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The Effect of Adding D-tagatose and Lemon Essential Oil on Quality of Chocolate

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ABSTRACT

Chocolate is a popular and widely consumed product in different ages, however, the demand of consumers has increased for healthy chocolate with free or low sugar in recent years. The objective of present research is to substitute the sucrose of chocolate with D-tagatose and lemon essential oil for improving biological functions. The seven formulations of chocolate included distinct percentages of D-tagatose (0, 13, 29 and 47 %) and lemon essential oil (0, 0.1 and 0.2 %). Afterwards, the tests were performed on chocolate treatments such as moisture level, fat, sugar content before and after hydrolysis, color perception, hardness, antioxidant, microbial and sensory evaluations. The results demonstrated that moisture of treatments (except CTL5 containing 47.80 % D-tagatose and 0.1 % lemon essential oil) was noticeably different from the control. A significant difference was observed between the average sugars of treatments before hydrolysis, however, there was no considerable difference in fat levels (33.39 to 33.41 %). The essential oil has led to a reduction in hardness, so CTL6 (including 47.90 % D-tagatose and 0.2 % lemon essential oil) with higher essential oil had a lower hardness (16.61 N). D-tagatose and lemon essential oil had an influence on color features, but antioxidant functions were affected only by essential oil and all treatments were within the standard range in terms of microbial load. In sensory assessment, the overall acceptance score was given to the control (4.96) and CTL5 (4.92). The conclusion of present research introduced CTL5 as the most suitable treatment for production of healthy chocolate.

1. Introduction

In recent years, the chocolate market and industry has increased under the influence of the increasing demand of consumers for healthy chocolate, i.e. low sugar or no sugar [1]. Chocolate is a source of energy with fast metabolism, which is rich in polyphenols such as catechin, anthocyanidin and proanthocyanidin. The unique taste and texture of chocolate and the feeling of pleasure after eating it are the main reasons for the growing consumption of this product. [2]. Consuming chocolate has positive effects on blood pressure, insulin and vascular function, it also increases antioxidant properties and nitric oxide production [3].

So far, extensive research has been done to reduce or replace sugar and fat in chocolate, for example, di-tagatose and inulin as a substitute for sucrose, hydrolyzed collagen as a part of cocoa butter fat, as well as date powder and soy flour as beneficial ingredients. It has been used in chocolate [4, 5, 6]. The results of using lemon marmalade, ditagatose, inulin, stevia and also Arabica coffee pulp in chocolate have been favorable [6, 7, 8]. The regenerating sugar ditagatose is a cetohexose and digalactose isomer, which is naturally isolated in low concentrations from some fruits and dairy products. This low-calorie and valuable sweetener can be used as a safe additive in dietary food products [9].

The variety of flavoring additives for food products is very wide, these substances may be natural, synthetic or a combination of them [11]. It has been reported that lemon essential oil has antibacterial, antioxidant and fungicidal properties and is listed as a safe additive.[2]It is recognized as GRAS and is also widely used as a preservative or flavoring in the food industry [12]. Its main flavoring compounds include citral[3], limonene[4], they arrive[5]Octanal[6] and gamma terpenes[7] is [13].

In this research, an attempt has been made to improve the quality and antioxidant properties of chocolate by reducing sucrose and replacing it with di-tagatose sweetener, as well as using lemon essential oil. Sensory evaluators should also be introduced. The review of previous researches has shown that so far the combination of different amounts of ditagatose (0, 13, 29 and 47%) and lemon essential oil (0.1 and 0.2%) has not been used in chocolate formulation, therefore the need for this The research was felt.

2- Materials and methods

2-1-Combinations used in the present research

Raw materials include cocoa powder (from Altinmarka company, Turkey), cocoa butter (with the brand name Africana from Shirin Assal, Iran), lecithin and vanillin (Taste Marker company, Dubai), sucrose (Shamami Sugar, Iran), Ditagatose (Damhert Company, Belgium) and lemon essential oil (Nikoshimi Company, Iran) were purchased. The production process was carried out in the chocolate workshop in Alborz province. All culture media and chemicals used in this research were obtained from Merck, Germany.

