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Investigating the Independent Effect of Different Levels of Chicken Feet Gelatin and Commercial Gelatin (Cow) with Cocoa Butter on the Physicochemical and Sensory Properties of Milk Chocolate

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1 - Introduction

Chocolate has many fans in all age groups due to its unique taste and texture and the feeling of pleasure and relaxation after consumption, and its consumption has been growing in recent years. Chocolate is a uniform and semi -solid mixture of sugar and cocoa particles and other ingredients, which depend on the formulation and type of chocolate. In the continuous phase, which usually includes cocoa butter. This product is the result of a complete and correct process of mixing one or more raw materials with cocoa beans. Among these raw materials, we can mention sugar or permitted edible sweeteners, powdered milk powder, flavorings and permitted edible essential oils [1]. One of the most popular types of chocolate in the world is milk chocolate. Milk chocolate is a complex rheological system that includes a solid phase (cocoa powder, sugar powder and non -fat dry milk powder) dispersed in the continuous phase of cocoa butter. One of the main factors that causes the thermal instability of chocolate is the initial softening of cocoa butter at a temperature of 30 –32 degrees Celsius. Therefore, its consumption in tropical regions is associated with the problem of melting [2]. When consumption of chocolate is moderate, it will be a strong and useful food. Chocolate is a very pleasant dessert and increases the acceptance of other nutrients. Chocolate is an emergency ration for the armed forces. One pound of chocolate (435.59 grams) will provide about 2515 kcal of energy. The average composition of chocolate is as follows: proteins (4.8 percent), fats (32.5 percent), carbohydrates (59.5 percent), and theobromine (1.25 percent). Chocolate is a nutrient, for example, a small piece of 100 grams of milk chocolate contains about 220 milligrams of calcium and 9 grams of protein [3]. Any change in the quality of food compared to its original quality causes a loss of product quality. The physical characteristics of chocolate have a lot to do with its textural characteristics, such as the size of chocolate particles, which is related to its favorable mouthfeel, and factors such as bloom, which reduces the appearance and texture quality of chocolate and is known as fat and sugar bloom. Some chemical characteristics, such as water activity and pH, are some of the things that contribute to the helplessness of the product [4]. Gelatin is the main protein in bones and cartilage. One of the factors affecting the

properties of gelatin is the age of the animal and the source and type of collagen. So far, more than 27 types of gelatin have been identified. Depending on the pretreatment method, two types of gelatin can be obtained, which are commercially divided into type A gelatin and type B gelatin. Type A gelatin was obtained under acidic treatment conditions, and type B gelatin was obtained under alkaline treatment conditions [5]. Chicken feet are rich in collagen, which is an excellent source of gelatin and is used in food and pharmaceutical products [6]. The use of chicken feet as a raw material for extracting gelatin will not only benefit the poultry industry but will also lead to the production of a relatively cheap and widespread substitute for mammalian gelatin [7]. By examining and comparing the structure of chicken foot collagen and commercial cow gelatin using FTIR spectroscopy, it was observed that the FTIR spectrum of chicken foot gelatin has more amino acids than commercial cow gelatin, therefore, the production of gel using chicken feet is suggested by the poultry industry, which also announced that the strength and nutritional quality of chicken foot gelatin are higher than commercial cow gelatin [8]. The texture of chocolate is its most complex physical characteristic, and, along with taste, it is the quality that often comes to mind when choosing to prefer products. In general, texture is a qualitative parameter that refers to the feeling of food in the mouth and the perception that a person has of its physical properties as a result of biting and chewing. Different words are used to describe the texture of chocolate, depending on whether the emphasis is on structure, consistency, or mouthfeel. Three texture sensory characteristics are very important in the perception of chocolate: softness, melting and hardness [9]. The texture and appearance of chocolate are key features in the consumer's choice to identify the product [10]. Although texture perception is a dynamic oral process before and during chewing, people also perceive texture through sight, touch, and hearing [11]. Chocolate texture can be evaluated with efficient and objective instrumental measurements or supplements for sensory evaluations [12], with statistically significant correlations [13]. Determining visual information, gloss, color, shape,

roughness, surface texture, and transparency are summarized in appearance characteristics [14]. Texture, sweetness, how it melts in the mouth, color and overall acceptance are the sensory characteristics of chocolate samples [15]. In the sensory test, the 5 -point hedonic method has been used. The purpose of this test is to check the sensory acceptance of the samples by trained evaluators [16]. The desired adjectives were scored by evaluators from one (bad) to five (excellent). Taste is the most important sensory attribute of chocolate, as consumers are influenced by aroma, taste and texture during consumption. In a more or less conscious way, chocolate consumers judge based on the quality characteristics inherent in the products. Today, chocolate is not a rare or premium product. The recognition of its values certainly involves previous experience and future expectations that are promoted by advertising. However, what makes it so desirable is its perceived sensory quality. Chocolate has many fans in all age groups due to its unique taste and texture and the feeling of pleasure and relaxation after consumption, and its consumption has been growing in recent years [1]. The melting of chocolate from a solid state at room temperature to a soft, dense suspension in the mouth at body temperature shows its unique properties. Melting of chocolate in the mouth is defined by lipid phase characteristics [10] and taste perception, and characteristic Tissues facilitate it. Perceived flavor intensity changes dynamically over time as chocolate is melted, manipulated, and mixed with saliva for swallowing [17].

