IMPROVEMENT OF FATTY ACIDS COMPOSITION AND REDUCTION OF CHOLESTEROL CONTENTS IN TRADITIONALLY PREPARED BUTTER AND OIL IN WEST OF IRAN

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Abstract

BACKGROUND: Industrial method of butter production involves direct separation of butter fat from the milk, however in traditional-making process of butter and oil in rural regions of Kermanshah province in west of Iran, the milk is converted to the yoghourt by the fermentation and following a few days maintenance of the yoghourt at room temperature, the butter fat is separated from the resulted yoghourt using several hours vigorous shaking. After melting of the butter and separation of its aqueous contents, traditional oil is prepared. A large body of evidence indicate that the type of fat has greater importance than total amounts of consumed fats with respect to risk of coronary artery disease (CAD), hence this study aimed to evaluate trend of fatty acids changes and cholesterol content from milk to oil, during the traditional method.

METHODS: Samples of milk, yoghourt, butter and oil prepared from the same bulk of milk were collected from different rural regions of Kermanshah province. To compare the traditional and industrial methods, samples of the industrially–prepared butter were purchased commercially. The total lipids of samples were extracted and subjected to fatty acids analysis by high performance liquid chromatography (HPLC).

RESULTS: We found that significant reduction is taking place in the cholesterol and long chain fatty acids contents of butter and oil during the traditional method, while short- and medium-chain fatty acids are significantly increased. The fatty acid composition of industrially-made butter in our study however, was the same as that of the milk samples.

CONCLUSION: Compared with the industrial method, the fatty acid composition of butter in the traditional method has better nutritional value. Some aspects of this process may be recommended for improvement of the commercial methods of butter production.

Introduction

Fatty acids and cholesterol are the most important components of the lipids. During the past several decades, reduction in fat intake has been the main focus of national dietary recommendations to decrease risk ofcoronary artery diseases (CAD). However, it has been cleared that types of fat have a more important role in determining risk of CAD than total amount of fat in the diet [1-2]. Various fatty acids have different effects on plasma lipids .While short and medium chain fatty acids do not affect plasma lipoproteins, consumption of saturated fatty acids (SFA) specifically, saturated fats with 12–16 carbon atoms tend to increase plasma total and low density lipoprotein (LDL) cholesterol levels [2-3]. Hence, reduction of long chain saturated fats in the diet, is an important recommendation to improve lipoprotein profile and decrease CAD incidence [2]. In industrial method of butter production, the milk fat is separated mainly by centrifugation, and after processing, the resulted fat is supplied as the butter. Published data showed that the same fatty acids profile is present in the milk and its resulted butter [4-6]. However in the traditional method of butter and oil production in rural regions of Kermanshah in west of Iran, after fermentation of the milk, the resulted yoghourt is maintained for a few days (1-3

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days) at room temperature (15-35 °C) and then transferred to a container. Following vigorous shaking for at least 2 hours the separated fat is removed, supplied and consumed as traditional butter. After melting of the butter, its aqueous content (named as Dogh) is separated physically or adsorbed on the flour which is added to the melted butter and remained fat is consumed as Kermanshahian oil. The aim of this study was to evaluate the trend of fatty acid changes from the milk to the resulted oil in the traditional method. Cholesterol content and fatty acid composition of the traditionally-prepared butter was compared with the industrial method too.

Materials and Methods

Samples
120 samples of the milk, yoghourt, butter and oil prepared from the same bulk of milk were collected from different rural regions of Kermanshah in west of Iran. Samples of the industrially–prepared butter (10 samples) were purchased commercially.

Analytical procedure
The total lipids of the samples were isolated and purified based on the Folch method [7]. Briefly the samples were homogenized and diluted with chloroform: methanol mixture (2:1 V/V) at a final dilution of 20-fold. Following filtration and washing of the extract with distilled water (2 mL) and centrifugation, the supernatant was discarded and the remaining organic medium was washed three times with a upper phase solvent (appropriate concentrations of $CaCl_2$, $MgCl_2$ and KCl in water: methanol: chloroform; 3:48:47; V/V). The organic phase was separated and evaporated to dryness under gentle stream of nitrogen at 45 °C. The residue kept closed and stored frozen at - 80 C° until the assay. Analysis of the samples was performed as soon as possible. Although gas chromatography (GC) is method of choice for separation and analysis of fatty acids however, due to less loss of short chain fatty acids, an HPLC method [8] was used for assay of the samples. Analysis of different fatty acids including short, medium and long chain fats as well as unsaturated fatty acids and their geometrical isomers (Elaidic and Linoelaidc acid) was carried–out using 2-nitrophenylhydrazide as pre-column labeling agent by the method which has been described in detail elsewhere with some modification in the mobile phase composition and after validation in the dairy samples [8]. Briefly the residue was dissolved in 200 µl absolute ethanol and and saponified using 10% ethanolic potassium hydroxide for 20 min at 80 °C. The saponified sample was subjected to the pre-column derivatization with 2-nitrophenylhydrazide (200 µl, 0.02M in HCl

