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Physicochemical, microbial, and textural properties of an imitation cheese based on rice milk, chia seed and hazelnut oil

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ABSTRACT

Imitation cheeses (IMC) attracted the attention of consumers due to their health benefits and cost-effectiveness. The purpose of this study was to formulate a new imitation cheese based on rice milk, chia seed and hazelnut oil. The imitation cheese was prepared using carboxymethyl cellulose and lecithin at three concentrations of 0, 1 % and 2% (v/w) which was added to the IMC samples contained rice milk, chia seeds and hazelnut oil. Then, physicochemical, microbial and textural characteristics and fatty acids profile of the cheese samples were evaluated. The addition of carboxymethyl cellulose and lecithin in the range of 0 to 2% increased the moisture content of samples (60.45 to 71.7%). The pH of the samples increased (4.41 to 4.57) with increasing the concentration of carboxymethyl cellulose and lecithin, while the peroxide value showed a decreasing trend (1.37 to 1.12 mLEq O_2/kg oil). Besides, during the storage time peroxide value showed an increasing trend. According to the total count results, the samples contained carboxymethyl cellulose and lecithin showed a dose-dependent inhibitory effect against microbial growth. The hardness, cohesiveness, springiness, gumminess of samples containing carboxymethyl cellulose and lecithin were higher than the control sample. The analysis of fatty acids profile in the selected sample of imitation cheese showed that it contained high amounts of unsaturated fatty acids. In general, the formulated imitation cheese with using carboxymethyl cellulose and lecithin showed proper microbial, physicochemical and textural properties and could be a suitable substitute for cheeses made from animal sources.

Contribution/Originality: The imitation cheese is produced for consumers suffer from phenyl-ketonuria and contain low levels of saturated fat and cholesterol. Also, their production process is simple, and milk components are replaced by inexpensive vegetable proteins which reduces the production costs. This paper evaluates and present a new vegetable-based formulation of Imitation cheese.

1. INTRODUCTION

Imitation cheese (IMC) is an alternative product for dairy cheese that is similar to common cheeses in terms of its structure, appearance and characteristics. In imitation cheese protein and fat milk are slightly or completely substituted with proteins from vegetable sources, such as proteins obtained from peanuts and soybeans, and fats and oils from vegetable sources, such as partially hydrogenated vegetable fat such as olives and sunflowers (Fox, Guinee, Cogan, & McSweeney, 2017). Imitation cheeses are formulated and prepared to achieve proper nutritional, textural, and organoleptic properties with favorable marketability and consumer requirements (Kamath, Basak, & Gokhale,

2022). These products are produced for consumers who suffer from phenyl-ketonuria and have a low protein content. The market for imitation cheeses has increased recently because their production process is simple, and milk components are replaced by inexpensive vegetable protein sources, a parameter that decreases product manufacturing costs (Sandrou & Arvanitoyannis, 2000; Zoidou, Andreadaki, Massouras, & Kaminarides, 2016). In addition, Imitation cheeses provide a new way to obtain products that contain low levels of saturated fat and cholesterol (Giha, Ordoñez, & Villamil, 2021). In recent years, low-fat products have gained popularity worldwide. Owing to the role of fat in the incidence of several chronic diseases, such as cancer and obesity, consumers prefer to use products with reduced fat content. On the other hand, a reduction in cheese fat adversely affects the sensory and textural properties of cheese and causes defects such as a lack of flavor, bitterness, a soft texture and an off-flavor (Soukoulis, Lyroni, & Tzia, 2010). Therefore, researchers have aimed to improve the textural and flavor attributes of low-fat imitation cheese (Liu, Xu, & Guo, 2008).

Table 1. Examples of research on imitation cheese formulations.

Product	Main constituent	Concentration	Ref.
Low-fat Domiati cheese	CMC	0–1 (%)	Abd Elhamid (2013)
Cheese	Modified starch	8–25 (%)	Ørskov, Christensen, Wiking, Hannibal, and Hammershøj (2021)
Tofu	Chia seed	1-2 (%)	Hsieh, Lin, and Kuo (2022)
Symbiotic cheddar cheese analog	Inulin	0-4 (%)	Islam et al. (2022)
Cheddar cheese analog	Canola oil	0-2 (%)	Leong et al. (2020)
Cheese	Maltodextrin	10-20 (%)	Aini, Prihananto, Sustriawan, Romadhon, and Ramadhan (2019)
Processed cheese analog	Lupine	25-100(%)	Awad, Salama, and Farahat (2014)
Tofu	Buffalo milk	10-50(%)	Mitra et al. (2013)
Processed cheese analog	Vegetable fat	0-50(%)	Cunha, Dias, and Viotto (2010)
Plant-based cheese analogs	Boiled peas and water	At ratio of 1:3, 1:3.5, 1:4, 1:4.5, and 1:5 (w/w)	Ferawati, Hefni, Östbring, and Witthöft (2021)
Probiotic soy-based product	MSP: Mixed product with milk cream and soy	-	Matias, Bedani, Castro, and Saad (2014)
Soy cream cheese	Tofu, palm oil	Hard tofu with 5% of palm oil (w/w)	Lim, Easa, Karim, Bhat, and Liong (2011)
Whey less feta Cheese	Milk protein concentrate and Soy protein isolate	Levels of 12:0, 10:2, 9:3, 8:4, 7:5, 6:6 w/v dry basis	Khiabanian, Motamedzadegan, Raisi, and Alimi (2022)

