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**The effect of using different ratios of *Lactobacillus helveticus* and mesophilic starter on the volatile compounds and sensory properties of UF white cheese**

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ABSTRACT

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Ultrafiltration is a technique used for concentration of milk in order to produce cheese with more desirable physicochemical and nutritional properties. On the other hand, use of combined starter cultures for cheese production can lead to improve the sensory characteristics and overall acceptability of final product. The objective of the present study was to investigate the effect of using different combinations of *Lactobacillus helveticus* (*L. helveticus*) and mesophilic starter culture (*Lactobacillus lactis* ssp. *lactis* and *Lactobacillus lactis* ssp. *cremoris*) on the volatile compounds and sensory characteristics of ultrafiltered white cheese during ripening. Five ultrafiltered white cheeses were produced using mesophilic starter culture and *L. helveticus* at different ratios (100:0, 75:25, 50:50, 25:75, and 0:100) and kept in refrigerator ( $9 \pm 0.1^\circ\text{C}$ ) for 90 days. The related analysis was performed on 1, 30, 60 and 90 days of ripening. The results revealed that an increasing in *L. helveticus* ratio caused a significant increasing in the  $\text{CO}_2$ , ethanol, ethylene oxide and a ( $p < 0.05$ ). Regarding sensory significant decreasing in the acetone properties, lower scores of body and texture, and higher scores of odor and flavor were assigned to the cheeses produced using higher ratios of ( $p < 0.05$ ). In conclusion, the use of combinations of *L. helveticus* mesophilic starter culture and *L. helveticus* at specific ratios (75:25 and 25:75) led to improve the volatile compounds in the final product and production an ultrafiltered cheese with desirable sensory characteristics.

## 1-Introduction

White cheese is one of the most consumed dairy products produced in the world and is classified in the semi-hard cheese group. This product has a cutable texture, a mild acidic taste, and a yellowish white color, and various types of milk, including cow, sheep, goat, and buffalo milk, are used in its production [1 and 2]. ultra-refining<sup>1</sup>It is a mechanism that is used to standardize the cheese production process and produce a product with industrial safety and the same quality characteristics. In this method, the milk is condensed before the formation and processing of the clot, and the outflow of whey is prevented during cheese production. The use of ultra-refining technology in the cheese industry produces cheeses with high production efficiency due to the preservation of whey proteins in the curd and the increase in the ratio of protein and fat in the final product. However, due to the high concentration of whey proteins in the final product and their high water holding capacity, ultra-refined white cheeses have high moisture content. Also, due to the inhibitory effect of water cheese proteins on the coagulating enzyme activity, cheeses produced with ultra-refining technology have a slower ripening and weaker aroma compared to salt water cheeses [1 and 3].

The use of primers has significant effects on the aroma producing compounds and the sensory characteristics of cheese due to the production of acid during the fermentation process, participation in enzymatic proteolysis, conversion of amino acids and fatty acids into aroma producing compounds and preventing the activity of undesirable microorganisms due to the production of compounds. Such as bacteriocins, organic acids and hydrogen peroxide [4 and 5]. Common starters for cheese production are mainly lactic acid bacteria strains. Among the mentioned strains, *Lactobacillus heloticus*<sup>2</sup> It is a thermophilic lactic acid bacterium that plays an important role in the production of fermented dairy products and is mainly used in the production

of Italian and Swiss cheese [6]. This bacterium has a proteinase attached to the cell wall, which is released from whole cells without any leakage of intracellular enzymes [7]. In addition to extracellular proteinase, strains *Lactobacillus heloticus* They have peptidases and lipases that are generally intracellular. Some strains *Lactobacillus heloticus* They release intracellular enzymes through autolysis, which play a major role in flavor development as a result of the catabolic conversion of amino acids into aromatic compounds. Hence the effects *Lactobacillus heloticus* Many researchers are interested in improving the aroma, reducing bitterness and accelerating the proteolysis process, especially in cheese [8]. On the other hand, along with high acid resistance, *Lactobacillus heloticus* It is able to produce lactic acid in significant amounts as a result of consuming glucose and galactose. So compared to *Streptococcus thermophilus* And *Lactobacillus acidophilus* which are only capable of metabolizing glucose, *Lactobacillus heloticus* It has the ability to metabolize glucose and galactose and produce higher amounts of acid [9 and 10]. For these reasons, bacteria *Lactoyacilus heloticus* It has been used as an auxiliary starter in the production of Cheddar [11], Ross [12] and Edam [13] cheeses. In this regard, Sichramaz et al. (2022) the effects of using starter cultures *Streptococcus thermophilus* with and auxiliary cultivation *Lactobacillus heloticus* investigated the quality characteristics of fresh Kashar cheese of pasta filata type. According to the results obtained, *Lactobacillus heloticus* It affected the aroma producing compounds of fresh Kashar cheese and reduced the content of diacetyl. In addition, the amounts of acetaldehyde and other aromatic components in cheese produced by addn *Lactobacillus heloticus* It was higher compared to control cheeses. Also, with the increase of shelf life, the share of alcohols and hydrocarbons in the aroma generating compounds decreased and the share of ketones increased. In general, the results of the sensory test showed that adding *Lactobacillus heloticus* As an auxiliary starter, it increases the taste and smell scores in the tested cheese [14]. On the other hand, Kofia et al. (2018) three-way capacity *Lactobacillus heloticus* including two

1-Ultrafiltration

2-lactobacillus helveticus

native strains<sup>3</sup> (*Lactobacillus heloticus* 138 and 209) and a commercial strain (*Lactobacillus heloticus* 02B) were investigated in the production and development of aroma generating compounds in hard cheese extract. Profile of volatile compounds during incubation of extracts at temperature °C 37 were analyzed during 14 days. According to the obtained results, the escape patterns were dependent on the strains, which reflected their distinct enzyme system. *Lactobacillus heloticus* 209 and 02B produced a greater variety of compounds, whereas *Lactobacillus heloticus* 138 was the least productive strain. In extracts inoculated with *Lactobacillus heloticus* 209 and 02B aroma producing compounds mainly belonged to ketones, esters, alcohols, aldehydes and acids. At the same time, in extracts containing *Lactobacillus heloticus* 138 main aroma producing compounds belonged to the groups of aldehydes and ketones. According to the results of the aforementioned study, *Lactobacillus heloticus* 209 and 02B can be among the best cheese starters to improve and intensify the taste [15].

