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Effect of xanthan, carrageenan and mono-diglyceride stabilizers on the sensory and physicochemical characteristics of cocoa milk using the response surface methodology

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ABSTRACT

Phase separation and cocoa precipitation at the bottom of the bottle of cocoa milk is unpleasant from the consumer's point of view. Usually the amount of cocoa particles precipitate at the bottom of the bottle is of most importance. The aim of this research was to investigate the effect of five levels of stabilizers including: xanthan, κ -carrageenan and mono-diglyceride on the sensory and physicochemical characteristics of cocoa milk using the response surface method (RSM). Therefore, 20 treatments were produced in three blocks with six replications at the central point, then physicochemical properties and sensory evaluation tests were performed. Numerical optimization method has been applied to produce optimal cocoa milk with a suitable viscosity of 110 centipoise and the lowest amount of phase separation and precipitation. The suggested samples of the software were compared in the production laboratory with the real results of cocoa milk with a one-sample T-test software at a 95% confidence level, which confirmed the correctness of the predicted model due to the absence of significant differences. Based on the results, it was found that the increase of hydrocolloids caused an increase in viscosity as a confidence level ($p < 0.001$), as well as an increase in product stability and a decrease in the amount of sediment formation. Sensory evaluation showed that κ -carrageenan had the greatest effect on overall acceptance, followed by xanthan and mono-diglyceride ($p < 0.001$).

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1- Introduction

Milk is a healthy, nutritious, delicious and thirst-quenching drink that is consumed by a large group of people. Despite this, the taste of milk is not compatible with the taste of some consumers, especially children, when they need it the most. Therefore, in order to create interest in milk consumption and solve this problem, a large part of the production of drinking milk is dedicated to the production of flavored milk] 2,1 and 3[. « There is a high correlation between the consumption of dairy products and the health level of people in the society in terms of efficiency and IQ, the rate of contracting infectious diseases and regulating the body's metabolic activities, lowering blood pressure, preventing colon cancer and preventing osteoporosis.]4[. « Flavored dairy drinks including Flavored milks are popular and widely consumed dairy products all over the world which have enjoyed increasing growth"]5[. « Fresh milk is recommended with acidity of 0.13 to 0.16 (percentage in terms of lactic acid, 13 until the 16 degree on the Drenick scale) Used in preparing cocoa milk the river High acidity 0.16% on the rheological properties of milk Cocoa has an effect and may accelerate the formation of hard gel during storage and make the product unusable. Milk contains a small amount of other substances such as pigments, enzymes, vitamins, phospholipids and gases. But The compounds in milk that are more effective on cocoa milk than other components are fat and protein.]6[. Flavored milk is one of the most popular milk products around the world and includes a wide variety of flavors including: cocoa milk, coffee milk, honey milk, banana milk, strawberry milk, etc. The most popular of them is cocoa milk. This type of milk can be used as a substitute for soft drinks and sweet fruit juices by a large group of people. Compared to plain milk, flavored milks are sweet and have more

sugar and calories. In recent years, due to the increase in society's level of awareness in the field of health and nutrition, most people have concerns about their diet. Therefore, the great acceptance of consumers has caused dairy industry producers to make a considerable effort in order to control and maintain its characteristics, including preventing physical instability.[2 and 7]. To prepare cocoa powder, cocoa beans are ground and cocoa paste is obtained. In the next stage, the cocoa paste is subjected to mechanical pressure, and with the removal of part of its fat, cocoa cake is obtained. As a result of pulverizing the rest of the cocoa cake by mechanical method, cocoa powder is obtained [8]. "Cocoa milk is a popular type of dairy drink based on milk, the expansion of which can play an effective role in approaching the appropriate per capita consumption of dairy products in the country.. For the stability of this product, different stabilizers are used in the industry, the most common of which are carrageenans. Because this product is imported, it also affects the final price of the product.]6[. also It is one of the most popular flavored milk drinks among children, teenagers and young people Using milk, sugar, powder Cocoa and some hydrocolloids to improve consistency Preventing the deposition of cocoa particles and pasteurized or It is sterilized]3[. "According to the definition of Iran's national standard, cocoa milk is a milk product that has been prepared using cocoa powder and stabilizers, and this milk has been produced and made healthy by using pasteurization or high-temperature processes."]9[. Although the taste, taste and color, component The quality characteristics of cocoa milk are important, the presence of a layer of cocoa at the bottom of the bottle is unpleasant from the consumer's point of

view, and usually the amount of cocoa particles deposited at the bottom of the bottle is the first thing that attracts the attention of the consumer [10].

Hydrocolloids or gums are high molecular weight polysaccharides that improve texture by providing structure in liquid formulations and gelling milk in flavored milks and yogurt drinks.. They are hydrophilic biopolymers with a polysaccharide or protein structure that, according to Stoke's law, causes stability by delaying the movement of dispersed phase droplets and increasing the viscosity of the continuous phase. They are emulsified. It increases the shelf life of chocolate milk and acidic fruit drinks and protects against the settling of scattered particles, creaminess and coagulation of emulsion drops. Improving rheological and textural properties· Creating consistency in cocoa milk, better mouth feel, increasing stability, preventing precipitation and two-phase formation during storage, Also, the addition of emulsifiers to create a better emulsion and, as a result, the cocoa does not settle, are things that are achieved by adding stabilizers such as xanthan, carrageenan, pectin, alginate, guar, and ketira to cocoa milk [9, 2, 11]. Polysaccharides are not good emulsifiers, but they can contribute to the formation of a network structure due to their thickening and water retention properties in the continuous phase. Also, by covering the surface of the protein layer on the fat globules, they can increase the stability of the emulsion [12]. These effects depend on the protein-polysaccharide interaction. The synergistic effect between proteins and polysaccharides can be influenced by the type of biopolymer, mixing ratio and total concentration. Also, by increasing the apparent viscosity of the product or as a result of colloidal interaction of the type of spatial hindrance and electrostatic repulsion, they cause the stability of some food

systems]2 and 13]. Sugar and cocoa play a role in increasing the viscosity of cocoa milk, but the main responsible for the appearance of viscosity is the action of stabilizers. For this reason, research and investigation should be done in relation to the type, amount and correct method of their use. Among the most important stabilizers used in food systems are carrageenan, alginate, guar, tragacanth, xanthan, locust bean gum, gellan, mono and diglyceride.]14[. Among the stabilizers used in cocoa milk, carrageenan is more useful because it is effective in increasing the stability and reducing the amount of precipitation of cocoa milk, and the reason for that is the unique properties of the mixture of carrageenan and milk resulting from the interaction of carrageenan molecules with casein micelles.]15[. Carrageenans are natural polysaccharides from edible red seaweed (Irish moss) of the family *Rhodophyceae*¹ are extracted and divided into different types such as κ - (kappa), ι - (iota), λ (lambda), θ - (theta), μ - (mu), and ν - (nu). They are classified as natural and biodegradable polysaccharides with gelling, thickening, emulsifying and stabilizing properties. Although the role of caparrageenan is prominent at its lower concentration, gelation has been found to be less at high concentrations in the presence of milk proteins, in particular, when the caparrageenan concentration is less than 0.018% by weight, it becomes important.[17, 16 and 18]. Xanthan gum is mainly composed of glucose, mannose, and galactronic acid, and is a microbial extracellular heteropolysaccharide derived from *Xanthomonas Compress*² it is produced. At low concentrations, the solution becomes viscous, and its viscosity is not significantly affected by pH changes,