In recent decades, many articles have been published regarding the production of imitation cheese formulations (Table 1). Imitation cheeses have a softer texture than animal cheeses do because of the absence of casein in their structure) (Fox et al., 2017). As a result, the use of different hydrocolloids in the formulation of these products was investigated to help prepare a product with suitable textural properties. Carboxymethyl cellulose (CMC) has been used in different food and pharmaceutical formulations because of its thickening and swelling characteristics (Behra et al., 2019). In addition, lecithin (LEC) has been used as a food additive and is commercially applied as a suitable natural emulsifier in the food industry. Its food application reduces viscosity, increasing the stability of the final product (Orthoefer & Kim, 2019). Many studies have used different ingredients for the production of imitation cheese, and some properties of the produced cheese have been reported. However, there is little research on the use of CMCs and LECs in the formulation of imitation cheese in published articles. Golchin, Jafarian, Ghaboos, and Nasiraie (2023) studied the optimization of imitation cheese formulations containing hazelnut oil, rice milk and chia flour.

Additionally, Abd Elhamid (2013) prepared low-fat Domiati cheese using carboxyl methylcellulose and investigated its rheological, physicochemical and sensory attributes during the ripening period. On the basis of their results, the sensory attributes of cheese samples made with 1% (w/w) CMC yielded the highest scores. To the best of our knowledge, no studies have investigated the effects of adding carboxymethyl cellulose and lecithin on the physicochemical and textural properties of imitation cheese containing rice milk, chia flour or hazelnut oil. Therefore, the present study aimed to 1) produce imitation cheese from rice milk, chia flour and hazelnut oil via carboxymethyl cellulose and lecithin and 2) investigate the effects of CMC and LEC on the physicochemical, textural, and organoleptic properties of the product.

2. MATERIALS AND METHODS

2.1. Materials

Chia seeds and hazel nuts were purchased from a local market (Sabzevar city, Khorasan Razavi Province, Iran). These ingredients were dried in an oven until the moisture content reached 10%. Lactic acid, lecithin, and carboxymethyl cellulose (CMC) were obtained from Sigma–Aldrich (St. Luis, MO, USA), and hydrogen peroxide, sodium thiosulfate, acetic acid, chloroform, sodium methoxide, sulfuric acid, isoctane, sodium hydroxide and plate count agar were purchased from Merck (Germany). All reagents and chemical solutions were of analytical grade.

2.2. Preparation of Hazelnut Oil

First, the hazelnuts were cleaned, and all damaged seeds and other impurities (such as stems and skin) were removed. A cold press oil extraction system (Berkdaneh Company, Iran) without heat treatment was subsequently used for oil extraction. Next, the hazelnut oil was purified from solid impurities by sedimentation for 4 days, followed by filtration. The obtained oil was stored at 5°C in a colored bottle in a refrigerator until use (Al Juhaimi, Özcan, Ghafoor, Babiker, & Hussain, 2018).

2.3. Preparation of Rice Milk

For the extraction of rice milk, the method purposed by Al Tamimi (2016) was used with minor modifications. In the first step, rice samples (a variety of Hashemi) were prepared from a local market located in Rasht city, Gilan Province, Iran. To prepare the rice milk, 100 g rice samples were placed in 300 mL of 50°C water (rice-to-water ratio of 1:3) and kept at room temperature overnight. For 18 hours, the rice milk mixture was stirred several times with a mortar to separate the maximum amount of starch, protein, and structural fibers. In the next step, the mixture was passed through cheesecloth and then filtered through a Whatman No. 4 filter to remove all the shells and impurities. The mixture was then thoroughly mixed for 5 minutes via a laboratory mixer. The obtained rice milk was stored in a refrigerator at 5°C until use.

