly consist of products that are not fermented. Some of these products are soybean oil and its processed products, such as soy flour, isolate and soy protein concentrate, as well as synthetic meat or TVP (Texturized vegetable protein) [16]. Soybeans contain protein reaching 35-38% greater than green beans. In addition to high protein, soybeans also contain vitamins B1, B2, niacin, pyridoxine, vitamins E and K [17]. Soy milk has a protein content equivalent to cow's milk, at around 3.5 g/100 g, This amount is the content before being processed into vegetable milk [18]. Furthermore, it is lactose-free and has a lower fat content, at 2.5 g/100 g [19]. Soy milk is a highly nutritious beverage, primarily due to its protein content, which is equivalent to cow's milk, at approximately 3.5g/100g, and slightly lower in vitamins and minerals.

Furthermore, soy milk is lactose-free and has a lower fat content (2.5g/100g), making it suitable for those on a low-fat diet. Soy milk contains a small amount of calcium and phosphorus, which play a role in bone and tooth formation [20]. In this research, the production of soy milk has been carried out to serve as a raw material for plant-based yogurt. (*alcosoy* yogurt).

Alcosoy milk is a mixture of almond milk, corn milk, and soy milk. *Alcosoy* yogurt is a product resulting from the fermentation of *alcosoy* milk by lactic acid bacteria (LAB), namely Lactobacillus bulgaricus and Streptococcus thermophilus. *Alcosoy* yogurt contains complementary plant-based proteins from essential amino acid sources derived from almonds, soybeans, and corn. In addition to being rich in plant-based protein, *Alcosoy* yogurt is also abundant in essential fatty acids, minerals, and vitamins, making it a probiotic health drink.

2. Materials and Methods

2.1. Materials and Equipment

The ingredients used in the process of making *alcosoy* yogurt are almonds, soybeans, corn, lactose, water, and the starters Lactobacillus bulgaricus FNCC 0041 and Streptococcus thermophilus FNCC 0040. The materials used for the analysis are water (*aquadest*), De Mann *Ragoso* Sharp Broth (MRSB) and De Mann *Ragoso* Sharp Agar (MRSA) for culture growth, 0.1 N NaOH solution, pp indicator, alcohol, and NaCl. The tools used are a blender, filter paper, chemical glassware, Erlenmeyer flask, incubator (*Memmert* 50Hz), weighing and volume measuring instruments, pH meter (HI 8424), autoclave (*Witeclave Daihan* Scientific 1 atm.), viscometer, spatula, stainless steel knife, aluminium foil, cotton, test tubes, petri dishes, Erlenmeyer flasks, micropipettes, graduated cylinders, Bunsen burner, and colony counter. (Stuart Scientific). and others.

2.2. Method

This research uses a factorial Randomized Block Design (RBD) with the treatment factor of plant-based milk formulation (F) composed of almond, corn, and soy, consisting of 4 levels: f_1 (1:1:1), f_2 (2:1:1), f_3 (1:2:1), and f_4 (1:1:2), and the factor of lactose concentration (C) consisting of c_1 (4%), c_2 (5%), and c_3 (6%). This treatment is repeated three times, resulting in a total of 36 experimental units. The data obtained were analyzed using analysis of variance to determine the effects on the observed or tested response parameters, namely the response of lactic acid levels, total LAB, and viscosity. If there are significant differences, whether from independent treatment factors or interactions of treatment factors, further tests will be conducted using the Duncan test [21].

2.3. Data Collection

2.3.1. Starter Preparation

The culture refreshment is carried out by adding one loop of Lactic Acid Bacteria (LAB) isolate (Lactobacillus bulgaricus FNCC 0041 and Streptococcus thermophilus FNCC 0040) from slant agar into 10 ml of MRS Broth media in a sterile test tube. The tube was then vortexed until homogeneous and incubated for 18 hours at 37 °C. The refreshed culture can then be used as inoculum.

2.3.2. Preparation of Alcosoy Milk

Preparation of making alcosoy refers to research [22].

1. Almonds are soaked for 12 hours in clean water (until fully submerged), then drained.
2. Whole corn is boiled until cooked, cooled, then shelled, until cooked corn kernels are obtained.
3. Soybeans are soaked in a 0.5% $NaHCO_3$ solution for 12 hours, boiled in a 0.5% $NaHCO_3$ solution for 30 minutes, skins are removed and washed, then drained.
4. Each of the ingredients almond milk, corn milk, and soybeans milk should be weighed according to the compositions of 1:1:1; 2:1:1; 1:2:1; and 1:1:2, so that the total amounts to 600 grams.

