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Influence of processing and storage on chemical and biochemical characteristics of Mish cheese traditionally produced in Jordan

L.M. Ahmad[1], K.M. Al-Ismail[2*] and M.I. Yamani[2]

[1]Directorate of Laboratories, Amman Food Laboratory, Jordan Food and Drug Administration, 11181 Amman, Jordan;
[2]Department of Nutrition and Food Technology, Faculty of Agriculture, The University of Jordan, 11942 Amman, Jordan;
kh.ismail@ju.edu.jo

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RESEARCH ARTICLE

Abstract

Mish is a soft pickled cheese produced traditionally in Jordan and Palestine. The influence of Mish cheese processing and storage on fat and cholesterol oxidation and hydrolytic rancidity was evaluated by determining peroxide value (PV), 7-ketocholesterol, and free fatty acid (FFA) content. Changes in Mish cheese acidity, salt, moisture, the antioxidant activity of water soluble nitrogen (WSN) measured by a super oxide dismutase (SOD) test and organoleptic evaluation were also evaluated. The results indicate that the extent of oxidation of fat and cholesterol was very low, fine PV and 7-ketocholesterol content after 20 weeks of storage were in the ranges 1.42-2.88 meqO_2/kg and 2.02-4.81 µg/g, respectively. The effect of storage was greater on hydrolytic rancidity i.e. the FFA content at the end period of storage ranged from 1.87-3.64%. There was a significant decrease in moisture and salt contents throughout the storage period, i.e. moisture decreased by 9-14% and salt decreased by approximately 5%. The WSN increased significantly with storage period, which increased the antioxidant activity. The aforementioned changes had no significant effect on the characteristic flavour and colour of the Mish cheese during the 20 months of storage.

Keywords: cholesterol oxidation, fat oxidation, free fatty acids, Mish cheese, organoleptic evaluation

1. Introduction

Cheese is produced throughout the world in a wide-range of flavours, textures, and forms (Milica *et al.*, 2008). White soft cheese is common in different parts of the world, particularly in the Mediterranean region. Several types of white cheese are known, such as Domiati in Egypt, Queso Blanco in Mexico, and Feta and Halloumi in various countries (Papademas and Robinson, 2001). Such cheeses are characterised by their relative high salt content and by being traditionally produced from raw milk without the use of a starter culture. Some of these cheeses are currently produced from pasteurised milk with or without the use of starter cultures. These cheeses are usually boiled in their whey (Halloumi), or in brine (Nabulsi), a treatment which can be considered equivalent to pasteurisation process which reduces their microflora content (Haddadin and Shahin, 1995).

During processing and storage of cheese, initial lactic acid bacteria (LAB) fermentation usually takes place affecting the major solid constituent of milk, lactose, protein, and fat (Humied *et al.*, 1990). Furthermore, cheese lipids may undergo lipolysis and oxidation which lead to the formation of various oxidation products such as aldehydes, ketones, acids, etc. (Laing and Jinks, 1996). These oxidation products are responsible for development of food rancidity, loss of nutritional value, and possible formation of compounds such as hydroperoxides, epoxides and cholesterol oxidation products (COPs), which can become toxic at high concentrations (Al-Ismail, 2002). The extant of lipid oxidation in milk products are affected by the severity of processing steps and storage conditions (Sander *et al.*, 1988). Al-Ismail and Humied (2003) found that the processing steps such as salting, and boiling in brine white cheese did not affect the peroxide value, free fatty acid (FFA) content or COPs; however, these attributes increased during the 9 months storage.

Peptides are short oligomers of amino acids linked by peptide bonds, but are commonly shorter in length than proteins. These peptides can be produced in protein containing food such as cheese by proteolytic processes with exogenous proteases (Bougatef *et al.*, 2010). Naqash and Nazeer (2010, 2011) observed the *in vitro* antioxidant activity of peptides purified from various marine sources. Abubakr *et al.* (2012) observed the antioxidant activity of peptides generated from fermented milk by LAB using diphenylpicrylhydrazyl test.