The starter used in the production of ultra-refined white cheese, a combination of two bacteria *Lactococcus lactis subspecies lactis* And *Lactococcus lactis subspecies Cremoris* is. In this research, the effect of combining the above primers with *Lactobacillus heloticus* In different ratios, the amounts of aroma generating compounds and the sensory characteristics of ultra-refined white cheese were investigated during the 90 days of ripening period.

## 2- Materials and methods

### 2-1 Materials used

Lion Cow raw materials and necessary equipment for cheese production were provided by Pegah pasteurized milk factory (Kerman, Iran). Homofermentative mesophilic starter (incl *Lactococcus lactis subspecies Lactis* And *Lactococcus lactis subspecies Cremoris*, DM-230) and *Lactobacillus heloticus* (LH-100) was obtained from Danisco (Danisco Deutschland GmbH, Niebüll, Germany). *Rhizomucor miehei* as a protease enzyme used to produce ultra-refined white cheeses (Framose 2200 TL Granualte, ≥2200 international milk clotting units/g) was provided by

DSM (DSM Food Specialties, Seclin, Cedex, France).

### 2-2 Method of producing cheese samples

All cheese samples used in this research were produced in Pegah pasteurized milk company (Kerman, Iran) in three consecutive weeks. The raw milk used after performing the necessary physicochemical tests and standardizing the fat (2.3%) was used to produce cheeses (ultra-refined white cheese). An ultra-refining system with a capacity of 5 tons per hour was used to produce retentite. A spiral membrane (Wound-type, SPIRA-CEL-Modules, MICRODYN-NADIR, GmbH, Germany) including 3 modules and a total area of 427 square meters was used in the ultra-refining process. Raw milk with standard fat, respectively, under pasteurization processes (°C 76, 15 seconds) and ultrapurified. Ultra-refining process at temperature °C 52 ± 1 and pressure 140 kPa and duration 900 seconds were performed with input pressure 520-540 kPa and output pressure 140-160 kPa.

At the end of the ultra-refining process, 1 kg of retentate was obtained from 1.5 kg of milk. After applying the heat process (°C 78, 1 minute) and homogenization (5 MPa), 25 kg of retentite obtained was divided into 5 equal parts and different proportions of mesophilic initiator and *Lactobacillus heloticus* As a culture starter (20 grams per 1000 kg of retentite) was added to retentite according to the strategy shown in Table No. 1. In the next step, retentite at temperature °35 C was kept until reaching a pH of 6.3-6.4, and then the protease enzyme *Rhizomucor miehei* It was added to retentite (30 grams per 1000 grams of retentite). The resulting mixture was quickly transferred into plastic containers (100 grams) and sent to the coagulation tunnel (°C 35, 20 minutes). After leaving the cheese samples from the coagulation tunnel, parchment paper was placed on the surface of the product and salt (3%) was added on it. In the final stage, containers containing cheese samples are covered with aluminum foil and kept at a temperature of 4.7-8.4 for 24 hours until the pH is reached. °C were 26 ± 1. Produced cheeses for cold storage °9 ± 1 C was transferred and the necessary analyzes were performed on the 1st,

30th, 60th and 90th days of the processing period [3].

Table1- Combination of starter treatments in terms of type and percentage of mixing of each culture

Type of treatment	Culture type and mixing percentage
A	100% Mesophilic starter+ 0% <i>lactobacillus helveticus</i>
B	75% Mesophilic starter+ 25% <i>lactobacillus helveticus</i>
C	50% Mesophilic starter+ 50% <i>lactobacillus helveticus</i>
D	25% Mesophilic starter+ 75% <i>lactobacillus helveticus</i>
AND	0% Mesophilic starter+ 100% <i>lactobacillus helveticus</i>

## 2-3 tests

### 2-3-1 Measurement of effective compounds in aroma

In order to extract effective compounds on the aroma of cheese, the method of Wang et al. (2021) was used with some modifications to optimize it. Based on this, 6 grams of each of the cheese samples were cut into small cubes (each side 3 cm) and placed inside a 40 ml vial, and then kept at room temperature for 30 minutes. °C 20 was placed. Then for 5 minutes enter the injection site of gas chromatography machine with temperature °C was 230 until the compounds were adsorbed, desorbed and entered the DB-wax capillary column (30m×250mm×0.5µm film) and HP-5 (30m×0.25mm×0.25µm film). Pure helium gas was selected at a speed of 1.2 ml/min. The initial temperature of the device's greenhouse °C was 35 for 5 minutes and then up °100 C, its temperature was increased at a rate of 5 °C/min and kept at the same temperature for 2 minutes. In the continuation of the temperature up to °180 C was increased at a rate of 6 °C/min and the holding time at this temperature was 2 min. Finally, the temperature up to °230 C was increased at a rate of 8°C/min and kept at this temperature for 2 minutes. The device was equipped with a mass selective detector that detected compounds from 30 to 350 m/z with an ionization voltage of 70 electron volts.

Volatile index compounds were selected from among the compounds with the highest percentage values and by SPSS version 20 software Statistical analysis was done to determine the effect of the type of starter in each treatment and the duration of shelf life of the superior cheese aroma and flavor compounds [16].

### 2-3-2 Sensory evaluation

Sensory evaluation of cheeses was done using an evaluation form on the 1st, 30th, 60th, and 90th days of the processing period by 9 female and male sensory evaluators in the age range of 24-40 years who are familiar with the product. For sensory evaluation of cheese samples, 0 to 5 points were considered for appearance and color, 0 to 5 points for texture, and 0 to 10 points for smell and taste. Also, the total points obtained for each cheese sample were included. To perform the test, half an hour before sensory evaluation, cheese samples with 3-digit random codes and in 100 gram containers were taken out of the refrigerator and given to the evaluators separately. Among the sensory evaluation of different samples, drinking water was provided to the evaluators for rinsing the mouth [17].

