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Investigation of physicochemical and color characteristics of low-fat whey-less ultrafiltrated cheese and optimizing of production formulation

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ABSTRACT

Whey is a precious byproduct of the cheese industry, which leads to a decrease in production yield, environmental problems, and loss of high nutritional value proteins. Innovation in the cheese industry and the use of novel methods are particularly important in retaining the whey and producing whey-less cheese with high protein value. The current research aimed to produce low-fat whey-less cheese, evaluate its characteristics, and optimize the formulation of this valuable product. Whey-less cheese treatments were determined using a mixture design. Independent variables included cream (20-30%), milk protein concentrate powder (MPC: 5-15%), and whey protein concentrate powder (WPC: 10-20%). Responses included pH, syneresis, and color factors (L*, a*, and b*). According to the results, pH decreased with increasing the amount of cream, but it represented an increasing trend with increasing MPC and WPC percentages. The syneresis of the product significantly increased by increasing the amount of cream, but this factor significantly decreased by increasing the amount of WPC. All samples were at an acceptable level in terms of color factors (lightness, greenness, yellowness). The optimal formula of whey-less cheese was predicted as 22.60% cream, 9.40% MPC, and 13% WPC through Design Expert software. The response factors consisted of pH 4.65, syneresis 10.3 ml, L* 70.17, a* 2.62 and b* 18.72.

1- Introduction

The production of various types of cheese and their high nutritional value have made this product have a special place in the food basket of people around the world [1]. Whey is a by-product of the cheese-making industry, which reduces the yield of the production process, loses valuable cheese proteins, and imposes additional costs for wastewater treatment [2]. About 100 million tons of whey are produced annually in the world, and since most cheese factories do not have appropriate whey recycling systems, most of it is discharged into rivers, farmland, or sewage, which causes environmental pollution [3]. Given the production of significant amounts of whey during the production of traditional or ultrafiltrated cheese, today, producers have drawn attention to alternative methods such as the production of whey-free or whey-less cheese. In fact, the production of whey-less cheese increases the yield of the production process by 100 percent, increases the capacity of the production line, reduces milk consumption by up to 70 percent, reduces CO_2 emissions, and also produces a high-quality product [4]. To produce this type of cheese, using ingredients such as milk protein concentrate powder (MPC) or whey protein powder (WPC), the milk solids are increased to the required level and after the production of curd, like in ultrafiltrated cheese, no more whey is released from the curd. The use of whey protein concentrate improves the rheological properties of cheese on the one hand [5] and on the other hand creates an unusual taste in the product [6]. In addition, the use of milk protein concentrate in cheese formulation reduces the bitterness [7]. Therefore, by using them simultaneously, a product with better rheological properties and a more desirable taste can be produced.

Cheese is one of the most consumed products, and today, with the increasing awareness of the harmful effects of high fat consumption, the demand for low-fat and low-calorie versions has also increased [8, 9]. However, reducing the fat content in cheese leads to a significant decrease in its texture and sensory quality [10]. Accordingly, the production of a low-fat cheese without the aforementioned problems is of great importance.

One of the most common methods for producing low-fat cheeses with desirable textural and sensory properties is the use of fat substitutes. The use of these compounds improves the texture, increases functional properties, increases production efficiency, and provides desirable organoleptic properties of low-fat cheese [11].

Whey proteins have various functional properties, especially increasing the water retention capacity in foods, and in this regard, they can be used as a fat substitute in the production of dairy products [12]. In fact, one of the most important approaches in the production of low-fat cheeses to improve their properties is to increase the moisture content to such an extent that the moisture to protein ratio in low-fat cheeses is equal to or greater than that of the full-fat type [13]. The ability to form gels in WPC and WPI solutions under the influence of heat treatment at 70 to 90 °C is one of the most important functional properties of whey protein, which is an effective factor in improving the texture of dairy products and water retention capacity. The use of whey protein also increases the yield and nutritional value of cheese [14]. In addition to their excellent functional properties, whey proteins have a high biological value and are a rich source of the amino acids methionine and cysteine, which neutralize toxins in the body and are precursors of the most powerful antioxidants in the body [15]. For this reason, in recent decades, many efforts have been made to use whey in food products as much as possible, and by applying various separation and concentration processes, products such as WPC and whey protein isolate (WPI) have been produced [16].