2. Materials and methods

2.1. Materials

In this research, to prepare milk chocolate from cocoa powder (Parand Company), cocoa butter (Latamarco Africa Company), sugar (Dena Diamond Company), powdered milk (Pak Company), vanilla (Gholha Food Industries), and liquid lecithin (Gujarat, India). A silicone mold was purchased from a confectionery store in Mazandaran -Qaemshahr, chicken Feets from a poultry slaughterhouse in Mazandaran - Qaemshahr, and hydrochloric acid was purchased from Merck, Germany.

2.2. Methods

2.2.1. Preparation of gelatin from chicken Feet

After weighing the chicken Feet and separating its nail and skin without heating, it was washed with cold water, cut into pieces of 3 –4 cm with a knife, and finely chopped by a model laboratory grinder (Bosch, Germany) for 4 minutes. In the next step, the chopped Feet was washed with water at a ratio of 11:2 (weight/volume) to remove blood and other residues. 0.5 normal hydrochloric acid was added to the shredded chicken feet in a ratio of 22.3:3 (weight/volume), and it was stirred with a mechanical magnet for 2 hours until its pH reached about 1. After this time, the oscein (scum) was separated and washed with distilled water at a ratio of 1:12/16 (weight/volume) in two stages of 10 minutes to reduce the acidity of the oscein and bring the pH to about 3. Distilled water in a ratio of 1:1 (weight/volume) was heated to 45 degrees Celsius, and oscein was added to it and stirred for 1 hour at the same temperature with a mechanical stirrer. The obtained gelatin was filtered by cloth, and its pH was adjusted to 7 with normal soda. Then the clear liquid in the container was separated and stored in the refrigerator, and its pH was measured for 4 days. Then it was melted at 40°C and dried in an oven at 45°C for 28 hours, and the dried gelatin, after being powdered by The grinding machine (Sapor, SCG -420), was stored in moisture -proof plastic bags (zip -cap) in the freezer at -18° C [18].

2 - 2 - 2 - Preparation of milk chocolate

In the first step, sugar and milk powder were ground with a grinder (Sapor, SCG -420) and then passed through a laboratory sieve (mesh 40). In general, 9 grams of cocoa powder, 37.56 grams of powdered sugar, 14.34 grams of non fat dry milk powder, 37 grams of cocoa butter, 1.5 grams of lecithin, and a small amount of vanilla were considered for each 100 -gram treatment. In order to prepare chocolate samples, cocoa powder and melted cocoa butter were mixed by a mixer in the laboratory at a speed of 50 rpm for 30 minutes in the same conditions (in a water bath). Mixed. Sugar powder and non -fat dry milk powder were added to the mixture with vanilla, and the chocolate dough was stirred for 1 hour. Lecithin was added to the chocolate mixture in 3 steps and stirred in a water bath at 65°C for 1 hour in

each step. For better mixing of materials, a number of anti -rust and anti -wear balls with a diameter of 8 mm were used. The samples were kept in a 60°C water bath for 24 hours. Then, it remained the same for 30 minutes at a temperature of 55°C. To condition the temperature during mixing, the temperature of the samples was brought to 28 degrees in 20 to 25 minutes and kept at this temperature for 10 minutes. To prepare samples containing different concentrations of gelatin (chicken feet

and commercial), gelatin is added before conditioning. Then, the samples were transferred to silicone molds with an approximate volume of 7 cc and kept for 30 minutes at 15°C. After leaving the molds, the samples were placed on aluminum sheets and kept in the refrigerator until the tests [15, 16, 19, 20].

Table 1. Combinations and quantities used to make Types of milk chocolate

*SH (control sample, without gelatin), T1 (milk chocolate containing 3% commercial gelatin), T2 (milk chocolate containing 5% commercial gelatin), T3 (milk chocolate containing 7% commercial gelatin), T4 (milk chocolate containing 3% Chicken Feet gelatin), T5 (milk chocolate containing 5% chicken Feet gelatin), T6 (milk chocolate containing 7% chicken Feet gelatin)

2.2.3. Experiments related to chicken Feet gelatin and commercial (cow) gelatin 2.2.3.1. Measuring the strength of gelatin

gel

6.67 grams of gelatin were mixed with 100 ml of deionized water. This mixture is left at room temperature for 3 hours until the gelatin swells. Then it was heated for 20 minutes in a 60°C water bath to dissolve the gelatin. After cooling to room temperature, it was placed in a cold water bath at a temperature of 10°C for 16 to 18 hours. Gelatin gel strength was measured with a Brookfield Engineering Lab Inc. device, made in the United States, with a cylindrical probe coded TA10 [21].

2.2.3.2. Gelatin pH measurement

To measure the pH, one gram of gelatin was dissolved in 99 ml of distilled water and heated for 5 minutes at a temperature of 45 degrees Celsius. After the solution was dissolved and reached room temperature, the pH was measured by a pH meter (Jenway, UK model). Became [22].