### 2-3-3 statistical analysis

Statistical analysis of the obtained data using analysis of variance in the software SPSS Version 20 done. A significant difference between the average data was also determined using Duncan's test at the 5% probability level. The tests were performed on the treatments produced on the 1st, 30th, 60th and 90th days of the treatment period.

## 3- Results

### 3-1 aroma generating compounds

The taste of cheese is caused by the microbial, enzymatic and chemical changes of the product during the ripening period. Decomposition of protein, fat, lactose and citrate during the ripening period creates a wide range of aroma generating compounds that affect the taste and smell of cheese. Proteolysis affects the taste of cheese by producing peptides and free amino acids. Large peptides do not directly contribute to cheese flavor, but can be hydrolyzed to shorter peptides that may be bitter [18]. Free amino acids are the end products of proteolysis. Also, fatty acids are important components of creating flavor in many types of cheeses, which originate from lipolysis and can also be obtained from ketones, esters and aldehydes by oxidation [19 and 20].

Based on the results of the chromatograms of the GC-MS device, the compounds identified during the 90-day treatment period along with their percentages are shown in Table 2. Based on the obtained results, the dominant compound present in all ultra-refined white cheese samples, including the control sample and the sample containing different proportions of bacteria *Lactobacillus heloticus*, carbon dioxide, ethanol, ethane and ethylene oxide.

#### 3-1-1 carbon dioxide

The pH of ultra-refined white cheese during storage is mainly in the range of 3.4-4.6 [21]. In this pH range, as well as in high salt concentrations, the metabolism of lactic acid bacteria is relatively low. Therefore, only the metabolism of lactose and galactose is considered and the carbon dioxide produced by other substrates such as lactic acid and amino acids is ignored. In a sample of cheese, lactose and galactose are metabolized through several pathways. The main one is Embden Meyerhoff<sup>4</sup> Is. If the yeasts do not reproduce and continue to produce biomass, pyruvic acid is completely converted to CO in the Krebs cycle.<sub>2</sub> and

H<sub>2</sub>O is oxidized and about 16 liters of CO<sub>2</sub> It is produced from 2 kg of cheese. Under anaerobic conditions (fermentation), sugar metabolism leads to the production of ethanol and about 10.5 liters of CO<sub>2</sub> It is possible [22].

The data results of this study indicated that with an increase in the proportion of bacteria *Lactobacillus heloticus* The percentage of carbon dioxide increased ( $p < 0.05$ ) so that the ultra-refined white cheese sample produced with 100% of the above bacteria had the highest percentage of carbon dioxide (87.30%). It has been shown in previous research that *Lactobacillus heloticus* Able to metabolize *to do* Lactose remaining in D.L – It is lactate, which can be converted to acetate and CO depending on the availability of oxygen<sub>2</sub> become [23]. Also with increasing ratio *Lactobacillus heloticus* The process of proteolysis is intensified during the ripening period, followed by the confinement of CO gas<sub>2</sub> In the matrix of cheese, as one of the metabolites of product fermentation, it increases due to the absence of pressing and dewatering steps from the cheese clot [1].

The effect of shelf life on the percentage of carbon dioxide gas was significant and with the continuation of the curing period in all samples, including the control sample and samples containing different ratios. *Lactobacillus heloticus* The percentage of carbon dioxide increased ( $p < 0.05$ ). So that the highest percentage of the above parameter was found on the 90th day of the treatment period. This can be caused by the metabolism of lactose in cheese by yeasts, which causes the release of carbon dioxide during storage [21].

#### 3-1-2 ethanol

The results of ethanol percentage indicated that the use of different proportions of bacteria *Lactobacillus heloticus* It caused a significant increase in the percentage of ethanol compared to the control sample *Lactobacillus heloticus* It had a direct relationship ( $p < 0.05$ ). Among the samples, sample E had the highest (4.36±0.17%) and sample A had the lowest ethanol percentage (1.03±0.11%) on the 90th day of the curing period. In this way, samples containing higher percentages *Lactobacillus heloticus* Due to having higher proteolytic activity, they had the highest level of ethanol. also *Lactobacillus heloticus* Lactose fermentation and alanine catabolism can lead to ethanol production [24].

4-Embden Meyerhof



According to the results of Table 2, the percentage of ethanol increased with the passage of time in all samples of produced cheese. In all the samples, there was a significant difference between the samples with the passage of time, and on the 90th day of the curing period, the highest percentage of ethanol was recorded in all the samples ( $p < 0.05$ ). Continuous degradation by different metabolic pathways during cheese ripening, especially the catabolism of aldehydes, can cause the formation of alcoholic compounds in different concentrations [24].

### 3-1-3 Stan

Ethane (from the category of diacetyl and generator of aromatic aromas) by enzymatic oxidation of free fatty acids and then decarboxylation  $\beta$ Ketoacids are produced. Ketones are intermediate compounds that can be converted into alcohol and play a role in creating aroma in cheeses such as Roquefort and Camembert [25]. Among the production samples, the control cheese had the highest percentage of cetane and with an increase in the ratio *Lactobacillus heloticus* Acetane percentage decreased significantly ( $p < 0.05$ ). So that the sample of cheese produced with 100% *Lactobacillus heloticus* On the 90th day of the treatment period, the lowest percentage of stannate ( $1.26 \pm 1.10\%$ ) was recorded. According to the research conducted by Kofia et al. (2018), cheese samples contain *Lactobacillus heloticus* They had similar and lower amounts of methyl ketones such as 2-propanone (acetone) compared to the control sample [15]. In this regard, Puganik et al. (2015), reported that the levels of methyl ketones detected in the aqueous medium of curd inoculated with *Lactobacillus heloticus* There was no significant difference with the control sample [26]. Also, Petersen et al. (2010) also reported no significant change of methyl ketones in semi-hard cheeses produced with *Lactobacillus heloticus* compared to the control sample. The reason for the existence of these compounds is the effect of the lipase/esterase activity of cheese

microorganisms on the fatty acids releasing acyl-lipid fatty acids, which are mostly catabolized by a  $\beta$ -oxidation pathway [27].