Mish is a soft pickled cheese produced traditionally in Jordan and Palestine. A similar type of cheese is still produced traditionally in Egypt and Sudan (Sulieman *et al.*, 2011). In Jordan, Mish cheese is seasonally produced at household level from soft white cheese (made from raw sheep, goat milk), green pepper, and shanenah (salted and diluted fermented skim milk obtained as a by-product in the process of traditionally produced butter). The cheese is prepared by placing alternate layers of fresh soft cheese and chopped green hot pepper, topped with shanenah, in a closed container, which is then stored at ambient temperature for several months. Fermentation by LAB, present in the ingredients, occurs during storage, resulting in the development of the distinctive sharp and salty flavour of Mish cheese.

Limited information is available regarding chemical and biochemical changes in Mish cheese. The goal of this study was to determine the influence of Mish cheese production steps and storage conditions on fat and cholesterol oxidation, hydrolytic rancidity, moisture and salt, lactic acid formation, and water soluble nitrogen (WSN) and their antioxidant activity.

2. Materials and methods

Production of Mish cheese

Three batches of Mish cheese were prepared in three cities in Jordan: Amman (T1), Madaba (T2), and Irbid (T3). The Mish cheese was prepared following the traditional method, in which no starter cultures is used. This method can be summarised as follows: sheep milk was heated to about 35 °C and inoculated with rennin (0.0025%) for about 2 h, the resulting curd was pressed into small portions (about 200 g) in cheese cloth, and then sprinkled with salt. Alternating layers of cheese pieces and chopped hot green pepper were placed in 2 l glass jars. The glass jars were topped with salted shanenah (diluted skimmed yoghurt with about 1% salt), hermetically sealed and stored at room temperature for 5 months. Samples were drawn at 0.5, 1, 2, 3, 4, and 5 month interval for analysis.

Chemical analysis of whole cheese samples

Moisture, titratable acidity, salt and total WSN in Mish cheese samples were determined following the Association of Official Analytical Chemists methods 926.08, 920.124, 935.43 and 2001.14, respectively (AOAC, 2011).

Diacetyl analysis

Diacetyl was measured following Macciola *et al.* (2008), where 2 g of Mish cheese were shaken for 30 s in 2 ml of acetone. 50 µl/ml of 2,3-pentanedione (99%) (ACROS, Bridgewater, NJ, USA) was used as an internal standard. The supernatant was filtered through a 0.02 µm disposable syringe membrane (Sartorius AG, Göttingen, Germany) and analysed using a gas-chromatograph (GC-2010; Shimadzu, Kyoto, Japan) equipped with a flame ionisation detector. A 30 m MEGA-Wax (MEGA, Legnano, Italy) capillary column, with an internal diameter of 0.32 mm, and 0.5 µm film thickness was used. Gas-chromatographic parameters were: carrier gas helium at 50 kPa flow, injection amount of 1 µl, injector and detector temperatures of 250 and 260 °C, respectively, and oven temperature from 50 to 240 °C at 7 °C/min (Macciola *et al.*, 2008).

Measurement of superoxide dismutase activity

The super oxide dismutase (SOD) activity in Mish cheese was measured using an EnzyChromTM Superoxide Dismutase Assay Kit (ESOD-100; BioAssay Systems, Hayward, CA, USA), following manufacturer's protocol. The BioAssay Systems' SOD provides a convenient colorimetric means for the quantitative determination of SOD enzyme activity in biological samples. It can be used to detect all 3 types of SODs (Cu/Zn, Fe/Mn, and the Ni type SOD), with a dynamic range of 0.05-3.00 units/ml SOD. SOD activity was expressed as units/mg (Janknegt *et al.*, 2007).

