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Effect of Calf and Goat Lipases on Color Parameters and Some Physicochemical Properties of UF-White Cheese During Storage Period

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ABSTRACT

The process of cheese production and ripening takes place with the help of an inclusive range of diverse and continuous biochemical reactions, which, if balanced, lead to the production of products with very desirable quality characteristics, including color and physicochemical properties. In this study, the kid goat and calf lipases at the levels of 0.1, 0.2, 0.3, and 0.4 g (per 100 Kg retentate) were used in the production of ultrufiltrated Iranian white cheeses. The effect of lipase treatment on color parameters (L^*, a^* and b^* values) and some physicochemical properties (acidity, moisture, protein and fat) of the product were evaluated. The cheese sample without enzymatic treatment considered as control and its color and physicochemical properties was compared with other cheese samples during 90 days of storage period. Analysis of variance showed that the lipase enzyme significantly increased L^* and b^* index values of the cheese samples but it had not significant effect on the a^* index. Furthermore, the time of storage caused a significant increase in these color parameters. Treatment with lipase and storage time had also significant effect on the physicochemical parameters. In general, by increasing the amount of lipase, and storage period, the amounts of acidity, moisture and protein increased and fat content decreased meaningfully. The results of this study revealed that by using 0.3-0.4 g lipase, particularly kid goat lipase, an ultrafiltrated Iranian white cheese with an acceptable quality can be produced.

1.Introduction

Cheese can be consumed as a food item, a secondary or main component of food, or as a dessert without the need for preparation or after various cooking processes. In different sources/documents, the number of types of cheese have been mentioned differently, in some sources 1000 to 1500 types of cheese have been mentioned [1]. The quality of cheese, especially the texture and taste of the product, is very important, because it plays an important role in consumer acceptability. Important factors such as the type of milk and as a result, the amount of components and its quality, the method of cheese preparation (enzymatic, acidic, etc.), the additives used (type of starter, type of enzyme, etc.), and the conditions of ripening and keeping of the cheese curde have an effect on the quality of the final product because it significantly affects the quantitative and qualitative properties of the cheese. Iranian white cheese is a type of brined cheese, produced thru traditional and ultrafiltrated (UF) ways, is the most consumed cheese in the breakfast meal of Iranian people [2]. In the production of white cheese produced by ultrafiltrated method, the operation with using membrane separation is used, which selectively concentrates milk protein and fat. Ultrafiltration, as a technology for cheese production, was introduced and widely used in Denmark in 1970. In this process, the milk is separated into two separate parts, the diluted portion or permeate and the concentrated part called retentate. In fact, the concentration of milk before cheese production reduces costs and speeds up all the stages of its production. For the production of UF cheese, the concentrated part is used, and unlike traditional cheese, during the production of this type of cheese, whey does not come out of the curd. The higher nutritional value and cost-effectiveness of UF cheese due to the maintenance of whey proteins [3] has caused its consumption in our country to surpass from its main competitor, i.e. the traditional white brined cheese.

The breaking of fat into one molecule of glycerol and three free fatty acids is called lipolysis, which is derived from the Greek root lipo meaning fat and lysis meaning decomposition. Lipases are part of hydrolases group of enzymes and their main biological function is to convert insoluble triglycerides into fatty acids and glycerol [4 and 5]. Lipases are among the most important group of biocatalysts. The use of lipases has increased since the 1980s, and by allocating 5% of the global enzyme market, it is placed after proteases and carbodases [6]. Among the types of lipase with different origins, microbial lipase has more and wider applications [5]. Triglyceride is broken down into glycerol and fatty acids step by step by lipases. The rate of hydrolysis depends on the amount of moisture or water activity. Also, lipase line up triglycerides that have fatty acids with short and unsaturated chains. Lipase activity may also contribute to the flavor and rheological properties of dairy products. Heating milk up to 80°C for 20 seconds causes inactivation and destruction of lipase in milk, while the maximum activity of lipase has been observed at pH 7.2 and temperature of 37°C [7]. Lipase activity is inhibited in the presence of mercury and silver ions and stimulated by calcium and magnesium ions [8]. Accurate control of lipase concentration, pH, temperature and emulsion characteristics play an important role in the amount of lipolysis and, as a result, the amount of aroma and flavor produced in cheese [9]. Pasteurization of milk deactivates most of the inherent lipase in milk, so there is a significant difference between cheese made from raw milk and pasteurized cheese in terms of lipolysis. In other words, the amount of lipolysis depends on the type of cheese and its range is very inclusive. In all kinds of brined and salted cheeses, the amount of lipolysis is very small, while in some types of Italian ripened cheese, lipolysis takes place extensively [10]. During cheese ripening, as a result of lipolysis and the creation of free fatty acids and subsequent

breakdown of the released fatty acids, the aroma and taste of the product improves [5].

Although various researches have been conducted in the field of the effect of lipases on the cheese characteristics, so far no research has been reported on the effect of goat and cow lipase enzymes on the characteristics of UF cheese. In a research on the effect of goat and cow lipase enzyme on Iranian white cheese during 90 days of storage in the refrigerator, Jooyandeh and Hojjati showed that using 4.5 grams of lipase enzyme (goat or calf, per 100 kg of milk), a cheese with more favorable characteristics could be produced [11]. Aydemir et al. [12] and Akın et al. [13] in the study of the effect of adding pregastric commercial lipase to milk used in the production of white brined cheese reported that the enzyme treatment caused a slight increase ($p>0.05$) of the fundamental cheese compounds includes pH, titratable acidity, total solids, fat, total nitrogen and cheese salt during the ripening period. Aminifar and Emam-Djomeh also reported that the addition of microbial lipase (zero to 8 grams of lipase per 100 kg of milk) had no significant effect on the pH, acidity, moisture and salt content of Liqvan cheese [14].