During the curing period, the amounts of stane in all ultra-refined white cheese samples gradually decreased and in all samples, both the control sample and the sample containing different percentages. *Lactobacillus heloticus* There was a significant difference between different days of treatment ( $p < 0.05$ ).

### 3-1-4 ethylene oxide

Only one aldehyde named ethylene oxide was detected in cheese samples. As can be seen in Table 2, there was a significant difference in the amount of ethylene oxide of the produced cheese samples during the ripening period ( $p < 0.05$ ). Adding different ratios *Lactobacillus heloticus* It caused a significant increase in the amount of ethylene oxide in ultra-refined white cheese ( $p < 0.05$ ). The highest and lowest amounts of ethylene oxide belonged to samples E and A, respectively. Lactic acid bacteria can produce acetaldehyde. Also, acetaldehyde has the ability to be metabolized into diacetyl and acetoin. It has been found that acetaldehyde obtained from glucose metabolism mainly originates from yogurt bacteria, while *Lactobacillus heloticus* It can synthesize acetaldehyde directly through threonine metabolism [14]. On the other hand, with the increase of the ripening period, the amount of ethylene oxide in all cheese samples produced increased texture and the highest amount of the mentioned compound was recorded in all the cheeses produced on the last day of the ripening period ( $p < 0.05$ ). Soltani et al. (2016) investigated the volatile compounds of ultra-refined white cheese and showed that acetaldehyde levels in all cheeses reached their highest level at the end of the ripening period. Lactate metabolism or ethanol oxidation can lead to the formation of acetaldehyde in cheese samples [24].

Table 2. Volatile compounds resulting from the ripening of various samples of ultrafiltered white cheese manufactured with different ratios of Mesophilic starter or *lactobacillus helveticus* and combinations during the ripening period

Sample	Carbon dioxide (%)			
	Day 1	Day 30	Day 60	Day 90
A	<sup>eD</sup> 46.52±0.82	<sup>eC</sup> ±1.01 59.90	<sup>eB</sup> 70.03±1.31	<sup>of A</sup> 75.50±1.24
B	<sup>dD</sup> 49.41±1.30	<sup>dC</sup> 63.08±1.33	<sup>dB</sup> 72.73±2.04	<sup>and</sup> 77.71±0.95
C	<sup>cD</sup> 51.50±1.13	<sup>cC</sup> 64.91±2.19	<sup>cB</sup> 75.26±1.62	<sup>that</sup> 80.56±1.44
D	<sup>bD</sup> 52.79±1.47	<sup>bC</sup> 66.25±1.88	<sup>bB</sup> 77.11±1.18	<sup>not</sup> 83.65±1.73
AND	<sup>aD</sup> 54.36±0.86	<sup>BC</sup> 70.21±1.40	<sup>aB</sup> 82.51±1.07	<sup>aA</sup> 87.30±1.57

Ethanol (%)

A	<sup>dD</sup> 1.03±0.11	<sup>cC</sup> 1.24±0.14	<sup>dB</sup> 2.01±0.10	<sup>of A</sup> 3.11±0.13
B	<sup>cD</sup> 1.10±0.14	<sup>cC</sup> 1.28±0.11	<sup>cB</sup> 2.12±0.15	<sup>and</sup> 3.32±0.19
C	<sup>cC</sup> 1.18±0.06	<sup>bcC</sup> 1.30±0.20	<sup>cB</sup> 2.20±0.12	<sup>that</sup> 3.54±0.25
D	<sup>bD</sup> 1.27±0.19	<sup>bC</sup> 1.46±0.09	<sup>bB</sup> 2.33±0.19	<sup>not</sup> 3.82±0.21
AND	<sup>aD</sup> 2.10±0.17	<sup>BC</sup> 2.51±0.15	<sup>aB</sup> 3.91±0.24	<sup>aA</sup> 4.36±0.17
Acetone (%)				
A	<sup>aA</sup> 3.04±0.16	<sup>aB</sup> 2.71±0.15	<sup>BC</sup> 2.50±0.22	<sup>aD</sup> 2.07±0.15
B	<sup>not</sup> 2.80±0.12	<sup>bB</sup> 2.42±0.18	<sup>bC</sup> 2.18±0.20	<sup>bD</sup> 1.89±0.10
C	<sup>that</sup> 2.57±0.19	<sup>cB</sup> 2.20±0.11	<sup>cC</sup> 1.96±0.17	<sup>cD</sup> 1.60±0.08
D	<sup>and</sup> 2.33±0.12	<sup>dB</sup> 2.01±0.07	<sup>dC</sup> 1.77±0.10	<sup>dD</sup> 1.41±0.04
AND	<sup>of A</sup> 2.15±0.09	<sup>eB</sup> 1.84±0.11	<sup>eC</sup> 1.58±0.13	<sup>eD</sup> 1.26±1.10
Ethylene oxide (%)				
A	<sup>eD</sup> 22.77±0.62	<sup>eC</sup> 26.42±0.30	<sup>eB</sup> 28.52±1.07	<sup>of A</sup> 31.44±1.60
B	<sup>dD</sup> 24.16±0.51	<sup>dC</sup> 28.87±0.90	<sup>dB</sup> 31.30±0.93	<sup>and</sup> 34.22±1.19
C	<sup>cD</sup> 26.05±0.47	<sup>cC</sup> 31.18±1.30	<sup>cB</sup> 34.87±1.36	<sup>that</sup> 36.70±0.85
D	<sup>bD</sup> 28.80±1.38	<sup>bC</sup> 33.56±1.41	<sup>bB</sup> 36.85±1.70	<sup>not</sup> 38.68±1.53
AND	<sup>aD</sup> 30.81±0.66	<sup>BC</sup> 35.95±1.44	<sup>aB</sup> 38.70±2.09	<sup>aA</sup> 42.12±1.88