2.4. Formulation of Cheese with Carboxymethyl Cellulose and Lecithin

For the formulation of cheese, the method described by Awad et al. (2014) with minor changes was used. Additionally, the formula optimized in our previous work (Golchin et al., 2023) was used for the preparation of cheese samples (71.85% rice milk, 17.61% chia seed and 4.85% hazel nut oil). In the first stage, according to the formulation instructions for each sample, carboxymethyl cellulose and lecithin, rice milk, chia seeds, hazelnut oil and salt (1%) were carefully weighed and mixed thoroughly. In the next step, a homogenizer (Bosch, Germany) was used to homogenize the mixture completely for 20 minutes. The mixture was subsequently transferred to the oven at 45°C for 10 minutes to remove additional moisture. The cheese samples were then pasteurized at 80°C for 60 seconds. Finally, lactic acid was added to the mixture, and the cheese samples were subsequently stored at 5°C in a refrigerator until use (Awad et al., 2014).

2.5. Proximate Analysis

The Soxhlet method was used for determination of crude oil (Soxtec System-Texator, Sweden), and the protein content was measured via the Kjeldahl method (Kjeltex System-Texator, Hagonas, Sweden). Additionally, crude fiber and total calorimetry were determined in the cheese samples according to the AACC (2000).

2.6. Trace Metal Ions

2.6.1. Sample preparation

First the cheese samples were ground and kept in colored bottle for analysis. 2 g of cheese sample was mixed with concentrated HNO_3 (65% w/w) and heated at a temperature of 130°C for 30 minutes until sample dry completely. The sample was allowed to cool down to room temperature and then 10 mL of concentrated hydrogen peroxide (30%, w/w) was added to the sample and the mixture was heated again to dry again. The obtained sample was diluted to 50 ml with deionized water and filtered through a 0.45-µm pore cellulose membrane filter (Millipore) and filtrate diluted to 100 mL with deionized water. Finally, the samples were acidified using 1% concentrated HNO_3 and kept in polyethylene flasks in the dark condition at temperature 4°C.

2.6.2. Flame Atomic Absorption Spectrometer

To measure the trace metal ions, a PG-990 flame atomic absorption spectrometer (PG instrument Ltd., United Kingdom) was used. The assessments were carried out under the following conditions: spectral resolution of 0.2 nm, lamp current of 5.0 mA, λ = 232.0 nm, and flow rates of 7.0 and 1.2 L min^{-1} for air and acetylene, respectively. A Metrohm digital pH meter (model: 827) was used for pH control and adjustment (Tavakoli, Jamali, & Nezhadali, 2021).

2.7. Moisture Content

Small pieces of cheese samples (5 g) were placed in an oven at 105°C for 10 h for moisture content determination. The weight of each sample before and after drying in the oven was recorded, and the moisture content was subsequently the Equation 1 was used for calculation of moisture content of sample (Møller, Rattray, Bredie, Høier, & Ardö, 2013):

$$Moisture\ content = \frac{initial\ sample\ weight - sample\ dry\ weight}{initial\ sample\ weight} \times 100 \qquad (1)$$

2.8. Textural Analysis

A texture profile analyzer (Brookfield CT3 model) was used to study the textural changes due to the addition of carboxymethyl cellulose and lecithin to cheese samples. The texture profile analysis (TPA) test was used. The maximum force required as an indicator of hardness was considered. The target value, trigger load, and test speed were 10 mm, 5 g, and 1 mm/s, respectively. A TA10 probe was used for determination of hardness, cohesiveness, springiness, and gumminess parameters (AACC74-09).

2.9. Total Count

To determine the total bacterial count, the method of Boreczek et al. (2020) with minor modifications, was used. Initially, 10 grams of the cheese samples were mixed and homogenized with 90 milliliters of 0.85 sodium chloride solution under sterile conditions. In the next step, bacterial enumeration was performed via the pour plate method with 1 mL of each diluted sample. The number of total bacteria was measured via plate count agar medium at 37°C for 30 days. All counts are reported as log CFU[1]/g (Boreczek et al., 2020).

[1] Colony Forming Unit.

2.10. pH

A Metrohm (model: 827) digital pH meter was used for the pH measurement. All the analyses were performed at room temperature, and the pH values of the samples are reported as the means ± SDs of the four replications.