2.2.3.3. Determining the melting profile of gelatin

The melting profile of gelatin was investigated using the differential scanning calorimetry model (DSC -500). Gelatin powder from chicken feet was weighed accurately in aluminum pans in the amount of 15 mg using a scale, and its melting temperature was calculated from 30 to 90 degrees Celsius with a heating rate of 10 degrees Celsius per minute [23].

2.2.3.4. Gelatin viscosity evaluation

6.67% gelatin solution was placed in a 45°C water bath until it was completely dissolved, and then this mixture was poured into a viscometer vessel connected to a 35°C water bath. Viscosity was measured by a Brookfield viscometer made in the United States [22].

2.2.4. Tests related to milk chocolate samples 2.2.4.1. Chocolate density measurement

The density (specific gravity) of the products is determined in three replicates by filling a beaker (150 ml), taking into account the available mass (g) . The density (g/mL) of the samples was calculated as the ratio of mass to human volume [24].

2.2.4.2. Measuring the hardness of chocolate texture

The texture test was performed by a Brookfield Engineering Lab Inc. device made in the USA with a cylindrical probe coded TA39. Milk chocolate with dimensions of length and width of 15 mm, height of 6 mm, and movement speed of 1 mm/s was done [25].

2.2.4.3. Surface roughness measurement

The imaging conditions should be such as to minimize factors such as light and reflection, which may eventually interfere with the analysis, so the imaging was taken by a smartphone camera with a 108 megapixel and ultra -wide lens sensor, and then the photos were transferred to the computer via USB port. Connected, done. For this purpose, the samples were placed on a clean white screen, and the samples were photographed vertically by a camera from a fixed distance of one meter. It was saved in JPG format from the imaging samples with dimensions of 1792 x 3904 pixels and a resolution of 300 dpi. To calculate image parameters after 8 -bit conversion, gamma correction and contrast enhancement were used for image processing. Also, for image processing, background subtraction and differentiation enhancement were repeated, and finally threshold will be done in the gray color channel [26, 27].

2.2.4.4. Evaluation of apparent viscosity

This feature was measured using a viscometer. First, the chocolates were stirred at a temperature of 32 degrees Celsius for 10 minutes and then homogenized. Then a 150 -ml beaker was filled with chocolate, and the rotating axis of the device was placed in it to measure viscosity. The number on the display of the device indicates the apparent viscosity of the samples [1].

2.2.4.5. determination of chocolate melting profile

To check the melting profile of chocolate samples, a differential scanning calorimeter (DSC -500/Ci) equipped with a thermal analysis base was used. The central part of the chocolate was used for analysis, and heat was applied with a heating rate of 5°C/min from 5 to 50°C [28].

2.2.4.6. Measurement of chocolate water activity

The water activity of chocolate samples was determined with a device (Novasina Sprint) [29]. We put some of the prepared samples in the special place of the device, and the water activity of the samples was measured.

2.2.4.7. Measurement of chocolate color indicators

The color of the chocolate treatments and the control sample was measured by the IMG Pardazesh Cam -System XI device and the L, a and b indices were analyzed. This model has factored (L) including black to white spectrum with a range from zero to 100 and two color factors (a) including green to red color spectrum with a range of $+120$ to -120 and (b) including a blue to yellow color spectrum with a range of $120 +$ to -120 [30].

2.2.4.8. Sensory evaluation

In order to check the sensory characteristics of the samples, which means the feeling that is felt in the mouth after eating chocolate and is more related to the melting in the mouth characteristic, 10 evaluators who had received the necessary basic training were used to evaluate the samples using a 5 -point hedonic numerical scale. Was used [28].

2.3. Data analysis

The results were based on a completely randomized design. To check the averages, one way analysis of variance (ANOVA) was used at the 0.05 level, and Duncan's test was used to determine the difference between the averages. Data analysis was done with SAS software.

3. Results and Discussion

3.1. Gelatin

The physicochemical characteristics of gelatin extracted from chicken feet are shown in Table 2. The numerical value of pH indicates the amount of washing necessary to eliminate the remaining acid and finally reach the desired pH. The strength of the gel depends on the molecular weight, concentration and extraction temperature [18]. The strength of chicken foot gelatin in the present study is 378.48 grams, compared to other sources such as commercial (cow) gelatin, which is 299 grams, and chicken skin gelatin, which is 355 grams [21]. It can be said that the high strength of chicken Feet gelatin gel is due to the high amount of hydroxyproline, strong hydrogen bond and low extraction temperature [18].

Commercial gelatin	Chicken Feet gelatin	Test
170.6 ± 0.9	378.48 ± 0.5	Bloom Gel
162.5 ± 0.3	220.75 ± 0.2	Viscosity
44.7 ± 0.01	70.5 ± 0.01	The temperature at the beginning of the melting point
83.5 ± 0.01	85.4 ± 0.01	The temperature at the end of the melting point
3.2 ± 0.6	3.31 ± 0.8	pH before drying
6.61 ± 0.01	6.65 ± 0.02	pH after drying

