



## Evaluation of physicochemical, rheological and microstructure properties of Kope cheese produced from bovine milk in different conditions of ripening

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### ABSTRACT

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In this research, physicochemical properties, texture and microstructure of traditional Kope cheese prepared in two different types of packaging (jar and plastic) and at two different ripening temperatures including soil (8-12 °C) and refrigerator (5-8 °C) During the ripening period (60 days) was examined by factorial experiment in a completely randomized design. The results showed that the amount of acidity, moisture, ratio of total nitrogen to dry matter and water-soluble nitrogen to total nitrogen in ripened cheeses in plastic packaging was significantly higher ( $P < 0.05$ ). Regardless of the type of packaging, samples ripened at soil temperature had higher acidity, total nitrogen to dry matter ratio, and water-soluble nitrogen to total nitrogen. Investigation of the rheological properties and microstructure of Kope cheese showed that the packaged treatments in jars at both soil and refrigerator temperatures have less moisture and therefore a firmer texture. While plastic-packed treatments at both soil and refrigerator temperatures have more and smaller pores and as a result have a spongy structure and softer texture compared to the other two treatments.

## 1. Introduction

Jug cheese, which is also known as Jug cheese or Pot cheese, is prepared in the western regions of Iran in a traditional way inside a clay jug or tanned sheep skin. Or they use metal instead of a clay jar due to its easier application [1, 2 and 3]. Paying attention to traditional cheeses has caused the producers to pay attention to new methods of packaging and storage conditions, and research has been done in this field, including the effect of packaging materials (jars and plastic) and The filling method on the characteristics of Atlo Turkish cheese by Taraki [4], the investigation of the ripening characteristics of Kulk Turkish cheese in wooden and plastic packaging by Dervisaglu and Yaziki [5], the effect of different packaging, culture Initiators and pasteurization on the chemical, textural and sensory characteristics of Tulum Turkish cheese by Bayar and Ozerk [6], the effect of pasteurization, type of packaging and storage temperatures on the physicochemical and sensory characteristics of jar cheese by Sarbazi et al. [7] and investigation of physicochemical characteristics of 30 samples of Kara jar cheese collected from the market was done by Kunar and Güler [8]. So far, many researchers have investigated the texture of cheese, including the studies that investigated the rheological behavior in Mozzarella analog cheeses by Nolan et al. [9], in Cheshire and Cheddar cheeses by Goine et al. [10] Halloumi cheese by Rafaeldes et al. [11] Milesi et al [12], and it was done in Kashar cheese by Taraksi and Kokoner [13], pointed out. So far, no study has been conducted on the rheological and microstructural characteristics of traditional Koze cheese. The type of packaging and the storage conditions of cheese during the ripening period have an important effect on the quality characteristics of cheese. In this research, the physicochemical characteristics, texture and microstructure of traditional jar cheese prepared in two different types of packaging (jar and plastic) and at two different ripening temperatures (in the soil and in the refrigerator) during the ripening period (three months) were investigated.

## 2- Materials and methods

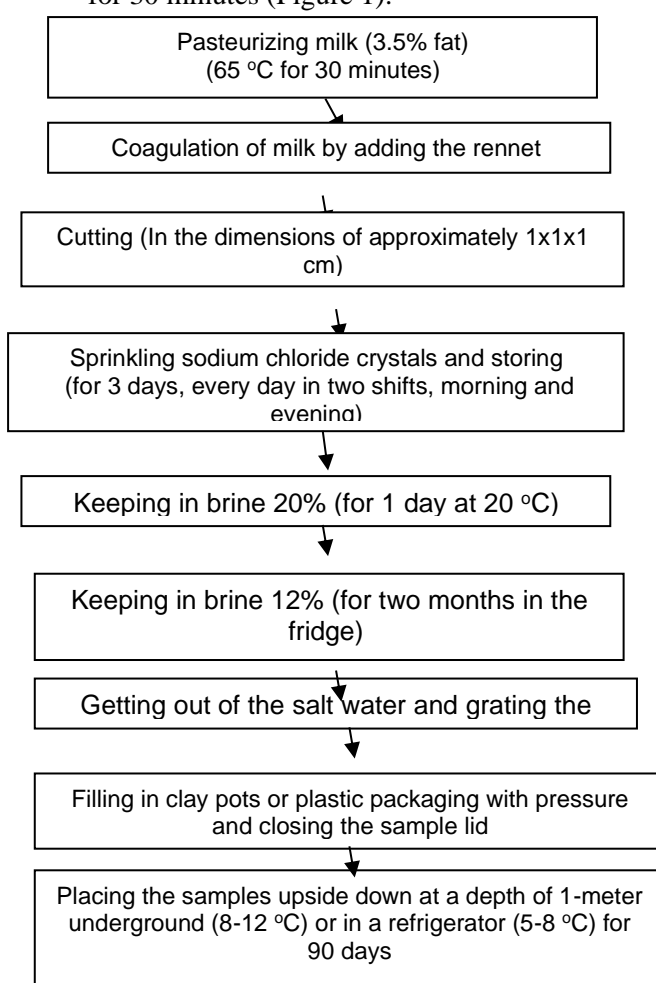
### 2-1- Materials

For cheese production, fresh cow's milk was obtained from Pegah Tehran company and

pasteurized at the factory. The starter was freeze-dried starter culture, which includes bacteria *Lactobacillus bulgaricus* And *Streptococcus thermophilus* is. Mushroom cheese sauce with the trade name Mito, made by the Sangiu company in Japan, and all the chemicals used in this research were produced by Merck, Germany, with analytical purity from reputable companies. Clay jars and polyethylene containers were procured from the market.

