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The Feasibility Study of Cheese Production from Whey and Evaluation of Physicochemical, Textural, and Sensory Properties of the Whey Cheese

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A B S T R A C T

This study aimed to produce cheese from whey as the main ingredient. The physicochemical, textural, and sensory properties of the whey cheese were evaluated over 16 pretreatments, and three formulations were obtained. The results showed that the cheese made from acid whey, lactic acid, milk, and doogh had the highest sensory score, protein content, and the best rheological properties. It was concluded that the lowest hardness obtained for this sample, despite its high protein content, was due to the high acidity induced by lactic acid. Presumably, the presence of lactic acid caused the acidity to the isoelectric point of proteins and decreased the net charge. Therefore, the repulsion between fat globules decreased, leading to a decline in emulsion stability. According to the results, the obtained formulation can be used to produce proper cheese made with whey as a primary ingredient.

Keywords: Whey, Cheese, Sensory Evaluation, Physicochemical Properties

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1. Introduction

Whey as a by-product of cheese making or casein production has high nutritional value and has been used as a medicine to treat many chronic diseases since the Middle Ages. Thousands of tons of whey are produced annually, which enters the environment and causes pollution (Shershenkov & Suchkova., 2015; Beik Mohammadi & Bolandi., 2011). Whey is a non-transparent liquid with a yellowish-green color that comes out of the clot after cutting the coagulated milk by applying heat and adding acid or enzyme. Whey should be processed as soon as it is collected because it is a suitable liquid medium for the growth of bacteria (Charles et al., 2008). Since whey proteins have nutritional and therapeutic properties such as increasing the body's immune system, weight loss, and cancer prevention, its application in cheese making has been considered, recently. Whey proteins contain all the essential amino acids that the human body needs. Whey proteins are a rich source of the branched-chain amino acids leucine, isoleucine, and valine. This high-quality source of protein is found naturally in dairy products, which are consumed as dietary supplements (Horne, 1998). At present, the reuse of whey for two main reasons has been considered in the dairy industry. The first reason is the presence of valuable compounds, which are significant both in terms of quantity and quality. Another reason is that whey's BOD is more than 40,000 milligrams of oxygen per liter, and its release into the environment causes environmental pollution. The separation of fats, proteins, and lactose from whey greatly reduces BOD. More than 90% of whey is obtained from the cheese production process, and less than 10% is obtained from the by-product of casein production (Sturaro et al., 2015). Whey protein has many functional properties that are useful in preparing various food products. Whey protein is used as a substitute for egg protein in confectionery and bakery products and as a substitute in dairy products such as ice cream. The functional properties of the protein in whey have the following properties: solubility, acid stability, and the ability to form stable emulsions, the ability to bond with water,

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gel formation, foaming, concentration control, brown color, adhesion, and fluidity. Whey protein is one of the few proteins that can be soluble at low pH and is used in various salads and fermented products such as yogurt. Whey proteins act as emulsifiers by coating around oil cells or water droplets and preventing them from sticking together. Whey proteins have two parts, hydrophilic and hydrophobic, which reduce surface tension and lead to emulsifying properties. Whey proteins can be used in various products such as sausages, soups, cakes, baby food formulations, salads, fruit drinks, and sports drinks (Charles et al., 2008).

Sturaro et al. (2015) examined the effect of micro whey particle (MWP) concentration and protein-to-fat ratio on the efficiency and composition of Caciotta cheese. This paper aims to evaluate the effect of different concentrations of MWP up to 40% and different ratios of protein to fat on the milk coagulation process, cheese yield, and Caciotta cheese composition. Cheese samples were examined after ten days of ripening period. Cheese yield decreased with increasing fat content, and the yield was higher in low-fat cheeses containing 4% whey microparticles. The stable composition of low-fat Caciotta cheese indicates that MWP can be used as a fat substitute (Sturaro et al., 2015). Salvatore (2014) examined the effect of whey concentration on protein recycling in fresh sheep ricotta cheese. The whey obtained from cheese making was heated to 63°C and quickly cooled to 40 °C. Its fat was removed and concentrated up to 6 times. Concentrated whey was heated to 78-80 °C with stirring. When the proteins began to clot, the stirring was stopped to form large clumps. The results showed that recycling alpha-lactalbumin and beta-lactoglobulin in cheese increased the production efficiency of ricotta cheese (Salvatore et al., 2014).