2-2- Preparation of samples

2-2-1- Preparation of chocolate samples

Seven treatments were prepared according to the formulation in Table 1, so that all compounds (except lemon essential oil) were mixed together in a laboratory ball mill (chocolate mill), reducing the particle size and kneading.[8] Chocolate was done at the same time. Chocolate was prepared at a temperature of 50 degrees Celsius for 3 hours at a speed of 85 rpm, then the samples were kept in an incubator at a temperature of 50 degrees Celsius for 12 hours. In the next step, lemon essential oil was added and the samples were temperature-conditioned by laboratory tempering at a speed of 75 rpm, at the end, the chocolates were wrapped in aluminum foil molds and kept in closed containers until the tests were carried out at a temperature of 10 degrees Celsius (refrigerator). were kept [9].

2. Generally recognized as safe
3. Citral
4. Limonene
5. Myrcene
6. Octanal
7. The gamma terpins
8. Conching

Table 1 Formulations of chocolate treatments in the present study

Code	Sugar	Cocoa butter	Cocoa powder	Lecithin	Vanillin	Lemon essential oil	D-Tagatos
CC	52.30	31	16	0.6	0.1	0	0
CTL_1	22.36	31	16	0.6	0.1	0.1	29.84
CTL_2	22.36	31	16	0.6	0.1	0.2	29.74
CTL_3	38.84	31	16	0.6	0.1	0.1	13.36
CTL_4	38.84	31	16	0.6	0.1	0.2	13.26
CTL_5	0	31	16	0.6	0.1	0.1	47.80
CTL_6	0	31	16	0.6	0.1	0.2	47.90

CC: Control Chocolate

CTL: Chocolate containing D-tagatose and lemon essential oil

2-3- Exams under review

2-3-1- Physical and chemical tests including determination of moisture, sugar and fat

The percentage of moisture was measured according to the Karl Fischer method according to the AOAC standard No. 10-9772 grams of chocolate samplesOven (made by Memmert, Germany)with a temperature of 100 degreesCelsius Dryness and weight loss were reported as moisture content.

The amount of sugar before and after dehydrating the samples using the Linn-Inon-Fisher method according to the national standard of Iran No. 759, the summary of the method was that 5 grams of chopped chocolate was mixed with some lukewarm water. A period of time was given to the solution for the insoluble compounds to precipitate, then the supernatant was slowly passed through a strainer and transferred to a 100 ml volumetric flask. This operation was done several times to cool and reach a volume of 100 ml. became. The desired solution was used to measure the amount of sugar before and after water extraction. Grading was done by using A and B Fehlings. Also, sugar solutions were prepared with sequential concentrations according to the target standard.

Level Total fat according to AOAC standard No. 963-15, in short, 5 grams of the homogenized sample was poured into filter paper and its weight was recorded, then it was placed inside the extractor and about 300 ml of n-hexane solution was added to the Soxhlet flask. which had already reached a fixed weight was added. The refrigerant was connected to the cold water and the heater temperature was adjusted so that 7 to 8 times of circulation were done within 1 hour. After a few hours, the solvent was recovered and the balloon was separated from the device and placed on a Bain-Marie device until the hexane evaporated, then the balloon was placed in a 100 degree oven until it reached a constant weight, then it was cooled in a desiccator and weighed, using Results were obtained from relationships 1 and 2 [14].

: $(IN_1 - IN_0) / M \times 100\%$ total fat: relation 1

IN_1 : BALLOON WEIGHT + EXTRACTED FAT WEIGHT: Relationship 2

M: SAMPLE WEIGHT, W_0 : INITIAL WEIGHT OF EMPTY BALLOON

2-3-2-Hardness level of tissue

Chocolate pieces with dimensions of 10 x 20 x 80 mm were kept at a temperature of 20 degrees Celsius for 6 hours in a refrigerated incubator (manufactured by Shimaz Company, Iran). The penetration test was performed with a texture tester (Hounsfield H50KS, England), equipped with a 1.6 mm flat bottom and a penetration speed of 1.5 mm/s, and the maximum force at a depth of 6 mm was reported as a hardness index [8].

2-3-3-Measurement of color characteristics

The measurement of color characteristics was done using a HunterLab device (Hunter Lab-D25-9000, Germany). For this purpose, chocolate samples were prepared in the form of circles with a diameter of 90 mm and a thickness of 10 mm. * ،b^* And L^* They were measured [15].

