

CHANGES ON PHYSICOCHEMICAL, LIPOLYSIS AND PROTEOLYSIS OF VACUUM-PACKED TURKISH KASHAR CHEESE DURING RIPENING

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ABSTRACT

The vacuum-packed Turkish Kashar cheese ripening was evaluated in the medium and internal zones of cheese throughout a 90 day storage period. The parameters analyzed were; lipolysis level, water soluble nitrogen (WSN), total nitrogen, ripening index (RI), trichloroacetic acid-soluble nitrogen (TCA-SN), moisture, salt and salt in moisture, fat values and sensorial properties. The moisture values, acidity, salt and salt in moisture was less internal section. The lipolysis level, RI, TCA-SN ratios of internal section of cheese was less than medium section. Lipolysis level, RI, TCA-SN values, salt content and salt in moisture of the cheese samples increased continuously until end of the ripening but total nitrogen and fat content shown no significant changes with ripening, as moisture values decreased slightly. The ripening period was the main factor affecting sensory properties of cheese in medium and internal zones.

KEYWORDS: Kashar cheese, lipolysis and proteolysis.

INTRODUCTION

Kashar cheese which is a semi-hard cheese is one of the most important cheese varieties manufactured in Turkey. After ripening period it has a unique flavour, taste and aroma. Cheeses similar to this type are extensively manufactured in Balkan countries known as Kashkaval and Kasserli [11].

Cheese ripening clearly involves a very complex series of interrelated events, resulting in the development of the flavour and texture characteristic of the cheese variety. Proteolysis level of cheese is also influenced by several factors as pH, salt and moisture content of curds, ripening temperature and time. Proteolysis is probably the most important biochemical event, having a major impact on flavour and texture of most cheese varieties. The biochemical pathways through which lactose, lactate, milk fat and caseins are converted to flavour compounds are now known in general terms, although much remains to be discovered, particularly with respect to secondary, reaction, amino acid catabolism, and in the area of the interactions of products of various secondary reactions [15]. Proteolysis affects the level of intact casein, which is a major determinant of the firmness and fracture properties of cheese [3]. Lipids in foods may undergo hydrolytic or oxidative degradation. However, in cheese, oxidative changes are very limited due to the low oxidation reaction potential. The triglycerides in all cheese varieties undergo hydrolysis by the action of indigenous, endogenous lipases, which result in the liberation of fatty acids in cheese during maturation [20]. This study was conducted with the objective of observing the changes on some physicochemical properties, lipolysis and proteolysis levels of vacuum-packed Kashar cheese during ripening.

MATERIALS AND METHODS

Cow's milk was supplied from in a commercial dairy plant in Van and Kashar cheeses produced in same place. The microbial enzyme was obtained from Mayasan Company®, Istanbul. Raw cow's milk was pasteurized at 65°C for 30 min, cooled to 32°C. Milk was coagulated with rennet for 45 min. After coagulation, curds were pressed. The cheese curds were fermented in 15±2°C temperature with extension 12 h (night) and lather cut into small slices (about 5x10 cm). The small slices curds were hand-stretched in to the 85°C hot water. They were put into cylindrical plastic molds. The cheeses in molds were removed. The cheeses were salted with dry salt (the amount of dry salt used was 5 % of cheese weight) and kept 5 days at 15±2°C. the fresh Kashar cheese was approximately 500g. At the end of this time, cheese

samples were vacuum packed in plastic bags. After that, cheese samples were ripened at 4±1°C for 90 days.

Lipolysis analysis

Lipolysis was done using the BDI method and measured as Acid Degree Value (ADV). For this test, 10 g of finely ground sample were placed in a lipolysis butyrometer. Twenty ml of BDI reagent (30 g triton X-100 70 g sodium tetra phosphate in 1 L distilled water) were added and the butyrometers were placed in a boiling water bath for 20 min to extract the fat. The mixture was centrifuged for 1min. and enough aqueous methanol was added to bring the fat into neck of butyrometer and centrifuged for 1 another min. Then, the fraction of liquid fat was transferred into a 50 ml glass and weighed. Five ml fat solvent (4:1 petroleum ether and n-propanal) were added to the flaks. This was titrated with 0.02 KOH and total free fatty were calculated [2].