5. The mixture of 600 grams is then added to 3000 ml of water and blended using a blender, followed by straining with a double-layered cloth, resulting in *alcosoy* milk.

2.3.3. Fermentation of Alcosoy Milk

Alcosoy milk in each treatment was inoculated with a mixture of lactic acid bacteria starter prepared at 5% (30 ml) of the volume of *Alcosoy* milk, while stirring until all starter clumps dissolved. The milk was then incubated at a temperature of 37 °C for 24 hours.

2.3.4. Variable Response Analysis

1. Analysis of Lactic Acid Levels. Lactate levels were measured every 4 hours for 24 hours of incubation using the Manns Acid Test method [23].
2. Calculation of Total Bacteria. The count of Lactobacillus bulgaricus bacteria was performed using the pour plate method (MRSA) [24] on MRSA medium, conducted in duplicate.
3. Measurement of Viscosity using a *falling ball viscometer*.
4. Measurement of antioxidant activity using the DPPH (*2.2-diphenyl-1-picrylhydrazyl*) oxidation method.
5. Vitamin B_1 using UV-VIS Spectrophotometry method.

3. Results and Discussion

3.1. Response to the Lactic Acid Levels (%) of Alcosoy Yogurt after 24 Hours of Incubation.

Through the results of the Two Way ANOVA test, it is stated that the interaction between the composition of *alkosoy* milk and lactose concentration shows a significant difference in the response of lactic acid levels. More clearly, as shown in the table below.

The composition of *alcosoy* with varying compositions results in different nutritional content, especially in sugar content. Similarly, the addition of varying amounts of lactose further enhances the nutritional composition of *alcosoy* milk, leading to the production of lactic acid metabolites that show significant differences.

Table 1.
Response of lactic acid levels (%) from *alcosoy* yogurt after 24 hours of incubation.

Factor (F), Composition of Almond milk: Corn milk: Soy milk.	Factor (C), Lactose Concentration		
	c_1 (4 %)	c_2 (5 %)	c_3 (6 %)
f_1 (1:1:1)	1.65 a	1.94 b	2.12 c
	A	B	B
f_2 (2:1:1)	1.63 a	1.72 b	1.86 c
	A	A	A
f_3 (1:2:1)	2.15 a	2.22 ab	2.28 b
	B	C	C
f_4 (1:1:2)	1.62 a	1.78 b	1.98 c
	A	AB	B

Note: Lowercase notation compared horizontally, the same lowercase notation indicates no significant difference in response. Uppercase notation compared vertically, the same uppercase notation indicates no significant difference in response.

Table 1 shows that the composition of *alcosoy* milk (almond milk, corn milk, soy milk 1:2:1) indicates a higher level of lactic acid compared to the others. This occurs because the sugar content in this composition is greater, leading to a higher production of lactic acid through fermentation by lactic acid bacteria (LAB).

Table 1 also reveals that the fermentation results of lactic acid at a lactose concentration of 6% are higher than those at lactose concentrations of 4% and 5%. This indicates that a greater amount of added lactose will yield a higher metabolite product of lactic acid; however, there is certainly a limit and an optimal level for lactose content. Lactic acid fermentation by lactic acid bacteria (LAB) can occur from lactose, glucose, and sucrose substrates; therefore, sugar substrates can influence the amount of lactic acid metabolite produced. In general, the substrate utilized by microorganisms is primarily for biomass growth. The growth of LAB isolates is characterized by an increase in cell biomass, which is also accompanied by an increase in secondary metabolites in the form of lactic acid and other organic acids. [25].

The mechanism of lactate formation from lactose into lactic acid begins with the hydrolysis of lactose in bacterial cells by β-D-galactosidase into glucose and galactose, and then by the enzyme β-D-phosphogalactosidase into glucose and galactose-6-phosphate. Subsequently, the glucose produced through the EMP pathway is converted into pyruvic acid, and finally, the enzyme lactate dehydrogenase converts pyruvic acid into lactic acid. The breakdown of lactose into lactic acid by homofermentative lactic acid bacteria occurs through the glycolysis process (*Embden-Mayerhoff Parnass* pathway/EMP) by converting lactose into pyruvic acid, which is then split into lactic acid. EMP is one of the pathways for the breakdown of glucose into pyruvic acid in microorganisms. EMP does not require oxygen in its process and occurs in the cytoplasm.