2-3-4- Microbiological test

Before weighing, the chocolate samples were heated and melted for 1 hour at 37 degrees Celsius, then 10 grams of each sample was placed aseptically into plastic bags containing 90

ml of sterile sodium chloride solution of 0.85% (weight by volume) and peptone (0.1 percent) were transferred and homogenized for 2 minutes at 37 degrees Celsius. Each sample was appropriately diluted by successive dilutions prepared from sterile sodium chloride solution of 0.85% (w/v) and peptone (0.1%). Counting groups of microorganisms such as living bacteria, spore-forming bacteria, yeasts and molds, xerophilic mold, Enterobacteriaceae species, Enterococcus and Staphylococcus coagulase recorded on chocolate samples using the conventional pour-plate method.[9] was investigated. Plates were examined after incubation in terms of the types of colonies and morphological characteristics associated with each growth medium, and the results were expressed as colony-forming units per gram of sample (CFU/g) [16].

2-3-5- DPPH free radical inhibition test

DPPH radical solution was prepared by dissolving 0.005 g of DPPH free radical powder in a volumetric flask (100 ml) containing methanol. 1 to 2.5 g of the sample was weighed and mixed with 80% (w/v) methanol in a volumetric flask (50 ml), then extraction was performed by vigorous manual shaking. Then the samples were filtered through Whatman No. 4 filter paper to remove insoluble compounds, followed by dilutions (samples from 50 ml volumetric flask: distilled water) 1:1, 2:1, 5:1, 10:1 and 20:1 were prepared. The reaction was carried out between 0.2 ml of suspension and 7.8 ml of methanolic radical solution, from methanol as the only sample (0.2 ml of distilled water with 7.8 ml of 80% methanol) and from 0.2 ml of A liter of water with 7.8 ml of methanolic radical solution was used as a control sample. The absorbance of the tested samples at 517 nm after the reaction of 30 minutes in the dark was measured and the value of IC_{50} was calculated [17].

2-3-6- Sensory evaluation

Sensory evaluation was done by 25 trained evaluators, in order to avoid common errors in sensory evaluation, such as the error resulting from the evaluators' expectation or the error resulting from the stimulus, samples in similar sizes and special containers that are randomly coded with three-digit numbers. were provided to the evaluators. The samples were evaluated in terms of color, taste, smell, taste and texture, and each of the sensory evaluators gave a score based on a five-point scale from 1 for "unacceptable" and 5 for "excellent" [18].

2-4- Data analysis method

In order to statistically analyze the data and examine the obtained information, a factorial experiment was used in the form of a completely random design. All experiments were performed in triplicate, then the mean and standard deviation were obtained. In order to determine the difference between the means, analysis of variance was used and Duncan's multiple range test was used to group them. Minitab version 21 software was used in all stages of statistical data analysis.

3. Results and Discussion

3-1- Humidity level

The results of Table 2 showed that the average moisture content of all chocolate samples had a significant difference with each other, except for the CTL sample $_5$ (1.05 percent) with the control (99.9 percent) that no significant difference was observed in their moisture content. The results showed that the amount of moisture decreased with the decrease in the amount of lemon essential oil and the increase in the amount of tagatose in the samples. Among the examples are CTL_4 (1.61 percent) had the highest amount of moisture with a significant difference.

In the present study, all the samples had a moisture content of less than 2%, and the reason for the increase in moisture content with the increase in the percentage of lemon essential oil can be attributed to the presence of hydrophilic groups and its moisture-absorbing nature [12, 13]. The sample with the highest amount of tagatose did not have a significant difference in the moisture factor with the control sample, which indicates the low tendency of tagatose to absorb and retain moisture, the results obtained were similar to the results of other researchers [4, 5, 19].