Kaminaridessk (2015) produced modified Mizithra cheese by replacing fresh whey with 65% dried whey concentrate. The proportion of raw materials used in the production of Mizithra modified cheese included 16.1% WPC, 49.9% water, 8% sheep milk, and 26% sheep cream. A 90 °C heat process was used for 30 minutes, and the final product was placed in polyethylene packaging to produce the product. A sample of Mizithra cheese was also produced traditionally and subjected to microbial, physicochemical, tissue, and sensory evaluation one day after production and 25 days after storage in refrigerated conditions. Mizithra cheese was higher in lactose, potassium, and sodium and had lower microbial, protein, ash, calcium, hardness, and adhesion than the control sample while free of mold and yeast. Also, the sensory and textural characteristics of the modified type of cheese did not change during storage in the refrigerator, while the control sample decreased. Sensory evaluation showed that the modified cheese of Mizithra cheese was of good quality (Kaminaridessk, 2015). Due to the importance of whey application as a by-product of cheese making or casein production, in this study, whey was used for cheese making to achieve the best quality, and physicochemical, textural, and sensory properties of the product were determined.

2. Material and Methods

The chemicals and culture media used in this study were provided by the German company Merck.

2.1. Preparation of test treatments

First, 16 types of cheese with different formulations were produced in the pre-test stage (Table 1), and the panelists evaluated their sensory characteristics. Then, the samples with higher scores were produced and tested.

Table1. Formulation of cheeses produced in pre-test section

Treatment	Formulation of cheeses produced
1	Acidic whey + Skim milk+ Cream (18g)+Salt
2	Acidic whey + Skim milk+ Cream (32g) +Salt
3	Acidic whey + Skim milk+ Cream (60g)+Salt
4	Milk permeate+ Skim milk+ Salt
5	Sweet whey + dough (water, High fat and low fat yogurt)
6	Acidic whey + dough (water, High fat and low fat yogurt)
7	Acidic whey + Skim milk+ Salt+ Without cream
8	Whey permeate+ Skim milk + Salt+ Cream (3.5g)
9	Whey permeate+ Skim milk + Salt + Cream (6.5g)
10	Whey permeate+ Skim milk + Salt + Cream (10g)
11	Sweet whey + Skim milk+ Salt + Cream (10.5g)
12	Sweet whey + Skim milk+ Salt + Cream (17.5g)
13	Sweet whey + Skim milk+ Salt + Cream (35.5g)
14	Milk permeate + dough (water, High fat and low fat yogurt)
15	Acidic whey + milk +dough +lactic acid+ salt
16	Acidic whey+ milk + dough+lactic acid+ Long-chain inulin

2.2. Cheese production process

To produce the cheeses in Table 1, heat processing at 85 °C and acidic conditions (using lactic acid) was used. After mixing the raw materials with acidic whey, a thermal process was applied. Then lactic acid was added and homogenized. The clots obtained were transferred to a mesh container to lose the moisture to minimize the moisture in the clot (Pisponen et al., 2013). Then, physicochemical and textural, and sensory evaluations were performed.

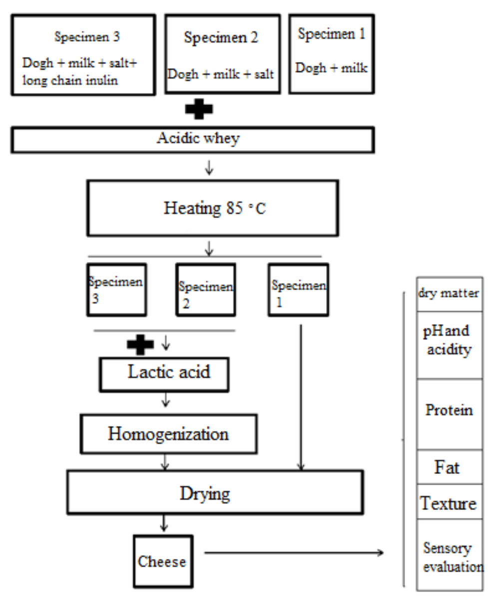

Fig. 1. Cheese making process from whey

2.3. Physicochemical properties

2.3.1 Moisture

The moisture content of the cheese was determined according to the Iranian National Standard No. 1753 after drying in an oven at a temperature of 102 ± 2 °C for three hours. Humidity was calculated from the difference in sample weight before and after repositioning (ISIRI, 2002).