Although cheese production is an ancient art, today, to produce high-quality cheese, one must use modern science and technology and be fully aware of complex chemical and biochemical processes, including complex fermentation reactions during the ripening period. The most important effective factors in lipolysis and creation of aroma and flavor in cheese are the activity originated from the inherent enzymes of milk, rennet and starter [15]. Nowadays, commercial lipases are used instead of milk's inherent lipase due to the better control of the product's aroma and flavor and the faster speed of cheese making. Commercial animal lipase, which is used to increase the aroma and flavor of cheese, is often obtained from the pancreas of cows and pigs, as well as the pre-stomach of young ruminants (goats, lambs and calves) [9]. Therefore, this research was carried out in order to investigate the effect of lipase enzyme of cow and goat origin on color parameters and some physicochemical characteristics of the Iranian white brined cheese.

2.Materials and methods

2-1- Materials

The UF cheese samples were made in Pegah factory (Khuzestan, Iran) with using fresh cow milk (0.14% lactic acid, 11.87% dry matter, 3.4% fat and 3.17% protein). Mesophilic cheese starter powder CHOOZIT 230 contains a mixture of *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* and thermophilic starter YO-MIX 532 contains *Streptococcus thermophilus* and *Lactobacillus delbruckii* subsp. *bulgaricus* from German Company Danisco and rennet with the brand name of Chey-Max in the form of recombinant chymosin type, expressed by *Aspergillus niger var*. *awamori* was acquired from Christian Hansen, Denmark. Also, lipase enzymes originating from the epiglottis glands of calves and goats manufactured by the Caglificio Clerici Company in Italy were used. These enzymes were purchased in the 500 grams white-yellowish color powder form and were kept in the refrigerator until utilization. According to the package information, the strength of each of the mentioned enzymes was 10 units per gram of enzyme, and its composition included lipase enzyme, sodium chloride, whey protein powder, milk cheese, and casein. Other chemicals used in this research were of high purity and were purchased from Merck, Germany.

2-2- Production of Iranian white UF-cheese

UF-cheese was produced according to the method of Danesh et al. The high-quality milk in terms of microbiology and physicochemistry, was cooled by a heat exchanger at temperature of 4-6 °C, and transferred to the raw milk storage tank regarded for the production of UF-cheese. After

standardizing the amount of milk fat in the separator, the milk with standardized fat was subjected to bactofugation in two stages to reduce more than 99% of its microbial load. Then the milk was pasteurized in a plate heat exchanger at a temperature of 76 °C for 15 seconds and sent to the pasteurized milk storage tanks for cooling to a temperature of 6 °C. In order to UF process, the pasteurized milk was first heated to 50 °C by a plate heat exchanger and after passing through the UF membrane, it was divided into two parts, permeate and retentate. Then the retentate was pasteurized at 78°C for 16 seconds, homogenized at 50 bar pressure, and finally it was stored in the UF-storage tank. In order to produce UF-cheese, the temperature of retentate was set to 31 ± 1 °C and after adding 0.01% of mixture of mesophilic and thermophilic starter powder and 0.03 grams of rennet per kilogram of retentate, it were poured inside the 400 CC cheese packages. Enzyme treatment of goat or cow lipase in different concentrations (0.1, 0.2, 0.3 and 0.4 grams of enzyme per kilogram of retentate) was performed simultaneously with the addition of rennet. Then the containers of cheese were transferred to the coagulation tunnel with a temperature of about 31 °C. After 30 minutes, the clot was formed in the coagulator and 4% salt (w/w) was poured on the parchment paper, which was on the upper surface of the cheese. Then, the containers were sealed using aluminum foil sealing machine. At the end, after being kept in an incubator (temperature 37 ± 1 °C) and reaching the pH upto 4.8, the cheese samples were transferred to a cold storage with a temperature of 5 °C. The physicochemical characteristics of the cheese samples were examined during 90 days of cold storage.

2-3- Color evaluation

The color of the cheese samples was measured using a colorimeter (Chroma meter, series CR-400, made in Japan), where L*, a*, and b* represent lightness, redness, and yellowness, respectively [17].

2-4- Physicochemical evaluation

pH of the cheese samples was measured using a digital pH meter (AZ, model 86502, Taiwan). Acidity of the cheeses was measured thru NaOH N/9 in the presence of *phenolphthalein* reagent and expressed as Dornic degree (°D). Furthermore, the fat content by the Gerber volumetric method and the use of a butyrometer; moisture content by oven method at 105 °C for about 2 hours until a constant weight was reached; and the amount of total protein was measured through the product of the amount of nitrogen obtained by the micro Kjeldahl method in the factor of 6.38 according to the methods of AOAC [18].

2-5- Data analysis

In this research, two types of lipase enzymes (of cow and goat origin) at four levels (0.1, 0.2, 0.3 and 0.4 grams of enzyme per kilogram of raw material) were used. Therefore, in the current study, 8 cheese treatments containing lipase enzyme were produced. The color parameters and physicochemical characteristics of the cheese samples during three months of storage (after sample production, and 30, 60 and 90 days) were compared with control (sample without added animal lipase enzyme). The 9 investigated treatments were produced in 3 replications. To investigate the main effects and interaction between the variables on the investigated characteristics, two-way analysis (factorial test in the form of a completely randomized design) was applied. Also, to compare the mean values of the treatments, one-way ANOVA analysis was used [19]. The obtained results were analyzed with the help of statistical program SPSS version 24 and the average results were compared with the help of Duncan's test at the 5% level. Excel 2013 software was used to draw graphs. It is worth mentioning that the different concentration levels of lipase enzyme used in this research were selected after conducting preliminary tests.