9 . For flat

Table 2. The comparison effects of D-tagatose and lemon essential oil on moisture (%), fat (%), and sugar (%) before and after hydrolysis in chocolate treatments

Treatments	CC	CTL_1	CTL_2	CTL_3	CTL_4	CTL_5	CTL_6
Moisture (%)	$0.99^f \pm 0.05$	$1.20^d \pm 0.11$	$1.34^c \pm 0.09$	$1.50^b \pm 0.03$	$1.61^a \pm 0.07$	$1.05^f \pm 0.12$	$1.13^{lt\ is} \pm 0.05$
Fat (%)	$33.40^a \pm 0.03$	$33.41^a \pm 0.02$	$33.39^a \pm 0.04$	$33.40^a \pm 0.02$	$33.41^a \pm 0.01$	$33.40^a \pm 0.05$	$33.39^a \pm 0.01$
Sugar (%) before hydrolysis	$8.29^d \pm 0.02$	$15.60^b \pm 0.021$	$15.41^b \pm 0.05$	$10.74^c \pm 0.33$	$10.39^c \pm 0.06$	$22.11^a \pm 0.09$	$22.00^a \pm 0.10$
Sugar (%) after hydrolysis	$50.91^a \pm 0.53$	$50.10^b \pm 0.86$	$50.00^b \pm 1.01$	$49.84^b \pm 0.89$	$49.80^b \pm 1.06$	$50.37^b \pm 0.78$	$50.40^b \pm 0.91$

±Standard deviation (a-f): Different uppercase letters with statistical differences in the studied level ($p<0.05$) related to the studied times

CC: Control chocolate, $CTL_{1:}$ Chocolate treated with 29.84 (%) D-tagatose and 0.1 (%) lemon essential oil, $CTL_{2:}$ Chocolate treated with 29.74 (%) D-tagatose and 0.2 (%) lemon essential oil, $CTL_{3:}$ Chocolate treated with 13.36 (%) D-tagatose and 0.1 (%) lemon essential oil, $CTL_{4:}$ Chocolate treated with 13.26 (%) D-tagatose and 0.2 (%) lemon essential oil, $CTL_{5:}$ Chocolate treated with 47.80 (%) D-tagatose and 0.1 (%) lemon essential oil and $CTL_{6:}$ Chocolate treated with 47.90 (%) D-tagatose and 0.2 (%) lemon essential oil.

2-3- The amount of fat

The results of the values related to fat percentage were shown in the range of 33.39 to 33.41 (Table 2), chocolate samples containing ditagatose and lemon essence were not significantly different from the control sample in terms of fat ($p<0.05$). Because there was no change in the amount of fatty compounds and fat replacement in chocolate samples and it was similar to the previous research [6].

3-3- The amount of sugar before and after water extraction

As seen in Table 2, the average sugar before dehydration of the chocolate samples had a significant difference, and the control sample (8.29%) had the lowest sugar before dehydration. The samples containing the highest amount of tagatose had the highest amount of sugar before dehydration, and the amount of lemon essential oil had no effect on it. The amount of sugar before dehydration in the samples is related to the amount of reducing sugar, which increased significantly with the increase in the amount of reducing sugar tagatose [4 and 25]. The results of the sugar level after water extraction showed that this factor was significantly higher in the control sample (50.91%) than in other samples. The amount of tagatose and lemon essential oil had no effect on sugar after dewatering, and the results showed that acid dewatering was done well [4].

Previous researchers investigated the sugar content of dark and milk chocolate, and their results showed that in milk chocolate, due to the presence of the reducing sugar lactose, the percentage of sugar was higher before boiling [5, 19]. In line with the current research, the reduction of sucrose sugar and the increase of glucose and fructose by replacing date powder in low-calorie dark chocolate led to the reduction of sugar after juicing [6].

3-4-color characteristics

The color characteristics provide the first impression in expressing the quality of the product to the consumer, as a result, the intensity of the product's acceptability is affected [18]. The results of Table 3 showed that both tagatose and essential oil had an effect on the amount of color factors, so that with the increase of tagatose, the intensity of L* and a* of the samples increased and it was in reverse ratio with the amount of lemon essential oil. Among the samples, Roshni CC (16/28) with CTL_4 (16/30) had no significant difference. CTL_5 (20/56) had the highest amount of clarity because these samples had the lowest and the highest amount of tagatose, respectively. a* It was the lowest value in CC (7/01) and CTL $sample_4$ With the lowest tagatose and the highest

essential oil after the control sample, it had the lowest a* (7.44), on the other hand, CTL_5 It had the highest amount of this factor (9.52). The results of Table 3 showed that by increasing the amount of tagatose and lemon essential oil, b* Increased. among samples b* The lowest in CC (6/00) and in CTL_6 The highest amount of this factor (8.80) was observed, because in this sample, the highest amount of tagatose and essential oil were present, both of which were positive influencing factors on b*.

