



## Investigating the physicochemical and sensory properties of synbiotic cocoa milk containing "inulin, stevia and lactobacillus plantarum"

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### ABSTRACT

Today, there is a great desire to consume superfoods. Among them, foods and drinks containing probiotics and prebiotic compounds are also important. Probiotics are known as live microorganisms that balance the host's microbial flora in sufficient quantities. Most probiotic microorganisms are lactic acid bacteria such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Streptococcus lactis*. Inulin is a sugar compound and is indigestible or with little digestibility and has prebiotic properties, and as a source of carbon or energy, it selectively causes the growth or activity of probiotics. Cocoa milk is one of the flavored milks and high amounts of sugar are used in its formulation. Using other sweeteners as a substitute can lower the amount of calories consumed by the consumer and prevent some diseases such as diabetes and obesity. Stevia is a di-terpene glycoside, which is 300 times sweeter than sugar. In this research, stevia was used as a substitute for regular sugar and inulin as a prebiotic in the production of synbiotic cocoa milk with *Lactobacillus plantarum*, and 6 samples were prepared and coded; Samples include T1 (%8 of stevia); T2: (%4 of stevia ); T3: (%8 of inulin %8 of sugar); T4: (%4 of inulin + %8 of sugar); T5: (%4 of stevia + %4 of inulin); T6: (%8 of sugar, control sample) per amount of milk consumed (3 liters of milk). The viability of probiotics, pH, acidity, Brix, viscosity, sedimentation rate and sensory evaluations of the samples were investigated on the first, third and seventh day of production. According to the results obtained from the research, samples containing inulin, especially T5 and T4 samples, had more live probiotics after 3 and 7 days of storage. In addition, the pH and acidity of these samples were lower and higher than other samples, respectively. The highest Brix was related to sample T6 (control) and the viscosity of sample T6 on the first day of storage and sample T3 after 7 days of storage was higher than other samples. The lowest amount of sedimentation was related to samples T3, T4, T5 on the first day and after the storage period. Sensory evaluations (aroma, taste, color, texture and Total acceptance) showed that the samples did not have statistically significant differences, but the T1 sample had a lower score than the other samples and the control sample had the highest score.

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## 1. Introduction

Foods that improve and improve the health level of society and provide essential nutrients to the body are called super-beneficial foods. Currently, there is a great desire to consume super-beneficial food. Among these, foods and drinks containing probiotics and prebiotic compounds are among these examples [1] Probiotics are considered as live microorganisms that balance the host's microbial flora in sufficient quantities. Although there is still no general agreement on the minimum number of viable probiotic bacteria in the final product, generally the range of  $10^7$ - $10^6$  The number of probiotics in each gram has been considered necessary for the occurrence of health-giving effects [2].

Most probiotic microorganisms are lactic acid bacteria such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Streptococcus lactis*. Research has shown that adding probiotics to food can have many beneficial functions for the health of the consumer, such as lowering blood cholesterol, improving the function of the digestive system, strengthening the immune system and reducing the incidence of cancer [3] Lactic acid bacteria are often commercially used in the production of products. Probiotic dairy products are used. Probiotics are usually added to yogurt and other fermented milk products. In recent years, the need to consume probiotic dairy products has been increasing, and the production of probiotic products has become especially important in the majority of beverages. These drinks are rich in functional food compounds including minerals, vitamins, fiber and antioxidants [4] In this study, the aim is to enrich the produced probiotic drink (cocoa milk) with stevia and inulin as a beneficial compound and the effect Let's examine it on the physico-chemical and sensory properties of the drink.

Inulin is a sugar compound (of the oligosaccharide type) and is indigestible or with little digestibility, which is found in more than 30,000 different plants [5] Inulin is a compound that is soluble in water and its solubility depends on temperature, such as its solubility at a temperature of 10 degrees Celsius is 6%, and at 90 degrees Celsius it is 35% [6] After reaching

the intestinal environment as a source of carbon or energy, inulin selectively causes the growth or activity of probiotics (beneficial intestinal bacteria containing *Lactobacillus* and *Bifidobacterium*). 5] Prebiotics are considered as a food additive with high nutritional value and also as an agent for increasing the activity of beneficial intestinal bacteria (*Bifidobacterium* and *Lactobacillus*) [7] lowering blood cholesterol levels, preventing constipation and infectious diarrhea, and... It is about the health-giving properties of prebiotics [8]