### 2-2- Preparing jar cheese

All the preparation steps of the jar cheese were done according to the mentioned sources [1, 2 and 3]. with the difference that cow's milk was used in the preparation of the tested cheese and a heat process of 65 degrees Celsius was used for 30 minutes (Figure 1).



**Fig 1.** Flow diagram of production of Kope cheese.

### 2-3-Physicochemical characteristics

The physicochemical characteristics that were evaluated 1, 30, 60 and 90 days after cheese production are: pH, acidity, dry matter, moisture, fat, total nitrogen (TN) and water soluble nitrogen (WSN). To measure the

humidity, the method of heating the cheese samples until reaching a constant weight was used using a model hygrometer (Sartorius Ltd., UK) and to measure titratable acidity, the AOAC method (1980) was used. was used [14]. Dry matter was measured by the Marshall method [15] and the pH of the samples was measured by a pH meter (Model Metrohm, 601). Fat measurement was done according to ISO 1735 standard (2012) [16]. To measure TN, Keldall's method [17] was used, and to measure WSN, Kuchero and Fox's method was used [18]. In this method, 20 grams of cheese were homogenized with 40 grams of water for 10 minutes at 20 degrees Celsius and then the suspension was kept at 40 degrees Celsius for one hour. Then the resulting suspension was centrifuged at 3000 x g for 30 minutes at 4 degrees Celsius. The centrifuged supernatant was passed through glass wool and its nitrogen was measured by Keldahl method. All the laboratory materials used in this research were manufactured by Merck, Germany and of analytical grade, and all experiments were performed in three replicates.

#### 4-2- Rheological tests

Rheological tests including strain sweep and frequency sweep were performed by a rheometer device (MC301, Anton Paar Country, Austria), the tests were performed at an angular frequency of 0.01-100 Hz and strain  $\approx 0.1$ , in which the structures formed in the system are preserved. remained, and the loss modulus ( $G''$ ), storage modulus ( $G'$ ) and loss tangent ( $\tan\delta$ ) were investigated [19].

#### 5-2- Measuring the microstructure of cheese samples

After preparing the luminizer, the cheese samples were dried under vacuum and gilded for 300 seconds in a device (Balzers, BalTec Inc., Type SCD 005, Switzerland), then examined by an electron microscope (XL30, Philips, Netherlands) under vacuum. , were bombarded with KV 15 electrons and photographed with a magnification of 2000, the photos were analyzed with Image J software [20].

#### 6-2- Statistical analysis

The experimental design used was a factorial type based on a randomized complete block design, in order to investigate the effect of each factor alone and their mutual effect using the analysis of variance table, SPSS statistical software, ver(17) was used. became. Then, the

averages were compared using Duncan's test at the level of probability ( $P<0.05$ ). All measurements were done in three repetitions and graphs were drawn with Microsoft Excel 2010 software.

### 3- Results and discussion

#### 3-1- Changes in the physicochemical properties of Koze cheese in different ripening conditions

The results of data variance analysis in table (1) showed that the effect of packaging type and time on all the measured indicators is significant ( $p<0.05$ ). Also, the effect of temperature on all indicators except acidity and water-soluble nitrogen index to total nitrogen (WSN/TN) is significant ( $p<0.05$ ). The double and triple interaction effects are also significant on the changes of all the measured indices except acidity level ( $p<0.05$ ).

According to the comparison of the least mean square of the triple interaction effect (Table 2), it can be concluded that the type of packaging and different storage conditions of cheese during the ripening period have a significant effect on the physicochemical characteristics of the jar cheese ( $05/ 0P<$ ). Similarly, the significant effect of the type of packaging and ripening period on the physicochemical properties of Telmeh cheese was also reported [21 and 22]. The pH of all treatments significantly decreases during the ripening period and their acidity increases ( $P<0.05$ ), which can be caused by the fermentation of lactose by the lactic flora of cheese and the production of lactic acid [ 23, 24, 25 and 26]. The trend of decreasing pH and increasing acidity during the ripening period is consistent with the results of other researchers' studies [23 and 27].

**Table 1** The results of the analysis of variance of the characteristics measured in Koze cheese in different treatments (least square mean)

Source of changes	pH	Acidity (Dornic degree)	Moisture content (%w/w)	Fat (%w/w)	Fat in dry matter (%w/w)	WSN/TN (%w/w)	$\beta$ -casein stain level (%)	$\alpha$ -casein stain level (%)
Temperature	0/04*	0/001	8/501*	0/98*	323/960*	0/002*	0/022*	0/312*
Time	0/04*	0/821*	21/864*	15/224*	199/215*	3/412*	1071/365*	3351/453*
Packaging type	0/335*	0/350*	43/510*	8/192*	217/260*	1/290*	0/044*	0/216*
Temperature $\times$ time	0/215*	0/037	0/939*	0/273*	304/166*	0/004*	6/399*	253/974*
Temperature $\times$ packaging	0/005*	0/008	1/027*	0/809*	255/210*	0/029*	0/020*	0/272*
Time $\times$ packaging	0/002*	0/030	4/974*	1/245*	269/695*	0/211*	104/557*	223/048*
Temperature $\times$ time $\times$ packaging	0/012*	0/025	1/407*	0/759*	328/657*	0/005*	75/239*	36/316*
Error	0/000	0/016	0/001	0/002	0/001	0/001	0/017	0/001

