

## Effect of Processing Methods on Cholesterol Contents and Cholesterol Oxides Formation in Some Dairy Products

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### Abstract

The effects of pasteurization, boiling, microwaving, processing and storage of milk and some locally produced dairy products on cholesterol contents and cholesterol oxides formation were studied and evaluated. The 7-ketocholesterol were not detected (ND) in all raw milk samples. On the contrary, heating of milk led to formation of cholesterol oxidation products (COPs), mostly, 7-ketocholesterol in different quantities. No significant effect of heating of milk on cholesterol level was observed with the exception of the ultra-high temperature (UHT) milk prepared from milk powder heated at  $140 \pm 1.0^\circ\text{C}$  for 4 sec showed the highest value of 7-ketocholesterol ( $80.97 \text{ mgg}^{-1}$ ), followed by microwave heated milk for 5 min ( $31.29 \text{ mgg}^{-1}$ ), whereas the lowest value was in milk pasteurized at  $85 \pm 1.0^\circ\text{C}$  for 16 sec ( $3.125 \text{ mgg}^{-1}$ ). Commercial storage showed no significant effect on cholesterol and 7-ketocholesterol but lowered cholesterol concentration and increased 7-ketocholesterol level of UHT reconstituted milk. Cholesterol content of both yogurt and labaneh strained by centrifugal separator showed significant decrease while 7-ketocholesterol level was increased significantly with refrigerated storage. The findings are discussed in the context with the results of previous similar studies.

**Key words:** Fat oxidation; cholesterol; ultra-high temperature; pasteurization; 7-ketocholesterol; microwaving; milk products.

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### Introduction

Dairy products are an important group in human nutrition. They are consumed as such or used in preparation of many food items such as pastries, pies, cakes,...etc to provide specific functional properties (e.g., texture, crust color, flavor,...etc.). Milk lipids may undergo chemical and physical changes during processing and storage such as autoxidation and formation of trans fatty acids (Semma, 2002). Cholesterol oxidation products (COPs) have been found in many foods due to the autoxidation of cholesterol in presence of light, heat and pro-oxidants (Kummar and Singhal, 1991). Many of them showed some biological activities such as enzymatic inhibition of cholesterol biosynthesis, mutagenicity and atherosclerosis (Kummar and Singhal, 1991; Tavani *et al.*, 1997). Milk and milk products usually undergo different changes during their

preparation (boiling and microwaving) or processing, which may include moderate or severe heat treatments that can lead to undesirable changes in lipids or proteins. Cooking and reheating of foods by microwave ovens are widely used in food preparation in millions of kitchens throughout the world. Food heating by microwave results from the conversion of microwave energy into heat by friction of water molecules vibration due to rapid fluctuation in the electromagnetic field (Potter and Hotchkiss, 1996; decareau, 1992). The trend of using the microwave oven in food processing could be attributed to the speed of heating and energy saving. Although microwave oven is widely used as a means of food preparation, insufficient information is available on the consequences of microwave heating on the composition and nutritional quality of the food. Some studies revealed that microwave heating affect fat oxidation

and fatty acid isomer formations (Albi *et al*, 1997).

Variations in heating treatments may have different effects on COPs in dairy products. Therefore, the objective of this study is to evaluate the effect of the conventional heating methods of milk, processing of milk into boiled white brined cheese, yogurt and labaneh, microwave heating of milk and white brined cheese and storage conditions of pasteurized milk, yogurt, labaneh and cheese on their cholesterol contents and cholesterol oxides formation.

## Materials and Methods

Raw Cow's milk used in the study was obtained from the bulk tank of three dairy plants: Danish Jordan Dairy Company (DJD), Jordan University Dairy Plant (JUDP) and Al-Sanabel Dairy Co. (SDC). The milk was produced by Cow's Breeder Society, Jordan University farm and from Haj Mustafa Farm, respectively. Ewe's milk used for white-brined cheese production was obtained from SDC and yogurt and labaneh were from DJD and JUDP. The UHT (at  $140\pm 1.0^\circ$ ) and Pasteurized (at  $85.0\pm 1.0^\circ\text{C}$ ) milk were obtained from DJD Co.

### Heat Treatments of Milk and Milk Products

The raw cow's milk obtained from the three selected sources was subjected to different heat treatments as shown in Table 1.

### Yogurt Production

Set yogurt was produced from milk pasteurized at  $95 \pm 1.0^\circ\text{C}$  for 5 minutes (tube pasteurization),  $95 \pm 1.0^\circ\text{C}$  for 15 (plate pasteurization) or  $85-90^\circ\text{C}$  for 2 min (batch pasteurization). The milk was cooled to  $45\pm 1.0^\circ\text{C}$  and inoculated with 2-3% freeze-dried mixed starter culture (Danisco, Denmark), then filled into plastic containers of different sizes, incubated at  $42\pm 1.0^\circ\text{C}$  up to 2 to 2.5 hr. When the desired acidity of 0.7% or a pH of 4.5-4.6 was reached, the yogurt was cooled at  $4\pm 1.0^\circ\text{C}$  (Smit, 2003).

### Labaneh Production

Labaneh was produced by the following two methods (Ozer, 2006):

**Traditional Method (cloth sacks):** The set yogurt after cooling was stirred and then poured in a cloth sacks overnight to drain off the whey for 12 to 24 hrs. The drained yogurt labaneh was salted with NaCl 1%, blended, filled into suitable plastic containers and refrigerated at  $4^\circ\text{C}$ . The produced labaneh was of 23-25 percent total soluble solids and a pH of 5 to 5.5.

