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The impact of elevated temperatures on accelerating the ripening process of kachcaval cheese manufactured in Syria

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Abstract

This research was carried out at the laboratories of Food Science Department, Faculty of Agriculture, Damascus University, to investigate the effect of high temperatures on accelerating the ripening of kachcaval cheese, through determining the chemical and sensory indicators of ripened cheese using three temperatures (16°C, 20°C for a week) then completing the ripening process up to 60 days at 10°C and comparing the results a control cheese sample ripened at 10°C for 60 days. The results showed that using elevated temperatures led to reduction in the moisture content, accelerated the proteolysis and significantly increased the ripening index comparing with the control cheese sample. The sensory evaluation indicated that using elevated temperature at 16°C for one week then completing the ripening up to 60 days at 10°C decreased the ripening 15 days, and the cheese possessed a distinctive flavor and similar to the control cheese. Using 20°C for one week then complete the ripening up to 60 days at 10°C negatively affected the sensory properties of the ripened cheese at the day 30, where the cheese had strong undesirable flavor and taste and the texture was more tender.

Introduction

The ripening and formation of flavor in cheese is considered a complicated and slow process which include different chemical and biochemical changes of milk components, and hence determine the distinguish characters of every kind of cheese (Singh *et al*., 2003; Weimer, 2007). The process of cheese ripening can take two weeks and up to two years according to the cheese kind (Mcsweeney and Sousa, 2000). Moreover, this process is hard, expensive and it is not completely accurate (Yvon and Rijnen, 2001; Smit *et al*., 2002). The researchers worldwide have seeked to find solutions to accelerate the ripening of cheese using different means and methods in order to reduce the cost of production and accelerate the capital cycle.

There have been several means and methods to accelerate the ripening of cheese such as, using higher temperatures for ripening, adding attenuated starters, using adjunct starter, adding external enzymes, using starter of genetic modified bacteria, applying high pressure treatments, adding ripening enzymes capsules and recombined enzymes (Saldo *et al*., 2000; Azarnia *et al.*, 2006). Using elevated temperatures have been considered one of the easiest methods in accelerating the ripening of cheese due the low cost of cooling and reducing ripening time to preserve the products (Hannon *et al*., 2005). However, many complicated biochemical reactions which occur during ripening period cannot be accelerated equally during using high ripening temperatures, and undesirable flavor might be produced (Fox *et al.*, 1996). Therefore, different reactions related to cheese ripening must be evaluated (Sihufe *et al*., 2010).

The results of Folkertsma and Fox (1992) showed the hastening pattern of proteolysis and lipolysis with increasing the temperature of ripening and slow cooling of cheese, and noticed that the ripening speed increased or decreased due to changes in temperatures, where cheese ripened at 16°C obtained higher degree of flavor especially during the early stages of ripening, but the structure deteriorated during the long period of ripening at temperature 16°C, and hence concluded that 12°C was the optimum for accelerating the ripening of cheddar cheese.

Hannon *et al*. (2005) in a study on cheddar cheese showed that the time of exposing the cheese to high temperatures had an influence on cheese properties, and concluded that ripening cheese at 20°C for one week and 12°C for six weeks and 8°C for eight months of ripening helped in reducing the time for two months as well as maintained the desirable properties of taste and flavor of cheddar cheese, and noticed the variation of proteolysis and lipolysis indicators depending on the exposure time of ripening at higher temperatures, where the values of these indicators

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increased with increasing the ripening temperature.

Sihufe *et al.* (2007) investigated the effect of elevated temperatures on Reggianito cheese in Argentina, which was at 12°C and 18°C and 85% relative humidity, and revealed a decrease in the moisture content during ripening the cheese, high temperatures for 186 days, whereas the pH increased. Similar results were reported by (Perotti *et al*., 2004; Sihufe *et al*., 2010). The ripening index is influenced by the applied temperature and time of ripening, where the index increases by increasing the ripening temperature. It has been noticed that the effect of ripening temperature was clearly during the last two months of ripening (Sihufe *et al*., 2007).