The stevia plant was first known in the north of Paraguay. This plant is native to the northern regions of South America and grows wildly in the high lands of the border areas between Brazil and Paraguay (Amambi) and is known as the honey leaf plant in those areas. The results of the research show that diterpene glycosides are compounds that have been identified as the main factor in creating a very sweet taste in the extracts of the stevia plant, so their sweetness is estimated to be 300 times that of sugar. Stevioside has been identified as a dominant compound in the extract of *Stevia rabadiana* leaves, and Ribioside A is the most desirable compound, which gives it its sweetening power and excellent taste characteristics. Due to the presence of flavonoids, alkaloids, xanthophylls and hydroxycinnamic acids, stevia shows significant antioxidant properties [9].

## 2- Materials and methods

### 1-2- Raw materials

Cow's milk with 2.1% fat used in this research was obtained from Pegah factory in Tehran. *Lactobacillus plantarum* bacterium of DVS type was freeze-dried from Christian Hansen company and inulin was purchased from Foodcom company and Stevia was purchased from Stevia Iran company. The use for the microbial test was also prepared from the German company Merk. The raw materials and microorganism and culture media used in the formulation of Farsamand cocoa milk are listed in Table 1.

**Table 1. Microorganism, culture medium and raw materials needed in the research**

Company and country of manufacture	Raw material
Christian Hansen Denmark	lactobacillus plants
Brand .germany	MRS <sup>1</sup> broth
Brand .germany	MRS agar
Foodcom.china	Inulin
Stevira, Iran	Stevia
Farman, Iran	Cocoa powder
Fooding, china	Carrageenan
Melody, United Arab Emirates	Vanilla
Golha.iran	Salt

### 2-3-preparation of useful cocoa milk

In the formulation of Farasamand cocoa milk, inulin was used as a prebiotic and stevia was used as a sugar substitute (0, 4 and 8%). In Shahid cocoa milk, 240 grams of sugar (per 3 liters of milk) were used to sweeten the milk and give it a good taste. As a result, sugar substitutes equivalent to 240 grams of sugar were considered; which was added to the formulation in different proportions according to the amount of sweetness. 8% cocoa powder, 0.02% to all treatments

### 2-2- Preparation of bacteria *Lactobacillus plantarum*

*Lactobacillus plantarum* bacteria was purchased from Christian Hansen, Denmark. Packages containing bacteria were carefully stored in sterile conditions and after activation, in special cryotypes at -18 degrees Celsius. In each step, a granule of bacteria was mixed uniformly in 100 ml of MRS broth medium and kept in a greenhouse at 37 degrees Celsius for 24 hours. Then, one milliliter of the resulting culture was added to 9 milliliters of fresh culture medium and placed in an incubator at 37 degrees Celsius for 24 hours. Next, the bacterial suspension was centrifuged at 4500 revolutions per minute for 5 minutes, the sediment obtained from the centrifuge was separated and washed in two steps with 0.1% peptone water solution and the bacterial sediment was dissolved in 0.1% peptone water. , then the optical absorption of bacteria was determined using a spectrophotometer at a wavelength of 595 nm. Simultaneously with the determination of light absorption with a spectrophotometer, bacterial counting should be done by the surface plate counting method [1].

<sup>1</sup>-Man rogosa sharp agar

Carrageenan, 0.001% vanilla and 0.025% salt were added. In order to prepare the treatments, first the milk prepared in a hot water bath was heated to a temperature of 50-60 degrees Celsius for 15 minutes, then carrageenan gum was added to the hot milk. In the continuation of the amount *Lactobacillus plantarum* (probiotic) The addition of cocoa powder was considered the same in all treatments and was added to the desired samples. The studied treatments were homogenized and uniformed by the same woman and then subjected to the Pasteur process in a hot water bath with a temperature of 75 degrees Celsius for 15 minutes. At the end, the prepared cocoa milk was packed in sterile containers and stored in the refrigerator to perform the desired tests. The storage period will be 7 days, and the samples were tested on days 1, 3 and 7.[3] The number and types of treatments produced and examined are listed in Table 2.

