ELSEVIER

Food Chemistry 70 (2000) 371–375

Food Chemistry

www.elsevier.com/locate/foodchem

Changes in membrane fatty acid composition of human erythrocytes obtained from dietary margarine users and non-users

Necmettin Yilmaz [a], Ayhan Demirbaş [b,*], Ayşe Şahin [b]

[a] *Department of Biology, Gaziosmanpaşa University, Tokat, Turkey*
[b] *Department of Chemical Education, Karadeniz Technical University, 61335, Akçaabat, Trabzon, Turkey*

Received 28 April 1999; received in revised form 17 February 2000; accepted 17 February 2000

Abstract

Concentrations of *trans* fatty acids (TFA) were determined in 6 soft-type and 6 hard-type Turkish margarines. Methyl esters of the fatty acids were analyzed on a Packard 427 model gas chromatograph equipped with a 32 m×0.3 mm capillary column containing a bonded 0.25 μm film of BDS liquid phase. The samples in soft-type users had a total TFA concentration of 5.28%, while margarines in hard-type users had a total TFA concentration of 12.4%. Average *trans* monoenoic-acid concentrations in soft-and hard-type margarine users, and non-margarine users were 2.94, 11.7 and 8.68%, respectively. Average *trans* dienoic-acid concentrations in soft- and hard-type margarine users, and non-margarine users were 2.18, 0.50 and 1.84%, respectively.

1. Introduction

The simple lipids contain only fatty acid and alcohol components. The alcohol is usually glycerol but may also be a long-chain alcohol or a sterol. Unesterified or free fatty acids, which occur in small amounts in most animal and plant tissues, and free sterols, the most abundant of which is cholesterol, are also classed as simple lipids (Christie, 1973).

There are many reasons to include fats and oils in foods in the diet and fats with different combinations of fatty acids may be desirable. Fats have important nutritional functions in addition to supplying a concentrated energy source. The fatty acid composition of dietary fat markedly influences the fatty acid composition of lipids of blood components and of adipose tissue (Glatz, Sofferz & Katan, 1989). The role of unsaturated fatty acids in human nutrition is a topic of rapidly increasing popularity in both scientific and lay publications. It has been demonstrated, in man and animals, that the fatty acid composition of serum, erythrocyte, and plateospholipids reflects the fatty acid composition of the dietary fat (Aktaş, Keha, Yilmaz & Demirbaş, 1999; Doughert, Galli, Ferro-Luzzi & Iacono, 1987; Hanahan, Watts & Pappajohn, 1960; Manku, Horrobin, Huang & Mors, 1983; Nordoy, 1974; Phillps & Dodge, 1967; Renaud, Morazain & Godsey, 1981). There is still, however, limited information available about the relationship of dietary fats to platelet-lipid composition. The fatty acid pattern of lipid components is being used increasingly as an objective biochemical indicator of adherence to experimental diets during dietary intervention studies, especially to assigned cholesterol-lowering diets rich in polyunsaturated fatty acids (Angeligo, Amodeo, Borgogelli, Catafora, Monkali & Ricci, 1980).

During margarine processing, isomerization of the naturally occurring *cis* unsaturated fatty acids to the *trans* configuration and positional shifts of the double bonds may occur. Partial hydrogenation yields a wide range of both geometric (*cis* and *trans*) and positional isomers of oleic and linoleic acids in which double bonds may be shifted anywhere from C-3 through C-15 in the case of 18:2 (Dutton, 1971). The distribution of positional isomers in various commercial products has been documented by Dutton (1979). The concentration of *trans* fatty acids varies with the extent and type of processing of the oil. Shortenings, margarine, and salad oils may contain from 14 to 60, 16 to 70 and 8 to 17% *trans* fatty acids, respectively (Kinsella, Bruckner, Mai & Shimp, 1981).

Polyunsaturated fat feeding alters the fatty acid composition of cellular membranes; this change can occur rapidly. In order to restore the original membrane

* Corresponding author.

0308-8146/00/$ - see front matter
PII: S0308-8146(00)00106-0

fluidity, a change in membrane fatty acid composition might be counteracted by changes in cholesterol/phospholipid ratio or phospholipid class distribution (Pop-Snijders, Schouten, van Blitterswijk & Van der Veen, 1986).

The aim of this study was to discover the fatty acid contents of erythrocyte membranes obtained from margarine users and non-users.