The results of the previous researches were in line with the results of the present research, in that the lowest values of L*, a* and b* were observed in the chocolate sample with 100% inulin without tagatose, and with the increase of tagatose, all three color factors increased [4]. Different percentages of roasted and unroasted cocoa beans were used to produce chocolate and the results showed that increasing the use of unroasted cocoa liquid led to an increase in L*, a* and b* of the samples [16]. Addition of cinnamon essential oil to chocolate formulation leads to a slight decrease in color factors L* and a* and an increase in b* which is consistent with the effect of lemon essential oil in the present study [20, 21].

Table 3. The comparison effects of D-tagatose and lemon essential oil on color, hardness and antioxidant attributes in chocolate treatments

Treatments	CC	CTL_1	CTL_2	CTL_3	CTL_4	CTL_5	CTL_6
L*	16.28^{f} ± 0.01	19.39^{c} ± 0.09	18.75^{d} ± 0.07	$17.50^{It\ is}$ ± 0.05	16.30^{f} ± 0.02	20.56^{a} ± 0.11	20.10^{b} ± 0.14
a*	7.01^{f} ± 0.03	8.50^{c} ± 0.10	7.92^{d} ± 0.15	7.73^{of} ± 0.06	$7.44^{It\ is}$ ± 0.17	9.52^{a} ± 0.20	9.10^{b} ± 0.09
b*	6.00^{f} ± 0.17	7.30^{d}± 0.09	6.97^{c} ± 0.10	6.20^{f} ± 0.19	$6.72^{It\ is}$ ± 0.36	8.61^{b} ± 0.06	8.80^{a} ± 0.11
Maximum compression force (N)	17.10^{a} ± 0.34	15.23^{b} ± 0.07	15.05^{bc} ± 1.16	14.90^{c} ± 0.19	14.87^{c} ± 0.03	16.85^{a} ± 0.25	16.61^{not} ± 1.01
IC50 (mg/mgDPPH)	11.32^{a} ± 0.12	7.41^{b} ± 0.14	2.20^{c} ± 0.71	7.54^{b} ± 0.15	2.14^{c} ± 0.31	7.48^{b} ± 0.50	1.27^{c} ± 0.11

±Standard deviation (a-f): Different uppercase letters with statistical differences in the studied level (p<0.05) related to the studied times

CC: Control chocolate, $CTL_{1:}$ Chocolate treated with 29.84 (%) D-tagatose and 0.1 (%) lemon essential oil, $CTL_{2:}$ Chocolate treated with 29.74 (%) D-tagatose and 0.2 (%) lemon essential oil, $CTL_{3:}$ Chocolate treated with 13.36 (%) D-tagatose and 0.1 (%) lemon essential oil, $CTL_{4:}$ Chocolate treated with 13.26 (%) D-tagatose and 0.2 (%) lemon essential oil, $CTL_{5:}$ Chocolate treated with 47.80 (%) D-tagatose and 0.1 (%) lemon essential oil and $CTL_{6:}$ Chocolate treated with 47.90 (%) D-tagatose and 0.2 (%) lemon essential oil

3-5- Texture hardness

Table 3 shows the significant effect of tagatose and lemon essential oil on the hardness of chocolate texture. The control sample had the highest level of hardness, and in the treated samples, the hardness of the tissue increased with the increase of tagatose, but the presence of lemon essential oil had an effect on reducing this factor. Examples of CC and CTL_5 (respectively 15.23 and 16.85 N) without significant difference, they had similar tissue hardness, but CTL_6 Having a high amount of tagatose (47.90%) and essential oil (0.2%), lower tissue hardness (16.61 N) than CTL_5 (N 16.85) had.

In a similar study, it has been shown that the hardness of the tissue samples increased with the increase in the amount of tagatose, and the formulas of 50% inulin-50% tagatose, 25% inulin-75% tagatose, and 100% tagatose were suitable for tissue improvement [5]. The lowest viscosity and yield stress were observed in chocolate samples containing high amounts of tagatose [22]. In another research, the hardness, thixotropy, plastic viscosity and appearance of chocolate increased with the increase of sucrose substitution percentage, as a result, the samples with the least substitution amount (15 and 20%) had less difference with the control sample [6]. The chocolate sample containing the highest amount of Arabica coffee grounds (60%) had the highest texture hardness due to its fiber and moisture content [8].