3.Results and disscution

3-1- Color indexes/values

Color is one of the sensory parameters effective in product acceptance by consumers. The scattering of light in any system depends on the uniformity of the molecules and the levels of its microstructure. In solid materials such as cheese, light penetrates through the surface layers and its upper part is scattered by milk fat globules as well as cheese water cavities [20]. The results of the statistical analysis of the variable effect of lipase enzymes and storage period on the color parameters including brightness (L*), redness (a*), yellowness (b*) of Iranian white UF-cheese are presented in table (1). As can be seen in Table 1, both variables had a significant effect on all the investigated color indices.

3-1-1- Lightness (L^*)

The results showed that the type of lipase enzyme ($p<0.05$) and its amount ($p<0.01$) had a significant effect on the amount of L* index. According to Table 1, the amount of brightness in cheese samples containing goat lipase enzyme was higher than cow lipase; meanwhile, with the increase in the amount of enzyme in both types of cow and goat lipase enzymes, the amount of brightness increased. Although there is no significant difference in this regard between the control cheese samples produced with the samples containing low levels of goat or cow lipase enzyme, especially the sample containing 0.1 gram per kilogram of bovine lipase enzyme at the beginning of the storage time, but these differences became significant after the storage time, and the samples of UF-cheese containing low levels of enzyme had a higher brightness index than the control sample. The average brightness index value of control cheese and samples containing goat and cow lipase enzymes were determined as 86.92, 88.46 and 88.84, respectively. The reason for the lower L* index in the control sample compared to the samples containing the lipase enzyme can be due to the higher humidity and the increase in the cheese water holes in the samples containing the enzyme, because the hydration of the proteins can to increase light diffusion and whiteness of cheese [20]. In similar results, Ghanbari et al. [21] while investigating color parameters in low-fat white cheese samples with fat substitute content reported that fat reduction causes a significant decrease in L* values in cheese. Rostamabadi et al. (2016) also showed that reducing the amount of fat in the samples containing Persian gum caused an increase in the moisture content of the cheese and, as a result, an increase in the lightness of the samples [20]. However, Koca and Metin [22] and Juan et al. [23], contrary to the present results, reported a decrease in brightness and dullness of cheese samples as a result of fat reduction. Also, with the passage of storage time, the brightness of the cheese samples increased significantly ($p<0.01$), so that the average brightness on days 0, 30, 60, and 90 days of storage were determined 85.18, 86.54, 88.38 and 93.75, respectively. Based on the obtained results (Table 1), the highest amount of L* index with a value of 95.42 corresponds to the sample containing the highest level of goat lipase enzyme at the end of 90 days of storage and the lowest value with a value of 24.84 corresponds to the control sample was determined at the beginning the storage period.

3-1-2- Red-green value (a^*)

The a* index indicates the color changes from red (positive values) to green (negative values). According to Table 1, the a* parameter was observed with negative values for all the samples, which is most likely due to the presence of riboflavin in the cheese samples [24]. However, as can be seen in Table 1, the results indicated the absence of significant effects of the lipase enzyme variable on the a* index of the samples. The average value of a* index in control cheese samples and containing goat lipase enzyme and cow enzyme was determined as -2.15, -2.16 and -2.17, respectively.

Unlike lipase enzyme, the storage time had a significant effect on a* values of the cheese samples and this index increased significantly with the passage of storage time. The average value of a* index on days 0, 30, 60 and 90 of storage was determined as -1.82, -1.92, -2.36 and -2.56, respectively. Also, according to the obtained results (Table 1), the highest amount of green index with a value of -2.61 corresponds to the sample containing the highest level of goat lipase enzyme (0.4 grams of enzyme per kilogram of raw material) at the end of 90 days of storage and the lowest amount was determined with the value of -1.78 corresponding to the sample containing 0.2 grams of bovine lipase enzyme at the beginning of the storage period. The reason for the increase in greenness index during the storage period of UF-cheese samples during the storage period can be due to the increase of syneresis of the samples [25]. Syneresis in yogurt causes the release of serum containing riboflavin and as a result increases the greenness of the product [24]. In accordance with the results obtained in this research, Kang et al. [26] reported a decrease in redness or an increase in greenness in control yogurt samples containing fermented extracts of green and hot red peppers during storage. The reason for these color changes in the product is probably the oxidation of the carotenoid compounds responsible for the red color of the product during the storage period [27, 28].

Table 1. Effect of calf and kid goat lipases and their concentrations on color parameters of ultrafiltrated white cheeses during 90 days of cold storage