Table 2. Physicochemical Analysis of Chicken Feet Gelatin and Commercial Gelatin

2.3. Chocolate 3.2.1. Density of chocolate

According to graph 1, there is a significant difference between the control sample and the treatments containing different levels of chicken feet gelatin and the treatments containing different levels of commercial gelatin, so that the lowest density corresponds to sample T4 (a milk chocolate sample containing 3% chicken feet gelatin) and the highest density to Sample T3 (a milk chocolate sample containing 7% commercial gelatin). In both groups, milk chocolate containing different levels of chicken feet gelatin and milk chocolate containing different levels of commercial gelatin have increased density with increasing gelatin concentration. The density of milk chocolate samples containing different levels of commercial gelatin was higher than that of milk chocolate samples containing different levels of chicken foot gelatin. In 2013, Fernandes et al. reported that increasing the

amount of albumin decreased the density of tomato paste [31]. In a study in 2005, researchers showed that fat substitutes including guar resin, xanthan gum, and starch in a mixture of rice flour and date oil led to an increase in density [32]. During the research conducted in 2016, the researchers came to the conclusion that with the increase in the concentration of grape concentrate due to the increase in Brix, the density of the tissue increased, which ultimately increased the chewability of pastilles [33]. In 2013, Mir Arab Razi et al., during their studies that added different amounts of albumin proteins, sodium caseinate, and cheese juice concentrate to chocolate dessert, concluded that, in general, with the increase in the amount of protein, the amount of density decreased, but the reverse trend was observed with gelatin. And with its increase, the density increased [26].

Figure 1. Density Chart of Milk Chocolate Samples Containing Different Levels of Chicken Feet Gelatin and milk chocolate samples containing different levels of commercial gelatin

3.2.2. Measuring the hardness of chocolate texture

In graph 2, the hardness of the control sample tissue, the treatments containing chicken foot gelatin, and the treatments containing commercial gelatin are given. According to the obtained results, the highest hardness is related to the control sample, and the lowest is related to the T6 sample (the sample containing 7% chicken feet gelatin). In both groups of treatments (milk chocolate samples containing different concentrations of chicken foot gelatin and milk chocolate samples containing different concentrations of commercial gelatin), tissue hardness decreases with increasing gelatin. Statistically, there is a significant difference between the control sample,

treatments containing different concentrations of chicken foot gelatin, and treatments containing different concentrations of commercial gelatin. In general, the hardness of the prepared treatments was lower than the control sample, which may be attributed to their higher humidity. During a study conducted by Bitraf et al., they concluded that chocolate samples containing a high percentage of maltodextrin had less hardness than the control sample, which may be due to the absorption of moisture by maltodextrin [15]. During the research conducted in 2014 on the texture of cantaloupe jelly, the results showed that with an increase in the percentage of gelatin, the hardness of the texture of cantaloupe jelly increases [18].

Figure 2. Hardness chart of milk chocolate samples containing different levels of chicken Feet gelatin and milk chocolate samples containing different levels of commercial gelatin

3.2.3. Image processing

According to the results obtained in graph no. 3, during imaging of chocolate samples, the surface roughness of the samples was compared. The data show that there is a significant difference between the samples containing chicken Feet gelatin and the samples containing commercial (cow) gelatin together, as well as with the control sample. The lowest unevenness is related to the T6 sample (the sample containing 7% chicken Feet gelatin), and the highest unevenness is related to the control sample. In fact, by increasing the concentration of chicken foot gelatin in samples containing different levels of chicken foot gelatin and by increasing the concentration of commercial gelatin in samples containing different levels of commercial gelatin, the surface roughness of the samples decreases.

Figure 3. Surface roughness chart of milk chocolate samples containing different levels of chicken Feet gelatin and milk chocolate samples containing different levels of commercial gelatin

3.2.4. Apparent viscosity

Figure 4 shows the apparent viscosity diagram of chocolate samples. According to this graph, there is a significant difference between the samples. The viscosity of samples containing different levels of chicken feet gelatin and samples containing different levels of commercial gelatin is higher than that of the control sample, so the lowest viscosity is assigned to the control sample. In the samples containing chicken feet gelatin and the samples containing commercial gelatin, the viscosity increases with the increase in gelatin concentration, and this increase in viscosity is greater in the treatments containing different concentrations of chicken feet gelatin. Therefore, the highest amount of viscosity belongs to sample T6 (a sample containing 7% chicken foot gelatin). Ashrafieh et al.'s (2014) research showed that the apparent viscosity of chocolate treatments prepared with hydrolyzed collagen increased with an increase in the level of hydrolyzed collagen. In another study, the researchers came to the conclusion that starch polymers, milk protein, beta glucan and gelatin increase the viscosity of chocolate and that this increase in viscosity is responsible for the ability to maintain the shape of chocolate at high temperatures [34]. Types of stabilizers such as gelatin are gelling or thickening agents, and the effect of these agents is to increase the viscosity of the continuous phase or form a three -dimensional network that delays the movement of components, which results in improved stability [35]. In a 2005 study, researchers concluded that, in general, the viscosity of a fluid is affected by temperature, so temperature control during processing is essential. The effect of temperature on fluid behavior can be described in such a way that fluidity is affected by intermolecular forces, so that the smaller the distance between molecules, the greater the intermolecular forces. Therefore, the movement of particles decreases and their viscosity increases. On the other hand, the increase in temperature increases the thermal energy of fluid molecules as well as their movement, and as a result, the distance between particles increases. This leads to a decrease in intermolecular forces and an increase in final fluidity [36].