6-3- Antioxidant properties

The ability to inhibit free radicals of the samples is shown in Table 3, that the amount of tagatose was not significant on this property, but the amount of essential oil was significantly on $IC._{50}$ It was impressive. The control sample (11.32 mg/mg DPPH) has the lowest amount of antioxidant properties and the highest IC_{50} was, on the other hand, the samples containing lemon essential oil had more free radicals inhibiting properties with a significant difference and CTL_2، CTL_4 And CTL_6 without significant difference from each other, they showed the best antioxidant properties.

The biggest decrease in free radical inhibition rate is related to the decrease in the content of polyphenol compounds, which occurs when baking (roasting) chocolate, other production processes also decrease this activity (although to a lesser extent) [8, 23]. Lemon essential oil has a phenolic structure and bioactive compounds that lead to free radical inhibitory properties, and when this essential oil was used in the production of chocolate, according to the amounts used, the antioxidant properties increased [12, 13]. The results of previous researches showed that chocolate samples containing 100% roasted coca seeds have the greatest ability to inhibit free radicals (IC = 9.41 mg/mg $DPPH_{50}$) and the weakest by chocolate containing 100% unroasted coca seeds (IC = 11.49 mg/mg $DPPH_{50}$) was determined [16]. Cinnamon essential oil was added to chocolate at the level of 0.1% (weight/weight) because at this concentration, the taste of chocolate enriched with it was acceptable to the consumer and it increased the antioxidant properties of chocolate samples [23].

7-3-Evaluating the effect of ditagatose and lemon essential oil on the microbial population

The presence of undesirable microorganisms in food products endangers the health of consumers, so their control is very important [15]. Table 4 shows the effect of ditagatose and lemon essential oil on the total number of bacteria, the number of spore-forming bacteria, yeasts and molds, dry-loving molds, Enterobacteriaceae, Enterococcus and coagulase-positive Staphylococcus. The total number of bacteria in the control sample is more than other samples and the presence of lemon essential oil was effective in reducing this value, so that the samples containing 0.2% essential oil $(CTL)_2$، CTL_4 And CTL_6) had the lowest microbial load. The amount of spore-forming bacteria in all samples was the same without any significant difference, which indicated the lack of effect of essential oil on the reduction of spores. Yeast, mold, dry-loving molds and staphylococcus coagulase positive microorganisms were not observed in the culture medium of any of the samples. The highest amount of Enterobacteriaceae and Enterococcus was observed in control samples, but in CTL $samples_1$، CTL_3 And CTL_5 The intensity of infection decreased in CTL $samples_2$، CTL_4 And CTL_6 No growth of these two bacteria was observed. The current study showed that in seven formulations, the amount of microorganisms present was within the standard limit, so that according to the national standard of Iran, the maximum number of $Enterobacteriaceae^2$ 10 per gram, Bacillus $cereus^2$ 10 per gram, $mold^2$ 10 x 3 per gram and $yeast^3$ 10 per gram [24].

Constituent compounds, type of fermentation process of cocoa beans and storage conditions of chocolate are considered to be essential factors affecting microbial contamination [25]. Cocoa beans are rich in copper, sulfur, vitamin C and two flavonoids catechin and epicatechin [26]. These compounds can be effective against Gram-positive bacteria and have also shown better bactericidal activity against Gram-negative bacteria [26, 27]. The amount of microbial contamination of traditional sweets offered in confectioneries in Yazd city showed that 33.8% of the contamination of the samples belonged to Enterobacteriaceae, Escherichia coli, mold and yeast, respectively, 13.2%, 5%, 21.7% and 11.4%. The highest level of contamination was related to "Loz Pistachio (8.88%)" candy and the lowest was related to "Haji Badam" candy, without any microbial contamination [28]. By adding omega-3 to milk chocolate, the results of the microbial test showed that the treatments did not contain any contamination related to salmonella.*Staphylococcus aureus* and Astercia were [29]. In another study, the highest level of

the total number of bacteria in the control chocolate was 10 CFU/g^6 was 2.3 × and for other samples treated with roasted and unroasted coca seeds, the amount of total bacteria was similar and reached 10 CFU/g^4 × 1.4-7.2 reached, although the statistical difference between the results was noted. Spore-forming bacteria in all chocolate samples at the level of 10 CFU/g^3 × 3.3-5.1 regardless of roasted and unroasted coca seeds [16].