**Separator method (centrifugal separator):** Cream –separated milk was pasteurized at  $83$  to  $85^\circ\text{C}$  for 16

**Table 1.** Cow's milk and milk products produced by different heat treatments.

Milk		Heating Treatment			Product
Producer	Source	Type	Temperature ( $^\circ\text{C}$ )	Time	
CBS	DJD	Tube pasteurization	85	16sec	Pasteurized milk
			95	5 min	Yogurt
			82	16 sec	labaneh
			140	4 sec	UHT
		Lab scale pasteurization	$63\pm 1.0$	30 min	Pasteurized milk
		Lab scale boiling	$97.5\pm 1.0$	5 min	Boiled milk
		Microwave boiling	$96.8\pm 1.0$	5 min	Microwave boiled milk
HMDF	SDC	Plate pasteurization	95	15 min	Yogurt
			95	15 min	labaneh
		Lab scale pasteurization	$63\pm 1.0$	30 min	Pasteurized milk
		Lab scale boiling	$97.5\pm 1.0$	5 min	Boiled milk
		Microwave boiling	$96.8\pm 1.0$	5 min	Microwave boiled milk
JUDF	JUDP	Batch pasteurization	85-90	2 min	Yogurt
			85-90	2 min	Labaneh
		Lab scale pasteurization	$63\pm 1.0$	30 min	Pasteurized milk
		Lab scale boiling	$97.5\pm 1.0$	5 min	Boiled milk
		Microwave boiling	$96.8\pm 1.0$	5 min	Microwave boiled milk

sec, NaCl 1% was added, the pasteurized skim milk was inoculated with ca 0.002% (w/v) powder freeze dried mixed starter culture (Danisco, Denmark) and kept for 15-17 hr at 42-44°C. When the pH of the yogurt reached 4.5 to 4.6, the product was stirred and the whey was separated via separator at 40°C (Ozer, 2006). The produced labaneh was filled into suitable plastic containers and stored at 4°C while the concentrated skim yogurt and the cream (40% butter) were mixed to have a total solid not less than 23% and 10% fat.

### Cheese Production

The brined white cheese (Nabulsi) was produced according to the method described by Humeid and Tukan (1986) and Humeid *et al.* (1990). Cheese samples were subjected to the following heat treatments as follows:

**Microwave heat treatments:** Two desalted, grated cheese samples of approximately 200 g each were heated in a microwave oven (Galanz, 800 Watts, WD800B, Korea) at 80% power. The first one was heated at  $96.3 \pm 1.0^\circ\text{C}$  until browning (ca. 10 min), while the second was filled in a polyethylene bag, placed in a Pyrex saucepan, filled with distilled water and boiled while floating in the microwave oven at  $96.3 \pm 1.0^\circ\text{C}$  for 5 min.

**Conventional heat treatments (gas cooker):** Desalted grated cheese sample (ca. 100g) was placed in polyethylene bags in Pyrex saucepan, covered with distilled water and boiled on a gas cooker for 5 min at  $95.5 \pm 1.0^\circ\text{C}$ .

**UHT Reconstituted Milk:** UHT milk sample prepared from powder milk (reconstituted) produced by Kuwaiti Danish Dairy Co., Kuwait (KDD) were purchased from the local market for comparison (production date 09/09/02 and expired on 09/03/03).

### Storage of Milk and Milk Products

The milk and milk products used in the present study; (pasteurized milk, UHT milk, yogurt and labaneh) were stored at  $5.0 \pm 1.0^\circ\text{C}$  and analyzed after a storage period of 3, 5, 7, 15 days for pasteurized milk, UHT milk, yogurt and labaneh, respectively, as indicated on the package label (commercial shelf life). On the other hand, the produced white brined cheese (Nabulsi) was evaluated after one month of storage in tins at room temperature at  $18 \pm 1.0^\circ\text{C}$ .

### Milk Fat Extraction and Analysis

Lipids were extracted from the milk and milk products samples with chloroform and methanol as described by Bligh and Dyer (1959) with some modifications regarding sample weight, solvent volume and centrifugation speed and time. Approximately 70 g of cheese, yogurt or labaneh and 100 ml of fluid milk products were homogenized with 100 ml methanol and 100 ml chloroform using a Hamilton Beach Scovel homogenizer (NSF, USA) for 2 min at a medium speed. Chloroform (100 ml) was added and the mixture was rehomogenized for an additional 2 min. The homogenate was centrifuged at 4000 rpm for 20 min using Haeraeus centrifuge (Haeraeus Christ, GmbH, Osterode/Harz, OJ3, Germany). The upper layer (methanol and water layer) was removed through aspiration. The middle and the lower layer (chloroform layer and precipitated protein layer) were filtered through a filter paper to separate precipitate particles. The chloroform-lipid extracts were again filtered through anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and the  $\text{Na}_2\text{SO}_4$  was rinsed 3 times with 30 ml chloroform 10 ml each. The lipid extracts were dried under nitrogen using rotoevaporator (LABOROTA, 4001 WB, Heidolph, Germany) with 150 rpm at  $50^\circ\text{C}$  and stored for analysis in 5 ml vials (brown glass) under nitrogen at  $-18^\circ\text{C}$ . The lipid samples were then used for the analysis of cholesterol content and cholesterol oxidation products (COPs) mainly 7-ketocholesterol.