Color parameter	Storage time (Day)	Control	Calf lipase (g/Kg retentate)				Kid goat lipase (g/Kg retentate)			
		0	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4
L^*	0	84.24 ±0.46^{Dd}	84.43 ±0.30^{Dd}	85.04±0.39^{Dbcd}	85.51±0.49^{Dabc}	85.73 ±0.33^{Dab}	84.71 ±0.64^{Dcd}	85.02 ±0.26^{Dbcd}	85.61 ±0.59^{Dab}	86.29 ±0.23^{Da}
	30	85.56 ±0.48^{Cd}	85.71 ±0.70^{Cd}	86.72±0.39^{Cabc}	86.99 ±0.56^{Cd}	87.33 ±0.58^{Ca}	85.91 ±0.23^{Ccd}	86.38 ±0.61^{Cbcd}	87.02 ±0.04^{Cab}	87.25 ±0.44^{Cab}
	60	86.67 ±0.29^{Bg}	87.22 ±0.60^{Bfg}	87.73 ±0.29^{Bef}	88.38 ±0.43^{Bcd}	88.96 ±0.32^{Bbc}	88.12 ±0.31^{Bde}	88.95 ±0.05^{Bbc}	89.47 ±0.27^{Bab}	89.89 ±0.43^{Ba}
	90	91.21 ±0.17^{Ae}	93.06 ±0.30^{Ad}	93.80 ±0.32^{Ac}	94.25 ±0.35^{Abc}	94.56 ±0.59^{Ab}	93.20 ±0.27^{Ad}	93.90 ±0.14^{Ac}	94.30 ±0.32^{Abc}	95.42 ±0.29^{Aa}
a^*	0	-1.83 ±0.11^{Ba}	-1.79 ±0.08^{Ba}	-1.78 ±0.09^{Da}	-1.80 ±0.12^{Ca}	-1.80 ±0.06^{Da}	-1.82 ±0.07^{Ba}	-1.82 ±0.10^{Ba}	-1.85 ±0.08^{Ca}	-1.85 ±0.09^{Ca}
	30	-1.91 ±0.10^{Ba}	-1.90 ±0.06^{Ba}	-1.92 ±0.03^{Ca}	-1.93 ±0.06^{Ca}	-1.95 ±0.09^{Ca}	-1.91 ±0.16^{Ba}	-1.86 ±0.16^{Ba}	-1.93 ±0.14^{Ca}	-1.94 ±0.11^{Ca}
	60	-2.34 ±0.13^{Aa}	-2.34 ±0.14^{Aa}	-2.35 ±0.05^{Ba}	-2.37 ±0.07^{Ba}	-2.37 ±0.05^{Ba}	-2.33 ±0.12^{Aa}	-2.37 ±0.09^{Aa}	-2.38 ±0.06^{Ba}	-2.38 ±0.10^{Ba}
	90	-2.53 ±0.14^{Aa}	-2.52 ±0.14^{Aa}	-2.55 ±0.08^{Aa}	-2.56 ±0.08^{Aa}	-2.57 ±0.04^{Aa}	-2.54 ±0.16^{Aa}	-2.56 ±0.12^{Aa}	-2.58 ±0.05^{Aa}	-2.61 ±0.12^{Aa}
b^*	0	9.41 ±0.52^{Da}	9.20 ±0.21^{Dab}	9.08 ±0.24^{Dab}	8.87 ±0.32^{Dab}	8.68 ±0.19^{Dbc}	9.11 ±0.22^{Dab}	8.98 ±0.33^{Dab}	8.55 ±0.14^{Dbc}	8.22 ±0.22^{Dc}
	30	10.45 ±0.20^{Ca}	11.03 ±0.61^{Ca}	10.22 ±0.22^{Ca}	9.96 ±0.07^{Ca}	9.90 ±0.12^{Ca}	10.22 ±0.22^{Ca}	10.06 ±0.26^{Ca}	10.00 ±0.15^{Ca}	9.99 ±0.34^{Ca}
	60	11.78 ±0.35^{Ba}	11.56 ±0.35^{Bab}	11.44 ±0.44^{Bab}	11.22 ±0.23^{Babc}	11.05 ±0.24^{Bbc}	11.37 ±0.38^{Bab}	11.18 ±0.19^{Bbc}	10.91 ±0.16^{Bbc}	10.78 ±0.23^{Bc}
	90	12.49 ±0.19^{Aa}	12.43 ±0.22^{Aab}	12.28 ±0.36^{Aab}	12.12 ±0.38^{Aab}	12.04 ±0.31^{Aab}	12.33 ±0.35^{Aab}	12.11 ±0.34^{Aab}	11.88 ±0.33^{Aab}	11.89 ±0.19^{Ab}

Different small and capital letters indicate significant differences ($p<0.05$) in each row (treatments) and column (days) for each cheese characteristics, respectively.

3-1-2- Yellow-blue value (b^*)

The values of index b* of UF-cheese samples containing different amounts of two types of cow and goat lipase enzymes during the storage period are shown in Table 1. Since the positive values of b* index are equivalent to yellow color and the negative values of this quantity are equivalent to blue color, all the samples of UF-cheese have a yellow color during 90 days of storage. Also, according to Table 1, both investigated variables had a significant effect on the b* index of the samples ($p < 0.01$). The value of b* index in lipase-containing samples was lower than the control sample, and with the increase in lipase concentration, the b* index of the samples decreased. Meanwhile, although the value of b* index of cheese samples containing bovine lipase enzyme was higher than that of goat lipase enzyme, no significant difference was observed. The average yellowness of control cheese samples, sample containing bovine lipase enzyme and goat enzyme, was determined as 11.03, 10.69 and 10.51, respectively. In accordance with the results obtained in this research, Rani and Jagtap [29] in investigating the effect of adding 200 and 800 units of lipase enzyme in the production of Swiss cheese reported that the use of the enzyme caused a relative reduction in the yellowness of the samples during 60 days of storage. Yüceer and Asik [30] also reported a significant reduction in the yellowness of meringue dough as a result of lipase enzyme treatment. The decrease in the yellow color index as a result of lipase treatment, as mentioned before, can be due to the oxidation of carotenoid compounds and also the breakdown of cheese fat through the lipolysis reaction.

In addition, the results of this research showed that the yellowness parameter or b* of the samples increased significantly during the 90-day storage period. The average value of b* index on days 0, 30, 60 and 90 of storage was determined as 8.91, 10.22, 11.27 and 12.19, respectively. According to the results obtained in Table 1, the highest amount of yellowness index with a value of 12.49 corresponds to the control sample at the beginning of the storage period and the lowest value with a value of 8.22 corresponds to the cheese sample containing the highest level of goat lipase enzyme (0.4 gram of enzyme per kilogram of raw material) was determined at the end of 90 days of storage. The increase in yellowness of cheese during the storage period has been reported by other researchers such as Rohm and Jaros in Emmental cheese [31], Sert et al. in Tulum cheese [32] and Fernandes et al. in Serrana cheese [33].

