

Effect of curd freezing and packaging methods on the organic acid contents of goat cheeses during storage

A. Kemal Seçkin¹, Özlem Kizilirmak Esmer²,
Pınar Balkir³, Pelin Günç Ergönül^{1*}

¹Celal Bayar University Faculty of Engineering Food Engineering
Department Muradiye, Manisa, Turkey

²Ege University Faculty of Engineering Food Engineering
Department Bornova, İzmir, Turkey

³Ege University Technical and Business College Bornova, İzmir, Turkey

Received - Prispjelo: 23.12.2010.

Accepted - Prihvaćeno: 19.07.2011.

Summary

Effects of freezing and packaging methods on organic acid content of goat cheese during 12 weeks of storage were determined. Goat cheese milk curds were divided into two batches; one of the batches was directly processed into goat cheese while the other was frozen at -18 °C and stored for six months and processed into cheese after being thawed. Cheese samples were packed in three parts and stored at 4 °C refrigerated control sample and at -18 °C for six months frozen experimental samples. Cheese samples were packed in three different packaging methods: aerobic, vacuum or modified atmosphere. Citric, malic, fumaric, acetic, lactic, pyruvic and propionic acids were analyzed using HPLC method after 1st, 3rd, 6th, 9th and 12th week of storage period. Lactic acid was the main organic acids while pyruvic acid had the lowest content in all cheese samples. Citric and fumaric acid levels of frozen samples increased during storage whereas malic, acetic, pyruvic and propionic acid amounts were decreased compared to the beginning of storage. Packaging methods and freezing process also effected lactic acid levels statistically ($p < 0.05$). Fumaric, acetic and lactic acid concentration of refrigerated samples were increased but citric, malic and propionic acids decreased during storage. Pyruvic acid level did not change significantly. It was determined that organic acid concentrations were effected by freezing process, storage time and packaging methods significantly ($p < 0.05$).

Key words: organic acids, goat cheese, freezing, packaging

Introduction

Organic acids occur in dairy products as a result of normal animal metabolism and break down of milk protein, fat, lactose and citrate during manufacture and storage. They play important role in the flavor of dairy products and so, they contribute to cheese quality (Califano and Bevilacqua, 1999). Many researchers use the level of some organic acids as indicator of starter activity and bacterial growth during cheese ripening (Careri et al, 1996). Organic acids can reflect the kind of fermentation and indicate deviations of the expected course of maturation, potentially leading to defects (Careri et al, 1996;

de Llano et al., 1996). The organic acid profile was found to differ among cheese varieties and some organic acids are of importance for the typical flavor of some cheeses (Manolahi et al., 2006) as acetic acid is a major contributor to the flavor of Feta cheese (Abd El-Salam and Alichanidis, 2004). The level of individual organic acid was found to vary according to the processing procedure, the ripening temperature and duration, the production season and type of a starter (Skeie, Linberg and Narvhus, 2001). Freezing is a suitable procedure to prolong stability and shelf-life of cheeses although some authors pointed out the lack of unanimity on the extent of damage caused by freezing. Damage depends

*Corresponding author/Dopisni autor: Phone/Tel.: +90 532 7087039; E-mail: pelingunc81@hotmail.com

on the type of cheese (composition and manufacturing procedures) the freezing conditions and parameters chosen to evaluate the damage (Cervantes et al., 1983; Diefes et al., 1993). Goat milk is not obtained in each month of year. The seasonality of goat milk production necessitates a food technological approach to alternative methods of year-round marketing such as frozen-storage of the goat products. Goat cheeses have a high economic value and so it is important to determine its properties under different packaging conditions to be able to increase the shelf life of cheese. Wendorff (2001) observed a large ice crystal formation in the ovine milk frozen at $-15\text{ }^{\circ}\text{C}$ compared to $-27\text{ }^{\circ}\text{C}$.

Modified atmosphere packaging (MAP) and vacuum packaging (VP) can be used to increase the shelf life of cheeses. Shelf life of unpacked fresh cheese changes according to type of cheese and storage temperature. Generally they are no more than 10-12 days at refrigerator temperature because of the effect of O_2 on microbial growth and sensory characteristics. The potential of MAP and VP for extending the shelf-life of cheese has been demonstrated by various researchers (Favati et al., 2007; Oliszewski et al., 2007; Papaioannou et al., 2007). Numerous varieties of goat milk cheeses are produced world wide and goat milk cheese has recently gained popularity among many consumer groups and farmers in the world (Park and Jin, 1998). Very few studies have been conducted on storage stability of goat milk cheeses and freezing process and packaging method in relation to organic acid contents. Thus, aims of this study were to determine organic acid profiles of plain goat cheeses, to compare changes in organic acids content of fresh and frozen-thawed plain soft goat cheeses and to evaluate and to compare the effect of storage on organic contents of freezing and refrigerated storage cheeses.