The presence of different lowercase letters in a column indicates a significant difference between the means ( $p < 0.05$ )

The presence of different capital letters in a row indicates a significant difference between the means ( $p < 0.05$ )

### 3-2 sensory characteristics

Sensory properties of food products refer to all the characteristics of food that can be understood by the five properties. Sensory properties are one of the basic factors in the acceptance of products by the consumer and obtaining satisfaction from their consumption. These properties include appearance (color or effect), structure (texture) and taste and smell. Considering the importance of these properties, it seems necessary to investigate and recognize the factors affecting them in order to achieve optimal sensory properties [28]. The results of sensory evaluation of ultra-refined white cheese samples produced with different proportions of bacteria *Lactobacillus heloticus* and mesophyll stars are shown in Figure 1. to the aforementioned results In short, in the research in line with the present article [29] It is noted that it is discussed in detail here.

### 3-2-1 Appearance and color

The appearance of food is one of its sensory aspects, which includes shape, geometric properties and color. The color and appearance of food is the first parameter in their quality assessment [28]. According to the results presented in Figure 1, with increasing ratio *Lactobacillus heloticus* And the ripening period, appearance score and color decreased in the produced cheese samples, which was not significant ( $p < 0.05$ ). Krishna et al. (2020) Physicochemical and sensory characteristics of reduced-fat raw cheese produced with different ratios *Lactobacillus heloticus* were examined. According to the obtained results, add *Lactobacillus heloticus* At the level of 1 and 2%, it caused a decrease in the appearance and color score compared to the control sample, but this decrease in terms of

It was not statistically significant, which is in line with the results of the present study [30].

### 3-2-2 texture

Texture characteristics are important factors in the quality acceptance of the consumer and are described at the molecular level as the interactions of proteins, fat and water in the food matrix [28]. According to the results, with an increase in the proportion of bacteria *Lactobacillus heloticus*, The texture score of the produced cheeses decreased significantly ( $p < 0.05$ ). Using higher ratios of bacteria *Lactobacillus heloticus* Due to its proteolytic property leading to further decomposition of  $\alpha$ -S<sub>1</sub> Casein and the formation of a protein network with less compression and a softer texture are found in cheese [31]. Accordingly, cheeses produced with higher proportions of bacteria *Lactobacillus heloticus* (Samples D and E) had a softer texture and obtained lower scores. On the other hand, with the increase of the processing period, the texture score decreased in all the produced cheese samples, which was not significant according to the evaluators.

### 3-2-3 smell and taste

According to the results presented in Figure 1, the addition of different proportions of bacteria *Lactobacillus heloticus* In the production of ultra-refined white cheese, there was a significant increase in the smell and taste score compared to the sample, and this increase was directly related to the increase in the proportion of the above-mentioned bacteria ( $p < 0.05$ ). Sample E with  $(9.71 \pm 0.16)$  on the first day and sample A with  $(8.11 \pm 0.11)$  on the 90th day had the lowest smell and taste scores respectively ( $p < 0.05$ ). Probiotic bacteria such as *Lactobacillus heloticus* origin of proteolytic enzymes and It is lipolytic during the ripening period of cheese through participation in proteolysis and lipolysis, and they are effective in creating the smell and taste of ultra-refined white cheese. These enzymes produce large and medium peptides by hydrolyzing casein. Peptides may be broken down into small

peptides and free amino acids under the influence of proteolytic enzymes obtained from the microphora of initiator bacteria, non-lactic acid bacteria and probiotics, which is the most important factor in creating the smell and taste of cheese [32]. Also, fat lipolysis and the increase in the percentage of free fatty acids and subsequently their biochemical reactions lead to strengthening the aroma and taste of cheese samples [33]. The results of the present study are in line with the results obtained by Mahdavi-pour et al. regarding the positive effect of probiotic strains on the sensory characteristics of cheese, including aroma, smell, and taste [34].

In this context, it has been reported that the further breakdown of caseins and the production of aroma-increasing peptides lead to the development of smell and taste in Cheddar, Swiss and Pasta Filata cheeses produced using *Lactobacillus heloticus* It has been used as a primer [35 and 36]. During 90 days of curing period, smell and taste score of all cheese samples containing different ratios *Lactobacillus heloticus* And the control sample had a significant difference and a downward trend ( $p < 0.05$ ). The possible reason for this is the continued proteolytic activity of the starters and enzymes used to produce cheeses and the production of bitter peptides as a result of increasing the intensity of proteolysis during the ripening period [21].

### 3-2-4 General acceptance

According to the obtained results, the type and proportion of starter used had a significant effect on the overall acceptance scores of ultra-refined white cheeses. Ultra-refined white cheeses produced with ratios of 25% and 75% of bacteria *Lactobacillus heloticus* had higher overall acceptance scores compared to other cheeses during the curing period ( $p < 0.05$ ). On the other hand, with the increase of the processing period, the overall acceptance scores in all ultra-refined white cheeses produced decreased significantly and reached their lowest level on the last day of the processing period ( $p < 0.05$ ).