**Table 2. The number and type of treatments produced in the research**

Sugar (%)	Inulin (%)	Stevia (%)	sample
0	0	%8	T1
0	0	%4	T2
%8	%8	0	T3
%8	%4	0	T4
0	%4	%4	T5
%8	0	0	T6(Control)

## 2-4- Synbiotic cocoa milk tests

### 1-4-2- Checking the viability of probiotics

Porplate method was used to count live cells. Samples were diluted with sterile serum solution ( $10^{-8}$  -  $10^{-10}$ ) and cultured in MRS Agar culture medium and incubated in 37 degree Celsius incubator for 48 hours. colony The identified

ones were counted by the colony counter and reported in the form of cfu/ml.[10]

### 2-4-2-pH measurement

To determine the pH, after calibrating the pH meter with standard buffer 4 and 7, the electrode of the pH meter was placed directly inside the samples and its pH was read [11].

The image of the pH meter is shown in Figure 1.



**Figure 1.pH measures**

### 3-4-2 Measuring acidity

In order to measure the acidity of cocoa milk samples, add 25 ml of distilled water to 5 grams of the sample, and then add 0.1 normal soda solution in the vicinity of the phenolphthalein indicator, until a stable pale pink color is created (for 30 seconds). , became the headline. Acidity was expressed as Dronic percentage [12].

### 4-4-2- Determination of BRICS

To determine the brix of the samples, after setting the refractometer with distilled water, a few drops of the sample with a temperature of 20 degrees Celsius were placed on the prism of the refractometer. After removing light scattering and creating two equal parts, bright and dark, the concentration of dissolved solids in water was read in terms of Brix. The result was expressed in grams per hundred grams of the sample. Figure 2 shows the image of the refractometer.



**Figure2. refractometer**

#### 5-4-2-viscosity

The viscosity of the production samples was measured using a viscometer. For this purpose, all cocoa milk samples were subjected to a shear speed of 50 l/s at a temperature of 25 degrees Celsius and their viscosity was measured. The amount of viscosity obtained was expressed in millipascal seconds (mPa.S) [13, 14, 15]. Figure 3 shows the image of the viscometer.



**Figure3.viscomete**

#### 6-4-2- Sediment measurement

In order to measure the amount of sediment, 20 grams of the sample was weighed in centrifuge tubes and placed in a centrifuge, and it was centrifuged at a speed of 5600 rpm for 15 minutes at a temperature of 20 degrees Celsius, then the soluble part was separated and divided

The sediment settled in the test tubes was first dried in an oven at 120 degrees Celsius for 36 hours and finally weighed in a desiccator after cooling. The results were reported in terms of grams of sediment per hundred grams of ultra-beneficial milk drink [16].

Figure 4 shows the centrifuge used.



**Figure 4. Centrifuge**

### 7-4-2- Sensory evaluations

Probiotic dairy drink samples that were coded randomly were accepted and evaluated by a sensory group of 10 people. Before the evaluation, people were asked to fill out a questionnaire containing questions about gender, age, and frequency of consumption of probiotic milk drink (no consumption, less than once a month, 4-5 times a month, and more than 6 times a month). Fill in the evaluators whose intake of probiotic milk drink 2-4 times per month or less were excluded from the data analysis. Probiotic dairy drink was evaluated from the point of view of color, taste, aroma, and overall acceptance based on a 5-point hedonic scale (1 = most unfavorable, 5 = most favorable). A standard-sized amount of probiotic dairy drink was placed in

air-tight plastic containers to equilibrate for 2 hours at room temperature before evaluation. Assessors use distilled water at ambient temperature to rinse the mouth between samples [17].

### 5-2-Statistical analysis

Data analysis was done using one-way ANOVA and comparison of significant differences between means was done using Duncan's multi-range post hoc test with a confidence level of 95%. Data analysis was done using SPSS version 22 software.