Materials and method

Cheese making procedure

Cheese productions were carried out at the pilot plant of Pınar Dairy Products Inc (İzmir, Turkey). The manufacturing procedure of acid goat cheese is as described in Figure 1.

Packaging of goat cheese

Goat cheese samples were packaged by vacuum packaging (VP) and modified atmosphere packaging (MAP). For VP, a polyamide/low density polyethylene (PA/LDPE) film in $100\mu\text{m}$ thickness was used. For MAP, polypropylene/ethylenevinylalcohol/lowdensity polyethylene (PP/EVOH/LDPE) film combination was used for top part of package and polyethylene terephthalate/ethylenevinylalcohol/lowdensitypolyethylene (PET/EVOH/LDPE) was used for bottom part of package. In MAP applications, a barrier layer with excellent impermeable properties is required. In this film combinations, EVOH functioned as a barrier layer. It was used for MAP application 20 % CO_2 and 80 % N_2 as it is the combination of soft cheese. And as a control, aerobic air packaging (AP) is also applied. These packagings are used in dairy industry widely.

Experimental design

After production of two batches of experimental cheese curd (frozen at $-18\text{ }^{\circ}\text{C}$ for six months and refrigerated at $4\text{ }^{\circ}\text{C}$ for 12 weeks), organic acid compositions of cheeses were determined and the batch was divided into 3 groups for VP, for MAP and for aerobic packaging (AP). These groups were stored at $+4\text{ }^{\circ}\text{C}$ for 12 weeks. Organic acid analyses were performed after 1st, 3rd, 6th, 9th and 12th weeks of storage. Productions were done in triplicate. At each analyses period three different packed cheeses were analyzed and analyses were done in duplicate.

Organic acid determination

Extraction of organic acids

Organic acids were determined according to Buffa et al. (2004). Seven grams of goat cheese was taken and than 40 ml mobile phase (0.1 % H_3PO_4) was added and mixed by ultraturax for 1 minute. Mixture was held in water bath ($40\text{ }^{\circ}\text{C}$) for 1 hour, than centrifuged at 6000 rpm for 5 minutes. Upper phase was filtered through filter paper (Whatman No.1).