Table 4 The effect of D-tagatose and lemon essential oil on the microbial population (CFU/g) of chocolate treatments

Microorganism	CC	CTL_1	CTL_2	CTL_3	CTL_4	CTL_5	CTL_6
Total viable count	3.10×10^5 a	1.07×10^4 b	1.24×10^2 c	4.10×10^4 b	2.40×10^2 c	1.06×10^4 b	1.65×10^2 c
Spore-forming bacteria	1.50×10^1 a	1.09×10^1 a	1.00×10^1 a	1.21×10^1 a	1.02×10^1 a	1.14×10^1 a	1.05×10^1 a
Yeasts and molds	-	-	-	-	-	-	-
Xerophilic molds	-	-	-	-	-	-	-
Enterobacteriaceae	4.07×10^2 a	6.34×10^1 b	-	5.27×10^1 b	-	3.45×10^1 b	
Enterococcus spp.	2.23×10^2 a	1.06×10^1 b	-	2.10×10^1 b	-	2.58×10^1 b	
Staphylococcus spp. coagulase-positive	-	-	-	-	-	-	-

±Standard deviation (a-f): Different uppercase letters with statistical differences in the studied level (p<0.05) related to the studied times

CC: Control chocolate, $CTL_{1:}$ Chocolate treated with 29.84 (%) D-tagatose and 0.1 (%) lemon essential oil, $CTL_{2:}$ Chocolate treated with 29.74 (%) D-tagatose and 0.2 (%) lemon essential oil, $CTL_{3:}$ Chocolate treated with 13.36 (%) D-tagatose and 0.1 (%) lemon essential oil, $CTL_{4:}$ Chocolate treated with 13.26 (%) D-tagatose and 0.2 (%) lemon essential oil, $CTL_{5:}$ Chocolate treated with 47.80 (%) D-tagatose and 0.1 (%) lemon essential oil and $CTL_{6:}$ Chocolate treated with 47.90 (%) D-tagatose and 0.2 (%) lemon essential oil

8-3- Evaluation of the effect of ditagatose and lemon essential oil on sensory characteristics

The effect of ditagatose and lemon essential oil on sensory evaluation was carried out by investigating the factors of texture, smell, taste, mouthfeel, stickiness and overall acceptance (Figure 1). As it is known, the chocolate samples treated with ditagatose and lemon essential oil were different from the control sample in terms of texture, taste and mouthfeel (p<0.05). The tissue results showed that increasing the amount of lemon essential oil led to a decrease in tissue quality, which was in line with the results of this factor. The taste and stickiness factors of the control and treated samples were not significantly different from each other, but the mouthfeel of the control sample received a higher score, which was probably due to the lack of acceptance of the taste of lemon essence in chocolate. Among chocolates, the control sample had the most favorable overall acceptance score according to the evaluators, and in the control sample it was equal to 4.96, which is the most similar to $CTL._5$ It was identified with the number 4.92.

In a research, the results of the sensory evaluation of chocolate samples in terms of texture, color, aroma, taste and glossiness of the product showed that the highest sensory acceptability was related to the sample containing 20% Arabica coffee grounds and the lowest acceptance due to the rough taste was related to the sample containing 60% grounds [8]. Sensory characteristics of chocolate samples showed that the reduction of 7% fat (soybean flour) and 10% sugar (date powder) had the highest evaluation score [7]. Mannitol and D-tagatose as sweeteners can increase the amount of bloom, color, crispness, fluidity, bitter taste and powdery feeling after consuming chocolate, but the hardness, chewiness, sweetness of the taste, intensity of the overall taste and cocoa taste will decrease with the process. decreased [30]. The average scores of sensory evaluation showed that the chocolate prepared by replacing sugar with stevia by 50% compared to other treatments had a higher sensory score, although the evaluators accepted all chocolate samples up

to 75% of replacing sugar with stevia [16].