2.11. Peroxide Value

100 g cheese sample was mixed with 200 mL of n-hexane and stirred well and filtered through Whatman No.1 filter paper. The solvent was removed using rotary evaporator at temperature 50°C for 30 min to obtain the cheese fat for next analysis. 5 g of obtained fat was mixed with 30 ml of acetic acid-chloroform mixture (3:2 ratio) and then 0.5 ml of saturated potassium iodide solution was added and the mixture was kept in the dark for 1 minute. In the next step, 30 ml of distilled water was added, and the mixture was titrated against 0.1 N sodium thiosulfate in the presence of starch reagent until the blue color disappeared. The Equation 2 was used to calculate the peroxide value as follow (Mortensen, Sørensen, & Stapelfeldt, 2002):

$$P = 1000 \times N \times V/W \quad (2)$$

Where P is the peroxide value per kilogram of extracted oil (mLeq o_2/kg oil), V is the volume of thiosulfate used in the titration (ml), N is the normality of the sodium thiosulfate solution, and W represents the weight of fat (g).

2.12. Fatty Acid Profile

To measure the fatty acid profile of the oil, a flame ionization detector equipped with a Shimizu GC system (Shimadzu, Kyoto, Japan) was used. First, 50 mg of the sample was carefully weighed, and 2 cc of sodium methoxide was added. The mixture was shaken carefully, some boiling stones were applied to the mixture, and the samples were refluxed several times. In the next step, 2 mL of phenol and 1 mL of sulfuric acid were added, and the mixture was refluxed again for 5 minutes. Then, the solution was cooled with cold water and mixed properly with 4 ml of saturated sodium chloride. Next, the mixture was shaken vigorously for 15 s after the addition of 1 mL of isooctane. The container is held in a stationary state so that the mixture becomes two phases. Finally, saturated sodium chloride was added until the aqueous phase reached the neck of the decanter. The upper phase contained fatty acid methyl ester, which was injected into the GC system for fatty acid analysis. The gas chromatography-flame ionization detection system was as follows: a Rtx®-Wax GC Column with dimensions of 30 m, 25 mm, and 0.25 µm was used as the carrier gas at a flow rate of 1.26 ml/min. The initial temperature of the oven was 120°C, which was maintained for 3 minutes. Then, at a rate of 20°C/min, the temperature was increased to 220°C, and the temperature was maintained at this temperature for 330 minutes. The temperature of the injection chamber was set to 250°C, and the temperature of the detector was set to 300°C.

2.13. Statistical Analysis

To compare the physicochemical, textural and antimicrobial properties of the samples, SPSS software (SPSS 17.0 for Windows, SPSS Inc., Chicago, IL, USA) was used. The significant difference between different measurements was determined by one-way analysis of variance (ANOVA), and Duncan's multiple range test was used for comparisons between the mean values. All the measurements were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Moisture

The moisture content of cheese is among the most important physical characteristics of this product and contributes to its marketability and bacterial growth (Possas, Bonilla-Luque, & Valero, 2021). According to the obtained results, the lowest moisture content was observed in the control sample, and with increasing concentrations of carboxymethyl cellulose and lecithin, the moisture content tended to increase (Table 2). The reason for this can be that carboxymethyl cellulose can be linked with free water in the mixture by creating hydrogen bonds; therefore,

more water remains in the conformation of the cheese mixture (Masotti, Cattaneo, Stuknytė, & De Noni, 2018). These results are in agreement with the results of Abd Elhamid (2013) who reported that the moisture content increased with increasing repining period and CMC concentration. Additionally, the moisture content of the cheese samples decreased during the 30-day storage period, which may be related to the evaporation of moisture from the cheese surface during storage. Additionally, the samples enriched with 2% CMC and 2% lecithin presented the highest moisture content during 30 days of storage. The reason for this is related to the strong link between these compounds and water in cheese samples, which effectively prevents the transfer of moisture from the center to the surface of cheese samples (Abou-Soliman, Awad, & El-Sayed, 2020). Lecithin, as an emulsifier, interacts with hydrophobic (Oil molecules in cheese samples) and hydrophilic (Proteins and carbohydrates) molecules and creates cross-links, increasing the amount of water entrapped by the samples.

Table 2. Moisture content (%DW basis) of imitation cheese samples during the storage period.

Type	1th day	10th day	20th day	30th day
Control	60.43 ±0.03 c	60. 22±0.024 c	59. 87±0.016 c	59. 35±0.029 c
CMC 1 %	63.66±0.01 b	63.49±0.017 b	63.15±0.029 b	62.69±0.052 b
CMC 2 %	71.70±0.03 a	71.65±0.040 a	71.12±0.034 a	70.77±0.023 a
LEC 1 %	63.48±0.011 b	63.32±0.026 b	62.99±0.035 b	61.58±0.017 b
LEC 2 %	71.54±0.056 a	71.38±0.013 a	71.08±0.017 a	70.69±0.084 a

Note: The values are presented as the means with standard deviations (n=3). Different letters (a, b, c) in the same columns indicate significant differences between the data ($p < 0.05$).