\* Significant at the 5% probability level

During the sampling that was done in each ripening period, the cheeses ripened in plastic packaging had a lower pH than the cheeses ripened in jars, and among them, the cheeses ripened in plastic containers that were stored in soil had the lowest pH. were pH, the cause of higher pH and, as a result, lower acidity of jar cheeses can be due to the semi-permeable membrane of the jar, which causes the exit of hydrogen ions along with cheese juice from its pores [28]. Regardless of the type of packaging, the samples ripened in soil temperature at the end of the ripening period had a lower pH than the samples ripened in refrigerator temperature, which could be due to the higher soil temperature than the refrigerator temperature. Researches showed that the higher the cheese storage temperature, the higher the lactose fermentation [29], and the cheese samples that were at room temperature had more acidity than the samples that were kept in the refrigerator. Also, research has shown that the temperature of the refrigerator reduces the activity of lactic acid bacteria, and as a result, it reduces the development of lactic acid in cheese [30 and 31]. As can be seen in table (2), the acidity of cheeses ripened in plastic containers is significantly higher than that of cheeses ripened in jars, which is due to the presence of pores in the walls of the jars, as mentioned before. Clay and the release of hydrogen ions are accompanied by cheese juice, which causes the acidity of ripened cheeses in the jar to be lower. Regardless of the type of packaging, the acidity of cheese ripened at soil temperature is significantly higher than that of cheeses ripened at refrigerator temperature. It seems that higher ripening temperature increases the activity of lactic acid bacteria and as a result, acid

production. It has been reported by other researchers [23 and 32]. The moisture content of all samples decreased significantly during ripening. The moisture content of the cheeses ripened in plastic containers (at both soil and refrigerator temperatures) was higher than the cheeses ripened in jars. The lower humidity of ripened cheeses in the jar is probably caused by the moisture coming out of the semi-permeable wall of the clay jar [33]. Regardless of the type of packaging, the cheeses ripened at ground temperature had more moisture than the cheeses ripened at refrigerator temperature, which is due to the absorption of moisture from proteolysis products during the activity of proteolytic microorganisms, which activity of this microorganism are more at soil temperature than at refrigerator temperature [34 and 35]. The percentage of fat and the percentage of fat in dry matter of all samples increased significantly during ripening, which can be caused by a significant decrease in moisture during this period. The percentage of fat related to cheeses ripened in jars (at both soil and refrigerator temperatures) was significantly higher than cheeses ripened in plastic containers. In all samples, the cheese ripened in soil temperature had a lower fat percentage than the cheese ripened in refrigerator temperature. Research has shown that the activity of lipolytic and proteolytic microorganisms is reduced in the refrigerator temperature and as a result, less fat will be decomposed in the refrigerator temperature compared to higher storage temperatures [34 and 36]. During the ripening period, the ratio of total nitrogen to dry matter (TN/DM) decreases significantly. The decrease in the ratio of total nitrogen to dry matter during ripening is caused by the proteolysis of cold-

loving microbes, the production of water-soluble nitrogen compounds and the release of these compounds into the aquatic environment [31, 36 and 37]. (TN/DM) related to cheeses ripened in plastic is higher than cheeses ripened in jars, which can be due to the permeable membrane of jars and as a result, these nitrogenous compounds dissolved in water are more easily removed from it [33]. . The ratio of water-soluble nitrogen to total nitrogen (WSN/TN) of all cheese samples increased significantly during the ripening period, which could be related to the activity of rennet or microbial proteases and the production of water-soluble nitrogen compounds [38]. . The increasing trend of the ratio of water-soluble nitrogen to total nitrogen during the ripening period has been reported by many researchers that proteolysis plays a key role in increasing

this ratio [39, 40, 41 and 42]. Cheeses ripened in plastic (WSN/TN) were higher compared to cheeses ripened in jars, which can be caused by the permeable membrane of jar containers and as a result of absorption of these compounds along with moisture in the tissue of the jar and its surrounding environment. Also, the higher humidity of cheeses ripened in plastic causes an increase in the activity of lipolytic and proteolytic microorganisms and as a result increases the nitrogen compounds dissolved in water [43 and 44]. In all the samples, the cheeses ripened at soil temperature had a higher amount (WSN/TN) than the cheeses ripened at refrigerator temperature, which is due to the increase in the activity of proteolytic microorganisms with temperature. The results are consistent with the results of other researchers. [28].

**Table 2** Least Square Mean (LSM) comparison of the triple interaction effect of storage conditions, packaging type and time on the physicochemical properties of Kope cheese<sup>1</sup>