1- *Rhodophyceae*

2- *Xanthomonas campestris*

and although it has a high molecular weight, it easily dissolves in hot water. Xanthan is also soluble in cold water, and the solutions created are highly pseudoplastic, and this property of xanthan gum makes foods, especially citrus fruits and fruit-flavored drinks, have a better mouth feel, and with the good effects it has on foods, it makes Products maintain their original quality better. In addition, due to characteristics such as good thermal stability, high compatibility with a wide range of salts and sugars, and stability in acidic systems, it can be used to improve texture, prevent biphasing and precipitation in products such as yogurt. "In the bread industry, this gum makes the dough soft, air-retaining, and resistant for cakes, muffins, biscuits, and bread mixes." [20, 19 and 21]. Monodiglycerides are a mixture of mono-, di-, and tri-ester fatty acids with small amounts of glycerol and free fatty acids. By heating oils and glycerol or esterification³ Glycerol and fatty acids are directly produced at temperatures above 200°C in the presence of a food-grade catalyst. It is the most common emulsifier used in the food and pharmaceutical industries, and the European Union regulates it under the food additive number E471, and it is divided into two types, saturated and unsaturated. [22].

2- Materials and methods

1-2) materials

In order to prevent possible fluctuations in the amount of protein percentage and also to accurately adjust the final fat percentage of the samples, reconstituted milk was used using non-fat dry milk and cream, a product of Pak Dairy Company (Iran) and purified water. sugar (Isfahan sugar, Iran), cocoa powder (Cargill GP50 Xanthan,

Netherlands Phew, China), monodiglyceride (Dmg 0093, Denmark) and Kapakarginan (GPI 106, Canada) was prepared. In addition, the chemicals required for cocoa milk tests such as amyl alcohol, hydrochloric acid, soda and phenolphthalein reagent were all obtained from Merck (Germany).

2-2) Methods

1-2-2) Preparation of reconstituted milk

To prepare reconstituted milk, the method of Bakhshi et al. was used with some changes [1]. In this way, according to the volume of milk produced according to the design of the experiments and also to achieve a milk fat of 1.5%, first non-fat dry milk with a moisture content of 4.5-4% and purified water with a ratio of 9.5% to 90.5% and with 40% fat cream according to Pearson's table in stainless steel weighing containers with an electric mixer (model ST1400M, made in USA) were mixed together for 10 minutes.

2-2-2) Preparation of cocoa milk samples

The preparation of cocoa milk was done by the method of Alipour et al. with a slight change [3]. First, sugar (6%) and cocoa powder (1%) were weighed in a constant amount in all samples, then triple hydrocolloids were also weighed according to the experiment plan.

³-Esterification

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were weighed (Table 2). Then it was slowly added to the reconstituted milk containing 1.5% fat that had already reached 55 degrees Celsius in a hot water bath and mixed with an electric stirrer (modelST1400M, made in America) were mixed for five minutes. Cocoa milk produced with a manual homogenizer (modelAPV, made in Denmark) were homogenized with a pressure of 200 bar and then pasteurized to a temperature of 85 degrees Celsius for 15 seconds and immediately cooled to a temperature less than 10 degrees Celsius in a cold water bath and finally packed in 250 ml polyethylene bottles. The range of concentrations used for hydrocolloids based on preliminary studies in various articles, datasheet⁴ Their manufacturing companies and finally various pre-tests were obtained from their various levels. Physicochemical and sensory tests, including the amount of viscosity and percentage of precipitation after 24 hours of production and the amount of biphasing after 7 days, were performed.

3-2-2 Viscosity measurement

Viscosity using a Brookfield viscometer (modelRV-DV2T, made in America) at a temperature of 25 degrees Celsius and using spindle number 02 and with 5 rotation speeds of 30, 70, 100, 150 and 200 revolutions per minute in centipoise, and due to reaching a higher efficiency at 200 revolutions, the numbers obtained It was reported from this speed [23].

4-2-2 Measurement of sediment amount

To measure the sedimentation index, Abedini et al.'s method was used with some modifications [24]. In this way, after mixing, pour 20 grams of cocoa milk samples into special 25 ml tubes and then for 15 minutes at a temperature of 20 degrees Celsius and a speed of 1500 rpm. centrifuge (ModelNova Safety, made in

Germany) became. After centrifugation, the released serum that was on top of the test tube was separated and the rest of the contents were weighed along with the test tube. Sedimentation rate was measured from the serum difference and the total weight of the sample. The results were reported in grams per hundred grams of cocoa milk.

2-5-2-2 Measuring the degree of biphasing

To check the degree of biphasing, the method of Ostadzadeh et al. was used with a little modification [10]. The produced cocoa milks were stored in sterile bottles at a temperature of four degrees Celsius for seven days. Then, the amount of the upper phase (serum) was measured by a ruler and divided by the total amount of the sample inside the bottle and multiplied by 100. The results were reported in terms of percentage of biphasing.

6-2-2 Sensory test

Aroma, taste, color, texture and general acceptability of cocoa milk samples were evaluated by 12 semi-trained evaluators in a five-point hedonic test. A three-digit code was randomly assigned to each sample and the samples were randomly provided to the evaluators. Then, the average scores were reported as Rand integers. For each of the mentioned characteristics, scoring was from 1 to 5 as 1=unacceptable, 2=low satisfaction, 3=moderate, 4=good, and 5=excellent. Due to the fact that during this study, some samples lost their tissue stability and were strongly biphasic, they were not subjected to sensory evaluation and received a score of 1.]25[.