3.2. pH

According to the results obtained from the pH changes in the samples (Figure 1), the concentrations of carboxymethyl cellulose and lecithin significantly affected the pH of the samples ($p<0.05$). An increase in the concentration of CMC (up to 2%) increased the pH value of the sample. The results revealed that in the control sample, the pH value was slightly lower than that in the cheese samples containing CMC and lecithin, and the pH decreased during the 30-day storage period. The reason for this can be related to the growth of bacteria or the breakdown of fat and the production of hydroperoxides (Tometri, Ahmady, Ariaii, & Soltani, 2020). The activity of microorganisms and breakdown of carbohydrates and sugars during storage also lead to the production of metabolites such as lactic acid, which reduces the pH during storage (Nwamba et al., 2021).

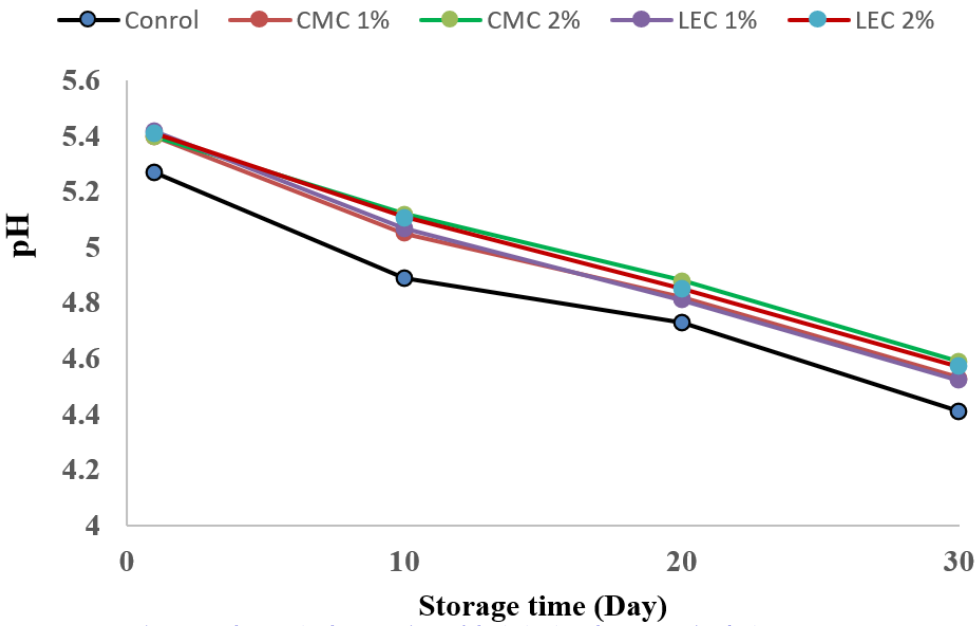

Figure 1. Changes in the pH values of the imitation cheese samples during storage.

High concentrations of CMC and lecithin resulted in the lowest pH decrease during storage, which may be due to their inhibitory effects on bacterial growth. The high ability of carboxymethyl cellulose to block moisture and reduce water activity, as well as the antimicrobial properties of lecithin due to the presence of fatty acids and diglycerides in its structure, may explain this finding (Mahmood, 2015; Ramadan & Selim Asker, 2009).

3.3. Peroxide Value

The peroxide index is a good indicator of the oxidation progress in oils, fats, and fat-based foods. The peroxides are created during the first stage of autooxidation, which further decompose into free radicals, aldehydes, ketones and formic and lactic acids (Arvanitoyannis, Varzakas, Kiokias, & Labropoulos, 2010). The oxidation process of triglycerides can be reduced by various compounds, which is correlated with the antioxidant characteristics of these compounds (Galus & Kadzińska, 2015). The peroxide values of the cheese samples decreased significantly with increasing concentrations of CMC and LEC (Figure 2). This reduction was dose dependent ($p<0.05$). Additionally, during the 30-day storage period, this factor tended to increase. In the control sample, the highest peroxide value was observed on the 30th day of storage. The mechanism of action is likely that the presence of carboxymethyl cellulose and lecithin in the cheese structure prevents the formation of hydroperoxide due to their decreasing effect on the autooxidation chain reaction. By creating bonds with fat molecules in the cheese matrix, lecithin does not allow these compounds to participate in chemical reactions such as autoxidation and therefore prevents the production of hydroperoxides (Benbettaïeb, Debeaufort, & Karbowiak, 2019). Additionally, different phenolic compounds present in hazelnut oil (such as ferulic acid, naringenin, hydroxybenzoic acid, vanillic acid, catechin, protocatechuic acid, luteolin and caffeic acid) create high antioxidant capacity in the cheese formula (Selli, Guclu, Sevindik, & Kelebek, 2022).