3-2- Physicochemical Characteristics

The results of analysis of variance of the effect of two variables, enzyme type and storage time, on the physicochemical properties of UF Iranian white cheese are presented in Table 2. As can be seen in this table, both variables have had a significant effect on all the examined characteristics. In addition, apart from protein, in other cases, a significant interaction between the two tested variables was determined.

3-2-1- Acidity

The effect of two variables of lipase enzyme and storage time on the acidity values of UF-cheese samples are shown in Figures 1 and 2, respectively. As can be seen in Table 2, both investigated variables significantly ($p<0.01$) affected the acidity values of produced cheeses. The amount of acidity in all the samples containing lipase was higher than the control sample, and with the increase in the concentration of lipase, the acidity of the samples was more increased. Meanwhile, the acidity of cheese samples containing cow lipase enzyme was lower than goat lipase enzyme, which indicates the higher activity of goat lipase enzyme. The average acidity values of the control cheese samples and those containing bovine and goat lipase enzymes were determined to be 87.56, 97.47 and 101.71, respectively. The increase in acidity is mainly due to lactic fermentations and lactic acid

production. Also, the activity of the lipase enzyme leads to the production of a large amount of free fatty acids. This factor can lead to an increase in acidity.

Table 2. The results of analysis of variance (ANOVA) of the effect of lipase addition and storage time on some physicochemical characteristics of ultrafiltrated cheeses.

Treatments	df	Mean Square			
		Acidity	Moisture	Fat	Protein
Lipase	2	389.5^{**}	7.63^{*}	0.70^{*}	3.72^{*}
Storage Time (Day)	2	1041^{**}	129.1^{**}	4.47^{**}	4.39^{**}
Lipase × Storage Time	2	234.1^{*}	3.55^{*}	0.10^{*}	0.071^{NS}
Error	4	13.36	0.539	0.006	3.505
Coefficeint variation	2	3.737	1.114	0.618	1.345

NS, * and ** respectively indicate: non-significance, and significance at $p<0.05$ and $p<0.01$ levels.

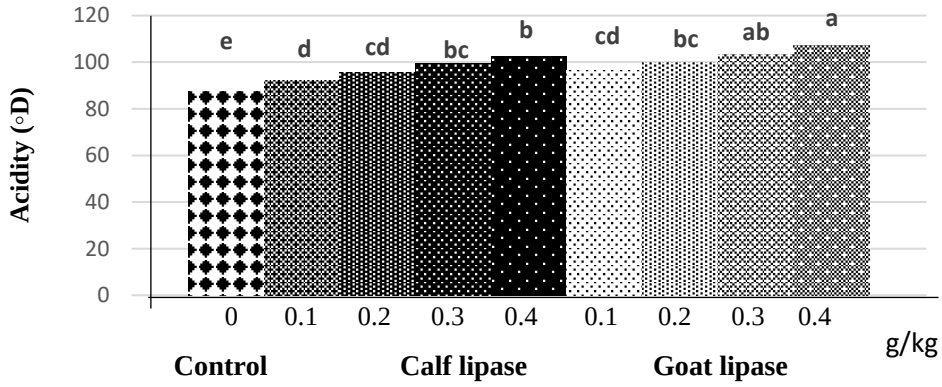

FIG. 1. Effect of lipase addition on acidity of ultrafiltrated cheeses during cold storage period.

According to Figure 2, the acidity of the samples of manufactured UF-cheese increased during the storage period from day 0 to 60 and then decreased significantly until the 90th day of storage. The increase in cheese acidity during the storage period is due to the partial completion of lactose fermentation by starter bacteria and cheese microbial flora and the production of hydrogen, amino acids and fatty acids [14]. According to the results of this research, the cheese sample containing 0.4% goat lipase enzyme had the highest acidity on the 60th day of the storage period with a value of 108.84, and the control sample had the lowest acidity at the beginning of the storage period with a value of 62.16. In accordance with the results of this research, Yilmaz et al [34] reported in a research that increasing the concentration of microbial lipase during the ripening of Tulum cheese decreases pH and increases acidity.

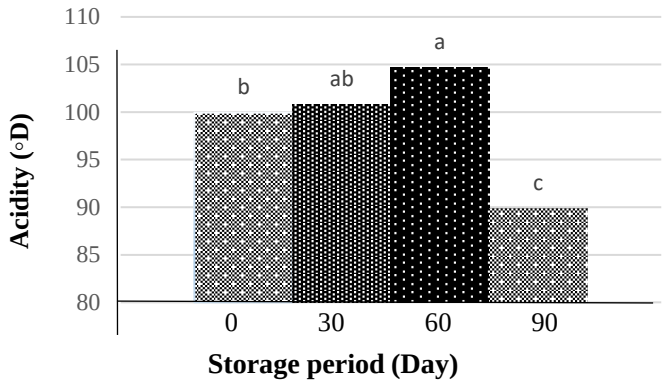

FIG. 2. Effect of storage period on acidity of ultrafiltrated cheeses treated with animal lipases.