Proteolysis analysis

Water soluble nitrogen (WSN) and trichloroacetic acid-soluble nitrogen (TCA-SN) was determined by the Kjeldahl method described as follows [21]; 10 g sample was homogenized with 100 ml distilled water and filtered. The nitrogen content of the extracted cheese was expressed as a percentage of total nitrogen (WSN/TN, %), which was described as a ripening index. Trichloroacetic acid-soluble nitrogen (TCA-SN) was determined in the same cheese as extract described above. Ten ml 24% TCA was added to 10 ml cheese extract. After mixing, it was incubated at 4°C for 2 h. Then, precipitate was filtered through Whatman no 40 paper. Filtrate of the nitrogen was determined by Kjeldahl method.

Cheese samples were analyzed for dry matter, total nitrogen, fat, salt, titratable acidity as lactic acid (LA %) and pH values were determined according to the methods described by Case et al. [2].

Sensorial analysis

Organoleptic assessment of the cheeses after 5, 30, 60, and 90 d of ripening was carried out by a five-member panel of the selected on the basis of interest in sensory evaluation of Turkish Kashar cheeses. The samples were presented to panellists in randomized order after having stood for 4 h at room temperature and were graded between 1-10 (1: very bad and 10: very good) for appearance and colour, body and texture, flavour and acceptability [13]. Panel members were also instructed to report any defects in appearance, body and texture, flavour and acceptability. Water was provided for mouth washing between evaluations of samples.

Statistical analysis

In this study, the production and storage of Kashar cheese together with all due analyses were performed in duplicate. All statistical calculations were performed using SAS Statistical Software [19], and the obtained values are presented as mean \pm standard deviation. Evaluation of significance was performed by analysis of variance, followed by Duncan's multiple range tests. The significance levels of $P < 0.05$ was used for statistical differences.

RESULT AND DISCUSSION

Changes on lipolysis and proteolysis

The lipolysis and proteolysis values of vacuum-Kashar cheese samples during ripening are shown in Table 1. The lipolysis level of internal section of cheese was importantly less than medium section. Differences in the values of lipolysis among the cheeses were significant ($P < 0.05$). As the ripening advanced, differences between the cheese samples in terms of fat acidity became clearer. In the Kashar cheeses, the lipolysis values showed increase linearly at a significant level during ripening ($P < 0.05$). These values are lower than the results reported by Guler and Uraz [6] on commercial Kashar cheeses, but similar to those reported by Dervisoglu and Yazici [4] on Kulek cheese produced with non-starter. These differences might be due to packed or unpacked positions, differences of the milk, manufacturing procedure, and ripening conditions etc. Lipolytic agents in cheese generally originate from the milk, the coagulant (in the case of rennet paste) and the cheese microflora (starter, non-starter and adjunct microorganism). Milk contains a potent indigenous lipase, lipoprotein lipase (LPL). LPL activity is of most significance in raw milk cheeses as

the enzyme is largely inactivated by pasteurization, although $78^{\circ}\text{C} \times 10 \text{ s}$ is required to inactivate this enzyme completely [15].

The total nitrogen values of cheeses were between 4.09 to 4.22% (Table 1). There were no significant differences between the cheese samples in terms of total nitrogen content during ripening ($P > 0.05$). The higher mean nitrogen content was obtained in the cheeses in medium zones; a nitrogen content of other in cheese zones was found lower. It can be concluded that the nitrogen contents were slightly affected by moisture contents of the cheeses.

The WSN fraction contains small molecules of proteins (non-casein), peptides and free amino acids, and commonly used as an index of ripening [7]. A comparison of the proteolysis data for the cheeses belonging to days of 5, 30, 60, and 90 of ripening were given in Table 1. The WSN values of the cheeses were affected significantly ($P < 0.05$) from the ripening period. The proteolysis values increased both internal and medium zones of cheeses throughout ripening, but level of internal cheese was significantly ($P < 0.05$) higher than medium cheese end of the ripening. The formation of water soluble nitrogen compounds during ripening is an index of the rate and extent of proteolysis, in that it is an indicator of casein hydrolysis brought about by the action of the rennet and the milk proteases present at the start of ripening [10]. The proteolysis values were similar to the values already observed by Tuncturk [22] for no packed Kashar cheese, Gulec et al. [5] for Kashar packed with different materials and, Pavia et al. [17] on vacuum packed Manchego-type cheese.