### Cholesterol and cholesterol oxides determination:

Cholesterol, 7-ketocholesterol and  $5\alpha$ -cholestane standards were obtained from SIGMA, Inc., Ethyl acetate was HPLC grade (J.T. Baker Chemical Co. Phillipsburg, N.J.), potassium hydroxide was from GCC Laboratory Reagent (85%), anhydrous sodium sulfate from SDS (fine chemical limited, Boisar), and methanol HPLC grade from Lab Scan, UK. Chloroform was from GCC (Gainland chemical Co., UK), pyridine (analytical reagent grade) from CBH, Chlorotrimethyl silane ( $\text{CH}_3$ )<sub>3</sub>SiCl was obtained from Fluka (Switzerland) and Hexamethyl disilazane  $\text{C}_6\text{H}_{19}\text{NSi}_2$  was from Janssen, US. Accurately 60 to 200 mg of the lipid extract (3.8) was weighed into a 25 ml screw capped test tube; 10 ml of 1M KOH in methanol and 20  $\mu\text{l}$  of  $5\alpha$ -cholestane solution (4 $\mu\text{g}/\mu\text{l}$ ) as internal standard (IS) were added to the sample. The mixture was shaken until its free of dispersed fat particles and placed in a shaking water bath (Mettmert, Germany) set at  $27^\circ\text{C}$  for 18 to 24 hr. Ten

milliliters of distilled water were added to the saponified mixture, which was transferred to 50 ml separatory funnel fitted with a Teflon cap. Unsaponifiables were extracted three times with 10, 5, 5 ml of diethyl ether (98%, Laboratory grade, GCC, England), and the pooled diethyl ether extracts were washed once with 5ml of 0.5M KOH and 5 times with 5 ml distilled water. The ether extract was dried and filtered using Whatman No.1 filter paper and dried over anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ). The  $\text{Na}_2\text{SO}_4$  and the filter paper were washed twice with 5 ml diethyl ether to minimize the losses due to the transfer steps. The combined filtrates were concentrated under nitrogen in dark to about 1 ml and dried under nitrogen (ultra pure) after being transferred into a 5-ml vial and stored at  $-18^\circ\text{C}$  (Vlencuela *et al.*, 2003).

#### Trimethylsilylation of cholesterol and cholesterol oxides

The trimethylsilyl derivatives (TMS) of cholesterol and cholesterol oxides was carried out according to the method used by Pie *et al.* (1990) with some modification regarding the derivatization condition (time and temperature). The dried nonsaponifiables extracts were dissolved in 100  $\mu\text{l}$  pyridine (CBH, Nottingham, UK) and mixed for 30 sec by vortex mixer. A 100  $\mu\text{l}$  of each hexamethyldisilazane (Janssen, Belgium) and trimethylsilylchloride (Fluka, Switzerland) was added and mixed for another 20 sec. The vial was placed in a water bath for 40 min, and then cooled to room temperature. The mixture dissolved in 2ml distilled water and extracted 4 times with 1ml hexane (GC grade, Lab Scan, Dublin). The hexane layer was evaporated under extra pure nitrogen gas. The derivatized (TMS) cholesterol and cholesterol oxides were redissolved in 100  $\mu\text{l}$  of hexane (GC grade).

**Recovery determination:** Quantitative recovery of cholesterol,  $5\alpha$ cholestane and 7-ketocholesterol was determined for the above mentioned procedure using 10g starch sample previously washed with chloroform and precisely spiked with 100  $\mu\text{l}$  of  $5\alpha$ -cholestane ( $4\mu\text{g}/\mu\text{l}$ ), 7-Ketocholesterol and cholesterol ( $2\mu\text{g}/\mu\text{l}$ ) using 100  $\mu\text{l}$  Hamilton syringe (Hamilton, USA). The nonsaponifiables in the spiked and nonspiked starch samples were extracted and analyzed by the same procedure followed in sample analysis.

The detection limits and calibration curves of cholesterol, 7-ketocholesterol and  $5\alpha$ -cholestane were found to be about  $1\mu\text{g g}^{-1}$  for each cholesterol, 7-ketocholesterol and

$5\alpha$ -cholestane (Fig. 1). This concentration agreed with the results obtained by Regueirio and Maraschello (1997) who found values ranging between 0.1 to  $1\mu\text{g g}^{-1}$  for cholesterol oxides. The linearity of the calibration curves are shown in Figure (3-1). It is obvious that the relationship is linear with  $R^2$  of 0.9991, 0.9994 and 0.9913 for  $5\alpha$ -cholestane, cholesterol and 7-ketocholesterol, respectively.

The recoveries percentage of cholesterol and 7-ketocholesterol as shown in Table 2, were 99.5 and 95.2 respectively.

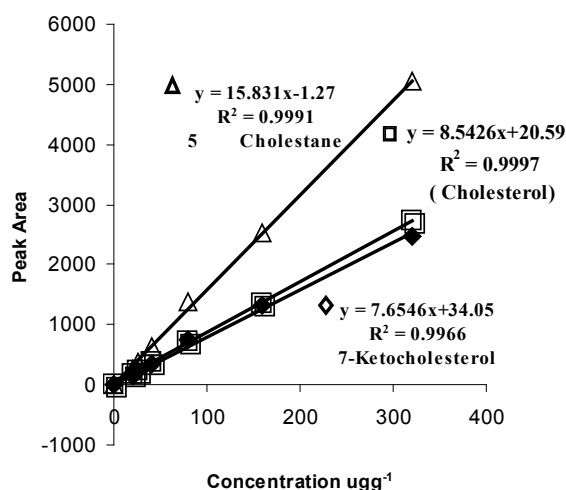


Figure 1: Calibration curves of the  $5\alpha$  cholestane, cholesterol and 7-ketocholesterol.