0.9 M) and 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide hydrochloride (100 µl, 0.25M). Following further heating of the sample at 80 ºC for 5 min, the fatty acid hydrazides were extracted using diethyl ether and a mixture of phosphate buffer-hydrochloric acid (.05 M;7:1 V/V). After evaporation of the organic phase the residue was reconstituted in 200 µl ethanol and 5 µl was injected into the system. Separations of the analytes were achieved by gradient elution of methanol-water on a C18 packing material analytical column. The signals were quantified by UV-VIS detector operated at 370 nm after correction of the response factor for the each fatty acid. The results were expressed as percent of fatty acids using corrected area normalization method. Determination of total cholesterol in the samples was performed by enzymatic method of cholesterol oxidase [9] after extraction of the total lipids by the Folch method. Fat content of different samples were estimated using the creamatocrit method. One way ANOVA with Tukey's test as a post hoc was used for statistical analysis of the data and a P- value level less than 0.05 was considered statistically significant.

Results
The averages fatty acids composition of the milk and resulted yoghourt, butter and oil prepared from the same bulk of milk by the traditional method, as well as industrially-produced butter samples have been shown in the Table 1. The total short and medium chain fatty acids as well as sum of long chain fatty acids in all samples have been presented in the figure 1A and B, respectively. Table 2 shows the average cholesterol and fat contents of different samples in the industrial and traditional methods. While, a similar fatty acid profile was seen in the milk and industrially-made butter samples, there was a definite significant trend of changes in fatty acids composition of the milk and resulted products. Statistical analysis of the data using ANOVA with Tukey's post-hoc comparison of the means showed that significant increasing in short and medium chain fatty acids (P<0.05) as well as reduction in cholesterol and long chain fatty acids (P<0.05) contents that are produced during the traditional method. Consistent with other reports [4-6], the fatty acid composition of industrial-made butter in our study was the same as that of the milk samples. Different factors may be involved in reducing of

cholesterol and long chain fats as well as increasing of short and medium chain fatty acids during the traditional method of butter and oil production. Bacterial activity and production of lactic acid during fermentation of the milk to the yoghourt, reduces pH of the medium. Due to higher solubility, likelihood of ionization and participation of short and medium chain fatty acids in resulting emulsion, is more than that of long chain fats at lower PH [11-12].

Furthermore it has been reported that duration and temperature of maintenance of the yoghourt, have important effects on the pH and solubility of various fatty acids [11-13], hence, a few days maintenance of the yoghourt in the traditional method may by involved in increasing of short and medium chain fatty acids as well as in reduction of the long chain fats in the resulted oil and butter. By activity of micro organisms in the yoghourt, esteric cholesterol is converted to the free cholesterol which can not participate in the resulting emulsion thus, compared with the industrial method, there is lower amount of cholesterol in the traditional-made butter.

In conclusion this study showed that in the traditional method of butter and oil production, several healthy beneficial changes are produced which lead to improvement of fatty acid composition and cholesterol contents of the resulted butter and oil. As increasing of the nutritional value and reduction of side effects on blood lipoproteins are expected by these changes, some aspects of the traditional procedure may be considered in industrial procedure.

Table 1: The average of different fatty acids in milk, resulted yoghourt, butter and oil-prepared from the same bulk of milk using the traditional method as well as industrial-made butter.