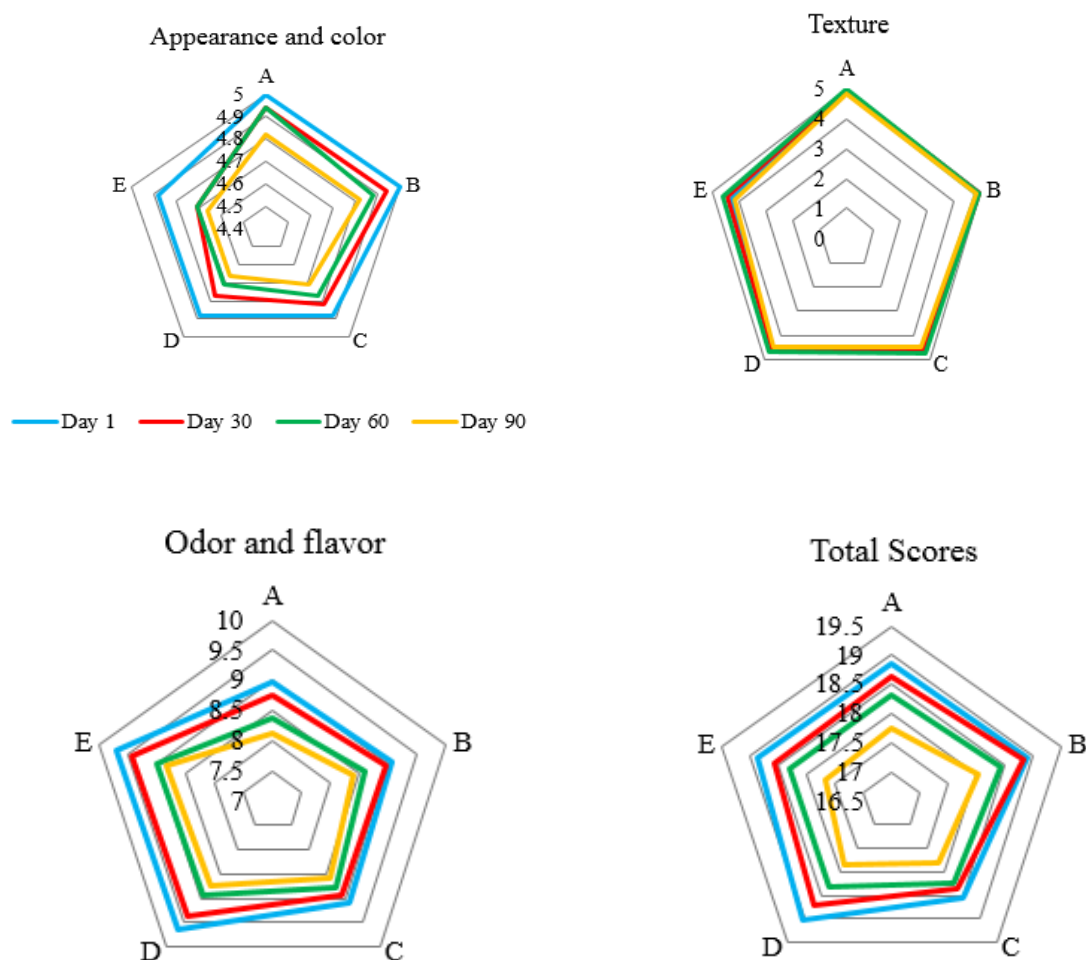


Figure 1. Sensory properties of ultrafiltered white cheese manufactured with different ratios of Mesophilic starter or *Lactobacillus helveticus* and combinations during the ripening period

#### 4- General conclusion

In this study, the mesophilic initiator and *Lactobacillus helveticus* It was used in 5 different ratios (0:100, 25:75, 50:50, 75:25 and 100:0) to produce ultra-refined white cheese, and the aroma producing compounds and sensory characteristics of the produced cheeses during 90 days of ripening period. evaluated. According to the results of the tests, the different ratios of the initiators used had significant effects on the volatile compounds and sensory characteristics of the produced cheeses. Due to higher proteolytic activity *Lactobacillus helveticus* Compared to the mesophilic starter, the cheese produced with higher proportions of mentioned bacteria had a higher percentage of carbon dioxide, ethanol and ethylene oxide, as well as a lower

percentage of stane compared to the control sample. Based on the sensory test results, although the cheeses produced with higher ratios *Lactobacillus helveticus* had more points in terms of smell and taste, the texture points in the mentioned cheeses were lower compared to other cheeses. Based on the overall acceptance results, cheeses produced with ratios of 25:75 and 75:25 of mesophilic starter and bacteria *Lactobacillus helveticus* They can be recommended for the production of ultra-refined white cheese on an industrial scale. The results of this research can provide a better understanding of the relationship between initiators

used to produce ultra-refined white cheese and help the quality characteristics of the final product. Also, the results of this study can be

## 5-Resources

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taken into consideration by producers in order to produce ultra-refined white cheese with appropriate texture and aroma.