3-2-2- Moisture

The changes in the moisture content of UF-cheese samples under the influence of the two investigated variables are shown in Figures 3 and 4, respectively. As can be seen in Table 2, different amounts of lipase enzyme ($p<0.05$) and storage period ($p<0.01$) significantly affected the moisture content of the produced cheeses. The amount of moisture in all the samples containing lipase was higher than the control sample, and with the increase in the concentration of lipase, the amount of moisture in the samples increased. However, there were no differences in moisture contents between cheese samples containing goat lipase enzyme and cow lipase enzyme. The average moisture values of control cheese samples, and samples containing bovine lipase enzyme and goat enzyme were determined as 63.04, 65.59 and 66.37, respectively. The increase in moisture in the samples containing the enzyme can probably be due to the breakdown of the fat in the samples by the enzyme lipase. The lipase enzyme causes the hydrolysis of triglycerides and the release of fatty acids, and in this way, the released free fatty acids migrate around the product, especially its surface, and thereby prevent water escaping, because of the interpolar agent of milk fat and water maintain serum phase in the cheese curd [35]. In confirmation of these results, Jooyandeh and Hojjati [11] reported a significant increase in free fatty acids as a result of lipase enzyme treatment, especially of goat origin compared to cow lipase. Also, the reduction of cheese fat increases the proportion of cheese protein and thus, the cheese moisture increases as the water absorption capacity of the protein matrix upsurges.

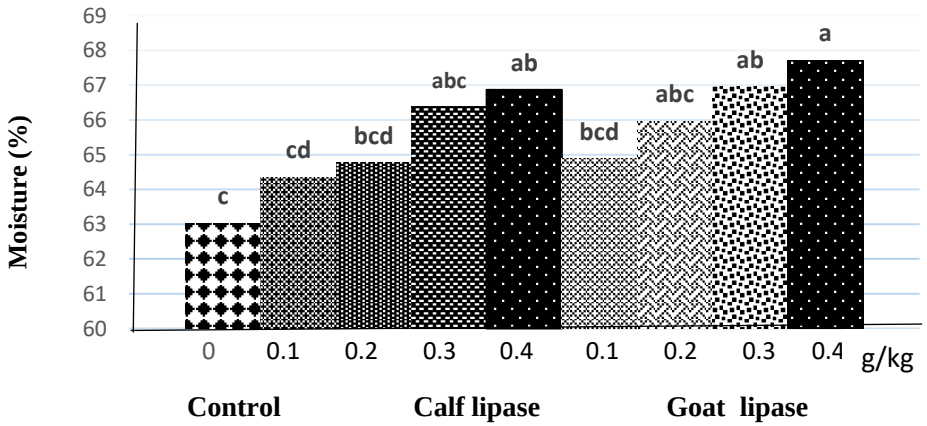

FIG. 3. Effect of lipase addition on moisture of ultrafiltrated cheeses during cold storage period.

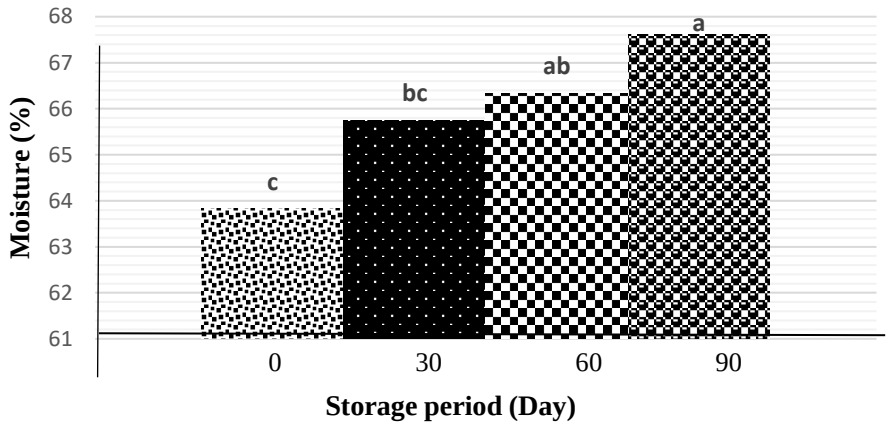

FIG. 4. Effect of storage period on moisture content of ultrafiltrated cheeses treated with animal lipases.

According to Table 2, there was a significant interaction effect between the two variables of lipase enzyme and storage time on the moisture content of UF Iranian white cheese samples ($p<0.05$). According to the results, the cheese sample containing 0.3% of goat lipase enzyme had the highest amount of moisture with 69.50% at the end of the storage period, and the control sample had the lowest moisture content at the beginning of the storage period with 60.63%. The existence of significant differences between the amount of moisture content of cheeses containing enzymes and those without enzymes is due to the difference in the amount of other cheese compounds, especially their protein. The high amount of protein in reduced-fat cheeses may be accompanied by an increase in water absorption in the protein network and, as a result, an increase in the amount of moisture in cheese samples [21]. The results of this study were consistent with the results of Solhi et al. [36], and contradicted the researches of Aydemir et al. [12] and Akin et al. [13].

3-2-3- Protein

As can be seen in table 2 and figures 5 and 6, lipase enzyme caused significant changes in the protein of the cheese samples during the storage period. The amount of protein in all samples containing lipase was higher than the control sample, and with the increase in lipase enzyme concentration, the amount of protein in the samples increased. The average protein values of control and cheese samples containing goat lipase enzyme and cow enzyme were determined as 15.57, 16.51 and 16.41%, respectively. When the amount of cheese fat is reduced due to lipolysis, protein plays a greater role in the structure and texture of cheese. These changes cause changes in sensory, functional, microbial and chemical characteristics of cheese [21]. Fat globules play an important role in the structure of cheese; therefore, with the reduction of fat globules, the cheese structure becomes more compact [37]. The results of this study were consistent with the results of Yazdanpanah et al. [38] and contrary to the results of Aydemir et al. [12].