2.3.2 Fat percentage

The fat percentage of the samples was determined according to the national standard No. 760 using Gerber method (ISIRI, 1991).

2.3.3 Protein

The protein content of the produced cheese samples was measured according to the national standard No. 1-9188 using the Kjeldahl method with a conversion factor of 6.38 (ISIRI, 2015).

2.3.4 pH

The pH of the samples was measured according to the national standard No. 2852 (ISIRI, 2006).

2.3.5 Acidity

The acidity of the samples was determined according to the national standard No. 2852 in terms of the percentage of lactic acid (ISIRI, 2006).

2.3.6 Texture

Texture properties were measured using a Texture Profile Analyser (TPA) device. A texture profile analyzer device with a cylindrical probe with a diameter of 35 mm was used for this test. The cheese samples were removed from the refrigerator one hour before the test, and cut into 15. 15 mm pieces and placed at room temperature. The basis of the device was compression force at a speed of 60 mm per minute. The specimens were compressed to 80% of the initial height. The hardness and adhesion properties were measured with 3 replications per sample [Hosseini et al., 2013).

2.3.7 Sensory evaluation

Sensory evaluation was performed by seven trained panelists using the 9-point hedonic method. Flavor indicators (by smelling the product and chewing and holding it in the mouth), appearance characteristics (evaluation of the visible internal and external properties of the product, including shape and color), and texture (with the senses of touch, especially the texture of the mouth and fingers) were determined so that the numbers assigned to the sensory indices of cheese samples from 1 to 9. The score 1 (very poor) was assigned to non-consumable samples, with the score 9 assigned to very satisfactory samples.

2.4 Statistical analysis

The experiments were performed in three replications. The data were analyzed based on one-way analysis of variance (One-Way ANOVA) followed by Duncan' test to compare mean at the level of 0.05 with SPSS software version 22.

3. Results and discussion

3.1 Physicochemical properties of whey

Titratable acidity and pH of different types of whey has been determined as reported in Table 2.

Table 2. A variety of whey was used for cheese production

Sample	pH	Titratable acidity (Lactic acid%)
Acidic Whey	< 5	0.4
Moderate acidity whey	5.8- 5	0.2-0.4
Sweet whey	5.8- 6.6	0.1-0.2

3.2. Sensory evaluation of whey cheeses

In the first step of the research, 16 different formulations were produced (Table 1) and their sensory properties were evaluated by panelists. Table 2 reveals the general characteristics of the different types of whey used in this study. Table 3 shows the sensory evaluation results of 16 different formulations of cheese produced with different types of whey. Samples 6, 15, and 16 were selected according to the sensory evaluation results in Table 3. As can be seen, there is no significant difference between the sensory evaluation results of most produced samples ($p \geq 0.05$). Sample 15 received the highest score on sensory evaluation, and samples 6 and 16 had the highest score after this sample ($p<0.05$). Samples 6, 15, and 16 were selected for the second phase of the research, and their physicochemical, sensory, and textural properties were examined. Whey is a soluble phase of milk that separates from the clot after coagulation of casein by the action of whey or lactic acid bacteria. The clot contains casein, fat, some nutrients, and various vitamins. However, whey contains lactose, soluble proteins, proteins, soluble nutrients, organic acids, vitamins, and enzymes (Charles et al., 2008). In addition, whey may also contain fine particles of casein. On the other hand, adding a culture medium to milk during the production process leads to the production of lactic acid-producing bacteria and aromatic substances in whey (Charles et al., 2008). All these compounds cause to produce taste, aroma, and overall color. The good color and taste of whey lead to the production of high-quality conversion products (Charles et al., 2008). Consistency is a texture characteristic that affects customer acceptance (Dimitreli et el., 2009). The release of flavor-producing compounds from food is a determining and complex factor in understanding flavor. High fats and volatile oils reduce hydrophobic aromatic substances, including long-chain aldehydes. Polysaccharides can bind to volatile compounds. For example, some carbohydrates can be linked to volatile compounds through hydrogen bonding between appropriate working groups. Some other compounds, such as starch, are composed of three-dimensional structures with hydrophobic regions and can form complexes with various volatile compounds. Flavor-volatile compounds react strongly with milk fat. Good color and whey taste can lead to products with the desired quality. Typically, the natural taste is mild and odorless, although whey is completely acidic (Hosseini et al., 2013).