So far, some different types of cheese have been produced using the method of whey-less cheese. Nazari [17] investigated the sensory and textural properties of feta cheese without whey. In this study, cheese produced without whey was introduced as an alternative to ultrafiltrated cheese. In this study, a mixture of cream and MPC and WPC powders was used instead of whey and the cheese production method was carried out in the same way as ultrafiltrated cheese. The results of this study showed that a product with desirable sensory properties can be produced by using a mixture of protein concentrates, and thus this method can well replace the conventional method of ultrafiltrated cheese production. Lashkari et al. [18] also managed to produce feta cheese without the need for dehydration by combining cream and WPC and MPC powders through a mixed experimental design. These researchers produced the cheese industrially and optimized its formulation as: 45.6% cream, 2.7% WPC, and 11.7% MPC with the help of a mixed experimental design. Mirhabibi et al. [19] also produced whey-free cream cheese using wheat fiber, skimmed milk, and sodium caseinate powder. The results of these researchers also showed that there was no difference between the whey-free cheese sample containing 4% sodium caseinate and the control sample. In another study, Gomah et al. [4] evaluated the production of soft white cheese using the whey-free method. In this study, different amounts of full-fat milk powder were added to milk to prepare cheese without whey, and finally, the cheese prepared containing 40% full-fat milk powder had the best results in terms of taste, cutability, texture, and overall product acceptance.

Although whey proteins have been used in the form of concentrates or isolates in the production of some types of cheese, especially traditional and ultrafiltrated white cheese, the use of these compounds along with milk protein concentrate in the production of whey-free cheese has been limited to some research conducted in this field, such as feta cheese. In the production of this type of cheese, unlike ultrafiltrated cheese, whey is not produced in this method due to the absence of use of membrane ultrafiltration, and therefore we will not face the problems resulting from the production of this by-product. Therefore, considering the importance of cheese consumption and its role in the nutrition of the Iranian people, this study was conducted to investigate the possibility of producing low-fat whey-free white cheese with a higher nutritional value than similar common cheeses on the market. In addition, this method will prevent the production of whey during the production of this product.

2- Materials and methods

2-1- Materials

To produce cheese samples, fresh milk, WPC powder, MPC powder, 30% fat cream, and mesophilic starters (CHOOZIT 230, containing a mixture of *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*) and thermophilic starters (Yo-Mix 532, containing *Streptococcus thermophilus* and *Lactobacillus delbrooki* subsp. *bulgaricus*) produced by Danisko, Germany were used. Also, for coagulation and cheese production, microbial rennet (rennilase) produced by Christian Hansen, Denmark was used.

2-2- Production of whey-less cheese

Whey-less cheese samples, were produced according to the method of Lashkari et al. [18] with some modifications. Cream and WPC and MPC powders were added to fresh milk according to the Mixture experimental design and the resulting mixture was heated at 45°C for 60 minutes to hydrate milk proteins. After single-stage homogenization at a pressure of 70 bar, the mixture was pasteurized at 63.5°C for 30 minutes. After cooling to 45°C, the mixture was transferred to a cheesemaking vat and a mixture of mesophilic and thermophilic starter (1:1) was added at a rate of 10 units per 1000 liters of milk and calcium chloride at a rate of 0.2 grams per liter of milk and incubated at 35°C for 30 minutes. In the next step, salt (15 g per 1000 liters of milk) was added to the milk before the formation of curd. After adding rennet at a rate of 0.04 g per 1000 liters of milk, the curd was transferred to 200 g plastic containers and kept at 35 °C for 25 minutes to form curd. Finally, the cheese containers were covered with aluminum foil. After being incubated at 30 °C for 12 hours, the cheese containers were transferred to a refrigerator at 7 °C and kept at this temperature for 45 days.