## 3-Result and discussion

### 1-3- Examination of the viability of probiotic bacteria

**Table 3** The survival rate of probiotic bacteria in 1, 3 and 7 days after production

Survival of probiotics (cfu/ml) - 7th day	Survival of probiotics (cfu/ml) - third day	Survival of probiotics (cfu/ml) -first day	Sample
0.01 <sup>aA</sup> ± 107 × 0.90	0.02 <sup>aB</sup> ± 107 × 0.96	0.01 <sup>BC</sup> ± 107 × 2.3	T1
0.01 <sup>aA</sup> ± 107 × 0.92	0.02 <sup>aB</sup> ± 107 × 0.96	0.01 <sup>BC</sup> ± 107 × 2.3	T2
0.01 <sup>aA</sup> ± 107 × 0.91	0.01 <sup>dB</sup> ± 107 × 1.6	0.01 <sup>bc</sup> ± 107 × 2.4	T3
0.01 <sup>not</sup> ± 107 × 0.95	0.01 <sup>bB</sup> ± 107 × 1.4	0.01 <sup>bc</sup> ± 107 × 2.4	T4
0.01 <sup>not</sup> ± 107 × 0.95	0.02 <sup>cB</sup> ± 107 × 1.5	0.01 <sup>bc</sup> ± 107 × 2.4	T5
0.01 <sup>aA</sup> ± 107 × 0.91	0.02 <sup>dB</sup> ± 107 × 1.6	0.01 <sup>BC</sup> ± 107 × 2.3	T6

Different lowercase Latin letters in each column and different uppercase letters in each row indicate differences at the 95% significance level.: T1 (%8 stevia); T2: (%4stevia); T3: (%8inulin + %8sugar); T4: (%4inulin + %8sugar); T5: (%4stevia+%4inulin); T6: (%8sugar, control sample).

According to Table 3, there is no statistically significant difference between the average viability of probiotic (*Lactobacillus plantarium*) on the first day of production in the control sample and T1 and T2 samples at the 95% confidence level ( $P > 0.05$ ). However, there is a statistically significant difference between the control sample and T3, T4, and T5 samples at the 95% confidence level ( $P < 0.05$ ). On the third day, there is no significant difference in the viability of probiotics in the control sample and the T3 sample, and these two samples showed the highest amount of probiotics on the third day. There is no statistically significant difference between the average viability of probiotics in T1 and T2 samples ( $P > 0.05$ ), but this difference is significant between T4 and T5 samples ( $P > 0.05$ ). On the seventh day of

production of cocoa milk samples, there is no significant difference between the average viability of probiotics in the control sample and T1, T2 and T3 samples, as well as between T4 and T5 samples ( $P > 0.05$ ). Considering the time factor in all samples The subject of investigation was the highest survival rate of probiotics related to the first day of production and the lowest was related to the seventh day. The difference between the average survival rate of probiotics in all 3 investigated times in all samples is significant at the 95% confidence level ( $P < 0.05$ ) and the survival rate decreases with the passage of time.

### 2-3-pH check

**Table 4**, pH rate of the samples in 1, 3 and 7 days after production

The seventh day	The third day	The first day	Sample
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0.02 <sup>aA</sup> ± 6.20	0.01 <sup>dB</sup> ± 6.62	0.01 <sup>bC</sup> ± 6.8	T1
0.01 <sup>aA</sup> ± 6.20	0.01 <sup>cB</sup> ± 6.64	0.01 <sup>bC</sup> ± 6.8	T2
0.02 <sup>aA</sup> ± 6.18	0.01 <sup>bB</sup> ± 6.60	0.01 <sup>bC</sup> ± 6.7	T3
0.02 <sup>aA</sup> ± 6.18	0.02 <sup>bB</sup> ± 6.60	0.01 <sup>bC</sup> ± 6.8	T4
0.01 <sup>aA</sup> ± 6.19	0.01 <sup>aB</sup> ± 6.57	0.01 <sup>bC</sup> ± 6.7	T5
0.02 <sup>aA</sup> ± 6.20	0.02 <sup>dB</sup> ± 6.69	0.01 <sup>bC</sup> ± 6.8	T6

Different lowercase Latin letters in each column and different uppercase letters in each row indicate differences at the 95% significance level.: T1 (%8 stevia); T2: (%4stevia); T3: (%8inulin + %8sugar); T4: (%4inulin + %8sugar); T5: (%4stevia+%4inulin); T6: (%8sugar, control sample)

According to Table 4, on the first day of production, there is no statistically significant difference between the average pH of T5 and T3 cocoa milk samples, as well as between T1, T2, T4 and T6 cocoa milk samples at the 95% confidence level ( $P > 0.05$ ). However, this difference between T3 and T5 samples and other samples is statistically significant at the 95% confidence level ( $P < 0.05$ ). On the third day of storing the samples, the T5 sample has the lowest pH and the control sample has the highest pH. Also, there is a significant difference between the average pH of the control sample and other samples ( $p < 0.05$ ).