Type of packaging	Storage conditions	Time	pH	Acidity (Dornic degree)	Moisture content (%w/w)	Fat (%w/w)	Fat in dry matter (%w/w)	TN/DM (%w/w)	WSN/TN (%w/w)
Polyethylene	Fridge	1	5.57 <sup>b</sup>	0.91 <sup>lt is</sup>	53.01 <sup>d</sup>	19.25 <sup>m</sup>	40.86 <sup>l</sup>	5.78 <sup>a</sup>	4.04 <sup>ij</sup>
		30	5.39 <sup>lt is</sup>	1.07 <sup>of</sup>	53.42 <sup>b</sup>	20.11 <sup>i</sup>	41.02 <sup>jk</sup>	5.72 <sup>c</sup>	4.36 <sup>f</sup>
		60	5.30 <sup>f</sup>	1.37 <sup>abc</sup>	52.02 <sup>g</sup>	20.73 <sup>g</sup>	42.14 <sup>g</sup>	5.50 <sup>lt is</sup>	4.80 <sup>lt is</sup>
		90	5.15 <sup>h</sup>	1.50 <sup>ab</sup>	51.76 <sup>h</sup>	21.19 <sup>lt is</sup>	43.25 <sup>d</sup>	5.47 <sup>g</sup>	5.41 <sup>b</sup>
	Underground	1	5.56 <sup>b</sup>	0.91 <sup>lt is</sup>	52.98 <sup>of</sup>	19.11 <sup>n</sup>	41.08 <sup>i</sup>	5.78 <sup>a</sup>	4.02 <sup>j</sup>
		30	5.39 <sup>lt is</sup>	1.09 <sup>of</sup>	55.01 <sup>a</sup>	19.54 <sup>l</sup>	42.11 <sup>g</sup>	5.71 <sup>c</sup>	4.37 <sup>f</sup>
		60	5.30 <sup>f</sup>	1.40 <sup>abc</sup>	53.11 <sup>c</sup>	20.16 <sup>i</sup>	43.17 <sup>lt is</sup>	5.57 <sup>d</sup>	4.92 <sup>d</sup>
		90	5.10 <sup>i</sup>	1.59 <sup>a</sup>	51.31 <sup>i</sup>	21.07 <sup>f</sup>	43.25 <sup>d</sup>	5.49 <sup>f</sup>	5.53 <sup>a</sup>
clay pot	Fridge	1	5.60 <sup>a</sup>	0.89 <sup>if</sup>	52.94 <sup>lt is</sup>	19.51 <sup>l</sup>	41.05 <sup>ij</sup>	5.74 <sup>b</sup>	4.04 <sup>ij</sup>
		30	5.53 <sup>c</sup>	0.98 <sup>lt is</sup>	51.24 <sup>j</sup>	19.62 <sup>k</sup>	42.77 <sup>f</sup>	5.01 <sup>i</sup>	4.13 <sup>h</sup>
		60	5.46 <sup>d</sup>	1.10 <sup>of</sup>	49.36 <sup>m</sup>	21.30 <sup>d</sup>	43.48 <sup>c</sup>	5.01 <sup>i</sup>	4.26 <sup>g</sup>
		90	5.30 <sup>f</sup>	1.30 <sup>bcd</sup>	47.88 <sup>n</sup>	23.12 <sup>a</sup>	45.01 <sup>a</sup>	4.90 <sup>k</sup>	5.05 <sup>c</sup>
	Underground	1	5.61 <sup>a</sup>	0.88 <sup>if</sup>	53.10 <sup>c</sup>	19.72 <sup>j</sup>	40.97 <sup>k</sup>	5.75 <sup>b</sup>	4.02 <sup>j</sup>
		30	5.55 <sup>b</sup>	0.68 <sup>f</sup>	52.11 <sup>f</sup>	20.31 <sup>h</sup>	41.16 <sup>h</sup>	5.05 <sup>h</sup>	4.08 <sup>hi</sup>
		60	5.47 <sup>d</sup>	1.23 <sup>cd</sup>	50.82 <sup>k</sup>	22.04 <sup>c</sup>	43.82 <sup>b</sup>	5.00 <sup>i</sup>	4.22 <sup>g</sup>
		90	5.25 <sup>g</sup>	1.40 <sup>abc</sup>	49.93 <sup>l</sup>	22.16 <sup>b</sup>	45.08 <sup>a</sup>	4.98 <sup>j</sup>	5.01 <sup>c</sup>

1. Means within the same rows with different common letters differ significantly (P<0.05)

### 3-2- Evaluation of texture (rheology) of Koze cheese in different ripening conditions

Chart of changes in storage modulus (G') and loss modulus (G'') of jar cheese samples stored in polyethylene packaging and underground (PS), polyethylene packaging and in the

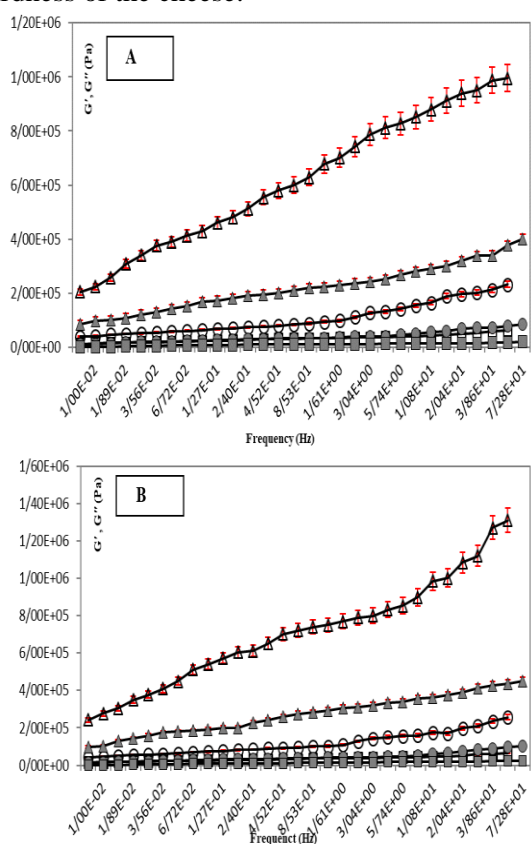
refrigerator (PR), jar packaging and underground (CJS) and jar packaging and in refrigerator (CJR) during arrival at angular frequency 0.01-100 Hz and temperature 25 °C are shown in figures (2) and (3). According to the figures, with the increase of ripening time, the storage modulus (G') and the loss modulus (G'') increase in all cheese samples, and the