Oils\Fatty acids	Name	Milk	Yoghourt	Butter	Traditional oil	*industrial butter*
C:4	Butyric Acid	3.3 (0.32) [a]	4.4 (0.38) [a]	6.8 (0.57) [b]	8.1 (1.0) [b]	3.6 (0.41)
C:6	Caproic Acid	1.7 (0.31),[a]	3.3 (0.30) [a]	7.1 (0.65)[b]	9.1 (1.1)[c]	2.1 (0.30)
C:8	Caprylic Acid	1.5 (0.18) [a]	1.9 (0.29) [a]	2.5 (0.32)[a]	3.7 (0.42) [b]	1.2 (0.17)
C:10	Capric Acid	2.8 (0.42) [a]	3.5 (0.36) [a,b]	4.5 (0.51) [b]	6.4 (0.65) [c]	2.7 (0.32)
C:12	Lauric Acid	3.9 (0.52)	4.4 (1.1)	4.5 (0.47)	4.3 (0.55)	4.0 (1.3)
C:14	Myristic Acid	12.9 (1.8) [a]	12.1 (2.2) [a]	9.3 (1.2)	7.9 (0.84)[b]	12.4 (2.3)
C:16	Palmitic Acid	33.0 (2.9) [a]	32.7 (3.1) [a]	29.3 (2.4)	24.8 (2.4) [b]	32.5 (3.6)
C:18	Stearic Acic	12.1 (1.3) [a]	11.7 (1.2) [a]	8.8 (0.82) [b]	7.2 (0.75),[b]	12.3 (1.9)
C:20	Arachidid Acid	0.8 (0.25)	0.7 (0.22)	0.5 (0.20)	0.5 (0.24)	0.8 (0.33)
C:183	Linolenic Acid	0.6 (0.21)	0.7 (0.24)	1.0 (0.31)	1.1 (0.3)	0.6 (0.28)
C:18(2)	Linoleic Acid	1.0 (0.20) [a]	1.0 (0.31) [a]	2.2 (0.25) [b]	2.5 (0.3) [b]	1.5 (0.40)
C:18(1)	Oleic Acid	20.2 (2.2	18.6 (2.0)	17.1 (2.1)	16.9 (1.9)	20.5 (2.9)
C18(1t)	Elaidic acid *	4.4 (0.9)	3.9 (1.0)	4.2 (1.1)	4.1 (1.2)	4.0 (1.2)

*Elaidic acid was not resolved from trans vaccinic acid in our study

The number in parentheses indicates one standard deviation

Values in the same row with different superscript (a, b, c, and d) are significantly different ($P < 0.05$) as determined by one-way ANOVA followed by the Tukey post hoc test.

Table 2: The means (SD) of cholesterol and fat contents in milk, resulted yoghourt, butter and oil-prepared from the same bulk of milk using the traditional method as well as industrial-made butter.

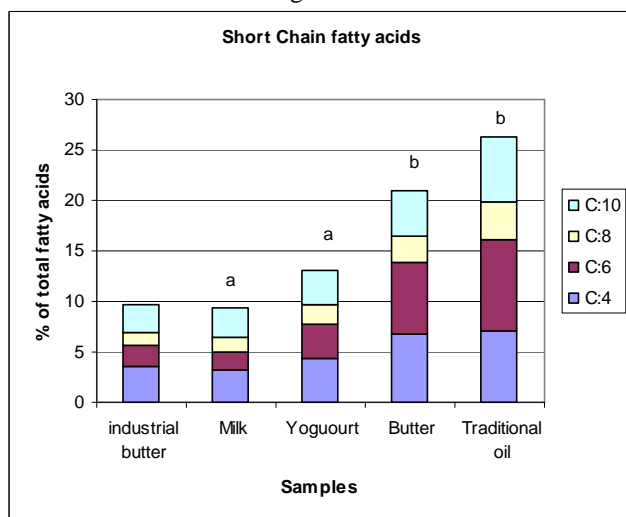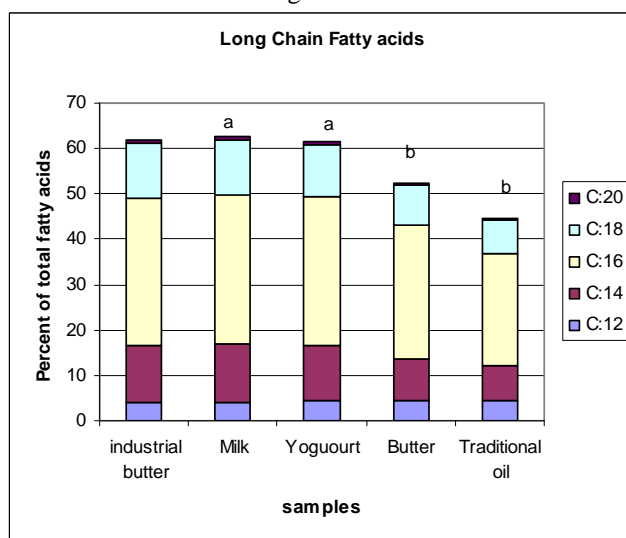| Parameters	Milk	Yoghourt	Butter	Traditional oil	*industrial butter*
Cholesterol	13 (2.4)	12 (2.2)	155 (12.7)	168 (12.9)	320 (44.7)
Fat percent	5.5 (1.1)	5.6 (1.0)	75.6 (3.4)	98.3 (2.11)	97.5 (2.2)