Trichloroacetic acid-soluble nitrogen ratio of internal cheese was higher slightly medium cheese ($P > 0.05$).

Table 1 Lipolysis and proteolysis of Kashar cheeses

		Ripening times (day)			
		5	30	60	90
Lipolysis (ADV)	Medium	1.91 \pm 0.20	2.11 \pm 0.14	2.75 \pm 0.10 a	3.20 \pm 0.14 a
	Internal	1.89 \pm 0.18	1.87 \pm 0.08	2.37 \pm 0.16 b	2.61 \pm 0.11 b
Total N (%)	Medium	4.18 \pm 0.15	4.22 \pm 0.11	4.18 \pm 0.11	4.19 \pm 0.08
	Internal	4.09 \pm 0.09	4.04 \pm 0.10	4.06 \pm 0.09	4.05 \pm 0.07
WSN (%)	Medium	0.44 \pm 0.04	0.51 \pm 0.03	0.74 \pm 0.06	0.88 \pm 0.05 a
	Internal	0.43 \pm 0.01	0.47 \pm 0.03	0.68 \pm 0.03	0.76 \pm 0.01 b
Ripening index (%)	Medium	10.50 \pm 1.06	12.18 \pm 0.61	17.71 \pm 0.89	20.95 \pm 1.44 a
	Internal	10.59 \pm 0.41	11.73 \pm 1.07	16.67 \pm 0.94	18.70 \pm 0.41 b
TCA-SN (%)	Medium	0.32 \pm 0.06	0.36 \pm 0.03	0.52 \pm 0.03	0.63 \pm 0.08
	Internal	0.31 \pm 0.02	0.35 \pm 0.04	0.50 \pm 0.02	0.60 \pm 0.03

^{ab} indicate different between medium-internal sections of cheese $P < 0.05$

Trichloroacetic acid-soluble nitrogen (TCA-SN) is known to be an indication of the amount of small peptides and amino acids present in cheese and its level is regarded as the ripening depth index. These peptides and amino acids are contributed mainly by the action of micro organisms on the caseins and their peptides [21]. The differences of the proteolysis between the different cheese zones were probably due to differences of microflora. The mean TCA-SN values significantly ($P<0.05$) increased until end of the ripening. Several authors have found that advancing the ripening time, leads to an increase in protein degradation in cheeses [9, 12 and 21].

McGuan et al. [14] and Aston and Creamer [1] studied the components of a water soluble fraction of cheddar cheese and linked those components to the flavour intensity of the cheese. Park et al. [16] found that the amount of WSN in cheese after manufacture was 6.78% and after 6 months of ripening for waxed and unwaxed cheeses this increased to 27.64% and 16.74%, respectively. Similarly, in this study the quantity of WSN also increased during maturation. The mean WSN value of medium cheese was higher than of the internal cheese zones.

Physicochemical changes

The chemical composition of Kashar cheeses at different ripening times are presented in Table 2. The moisture contents of internal cheeses were significantly higher than medium zones ($P<0.05$). The moisture ratios were not affected with ripening times. This is due to the fact that vacuum-wrapping with polyethylene film restricted the moisture loss [5]. The contrast moisture values, salt and salt in moisture was less internal zones. The rate of salt absorption was very high at the first month due to a movement of NaCl molecules as a result of the osmotic

pressure and difference moisture contents of cheeses [7]. These results agree with those reported by Pavia et al. [17]. The effects of salt include the control of microbial growth and activity, control of various enzyme activities in cheese, reduction of cheese moisture content, physical changes in cheese proteins that can influence cheese texture, and flavour development [8]. The fat content of samples was between 26.50 to 27.50%. The fat content in internal zones was decreased compare to the medium zone. The higher mean salt and fat contents were obtained in the cheeses in medium zones. It can be concluded that the fat and salt contents were affected by moisture contents of the cheeses.