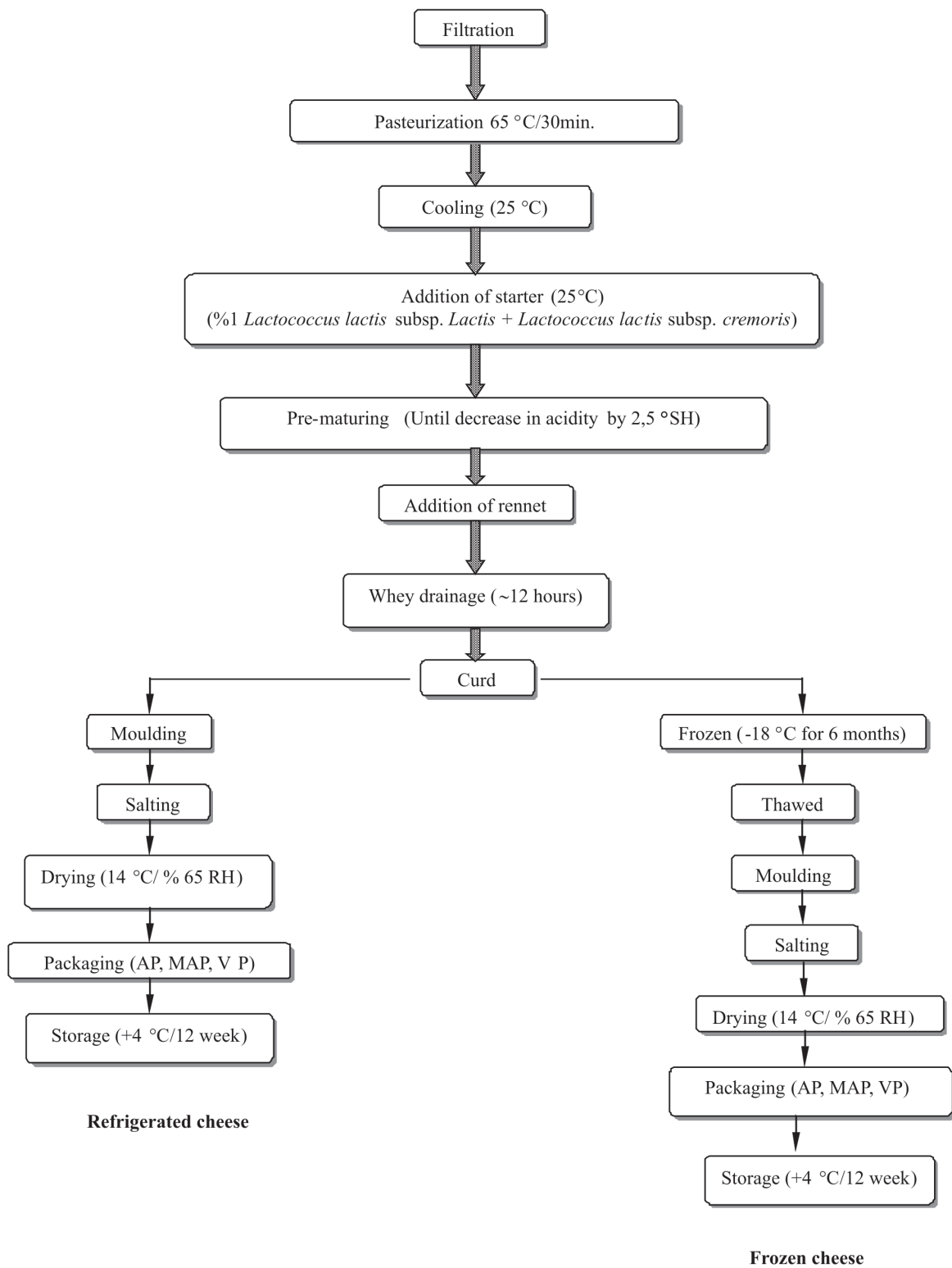


Figure 1. Cheese making procedures

Determination of organic acids by HPLC

20 μL aliquots of individual standards were injected to column and their retention times were determined. To obtain the calibration curves a mixture of standards of certain concentrations were also injected into HPLC and their chromatograms were obtained. After injection of the samples (20 μL) chromatographic peaks were identified by comparing retention times of samples to known standards. Results are presented as the area under peaks in mili absorption unit (mAU).

A Perkin Elmer Series 200 Model HPLC apparatus equipped with a UV absorbance detector set at 214 nm was used. Chromatographic separation was performed on a Shodex RSpak KC-118 model ion-exchange organic acid column (8x300 mm i.d.). The mobile phases was 0.1 % (w/v) of phosphoric acid in distilled water (HPLC grade) with a flow rate of 0.8 mL/min.

Statistical analyses

The treatment structure was completely randomized design. The analysis of variance was carried using the PROC GLM procedure of SAS (version 8.2, SAS Institute, Cary, NC, 2001). Least Squares of Means (LSMEANS) for treatments were generated.

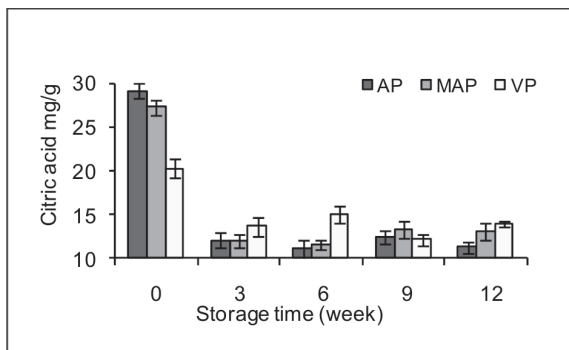
Results and discussion

Citrate in milk is metabolized by many lactic acid bacteria into flavor compounds such as acetate, acetaldehyde and diacetyl (Hugenholz, 1993). Citric acid level of refrigerated AP cheese was 29.22 mg/g at the beginning of storage. During the storage this level changed irregularly and at the end of ripening it decreased to 11.35 mg/g. At the beginning of storage, citric acid level of frozen AP sample was 12.84 mg/g while at the end of storage it was 14.67 mg/g cheese (Figure 2).