On the seventh day of storage, there is no statistically significant difference between the average pH of the samples at the 95% confidence level ( $p > 0.05$ ). According to the storage time of the produced cocoa milk samples, there is a statistically significant difference between the average pH of the samples in the three investigated times at the 95% confidence level ( $p < 0.05$ ) and on the seventh day of storage compared to the first and third day, The pH of the samples is lower. In fact, with the passage of storage time, the pH level decreases.

### 3-3- Checking acidity

**Table5** .Acidity rate of samples in 1, 3 and 7 days after production

The seventh day	The third day	The first day	Sample
0.002 <sup>BC</sup> ± 0.180	0.001 <sup>aB</sup> ± 0.154	0.001 <sup>aA</sup> ± 0.135	T1
0.000 <sup>BC</sup> ± 0.180	0.002 <sup>aB</sup> ± 0.154	0.001 <sup>aA</sup> ± 0.135	T2
0.002 <sup>cC</sup> ± 0.207	0.003 <sup>aB</sup> ± 0.155	0.002 <sup>aA</sup> ± 0.135	T3
0.001 <sup>cC</sup> ± 0.207	0.001 <sup>aB</sup> ± 0.153	0.00 <sup>aA</sup> ± 0.135	T4
0.003 <sup>bC</sup> ± 0.190	0.001 <sup>aB</sup> ± 0.153	0.001 <sup>aA</sup> ± 0.136	T5
0.001 <sup>BC</sup> ± 0.180	0.002 <sup>aB</sup> ± 0.152	0.001 <sup>aA</sup> ± 0.136	T6

Different lowercase Latin letters in each column and different uppercase letters in each row indicate differences at the 95% significance level.: T1 (%8 stevia); T2: (%4stevia); T3: (%8inulin + %8sugar); T4: (%4inulin + %8sugar); T5: (%4stevia+%4inulin); T6: (%8sugar, control sample).

According to Table 5, on the first day of production and the third day of storage, there is no statistically significant difference between the average acidity of cocoa milk samples at the 95% confidence level ( $P > 0.05$ ). However, this difference between T3 and T5 samples and other samples is statistically significant at the 95% confidence level ( $P < 0.05$ ). On the seventh day of storage, there is no statistically significant difference between the average acidity of control samples and T1 and T2 samples, as well as between T3 and T4 samples at the 95% confidence level ( $p > 0.05$ ). But there is a significant difference between T1 and T2 control samples, T3 and T4

samples and T5 sample at 95% confidence level ( $P < 0.05$ ). On the seventh day of storage, the highest acidity is related to samples T3 and T4 and the lowest acidity is related to samples T1 and T2.

According to the storage time of the produced cocoa milk samples, there is a statistically significant difference between the average acidity of the samples in the three investigated times at the 95% confidence level ( $p < 0.05$ ) and on the seventh day of storage compared to the first and third day, The acidity of the samples is higher, in fact, with the passage of storage time, the amount of acidity increases.

## 4-3- BRICS review

**Table 6**, Brix rate of the samples in 1-3-7 days after production

The seventh day	The third day	The first day	Sample
0.15 <sup>bb</sup> ± 15.20	0.05 <sup>bc</sup> ± 15.40	0.05 <sup>not</sup> ± 11.30	T1
0.1 <sup>ab</sup> ± 14.20	0.1 <sup>BC</sup> ± 14.80	0.05 <sup>aA</sup> ± 11.15	T2
0.01 <sup>ec</sup> ± 21.70	0.05 <sup>eb</sup> ± 20.70	0.1 <sup>of A</sup> ± 18.60	T3
0.01 <sup>dc</sup> ± 21.14	0.05 <sup>db</sup> ± 19.60	0.1 <sup>that</sup> ± 17.10	T4
0.1 <sup>cC</sup> ± 20.10	0.1 <sup>cb</sup> ± 19.30	0.05 <sup>of A</sup> ± 18.7	T5
0.1 <sup>ec</sup> ± 21.80	0.05 <sup>eb</sup> ± 20.60	0.05 <sup>and</sup> ± 18.30	T6

Different lowercase Latin letters in each column and different uppercase letters in each row indicate differences at the 95% significance level.: T1 (%8 stevia); T2: (%4stevia); T3: (%8inulin + %8sugar); T4: (%4inulin + %8sugar); T5: (%4stevia+%4inulin); T6: (%8sugar, control sample).