storage modulus is higher than the loss modulus for all four treatments at each frequency, which indicates the role of The characteristic of elasticity is dominant over viscosity, which is similar to the results of many researchers [45, 46, 47 and 48]. The research showed that with the increase in ripening time, due to the breakdown of fat cells and proteolysis, the protein matrix undergoes a structural rearrangement and a very dense tissue containing casein masses is formed. As a result, the elastic modulus of cottage cheese dominates its viscous modulus [39]. As the fermentation time increases and the pH of the cheese decreases, the storage modulus and the drop modulus increase at different rates, which is in accordance with the results of the study by Kerami et al. and the storage modulus both increase and the storage modulus is always more than the drop modulus, so the elastic property is more than the viscous property. Studies have shown that pH plays an important role in the viscoelastic property of cheese and the softness of cheese depends on its pH [40]. Referring to the statistical results of table (2), it can be seen that along with the decreasing pH during the ripening period, the drop modulus increased more strongly than the storage modulus, similar results have been reported in relation to the relationship between the pH decrease and the hardness of processed cheese. [50 and 51]. In this report, it is shown that the decrease in pH causes an increase in the ratio of lactose to protein in cheese tissue and increases its hardness. At low pH, there are coarse protein aggregates with fat that is not fully emulsified. With the increase in pH, the compression of proteins decreases, which indicates a decrease in protein-protein interactions. At pHs higher than the isoelectric pH, due to the reduction of electrostatic forces, the hydration of caseins increases and the hardness of cheese decreases [10]. In general, fat globules and moisture act as a filler phase in the casein matrix and make the cheese soft. [52]. During the ripening period, the percentage of fat and the percentage of fat in dry matter increased (Table 2), however, we saw an increase in storage modulus ( $G'$ ) during this period, which is probably due to the unfolded proteins that are involved with each other and cause Increasing the viscoelastic properties of cheese. is related. In general, the firmness of cheese during the storage period depends on two main factors: moisture reduction during storage, which

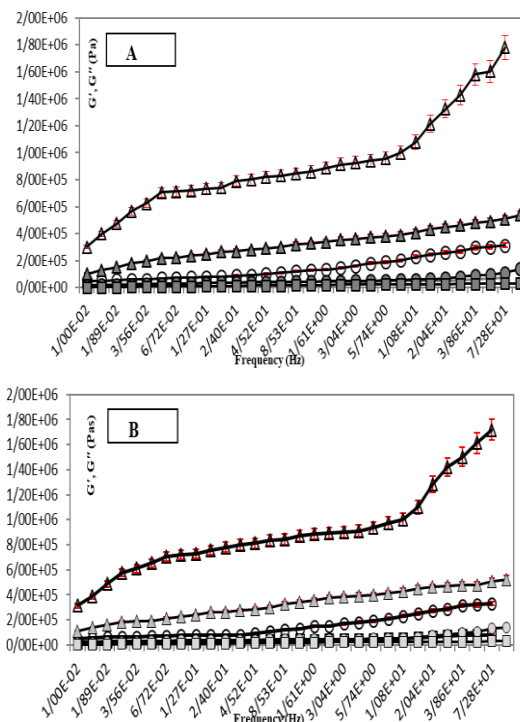
increases the firmness, and proteolysis, which reduces the firmness due to the breakdown of casein. The acidity and pH of the clot medium affect the amount of water released during the production stage, the activity of microbial flora and various enzymes during the production and ripening period, the consistency and taste of cheese [53]. The intensification of lipolysis due to the increase in the amount of moisture and the production of fatty acids, as well as the complete conversion of lactose into lactic acid, are also effective in reducing pH and increasing acidity [54]. On the other hand, micellar calcium phosphate does not only act as a bridge between small casein clusters, but also acts as a neutralizer due to having positive charges. These positive charges neutralize the negative charges of phosphoserine at the point of interaction between the hydrophobic areas of casein and thus make their connection to each other possible [37 and 55]. During the ripening of cheese, with a decrease in pH, calcium phosphate becomes soluble, and with a decrease in the amount of calcium attached to casein micelles, the repulsive forces between caseins increase [56] and lead to the weakening of structural bonds and, as a result, softening of the cheese. These are examples. Figure (4) part (A) and (B) Respectively It shows the changes of storage modulus ( $G'$ ) and loss modulus ( $G''$ ) on the 90th day of ripening related to cheese samples in different packaging and storage conditions. As can be seen in these figures, in all types of packaging, the storage modulus and loss modulus increased with increasing frequency. The amount of storage modulus increase and loss modulus of the studied samples on the 90th day of reaching is  $CJS << PR < CJR$  PS. Referring to the statistical results of table (2), it can be seen that the samples of CJR and CJS have less moisture and, as a result, a stiffer texture. The decrease in the amount of protein in polyethylene packaging compared to jar packaging during ripening (Table 2) can be the reason for the reduction of cheese hardness in these samples. As can be seen in pictures (C7) and (C8) as well as in figure (9), the protein network created in (PS) and (PR) treatments has more and smaller pores compared to the other two treatments, which The spongy structure keeps the moisture inside the tissue and increases the ratio of moisture to protein in the product and causes more softness of the tissue in polyethylene packaging samples. Less amount of cheese moisture leads

to less hydration of protein and as a result less free movement of protein molecules and more amount of intact casein remains and the stiffness of the casein network increases [49, 57 and 58]. The statistical results of Table (2) also showed that polyethylene packaging samples had higher moisture content, since the ratio of remaining rennet to casein in cheeses with high moisture content is higher than cheeses with low moisture content [59].

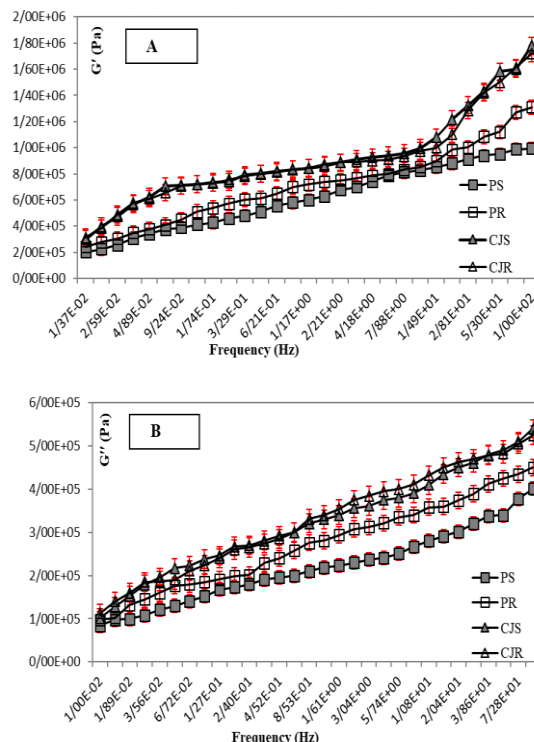
As a result, the degree of softening is also higher in them. This issue is due to the fact that the enzyme and microbial activity is more intense in higher humidity, it is quite natural that proteolysis is more and reduces the hardness of the cheese.