Chemical analysis of cheese fat

Lipid extraction

Total lipids were extracted following the modified Folch method (Rodriguez-Estrada *et al.*, 1997). A 30 g portion of Mish cheese was added to 200 ml of a $CHCL_3/CH_3OH$ solution (1:1, v:v) and homogenised for 30 s. 100 ml of chloroform was added to the homogenate, the mixture was homogenised for 1 m and then filtered. 100 ml of 1 M KCl solution was added to the filtrate and left to stand overnight at 40 °C. Following phase separation, the chloroform phase was evaporated using a Stuart RE300 rotary evaporator (Bibby Scientific Limited, Stone, UK) and the lipid fraction was stored at -20 °C.

Peroxide value and free fatty acids content

The peroxide value (PV) and FFAs were determined following methods 965.33 and 940.28, respectively, of AOAC (2011).

7-ketocholesterol enrichment and HPLC analysis

The enrichment of 7-ketocholesterol followed the method suggested by Penazzi *et al.* (1995) on a solid-phase extraction Isolute Florisil cartridge (International Sorbent Technology, Hengoed, UK). 0.02 g of the extracted lipids were dissolved in 0.5 ml of 2-propanol/n-heptane (2:98, v:v) and loaded onto a Florisil cartridge previously washed with heptane. The cartridge was then washed with 4 ml of 2-propanol/n-heptane to remove triglyceride and most of the cholesterol. The cholesterol oxides were eluted with 5 ml of acetone. The solvent was then evaporated under a flow of nitrogen at <50 °C. 7-ketocholesterol was quantified by normal phase high-performance liquid chromatography (HPLC) using a Pye-Unicam system equipped with a PU 4020 variable UV detector and a PU 4010 pump (Pye-Unicam, Cambridge, UK). The column used was a Thermo Hypersil type (silica) with a length of 150 mm and diameter of 4.6 mm, and packed with 5 µm particle size. HPLC elution was carried out under isocratic conditions with a mobile phase of 2-propanol/n-hexane (7%, v:v) at 1 ml/ min flow rate The wavelength for 7-ketocholesterol detection was 233 nm. All determinations were run in duplicates.

Organoleptic evaluation of Mish cheese

Samples of Mish cheese at week 20 of fermentation were cut into approximately 5×5 cm pieces and randomly presented at ambient temperature (20±2 °C) to untrained panellists consisting of both laboratory staff and students from the Food Science and Nutrition Department, University of Jordan who were familiar with Mish cheese. The panel evaluated the cheese samples for changes in characteristic flavour and colour.

Statistical analysis

Statistical analysis was performed using Statistical Analysis System (SAS, 2008), version 9. Analysis of variance (ANOVA) with t-test was used to determine significant differences between the means at $P<0.05$ (Steel and Torrie, 1980). Values in the tables are expressed as mean ± standard error of the mean. All experiments duplicated.

3. Results and discussion

Moisture content

Moisture is the major component of cheese, acting as a plasticiser in the protein matrix thus making it less elastic and more susceptible to fracture upon compression. Final product moisture content is an important quality parameter because variation can affect cheese textural properties and shelf-life (Fox, 1989). Results show that moisture content of Mish cheese decreased significantly after 5 months of ripening (Table 1). The greatest decrease was observed in T1 (13.8%), while the lowest decrease was in T3 (9.0%). Mallatou *et al.* (1994) reported similar findings where moisture decreased sharply within 20 days, causing shrinkage of the curd; the moisture reduction was attributed to acid development expelling whey from the cheese mass.

Titratable acidity

It was observed that titratable acidity (expresses as lactic acid % dry basis) increased in all cheese; the acidity ranged between 0.97 and 2.1% with an average of 1.69% at the onset of storage. A gradual and significant ($P<0.05$) increase in acidity was observed in all treatments until the 12th week of storage, after which, no further increase was observed

Table 1. The influence of processing and storage period of Mish cheese on moisture, titratable acidity, salt, water soluble nitrogen (WSN) and superoxide dismutase activity (SOD).[1,2]