3.2. Response to the Growth of Lactic Acid Bacteria (LAB) from Alcosoy Yogurt After 24 Hours of Incubation

Through the results of the Two Way ANOVA test, it is stated that the interaction between the composition factor of *alcosoy* milk and lactose concentration shows a significant difference in the response of Lactic Acid Bacteria (LAB) growth. More clearly, as shown in the table below.

Table 2.
Response to the growth of lactic acid bacteria (cfu/ml) from *alcosoy* yogurt after 24 hours of incubation.

Factor (F), Composition of Almond milk: Corn milk : Soy milk.	Factor (C), Lactose Concentration		
	c_1 (4 %)	c_2 (5 %)	c_3 (6 %)
f_1 (1:1:1)	1.48E6 a B	2.05E6 b B	2.28E6 b B
f_2 (2:1:1)	1.06E6 a A	1.36E6 b A	2.02E6 c A
f_3 (1:2:1)	2.18E7 a C	3.24E7 b C	4.03E7 c C
f_4 (1:1:2)	1.17E6 a A	1.52E6 b A	2.12E6 c AB

Note: Lowercase notation compared horizontally, the same lowercase notation indicates no significant difference in response. Uppercase notation compared vertically, the same uppercase notation indicates no significant difference in response.

Through the results of the Two Way ANOVA test, it is stated that the interaction between the factor of *alcosoy* milk composition and lactose concentration shows a significant difference in the response of the number of lactic acid bacteria (LAB) after fermentation for 24 hours. Similarly, the response to the metabolite product of lactic acid content shows that the reproduction of LAB in the almond milk, corn milk, and soybean milk with composition of 1:2:1 indicates a higher number of LAB than in other compositions, specifically 4.03 x 10^7 cfu/ml. Likewise, at a lactose concentration of 6%, it shows a higher number of LAB compared to concentrations of 4% and 5%. As mentioned regarding the lactic acid response, the content of glucose and lactose substrates determines the reproducibility of LAB formation. The curve in Figure 1 shows that the composition of almond milk, corn milk, and soybean milk at a ratio of 1:2:1 with a concentration of 6% indicates a greater number of LAB with a faster growth rate than the others.

Lactic Acid Bacteria / LAB (*Lactobacillus. bulgaricus* and *Streptococcus. thermophilus*) share the characteristic of being salt-sensitive and thermoduric (capable of surviving at high temperatures). Thermoduric bacteria grow optimally at temperatures of 20-37 °C, with a minimum growth temperature of 5-10 °C. Based on its need for oxygen, this bacterium is classified as a facultative anaerobe. (can live with or without oxygen). At optimal temperatures and supportive environments, S. *thermophilus* will grow earlier than L. *bulgaricus*, where S. *thermophilus* will stimulate the growth of L.

bulgaricus and lower the pH by producing lactic acid, formic acid, acetaldehyde, and acetic acid. Similarly, *L. bulgaricus* will release glycine, amino acids, and histidine that are necessary for S. *thermophilus.*