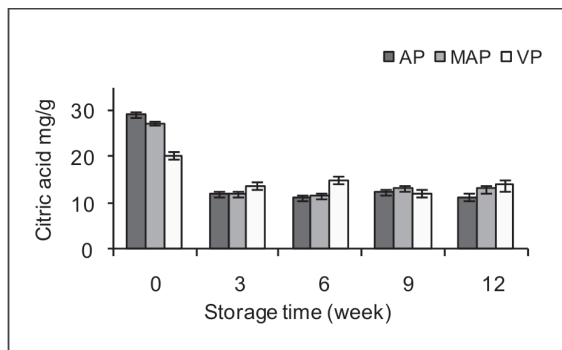
Storage process effected the citric acid level of frozen AP cheeses ($p < 0.05$) while it did not effect refrigerated AP cheese ($p > 0.05$). Freezing process effected the citric acid levels of AP samples statistically ($p < 0.05$). Citric acid content of refrigerated MAP samples changed during the storage significantly ($p < 0.05$). Citric acid content reached maximum

level at 6th week storage with 11.59 mg/g. Generally the citric acid levels of frozen MAP samples were lower than refrigerated samples of cheese. For frozen MAP samples, storage process did not affect citric acid levels statistically except 6th week of storage. Another storage days, this value did not change significantly ($p > 0.05$). The lowest level reached at 9th week of storage as 10.93 mg/g. Freezing process effected citric acid level of MAP samples significantly ($p < 0.05$). For both frozen and refrigerated VP samples, storage process also effected the citric acid concentrations ($p < 0.05$). Citric acid reached maximum level at 12th week of storage as 13.93 mg/g for refrigerated VP cheese while frozen VP samples had maximum level at 6th week of storage as 11.95 mg/g (Figure 2). Packaging type also effected the citric acid concentration for frozen and refrigerated cheeses. Citric acid level of refrigerated cheeses is higher than all of frozen samples. So, frozen process may effect the survival of *Lactococcus sp.*, *Leuconostoc sp.* and *Lactobacilli sp.* that are metabolized citrate. Monalaki et al. (2006) determined organic acid contents of Feta type cheese during the storage. They found that citric acid content of cheese was regularly decreased with storage time. Akalin et al. (2002) claimed that the citric acid contents of white cheese changed irregularly during the storage. But at the end of storage citric acid content increased compared to beginning level. These results are parallel with the results of Akalin et al (2002). Park and Lee (2006) researched freezing effect on organic acid content of goat milk cheese and they declared that citric acid content increased in frozen type while for refrigerated type cheese it decreased with storage. This result resembles with our results.

The highest malic acid levels of refrigerated and frozen AP were 32.36 and 21.15 mg/g respectively at the beginning of the storage. Malic acid level decreased strictly to 20.05 mg/g at 3rd week for refrigerated AP samples, than increased to 21.60 mg/g and then decreased. At the end of storage malic acid level decreased to 15.21 mg/g (Figure 3). For frozen cheese, malic acid level decreased regularly until 6th week and then increased slightly. At the end of storage malic acid level of AP was determined as 17.52 mg/g. At 1st week of storage the malic acid of MAP refrigerated sample was 21.89 mg/g while at the end of storage this value decreased to 15.42 mg/g.

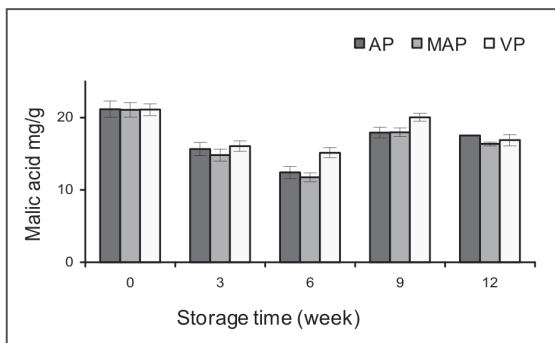


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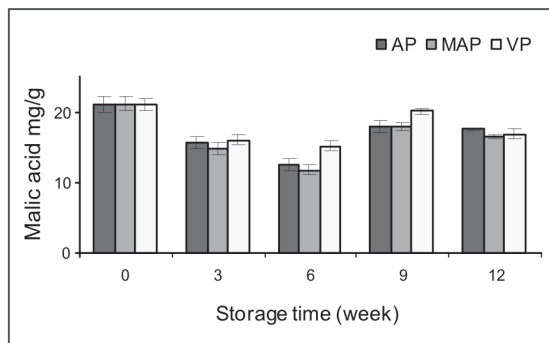