Table 2: Recoveries of cholesterol and 7-ketocholesterol. ( addition of 200  $\mu\text{g}$ )

Determination	Cholesterol Recovery (%)	CL <sup>1</sup>	7-ketocholesterol Recovery (%)	CL
1	97.9	$195 \pm 4.9$	96.5	$193 \pm 3.1$
2	96.0	$192 \pm 1.8$	99.0	$198 \pm 2.8$
3	108.0	$216 \pm 1.9$	96.3	$192 \pm 3.02$
4	96.0	$192 \pm 1.3$	89.0	$178 \pm 8.6$
Mean Recovery	99.5		95.2	
Standard deviation (SD)	5.6		4.3	
Coefficient of Variability (CV)	5.6		4.5	

<sup>1</sup> Confidence limits are calculated at 95% confidence level. ( $p > 0.05$ ).

**Gas chromatographic analysis:** The derivatized sterols (trimethylsilylated cholesterol and cholesterol oxides) were analyzed on gas chromatograph (Varian 3700) supplied with an split-splitless injector port and flame ionization detector. A TRB-5 (95% dimethyl-5% diphenyl polysiloxan) capillary column (25m x 0.25mm i.d.; phase thickness, 0.25 $\mu$ m ; Teknokroma, Barcelona, Spain). The GC conditions used were; 280°C oven temperature, 300°C injector port temperature, and 310°C detector temperature. One microliter of the derivatized sample was injected at a split ratio of 50:50 into the capillary column. Flow rate was set at 1.4 ml/min of N<sub>2</sub> carrier gas. The cholesterol oxidation products COPs peaks were-identified compared with the retention time of the reference standard. The COPs content of milk and milk products samples was determined using the internal standard techniques (IS) of 5 $\alpha$ -cholestane and the units of measurement were expressed as  $\mu$ g/g for the COPs and as percent (%) for the cholesterol (Lin *et al.*, 1995, Sander *et al.*, 1988).

#### Statistical Analysis of Experimental Data

Experiments were conducted using completely random design to find the effect of different treatments. Data were analyzed using the analysis of variance (ANOVA) procedure of SAS institute Inc., (Konda and Rajurkar, 2005) version seven software. Duncan's multiple range test was applied to determine significance among different treatments.

## Results and Discussion

### A-Effect of heating and processing of milk on cholesterol content and 7-ketocholesterol formation

Heating of milk showed the following effects on cholesterol content and cholesterol oxide (7-ketocholesterol) formation:

**Effect of pasteurization and boiling:** The effects of different heating methods of milk on cholesterol and 7-ketocholesterol levels are shown in Table 3. A chromatogram of cholesterol and cholesterol oxidation products of milk sample is given in Figure 2. In general, no significant effect of heating of milk on cholesterol level was observed with the exception of the UHT milk prepared from reconstituted powdered milk before and after storage. Along with that, commercial storage of milk had also no significant effect ( $p > 0.05$ ) with the exception of UHT reconstituted milk where storage significantly ( $p < 0.05$ )

lowered the cholesterol concentration. For example, the cholesterol percentages of raw, pasteurized milk (95  $\pm$  1.0°C for 15 min), microwave heated and UHT milk were 0.293, 0.283, 0.275, and 0.285%, respectively. The insignificant decrease in cholesterol content of the heat treated milk samples compared to that of raw milk is probably due to oxidation of cholesterol and formation of cholesterol oxides and to inactivation of heat labile antioxidants (Regueiro *et al.*, 1997, Lehtinen *et al.*, 2003)). The decrease in the cholesterol content of the reconstituted UHT compared to that of the fresh raw milk may be due to the fact that this product contains stabilizers and emulsifiers, as indicated on the label of the package (carageenan, guar gum, vegetable mono and diglycerides).

These compounds are probably able to bind (complex) some lipid components such as cholesterol and cholesterol oxides thus lowering their availability for solvent (chloroform) that is used to extract the milk fat (Valerizuela *et al.*, 2003). Furthermore, the significant ( $p < 0.05$ ) decrease in cholesterol contents of UHT milk prepared from reconstituted milk powder compared to that of UHT prepared from fresh cows milk (Table 3) could be partially explained by the increase in the level of 7-ketocholesterol. The values of 7-ketocholesterol for UHT reconstituted milk was 80.97  $\mu$ g g<sup>-1</sup> compared to 8.708  $\mu$ g g<sup>-1</sup> found in UHT from fresh cow milk. The high level of cholesterol oxides is probably due to the drying process including exposure to heat and oxygen (Rodriguez *et al.*, 1997).

The concentrations of 7-ketocholesterol of milk presented in Table 3 showed that 7-ketocholesterol is not detected in raw milk and is formed upon all types of heat treatments and during storage. The concentration of this indicator of cholesterol oxidation ranged between 3.125  $\mu$ g g<sup>-1</sup> in milk pasteurized at 85  $\pm$  1.0°C for 16 sec and 132.965  $\mu$ g g<sup>-1</sup> in UHT prepared from reconstituted milk powder at 140  $\pm$  1.0°C and stored for 5 days. The results showed that 7-ketocholesterol was significantly affected ( $p < 0.05$ ) by both heat treatment and refrigerated storage of the previously heated milk products. As shown in Table 3, the range of 7-ketocholesterol content in raw and heated milk samples before storage was < 1.0 (ND) to 34.538  $\mu$ g g<sup>-1</sup> and 5.52 to 132.965  $\mu$ g g<sup>-1</sup> after refrigerated storage for 3 and 5 days for the pasteurized and UHT milk respectively.

**Effect of microwave heating:** Microwave heating of raw cows milk samples show a significant ( $p < 0.05$ ) increase in

**Table 3:** Effect of heat treatment of and refrigerated storage of milk on the formation of 7-ketocholesterol <sup>1</sup>.