2.3- pH measurement

The pH of the Whey-less cheese samples was measured using a Metrohm-780 pH meter made in Switzerland [20].

2-4- Syneresis

The syneresis of the produced samples was assessed by draining the water in the cheese containers into a graduated cylinder. The volume of the separated aqueous phase was recorded in milliliters [21].

2.5- Cheese color evaluation

The color characteristics of the cheese samples produced were evaluated using a Hunter Lab Manitoba (model 400-CR), made in Japan. The parameters L* indicating the color brightness (between 0 and 100), a* (a+ red, a- green) and b* (b+ yellow, b- blue) were measured between -120 and +120 [22].

2-6- Statistical analysis

In this study, the effect of adding cream (20-30%), MPC powder (5-15%), and WPC powder (10-20%) to fresh milk (55%) on the properties of whey-less cheese was investigated using the mixture model in Design Expert software. Considering the effect of the three variables under investigation, according to Table 1, 12 mixtures were produced and the samples were evaluated during a 45-day storage period, on days 1, 23, and 45.

Table 1. Treatment coding

Run	Cream	MPC	**WPC**
1	20	10	**15**
2	20	5	**20**

3	20	15	**10**
4	21.67	6.67	**16.67**
5	23.33	8.33	**13.33**
6	30	5	**10**
7	21.67	11.67	**11.67**
8	20	15	**10**
9	26.67	6.67	**11.67**
10	25	5	**15**
11	30	5	**10**
12	25	10	**10**

3-Results and Discussion

3-1- Evaluation of the texture of yogurt samples

Analysis of variance was performed to evaluate the significant effects of the independent process variables on each of the response variables. Different models were compared based on R^2 and predicted R^2. In this way, the model with the highest R^2 and predicted R^2 will have high predictive power and accuracy.

3-1- Investigating the pH of the manufactured product

The proposed models along with the correlation coefficients for each of the response variables under investigation are given in Table 2. As can be seen in this table, the linear model was found to be significant ($p<0.05$) for describing the pH response variable and was suggested as the most appropriate model (R2=0.80). In fact, the significance of the linear mixture indicates the significance of the effect of the mixture components (cream, MPC and WPC). This indicates the expected changes in the response variable Y in terms of a unit change in X, when all other factors are constant. Therefore, it is a measure of the degree of influence of the relevant component relative to other components of the model on the pH response variable, and the higher this coefficient, the greater the effect of the term on the response variable. The linear regression model estimates the pH index with the following equation:

$$pH= 0.09 A + 0.12 B + 0.12 C \quad \text{(Equation 1)}$$

Where the letters A, B and C are related to the percentage of cream, MPC and WPC in the formulation, respectively.

According to Equation 1, it is observed that the MPC and WPC factors, compared to the cream factor, have a great impact on increasing the pH response variable.

Table 2. Selected models by software

Traits	R^2	Predicted R^2	P- value	STD	**Proposed model**
L*	0.86	0.77	0.0001	1.11	**Linear**
a*	0.91	0.88	<0.0001	0.25	**Linear**
b*	0.74	0.52	0.08	0.47	**Quadratic**
pH	0.80	0.64	0.0008	0.06	**Linear**
Syneresis	0.97	0.87	0.0001	0.48	**Quadratic**

The simultaneous effect of the independent variables of cream, MPC and WPC on the pH response variable of the whey cheese formulation samples is shown in Figure 1. As can be seen, with increasing cream content, pH decreased. The highest pH was observed in formulations with lower cream content and higher MPC and WPC content. In a study conducted by Omrani Khiabanian et al. [23], different amounts of chickpea protein isolate were substituted for MPC to produce whey feta cheese. The results showed that with increasing chickpea protein isolate and decreasing MPC content, the pH value also decreased significantly. Petridis et al. [24] also reported in a study that the acidity of yogurt increased and consequently its pH decreased in samples containing higher amounts of buffalo milk. Also, Jooyandeh et al. [25] reported that with increasing buffalo milk content, the pH of the yogurt samples decreased significantly and attributed the reason to the higher dry matter content of buffalo milk and greater production of lactic acid by starter bacteria.