7-2-2 Statistical analysis

response surface method (RSM⁵) is a set of statistical techniques used in process optimization. In this method, while the number of tests is significantly reduced, the

4- Data Sheet

5- Response Surface Methodology

desired answer is checked under the influence of a number of variables at the same time [26 and 27]. In this research according to preliminary tests and determination of the range of each of the independent variables, From the response surface method and the central composite design with three independent variables each in five levels and including hydrocolloids, capcarrageenan (0.008, 0.02, 0.031, 0.04)%, monodiglyceride (0.016, 0.04)% respectively. 0.08, 0.063 and 0.08)% and xanthan (0.075, 0.030, 0.075, 0.119 and 0.15)% with 20 treatments in three blocks and with six repetitions at the central point (to calculate error and reproducibility process), in order to investigate the effect of the mentioned stabilizers on the sensory and physicochemical properties of cocoa milk was used To check the relationship between the obtained answers and the process variables and optimize the variables, design and analyze the information and draw the relevant diagrams from the Design Expert software.⁶ Version 13.0.5.0 was used and then the independent effect of the factors and their mutual effects at the 95% confidence level ($P < 0.05$) was investigated. Numerical and factored values of independent variables including the percentage of capcarrageenan, monodiglyceride and xanthan are shown (Table 1). The number of tested samples and the amount of different responses are also provided (Table 2).. Optimal formulation conditions using numerical optimization technique⁷ The software was done. For this purpose, at first, the optimization goals and the upper and lower limits of the parameters, which include viscosity 110 centipoise and determined the minimum amount of biphasing and sedimentation and with Using the numerical optimization technique, the

best solutions were obtained (Table 4). Also, the statistical analysis of the optimal characteristics based on the completely random experiment design in three repetitions and comparing the averages using the test T-test In level of 95% In the form of a sample plan⁸ using software SPSS IBM Was performed [18 and 28.] According to the percentages obtained from the initial production tests, the upper and lower limits of the variables were obtained. Then with DesignExpert software, version 13.0.5.0 Response surface method in dominant central composite design with the amount $\alpha = 1.68$ And 6 central points were chosen for it. Determining the optimal operating conditions to achieve the best answers was done using the numerical optimization technique. In this technique, the response space was created by using the created models, in order to find the best conditions that meet the desired optimization goals [29].

6- Design Expert

7- Numerical Optimization

8- One Sample

Table 1) Independent process variables and their application levels

Independent variables	Factor	-a	-1	0	+1	+a
K-carrageenan	A	0	0.008	0.02	0.031	0.04
mono-diglyceride	B	0	0.016	0.04	0.063	0.08
Xanthan	C	0	0.030	0.075	0.119	0.15

Table 2) Suggested treatments by Design Expert software using the response surface methodology(rsm) and their related responses

Run	F 1 A: κ - Carrageenan %	F2 B:mono diglyceride %	F 3 C:xanthan %	R1 Viscosity cP	R2 Phase Separation %	R3 Precipitation %	R4 Overall Acceptance
1	0.008	0.063	0.119	97.8	14.47	5.202	1
2	0.031	0.0162	0.119	122.4	15.23	6.6	5
3	0.031	0.063	0.030	90.8	2.77	5.369	4
4	0.02	0.04	0.075	91.2	8.21	5.558	3
5	0.02	0.04	0.075	88.4	14.75	5.209	3
6	0.008	0.016	0.030	50.8	47	6.199	1
7	0.031	0.063	0.119	145.8	1.51	6.7	5
8	0.02	0.04	0.075	98.8	3.94	5.51	4
9	0.008	0.063	0.030	58	48	5.45	1
10	0.031	0.016	0.030	83	6.32	6.604	4
11	0.02	0.04	0.075	90	8.43	5.77	3
12	0.008	0.016	0.119	86.4	49.63	6.04	1
13	0.02	0.08	0.075	92	7.79	5.55	5
14	0.02	0	0.075	83.8	17.89	7	3
15	0	0.04	0.075	60	60	6.61	1
16	0.02	0.04	0.075	86	6.77	6.1	3
17	0.04	0.04	0.075	107.6	6.41	7.66	5
18	0.02	0.04	0	48.4	12.67	8.18	2
19	0.02	0.04	0.15	111	6.25	8.61	5
20	0.02	0.04	0.075	88	6.41	6.36	4

3. Results and Discussion

1-3 Choosing the right model and analyzing the fitted model

In order to obtain experimental models for fitting response prediction, linear and quadratic polynomial relationships were fitted to the data obtained from the experiments.. Then these models were subjected to statistical analysis to select the appropriate model. From a statistical point of view, it is a suitable model that the lack of fit test⁹ It is not significant and has the highest adjusted explanatory coefficient¹⁰ and the predicted coefficient of explanation¹¹ be Also, the difference of less than 0.2 between these two, The standard deviation (*Std.Dev*) and the estimated residual sum of squares (Press) low and The value of the correlation coefficient (R^2) Top, it shows the high power of the model in predicting that all the answers of this research included these characteristics. The results of the misfit test and the assessment of the accuracy of the fitted models are given. The misfit test related to the fitted model.(Second degree polynomial) The answer was not significant($P>0.05$). On the other hand, the quadratic polynomial model due to having high values of the explanatory coefficient (R^2) The adjusted explanatory coefficient and the predicted explanatory coefficient showed high power in fitting the data and It showed the high power of the model in forecasting. Corrected quadratic equations were determined after removing

non-significant variables that were statistically significant for all responses. ($P\leq 0.001$ (Table 3). The model proposed by the software for viscosity responses and sensory evaluation of the linear model.(Linear) and for the biphasic and sedimentation responses of the quadratic model¹² was]30.[

1- Lack of fit

¹⁰ - R^2 Adjusted

¹¹ - R^2 Predicted

¹² - Quadratic model

Table 3) Results of ANOVA (Analysis of variance) of RSM for responses($P \leq 0.05$)

Response	R^2	Adj- R^2	Pre- R^2	Press	p.Value Model	Lack of fit p.value	Std.De v	Sequential p.value
Viscosity	0.9622	0.9541	0.9131	874.4 2	<0.0001 _a Linear	0.3026 _{ns}	5.21	<0.0001 _a
Phase Separation	0.9723	0.9411	0.7488	1546. 52	<0.0001 _a Quadratic	0.2264 _{ns}	4.62	0.0003 _a
Precipitation	0.9557	0.9059	0.7082	7.16	0.0002 _a Quadratic	0.7989 _{ns}	0.3684	0.0024 _a
Overall acceptance	0.8386	0.8040	0.6471	15.12	<0.0001 _a Quadratic	0.3836 _{ns}	0.7027	<0.0001 _a