According to Table 6, on the first day of production, there is a statistically significant difference between the average Brix of cocoa milk samples at the 95% confidence level ( $P < 0.05$ ). However, this difference between T3 and T5 samples is not statistically significant at the 95% confidence level ( $P > 0.05$ ). The highest amount of brix on the first day of production is related to sample T5 and the lowest amount of brix is related to sample T2. On the third day of sample storage, there is a statistically significant difference between the average Brix of cocoa milk samples at the 95% confidence level ( $P < 0.05$ ). Sample T2 has the lowest amount of Brix and sample T3 has the highest amount of Brix. Also, there is no significant difference between the mean brix of the control sample and the T3 sample ( $p > 0.05$ ). On the seventh day of storage, there is a statistically significant

difference ( $p < 0.05$ ) between the mean Brix of the samples except for the control sample and T3 at the 95% confidence level.

According to the storage time of the produced cocoa milk samples, there is a statistically significant difference between the average Brix of the samples in the three investigated times at the 95% confidence level ( $p < 0.05$ ) and on the seventh day of storage compared to the first and third day, The Brix of the samples is higher, except for T1 and T2 samples, which have the highest amount of Brix on the third day of storage. In fact, in most samples of produced cocoa milk, with the passage of storage time, the amount of Brix increases.

## 5-3-viscosity check

**Table7**, Viscosity of the samples in 1, 3 and 7 days after production

The seventh day	The third day	The first day	Sample
0.2 <sup>aA</sup> ± 37.0	0.3 <sup>aA</sup> ± 36.80	0.4 <sup>aA</sup> ± 37.00	T1
0.5 <sup>aA</sup> ± 37.00	0.5 <sup>aA</sup> ± 37.00	0.2 <sup>aA</sup> ± 36.80	T2
0.2 <sup>dB</sup> ± 40.80	0.2 <sup>not</sup> ± 39.00	0.2 <sup>aA</sup> ± 36.80	T3
0.2 <sup>cC</sup> ± 40.0	0.1 <sup>bb</sup> ± 38.6	0.5 <sup>aA</sup> ± 37.00	T4
0.2 <sup>bC</sup> ± 39.20	0.2 <sup>bb</sup> ± 38.40	0.5 <sup>aA</sup> ± 37.00	T5
0.2 <sup>cb</sup> ± 39.80	0.5 <sup>bb</sup> ± 39.00	0.5 <sup>not</sup> ± 38.00	T6

Different lowercase Latin letters in each column and different uppercase letters in each row indicate differences at the 95% significance level.: T1 (%8 stevia); T2: (%4stevia); T3: (%8inulin + %8sugar); T4: (%4inulin + %8sugar); T5: (%4stevia+%4inulin); T6: (%8sugar, control sample)..

According to Table 7, on the first day of production, there is no statistically significant difference between the average viscosity of cocoa milk samples at the



95% confidence level ( $P>0.05$ ). Except for sample T6, which has a significant difference with other samples in terms of viscosity ( $P<0.05$ ). The highest amount of viscosity on the first day of production is related to the control sample. On the third day of sample storage, there is no statistically significant difference between the average viscosity of T1 and T2 samples as well as T3, T4, T5 and T6 samples at the 95% confidence level ( $P>0.05$ ). Sample T1 has the lowest viscosity and sample T3 and the control have the highest viscosity. On the seventh day of storage, there is a statistically significant difference at the 95% confidence level between the average

viscosity of most of the samples except the control and T4 samples and the T1 and T2 samples ( $p<0.05$ ).

According to the storage time of the produced cocoa milk samples, there is a statistically significant difference between the average viscosity of the samples, except T1 and T2 samples, at the 95% confidence level in the three investigated times ( $p<0.05$ ) and on the seventh day of storage compared to the day First and third, viscosity is higher in most samples.