Considering that the pH of the produced whey-less cheese is optimal in the range of 4.4-6.7, the results show that the optimal pH value is achieved by reducing the cream percentage to 22-25% and increasing MPC to 6-10% and WPC to 12-15%.

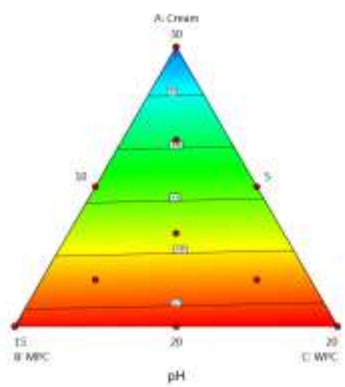

Figure 1. The pH of Whey less cheese with the addition of cream, MPC, and WPC

3-2- Syneresis of the product

According to the results obtained, the quadratic model for the response variable of waterlogging was significant ($p<0.05$) and with a correlation coefficient of 0.97, it was proposed as the most appropriate model to describe this response variable (Table 2). Also, according to the p value reported in Table 3, the model and effects of each of the formulation components including cream, MPC and WPC on the amount of waterlogging of whey-less cheese were significant. As mentioned earlier, the significance of the linear mixture indicates the significance of these three components. Among the interaction effects, the effect of cream-MPC was significant ($p<0.05$), which indicates the expected changes in the response variable Y in terms of unit change in X, when all other factors are constant. Therefore, it is a measure of the degree of influence of the relevant component relative to other components of the model on the response variable of flooding, and the higher this coefficient, the greater the effect of the term on the response variable. The quadratic model estimates the amount of flooding of the product with the following relationship:

$$\text{Syneresis} = 0.82\ A + 0.58\ B - 0.004\ C - 0.07\ AB \qquad \text{(Equation 2)}$$

Where the letters A, B and C are related to the percentage of cream, MPC and WPC in the formulation, respectively. Non-significant coefficients have also been removed from the equation.

In equation 2, as can be seen, the cream factor had the greatest effect on increasing the flood response variable, and the MPC factor had a lesser effect. Also, the cream-MPC interaction and the WPC factor showed the greatest effect on decreasing this response variable.

Table 3. Analysis of variance for effect of cream, MPC and WPC on L*, a*, b*, pH and syneresis of whey less cheese

Variable sources	Mean square					
	df	L*	a*	b*	pH	**Syneresis**
Model	2	34.79**	3.03**	0.74ns	0.05**	**9.55****
Linear mixture	2	34.79**	3.03**	0.10ns	0.05**	**21.57****
AB	-	-	-	0.48ns	-	**2.94***
AC	-	-	-	3.00**	-	**0.78ns**
BC	-	-	-	0.32ns	-	**0.80ns**
A2BC	-	-	-	-	-	**-**
AB2C	-	-	-	-	-	**-**
ABC2	-	-	-	-	-	**-**
ABC	-	-	-	-	-	**-**
Residual	9	1.22	0.06	0.22	0.003	**0.23**
Lack of fit	7	1.57	0.08	0.33	0.004	**0.35**
Error	2	0.00	0.00	0.00	0.00	**0.00**
CV (%)	-	1.56	10.18	2.47	1.20	**12.47**