Small letters include a and ns represent in order: a: ($P \leq 0.05$) at 95% level, ns(non-significant): ($P \geq 0.05$)

2-3 viscosity

The greatest impact was related to xanthan, capacarrageenan and monodiglyceride, respectively. It was found that assuming that xanthan is constant at 0.075%, viscosity increased with the increase of capcarrageenan in the minimal presence of monodiglyceride. However, with minimal capacarrageenan and increasing monodiglyceride, only 10 units were added to the viscosity and reached 80. With minimal monodiglyceride and increasing capcarrageenan from 0.008 to 0.031%, the viscosity value was 32 units, and with increasing monodiglyceride from 0.016 to 0.063%, it increased only about 10 units and reached 110. (Figure 1). Viscosity with xanthan 0.03% to about 0.07% was at a low level (>70), but gradually increased with the increase of xanthan (Figure 2). As the amount of capacarrageenan increased from zero to 0.015%, the viscosity was still low, but it increased with further increase. With xanthan (0.030%) and with the increase of capacarrageenan from 0.008% to 0.031%, the viscosity increased slightly, but with the

increase of xanthan from 0.030 to 0.119%, it reached above cP126. With capacarrageenan 0.008% and with the increase of xanthan from 0.030% to 0.119%, the viscosity reached from 51 to 93, now with the increase of capacarrageenan from 0.008% to 0.02%, the viscosity reached from 93 to 110 and with the increase Most capacarrageenan viscosity also increased. In general, the simultaneous increase of both hydrocolloids causes a noticeable increase in viscosity, so that the highest level of synergy among the three conditions created in this case has happened. The reason can be related to the chemical structure of these two stabilizers. Hydrocolloids are hydrophilic and hydrophilic compounds that, due to the hydroxide groups in their structure, have the ability to react with water molecules and establish hydrogen bonds, which leads to an increase in water absorption and retention, and as a result, an increase in the viscosity of the final product.][1]. Also, on the one hand, carrageenan is well hydrated in water due to having sulfate groups, and this hydrophilic property causes it to be

surrounded by water molecules, and on the other hand, its sulfated or anionic part reacts with casein and forms a gel. Therefore, the formation of a gel leads to an increase in viscosity [7, 14]. By examining the effect of capcarrageenan and monodiglyceride, as well as monodiglyceride and xanthan on viscosity, it was found that monodiglyceride had no significant effect on viscosity (Figures 1 and 3). This was while the increase of xanthan from 0.064% caused the growth of viscosity, and with the increase of monodiglyceride up to 0.04% and xanthan up to 0.054%, it reached 80 centipoise. With a minimum of 0.03% xanthan and an increase in monodiglyceride from 0.016 to about 0.042%, the viscosity went from 65 to 70, and from 0.042% to 0.063%, the viscosity reached from 70 to 75. With minimum monodiglyceride of 0.016% and increasing xanthan from 0.030 to 0.119%, the viscosity reached from 65 to 105 centipoise, but with the increase of monodiglyceride, little changes were obtained in viscosity and it reached from 105 to 115. According to the mentioned cases, xanthan, then capcarrageenan, and finally monodiglyceride had the greatest effect on viscosity. The results of statistical analysis showed that the linear effects of all three independent variables of capcarrageenan, monodiglyceride and xanthan on viscosity were significant and they led to an increase in the viscosity of cocoa milk in a highly significant manner ($P \leq 0.001$). However, the interaction effect of hydrocolloids in pairs on each other and their square effects were not significant. The meaningful equation for viscosity is as follows:

$$\text{Viscosity} = +89.50 + 16.77A + 4.66B + 20.14C$$

The results of this research are in line with the findings of other researchers, including Selimian et al reported that capcarrageenan causes an increase in the viscosity of cocoa milk and also increases the viscosity of

cream by 0.01% to 0.03% [15]. Zarabadipour et al. also stated in an article entitled "Optimization of coconut milk formulation with tragacanth gum" that the use of tragacanth gum increases the viscosity of coconut milk [31] and also in a similar study. The effect of two variables of cocoa powder and tragacanth gum in cocoa milk was investigated. They reported that the use of different concentrations of guar, carrageenan and xanthan increases the viscosity of flavored milks. [2]. Leila Natghi and colleagues also in the research. The effect of berry juice, basil seed gum and Katira on the physicochemical, antioxidant and sensory properties of cocoa milk was expressed that adding small concentrations of gums such as pectin, xanthan, guar, locust bean gum, gelatin and carrageenan, in order to stabilize acidic and non-acidic dairy drinks, has increased the viscosity of the product, which is in line with the results of this study. [24].