Table 3. Sensory evaluation of cheeses produced in pre-test section

Treatment	*Formulation of cheeses produced*	*Overall acceptability*
1	Acidic whey + Skim milk+ Cream (18g)+Salt	3.00±1.19^{c}
2	Acidic whey + Skim milk+ Cream (32g)+Salt	3.00±1.07^{c}
3	Acidic whey + Skim milk+ Cream (60g)+Salt	3.16±1.94^{c}
4	Milk permeate+ Skim milk+ Salt	2.83±1.00^{c}
5	Sweet whey + doogh(water, High fat and low fat yogurt)	3.16±1.94^{c}
6	Acidic whey + doogh(water, High fat and low fat yogurt)	5.00±2.20^{b}
7	Acidic whey + Skim milk+ Salt+ Without cream	2.83±1.04^{c}
8	Whey permeate+ Skim milk + Salt+ Cream (3.5g)	2.50±1.42^{c}
9	Whey permeate+ Skim milk + Salt + Cream (6.5g)	1.83±0.72^{c}
10	Whey permeate+ Skim milk + Salt + Cream (10g)	1.66±0.54^{c}
11	Sweet whey + Skim milk+ Salt + Cream (10.5g)	2.16±0.75^{c}
12	Sweet whey + Skim milk+ Salt + Cream (17.5g)	2.33±0.51^{c}
13	Sweet whey + Skim milk+ Salt + Cream (35.5g)	1.66±0.81^{c}
14	Milk permeate + doogh(water, High fat and low fat yogurt)	2.00±0.63^{c}
15	Acidic whey+ milk+ dough +lactic acid+ salt	7.50±0.54^{a}
16	Acidic whey+ milk+ doogh+ lactic acid+ Long-chain inulin	5.16±0.75^{b}

1. Results are shown as mean±SD
2. Small letters (a, b, c, d) in the column indicate significant differences (p<0.05) of treatment.

3.3. Properties of selected whey cheese

Treatments 6, 15, and 16 scored the highest in sensory evaluations. Table 4 shows the formulation of these three treatments. The raw materials were processed at 85 °C and immediately introduced into a homogeneous apparatus and then dewatered. Then the physicochemical properties and textural properties of the produced cheeses were investigated.

Table 4. Samples produced in the second phase

No	**Formulation of cheeses produced**
Sample (1)	Acidic whey + Milk (3.5 % fat) + Doogh(Water, High fat and Low fat yogurt)
Sample (2)	Acidic whey + Milk (3.5 % fat)+ Doogh(water, High fat and Low fat yogurt)+ Salt+ Lactic Acid
Sample (3)	Acidic whey + Milk (3.5 % fat)+ Doogh(water, High fat and Low fat yogurt)+ Salt+ Long-chain inulin

3.3.1 Physicochemical properties of cheese

Table 5 shows the physicochemical properties of the produced cheeses.