***and ** means significant at 5 and 1% and ns means non-significant**

Figure 2 shows the simultaneous effect of the independent variables cream, MPC and WPC on the response variable of syneresis of the whey cheese formulation samples. According to Figure 2, it can be seen that the highest amount of syneresis of the product (1.8 ml) occurred when the amount of cream used was at its maximum (red area) and by reducing the amount of cream to 20%, the amount of syneresis also decreased to 1.8-15.2 ml. In fact, increasing the percentage of cream in the formulation significantly increased ($p<0.05$) the amount of syneresis of the whey cheese produced. Since, according to the results of this study, increasing the percentage of cream in the formulation caused a decrease in the pH of the produced samples, it is likely that the decrease in pH led to an increase in waterlogging in formulations containing high percentages of cream [25 and 26]. On the other hand, the lowest amount of syneresis occurred when the WPC content was at its maximum (20%) and in fact, with increasing WPC percentage, the product syneresis decreased significantly ($p<0.05$). This decrease may be due to the formation of a strong protein network that leads to reduced whey outflow. Other researchers have also reported that with increasing milk solids, the amount of cheese run-off decreases [27]. Omrani-Khiabanian et al. [23] also reported that increasing the amount of chickpea protein isolate and decreasing MPC increased the run-off of feta cheese, and they reported that the reason for this was the formation of a weak protein network between chickpea and cow's milk proteins, resulting in increased run-off of cheese.

Considering that syneresis is desirable in the range of 4-5 ml, the results show that the desirable syneresis value is achieved by reducing the cream percentage to 25-26% and increasing MPC to 5-6.5% and WPC to 12-13%.

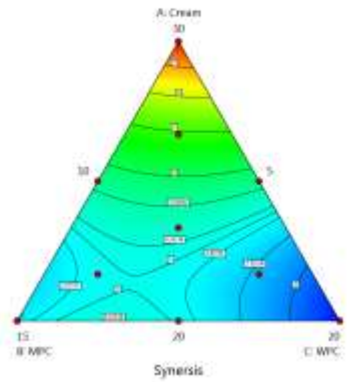

Figure 2. The Syneresis of Whey less cheese with the addition of cream, MPC, and WPC

3-3- Colorimetric factors of the produced product

In cheese production, as in other food products, the color of the final product is an important factor in its evaluation by the consumer. In the present study, all samples of whey-less cheese produced were at a good and acceptable level in terms of brightness, greenness and yellowness factors. According to the results seen in Table 2, the linear model for the factors L* (brightness) and a* (red-greenness) and the quadratic model for the factor b* (blue-yellow), were fitted with correlation coefficients of 0.86, 0.91 and 0.74, respectively.

According to the p value in Table 3, the model and the effects of the formulation components on the factors L* and a* are significant ($p<0.05$), which indicates the significance of the effect of the mixture components (cream, MPC and WPC) on the response variables L* and a*. As can be seen in Tables 2 and 3, the model and the effect of the formulation components on the b* factor were not significant ($p<0.05$) and only the cream-WPC interaction was significant ($p<0.05$). Therefore, it is a measure of the extent of the effect of the relevant term on the response variable b* compared to other terms in the model. As shown in Equation 5, the cream-WPC interaction has the greatest effect in reducing the b* factor.

The linear regression model estimates the color indices L*, a*, and b* with the following relationship:

$L^* = 1.89\ A + 1.49\ B + 1.04\ C$ (Equation 3)

$a^* = -0.04\ A + 0.10\ B + 0.20\ C$ (Equation 4)

$b^* = -0.07\ AC$ (Equation 5)

Where the letters A, B, and C are cream, MPC, and WPC, respectively. Only significant coefficients are included in the formula.

As shown in Figure 3-a, with increasing the amount of cream, the brightness of the produced whey-less cheese also increased significantly ($p<0.05$). Meanwhile, the amount of factor a* decreased significantly ($p<0.05$) with increasing the percentage of cream (Figure 3-b); however, according to Figure 3-c, although increasing the amount of cream increased the yellowness (b*) of the samples, this effect was not significant ($p<0.05$). With increasing the percentage of MPC, the yellowness (b*) decreased, although this decrease was not significant. In relation to the change in the percentage of WPC in the formulation, it was observed that with increasing this variable, the color factors L* and a* decreased and increased significantly ($p<0.05$), respectively (Figure 3); while this variable caused a non-significant increase in the factor b* ($p<0.05$).