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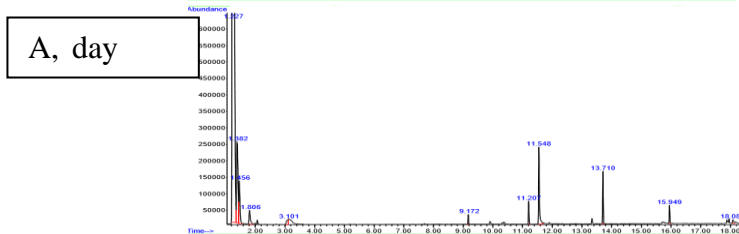
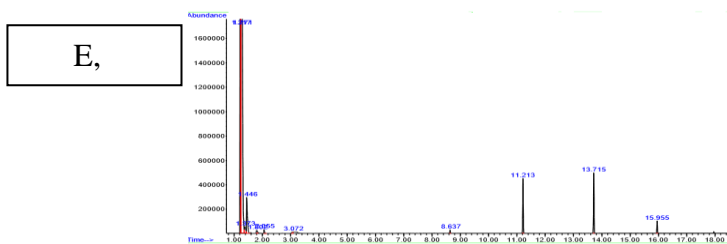
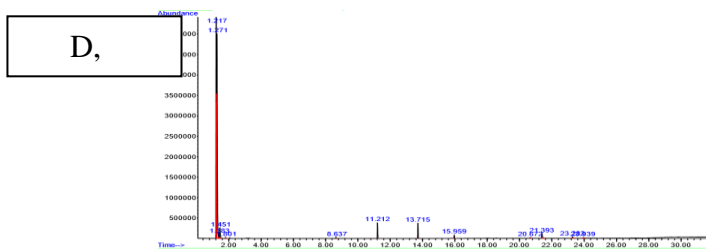
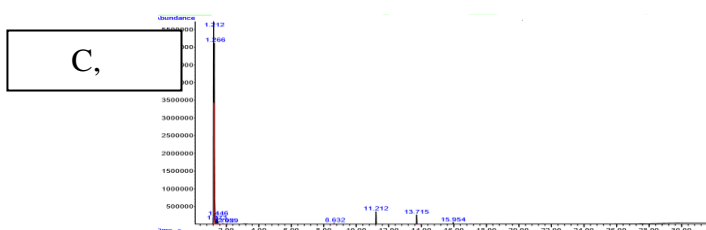
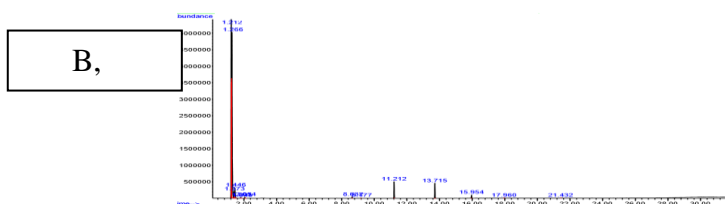
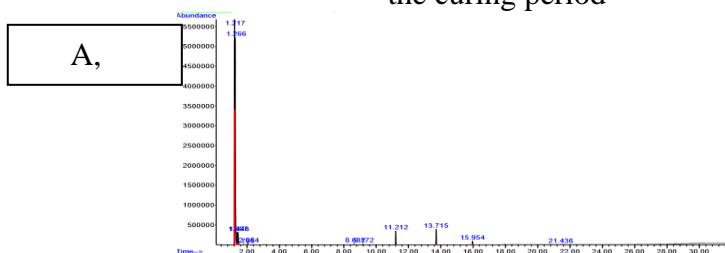
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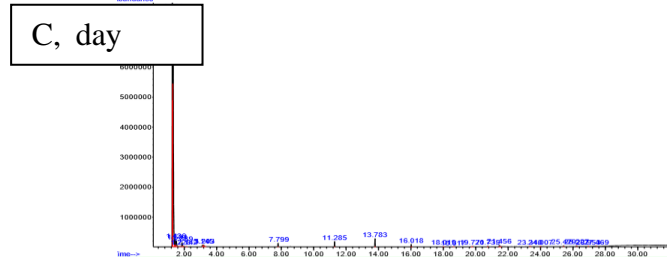
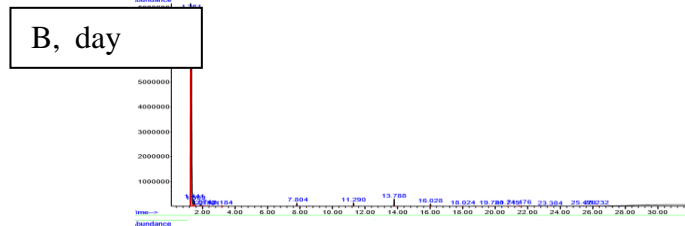
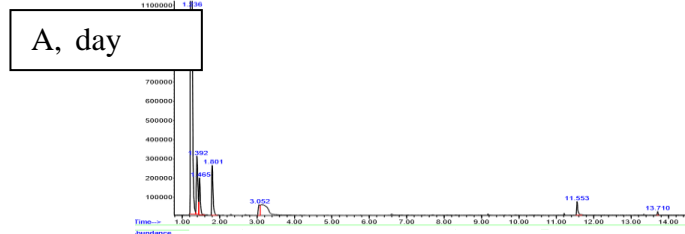
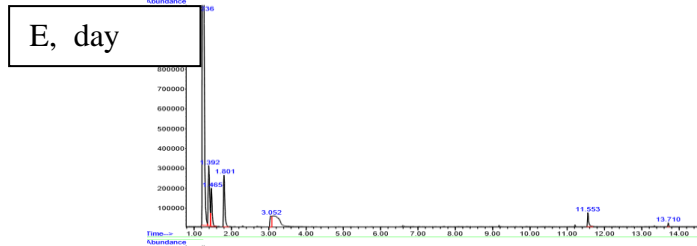
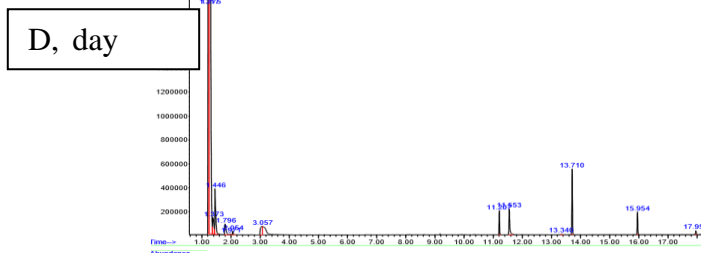
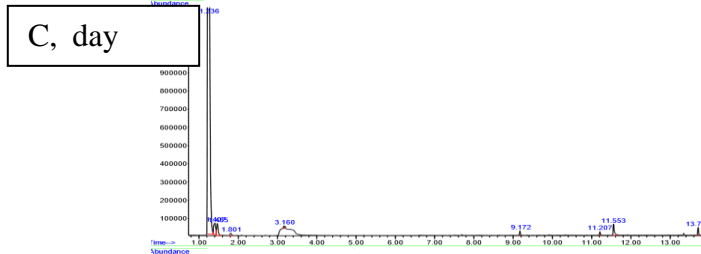
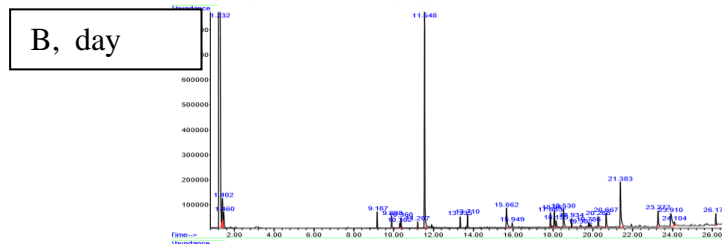
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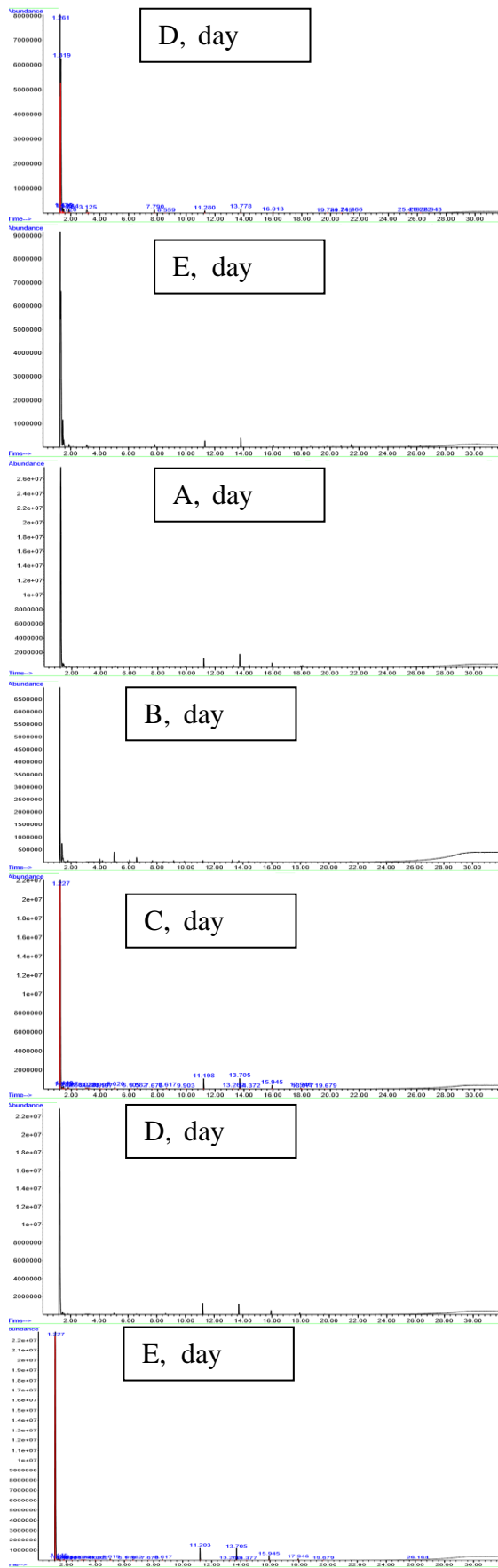
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Attachment: Chromatograms of volatile substances producing aroma and flavor in cheeses produced with different ratios *Lactobacillus heloticus* and mesophilic initiators during the curing period