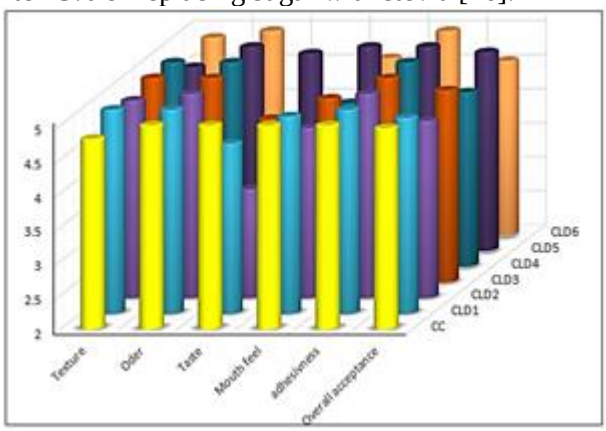

Fig 1 The effects of D-tagatose and lemon essential oil on sensory features

4 - Conclusion

According to the obtained results, it can be concluded that decreasing and increasing the amounts of tagatose and lemon essential oil have an effect on the increase in humidity, and also the increase of tagatose has an effect on the average sugar before dehydration of the treated samples, but no significant difference was observed in the fat content of the chocolates. . The presence of tagatose and lemon essential oil had a significant effect on the intensity of the color factors L*, a* and b* of the samples. Also, increasing the amount of lemon essential oil reduced the hardness of the chocolate texture. The antioxidant capacity of free radicals of chocolate samples treated with lemon essential oil increased, and according to the antimicrobial activity of its phenolic content, there was a reduction in contamination in the treated samples. Among CTL treated samples$_5$ With the most appropriate biological results and the score of sensory evaluators, it was identified as the superior formulation for chocolate formulation.

5- Resources

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ارزیابی تاثیر افزودن دی تاگاتوز و اسانس لیمو بر ویژگی‌های کیفی شکلات

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چکیده

شکلات محصولی پرطرفدار و پر مصرف در گروه‌های سنی مختلف است اما در سال‌های اخیر تحت تاثیر افزایش نیاز مصرف کنندگان به شکلات سالم یعنی کم قند یا بدون قند قرار گرفته است. هدف پژوهش حاضر جایگزین کردن ساکاروز موجود در شکلات با دی تاگاتوز و همچنین بکارگیری اسانس لیمو جهت بهبود خصوصیات بیولوژیک می‌باشد. هفت فرمولاسیون شکلات تولید شده شامل درصدهای مختلف تاگاتوز (۰، ۱۳، ۲۹ و ۴۷ درصد) و اسانس لیمو (۰، ۰/۱ و ۰/۲ درصد) بود، سپس آزمون‌های میزان رطوبت، چربی، قند قبل و بعد آبکافت، خصوصیات رنگی، سختی بافت، آنتی اکسیدانی، میکروبی و حسی بر روی نمونه‌ها انجام شد. نتایج نشان داد رطوبت نمونه‌ها (بجز CTL_5: حاوی ۴۷/۸۰ درصد تاگاتوز و ۰/۱ درصد اسانس لیمو) اختلاف معنی داری با شاهد داشتند همچنین میانگین قند قبل از آبکافت نمونه‌ها با یکدیگر اختلاف معناداری داشت اما درصد چربی آنها (۳۳/۳۹ تا ۳۳/۴۱ درصد) بدون تفاوت معنی دار بود. حضور اسانس منجر به کاهش سختی نمونه‌ها شده است بطوریکه CTL_6 (نمونه حاوی ۴۷/۹۰ درصد تاگاتوز و ۰/۲ درصد اسانس لیمو) با داشتن مقادیر اسانس بیشتر دارای سختی بافت کمتری (۱۶/۶۱ N) بود. دو عامل تاگاتوز و اسانس لیمو بر خصوصیات رنگی تاثیر گذار بودند اما تنها اسانس لیمو بر خصوصیات آنتی اکسیدانی اثر داشت و همه نمونه‌ها از لحاظ بار میکروبی در حد استاندارد قرار داشتند. در ارزیابی حسی به نمونه شاهد (۴/۹۶) مشابه با نمونه CTL_5 (۴/۹۲) امتیاز پذیرش کلی داده شد. نتایج نهایی این پژوهش، CTL_5 را به عنوان مناسبترین فرمولاسیون جهت تولید شکلات سلامتی بخش معرفی کرد.