**Fig 2** Changes in storage modulus ( $G'$ ) on day 1 ( $\square$ ), day 45 ( $\circ$ ) and day 90 ( $\Delta$ ) and Loss modulus ( $G''$ ) on day 1 ( $\blacksquare$ ), day 45 ( $\bullet$ ) and day 90 ( $\blacktriangle$ ) in Kope cheese stored in polyethylene packaging and in the refrigerator (PR) (A) in polyethylene packaging and underground (PS) (B)



**Fig 3** Changes in storage modulus ( $G'$ ) on day 1 ( $\square$ ), day 45 ( $\circ$ ) and day 90 ( $\Delta$ ) and Loss modulus ( $G''$ ) on day 1 ( $\blacksquare$ ), day 45 ( $\bullet$ ) and day 90 ( $\blacktriangle$ ) in Kope cheese stored in clay pot packaging and in the refrigerator (CJR) (A) in clay pot packaging and underground (CJS) (B)



**Fig 4** Changes in storage modulus ( $G'$ ) and Loss modulus ( $G''$ ) on day 90 in Kope cheese stored in Polyethylene packaging and in the refrigerator (PR) ( $\square$ ), in polyethylene packaging and underground (PS) ( $\blacksquare$ ), in clay pot packaging and in the refrigerator (CJR) ( $\Delta$ ) and in clay pot packaging and underground (CJS) ( $\blacktriangle$ ) ( $P < 0.05$ )

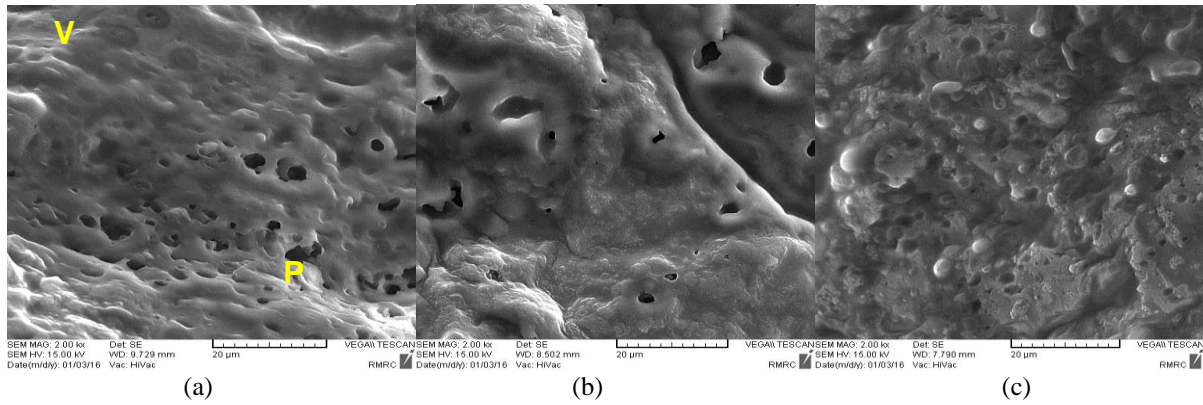
. The intensity of proteolysis in polyethylene and underground packaging was lower than other samples, which could be due to the higher humidity of the cheeses ripened in polyethylene containers (Table 2), on the other hand, the sensitivity of the cheeses ripened in the packaging is higher. Polyethylene to proteolysis can be caused by the higher acidity of these samples (Table 2), which causes greater instability of casein micelles against proteolytic degradation [60 and 61].

### **3-3- Evaluation of the microstructure characteristics of Koze cheese in packaging and different ripening conditions**

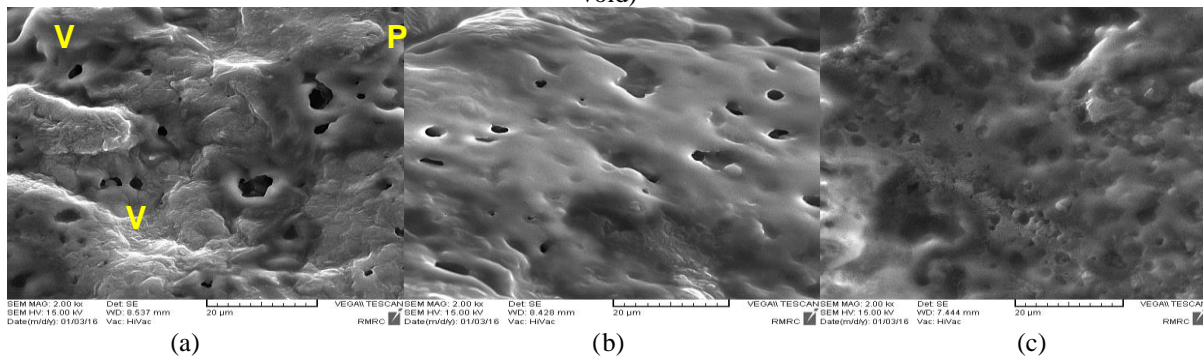
Electron microscope images of jar cheese samples stored in polyethylene packaging and underground (PS), polyethylene packaging and in the refrigerator (PR), jar packaging and underground (CJS) and jar packaging and It is shown in the refrigerator (CJR) during arrival with a magnification of 2000 in figures (5) to (9). According to these images, the presence of large protein aggregates with few holes was common in all samples on the first day of cheese storage (Figures (a5) to (a9)). After 45 days, more holes were formed in the cheese and the caseins became a network around the cheese holes (Figures (b5) to (b9)). During the period until the end of the 90th day, we see an increasing trend in the number of holes and the size of the holes in all samples. The results are consistent with the results of other researchers [20]. The large holes that are evident in all the images can be attributed to gas production by microorganisms [62] and also to the release of fat globules extracted by chloroform during the preparation stages [63]. The accumulation of fat globules act as a brittle factor of the casein matrix and in their presence, the compression of the casein matrix is reduced [64]. As it can be seen in figures (a5) to (a8), the micrographs taken from the cheese by electron microscope included two distinct structures, which include protein masses (P) and cavities in between them (V). . Cavities are the location of milk fat, which reduce the density of the protein matrix.