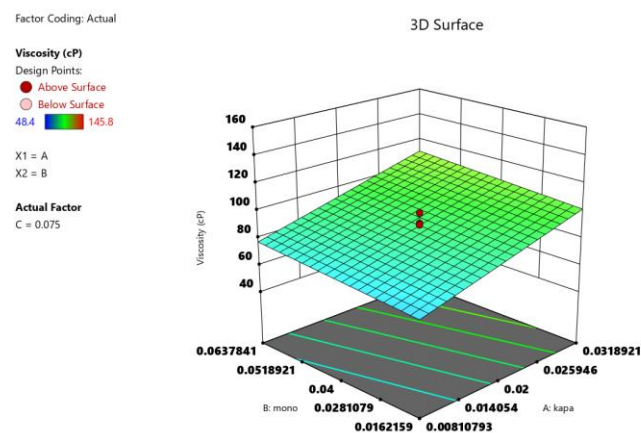


Fig 1) Effect of K-carrageenan and monodiglyceride on viscosity

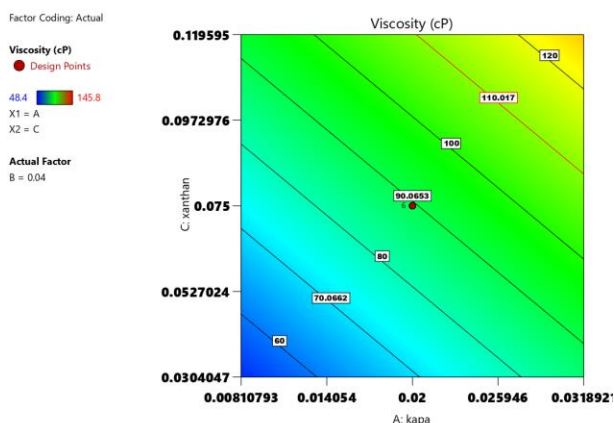


Fig 2) Effect of K-carrageenan and mono-diglyceride on viscosity

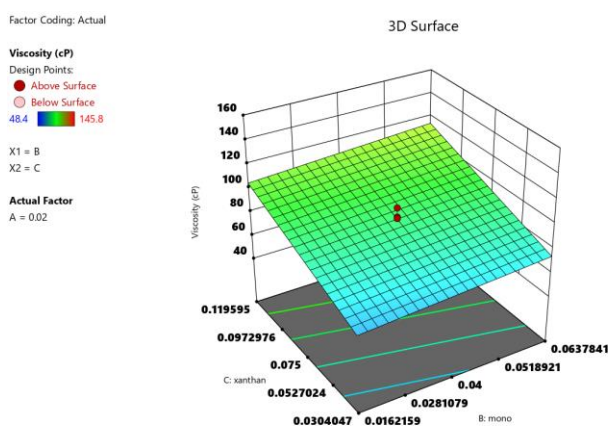


Fig 3) Effect of mono-diglyceride and xanthan on viscosity

3.3 Biphasing

The results showed that the effect of the quadratic equation of the independent variables caparrageenan (A (and monodiglyceride) B (with the simultaneous effect of monodiglyceride-xanthan) BC) and caparrageenan-xanthan (AC (and the effect of the second power of Kappakaragin) A^2) at a significant level They were. In addition, the quadratic effect of monodiglyceride (B^2 (with a level of 0.0671 very significant) $P \leq 0.05$) It was close. According to the statistical data, the lowest amount of biphasic was related to treatment number 7 with 1.51%, in their formula +1 level of hydrocolloids was used, and the maximum was for treatment number 15 with 60%

biphasic (completely two and three phased) which is from the level -a Caparrageenan and level 0 of monodiglyceride and xanthan were used (Table 2). Basically, carrageenan acts as an absorbent hydrocolloid. Therefore, if the amount of carrageenan is so low that this molecule is not enough to completely cover the casein particles (usually one molecule of this hydrocolloid is absorbed by the surface of two or more casein particles), as a result, it causes the particles to connect to each other by forming a bridge, which is unstable. Follows the system. While the use of carrageenan in the right concentration causes the stability of cocoa milk [10]. It was found that with the assumption of xanthan 0.075%, if monodiglyceride is 0.016%, the addition of caparrageenan decreased the biphasic amount by 36%. But the increase of caparrageenan from 0.008% to 0.02% and from 0.02 to 0.031% caused a decrease of 30% and 8% of biphasic (Figure 4).

If the amount of caparrageenan is 0.008%, the increase of monodiglyceride caused a decrease in biphasic hypoxia, so that the increase of monodiglyceride from 0.016% to 0.063% resulted in only 13% decrease. With caparrageenan content of 0.02%, the increase of monodiglyceride from 0.016% to 0.04% caused a decrease in biphasic from 15.5% to 8%, and with further increase of monodiglyceride to 0.063%, this number decreased from 8% to 5.5%. 5% receipt. If the amount of caparrageenan is constant at 0.031%, with the increase of monodiglyceride from 0.016 to 0.063%, the biphasic level increased from 7% to about 1%. According to this analysis, increasing monodiglyceride caused a slight decrease in biphasic, but increasing caparrageenan caused a significant decrease in biphasic. Another noteworthy point is that with the amount of caparrageenan at 0.029% and with the increase of monodiglyceride from 0.016 to 0.055%, biphasezation decreased,

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but by adding more monodiglyceride, biphasization also increased. Also, in the amount of monodiglyceride 0.055% and with the increase of capcarrageenan to 0.029%, biphasing decreased and then increased. Assuming that the amount of monodiglyceride is 0.02% and xanthan is 0.030%, the increase of capcarrageenan from 0.008 to 0.02% caused a noticeable decrease in biphasic, and then with the increase of capcarrageenan to 0.031%, biphasic from 12 to 055 0% receipt (Figure 5). If capcarrageenan and xanthan are used simultaneously in the cocoa milk formula, as long as the xanthan used is at the level of -1, with the increase of capcarrageenan up to 0.29%, biphasic will decrease, but if in If this amount of capcarrageenan, increasing xanthan occurs, it will not only decrease the biphasic, but also increase the biphasic. In the analysis of the mutual effect of monodiglyceride and xanthan on biphasic, if capcarrageenan is 0.02%, with minimum xanthan of 0.030% and increasing monodiglyceride from 0.016 to 0.036%, biphasic from 13.72 to 872. It reached 11% and had a slight decrease of about 2%, but with the increase of monodiglyceride from 0.036 to 0.063%, biphasic increased and grew about 3.5% and reached 15.22% from 11.872 to 0.063% (Figure 6). According to this analysis, the use of xanthan to reduce biphasic was effective when the amount of capcarrageenan used was at least 0.02% and monodiglyceride was 0.063%, in this case, with the increase of xanthan, the biphasic was greatly reduced and almost reached zero. Capcarrageenan (independent variable) has the greatest effect on diphasicA), Capacaraganin's square effectA²), the simultaneous effect of monodiglyceride-xanthanBC), the simultaneous effect of capcarrageenan-xanthan.AC(and the independent effect of monodiglyceride)B) they had.

$$\text{Phase Separation} = +8.07-16.36A-5.01B+4.82AC-5.79BC+9.78A^2$$

The increase of capcarrageenan and monodiglyceride alone, as well as the increase of xanthan, if the level of capcarrageenan is 0.02% and monodiglyceride is 0.04%, caused biphasic decrease. The lowest biphasic value was also obtained with +1 level of all three hydrocolloids. The data of this study are in line with the findings of Beta Bakhshi et al. [1] who reported that the use of stabilizers greatly reduces biphasing. Hydrocolloid stabilizers stabilize food systems by increasing the apparent viscosity of the product or by colloidal interaction of the type of spatial hindrance and electrostatic repulsion. Basically, carrageenan in low concentrations is not able to stabilize cocoa milk. Also, according to the findings of Ostadzadeh et al. [10], in the concentrations of 0.01% and 0.015% of capcarrageenan, cocoa particles were not precipitated, but the cocoa milk was two-phased and the amount of this two-phased in 0.015% was less than 0.01%. ; While in the concentration of 0.02% carrageenan, complete stability was established, which is also according to the data of this study.