Fig. 1A

Fig. 1 B

Figure 1: Comparison of total A) short and medium and B) long chain fatty acids in different samples.
Values in the same column with different superscript (a and b) are significantly different ($P < 0.05$) as determined by one-way ANOVA followed by the Tukey post hoc test.
Short medium chain fatty acids include: C: 4, C: 6, C: 8 and C: 10. Long chain fatty acids include: C: 12, C: 14, C: 16 and C: 18.

Discussion

Our results indicated that long chain fatty acid contents of canned fish show a great variation among four groups. Sum of EPA and DHA in Iranian canned fish was ranged from 4 to 25.5 g/100g food.

Different essential fatty acids not only increase nitric oxide(NO) generation but also react with NO to produce their respective nitroalkene derivatives that produce vascular relaxation, inhibit neutrophil degranulation and superoxide formation, inhibit platelet activation, and release NO, thus prevent thrombus formation, platelet aggregation, atherosclerosis, and cardiovascular diseases.[13]

The results from determining the fatty acid composition of tuna canned in oil and in water coming from three fishing areas of the Mexican Pacific showed evident variation exists in the content of FA among areas, and the tuna in water is a richer food in omega 3 and omega 6 fatty acids that the tuna in oil, independently of the fishery area.[1]

Three canned fish species-Pacific saury (Cololabis saira), Pacific herring (Clupea harengus) and Baltic sprat (Sprattus sprattus)-most common and popular in Russia, were analyzed for fatty acids. Those results showed that Sums of EPA and DHA in saury, herring and sprat were, on average, 2.42, 1.80 and 1.43 g/100 g product, respectively [14]. In Mexico, the fatty acid profiles in sardine canned in tomato sauce coming from different fishing areas of the Mexican Pacific were analyses by GC with a flame ionization detector.

In all the areas they were identified and quantified as three omega 3 fatty acids (linolenic acid, EPA and DHA) and two omega 6 fatty acids (linoleic and arachidonic acid); this source is rich in FA monounsaturated and also presents a considerable quantity of trans fatty acids (18:1n9t and 18:2n6t). The DHA was the most abundant fatty acids in all the areas (3.064-4.704 g/100 g). Sardine canned in tomato sauce of the Mexican Pacific is a rich food in omega-3 and omega-6 FA, independently of the processing area[5]. Also, In Iranian

canned fish, elaidic acid (C18:1 9t) was only TFA and other TFA isomers were not detectable. The distribution of the positional trans C18:1isomers may indicate that partially hydrogenated fats were used in production of these samples [15].

Evidence suggests that differences in fatty acid composition among various fish species may be due to differences in diet or to environmental factors such as temperature, salinity, and depth at which the fish are seized[4].The scientific evidence proposes "the omega-3" index as a new risk factor for sudden cardiac death. It is measured in red blood cells, and is defined as a percentage of EPA + DHA of total fatty acids. The omega-3 index can be used for treatment with EPA and DHA. An omega-3 index of >8% as compared to an omega-3 index of <4%, is associated with 90% less risk for sudden cardiac death.[16]

All the canned fish appeared to be highly valuable products for human nutrition concerning the content of EPA and DHA.[14]

To get the most omega 3 fats from your canned tuna, choose water-packed tuna rather than oil-packed. The oil mixes with some of the natural fat in canned tuna, so when you drain oil-packed tuna, some of its omega-3 fatty acids also go down the drain. Since oil and water don't mix, water-packed tuna won't leach any of its precious omega-3s.

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