These fats were separated from cheese by chloroform during the preparation process [65]. Research has shown that by reducing or increasing the amount of fat in cheese, the protein structure becomes denser and more open, respectively, which is due to the greater accumulation of fats in products with more fat than in low-fat products and as a result, larger holes are created. is [27]. During ripening from day 1 to day 90, the structure of the treatments was more open due to the increase of proteolysis and as a result the loosening of the protein network and better integration of fats [66]. The intensity of proteolysis increases during the ripening period in jar cheese, and dense casein communities were observed in cheeses packed in jars and under soil (CJS) and packaged in jars and in refrigerators (CJR), and obviously the protein matrix was compact and This explains the stiffer texture of these treatments compared to the other two treatments. As can be seen in the images (C7) and (C8), the protein network created in the treatments (PS) and (PR) has more and smaller pores compared to the other two treatments, which makes this spongy structure moisture inside the tissue. It preserves and increases the ratio of moisture to protein in the product and improves the softness of the texture. The obtained results are consistent with the results of figures (A4) and (B4), which showed that the increase of storage modulus and loss modulus of the studied samples on the 90th day of ripening is  $CJS < PR < CJR$  PS. Referring to the statistical results of table (2), it can be seen that the samples of CJR and CJS have less moisture and therefore a stiffer texture. The decrease in the amount of protein in polyethylene packaging compared to jar packaging during ripening can be the reason for the reduction of cheese hardness in these samples.

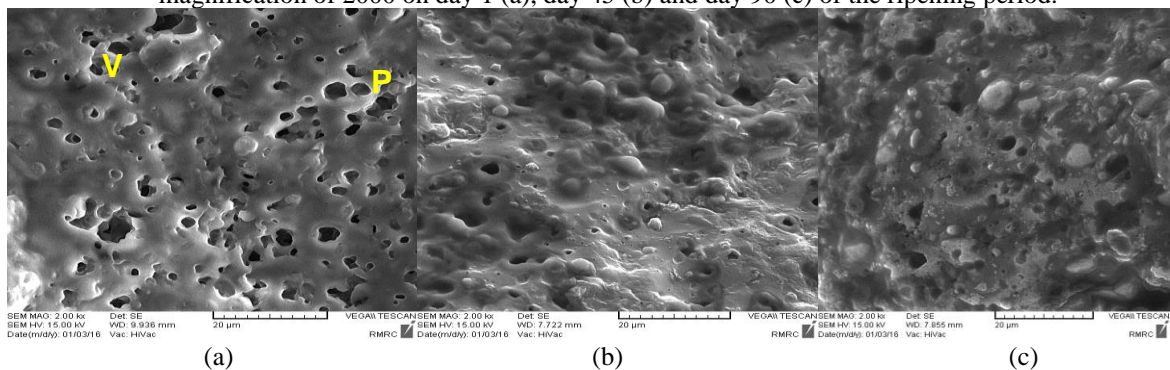




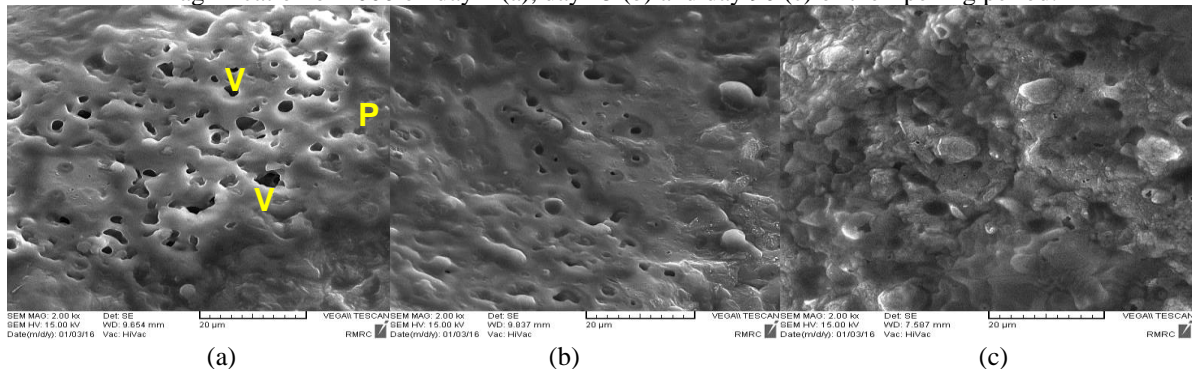
**Fig 5** Electron microscope images of Kope cheese kept in clay pot packaging and underground (CJS) with a magnification of 2000 on day 1 (a), day 45 (b) and day 90 (c) of the ripening period. (P: Protein aggregate, V: void)