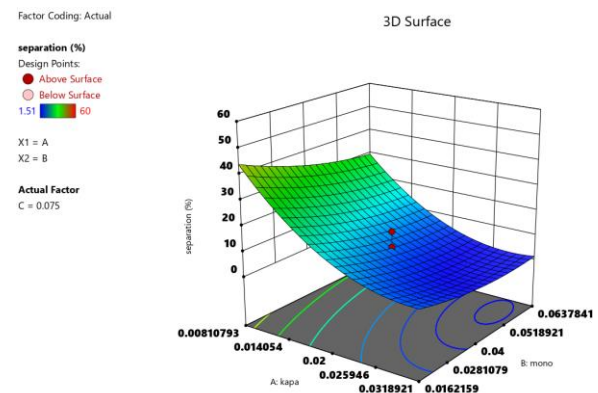


Fig 4) Effect of K-carrageenan and monodiglyceride on separation

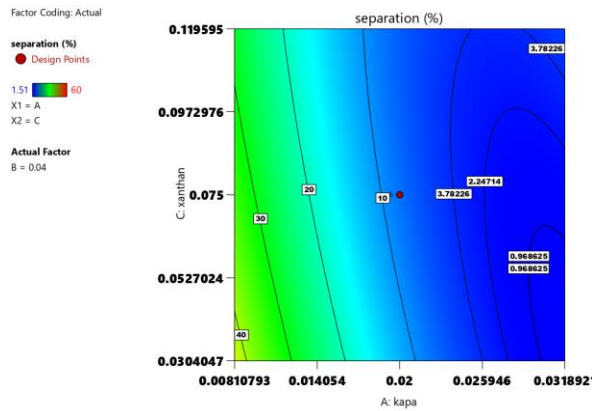


Fig 5) Effect of K-carrageenan and xanthan on separation

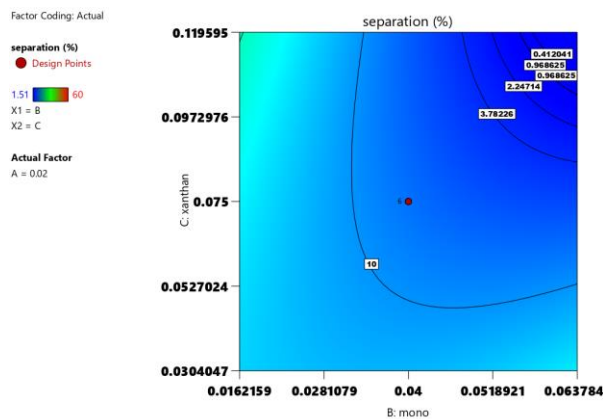


Fig 6) Effect of mono-diglyceride and xanthan on separation

4-3 deposition

The results showed that based on the analysis of variance table¹³, the effect of the quadratic equation of the independent variables of CapacarrageninA (and monodiglyceride)B), the simultaneous effect of capacarrageenan-xanthan.AC (and the simultaneous effect of monodiglyceride-xanthan)BC), Capacarragenin's square effectA² (and the square effect of monodiglyceride)B²) at a significant level(P≤0.05) They were.

¹³ - ANOVA

$$\text{Precipitation} = +5.69 - 0.9055A - 0.5517B + 0.3826AC - 0.4949BC + 0.5064A^2 + 0.3438B^2$$

The minimum amount of precipitation was 5.202% and the maximum was 8.61% (Table 2). With xanthan at least 0.075%, the increase of capacarrageenan and monodiglyceride both decreased the amount of precipitation (Figure 7). If monodiglyceride is equal to at least 0.04%, the increase of capacarrageenan decreased the amount of sediment, but with the increase of xanthan up to 0.02% of capacarrageenan, the sedimentation decreased and with the increase of capacarrageenan, the amount of sediment increased slightly (Figure 8). With 0.02% capacarrageenan, the increase of monodiglyceride decreased the precipitation. The increase of xanthan also increased the amount of monodiglyceride up to 0.04% and decreased the amount of monodiglyceride in higher amounts (Figure 9). Salimian et al. stated that the use of 0.1% commercial stabilizer caused a significant increase in the amount of precipitation of cocoa milk samples, and the reason for this may be the formation of a very strong gel, which is caused by the use of a high amount of this stabilizer, which increased the viscosity, but showed less resistance to biphasing.[15]. According to the report of Zarabadipour et al., the reason for the different effect of the percentage of hydrocolloids on the amount of deposition is due to the theory of Sirbeh et al., in which the phenomenon of discharge coagulation¹⁴ It also occurs when the concentration of free hydrocolloid is more than the required amount, and therefore the hydrocolloid cannot absorb water and shows itself as sediment. Also below Kappa and Landa-Karaginan stated¹⁵ in concentrations

¹⁴ - Depletion Flocculation

¹⁵ - λ-Carrageenan

of 0.1% and 0.3% caused stability and reduced the amount of sediment in cocoa milk, and the concentrations of 0.05 and 0.1% of carrageenan increased the precipitation of cocoa milk, which is in line with the results of this research [2]. In general, however, the results of this study showed that by taking into account different percentages of hydrocolloids and proportionality of their usage percentage in the formulation of cocoa milk, it is expected to increase the stability of the product and reduce the amount of sediment formation. Zarabadipour and colleagues are also in line with Bakhshi et al reported that they use hydrocolloids to prevent the sedimentation of cocoa particles in cocoa milk [1]. Salimian et al. stated that the increase of carrageenan by 2.0% Due to the production of gel Very strong, it increased the viscosity of the cream, but it was less resistant to two phases [15]. The findings of Leila Natghi et al. regarding the reduction of sedimentation by caparraganin in cocoa milk are also consistent with the data of the present study [24].