Figure 4. An apparent viscosity chart of milk chocolate samples containing different levels of chicken Feet gelatin and milk chocolate samples containing different levels of commercial gelatin

3.2.5. Chocolate melting profile

As shown in Figure 5, the results show that there is a significant difference between the control sample and the other samples. The highest temperature at the beginning of melting is related to samples T3 (containing 7% commercial gelatin) and T6 (containing 7% chicken feet gelatin), and the lowest is related to sample T4 (containing 3% chicken feet gelatin). The highest temperature at the end of melting is related to the T6 sample, and the lowest is related to the control sample. In both gelatin groups (chocolate samples containing different concentrations of chicken foot gelatin and chocolate samples containing different concentrations of commercial gelatin), as

expected, with the increase in gelatin (chicken foot and commercial), the temperature at the beginning and end of milk chocolate melting also increased. In a study, researchers came to the conclusion that polymers of starch, milk protein, B -glucan and gelatin increase the viscosity, and this increase in viscosity ultimately leads to the ability to maintain the shape of chocolate at high temperatures in such a way that these polymers trap fat, preventing the melting of fat at high temperatures [29]. In research that used corn flour and gelatin at levels of 2.5, 5, 7.5, and 10% in chocolate formulation, they concluded that increasing the levels of flour and gelatin increases the melting point of chocolate [2].

Figure 5. Melting profile chart of milk chocolate samples containing different levels of chicken Feet gelatin and milk chocolate samples containing different levels of commercial gelatin

*SH (control sample, without gelatin), T1 (milk chocolate containing 3% commercial gelatin), T2 (milk chocolate containing 5% commercial gelatin), T3 (milk chocolate containing 7% commercial gelatin), T4 (milk chocolate containing 3% Chicken Feet gelatin), T5 (milk chocolate containing 5% chicken Feet gelatin), T6 (milk chocolate containing 7% chicken Feet gelatin)

3.2.6. Water activity measurement

The results of this test, according to diagram 5, show that there is a significant difference between the control sample and other treatments. By increasing the concentration of chicken foot gelatin and increasing the concentration of commercial gelatin, the water activity of the samples decreases. According to the results, it can be stated that there is no significant difference between samples T3 (sample containing 7% commercial gelatin), T5 (sample containing 5% chicken feet gelatin), and T6 (sample containing 7% chicken feet gelatin). The lowest aw is related to T3, and the highest is related to sample T1 (a sample containing 3% commercial gelatin). In a study conducted by Herati et al. in 2017, they concluded that with the increase of gelatin and guar, the water activity increased in the barberry pastille sample [37]. According to research in 2014, in relation to the formulation of date jelly products based on condensed milk cheese, gelatin and xanthan gum in different

formulas, with the increase of these three variables, the water activity of the samples also increased [38]. In another study in 2014, they reported the effect of gelatin and guar on the water activity of white mulberry paste and concluded that with an increase in the percentage of gelatin and guar, the water activity of the samples decreased [39]. In 2012, Shurideh et al., while investigating the effect of vanillin tagatose as a sucrose substitute on the physical, chemical and rheological properties of milk chocolate, reported that with the increase in tagatose content, the amount of water activity in milk chocolate samples decreased [40]. According to research in 2011, researchers stated that the samples containing sucrose without the presence of liquid glucose had more water activity, and with the increase in the concentration of liquid glucose, the water activity decreased, while the opposite trend was observed with the increase in the concentration of sucrose [41].

Figure 6. Water activity chart of milk chocolate samples containing different levels of chicken Feet gelatin and milk chocolate samples containing different levels of commercial gelatin

*SH (control sample, without gelatin), T1 (milk chocolate containing 3% commercial gelatin), T2 (milk chocolate containing 5% commercial gelatin), T3 (milk chocolate containing 7% commercial gelatin), T4 (milk chocolate containing 3% Chicken Feet gelatin), T5 (milk chocolate containing 5% chicken Feet gelatin), T6 (milk chocolate containing 7% chicken Feet gelatin)

3.2.7. Color indicators

According to the results obtained in Table 3, the L, a and b indices are given. Regarding the L index, there is no significant difference between the control sample and the T4 sample (the sample containing 3% gelatin from chicken feet). If there is a significant difference between the control sample and other samples, in fact, the highest score is related to T6 (the sample containing 7% gelatin). Chicken feet), and the lowest is related to the control sample. In samples containing different levels of chicken feet gelatin and samples containing different levels of commercial gelatin, this index increases with the increase in gelatin concentration. Regarding factor A, there is a significant difference between the control sample and the treatments. According to these results, the lowest amount of this factor is related to sample T1 (sample containing 3% commercial gelatin) and the highest amount is related to sample T3 (a sample containing 7% commercial gelatin). In samples containing different levels of chicken feet gelatin and samples containing different levels of commercial gelatin, the amount of this factor is increasing with the increase in gelatin concentration. The obtained results show that in factor b, there is no significant difference between the control sample and the T1 sample,

as well as between the T4 and T5 samples (the latter containing 5% chicken feet gelatin), but a significant difference was observed between the others. The lowest value is related to the control sample, and the highest value is related to the T2 sample (the sample containing 5% commercial gelatin). The results of the research conducted on the date jelly product containing different ratios of xanthan, whey and gelatin in 2014 showed that the pastille formulation with the lowest percentage of hydrocolloid has the highest color intensity and the pastille sample containing the highest percentage of hydrocolloid has the lowest color intensity. By adding gelatin and xanthan, it reduced the transparency and color of the gel [38]. In 2014, researchers concluded that by increasing the percentage of gelatin, factor L in white berry pastille samples decreased and factors a and b increased [39]. During the research conducted in 2014, which was related to the replacement of sucrose with grape juice in sponge cake, they concluded that this replacement decreases factor L and increases factor b [42]. According to research in 2014, the researchers came to the conclusion that the control sample, the cantaloupe jelly that lacked gelatin, had the lowest transparency and the lowest color spectrum according to the L, a and b parameters [18].