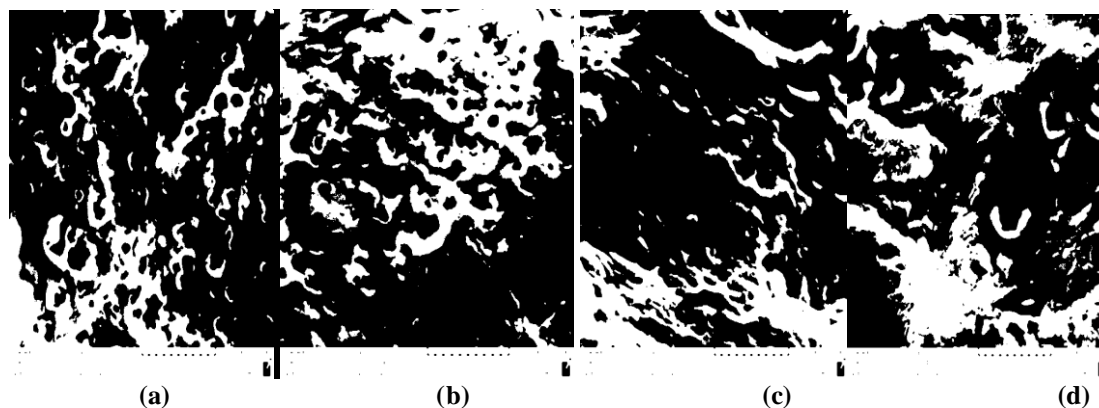
**Fig 6** Electron microscope images of Kope cheese kept in clay pot packaging and in the fridge (CJR) with a magnification of 2000 on day 1 (a), day 45 (b) and day 90 (c) of the ripening period.



**Fig 7** Electron microscope images of Kope cheese kept in polyethylene packaging and underground (PS) with a magnification of 2000 on day 1 (a), day 45 (b) and day 90 (c) of the ripening period.



**Fig 8** Electron microscope images of Kope cheese kept in polyethylene packaging and in the fridge (PR) with a magnification of 2000 on day 1 (a), day 45 (b) and day 90 (c) of the ripening period.



**Fig 9** Binariesd SEM micrographs of Kope cheese stored in polyethylene packaging and underground (a), in polyethylene packaging and in the fridge (b), in clay pot packaging and underground (c) and in clay pot packaging and in the fridge (d) on the 90th day of the ripening period.

#### 4- Conclusion

With increasing ripening period, acidity, fat, fat in dry matter and water-soluble nitrogen increased compared to total nitrogen, while pH, moisture and total nitrogen decreased compared to dry matter. The significant reduction of total nitrogen to dry matter (TN/DM) during the ripening period is caused by proteolysis, the production of water-soluble nitrogenous compounds and the release of these compounds into the surrounding environment. A significant increase in the ratio of water-soluble nitrogen to total nitrogen (WSN/TN) during the ripening period can be related to the activity of microbial proteases and the production of water-soluble nitrogen compounds. The results showed that the amount of acidity, moisture, the ratio of total nitrogen to dry matter and water-soluble nitrogen to total nitrogen was significantly higher in cheeses ripened in plastic packaging. Regardless of the type of packaging, the samples delivered at soil temperature had higher acidity, ratio of total nitrogen to dry matter and water-soluble nitrogen to total nitrogen. The amount of pH, fat and fat in the dry matter of cheese samples ripened in a jar was higher than the samples ripened in plastic containers, while the samples ripened in plastic containers had higher levels of acidity, moisture, ratio of total nitrogen to dry matter and soluble nitrogen. In water, they were higher than total nitrogen. In the comparison between the samples with similar packaging, the cheese samples ripened at soil temperature had the highest amount of acidity, the ratio of total nitrogen to dry matter and water-soluble nitrogen to total nitrogen. Examining the rheological characteristics and microstructure of the jar cheese showed that the treatments packed in the jar at both soil and refrigerator

temperatures have less moisture and as a result a firmer texture, while the treatments packaged in plastic at both soil and refrigerator temperatures have It has more and smaller pores and as a result it has a spongy structure and a softer texture compared to the other two treatments.

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#### 6- Resources

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## ارزیابی خصوصیات فیزیکوشیمیایی، رئولوژیکی و ریزساختار پنیر کوزه تهیه شده از شیر گاو در شرایط مختلف رسیدن

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### چکیده

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در این پژوهش ویژگی های فیزیکوشیمیایی، بافت و ریزساختار پنیر سنتی کوزه تهیه شده در دو نوع بسته بندی متفاوت (کوزه و پلاستیک) و در دو دمای رسیدن متفاوت که شامل خاک (°C) ۸-۱۲ و یخچال (°C) ۵-۸ می باشد، طی دوره رسیدن (۶۰ روز) با آزمایش فاکتوریل در قالب طرح کاملاً تصادفی مورد بررسی قرار گرفت. نتایج نشان داد که میزان اسیدیته، رطوبت، نسبت نیتروژن کل به ماده خشک و نیتروژن محلول در آب به نیتروژن کل در پنیرهای رسیده در بسته بندی پلاستیکی به صورت معنی داری بالاتر بود ( $P < 0/05$ ). صرف نظر از نوع بسته بندی، نمونه های رسانده شده در دمای خاک از اسیدیته، نسبت نیتروژن کل به ماده خشک و نیتروژن محلول در آب به نیتروژن کل بالاتری برخوردار بودند. بررسی ویژگی های رئولوژیکی و ریزساختار پنیر کوزه نشان داد که تیمارهای بسته بندی شده در هر دو دمای خاک و یخچال دارای رطوبت کمتر و در نتیجه بافت سفت تری می باشند درحالی که تیمارهای بسته بندی شده در پلاستیک در هر دو دمای خاک و یخچال دارای منافذ بیشتر و کوچک تر و در نتیجه دارای ساختار اسفنجی و بافت نرم تری در مقایسه با دو تیمار دیگر می باشد.

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