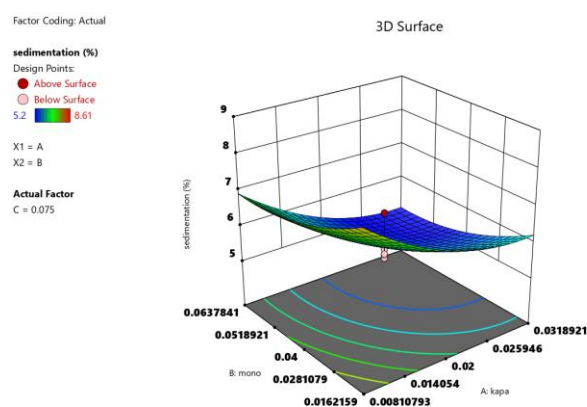


Fig 7) Effect of K-carrageenan and mono-diglyceride on precipitation

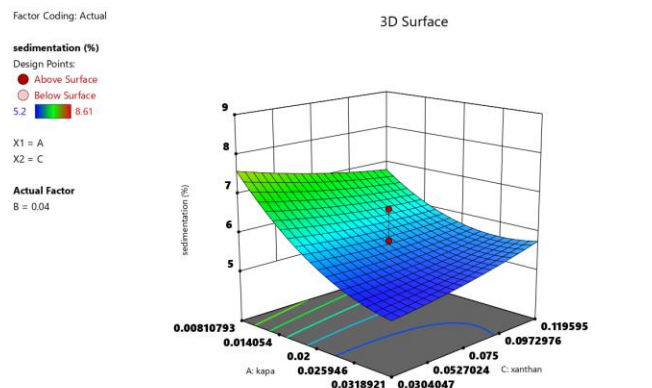


Fig 8) Effect of K-carrageenan and xanthan on precipitation

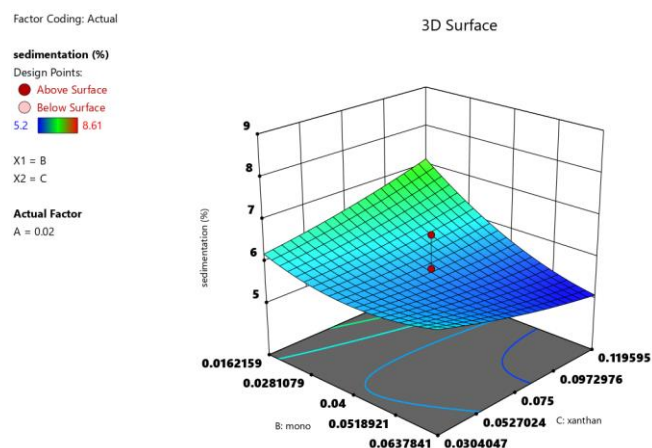


Fig 9) Effect of mono-diglyceride and xanthan on precipitation

5-3 sensory evaluation (general acceptance)

Overall acceptance is considered the most important factor in sensory evaluation, which evaluators rate by considering all product characteristics. These characteristics are largely related to consumer acceptance of that product. The results of statistical analysis showed that the linear effects of two independent variables of caparraganin (A and Xanthan) C (on overall acceptance) was significant and they were significantly ($P \leq 0.001$) in the formulation of cocoa milk led to an increase in overall acceptance. However, the interaction effect of hydrocolloids in pairs

on each other, as well as their quadratic effects, were not significant. Capcaraginan and then xanthan had the greatest impact on overall acceptance.

Overall acceptance= +3.11+1.52A+0.5159C
 With 0.075% xanthan and increasing capacarrageenan at any level of monodiglyceride, the sensory test score increased so that it reached a score of less than two with 0.008% capacarrageenan to a score of five at 0.031% capacarrageenan, but the increase of monodiglyceride showed a significant change. It did not cause overall satisfaction and only 0.5% increased satisfaction at each level of capacarrageenan (Figure 10). with 0.04% Monodiglyceride And with the increase of capacarrageenan at all levels of xanthan, the sensory test score increased, but with the increase of xanthan at each level of capacarrageenan, only one score improved (Figure 11). Without the presence of capacarrageenan, even in the highest amounts of monodiglyceride and xanthan, the sensory test score was not higher than 3.8. But with the presence of at least 0.025% of kapacaraginan, the score was at least four (Figure 12). These data are in line with the data of Beta Bakshi et al. who reported that the sensory test scores increased significantly with the increase of stabilizers [1]. The research results of Salimian et al showed that the use of stabilizers improved the sensory score due to the favorable effect it had on the physical and chemical properties of cocoa milk [15]. Natghi et al also reported The addition of agents improves the sensory characteristics of the drink in such a way that the addition of hydrocolloids increases the viscosity of the drink but reduces the acceptance of the taste of the drink [24]. Excessive use of gums causes a decrease in acceptability from the consumer's side, because with the increase in viscosity, the fluidity of the product, which is one of the parameters of desirability, decreases [32 and 33].

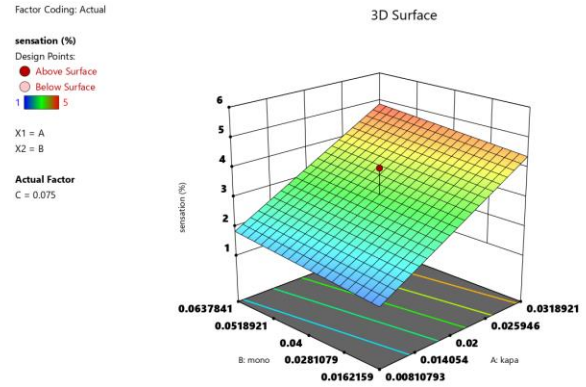


Fig 10) Effect of K-carrageenan and mono-diglyceride on overall acceptance

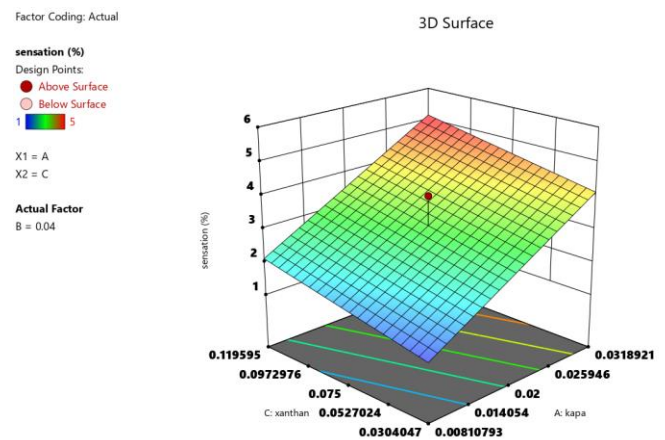


Fig 11) Effect of K-carrageenan and xanthan on overall acceptance

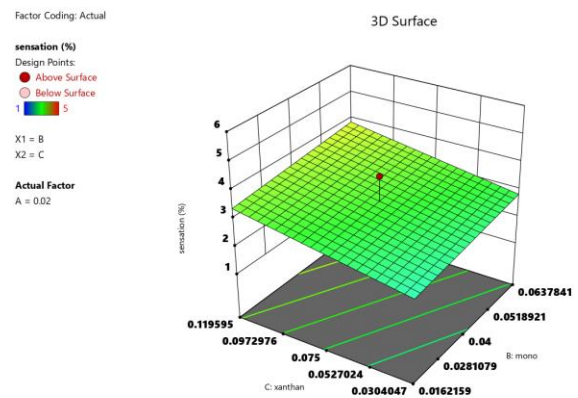


Fig 12) Effect of mono-diglyceride and xanthan on overall acceptance

6-3 optimization

Numerical optimization method was used to produce optimal cocoa milk containing triple hydrocolloids. The range of values obtained for the optimization process and its purpose are specified in Table 4. The level of desirability of the optimal samples including viscosity with 110 centipoise and the highest overall acceptance score with an importance level of 3 out of 5, as well as the lowest amount of precipitation and two-phase with an importance level of 5 out of 5 were considered in the software. The optimal examples suggested by the software included 26 formulas, but the number one formula is because it has the highest level Desirability¹⁶ It was selected which included 0.031% caparrageenan, 0.064% monodiglyceride and 0.075% xanthan. The optimal sample proposed by the software with the same conditions as other production treatments and with the actual results of the cocoa milk produced by the test-t-test An example with software Spss IBM Version 26 at the 95% confidence level ($P < 0.05$) was compared (Table 5) and considering that no significant difference was observed, the accuracy of the predicted model was confirmed as a result (Table 6).