Treatments		a	b
SH	7.9453 ± 0.19 e	0.3720 ± 0.02 ^f	3.4843 \pm 0.14 \degree
T ₁	8.8323 ± 0.14 ^{cd}	0.1753 ± 0.02 s	3.5490 \pm 0.01 \degree
T ₂	9.3963 \pm 0.35 $^{\rm b}$	1.2793 ± 0.007 c	5.1116 \pm 0.02 ^a
T ₃	9.6713 \pm 0.5 ab	$1.3823 + 0.02$ ^a	4.9830 \pm 0.03 b
T4	8.4313 ± 0.11 ^{ed}	0.4083 ± 0.005 ^e	4.2803 \pm 0.03 ^d
T ₅	9.2493 \pm 0.19 cb	0.7550 ± 0.01 ^d	4.2630 \pm 0.01 $^{\rm d}$
T ₆	10.0233 ± 0.31 ^a	1.3213 ± 0.01 b	4.3966 \pm 0.03 °

Table 3. Color index table of milk chocolate samples containing different levels of chicken Feet gelatin and milk chocolate samples containing different levels of commercial gelatin

3 . 2 .8. Sensory evaluation

Table 4 shows the sensory characteristics of milk chocolate samples. Color, smell, taste and texture of milk chocolate were evaluated by evaluators. There is no significant difference in texture measurement between the control sample and the T2 sample (sample containing 5% commercial gelatin), the highest value is related to the control sample, and the lowest value is related to the T3 sample (sample containing 7% commercial gelatin). In the evaluation of the taste of the samples, a significant difference was observed between all the treatments and the control sample, so that the highest score in terms of taste is the control sample and the lowest score is related to the T6 sample (the sample containing 7% chicken feet gelatin). Regarding the color of the chocolate samples between the control sample and the T4 sample (the sample containing 3% chicken feet gelatin) and between the T1 sample (the sample containing 3% commercial gelatin) and T6, as well as between the T3 and T5 samples (the sample containing 5% chicken feet gelatin), there is no significant difference. In the overall acceptance of the samples, the highest score was given to the control sample, and the lowest score is related to the T6 sample. The closest acceptance between chocolates containing commercial gelatin and chocolates containing chicken feet gelatin is related to sample T2. During a study, it was shown that the amount of

brightness depends on the size of the particles and the amount of fat and lecithin in chocolate [1]. In a research, chocolate enriched with quinoa was accepted by sensory evaluators. No significant difference was observed in the preferences of consumers for chocolate samples with different percentages of quinoa. All formulations showed acceptance above 70% [43]. The research conducted in 2007 shows that one of the reasons for the decrease in taste with increasing the content of gelatin and corn starch can be attributed to the high viscosity of starch and gelatin samples, which have a negative effect on the taste [44]. In 2010, researchers reported that the solubility of compounds added to chocolate has an effect on chocolate melting and mouthfeel scores. The sample containing inulin has little hardness compared to the samples containing simpler sugars such as sucrose and tagatose, and it creates a state of adhesion and aggregation in the mouth, which was clearly visible in the samples with higher amounts of inulin. This state has an effect on how the chocolate melts. Had [45].

4. Conclusion

The results of this research showed that the addition of gelatin (chicken Feet and commercial) independently increases the specific mass (density) of milk chocolate, and this density increase is greater in chocolates containing chicken Feet gelatin. The hardness of the samples decreased with the increase in gelatin. In examining the image of milk chocolate to determine the surface roughness of the samples, the roughness decreased with increasing the concentration of chicken foot gelatin in the samples containing chicken foot gelatin and increasing commercial gelatin in the samples containing commercial gelatin. Viscosity increased independently with the increase in gelatin concentration (chicken feet and commercial). By adding gelatin extracted from chicken feet and commercial gelatin in different concentrations to milk chocolate, its melting temperature increases, which can be attributed to the absorption of moisture by gelatin. By examining the water activity of milk chocolate, we came to the conclusion that with the increase of gelatin (chicken feet and commercial), it has a downward trend independently. The evaluation of the color parameters of the treatments showed that there was a significant difference between the treatments in factors a and b, and the highest score was given to the control sample in relation to factor L. Sensory factors such as color, smell, taste and texture were affected by different levels of gelatin and showed a significant difference with the control sample. In terms of overall acceptance of the samples, the sample with the highest score from the consumers' point of view was the sample containing 5%

commercial gelatin. Based on the results of this research, chicken foot gelatin can be used in milk chocolate formulations in hot regions to solve the problem of samples melting in the environment.