2. Materials and methods

All blood samples were supplied from each of the 30 female and 50 male volunteers between 18 and 23 years of age who had earlier participated in trials involving diet with and without margarine. After a 12 h fast, venous (8–10 ml) blood samples were drawn into tubes containing, per ml of blood, 5 mg of Na_2EDTA as anticoagulant. For each blood sample obtained from margarine users and non-users, the EDTA tubes were immediately centrifuged at low speed and packed in wet ice to separate the red blood cells. Plasma obtained from the latter collections was stored at $-20°C$ and used for the analysis of cholesteryl esters. The cells were washed twice with ice-cold isotonic saline to remove leucocytes and thrombocytes, transferred into stoppered glass tubes, and hemolyzed by freezing at $-20°C$. All further analyses were performed after the dietary trial was finished.

Phosphoglyceride classes were separated on a washed and dried (100°C, 45 min) silica gel HF 254 plate (Merck), using the solvent system, of chloroform:methanol:ammonia (65:35:5, v:v:v). The plate was dried under a stream of nitrogen, and individual phospholipid classes were made visible under UV light by spraying with a 0.01% solution of rhodamine in methanol. A small section of the developed plate was sprayed with a 50% solution of sulfuric acid and charred to demonstrate complete separation of phospholipid classes. Appropriate phospholipid bands from the uncharred portion of the plate were scraped of immediately and analyzed for fatty acids. One and a half millilitres of chloroform was added to the material obtained. Then it was centrifuged at 2500 rpm for 10 min to isolate the gel.

To prepare the methyl esters of the total and individual serum phospholipids, these fractions were separated by TLC and the fatty acids of each fraction converted to methyl esters with diazomethane (Schelenk & Gellerman, 1960). An ethereal solution of the acids was treated with diazomethane, generated by adding a 50% KOH solution to an ethereal solution of *N*-nitrosomethylurea. Glassware with smooth surfaces was used to prevent the formation of polymethylene.

Methyl esters of the fatty acids were analyzed on a Packard 427 model gas chromatograph equipped with a 32 m×0.3 mm fused silica capillary column containing a bonded 0.25 μm film of BDS liquid phase. Analyses were made at 183°C isothermally. Helium was used as carrier gas. This column resolves positional isomers with the same configuration (i.e. 9c-18:1 and 11c-18:1), *cis* and *trans* isomers with the double bond at the same position (i.e. 9c-18:1 and 9t-18:1), and *iso* and *anteiso* isomers (i.e. 16:0 and 16:0i, 15:0 and 15:0ai).

Mixtures of *cis* and *trans* isomers consisting of numerous positional isomers, found in most monoene fractions resulting from the partial hydrogenation of fats and oils, are not resolved sufficiently for quantitation. This requires separation of the *cis* and *trans* isomers by argentation thin-layer chromatography (TLC). To this end, Merck DC Fertigplatten Kieselgel 60 were dipped in 12% AgNO3 in methanol:water (2:1) for 30 s. The plates were dried for 2 h at 70°C in the dark and stored in an aluminium box. Fatty acid methyl esters were applied and the plates were developed in the dark as follows: (a) for 10 min in toluene and a 5:1 ethyl acetate:acetic acid mixture (40:3); (b) about 30 min in the same solvent but with a liner of 5 cm; (c) about 75 min in toluene and ethyl acetate (40:3) with a liner of 10 cm. The methyl esters were visualized after spraying with 2′,7′-dichlorofluorescein (0.5% in methanol) under ultraviolet light (350 nm). The bands were scraped off into counting vials.

Samples were analyzed in triplicate, and the results were given as the mean of triplicate analyses. A standard mixture of fatty acids was also quantitatively analyzed by gas chromatography under the same conditions. The percentage of relative peak area was compared with the weight of fatty acids, to obtain the detector response factors (all approximately 1.0).

3. Results and discussion

Oils and fats that are typically used in margarine manufacturing include, sunflower, cottonseed, corn, and soybean oils. Hydrogenated marine fish oils are used in some countries. Safflower and sunflower oils are more typically used in soft and liquid oil margarines because of their high polyunsaturated content. Margarines must have some crystalline structure to maintain a semisolid consistency at refrigerator and room temperatures. A sharp melting point is needed at body temperature so that the margarines will melt rapidly in the mouth leaving no waxy mouthfeel. Solidification of oils to produce margarines must be done by selective hydrogenation to reach the correct polyunsaturated fatty acids:monounsaturated fatty acids:saturated fatty acids ratios and to control conversion from *cis* bonds to *trans* bonds. Oleic acid (*cis*-9-octadecenoic acid) melts at 16.3°C whereas elaidic acid (*trans*-9-octadecenoic acid) melts at 43.7°C and vaccenic acid (*trans*-11-octadecenoic acid) melts at 44.0°C, so that the presence of some *trans* isomers may markedly raise the melting point and stability of a product. Stick-type margarines contain 10–29% *trans* fatty acids, and

tub-type margarines have 10–21% *trans* fatty acids (Weihrauch, Brignoli, Reeves & Iverxson, 1977). In addition to partial hydrogenation, the correct consistency of a margarine can be obtained by blending soft and hard fats.