### 6-3- Sediment investigation (settling)

**Table 8.** The sedimentation rate of the samples in 1, 3 and 7 days after production

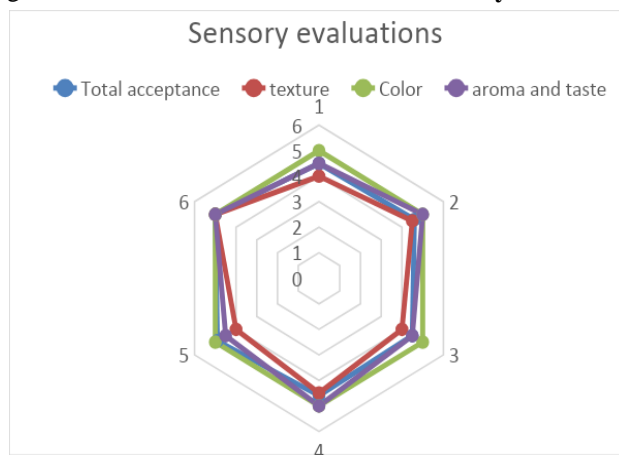
The seventh day	The third day	The first day	Sample
0.002 <sup>and</sup> ± 0.159	0.001 <sup>and</sup> ± 0.159	0.002 <sup>and</sup> ± 0.158	T1
0.002 <sup>that</sup> ± 0.154	0.001 <sup>that</sup> ± 0.152	0.001 <sup>that</sup> ± 0.152	T2
0.001 <sup>aA</sup> ± 0.133	0.001 <sup>aA</sup> ± 0.135	0.002 <sup>aA</sup> ± 0.135	T3
0.002 <sup>aA</sup> ± 0.135	0.002 <sup>aA</sup> ± 0.135	0.002 <sup>aA</sup> ± 0.135	T4
0.002 <sup>aA</sup> ± 0.134	0.001 <sup>aA</sup> ± 0.134	0.002 <sup>aA</sup> ± 0.135	T5
0.002 <sup>not</sup> ± 0.149	0.002 <sup>not</sup> ± 0.148	0.002 <sup>not</sup> ± 0.148	T6

Different lowercase Latin letters in each column and different uppercase letters in each row indicate differences at the 95% significance level.: T1 (%8 stevia); T2: (%4stevia); T3: (%8inulin + %8sugar); T4: (%4inulin + %8sugar); T5: (%4stevia+%4inulin); T6: (%8sugar, control sample).

According to Table 8, on the first day of production and the third and seventh days of storage, there is no statistically significant difference between the average sedimentation of T3, T4 and T5 samples of cocoa milk at the 95% confidence level ( $P>0.05$ ). These samples had lower sedimentation rate than other samples. However, there is a significant difference between the average sedimentation rate of

these samples and control samples, T1 and T2 ( $P<0.05$ ). The highest sedimentation rate on the first day of production is related to sample T1. According to the storage time of the produced cocoa milk samples, there is no statistically significant difference between the average sedimentation rate of the samples in the three investigated times at the 95% confidence level ( $p>0.05$ ).

### 3-7- Sensory evaluation



**Figure 6.** Sensory evaluation results of cocoa milk samples

Different lowercase Latin letters in each column and different uppercase letters in each row indicate differences at the 95% significance level: T1 (8% stevia); T2: (4% stevia); T3: (8% inulin + 8% sugar); T4: (4% inulin + 8% sugar); T5: (4% stevia+4% inulin); T6: (8% sugar, control sample)

According to chart 1, the best sample in terms of aroma, texture, color and overall acceptance is the control sample. Sample 4 and then sample 5 ranked second and third in terms of sensory characteristics, and samples 1 and 3 also had the lowest score in sensory characteristics. However, there was no statistically significant difference between the average scores of the cocoa milk samples and the control sample at the 95% confidence level ( $P < 0.05$ ).

#### 4 - Conclusion

Inulin is widely used in the food industry based on its technological nutritional properties. Fibrous and prebiotic properties of inulin are important, because they have positive effects on the human intestine and change its microflora and increase bifidobacteria in all ages.