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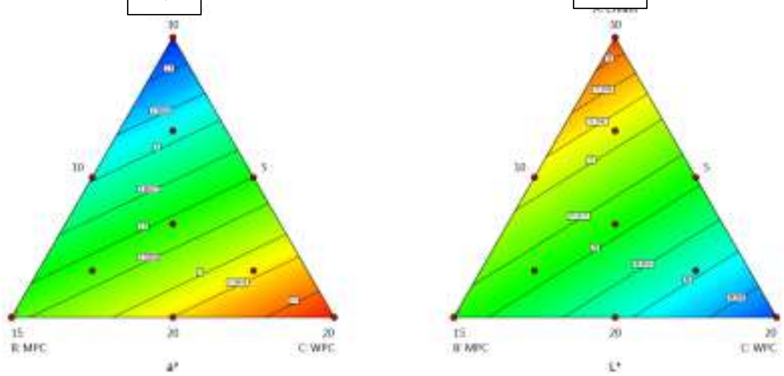

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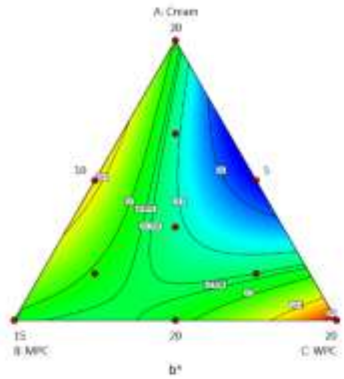

Figure 3. The L*, a*, and b* of Whey less cheese with the addition of cream, MPC, and WPC

The values obtained for the color factors examined were consistent with the results obtained for cheese by other researchers [22, 25 and 28]. Juan et al. [29] observed that the brightness factor of the produced cheese samples decreased with decreasing fat content. Nosrati et al. [30] evaluated the effects of different concentrations of demineralized ultrapure whey powder (DUWP) at levels of 0, 1 and 2% (W/V) and lactulose at levels of 0 and 1% (W/V) on the color characteristics of ultrapure synbiotic cheese as a functional food. The results of these researchers showed that DUWP powder significantly reduced the L* index of the samples ($p<0.001$), but this variable had no effect on the a* and b* indexes. Contrary to the present results, Danesh et al. [10] stated that the use of whey proteins in the formulation of traditional Iranian white cheese makes the cheese texture more porous and, due to the creation of more pores, the microstructure of the cheese resembles a honeycomb structure; a condition that certainly leads to increased light refraction and increased brightness of the samples. However, the reason for the lack of effect of DUWP powder on the brightness of the cheese in this study is probably the low level of whey proteins in DUWP powder. In addition, Danesh et al. [31] in another study investigated the effect of combining transglutaminase enzyme and whey proteins on Iranian ultrafiltrated cheese. These researchers also attributed the higher brightness of the samples in ultrafiltrated cheese to factors such as the increase in the number of serum pores and the creation of a porous texture in the cheese samples.

3.4- Optimization of the formulation of whey-less cheese

In order to achieve the optimal formulation of whey-less cheese using Design Expert software and the mixture design, the amount of cream in the formulation was set to its minimum, MPC to the range under study, and WPC to its maximum in the software. The pH response values were selected to be within the target range (4.65), syneresis to the target range (5), and colorimetric factors to be within the range obtained from the experiments. Finally, the software presented the best selected formulation with a desirability of 0.78 as shown in Figure 4. This point is shown in the peak area of the curve and is colored yellow.

The optimal formula for whey-less cheese was predicted using 60.22% cream, 40.9% MPC, and 13% WPC with a pH of 4.65, syneresis of 10.3, L* index of 17.70, a* of 2.62, and b* of 18.72 by Design Expert software.