Ceruti *et al.* (2012) studied the effect of temperature and time of ripening on proteolysis of Reggianito cheese in Argentina, where the control cheese stored at 12°C for six months and the treated cheese at 20°C for 2 or 4 weeks and afterward at 12°C for six months. The results showed an increase in proteolysis when elevated temperatures were used, where a higher level of proteolysis products in cheese stored at 20°C for four weeks. Moreover the cheese ripened for 124 days was similar in properties to the control cheese ripened for 180 days.

Kachcaval cheese is considered as one of the most important kinds of ripened and produced cheese in Syria, which requires to be ripened for sixty days at 10°C. The product during this period is exposed to various threats of spoilage due to undesirable fermentation. Consequently, the major aim of this research was to accelerate the ripening period using elevated temperature and investigate the effect of elevated ripening temperature on the chemical and sensory properties of ripened cheese.

Materials and Methods

Manufacturing of Kachcaval cheese

Cheeses were manufactured according to the standard procedure (Barthelemy and Sperat-Czar, 2004), milk was Pasteurized at 73°C for 15 seconds then cooled at 38°C for 30 minutes. 0.5 % of starter culture was added (*Lactococcus lactis subsp cremoris* and *Lactococcus lactis subsp lactis*). 10 % calcium chloride was added in the form of salt solution at a rate of 3 ml/ L of milk, the coagulant 100% Chymosin (Hansen) was added at the rate of 2 g/100 L of milk (according to the recommendation of the manufactured company). The milk was incubated at 38°C for one hour. The curd was suspended until the pH reached up to 6.0 and a part of the whey was disposed. The temperature of curd was increased up to 40°C with continues stirring of curd up to reaching the required pH 5.2, after that the whey was separated from curd by filtration. The cheese was pressed and the curd cut into thin slices and cooked with salt solution (10% sodium chloride) at 85°C until the curd reached 59°C during cooking. As the cooking process completed, the curd was placed in a matrix. The experiment was conducted in a complete randomized design with three replications.

Ripening process

The prepared cheese was divided into three parts, and every part was ripened at different temperature, as following: The first part (control): the control was ripened at 10°C for one week at 80–85% relative humidity. After drying the curd, the formed matrices were wrapped using special covers made of polyamid, polyethylene and cellulose, and the ripening process was completed for sixty days at the same temperature. The second part (first treatment): ripened at 16°C for one week at 80–85% relative humidity, and after drying the curd, the formed matrices were wrapped under evacuation using special covers made of polyamid, polyethylene and cellulose, and completing the ripening for sixty days at a temperature f 10°C. The third part (second treatment): ripened at 20°C for one week at 80–85% relative humidity, and after drying the curd, the formed matrices were wrapped under evacuation using special covers made of polyamid, polyethylene and cellulose, and completing the ripening for sixty days at 10°C.

Chemical analysis

Moisture, fat and titratable acidity content were estimated according to the approved method of (AOAC, 1999). Salt was measured using Mhor test according to the approved method of (AOAC, 1990). Total nitrogen content (TN) Water soluble nitrogen (WSN) and non-protein nitrogen (NPN) were determined by the micro- Kjeidahl method. WSN was obtained via fraction with water followed by acidification to pH 4.6 (Tarakci, 2004). NPN fraction, i.e. the nitrogen fraction soluble in trichloroacetic acid (TCA) 12% was estimated by blending the grated cheese sample in trichloroacetic acid followed by centrifugation at 3000× g for 30 minutes at 4°C in order to precipitate the protein compounds (Moatsou *et al*., 2001). Ripening index was expressed as a percentage of WSN of the cheese TN (WS*100/ TN) according to (Ardo and Polychroniadou, 1999). Evolution of free amino group was monitored according to (Folkertsma and Fox, 1992), using Cd Neinhydrine reagent (Sigma, USA). A sample (25-50 µl) of WSE (water soluble extract) was diluted to 1 ml