Sweeteners can be added as an additive to food products, which creates a sweet taste and a special oral taste in the product, and affects sensory properties such as volume density and porosity, physical, chemical, rheological, mechanical, firmness, ability. It affects the softening of the products. Also, sweeteners have an effect on volume and color activities and increase the nutritional value of the food.

According to the produced cocoa milk samples including: T1 (8% stevia); T2: (4% stevia); T3: (8% inulin + 8% sugar); T4: (4% inulin + 8% sugar); T5: (4% stevia+4% inulin); T6: (8% sugar, control sample)

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According to the amount of milk consumed (3 liters of milk), the general results can be expressed as follows:

- 1- Samples containing inulin, especially samples T5 and T4, had more live probiotics after 3 and 7 days of storage.
- 2- The pH and acidity of T4 and T5 samples were lower and higher than other samples, respectively.
- 3- The highest Brix was related to the sample T6 (control).
- 4- The viscosity of sample T6 on the first day of storage and sample T3 after 7 days of storage was higher than other samples.
- 5- The lowest amount of deposition was related to samples T3, T4, T5 on the first day and after the storage period.
- 6- Sensory evaluations (aroma, taste, color, texture and overall acceptance) showed that the samples did not have statistically significant differences, but the T1 sample had a lower score than the other samples and the control sample had the highest score.
- 7- Inulin can be used as a prebiotic and stevia can be used as a sugar substitute in the production of synbiotic cocoa milk.

#### 5- Resources

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## بررسی خواص فیزیکوشیمیایی و حسی شیر کاکائو سین بیوتیک حاوی " اینولین، استویا و لاکتوباسیلوس پلاتاروم "

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### چکیده

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بیشترین میکروارگانیزم های پروبیوتیک باکتری های اسید لاکتیک هستند مانند لاکتوباسیلوس پلاتاروم، لاکتوباسیلوس کازئی، لاکتوباسیلوس اسیدوفیلوس و استرپتوکوکوس لاکتیس. اینولین ترکیبی قندی و غیر قابل هضم یا با قابلیت هضم اندک است و دارای خواص پری بیوتیکی است و به عنوان منبع کربن یا انرژی، به طور انتخابی سبب رشد و یا فعالیت پروبیوتیک ها می شود. شیر کاکائو جزو شیرهای طعم دار می باشد و در فرمولاسیون آن از مقادیر بالایی شکر استفاده می شود. در تحقیق حاضر از استویا به عنوان جایگزین شکر معمولی و از اینولین به عنوان پری بیوتیک در تولید شیرکاکائو سین بیوتیک به کمک لاکتوباسیلوس پلاتاروم استفاده شد و تعداد ۶ نمونه تهیه و کدگذاری گردید؛ نمونه ها شامل T1 (۸% استویا)؛ T2 (4% استویا)؛ T3 (8% اینولین + 8% شکر)؛ T4 (4% اینولین + 8% شکر)؛ T5 (4% استویا + 4% اینولین)؛ T6 (8% شکر، نمونه شاهد) به ازای مقدار شیر مصرفی (۳ لیتر شیر) بود. طبق نتایج به دست آمده از تحقیق، نمونه های حاوی اینولین به ویژه نمونه T5 و T4 دارای پروبیوتیک های زنده بیشتری پس از ۳ و ۷ روز نگهداری بودند. علاوه بر آن میزان pH و اسیدیته این نمونه ها نیز نسبت به سایر نمونه ها به ترتیب کمتر و بیشتر بود. بیشترین بریکس مربوط به نمونه T6 (شاهد) بود و میزان ویسکوزیته نمونه T6 در روز اول نگهداری و نمونه T3 پس از ۷ روز نگهداری بیشتر از سایر نمونه ها بود. کمترین میزان رسوب گذاری نیز مربوط به نمونه های T3، T5، T4 در روز اول و پس از مدت زمان نگهداری مورد بررسی بود. ارزیابی های حسی (عطر و طعم، رنگ، بافت و پذیرش کلی) نشان داد که نمونه ها از نظر آماری دارای اختلاف معنی دار نبودند ولی نمونه T1 نسبت به سایر نمونه ها امتیاز کمتر و نمونه شاهد بیشترین امتیاز را داشت.