(b)

Figure 2. Citric acid change in refrigerated (a) and frozen (b) goat cheese

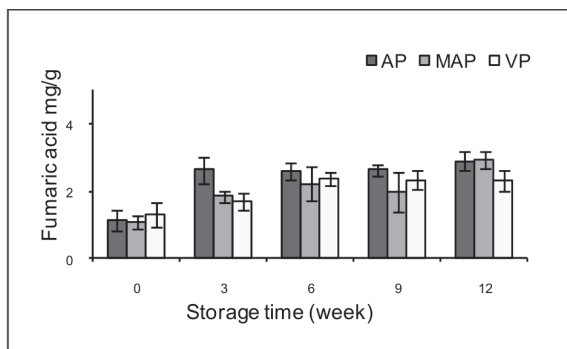


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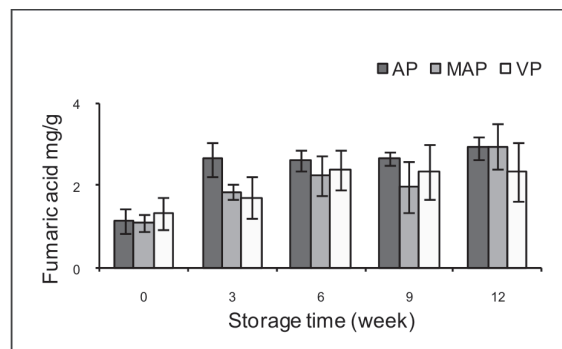


(b)

Figure 3. Malic acid change in refrigerated (a) and frozen (b) goat cheese



(a)



(b)

Figure 4. Fumaric acid change in refrigerated (a) and frozen (b) goat cheese

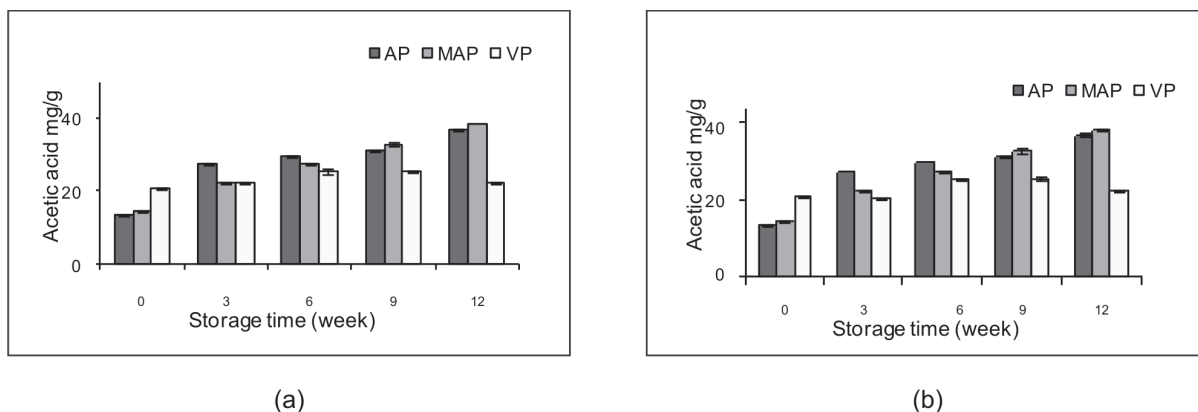


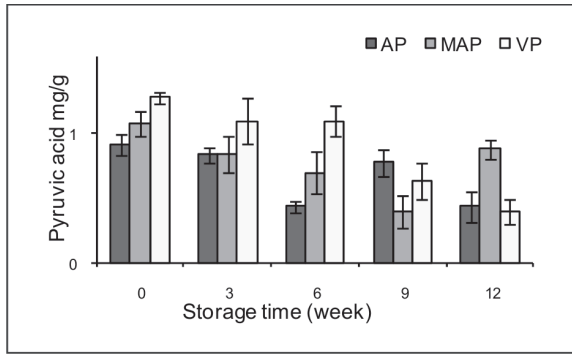
Figure 5. Acetic acid change in refrigerated (a) and frozen (b) goat cheese