Time of storage (weeks)	Moisture			Titratable acidity (as lactic acid %)[3]			NaCl%[3]			WSN (%)[3]			SOD (U/mg)[3]		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
0	72.7^{a}	72.7^{a}	64.0^{a}	2.0^{c}	2.1^{c}	0.97^{c}	14.8^{a}	14.8^{a}	17.7^{a}	0.88^{d}	0.81^{c}	0.55^{c}	33.7^{d}	34.6^{d}	36.6^{c}
4	68.6^{b}	69.3^{b}	60.1^{b}	2.9^{b}	3.2^{b}	1.13^{b}	14.9^{a}	13.5^{a}	15.3^{b}	1.07^{c}	0.85^{bc}	0.67^{bc}	40.1^{c}	37.8^{c}	39.6^{b}
12	65.4^{c}	65.2^{c}	56.4^{c}	3.9^{a}	3.5^{a}	2.7^{a}	15.0^{a}	11.8^{b}	13.8^{c}	1.29^{b}	0.94^{b}	0.71^{b}	48.5^{b}	40.8^{b}	44.5^{a}
20	58.9^{d}	61.1^{d}	55.0^{c}	3.4^{a}	3.8^{a}	2.7^{a}	11.0^{b}	9.7^{c}	13.3c	1.34^{a}	1.05^{a}	1.05^{a}	57.9^{a}	45.7^{a}	45.8^{a}

[1] Means within the same column without a common superscript are significantly different ($P<0.05$).

[2] T1 = cheese sample from Amman; T2 = cheese sample from Madaba; T3 = cheese sample from Irbid.

[3] The results of these parameters are expressed on a dry matter basis.

(Table 1). The highest and lowest acidities were observed in T2 and T3 cheese samples, respectively. There were significant differences in acidity (P<0.05) between cheese samples stored for the same periods. The increase in the titratable acidity was likely caused by the conversion of residual lactose to lactic and propionic acid by LAB (Lavermicocca *et al.*, 2000).

Salt percentage

The salt content of T3 was higher than that of T2 and T1 at the onset of storage, due to sprinkling variations. The salt content in T1 and T2 remained constant for the first 12 and 4 weeks, respectively, then significantly decreased for the remainder storage (Table 1). In case of T3, the decrease was observed during the first week and continued throughout storage. The decrease in salt might be due to the acid development by LAB bacteria causing shrinkage of the cured and expelling whey from the cheese in which the salt was dissolved.

The process of salting is an integral part of the cheese making process and serves distinct purposes: (1) salt acts as a preservative; (2) contributes directly to flavour; and (3) is a source of dietary sodium. Together with the desired pH, water activity and redox potential, salt assists in cheese preservation by minimising spoilage and preventing the growth of pathogens. In addition salt level has a major effect on cheese composition, microbial growth, enzymatic activities and biochemical changes such as glycolysis, proteolysis, and lipolysis during fermentation. Consequently, the salt level influences cheese flavour and aroma, rheological and textural properties (Guinee, 2004).

Water soluble nitrogen and superoxide dismutase activity

The increase in WSN mainly resulted from breakdown of proteins into soluble peptides and free amino acids by LAB (Tjwantan *et al.*, 1993). The WSN in the Mish cheese samples gradually increased during storage (Table 1). The increase was highest in T2 (0.5%) and lowest in T3 (0.21%) cheese samples. Elewa *et al.* (2009) reported similar findings where soluble nitrogen contents of white soft cheeses made with probiotics increased at the end of the storage period. Shehata *et al.* (2001) postulated that increase in WSN (%) of soft cheese resulted from enzymes released by starter cultures (*Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillusdel brueckii* subsp. *bulgaricus*) during pickling.

SOD is thought to play a very important role in protecting living cells against toxic oxygen derivatives. The enzyme catalyses the dismutation of two superoxide radicals (O_2^-) into O_2 and H_2O_2, which could convert active oxygen molecules into non-toxic compounds. The results of the antioxidant activity of the soluble peptides evaluated by measuring the SOD activity, which are presented in Table 1 as U/mg of cheese (on a dry matter basis), show that SOD activity significantly increased during storage, same as WSN. The results show that SOD activity may be greatly influenced by WSN.