Table 1. Chemical composition of Kachcaval cheese ripened on different temperatures

	Time (days)	Control 10°C/60 days	Treatment 1 16°C /week then 10°C till 60 days	Treatment 2 20°C /week then 10°C till 60 days
Moisture	7	45.80± 0.03 ^{Aa}	45.26 ± 0.02 ^{Aab}	44.50 ±0.02 ^{Ab}
	60	45.20± 0.03 ^{Aa}	44.66C± 0.03 ^{ab}	44.00± 0.03 ^{Bb}
Fat	7	23.50±0.01 ^{Aa}	23.90±0.02 ^{Ab}	24.40±0.01 ^{Ac}
	60	24.00±0.03 ^{Ba}	24.50±0.03 ^{Bb}	24.80±0.03 ^{Bc}
Protein	7	21.12±0.1 ^{Aa}	21.42A±0.15 ^{ab}	22.54±0.1 ^{Ab}
	60	22.01±0.2 ^{Aa}	22.51±0.2 ^{Aab}	23.80±0.1 ^{Bb}
Salt	7	1.90±0.05 ^{Aa}	2.00±0.07 ^{Aa}	2.10±0.05 ^{Aa}
	60	2.2±0.01 ^{Ba}	2.3±0.02 ^{Ba}	2.40± 0.03 ^{Ba}

Capital letters in the same column indicate significant differences between periods of each treatment at P<0.01.
Small letters in the same row indicate significant differences between treatments at P<0.01

with distilled water; to this, 2 ml of Cd Neinhydrine reagent were added. The mixture was heated at 84°C for 5 minutes, cooled to room temperature, and absorbance was read at 507nm.

Stock solutions of 2Mm glycine (Merck) were used to obtain calibration curves for this method. All analysis were performed in duplicate. The cheese were evaluated organoleptically by a team of experienced cheese graders. The cheese samples were characterized by appearance of color, cutting, texture and flavor (oder and taste) during ripening period according to Alizadeh *et al.* (2006) which depends on a scale of 30 grades distributed as follow, taste 10 grades, odor 5 grades, color 5 grades, structure 5 grades and cutting 5 grades.

Cheese samples were analyzed chemically and organoleptically after 7, 14, 30 , 45 and 60 days. The statistical analyses were performed using simple statistical procedure to calculate the means and the standard deviations. ANOVA and least significant differences were conducted among the means at a level of (P<0.01).

Results and Discussion

Effect of ripening temperatures on the chemical composition of Kachcaval cheese:

The results in Table 1 clearly indicated that increasing ripening temperature to 16°C and 20°C leaded to a significant decreasing (P>0.01) in the cheese moisture content and a significant increasing in fat and protein contents comparing with the control at the day seven of ripening . These results were in agreement with the findings of (Exterkate, 1987; Bertola *et al.*, 2000; Peta and Eva-Moria, 2000; Rasheed *et al.*, 2010). The results in Table 1 also referred to increasing in salt absorption under increasing the ripening temperatures, these results

Table 2. The estimation of the Non-Protein Nitrogen, Non-Casein Nitrogen and Free Amino Groups in Kachcaval cheese