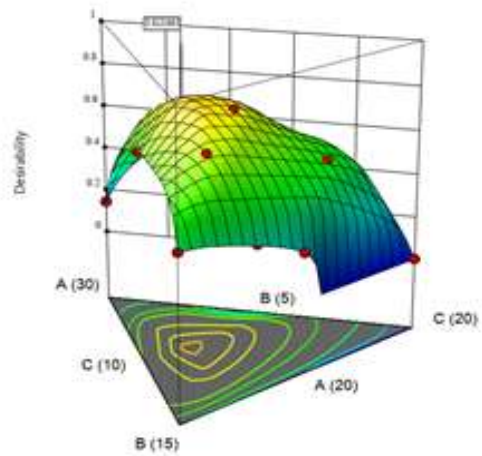

Figure 4. Contour plot of the optimization of whey less cheese formulation

4-Conclusion

In the present study, different levels of cream, MPC and WPC were evaluated for the production of whey-less cheese. The optimal whey-less cheese formula was obtained using 22.60% cream, 9.40% MPC and 13% WPC, indicating that by using milk protein concentrate and whey protein concentrate, a cheese with high protein value (suitable for athletes and patients) can be produced without producing whey and any undesirable changes in the characteristics of the product. In addition, the production of this product reduces the costs of processing cheese factory, wastewater and environmental pollution.

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Author Contributions

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Competing Interests

The author confirms that he / she has no financial conflicts of interest or competing interests in this study

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بررسی ویژگیهای فیزیکوشیمیایی و رنگ پنیر فرآپالودهٔ ویلس کم چرب و بهینه یابی فرمولاسیون تولید

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چکیده

آب پنیر محصول جانبی تولید پنیر است که منجر به کاهش بهره‌وری تولید، مشکلات زیست محیطی و همچنین از دست رفتن پروتئین‌های ارزشمند پنیر می‌شود. درواقع نوآوری در صنایع پنیرسازی و به‌کارگیری روش‌های نوین جهت حفظ آب پنیر در محصول نهایی و تولید پنیر وی‌لس با ارزش پروتئینی بالا از اهمیت ویژه‌ای برخوردار است. لذا تحقیق حاضر با هدف تولید پنیر وی‌لس کم‌چرب، ارزیابی ویژگی‌های آن و بهینه‌یابی فرمولاسیون تولید این محصول ارزشمند انجام شد. تیمارهای پنیر وی‌لس با استفاده از طرح آماری مخلوط تعیین شدند. متغیرهای مستقل شامل خامه در محدوده ۳۰-۲۰ درصد، پودر کنسانتره پروتئینی شیر (MPC) در محدوده ۱۵-۵ درصد و پودر کنسانتره پروتئینی آب پنیر (WPC) در محدوده ۲۰-۱۰ درصد بودند. متغیرهای پاسخ مورد بررسی نیز شامل pH، آب اندازی و فاکتورهای رنگی (*L، *a و *b) بودند. نتایج نشان داد که با افزایش میزان خامه، pH روند کاهشی داشته ولی با افزایش میزان MPC و WPC در فرمولاسیون، pH روند افزایشی نشان داد. آب اندازی محصول نیز با افزایش درصد خامه، افزایش معنی‌داری نشان داد ولی با افزایش درصد WPC کاهش معنی‌داری به همراه داشت. نمونه‌های پنیر وی‌لس تولید شده از نظر فاکتورهای رنگی (روشنایی، سبزی و زردی) در سطح مطلوبی بودند. فرمول بهینه پنیر وی‌لس با استفاده از ۲۲/۶۰ درصد خامه، ۹/۴۰ درصد MPC و ۱۳ درصد WPC با مقدار pH ۴/۶۵، آب اندازی ۳/۱۰ میلی‌لیتر، شاخص *L برابر با ۷۰/۱۷، *a برابر با ۲/۶۲ و *b برابر با ۱۸/۷۲ توسط نرم‌افزار دیزاین اکسپرت پیش‌بینی شد.