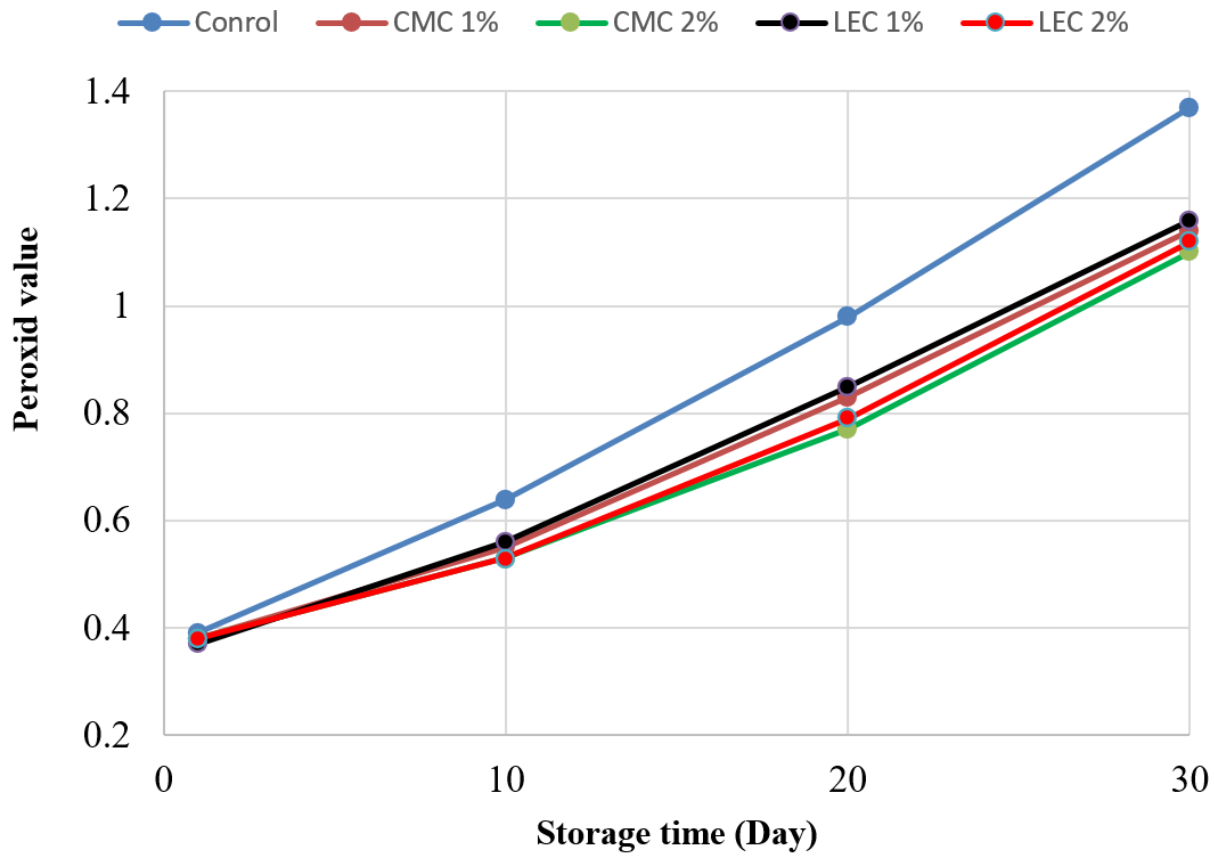

Figure 2. The peroxide values of the imitation cheese samples (mLEq. O_2/kg oil) during the storage period.

3.4. Total Count

The microbial counts for different cheese formulations are given in Table 3. The results showed that when carboxymethyl cellulose and lecithin were added to the cheese matrix, the microbial count of all the samples decreased significantly ($p<0.05$). The results indicated that the total microbial count in the control sample on the 30th day of storage was greater than that in the other samples. The antibacterial effect of carboxymethyl cellulose has been reported in many studies Benakatti et al. (2017) and Jiang et al. (2020). In addition, the inactivation of microorganisms in cheese samples can be related to the action of flavonoid and anthocyanin compounds present in chia seeds and the presence of metal ions in rice milk (Nikolić, Žilić, Simić, & Perić, 2020; Srivastava, Mall, & Mishra, 2008). The presence of high amounts of oleic, linoleic and linolenic acids in chia seeds and hazelnut oil is one of the main factors preventing the growth of microorganisms in cheese samples (Dilika, Bremner, & Meyer, 2000). The main role of carboxymethyl cellulose is to create high cross-link connections with the water in the 3D matrix to prevent the use of moisture by microorganisms, which can result in less water mobility in the cheese matrix.