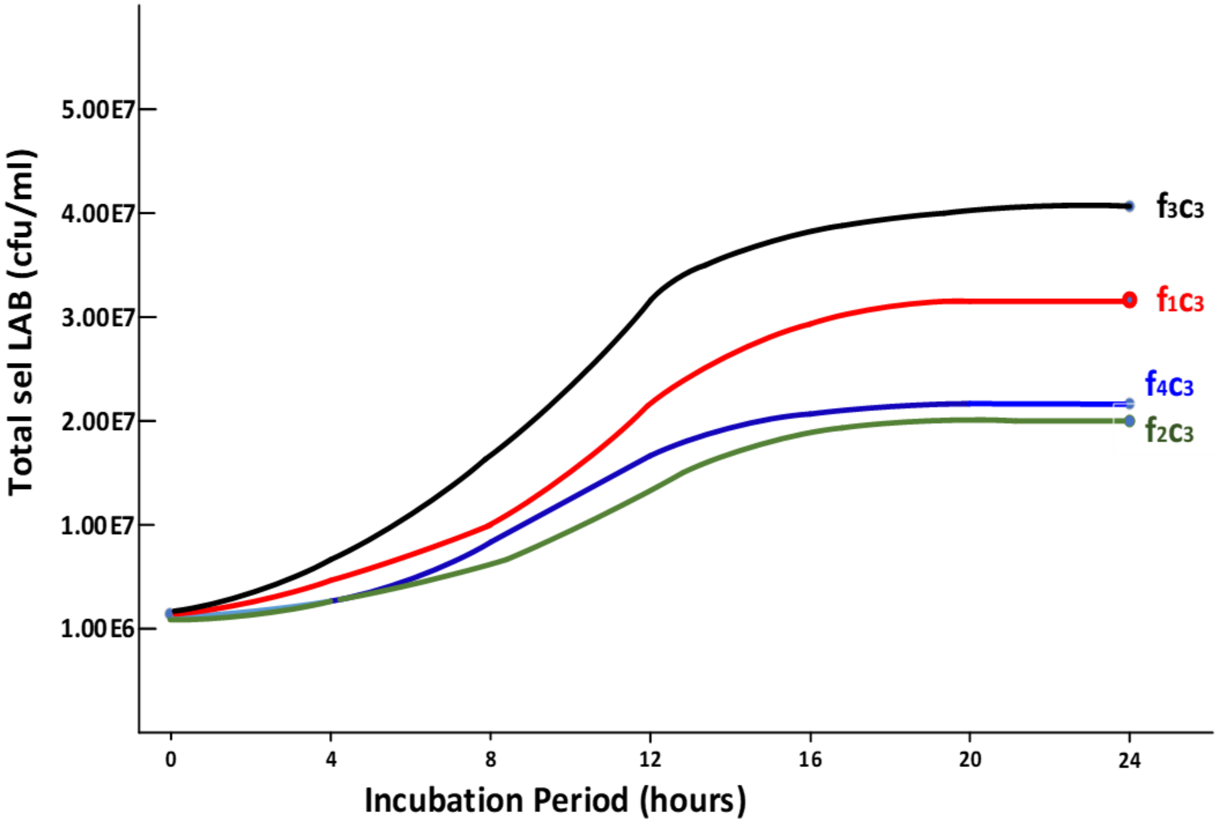

Figure 1.
Growth curve of LAB for composition f_1 with lactose concentrations c_1, c_2, and c_3.

Lactic acid fermentation is divided into two types: homofermentative (where the majority of the end product is lactic acid) and heterofermentative (where the end products include lactic acid, acetic acid, ethanol, and CO_2). Broadly speaking, both types share similarities in the mechanism of lactic acid formation, where pyruvate is converted into lactate (or lactic acid) followed by the process of electron transfer from NADH to NAD+. This fermentation pattern can be distinguished by identifying the presence of enzymes that play a role in the glycolysis metabolic pathway. Bacteria classified as BAL have several specific characteristics, including: lacking porphyrin and cytochrome, being catalase negative, not performing electron transport phosphorylation, and obtaining energy solely from substrate-level phosphorylation. Almost all BAL obtain energy solely from the metabolism of lactose/sugar, so their growth habitat is limited to environments that provide sufficient lactose/sugar, or what can be referred to as nutrient-rich environments. Their ability to produce compounds (biosynthesis) is also limited, and the complex nutritional requirements of BAL include amino acids, vitamins, purines, and pyrimidines.

3.3. Response to the Viscosity (cm^2/s) of Alcosoy Yogurt after 24 Hours of Incubation

Through the Two Way ANOVA test, it is stated that the interaction between the composition factor of *alcosoy* milk and lactose concentration shows a significant difference in the response of LAB growth. More clearly, as seen in the table below.

Table 3.
Response to the viscosity (cm^2/s) of alcosoy yogurt after 24 hours of incubation.

Factor (F), Composition of Almond milk : Corn milk : Soy milk.	**Factor (C), Lactose Concentration**		
	c_1 (4 %)	c_2 (5 %)	c_3 (6 %)
f_1 (1:1:1)	0.0684 a	0.0632 b	0.0551 c
	A	A	B
f_2 (2:1:1)	0.0696 a	0.0650 b	0.0614 c
	A	A	A
f_3 (1:2:1)	0.0528 a	0.0504 ab	0.0475 b
	B	B	C
f_4 (1:1:2)	0.0454 a	0.0388 ab	0.0354 b
	C	C	D

Note: Lowercase notation compared horizontally, the same lowercase notation indicates non significant difference in response. Uppercase notation compared vertically, the same uppercase notation indicates no significant difference in response.