Influence of storage on Mish cheese lipids

Peroxide value and 7-ketocholesterol

Factors that cause lipid oxidation include heat, light, radiation, moisture, low pH and storage (Al-Ismail and Humied, 2003; Savage *et al.*, 2002). The results of PV, which is a measure of the primary products of fat oxidation and 7-ketocholesterol, the two main products of cholesterol oxidation, are reported in Table 2. The results indicate that storage did significantly (P<0.05) increase PV in cheese samples and the increase in PV was relatively low. The data in Table 2 show that the level of 7-ketocholesterol formed during storage period for all treatment was very low i.e. not exceeding 5 µg/g. The low levels of 7-ketocholoesterol may have been due to the low level of oxygen in the shanenah solution. Since the glass containers used for storing the cheeses were completely filled/topped with shanenah and tightly closed, the only source of oxygen present in the glass

Table 2. The influence of processing and storage period of Mish cheese on some chemical and biochemical changes on its lipid components.[1,2]

Time of storage (weeks)	Peroxide value (meq O_2/kg oil)			7-ketocholesterol (µg/g fat)			Free fatty acids (as oleic acid %)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
0	0.72^b	0.72^c	1.37^a	0.73^d	1.13^d	1.22^d	1.76^d	0.74^c	0.96^c
4	0.83^c	1.69^b	1.60^a	1.09^c	1.53^c	1.51^c	2.91^c	0.87^c	1.02^c
12	1.40^a	1.68^b	1.91^a	1.83^b	2.64^b	2.06^b	3.18^b	1.45^b	2.59^b
20	1.42^a	2.88^a	2.37^a	2.02^a	4.88^a	4.08^a	3.64^a	1.87^a	2.84^a

[1] Means within the same column without a common superscript are significantly different (P<0.05).
[2] T1 = cheese sample from Amman; T2 = cheese sample from Madaba; T3 = cheese sample from Irbid.

containers was that dissolved in the shanenah. Moreover, the low surface/volume ratio of the cheese pieces and the low diffusion rate of the dissolved oxygen through the pieces could have inhibited the interaction of fat with oxygen. Al-Ismail and Humied (2003) found similar results i.e. low levels of fat and cholesterol oxidation in brined white cheese stored for 9 months in closed glass jars.

Free fatty acid content

The development of FFA was primarily attributed to the activity of lipase during storage (Table 2). The data show that the FFA% in the fat fraction of the freshly prepared cheeses was relatively high, which might be due to the enzyme activity during raw milk warming, curd formation and pressing. A gradual and significant increase in FFA% through the storage period was observed. The FFA% of all treatments exceeded the maximum permitted level (1%) set by the Jordanian Standards for the anhydrous butter fat after 12 weeks of storage.

Hydrolytic rancidity is, however, not a major problem in the storage of cheese (Al-Ismail and Humied, 2003). FFAs contribute to the desired flavour of most cheeses (Robinson and Tamime, 1991). The brined shanenah may have served as a protective barrier against fat oxidation

Diacetyl contents in Mish cheese during ripening

Diacetyl was not detected by the gas chromatographic technique with a flame ionisation detector among all Mish cheese samples. These results are in agreement with those obtained by Gűzel-Seydim *et al.* (2000), and Fix (1993). Solomons (1984) suggested that enzymatic reduction of diacetyl by yeast as a possible cause because yeast count increased during fermentation.

Organoleptic evaluation of Mish cheese

The results of the organoleptic evaluation indicated that no objectionable changes were observed on the characteristic flavour or colour of the cheese in all treatments after 20 weeks of storage. T1 samples were most preferred compared to T2 and T3 samples.

4. Conclusions

The study shows that the lack of standardised production methods mostly affected acidity of Mish cheese. Fat oxidation was not a major problem.

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