Table 3. Total count of imitation cheese samples (log CFU/g) during the storage period.

Type	1th day	10th day	20th day	30th day
Control	1.67±0.025 [a]	3.45±0.023 [a]	4.89±0.064 [a]	6.75±0.081 [a]
CMC 1 %	1.61±0.015 [b]	3.21±0.020 [b]	4.37±0.029 [b]	6.22±0.036 [b]
CMC 2 %	1.60±0.029[b]	3.07±0.040 [c]	4.11±0.033 [c]	6.07±0.040 [c]
LEC 1 %	1.61±0.035 [b]	3.19±0.031 [b]	4.34±0.063 [b]	6.25±0.018 [b]
LEC 2 %	1.60±0.019[b]	3.02±0.052 [c]	4.13±0.045 [c]	5.10±0.033 [d]

Note: The values are presented as the means with standard deviations (n=3). Different letters (a, b, c, ..) in the same columns indicate significant differences between the data ($p < 0.05$).

3.5. Textural Analysis

The results revealed that the control sample had very low hardness, springiness and gumminess, but with the addition of CMC and lecithin, the textural indicators of the cheese improved (Table 4). According to the results obtained from the textural evaluation of cheese samples, CMC and LEC increased the hardness of cheese samples. The reason for this is related to the increase in the number of hydrogen bonds in the matrix of cheeses (Mattice & Marangoni, 2020).

Table 4. Textural properties of different imitation cheese samples during storage.

Type	Springiness (mm)	Gumminess	Cohesiveness	Hardness (N)
Control	2.38±0.021 [b]	8.46±0.044 [b]	4.96±0.07[b]	11.64±0.052[b]
CMC 2 %	6.75±0.024 [a]	11.70±0.059 [a]	8.09±0.042 [a]	22.15±0.041 [a]
LEC 2 %	6.59±0.072 [a]	11.34±0.068[a]	7.96±0.013 [a]	21.86±0.057 [a]

Note: The values are presented as the means with standard deviations (n=3). Different letters (a, b) in the same columns indicate significant differences between the data ($p < 0.05$).

The same trend was observed for cohesiveness, gumminess and springiness. Lecithin, as an emulsifier, contains hydrophilic and hydrophobic side groups and is able to bond with matrix complexes, including proteins, carbohydrates, and oils, and improve the textural indicators of cheese, such as gumminess. On the other hand, carboxymethyl cellulose, with its ability to connect with water and matrix ingredients (protein and carbohydrate), can strengthen the tissue, thereby improving hardness and springiness (Dimitreli & Thomareis, 2009). Gampala and Brennan (2008) reported that the hardness of the final product increased when 1 or 2% starch was added to the processed cheese formula.

3.6. Chemical Composition and Trace Metal Ions

The chemical compositions and mineral contents of the rice milk, chia seeds and cheese samples are presented in Table 5. Compared with the chia seeds and cheese samples, the rice milk samples presented the lowest amounts of

protein, carbohydrates and total fiber. The amounts of dietary fiber in the rice milk, chia seeds, and cheese samples were 0.74, 32.59, and 5.64, respectively.

Table 5. Chemical composition and mineral content (mg/100) of the rice milk, chia seeds and imitation cheese samples.

Composition	Rice milk	Chia seed	Hazelnut oil	IM cheese
Dietary fiber (g/100)	0.74 ± 0.02^{c}	32.59 ± 0.26 ^{a}	-	5.64 ± 0.13^{b}
Protein (%)	2.4 ± 0.07^{c}	16.58 ± 0.31^{a}	-	5.37± 0.11 ^{b}
carbohydrate (g/100)	18.40 ± 0.34^{c}	45.71± 1.15 ^{a}	-	21.58± 0.98 ^{b}
Energy (kcal)	56.72± 1.6^{d}	473.82 ± 11.3 ^{c}	820.34 ± 14.8 ^{a}	176.69 ± 6.7 ^{b}
fat (%)	0.60 ± 0.24^{d}	30.98 ± 1.12 ^{b}	92.35 ± 1.87 ^{a}	14.34 ± 0.89 ^{c}
Sodium	39.23± 0.84 ^{d}	16.57± 0.73 ^{c}	-	30.82± 1.24^{b}
Potassium	65.48± 1.13 ^{c}	407.22± 2.35 ^{a}	-	121.56 ± 0.98^{b}
Calcium	118.55± 1.07 ^{c}	631.17 ± 2.37^{a}	-	199.04 ± 1.15^{b}
Iron	0.35± 0.03 ^{a}	7.72 ± 0.43^{a}	-	1.54± 0.18 ^{b}
Magnesium	11.46 ± 0.74^{c}	335.11± 2.12 ^{a}	-	67.54 ± 1.14^{b}
Manganese	0.27± 0.02^{c}	2.77 ± 0.04^{a}	-	0.84 ± 0.035^{b}
Zn	0.19 ± 0.01^{c}	4.67 ± 0.35^{a}	-	1.75± 0.13 ^{b}

Note: The values are presented as the means with standard deviations (n=3). Different letters (a, b, c, ..) in the same row indicate significant differences between the data ($p < 0.05$).