Table 5. Physicochemical properties of cheeses produced in pretest section

Treatment	*Moisture (%)*	*Fat (%)*	*Protein (%)*
1	66.47±0.07 ^{a*}	24.15±0.07^{c}	22.47±0.01^{a}
2	66.52±0.07^{a}	22.15±0.07 ^{b}	22.57±0.02^{a}
3	64.50±0.56^{c}	17.15±0.07 ^{a}	19.66±0.01^{b}

*Small letters in the column indicate significant differences (p<0.05) of the treatment

Based on the results of different treatments, there was no significant difference between the humidity of samples 1 and 2 (p≥0.05), while sample 3 showed the lowest humidity (p<0.05). There was a significant difference between the fats of all three samples, with the lowest fat in sample 3 and the highest fat in sample 1 (p<0.05). There was also a significant difference between the proteins of all three samples, with the lowest protein in sample 3 and the highest protein in sample 2 (p<0.05). The difference in the chemical composition of the cheeses produced is due to different raw materials in their preparation. The fat in cheese is leading its taste and texture, and for this reason, customers tend to consume high-fat cheeses. Cheeses vary greatly in fat content for the type of milk (whole milk, low-fat milk, and skim milk) and the raw materials used to make cheese (such as cream). The higher fat content of sample 1 compared to other samples was due to the use of high-fat yogurt in the formulation. Sample 3 had the lowest moisture. This can be attributed to the presence of inulin; long-chain inulin increases water absorption. The most crucial factor that can be mentioned concerning the reduction of moisture in cheese samples is the formation of microcrystals by inulin, which by trapping water inside its networks, a decrease moisture will be observed in the final product (Dimitreli et al., 2009). Inulin soluble fiber is a fructan obtained from chicory root by hot water extraction and enzymatic hydrolysis. From a structural point of view, inulin is a polyoxyethylene skeleton to which fructose groups are attached. Sometimes glucose is attached at the end. The degree of polymerization and the presence of branches and chains play an essential role in its functional characteristics. Its long-chain types have a natural taste and less water solubility, and its polymerization degree is between 2 and 60 (Hosseini et al., 2013). The whey used in the final whey samples was acidic, and therefore, the pH of the cheeses produced was less than 5. In 2015, researchers studied whey cheese's production method and physicochemical, sensory, and textural properties. In this study, whey cheese in both types (with milk and with buttermilk) was traditionally produced and evaluated in sensory, physicochemical, and rheological properties. The results showed that the moisture content of whey cheese with milk and whey cheese with buttermilk was 66.45% and 72.82%, respectively. The fat content of these two products was 22.33% and 13.70%, respectively, and whey cheese with milk (8.7%) contained less protein than whey cheese with buttermilk (9.73%) (Beik Mohammadi and Bolandi, 1390).

3.3.2 Acidity and pH of cheese

Figure 2 and Figure 3 show the pH and acidity of the cheese samples, respectively. There was no significant difference between the pH of the samples ($p \geq 0.05$). There was a significant difference between the acidity of all three samples. The highest acidity is in sample 2, and the lowest acidity is in sample 1 ($p<0.05$). The higher acidity of samples 2 and 3 is due to lactic acid in the formulation. Adding lactic acid lowers the pH and consequently increases the acidity. It has been reported that when the pH decreases and the acidity increases, the amount of colloidal calcium phosphate decreases, resulting in a decrease in cross-links in the casein network and a change in rheological properties. Lowering the pH leads to the dissolution of colloidal calcium phosphate and reduces the elastic properties of the product. This probably explains the reduction in stiffness in sample 2. Increased acidity dissolves calcium phosphate complexes and thus reduces the number of bonds between caseins and, consequently, the product's hardness (Fox and McSweeney, 1998).

Mizuno and Lucey (2007) state that the gel of milk protein concentrate solutions containing emulsifying salts is affected by pH. At low pH (below 6), the calcium phosphate crosslinkers dissolve in the milk protein concentrate solution.

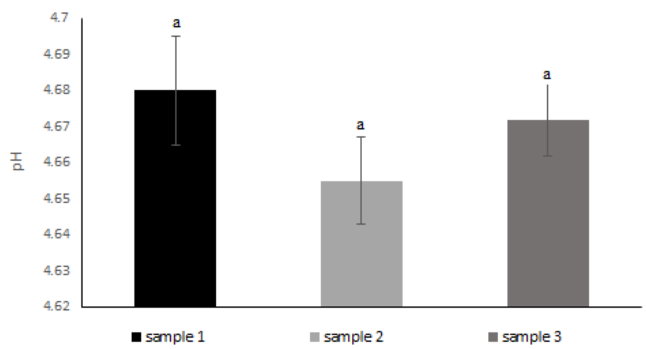

Fig 2. pH of produced cheese
Small letters in the column indicate significant differences ($p<0.05$) between treatments