In general, the interaction of the composition factor of alcosoy and lactose concentration on the viscosity response of the fermentation results shows a significant difference. The treatment with a composition of (1:1:2) indicates the thickest viscosity of the fermentation results, while a lactose concentration of 6% shows the thickest viscosity of the fermentation results. The increase in viscosity is caused by the coagulation of proteins due to a decrease in pH to 4.6. At this pH level, proteins are at their isoelectric point, where their solubility in water decreases, leading to the physical manifestation of protein coagulation. Therefore, the increase in viscosity occurs as a result of the reduced solubility of proteins in water.

The fermentation process occurs through the breakdown of the protein in *alcosoy* milk. (denaturation process). Bacterial cells use lactose from lactose to obtain carbon and energy, breaking down lactose into simple sugars, namely glucose and galactose, with the help of the enzyme β-galactosidase. The fermentation process ultimately converts glucose into the end product, lactic acid.

$$\text{Lactose} \rightarrow \text{Glucose} + \text{Galactose} \rightarrow \text{Pyruvic acid} \rightarrow \text{Lactic acid} + CO_2 + H_2O$$

The presence of lactic acid gives yogurt its sour taste. The fermentation of *alcosoy* milk changes its texture to a thick consistency. This is because the proteins in *alcosoy* milk coagulate in an acidic environment, forming clumps.

Denaturation is the process by which proteins lose their folded structure and cease to function. The denaturation process occurs when the folded structure of the protein stops functioning. The mechanism of protein denaturation is also a process that breaks the strong bonds that form the protein molecule. The protein molecule in its original form has strong bonds and a very orderly and stable structure. After the denaturation of the protein molecule occurs, these bonds weaken, and the molecule adopts a looser or more random structure, with most proteins becoming insoluble in water, or in other words, their solubility product decreases.

3.4. Results of the Antioxidant Activity Analysis

The antioxidant activity in this study was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with the indicator of % inhibition. Through the results of the Two Way ANOVA test, it is stated that the interaction between the factor of *alcosoy* milk composition and lactose concentration shows no significant difference in the response of antioxidant activity, although independently there are also some that show significant differences, namely the treatment f1 with f2 for each lactose concentration. More clearly, as shown in the table below.

Table 4.
Response to antioxidant activity (ppm) *alcosoy* yogurt after 24 hours of incubation.

Factor (F), Composition of Almond milk : Corn milk : Soy milk.	**Factor (C), Lactose Concentration**		
	C_1 (4 %)	**C_2 (5 %)**	**C_3 (^ %)**
f_1 (1:1:1)	86,45 a B	86,40 a B	85,80 a B
f_2 (2:1:1)	82,40 a A	82,00 a A	81,86 a A
f_3 (1:2:1)	84,62 a AB	84,06 a AB	83,50 a AB
f_4 (1:1:2)	84,28 a AB	84,10 a AB	83,72 a AB

Note: Lowercase notation compared horizontally, the same lowercase notation indicates no significant difference in response. Uppercase notation compared vertically, the same uppercase notation indicates no significant difference in response.

The results of the antioxidant activity testing of *alcosoy* yogurt mostly showed a strong category, which is between 50 – 100 ppm. Antioxidants are natural or synthetic substances that can prevent or delay certain types of cell damage caused by oxidation processes by oxidants. Oxygen itself is a free radical that exists in the environment, but it is also produced naturally in the body. Antioxidants are commonly found in foods, including fruits and vegetables. Each of us has antioxidants and free radicals present in our bodies at all times. Some antioxidants are produced by the body itself, in addition, these substances can also be obtained from food. Consuming foods high in antioxidants is a natural way to meet the body's antioxidant intake. Antioxidants play an important role in protecting the body from the effects of free radicals that can lead to various diseases. Here are some types of antioxidants along with foods that are high in those antioxidants, such as flavonoids, anthocyanins, beta-carotene, lycopene, polyphenols, and so on.