Treatment	Cholesterol (%)	7-Ketocholesterol ( $\mu\text{gg}^{-1}$ fat)
Raw cow's milk	0.293 <sup>a</sup> $\pm$ 0.017	ND
Milk Pasteurized at 85 $\pm$ 1.0°C for 16 sec.	0.291 <sup>a</sup> $\pm$ 0.01	3.125 <sup>e</sup> $\pm$ 0.806
Milk pasteurized at 85 $\pm$ 1.0°C for 16 sec and stored for 3 days <sup>2</sup> .	0.290 <sup>a</sup> $\pm$ 0.058	5.520 <sup>e</sup> $\pm$ 0.186
Milk pasteurized at 63 $\pm$ 1.0°C for 30 min.	0.284 <sup>a</sup> $\pm$ 0.009	9.383 <sup>e</sup> $\pm$ 1.005
Milk pasteurized at 95°C for 5 min.	0.290 <sup>a</sup> $\pm$ 0.023	11.733 <sup>e</sup> $\pm$ 8.119
Milk pasteurized at 95 $\pm$ 1.0°C for 15 min.	0.283 <sup>a</sup> $\pm$ 0.043	16.328 <sup>de</sup> $\pm$ 1.717
Milk pasteurized at 85-90°C for 2 min.	0.292 <sup>a</sup> $\pm$ 0.023	3.142 <sup>e</sup> $\pm$ 0.694
Milk boiled at 96.3 $\pm$ 1.0°C for 5min.	0.283 <sup>a</sup> $\pm$ 0.017	15.363 <sup>e</sup> $\pm$ 1.922
Milk boiled in microwave oven at (80% power) 95.8 $\pm$ 1.0°C for 5min.	0.275 <sup>a</sup> $\pm$ 0.036	31.029 <sup>e</sup> $\pm$ 1.089
Milk heated at 140 $\pm$ 1.0°C for 4 sec (UHT) <sup>3</sup> .	0.285 <sup>a</sup> $\pm$ 0.006	8.708 <sup>e</sup> $\pm$ 1.399
Milk heated at 140 $\pm$ 1.0°C for 4 sec (UHT) and stored for 5 days.	0.283 <sup>a</sup> $\pm$ 0.009	34.538 <sup>e</sup> $\pm$ 1.094
Reconstituted milk powder (UHT) <sup>4</sup> .	0.260 <sup>b</sup> $\pm$ 0.006	80.97 <sup>b</sup> $\pm$ 1.232
Reconstituted milk powder (UHT) and stored for 5 days.	0.205 <sup>c</sup> $\pm$ 0.016	132.965 <sup>a</sup> $\pm$ 1.523

Values represent means  $\pm$  SD (n = 4). Means values in the same column with different superscript letters are significantly different (p  $\leq$  0.05) according to (ANOVA) Duncan's multiple range test. <sup>2</sup> Commercial refrigeration at 5.0  $\pm$  1.0°C. <sup>3</sup> Ultra high pasteurization temperature of fresh cow's milk provided by Danish Jordan Dairy Company (DJD). <sup>4</sup> Milk prepared from cow's milk powder after reconstitution KDD brand name (Kuwaiti Danish Dairy Company), purchased from local market.

7-ketocholesterol compared to those heated by conventional methods (boiling and/or tube, plate or batch pasteurization) (Table 3). The values of 7-ketocholesterol of the pasteurized (85 $\pm$ 1.0°C for 16 sec), boiled (96.3 $\pm$ 1.0°C for 5min), UHT and microwave heated milk samples were 3.125, 15.363, 8.708 and 31.029  $\mu\text{gg}^{-1}$ , respectively. Microwave heating seems to be highly detrimental to quality compared to the other heating method due to its unique heating mechanism. These results were in agreement with those obtained by others where heating of oil samples and edible fat in microwave oven enhances lipid oxidation (Albi *et al.*, 1997 a&b; Yoshida and Kajimoto, 1994; Yoshida *et al.*, 1991&1992).

**Effect of storage:** Refrigerated storage of pasteurized milk for three days showed no significant effect on cholesterol or 7-ketocholesterol content, whereas, UHT heated milk prepared from reconstituted milk powder as well as its storage for five days had significantly (p<0.05) lowered cholesterol but increased 7-ketocholesterol levels as shown in Figure 3. The values of the 7-ketocholesterol for UHT milk were 8.708 and 34.538  $\mu\text{gg}^{-1}$  before and after storage, respectively. The increase in the 7-ketocholesterol content of UHT upon storage can be seen as continuation of the oxidation process that was indicated by heating under the

prevailing aerobic condition (Kumar and Singhal 1991). These results are consistent with those obtained by Nourooz-Zadeh and Appelqvist (1988) who studied the formation of COPs during storage of milk powder for one year in paper cans at about 20°C and found 7-ketocholesterol to be 5.6-9.2  $\mu\text{gg}^{-1}$ . The formation of COPs as a result of heating is expected because heating positively increases lipid oxidation in the presence of air, prooxidant and radicals that enhance the formation of cholesterol oxides (Kumar and Singhal 1991, Morgan and Armstrong 1992).

#### **B-Effect of heating and processing of yogurt and labaneh on cholesterol content and 7-ketocholesterol formation**

Cholesterol and 7-ketocholesterol content of yogurt and labaneh are shown in Table 4. The results indicate that processing of yogurt and labaneh had no significant effect on cholesterol content. On the contrary, processing steps produced significant (p<0.05) increase in 7-ketocholesterol levels compared with those values of the raw or pasteurized milk. For example, the values of 7-ketocholesterol content in the fat extracted from raw milk, yogurt (produced from milk pasteurized at 80-90°C for 2 min) and labaneh were ND (not detected), 3.78 and 5.16  $\mu\text{gg}^{-1}$ , respectively.