تاثیر استفاده از نسبت‌های مختلف لاکتوباسیلوس هلویتیکوس و آغازگر مزوفیل بر ترکیبات مولد آروما و ویژگی‌های

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### چکیده

### اطلاعات مقاله

فراپالایش از تکنیک‌های مورد استفاده برای تغلیظ شیر به منظور تولید پنیر با ویژگی‌های فیزیکی شیمیایی و تغذیه‌ای مطلوب‌تر می‌باشد. از سوی دیگر استفاده از آغازگرهای ترکیبی در تولید پنیر می‌تواند منجر به ارتقاء ویژگی‌های حسی و مطلوبیت کلی در محصول نهایی گردد. هدف این پژوهش بررسی اثر استفاده از نسبت‌های مختلف باکتری لاکتوباسیلوس هلویتیکوس و آغازگرهای مزوفیل (لاکتوباسیلوس لاکتیس زیرگونه لاکتیس و لاکتوباسیلوس لاکتیس زیرگونه کرموریس) بر ترکیبات مولد آروما و ویژگی‌های حسی پنیر سفید فراپالایش در طول دوره رسیدگی بود. بر این اساس ۵ نمونه پنیر سفید فراپالایش با استفاده از نسبت‌های مختلف آغازگرهای مزوفیل و لاکتوباسیلوس هلویتیکوس (۰:۱۰۰، ۲۵:۷۵، ۵۰:۵۰، ۷۵:۲۵ و ۱۰۰:۰) تولید شده و در سردخانه ( $9 \pm 1^\circ\text{C}$ ) به مدت ۹۰ روز نگهداری شدند. آزمون‌های لازم بر روی نمونه‌های تولید شده در روزهای ۱، ۳۰، ۶۰ و ۹۰ از دوره رسیدگی صورت پذیرفت. طبق نتایج بدست آمده استفاده از نسبت‌های بالاتر لاکتوباسیلوس هلویتیکوس در تولید پنیر سفید فراپالایش سبب افزایش معنی‌دار مقادیر کربن دی اکسید، اتانول و اتیلن اکساید و کاهش معنی‌دار میزان استن گردید. در ارتباط با ویژگی‌های حسی، افزایش میزان لاکتوباسیلوس هلویتیکوس منجر به دریافت امتیازات پایین‌تر در پارامتر قوام و بافت و امتیازات بالاتر در مزه و بو شد ( $p < 0.05$ ). بطور کلی استفاده از آغازگر مزوفیل و لاکتوباسیلوس هلویتیکوس با نسبت‌های ۲۵:۷۵ و ۷۵:۲۵ منجر به ارتقاء ترکیبات مولد آروما در محصول نهایی و تولید پنیر سفید فراپالایش با ویژگی‌های حسی مطلوب شد.

### تاریخ‌های مقاله:

تاریخ دریافت: ۱۴۰۲/۱۲/۱۹

تاریخ پذیرش: ۱۴۰۳/۲/۲

### کلمات کلیدی:

پنیر سفید فراپالایش، لاکتوباسیلوس هلویتیکوس، آغازگرهای مزوفیل، ترکیبات مولد آروما، ویژگی‌های حسی.

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