5. Resources

1. Afoakwa, E. O., (2010), Chocolate Science and Technology. Wiley – Blackwell Publishers, Oxford, U.K. 3 -22.

2. Ogunwolu SO and Jayeola CO,(2015). Development of non conventional thermo resistant chocolate for the tropics. J British Food; 108: 451 -455.

3. Beckett, S. T. (2000). The Science of Chocolate. London: Royal Society of Chemistry Paperbacks.

4. Wichchukit, S., & J. McCarthy, M. (2004). Flow behavior of milkchocolatemelt and the application to coating flow. Journal of Food Science, 70 (3): 165-171.

5. Gómez -Guillén, M.C., Giménez, B., López - Caballero, M.E. and Montero, M.P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food hydrocolloids. 25, 1813 -1827.

6. Dhakal, D.; Koomsap, P.; Lamichhane, A.; Sadiq, M.B.; Anal, A.K.(2018). Optimization of collagen extraction from chicken feet by papain hydrolysis and synthesis of chicken feet collagen based biopolymeric fibres. Food Biosci. 2018, 23, 23 –30.

7. Aykın -Dinçer, E.; Koç, A.; Erba¸s, M.(2017). Extraction and physicochemical characterization of broiler (Gallus gallus

domesticus) skin gelatin compared to commercial bovine gelatin. Poult Sci. 2017, 96, 4124 –4131.

8. Almeida, P., Silva Lannes, S., Calarge, F., Brito Farias, T . and Curvelo Santana, J.(2012). FTIR characterization of gelatin from chicken feet. Journal of chemistry and chemical engineering. 6(11): 1029-1032.

9. Urbanski, J. J. (1992). Chocolate flavor/origins and descriptions on the effects of process and bean source. The Manufacturing Confectioner, 11, 69 –82.

10. Beckett, S. T. (2003). Is the taste of British milk chocolate different? International Journal of Dairy Technology, 56(3), 139 –142 .

11. Wilkinson, C., Dijksterhuis, G. B. & Minekus, M. (2000). From food structure to texture. Trends in Food Science and Technology, 11, 442 –450.

12. Rosenthal, A. J. (1999). Relation between instrumental and sensory measurement of food texture. In Food Texture, Measurement and Perception. Rosenthal, A. J. (Ed.). USA: Chapman & Hall, Aspen, pp. 1 –17.

13. Mohamed, A. A. A., Jowitt, R. & Brennan, J. G. (1982). Instrumental and sensory evaluation of crispness: $I - in$ friable foods. Journal of Food Engineering, 1, 55 –75.

14. Briones, V., Aguilera, J. M. & Brown, C. (2006). Effect of surface topography on color and gloss of chocolate samples. Journal of Food Engineering, 77, 776 –783.

15. Bitaraf Sh , Abbasi S , Hamidi Z. (2012). Production of low -energy prebiotic dark chocolate using inulin, polydextrose, and maltodextrin : Vol. 8, No. 1, Spring 2013. Iranian Journal of Nutrition Sciences & Food Technology, 49-62.

16. N. Ravatab , S. Asadollahi , M. R. Eshaghi. (2018). Feasibility of producing milk chocolate containing flaxseed powder and evaluating its physicochemical and organoleptic properties: Vol. 15, No. 1, Apr. May. 2019. Iranian Food Science and Technology Research Journal, 107 -120.

17. Lee, W. E. & Pangborn, R. M. (1986). Time -intensity: the temporal aspects of sensory perception. Food Technology, 40(11), 71 –82.

18. A. Rezaee Zadeh, Z. Raftani Amiri. (2016). Extraction and characterization of gelatin from chicken feet and its application in cantaloupe jelly : Vol. 13, No. 2, June. July. 2017. Iranian Food Science and Technology Research Journal, 322 -332.

19. Farzanmehr H, Abbasi S, Sahari MA(2008). Effect of sugar replacer on some physicochemical, rheological and sensory properties of milk chocolate. Iranian J Nutr Sci Food Technol; 3(3):65 –82[in Persian].

20. Keogh, MK. Murray, CA, O'Kennedy, BT,(2003). Effect of selected properties of ultrafiltered spray -dried milk powders on some properties of chocolate. Int Dairy J; 13: 719 – 726.

21. Sarbon, N., Abdi, F. and Howell, N. (2013). Preparation and characterisation of chicken skin gelatin as an alternative to mammalian gelatin. Food hydrocolloids. 30, 143 -151.

22. Shyni, K., Hema, G.S., Ninan, G., Mathew, S., Joshy, C.G. and Lakshmanan, P.T.(2014). Isolation and characterization of gelatin from the skins of skipjack tuna (Katsuwonus pelamis), dog shark (Scoliodon sorrakowah), and rohu (Labeo rohita). Food hydrocolloids. 39, 68 -76.

23. Sarbon, N. Badii, F. Howell, NK. (2015). The effect of chicken skin gelatin and whey protein intraction on rhological and termal properties.Journal of food Hydrocolloid 45:83 - 29 .

24. Campbell, G. M. & Mougeot, E. (1999). Creation and characterisation of aerated food products. Trends in Food Science & Technology, 10, 283 -296 .