Additionally, the total carbohydrate contents in the rice milk, chia seeds and cheese samples were reported to be 18.40, 45.71 and 21.85, respectively. The highest amount of fat and total energy were observed in hazelnut oil. Among the fatty acids, oleic acid was present in the greatest amount in the cheese samples (Table 6). Additionally, many researchers have noted that oleic acid is the predominant fatty acid in hazelnut oil, chia seeds and rice milk (Kitta et al., 2005; Selli et al., 2022). Minerals should be considered compounds with pro-oxidant activity and favorable effects on health (OÈzdemir et al., 2001). Rice milk (39.23 mg/100) contained the highest amount of sodium, and the Na contents of cheese and chia seeds were 30.82 and 16.57 mg/100, respectively. The metal content of hazelnut oil was very low. The highest amount of potassium was detected in the chia seeds (407.22 mg/100), followed by the imitation cheese and rice milk samples. The highest amounts of calcium, iron, zinc, manganese and magnesium were reported in chia seeds. The results revealed the high nutritional value of cheese samples as rich sources of mineral elements.

3.7. Fatty Acid Profile

Foods based on edible oils are biological mixtures with different sources that consist of glycerol with fatty acid esters. The nutritional value of edible oils depends mainly on the content and characteristics of fatty acids, especially the unsaturated:saturated fatty acid (UFA:SFA) ratio. According to the fatty acid profile of the imitation cheese sample, different unsaturated fatty acids were present in the cheese sample (Table 6). Additionally, in this research, the UFA:SFA ratio was 28.77%.

Table 6. Fatty acid profile of the imitation cheese.

Fatty acid	Concentration (%)
C10:0	0.0093
C11:0	0.0103
C12:0	0.0123
C14:0	0.057
C16:0	0.999
C16:1	0.182
C17:0	0.0229
C17:1	0.0119
C18:0	2.04
C18:1	55.7
C18:2	14.8
C18:3	21.16
C20:0	0.050

The major unsaturated and saturated fatty acid in the cheese samples were oleic acid (18:1) and palmitic acid (16:0), respectively. Oleic acid (55.7%), linoleic acid (14.8%) and linolenic acid (21.16%) were the major fatty acids in the formulated imitation cheese sample. It was reported that the oleic acid (18:1 omega-6) accounts for more than 80% of the total fatty acids in hazelnut oil, while linoleic (18:2), palmitic (16:0) and stearic (18:1) acids accounts the lowest values (Selli et al., 2022). As previously reported by other researchers, the oleic acid and linoleic acid are the major fatty acid presented in the rice milk, chia seeds and hazelnut oil. The fatty acid profile of the cheese sample reported in the present work is in line with those reported by other researchers (OÈzdemir et al., 2001; Selli et al., 2022).

4. CONCLUSION

In recent years, nutritionists have emphasized the adverse effects of cheese consumption due to health implications such as coronary heart disease and anemia. Imitation cheese is produced to combat these adverse problems and opens a new door for the formulation of functional foods. The main challenge of imitation cheeses is the lack of proper firmness compared with cheeses produced from dairy sources, which is related to the presence of casein in the milk. On the basis of the results obtained in the present work, as the concentration of carboxymethyl cellulose and lecithin increased, the moisture content and pH of the sample increased. These two parameters decreased during the storage period. During the storage of the samples in the refrigerator, with increasing concentrations of carboxymethyl cellulose and lecithin, the peroxide value and total microbial load in the formulated cheese samples were lower than those in the control samples. With the addition of carboxymethyl cellulose and lecithin, the textural parameters of the cheese samples improved. The major fatty acids in the formulated cheese samples were oleic acid, linoleic acid and linolenic acid. According to the obtained results, the formulated IMC with the use of carboxymethyl cellulose and lecithin shows proper nutritional and textural characteristics with desirable mineral and fatty acid profiles. The oil content was highly unsaturated, and according to the nutritional properties of the formulated cheese, it is recommended as a suitable alternative for cheeses made with animal milk.

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