For frozen MAP cheese, malic acid decreased from 21.06 mg/g to 16.35 mg/g. Malic acid levels of MAP and AP refrigerated and frozen cheese decreased strictly at 3rd week from 21.89 and 21.06 to 15.92 and 14.81 mg/g cheese respectively and then both samples increased slightly. For VP refrigerated samples, malic acid of cheeses were 21.07 and 21.08 mg/g respectively. For frozen and refrigerated VP samples, malic acid contents changed irregularly during the storage (Figure 3). Packaging method effected malic acid samples for frozen and refrigerated cheeses importantly ($p < 0.05$). Statistically, storage process also effected malic acid level of frozen and refrigerated cheese for all type of packaging samples ($p < 0.05$). Malic acid level is affected by bacterial activity and this activity is effected by frozen and packaging process. So, these differences might originate for this reason. For both samples (frozen and refrigerated) storage process effected the malic acid levels significantly for AP, MAP and VP samples ($p < 0.05$). Park and Drake (2005) and Park and Lee (2006) found that malic acid content of refrigerated goat cheese increase while it decreased for frozen goat cheese. Buffa et al. (2004) claimed that malic acid level increased very strictly during ripening. In this study the malic acid concentration of frozen type of all cheese samples (AP, MAP and VP) increased, but for refrigerated cheese this was seen to be contrast. So, these results of frozen cheese resemble with Park and Drake (2005) and Park and Lee (2006) and Buffa et al. (2004).

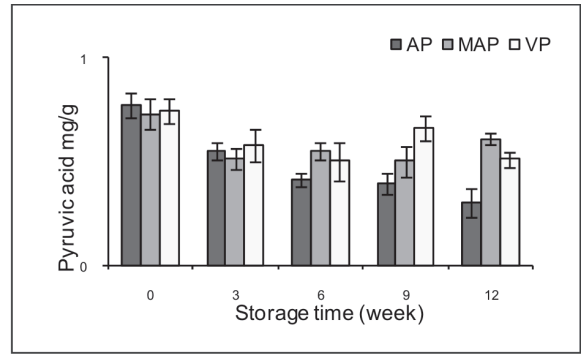
At the beginning of the storage, fumaric acid content of refrigerated and frozen AP, MAP and VP samples were 1.47, 1.34, 2.85, 1.14, 1.07 and 1.31

mg/g respectively. As it can be seen from Figure 4, generally at the end of storage fumaric acid concentration of the samples increased with respect to beginning levels. The highest fumaric acid level of refrigerated AP reached at 3rd week as 3.08 mg/g. For MAP and VP samples, the highest values were at 9th week 12st week of storage as 2.72 and 2.86 mg/g respectively. For frozen AP samples, the highest values were reached at 3th as 2.64 while for MAP and VP samples were at 6th week of storage as 2.23 and 2.38 respectively. Statistically storage time, packaging type and freezing process effected fumaric acid level of all cheese samples ($p < 0.05$).

Acetic acid is considered as a product of several biochemical pathways, such as fermentation of lactate and citrate or metabolism of amino acids by bacteria. It contributes greatly to the final flavor of cheese (Kandarakis et al., 2001). Acetic acid may provide an indication of the degree of heterofermentative metabolism that may have taken place in cheese (Bouzas et al., 1993). Acetic acid levels of frozen AP, MAP and VP were higher than refrigerated samples. Acetic acid levels of frozen cheese samples showed irregularly changing. Until 3rd week acetic acid levels were not changed importantly than increased at 6th week and then these levels increased again at 9th week and at the end of storage acetic acid levels decreased again. At 1st week the amount of acetic acid levels of refrigerated AP, MAP and VP were 13.46, 14.35 and 20.76 mg/g at the beginning of storage and these levels were increased to 36.83, 38.14 and 22.33 mg/g at the end of storage. It can be said that acetic acid levels of refrigerated cheeses increased during the storage.

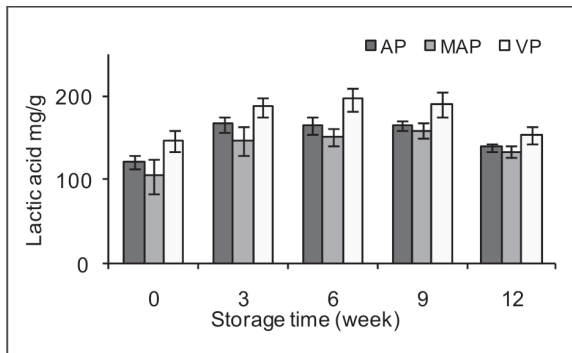


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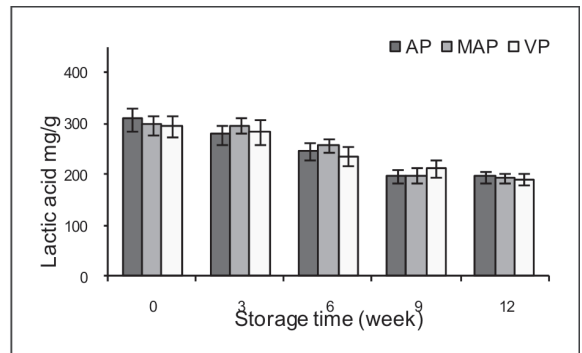