Refrigerated storage of yogurt or labaneh did not produce significant changes in cholesterol levels with the exception

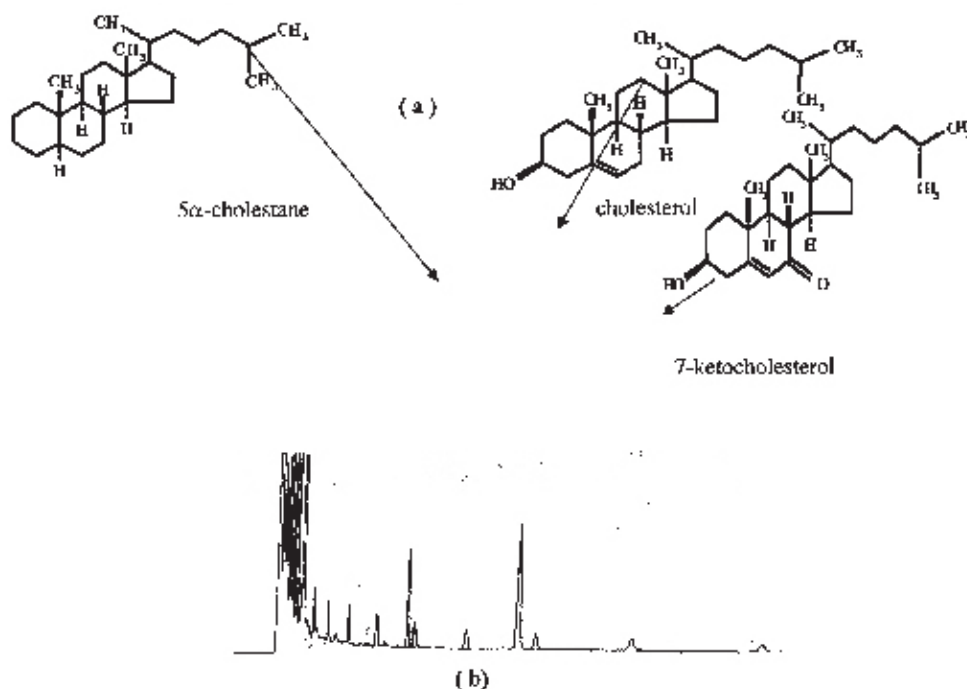


Fig. 2. (a)- Derivatives for standard mix of cholesterol and 7-ketocholesterol, including the internal standard, 5α-cholestane. (b)- chromatogram of COPs isolated from UHT reconstituted milk. GC conditions were as described under material and methods.

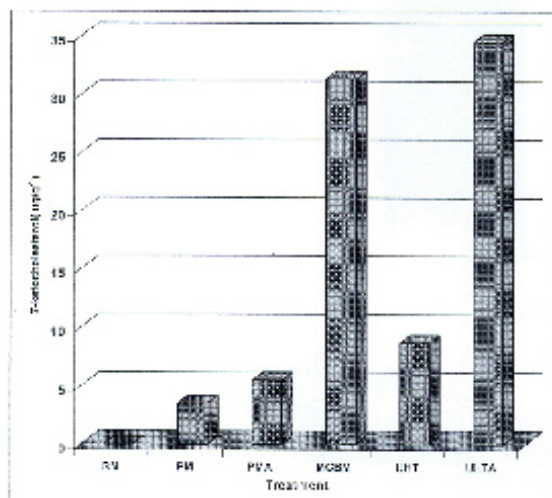


Figure 3. Effect of heat treatment and refrigerated storage on 7-ketocholesterol content of milk (RM; raw cows milk, PM; pasteurized milk at 85°C for 16 sec, PVA; pasteurized milk stored for 3 days, MCBM; microwave boiled milk, UHT; ultra-high-temperature and UHTA; ultra high temperature milk stored for 5 days).

of labaneh produced mechanically using a centrifugal separator, where the cholesterol content decreased significantly ( $p < 0.05$ ) after 15 days storage. On the other hand, 7-ketocholesterol content increased significantly with storage of both yogurt and labaneh for 7 or 15 days,

compared with those of fresh products. For example, the values of 7-ketocholesterol in yogurt and labaneh were 3.78 and 5.11  $\mu\text{g g}^{-1}$  in fresh product compared to 5.16 and 6.55  $\mu\text{g g}^{-1}$  after storage, respectively. The increase in 7-ketocholesterol content of labaneh or yogurt after storage is expected since almost aerobic condition is prevailing during fermentation. The time elapsed between pasteurization and analysis of the extracted fat did not exceed 24 hr in which the oxidation process could not produce significant difference of the values. In contrast prolonged storage of yogurt or labaneh for 7 or 15 days resulted in a significant increase in 7-ketocholesterol content. It is worth mentioning that the samples analyzed after storage period were taken from the same cups that were opened at the beginning of the analyses and stored after reclosure in refrigerator (at  $5 \pm 1.0^\circ\text{C}$ ). This implies that either yogurt or labaneh were exposed to air diffusion during storage, which might lead to pronounced aerobic condition that in turn facilitates oxidation.

Cholesterol content of labaneh strained by centrifugal separator showed significant ( $p < 0.05$ ) decrease in cholesterol content after refrigerated storage for 15 days compared with that of the labaneh strained in cloth. The

**Table 4:** Effect of different heat treatments, processing and refrigerated storage of milk, yogurt and labaneh on their cholesterol content and 7-ketocholesterol formation.