Indicator \ Treatment	Time (day)	Control 10°C/60 days	Treatment 1 16°C /week then 10°C till 60 days	Treatment 2 20°C /week then 10°C till 60 days
Non-Protein Nitrogen (NPN) (g N/ 100 g cheese).	7	0.018 ± 0.01 ^{Aa}	0.027 ±0.012 ^{Aa}	0.057 ±0.01 ^{Ab}
	15	0.037 ± 0.013 ^{ABa}	0.04 ± 0.011 ^{Aa}	0.09 ±0.01 ^{ABb}
	30	0.057 ±0.02 ^{BCa}	0.08 ±0.03 ^{Bb}	0.123 ±0.01 ^{Bc}
	45	0.083± 0.01 ^{Ca}	0.121 ±0.02 ^{Cb}	0.183 ±0.03 ^{Cc}
	60	0.153 ±0.012 ^{Da}	0.190 ± 0.01 ^{Db}	0.273±0.013 ^{Dc}
Non-Casein Nitrogen (NCN) (g N/ 100 g cheese)	7	0.3 ±0.033 ^{Aa}	0.397 ±0.023 ^{Ab}	0.567± 0.023 ^{Ac}
	15	0.507 ±0.02 ^{Ba}	0.606 ± 0.023 ^{Bb}	0.8 ± 0.013 ^{Bc}
	30	0.756 ±0.03 ^{Ca}	0.850± 0.03 ^{Cb}	1.01 ±0.03 ^{Cc}
	45	0.932 ±0.033 ^{Da}	1.072 ±0.034 ^{Db}	1.278 ±0.023 ^{Dc}
	60	1.066 ± 0.033 ^{Ea}	1.22 ±0.023 ^{Eb}	1.487± 0.013 ^{Ec}
Free Amino Groups (glycine equivalent/100g cheese).	7	0.33 ±0.09 ^{Aa}	0.456 ±0.09 ^{Ab}	0.533 ± 0.09 ^{Ac}
	15	0.672 ±0.08 ^{Ba}	0.749 ±0.06 ^{ABb}	0.784 ± 0.05 ^{Bb}
	30	0.781 ±0.09 ^{Ca}	0.814 ±0.07 ^{ABb}	1.08 ±0.07 ^{Cc}
	45	0.816 ± 0.04 ^{Da}	0.952± 0.08 ^{Bb}	1.850 ±0.05 ^{Dc}
	60	0.962± 0.07 ^{Ea}	1.940 ±0.03 ^{Cb}	2.20± 0.04 ^{Ec}

Capital letters in the same column indicate significant differences between periods of each treatment at P<0.01
Small letters in the same row indicate significant differences between treatments at P<0.01

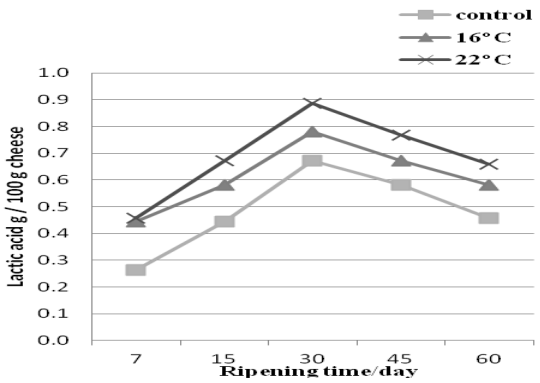

Figure 1. Titratable acidity (g lactic acid/100 g cheese)

agreed with the results of (Hannon *et al.*, 2005).

The results in Figure 1 showed the titratable acidity (g lactic acid/100 g cheese). It was noticed an increase in acidity percentage under higher temperatures of ripening, and it was 0.264% in control treatment whereas it was 0.440 and 0.456% in the first and the second treatments respectively during first week of ripening, these results were in conformity with the findings of (Sihufe *et al.*, 2007). Titratable acidity increased significantly during ripening period and reached during the day 30 up to 0.672% in the control cheese sample, 0.780% in the first treatment cheese and 0.888% in the second treatment, whereas it significantly decreased in the day 45 and 60 in all treatments. This may be due to the growth of some microorganisms like yeast which consumed lactic acid and the production of alkaline nitrogen compounds resulted from proteolysis and helped in decreasing the acidity, these results were

Table 3. Sensory characteristic of Kachcaval cheese ripened at different temperatures