Titrate acidity increased until 60 days of ripening and then in ripening period, made a little change, and this results is agree with those reported by Kurultay et al. [12] for Kashar cheese addition different hydrocolloids and Tarakci [21] for vacuum-packet herby cheese. The initial increase in acidity is due to lactic acid and hydrogen formation [4]. The following decrease in acidity may be happen due to the consequence of moulds and formation of alkaline nitrogenous compounds [7]. The pH values of cheese samples were shown similar changes.

Sensorial properties

The results of the sensory panel's assessment of cheese quality during ripening for 5, 30, 60 and 90 days are given in Table 3. The appearances and colour of the experimental cheese was considered good at medium zones, and did not significantly differ from the internal zones of the cheese. The body and texture scores for the internal and medium zones of the cheese were non significant ($P>0.05$). Appearances and colour, body and texture, flavour, acceptability scores increased

Table 2 Physicochemical composition of Kashar cheeses

		Ripening times (day)			
		5	30	60	90
Moisture (%)	Medium	40.83±1.50	39.80±1.74	40.17±1.47 b	39.15±1.76 b
	Internal	42.39±0.91	41.82±0.82	42.55±1.85 a	41.46±1.70 a
Salt (%)	Medium	3.41±0.35 a	3.89±0.10 a	3.94±0.22 a	3.91±0.19 a
	Internal	2.74±0.17 b	3.21±0.16 b	3.46±0.06 b	3.42±0.13 b
Salt in moisture (%)	Medium	8.36±0.72 a	9.78±0.58 a	9.83±0.74 a	10.01±0.96 a
	Internal	6.46±0.25 b	7.67±0.31 b	7.43±0.32 b	8.25±0.54 b
Fat (%)	Medium	27.05±0.75	27.08±0.38	27.35±1.04	27.50±0.50
	Internal	26.67±0.58	26.42±0.52	26.35±0.76	26.83±0.29
Acidity (%LA)	Medium	1.20±0.04	1.41±0.04	1.44±0.07	1.30±0.04
	Internal	1.14±0.03	1.30±0.08	1.40±0.03	1.32±0.05
pH	Medium	5.59±0.14	5.46±0.03	5.54±0.03	5.48±0.06
	Internal	5.48±0.04	5.42±0.04	5.61±0.04	5.44±0.05

^{ab} indicate different between medium-internal sections of cheese $P<0.05$

Table 3 Sensorial scores of Kashar cheeses

		Ripening times (day)			
		5	30	60	90
Appearances and colour	Medium	7.2±0.55	7.8±1.30	8.4±1.58	9.0±0.71
	Internal	7.5±0.89	7.2±1.23	8.0±1.64	8.5±0.84
Body and texture	Medium	7.2±0.71	7.4±1.14	8.0±0.55	8.5±1.34
	Internal	7.0±1.30	7.4±0.90	8.0±1.79	8.4±1.10
Flavour	Medium	7.0±0.84	7.4±1.52	8.2±1.30	9.0±1.23
	Internal	7.0±1.00	7.2±0.84	8.0±0.45	8.6±1.58
Acceptability	Medium	7.0±0.71	7.2±0.55	8.4±0.89	9.0±1.14
	Internal	7.0±0.45	7.4±0.89	8.0±0.71	8.0±0.45

generally during ripening. These scores of internal cheese zones were slightly lower medium scores and between section was no important ($P>0.05$). As regards to acceptability scores of medium cheeses were preferred more than that other section. The reason why panellists liked medium cheeses was it's to be perceive properties more taste, texture, colour and appearance.

CONCLUSION

It was observed that different zones had no influence on the salt, acidity and pH values of vacuum-Kashar cheese. As the moisture internal zones were higher than medium, salt, salt in moisture content were contrast shown. The total nitrogen and water soluble nitrogen in cheese increased during maturation. The proteolysis values and lipolysis level of medium cheese increased importantly, but was no presented different TCA-SN ratios and sensorial properties of samples increased with ripening. Appearances and colour, body and texture, flavour, acceptability properties of medium cheese zones were more preferred compared with internal cheeses.

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