According to Figure 6, the protein content in all UF-cheese samples during the storage period from day 3 to 60 increased significantly and then decreased until 90th day of storage. According to figure 6, the amount of protein varied from 15.57% (control sample) to 17.21% (treatment containing 0.4 grams of goat enzyme per kilogram of retentate). The decrease in protein during the storage period is probably due to the decomposition of cheese proteins and a small amount of them entering the brine cheese. During the cheese storage period, proteins are converted into compounds such as water-soluble nitrogen and amino acids, which leads to an increase in cheese nitrogen [38].

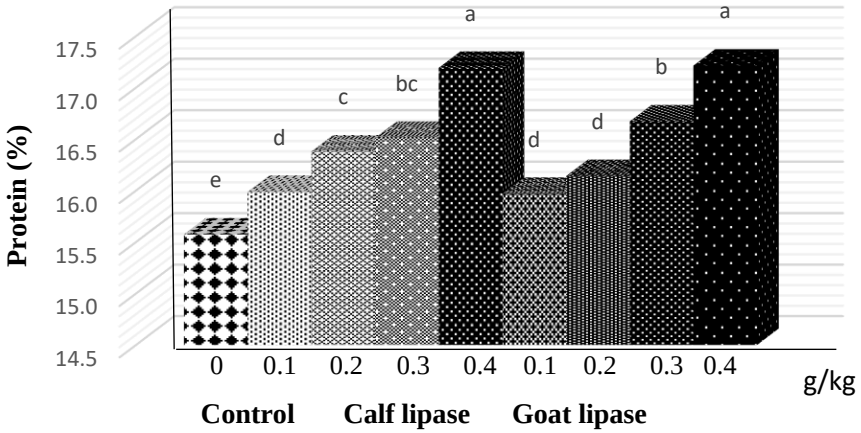

FIG. 5. Effect of lipase addition on protein content of ultrafiltrated cheeses during cold storage period.

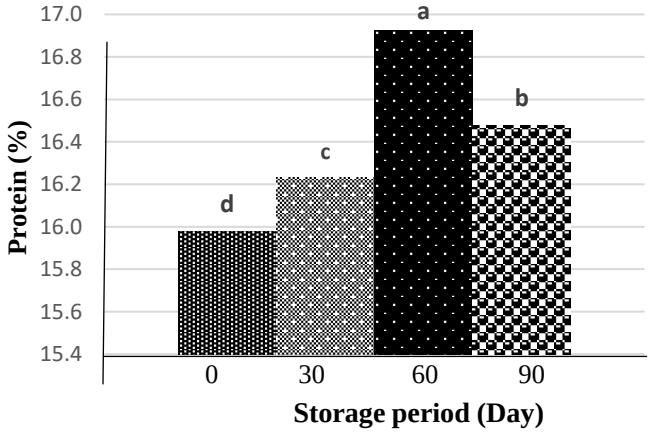

FIG. 6. Effect of storage period on protein content of ultrafiltrated cheeses treated with animal lipases.

3-2-4- Fat

The changes in the fat content of the UF-cheese samples containing different amounts of lipase enzyme and during the storage period are shown in Figures 7 and 8, respectively. As can be seen, both investigated variables significantly ($p<0.05$) affected the fat values of produced cheeses. The amount of fat in all samples containing lipase was lower than the control sample, and as the lipase concentration increased, the amount of fat in the samples decreased. Meanwhile, no difference was observed between the fat content of cheese samples containing bovine and goat lipase enzymes. The average fat values of the control cheese, and samples containing cow and goat lipase enzymes were determined as 13.385, 12.834 and 12.826%, respectively. The reduction of fat in enzyme-containing samples can be attributed to the intense lipolysis of fat in cheese samples.

According to Figure 8, the fat content in all the samples of the produced UF-cheese decreased significantly during the storage period from day 0 to day 90. The decrease in fat percentage during the storage period is due to lipolysis and fat breakdown, which subsequently increases the amount of free fatty acids. Jooyandeh and Hojjati, in the study of the effect of treatment of cheese milk with goat and cow lipases on brined white cheese, showed that among different cheese samples, the control sample (without lipase) with 132.91 g/100 kg of cheese had the lowest total free fatty acids and the sample treated with 6 grams of goat lipase enzyme (to per 100 kilos of milk) with 309.51 g per 100 Kg of cheese had the highest amount of total free fatty acids at the end of 90 days of cold storage [11].

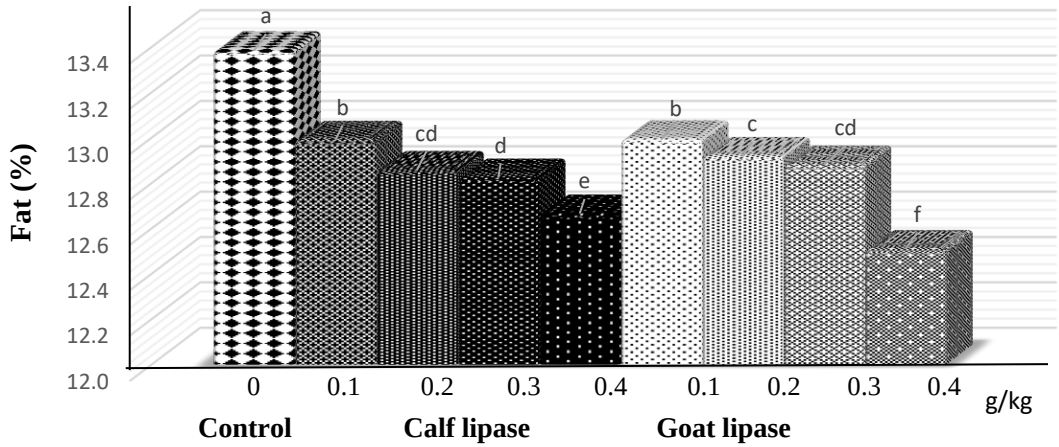

FIG. 7. Effect of lipase addition on fat content of ultrafiltrated cheeses during cold storage period.