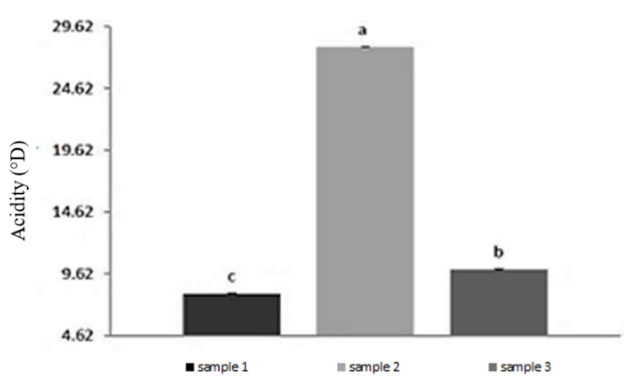

Fig 3. Acidity (°D) of produced cheese
Small letters in the column indicate significant differences ($p<0.05$) of the treatments

3.3.3 Texture

Texture properties were measured using a Texture Profile Analyzer (TPA) (Figures 4 and 5). The studied features included hardness and adhesion, done in 3 replications per sample.

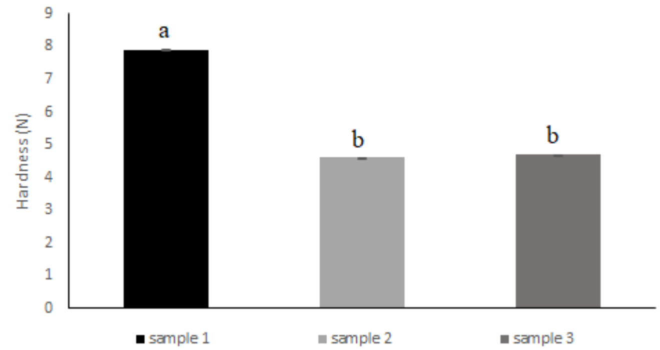

Fig 4. Hardness of produced cheese
Small letters in the column indicate significant differences ($p<0.05$) of the treatment

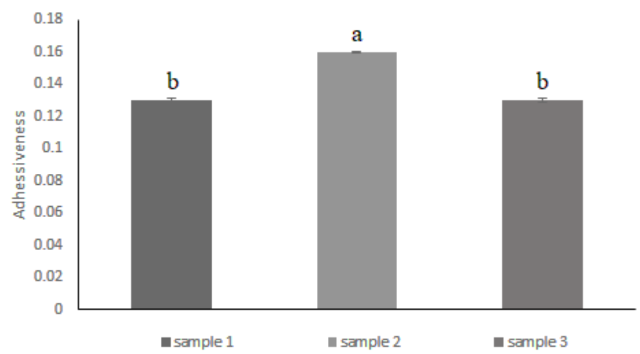

Fig 5. Adhesiveness of produced cheese
Small letters in the column indicate significant differences ($p<0.05$) in the treatment

The maximum force required to compress the sample is called the hardness, which is displayed in units of energy (N); the results showed that the highest hardness was in sample 1 and the lowest hardness was in sample 2 ($p<0.05$). After applying the compaction, when the probe of the device is separated from the sample, a negative peak is formed if the sample's surface is sticky when the probe separates. The area below the peak curve is called the surface adhesion of the required energy to separate the probe from the sample surface and is reported in units of energy. Also, sample 2 has the highest adhesion, and sample 1 has the lowest adhesion ($p<0.05$). There was no significant difference between the adhesion of samples 1 and 3 ($p \geq 0.05$). Hardness is the force required to compress a specimen between the teeth of a mill, or in other words, the force needed to achieve a certain deformation (Hosseini et al., 2013). Increasing the humidity reduces all texture features; this is because moisture increases the plasticity of the protein matrix and decreases its elasticity. As a result, the protein network becomes more prone to rupture during compression. Water molecules are located between the three-dimensional network of proteins and weaken the network's structure. Therefore, increasing the humidity reduces the protein network cohesion and makes the product softer (Dimitreli el., 2009).