(b)

Figure 6. Pyruvic acid change of refrigerated (a) and frozen (b) goat cheese

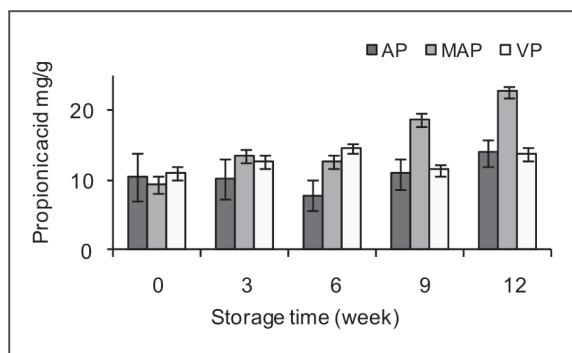


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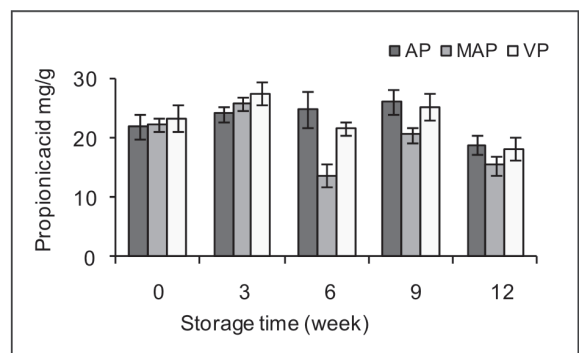


(b)

Figure 7. Lactic acid change of refrigerated (a) and frozen (b) goat cheese



(a)



(b)

Figure 8. Propionic acid change of refrigerated (a) and frozen cheese

For refrigerated cheeses at 1st week the acetic acid contents of AP, MAP and VP were 85.83, 84.28 and 84.77 mg/g respectively. The levels of acetic acid of refrigerated cheeses in all packages decreased at 12th week of storage (Figure 5).

Interaction between time and production method had been found important for all type samples. So, storage time and packaging method and freezing process effected the acetic acid levels of samples ($p < 0.05$). Results of Buffa et al. (2004), Kaminarides et al. (2007) resemble with these refrigerated cheese samples while the results of Monolaki et al. (2006) and Park and Drake (2005) showed parallel results with these frozen cheese samples.

Pyruvic acid levels were at the lowest level among the other organic acid of the sample. At 1st week of storage, the pyruvic acid level of refrigerated AP cheese was 0.46 mg/g. This level did not change importantly until end of storage (Figure 6). The pyruvic acid level of MAP and VP samples were 0.54 and 0.64 mg/g at the beginning of storage. These amounts did not change importantly until 6th week of storage like AP. Statistically storage time effected pyruvic acid concentration of refrigerated cheese samples ($p < 0.05$). The pyruvic acid level of frozen cheeses packed in AP, MAP and VP were 0.77, 0.73, 0.74 mg/g respectively at the beginning of storage. These results are different from Buffa et al. (2004) and Manolaki et al. (2006) results. This difference could come from production and packaging methods.

Lactic acid levels were at the highest level compared to the amounts of other organic acids of goat cheese. As it can be seen from Figure 7, lactic acid levels of refrigerated cheese samples in AP, MAP and VP package, were 120.76, 104.58 and 147.29 mg/g, respectively. These values of frozen cheese in AP, MAP and VP package were 308.57, 296.98 and 294.76 mg/g respectively. The contents of lactic acid of refrigerated cheese samples packed in AP, MAP and VP slightly increased ($p > 0.05$) during storage time and were 138.96, 133.54 and 154.04 mg/g, respectively at the end of storage. The frozen cheese samples in AP, MAP and VP differed by 196.43, 192.34 and 190.31 mg/g of lactic acid, respectively (Figure 7), while for frozen cheese showed the same status except 6th week of storage. For all samples, the storage process effected lactic acid level impor-