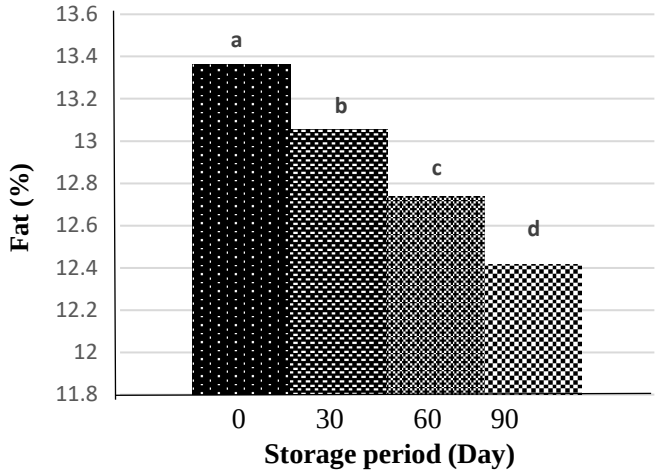

FIG. 8. Effect of storage period on fat content of ultrafiltrated cheeses treated with animal lipases.

According to Table 2, a significant interaction effect was observed between the two tested variables on the amount of cheese fat. Among UF-cheese samples, the cheese sample containing 0.4 grams of goat lipase enzyme had the lowest fat content at the end of the storage period with 11.42% fat, and the control sample had the highest fat content at the beginning of the storage period with 13.53% fat content. As mentioned, the reason for fat reduction over time can be attributed to the development of lipolysis and the production of short-chain and volatile fatty acids. In accordance with the results of this research, Jooyandeh et al. [39] and Nosrati et al. [40] reported the reduction of fat content in UF-cheese samples due to lipolysis during the storage period.

4- Conclusion

Today, various enzymes are used in the dairy industry, especially in the production of different cheeses. Among them, different types of hydrolases, especially protease and lipase, are the most important enzymes. These enzymes can play an important role in creating flavor and ripening of cheese. Although milk naturally contains different types of lipase, these enzymes do not have much thermal resistance and are easily deactivated during the pasteurization process. Therefore, in order to speed up the ripening time of cheese and to improve and increase the taste of cheese, lipase enzyme can be used and added to milk or fermented milk during cheese production. In this research, the effect of adding two types of goat and cow lipase enzymes on the color characteristics and some physicochemical properties of UF-white cheese during 90 days of storage was investigated. The results of this research showed that the treatment of lipase

enzyme has a significant effect on the examined characteristics and especially the use of high levels (0.4 grams of enzyme per 100 kilos of cheese) can improve the characteristics of the produced cheese. Although, in general, no significant differences were observed between the two enzymes of cow and goat lipases on the studied parameters of the cheeses, but the goat enzyme was better and caused more changes in the cheese than the cow type.

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مقاله علمی_پژوهشی

تأثیر تیمار آنزیمی لیپاز با منشاء گاوی و بزی بر ویژگی‌های رنگ و برخی خواص فیزیکوشیمیایی پنیر سفید فرآپالوده طی دوره نگهداری

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چکیده

فرآیند تولید و رسیدن پنیر با کمک مجموعه‌ی گسترده‌ای از واکنش‌های بیوشیمیایی مختلف و پیوسته صورت می‌پذیرد که در صورت متعادل بودن منجر به تولید فرآورده‌ای با ویژگی‌های کیفی بسیار مطلوب منجمله رنگ و خواص فیزیکوشیمیایی می‌شود. در این تحقیق، آنزیم لیپاز با دو منشاء بزغاله و گوساله در سطوح ۰/۱، ۰/۲، ۰/۳ و ۰/٤ گرم (به ازای ۱۰۰ کیلوگرم ناتراوه) در تولید پنیر سفید فرآپالوده ایرانی استفاده شد و تأثیر تیمار آنزیمی بر ویژگی‌های رنگ (روشنایی L^*، قرمزی/a^* و زردی/b^*)، و برخی خواص فیزیکوشیمیایی (اسیدیته، رطوبت، پروتئین و چربی) محصول بررسی گردید. نمونه پنیر فاقد آنزیم لیپاز به‌عنوان نمونه شاهد در نظر گرفته شد. پارامترهای رنگ و خواص فیزیکوشیمیایی پنیرهای تولیدی با نمونه شاهد طی مدت ۹۰ روز نگهداری مقایسه گردید. نتایج تجزیه واریانس نشان داد که آنزیم لیپاز سبب افزایش معنی‌دار شاخص L^* و b^* نمونه‌های پنیر شد، ولی بر شاخص a^* تأثیر معنی‌داری نداشت. زمان نگهداری نیز به‌شکل معنی‌داری سبب افزایش این پارامترها شد. همچنین تیمار آنزیمی لیپاز و زمان نگهداری سبب تغییرات معنی‌داری در خصوصیات شیمیایی مورد بررسی شد. به‌طور کلی، با افزایش غلظت آنزیم و گذشت زمان نگهداری، مقادیر اسیدیته، رطوبت و پروتئین افزایش و مقدار چربی کاهش یافت. نتایج این تحقیق نشان داد که در صورت استفاده از سطوح ۰/۳ یا ۰/٤ آنزیم لیپاز به‌ویژه لیپاز بزی می‌توان پنیر سفید فرآپالوده‌ای قابل قبول تولید نمود.