Treatment	Cholesterol (%)	7-Ketocholesterol ( $\mu\text{g g}^{-1}$ fat)
Raw cows milk <sup>2</sup> .	0.293 <sup>a</sup> $\pm$ 0.017	ND
Milk pasteurized at 85-90°C for 2 min <sup>3</sup> .	0.292 <sup>a</sup> $\pm$ 0.023	3.14 <sup>a</sup> $\pm$ 0.694
Pasteurized milk <sup>4</sup> at 85 $\pm$ 1.0°C for 16 sec.	0.291 <sup>a</sup> $\pm$ 0.01	3.125 <sup>d</sup> $\pm$ 0.806
Yogurt( produced from the milk pasteurized at 85-90°C for 2 min).	0.286 <sup>a</sup> $\pm$ 0.013	3.78 <sup>cd</sup> $\pm$ 0.664
Yogurt after 7 days storage <sup>5</sup> .	0.281 <sup>a</sup> $\pm$ 0.011	5.11 <sup>b</sup> $\pm$ 0.324
Yagurt produced from milk pasteurized at 85 $\pm$ 1.0°C for 16 sec.	0.281 <sup>a</sup> $\pm$ 0.013	4.18 <sup>bc</sup> $\pm$ 0.806
Yagurt produced from milk pasteurized at 85 $\pm$ 1.0°C for 16 sec. After 7 days storage	0.282 <sup>a</sup> $\pm$ 0.023	6.35 <sup>a</sup> $\pm$ 0.15
Labaneh produced by Separator <sup>6</sup> .	0.276 <sup>a</sup> $\pm$ 0.024	5.03 <sup>b</sup> $\pm$ 0.181
Labaneh produced by modern method after 15 days storage.	0.18 <sup>b</sup> $\pm$ 0.040	5.108 <sup>b</sup> $\pm$ 0.205
Labaneh produced by conventional method <sup>7</sup> .	0.288 <sup>a</sup> $\pm$ 0.010	5.16 <sup>b</sup> $\pm$ 0.119
Labaneh produced by conventional method after 15 days storage.	0.283 <sup>a</sup> $\pm$ 0.011	6.55 <sup>b</sup> $\pm$ 0.443

<sup>1</sup>Values represent means  $\pm$  SD (n =4). Means with different letters within a column are significantly different ( $p \leq 0.05$ ) according to (ANOVA) Duncan's multiple range test. <sup>2,3,4</sup> milk used for yogurt production and Labaneh. <sup>5</sup> Commercial storage at 5  $\pm$  1.0°C for 7 and 15 days for yogurt and labaneh respectively. <sup>6</sup> Straining by separator. <sup>7</sup> Straining in cloth.

cholesterol values for labaneh were 0.276 and 0.18% before and after storage, respectively. The decrease in cholesterol percentage could be due to the additional heat treatment of the cream before mixing with the concentrated yogurt (Rodriguez *et al*, 1997). Another explanation is the possible decrease in the extractability of cholesterol as a result of complex formation between cholesterol as lipid component and protein or carbohydrate (Min and Steenson, 1998). It is also worth to notice that, the cream added to the concentrated yogurt was mixed with stabilizers and emulsifiers such as guar gum and carageenan. These additives may interact with cholesterol during storage and made it unextractable by chloroform.

### C- Effect of heating, processing and storage of white brined (Nabulsi) cheese on cholesterol level and 7-ketocholesterol formation

Cholesterol and 7-ketocholesterol levels of processed cheese are presented in Table 5. The results indicate that boiling of cheese pieces in brine (17% NaCl) for 15 min caused a significant reduction ( $p < 0.05$ ) of the cholesterol content by 6%. Reboiling of the desalted and grated cheese samples for 5 minutes or its microwaving did not produce any significant ( $p > 0.05$ ) changes in cholesterol content (Table 5). However, heating of cheese for longer period (10 min) in a microwave resulted in a significant ( $p > 0.05$ )

decrease in cholesterol content. For example, the averaged cholesterol percentages for the cheese samples heated in a microwave oven for 5 and 10 min were 0.278 and 0.265 %, respectively. This is obviously due to the formation of cholesterol oxides as will be explained below.

Processing steps and reheating of the cheese by conventional heating method (boiling on gas cooker) had no significant effect on oxidation of cholesterol as shown in Table 5. However, reboiling of cheese using a microwave oven for 5 min had significantly increase 7-ketocholesterol content with clear effect of the heating duration.

Storage of the salted boiled cheese in polyethylene bags placed in a Pyrex saucepan at (5 $\pm$ 1.0°C) had no significant effect on the 7-ketocholesterol levels. On the other hand, the insignificant effect of processing on 7-ketocholesterol is in agreement with those obtained by Al-Ismail and Humeid (2002) in Nabulsi cheese.

The effect of microwaving on cholesterol oxidation of milk, is shown in table 5.

It can be concluded from the above findings that cholesterol oxides, in particular 7-ketocholesterol, which are considered carcinogenic were not detected in fresh milk, while all of the applied heating treatments led to the formation of cholesterol oxides at different levels. Conventional heating of milk (pasteurization and boiling) caused formation of these oxides with significant differences.

**Table 5:** Effect of microwave heating, processing steps and storage of one month of white brined cheese on cholesterol content and 7-ketocholesterol formation<sup>1</sup>.