25. Trinh, K. T. &Glasgow, s. (2012). On the texture profile analysis test. Quality of life through chemical engineering, Institute of Food Nutrition and Human Health Massey University Wellington, New Zealand, 23 -26.

26. Mir Arab Razi Saeed, Mohaibi Mohabbat, Haddad Khodaparast Mohammad Hossein, Kochaki Arash (2013), investigation of the rheological and sensory properties of chocolate mousse containing sodium caseinate and gelatin, Iran Research Journal of Food Science and Industry, Volume 12, Number 12. June 2014, pp. 339 -330.

27. Khosravi Farnaz, Taze Mehdi, Sarmi Nayini Mohammad Ali (2017), review and comparison of J Image and GIAS software with mechanical sieve in automatic granulation of surface particles, volume 9, number 2, autumn and winter.

28. Vasanthan T and Bhatty R S,(1996). Physicochemical properties of small and large granule starches of waxy, regular, and high amylose barleys. Cereal Chemistry J; 73: 199 – 207.

29. Afoakwa EO, Paterson A, Fowler M, Vieira J,(2008). Particle size distribution and compositional effects on textural properties and appearance of dark chocolates. J Food Eng ; 87: 181 –90 .

30. Farhanaki Asghar, Safari Zahra, Ahmadi Gurji Farzaneh, Mesbahi Gholamreza (2009), The use of gelatin as hydrocolloid to replace fat in the production of low -fat cream, Journal of Food Science and Industry, Volume 8, Number 31, Fall 2010.

31. Fernandes, V. A., Müller, A. J. and Sandoval, A. J., (2013), Thermal, structural and rheological characteristics of dark chocolate with different compositions. Journal of Food Engineering, 116, 97 -108.

32. Arslan E,Yener ME, Esin A.(2005) Rheological characteristics of tahin/pekmez blends. Journal of Food Engineering. 69, 167 - 172.

33. Seyedeh Prasto Mojarian, Zainab Fardani, Shahiri Tabarstani Hoda (2016), optimization of ginger paste formulation based on chicken leg gelatin and grape concentrate by response surface method (RSM), Iranian Journal of Food Science and Technology, Volume 15, Number 82 - (2017), pages 319 -334.

34. Tayefeh Ashrafiyeh, N., Azizi, M.H., Taslimi, A., Mohamadi Far, M.A., Shorideh, M., Mohammadi, M. (2014). The effect of collagen hydrolysate as a partial replacement of cocoa butter on the rheological and sensory properties of milk chocolate. Journal of Food Science and Technology, 42(11), 141-153 [in Persian].

35. Walsh, D.J., Russell, K. & FitzGerald, J.R. (2008). Stabilisation of sodium caseinate hydrolysate foams. Food Research International, 41: 43 –52.

36. Altay FL, AK MM (2005) Effects of temperature, shear rate and constituents on rheological properties of tahin. Journal of the science of Food and Agriculture. 85, 105-111.

37. Harati Farzaghi, M,. Sharifi, A,. Estiri, S H. (2017). Optimization of the production process of the functional Gummy candy from the fruit of barberry without seeds by the response surface method. Iranian Journal of food science and technology, 9 (1): 9 -10.

38. Fiyouzi, B,. Mazaheri tehrani, M,. Khazaii pool, A. (2014). Formulation of date jelly product and condensed whey and its physicochemical and sensory properties. Iranian food science and technology researche Journal, 12 (1): 78 -61.

39. Azimi, N,. Mortazavi, A,. Basiri, Sh. (2014). Effect of various concentrations of guar gum and gelatin on the moisture content and water activity of the fruit gummy candies based on white berries. The 12th and 2nd Congress of Iranian food science and technology. Gorgan University of Agricultural Sciences and Natural Resources.

40. Shourideh, M., Taslimi, A., Azizi, M. H., & Mohammadifar, M. A. (2012). Effects of D - Tagatose and Inulin on Some Physicochemical, Rheological and Sensory Properties of Dark Chocolate. International Journal of Bioscience, Biochemistry and Bioinformatics, 2(5), 314.

41. Shahidi, F. Khalilian, p. Mohabi, M. Fathi, M. (2011). Formulation of apple pastille and evaluation of different formulas based on sensory characteristics and water activity. Iranian Journal of Food Science and Industry Research, Volume 7(2): 136 -129.

42. Shahidi, b. Police station, M. Bostani, S. (2014). The effect of replacing sucrose with grape juice on the physical properties of sponge cake. Iranian Food Science and Industry Research, Volume 1(1): 99 -105.

43. Schumacher A. B, Brandelli A, Macedo F. C, Pieta L, Klug T. V, de Jong E. V. (2010). Chemical and sensory evaluation of dark chocolate with addition of quinoa (Chenopodium quinoa Willd.). Journal of Food Science Technology, 47(2): 202 –206.

44. Afoakwa EO, Paterson A and Fowler M.(2007) Factors influencing rheological and textural qualities in chocolate - a review. Trends in Food Sci and Tech. 2007; 18: 290 -298.

45. Shorideh M, Azizi M, Taslimi A.(2010) Effect of Tagatos, Inolin and stivia as replacer

od suger on physico - chemical, reological and sensory properties of dark chocolate. Food Sci J 2010; 5: 38 -29 [in Persian].

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