Treatment	days	odor	Taste	Color	Texture	cutting	Total
control 10°C/60 days	15	3±0. 3 Aa	6± 0.4 Ba	4±0.2 Aa	4± 0.3 Aa	4±0. 3 Aa	22± 0.2 Ba
	30	3.6±0. 3 Aa	6.8± 0.2 ABa	4±0.3 Aa	4± 0.3 Aa	4±0.1 Aa	22.4 ±0.3 ABa
	45	4 ± 0. 1 Aab	7.4±0.4 ABab	4±0.2 Aa	4±0.2 Aab	4±0. 1 Aab	23.4 ±0.1 ABab
	60	4 ± 0. 2 Aab	8±0.5 Aab	4.6±0.3 Aa	4±0. 2 Aa	4.6±0. 2 Aa	25.2 ± 0.3 Aab
Treatment 16°C /w then 10°C 60 days	15	3.8 ± 0.3 Aa	6.8±0.3 Ba	4.2±0.1 Aa	4.4±0.3 Aa	4.2±0. 3 Aa	23.4±0.3 Ba
	30	4.2 ±0.1 A a	8±0.2 ABa	4.2± 0.1 Aa	4.2± 0. 1 Aa	4.2±0.4 Aa	24.8±0.1 ABa
	45	4.6 ± 0.2 A a	9±0. 1 Aa	4.6±0.1 Aa	4.6±0. 3 Aa	4.6±0.2 Aa	27.4±0. 2 Aa
	60	4.6 ±0.2 Aa	9±0. 2 Aa	4.6 ±0.1 Aa	4.6± 0. 2 Aa	4.6±0.1 Aa	27.4±0. 3 Aa
Treatment 20°C/w then 10°C 60 days	15	4± 0.3 A a	7.6±0.6 Aa	4.4± 0.1 Aa	4.4±0.3 Aa	4.4±0.5 Aa	24.8±0.5 Aa
	30	4± 0.5 Aa	7.2±0.5 ABa	4.2±0.1 Aa	4 ±0.3 ABa	4±0.2 ABa	23.4±0.4 ABa
	45	3.2± 0.4 Bb	6.4±0.5 ABb	4±0.2 Aa	3±0. 3 ABb	3±0.1 ABb	19.6±0.3 ABb
	60	2.6± 0.4 Bb	6± 0.2 Bb	2.6±0.1 Ba	2.6±0. 2 Bb	2.6 ±0.1 Bb	16.4±0.1 Bb

Capital letters in the same column indicate significant differences between periods of each treatment at P<0.01
Small letters indicate significant differences between treatments at the same time of ripening at P<0.01

in conformity with the findings of (Kurultay *et al*., 2000; Novella *et al.*, 2002; Tarakci and Kucukoner, 2006; Sihufe *et al.*, 2007).

Effect of ripening temperatures on the proteolysis of Kachcaval cheese:

The results in Table 2 presented the content of non-protein nitrogen in the ripened Kachcaval cheese at different ripening temperatures. It was noticed that increasing the temperature of ripening from 10°C to 16°C for one week did not significantly affect the non-protein nitrogen percentage in the cheese during the first and the second week of ripening (0.027 and 0.040 g N/100 g cheese respectively) as compared to (0.018 and 0.037 g N/100 g cheese respectively) in the control treatment. However, there was a significant increase in non-protein nitrogen percentage in the cheese starting from the day 30 of ripening until the end of ripening. There was significant increase in non-protein nitrogen percentage in the cheese starting from the first week of ripening (0.057 g N/100 g cheese) when the ripening temperature increased to 20°C.

The percentage of non-protein nitrogen increased gradually and significantly with increasing the ripening time of the cheese in all treatments, the increase was more in the second treatment cheese during the day 60 of ripening (0.273 g N/100 g cheese), whereas it was (0.190 g N/100 g cheese) in the first treatment cheese and (0.153 g N/100 g cheese) in the control.