The antioxidants found in *alcosoy* fermented milk primarily come from the raw materials of almonds, corn, and soybeans, which already contain vitamin E in their raw form. Antioxidants can also be formed from the fermentation of *alcosoy* milk proteins into peptides, some of which are bioactive. According to Walther and Sieber [26] the main sources of bioactive peptides are milk and fermented products. The high protein content in *alcosoy* milk and the process of degrading *alcosoy* milk proteins into peptides consisting of 2-20 amino acids with the help of lactic acid bacteria are the two reasons why *alcosoy* milk and its fermented products can be considered primary sources of bioactive peptides. The bioactive peptides produced from the breakdown of *alcosoy* milk proteins have antioxidant activity. Antioxidant peptides typically consist of hydrophobic amino acids such as proline, histidine, tyrosine, or tryptophan in their sequence.

3.5. Results of Vitamin B_1 Analysis

The results of the Two Way ANOVA test indicate that the interaction between the composition of *alcosoy* milk and lactose concentration shows a significant difference in the response of Vitamin B1 levels. More clearly, as shown in the table below.

Table 5.
Response to Vitamin B_1 Levels (mg/100 ml) in *Alcosoy* Yogurt.

Factor (F). Composition of Almond milk: Corn milk: Soy milk.	Factor (C). Lactose Concentration		
	C_1 (4 %)	C_2 (5 %)	C_3 (6%)
f_1 (1:1:1)	0.21 a AB	0.22 ab A	0.24 b A
f_2 (2:1:1)	0.25 a B	0.28 b B	0.31 c B
f_3 (1:2:1)	0.18 a A	0.20 ab A	0.22 b A
f_4 (1:1:2)	0.28 a C	0.30 ab B	0.32 b B

Note: Lowercase notation compared horizontally, the same lowercase notation indicates no significant difference in response. Uppercase notation compared vertically, the same uppercase notation indicates no significant difference in response.

The results of the testing on the response to vitamin B_1 indicate that the factor of lactose concentration shows a significant difference. A lactose concentration of 6% shows a higher vitamin B_1 content compared to other lactose concentrations (4% and 5%). This occurs because the optimal lactose content (6%) can stimulate the formation of vitamin B_1. Meanwhile, the composition factor of *alcosoy* milk shows that the composition of almond milk: corn milk: soy milk (1:1:2) indicates a greater response of vitamin B_1 compared to other compositions (1:1:1, 2:1:1, and 1:2:1). The higher composition of soy milk shows a greater response of vitamin B_1 than the others, as the vitamin B1 content in soy milk is relatively higher at 0.084 mg/100 ml compared to almond milk at 0.065 mg/100 ml and corn milk at 0.036 mg/100 ml.

Vitamin B_1, or thiamine, is a vitamin that helps the body's cells convert carbohydrates into energy. This vitamin also helps maintain proper nerve function. The daily intake of vitamin B1 for the body can be obtained through food or supplements. Vitamin B_1 has various properties, including being water-soluble, stable in dry conditions, heat-resistant in acidic environments, easily damaged by heat or oxidation, and easily damaged by prolonged heating.

4. Conclusion

Based on the results of this study, it can be concluded that:

1. The treatment with the interaction of the *alcosoy* composition factor and variations in lactose concentration showed significantly different responses regarding lactic acid levels, the growth of Lactic Acid Bacteria (LAB) microorganisms, and viscosity.
2. The response regarding lactic acid levels indicated that the fermentation results with the *alcosoy* composition treatment (almond milk : corn milk : and soy milk, 1:2:1) showed the highest lactic acid levels, and similarly, a lactose concentration of 6% also showed the highest lactic acid levels.
3. The response to microorganism growth for the composition treatment (almond milk : corn milk : and soy milk,1:1:1) and a lactose concentration of 6% showed the highest and relatively faster growth.
4. The response regarding viscosity for the composition treatment (almond milk : corn milk : and soy milk,1:1:2) with a lactose concentration of 6% showed the thickest fermentation results.
5. The treatment factors of *alcosoy* milk composition and lactose concentration overall showed no significant difference in antioxidant activity.
6. The effect of *alcosoy* milk composition and lactose concentration overall showed a significant difference in the response of vitamin B_1 content. A higher composition of soy milk and a lactose concentration of 6% indicated a greater response of vitamin B_1 compared to the others.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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