Treatment	Cholesterol (%)	7-Ketocholesterol ( $\mu\text{gg}^{-1}$ fat)
Raw ewe milk	0.302 <sup>a</sup> $\pm$ 0.028	1.023 <sup>d</sup> $\pm$ 0.554
Fresh curd	0.296 <sup>a</sup> $\pm$ 0.008	1.132 <sup>d</sup> $\pm$ 1.640
Boiled cheese curd <sup>2</sup> .	0.283 <sup>b</sup> $\pm$ 0.009	4.025 <sup>d</sup> $\pm$ 0.306
Boiled cheese stored for one-month <sup>3</sup> .	0.282 <sup>b</sup> $\pm$ 0.010	2.355 <sup>d</sup> $\pm$ 1.414
Boiled cheese reheated on gas cooker <sup>4</sup> at 94.3 $\pm$ 1.0°C for 5min.	0.279 <sup>bc</sup> $\pm$ 0.031	8.17 <sup>d</sup> $\pm$ 1.153
Boiled cheese reheated on gas cooker after storage at 94.3 $\pm$ 1.0°C for 5min.	0.275 <sup>bcd</sup> $\pm$ 0.013	9.835 <sup>d</sup> $\pm$ 1.793
Boiled cheese reheated in microwave oven <sup>5</sup> at 94.3 $\pm$ 1.0°C for 5min.	0.280 <sup>b</sup> $\pm$ 0.015	24.94 <sup>c</sup> $\pm$ 2.130
Boiled cheese reheated in microwave oven within a liquid at 94.3 $\pm$ 1.0°C for 5min.	0.278 <sup>bc</sup> $\pm$ 0.018	27.705 <sup>c</sup> $\pm$ 1.383
Boiled cheese reheated in microwave oven after storage. at 94.3 $\pm$ 1.0°C for 5min.	0.282 <sup>b</sup> $\pm$ 0.018	23.084 <sup>c</sup> $\pm$ 1.361
Boiled cheese reheated in microwave oven within a liquid after storage. at 94.3 $\pm$ 1.0°C for 5min.	0.276 <sup>bc</sup> $\pm$ 0.010	24.053 <sup>c</sup> $\pm$ 1.206
Boiled cheese reheated in microwave oven at 94.3 $\pm$ 1.0°C for 10 min.	0.267 <sup>d</sup> $\pm$ 0.009	103.698 <sup>b</sup> $\pm$ 1.439
Boiled cheese reheated in microwave oven at 94.3 $\pm$ 1.0°C for 10 min after storage.	0.265 <sup>d</sup> $\pm$ 0.006	115.308 <sup>a</sup> $\pm$ 2.493

Values represent means  $\pm$  SD (n=4), means with different letters within a column are significantly different ( $p \leq 0.05$ ) according to (ANOVA) Duncan's multiple range test. <sup>2</sup> white brined cheese pieces boiled in brine (17% NaCl) at 94.3  $\pm$  1.0°C measured at the centre of the cheese pieces for 5min. <sup>3</sup> White brined boiled cheese pieces stored in tins at room temperature of 18  $\pm$  1.0°C for one month. <sup>4</sup> Desalted grated white-brined boiled cheese reheated in a liquid medium (distilled water). <sup>5</sup> Desalted grated white-brined boiled cheese reheated in a liquid medium (distilled water) in microwave oven at 80% power.

Flash pasteurization gave the lowest level followed by low temperature long time pasteurization without significant differences between them. Processing steps of milk, yogurt, labaneh and cheese did not produce any significant difference in COPs with the exception of cheese reboiled for 5 min, which showed a significant increase in the COPs formation. On the contrary, microwave heating of cheese (regardless of the heating time) caused tremendous increase in the level of the COPs, which puts a big question mark on the use of microwave oven in food processing and preparation. The oxidation level in UHT milk produced from reconstituted milk was significantly higher than those of UHT produced from fresh milk. This draws our attention towards unsuitability of the use of milk powder in the production of UHT milk (multi-heating effect).

The ability of cholesterol extraction from milk mixed with carageenan and guar gum, and labaneh mixed with locust bean gums was reduced. The reduction was also increased with refrigerated storage of UHT milk and labaneh for 5 and 15 days respectively. This is an indication of the formation of hydrophilic interaction (complexes),

which were not extractable with organic solvent.

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## تأثير التصنيع على محتوى الكوليستيرول وإنتاج أكاسيد الكوليستيرول في بعض منتجات الألبان

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### الملخص

تم في هذه البحث دراسة وتقييم تأثير كل من البسترة والغليان و الميكروويف و خطوات المعالجة وظروف التخزين على الحليب و بعض المنتجات المحلية من الألبان من حيث المحتوى من الكوليستيرول وتكوين أكاسيد الكوليستيرول. أثبتت نتائج هذا البحث عدم وجود ٧ كيتو كوليستيرول في كل عينات الحليب غير المعاملة حراريا بينما أدى تسخين الحليب إلى أكسدة الكوليستيرول خصوصا تكوين ٧- كيتو كوليستيرول بنسب مختلفة. احتوى الحليب المحضر من البودرة عن طريق التسخين عند درجة حرارة ١٤٠ درجة مئوية لمدة أربع ثوان على أعلى قيمة من ٧ كيتو كوليستيرول تلاه الحليب الذي تم تسخينه بواسطة الميكروويف لمدة خمس دقائق وكانت أقل قيمة من ٧ كيتو كوليستيرول في الحليب المبستر. لم يكن لظروف التخزين التجارية تأثير واضح على كل من الكوليستيرول و٧- كيتو كوليستيرول بينما تسبب في خفض تركيز الكوليستيرول وارتفاع مستوى ٧- كيتو كوليستيرول في الحليب المحضر من البودرة والذي كان قد تم تسخينه عند درجة حرارة عند درجة حرارة ١٤٠ درجة مئوية. وكما أدى التخزين المبرد إلى خفض مستويات الكوليستيرول ورفع مستويات ٧- كيتو كوليستيرول في عينات اللبن واللبننة تحت الدراسة والتي تم تحضيرها بطريقة الفصل بالطرد المركزي. نوقشت النتائج على ضوء نتائج دراسات مشابهة.