The data in Table 2 indicated to a significant increase in the percentage of non-casein nitrogen in Syrian Kachcaval with increasing the temperature of ripening. The percentage increased from 0.3 g N/ 100 g cheese in the control treatment and reached up to 0.397 and 0.567 g N/ 100 g cheese in the first and second treatment respectively in the day 7 of ripening, and later on increased gradually and

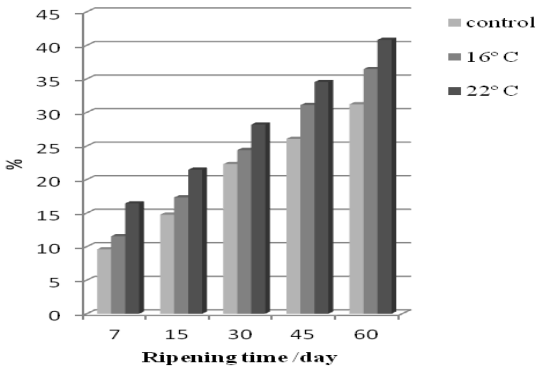

Figure 2. Ripening index of Kachcaval cheese

significantly until reaching 1.066 in the control and 1.220 and 1.487 g N/ 100 g cheese in the first and the second treatments respectively in the day 60 of ripening. These results were similar to the results of (Aston *et al*., 1985; Gaya *et al*., 1990; Hannon *et al*., 2005; Rasheed *et al.*, 2010).

Table 2 represented the free amino groups in Kachcaval cheese estimated by Ninhydrine method (glycine equivalent/100 g cheese). The results clearly indicated a significant increase in free amino groups with increasing the ripening temperature, where increased from 0.33 in the control cheese and reached 0.456 and 0.533 glycine equivalent/100 g cheese in the cheese of the first and the second treatment respectively. This increase coincided with increases in non-protein nitrogen and non-casein nitrogen. These values gradually increased by ripening, and it was observed that the first treatment at the day 45 reached the equivalent amount at the day 60 for the control sample, whereas the cheese in the second treatment surpassed these values in the day 30 of ripening. These results were in conformity with the findings of (Hannon *et al.*, 2005) in a study of free amino acids in cheddar cheese.

The ripening index was measured to estimate the effect of ripening temperature on proteolysis (Figure

2). The data in the figure clearly showed an increase in the ripening rate with increasing in the ripening temperature of cheese as compared with the control, and there was an increase in the ripening rate during the full period of cheese ripening, where the cheese in the first treatment (ripened at 16°C for one week) reached to the required ripening degree in the day 45 of ripening which was similar to the ripening rate of the control cheese in the day 60 of ripening. There was higher and faster increase in the ripening index in the second treatment cheese ripened at 20°C for one week, but this negatively affected the sensory properties of cheese.

Effect of ripening temperature on the sensory characteristics of cheese:

The data presented in Table 3 showed the results of sensory evaluation for cheese samples ripened at different ripening temperature. The results of sensory tests confirmed the results of chemical analysis that using relatively high ripening temperature for one week resulted in shortening the time of ripening with conserving the sensory properties of cheese. It was noticed that ripened cheese at 16°C reached to optimum sensory properties during 45 days of ripening as compared to 60 days in the control treatment. The results also indicated that increasing the ripening temperature to 20° C largely fastened the ripening of cheese which was reflected on the sensory properties of cheese, where rancidness taste and soft structure appeared due to proteolysis and lipolysis. These results were in agreement with the findings of (Hannon *et al*., 2005; Rasheed *et al*., 2010).

Conclusions

Using relatively elevated temperature (16°C for one week and completing ripening at 10°C) helped in fastening the ripening of Kachcaval cheese and shortening the time of ripening approximately 15 days and maintained the optimum sensory properties of cheese comparing with the control. But applying high ripening temperature (20°C for one week and completing ripening at 10°C) resulted in a high increase in the rate of ripening and a decline in the sensory properties of cheese starting from the day 30 of ripening.

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