tantly ($p < 0.05$). Packaging methods and freezing process also effected lactic acid levels statistically for all cheeses ($p < 0.05$). Park and Drake (2005) claimed that lactic acid concentration of soft goat cheese increased during the storage. Kaminarides et al. (2007) found lactic acid level increased very strictly during the ripening of Halloumi cheese. Buffa et al. (2004) determined that lactic acid of goat cheeses did not change importantly during the storage. In this study the lactic acid concentration increased at the end of storage for refrigerated (AP, MAP and VP) samples. So, this result was similar to the findings of Park and Drake (2005) and Kaminarides et al. (2007). For the frozen sample, it was observed that lactic acid concentration changed very slightly. Therefore, this result resembles to Buffa et al. (2004) results. Califano and Bevilacqua (1999) studied the effect of freezing process on changing organic acid content of Mozzarella cheese. They found that freezing process did not affect the lactic acid content of cheese. This result is different from the results of this research. In this study freezing affected the lactic acid concentration of all type of cheeses.

Propionic acid contents of frozen AP, MAP and VP samples were 21.90, 22.29 and 23.37 mg/g and 10.54, 9.37 and 11.09 mg/g of refrigerated cheese samples, respectively, after 1st week of storage (Figure 8). As it can be seen, propionic acid levels of frozen samples were 2-3 times higher than same of refrigerated cheeses. It was probably consumed by the microorganisms mainly during the period of their maximum propagation and activity (1-3 week). For both samples at 6th week, the propionic acid levels were decreased strictly than increased again. Statistically storage time affected propionic acid levels of all samples. Packaging method and freezing process also affected these organic acid levels. Akalin et al. (2002) and Monolaki et al. (2006), Park and Lee (2006) claimed to increase propionic acid contents of cheese during ripening. So, these results are not resembled with results of this research. These differences can become from production and packaging methods.

Conclusions

Organic acids are very important for quality of cheese and they can be used as indicator of starter activity during ripening. In this study, seven different organic acids were determined in frozen and refrigerated samples during storage. Citric, malic, fumaric, acetic, propionic acid levels of all samples were affected significantly with freezing, packaging and storage time. For all samples pyruvic acid was the lowest organic acid whereas lactic acid was the highest. On the other hand lactic acid levels of the samples were not affected by storage period and freezing process.

Organic acids play important role in the flavor of dairy products and they can reflect the kind of fermentation. The results of study indicate that freezing goat cheeses would be feasible for later marketing which can overcome the seasonality of goat milk production and enhance the sustainability of the dairy goat industry.

Utjecaj zamrzavanja i metoda ambalažiranja na udjel organskih kiselina kozjeg sira tijekom skladištenja

Sažetak

U istraživanju je utvrđen utjecaj zamrzavanja i metoda ambalažiranja na udjel organskih kiselina kozjeg sira tijekom 12 tjedana skladištenja. Gruš sira od kozjeg mlijeka bio je podijeljen u dva dijela - kao kontrolni uzorak pohranjen u hladnjaku na 4 °C i kao zamrznuti eksperimentalni uzorak na -18 °C tijekom šest mjeseci. Nakon toga, sirevi su pakirani trima različitim metodama pakiranja: aerobnom, vakuum ili modificiranom atmosferom. Citronska, jabučna, mravlja, octena, mliječna, pirogroždana i propionska kiselina analizirane su HPLC metodom nakon 1., 3., 6., 9. i 12. tjedna razdoblja čuvanja. Mliječna kiselina bila je najvažnija organska kiselina, dok je pirogroždana imala najniži udjel u svim uzorcima sira. Tijekom skladištenja došlo je do povećanja udjela citronske i mravlje kiseline smrznutih uzoraka, dok su udjeli jabučne, octene, pirogroždane i propionske kiseline opadali u odnosu na početak pohrane. Metode paki-

ranja i proces zamrzavanja također su statistički utjecali na udjel mliječne kiseline ($p < 0,05$). Koncentracije mravlje, octene i mliječne kiseline ohlađenih uzoraka bile su povećane, dok su citronske, jabučne i propionske kiseline opadale tijekom skladištenja. Razina pirogroždane kiseline nije se značajno promijenila. Utvrđen je statistički značajan utjecaj procesa zamrzavanja, vremena pohrane i metoda pakiranja na koncentracije organskih kiselina ($p < 0,05$).

Ključne riječi: organske kiseline, kozji sir, zamrzavanje, pakiranje

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