Table 4) Optimizing the percentage of physical and sensory characteristics

Name	Goal	Lower Limit	Upper Limit	Importance
A: name	is in range	0.008	0.031	3
B: mono	is in range	0.016	0.063	3
C: xanthan	is in range	0.030	0.119	3
Viscosity	is target = 110	48.4	145.8	3

¹⁶ - Desirability

Phase separation	Minimize	1.51	60	5
Precipitation	Minimize	5.202	8.61	5
Overall Acceptance	Maximize	1	5	3

Table 5) Evaluation of the average of optimal laboratory samples and the optimal sample proposed by the software

	Viscosity	Phase Separation	Precipitation	Overall Acceptance
Lab. Samples	110.00	0.533	5.266	4.33
Suggested	110.20	0.807	5.051	4.77

Table 6) No significant difference between the optimal sample proposed by the software and the experimental optimal samples

Number	Name	Mean	Test Value	Sig. (2-tailed)
1	Viscosity	110.2	111.933	0.489 _{ns}
2	Phase Separation	0.533	0.116694	0.103 _{ns}
3	Precipitation	5.26667	5.03	0.115 _{ns}
4	Overall Acceptance	4.33	4.6666	0.423 _{ns}

($P \geq 0.05$) at 95% level and ns= no significant difference

4 - Conclusion

The results of the current research showed that the response level statistical method is a reliable method for choosing the optimal

levels of conditions for the production of cocoa milk containing triple hydrocolloids. Considering the lack of significant differences between the predicted answers of the response level program by DesignExpert software and the actual (laboratory) results by the test T-test, there was a suitable efficiency for predicting and optimizing the evaluated parameters (Tables 5 and 6). As a result, the optimal formula including 0.031% caparrageenan, 0.064% monodiglyceride and 0.075% xanthan is suggested to produce cocoa milk with a suitable viscosity of 110 centipoise and the lowest amount of precipitation and biphasing. The results showed that the increase of hydrocolloids had a significant effect on increasing the viscosity of the treatments, so that the highest effect was related to xanthan and caparrageenan, respectively, and the lowest was related to monodiglyceride. The highest level of synergism was related to xanthan and caparrageenan. The results of statistical analysis showed that the linear effects of all three independent variables of caparrageenan, monodiglyceride and xanthan on viscosity were significant and they led to an increase in viscosity in cocoa milk formulation. However, the interaction effect of hydrocolloids in pairs on each other, as well as their quadratic effects, were not significant. The results of this study showed an increase in monodiglyceride caused a slight decrease in biphasic and an

increase in caparrageenan caused a significant decrease in biphasic. By considering different percentages of hydrocolloids and proportionality of their usage percentage in the formulation of cocoa milk, it is expected to increase the stability of the product and reduce the amount of sediment formation. The results of statistical analysis showed that the linear effects of the two independent variables of caparrageenan and xanthan on overall acceptance were significant and they led to an increase in overall acceptance. However, the interaction effect of hydrocolloids in pairs on each other, as well as their quadratic effects, were not significant. First, caparrageenan and then xanthan had the greatest impact on overall acceptance, but the increase of monodiglyceride did not cause a significant change in overall satisfaction, and only 0.5% increased satisfaction at each level of caparrageenan.

5) Gratitude

The authors of this article would like to express their gratitude to Professor Ali Motamedzadegan from Sari University of Agricultural Sciences and Natural Resources for his valuable guidance and also to Pak Pasteurized Milk Company for providing the necessary facilities to carry out this research.

6- Resources

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اثر پایدارکننده‌های زانتان، کاراگینان و مونودیگلیسیرید بر ویژگی‌های حسی و فیزیکوشیمیایی شیرکاکائو به

روش سطح پاسخ

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چکیده

اطلاعات مقاله

وجود یک لایه کاکائو در انتهای بطری شیرکاکائو از نظر مصرف‌کننده ناخوشایند است و معمولاً میزان رسوب ذرات کاکائو در انتهای بطری اولین موردی است که جلب توجه می‌کند. هدف از این پژوهش بررسی اثر پنج سطح از پایدارکننده‌ها شامل: زانتان، کاپاکاراگینان و مونودیگلیسیرید بر ویژگی‌های حسی و فیزیکوشیمیایی شیرکاکائو به روش سطح پاسخ (RSM) بود. از اینرو، ۲۰ تیمار در سه بلوک و با شش تکرار در نقطه‌ی مرکزی تولید شد. سپس آزمون‌های فیزیکوشیمیایی و حسی انجام پذیرفت. برای تولید شیرکاکائوی بهینه با ویسکوزیته‌ی مناسب ۱۱۰ سانتی‌پواز و کمترین میزان رسوب و دوفازشدن، از روش بهینه‌یابی عددی استفاده شد. نمونه‌های پیشنهادی نرم‌افزار در آزمایشگاه تولید و با نتایج واقعی شیرکاکائوها با آزمون T-test یک‌نمونه‌ای در سطح اطمینان ۹۵٪ مورد مقایسه قرارگرفت که باتوجه به عدم مشاهده تفاوت معنی‌دار، صحت مدل پیش‌گویی شده تایید شد. بر اساس نتایج مشخص شد که افزایش هیدروکلوئیدها در سطح اطمینان ($P < 0.001$) سبب افزایش ویسکوزیته و همینطور افزایش پایداری محصول و کاهش میزان تشکیل رسوب گردید. ارزیابی حسی نشان داد که کاپاکاراگینان بیشترین تاثیر را بر پذیرش کلی داشت و سپس به ترتیب زانتان و مونودیگلیسیرید نقش مثبت داشته‌اند ($P < 0.001$).

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