As observed, sample 2, which has high humidity, also has less hardness. On the other hand, lower protein content also reduces texture features. The higher concentration of casein in the protein matrix causes greater internal and interstitial bonds, and more elastic matrix, and resistance (Fox and McSweeney, 1998). Sturaro et al. (2015) examined the texture properties of the whey protein mixture and rice bran protein isolate. They reported that the higher hardness of some samples was due to higher amounts of whey protein and the formation of stronger transverse bonds in the gel structure. However, in this study regarding the reduction of hardness in sample 2, the highest amount of acidity (near the protein's isoelectric point) was considered. As a result, the fat cell's repulsion emulsified by the proteins is reduced, and the stability of the emulsion is impaired. When milk becomes acidic, colloidal calcium phosphate is released from casein micelles, and the amount of calcium in the serum increases. On the other hand, at alkaline pH, the intermolecular bonds of disulfide, which help maintain the secondary structure of whey proteins, are easily broken. The higher hardness of sample 3 than sample 2 is probably the presence of long-chain inulin. In a previous study has been reported that the hardness and creaminess of low-fat yogurts increase with inulin and fat. Also, low-fat yogurts containing 4% inulin were similar to high-fat yogurts (Pisponen et al., 2013). Adhesion is the force required to separate food from the roof of the mouth during eating. In other words, work is needed to overcome the adhesion forces between the surface of the food and the surface of different materials with which the food is in contact (Dimitreli el., 2009). Hosseini et al. (2013) stated that the main factor affecting the adhesion and cohesion of imitation cheese is the amount and type of fat, and the fat in the enzyme-modified cheese, which is milk fat, has been the main factor in increasing the adhesion of imitation cheese (Hosseini et al., 2013). However, in this study, sample 1, which contained the highest amount of fat, had the highest hardness and the lowest adhesion. The reason for the decrease in the firmness of the imitation cheese texture in samples 2 and 3, which contained lactic acid, is the weakening of the emulsion in the product, which was discussed earlier.

3.3.4 Sensory evaluation

The general acceptance of the produced cheeses was evaluated according to their appearance, texture, smell, and taste. Table 3 shows the total acceptance of cheese samples. According to the scores of sensory evaluation, cheese prepared with whey, buttermilk, milk, and lactic acid had higher score than other samples. The results of the texture test showed that this sample had the lowest hardness among different samples, which from the consumer's point of view; this hardness was more acceptable than other samples. No significant difference was observed between samples 1 and 3 ($p \geq 0.05$).

4. Conclusion

Three formulations using acidic whey were selected from 16 types of cheese produced. The higher total acceptance of cheeses produced with acidic whey and cheese clots had more suitable texture (Dimitreli el al., 2009). Cheese prepared with acidic whey, milk, buttermilk, salt, and lactic acid was more acceptable in sensory evaluation. This sample was also lower in fat content than the cheeses on the market and can be an excellent alternative to standard breakfast products such as cream and butter for people who care about their health. Despite having a high amount of protein, a slight hardness was observed in this cheese. It had a better dispersibility, which made it an ideal cheese among the evaluators. Based on the favorable results obtained in this study, the conditions for industrial and semi-industrial production of this cheese can be prepared. Athletes can be mentioned among the groups consuming this cheese due to its nutritional benefits and contains all the essential amino acids needed by the diet, the type of amino acids is also significant. For example, the amino acid leucine is essential for athletes because it plays a crucial role in muscle growth. Also, the role of whey proteins in maintaining health in old age, when muscles are depleted and negative, and harmful health effects increase, good nutrition and intake of sufficient amounts of high-quality protein such as whey proteins can help maintain muscle strength. Another primary reason that makes whey recycling and reuse necessary is the presence of large amounts of organic matter in it, and its release into the environment causes its pollution. That's why environmental laws prevent it. A cheese factory consumes 100 tons of milk a day, polluting the size of a city of 60,000 causes a severe reduction in BOD (Charles et al., 2008). The production of this cheese also plays an essential role in preserving the environment due to the significant reduction of wastewater from food factories.

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6. Conflict of interest

The authors declare that there is no conflict of interest.

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