The average daily consumptions of the major dietary fatty acids found in the test margarines are given in Table 1 and the average fatty acid compositions of the participants' serum lipids after 6 weeks on the test margarines are given in Table 2.

The ability of dietary saturated fatty acids, as a lipid class, to raise total cholesterol levels, compared to carbohydrate, is well established. LDL-cholesterol levels, as well as total cholesterol concentrations, are raised by saturated fatty acids in the diet (Grundy & Denke, 1990).

The principal saturated fatty acid in most diets is palmitic acid (Table 1). It has been shown clearly that palmitic acid increases LDL-cholesterol levels in parallel with total cholesterol concentration when it is substituted for carbohydrates or monounsaturates in the diet (Grundy & Denke, 1990). It has been suggested that myristic acid raises the total cholesterol even more than does palmitic acid (Grundy & Denke). Stearic acid, in contrast to palmitic acid, seemingly does not raise the total cholesterol level. Since stearic acid does not raise LDL-cholesterol levels, it might be used as a replacement for palmitic or myristic acid in diets designed to lower the cholesterol level. In spite of a common belief to the contrary, hydrogenation of vegetable oils does not necessarily transform them into cholesterol-raising fats because stearic acid, not palmitic acid, is the saturated acid produced by hydrogenation (Bonanome & Grundy, 1988). This assumes of course that *trans* unsaturated fatty acids, also formed during hydrogenation, do not raise cholesterol levels.

The major monounsaturated fatty acid in the diet is oleic acid (9c-18:1). Oleic acid has been considered "neutral" in its influence on total cholesterol levels. Hydrogenation of vegetable oils rich in polyunsaturated fatty acids produces considerable quantities of elaidic acid (9t-18:1), a *trans* monounsaturated fatty acid. Several other *trans*-unsaturated isomers are formed as well. It was found that *trans* monounsaturates, like oleic acid, are "neutral" and do not increase serum cholesterol levels, one study suggested that they raise cholesterol concentrations (Vergroesen, 1972).

Two types of polyunsaturated fatty acids occur in the diet, *n*-6 and *n*-3 polyunsaturates. The predominant *n*-6 fatty acid is linoleic acid (18:2) which comes mainly from plant oils. The parent *n*-3 polyunsaturate is linolenic acid (18:3), and it also occurs in certain vegetable oils. Soybean oil, rapeseed oil, and linseed oil are particularly rich sources of linolenic acid. The fish oils contain large amounts of very-long-chain polyunsaturates that have their origins from linolenic acid of plant sources. Major *n*-3 fatty acids in fish oils are eicosapentaenoic acid (20:5 *n*-3) and docosahexaenoic acid (22:6 *n*-3). Together they constitute about 26% of fish oil fatty acids. For many years, linoleic acid was considered a "cholesterol-lowering" fatty acid. In general, using oleic acid as a baseline, they (Bonanome & Grundy, 1988; Grundy & Denke, 1990; Kinsella et al., 1981; Vergroesen, 1972) estimated that linoleic acid lowers the cholesterol level about half as much as saturated fatty acids raise it. It was assumed that the cholesterol-lowering action of linoleic acid occurs mainly in the LDL fraction.

Average fatty acid compositions of different margarine samples consumed daily in Turkey are given in Table 3. The fatty acid compositions of total erythrocyte membranes of margarine users and non-margarine users are shown in Table 4. Concentrations of *trans* fatty acids (TFA) were determined in soft-, breakfast- (soft-type), hard-, and paste and cake-type (hard-type) Turkish margarines (Table 3). The samples of soft-type had TFA concentrations of 5.2–6.1% (average 5.65%), while margarines of the hard-type had

Table 1
Contents of fatty acids consumed daily from test margarine (wt% of total acid as mean and ±S.D. for three separate determinations)

Test fat	14:0	16:0	16:1	18:0	18:1	18:2
Soft margarine	2.0±0.7	12.7±3.4	0.8±0.3	6.7±2.5	21.0±5.2	38.8±12.1
Hard margarine	1.1±0.1	13.3±4.0	0.8±0.2	7.0±2.2	51.8±18.0	9.3±4.7

Table 2
Fatty acid composition of total serum lipids from participants on the margarine used diets (wt% of total acid as mean and ±S.D. for three separate determinations)

Test fat	14:0	16:0	16:1	18:0	18:1	18:2	20:4
Soft margarine	1.0±0.3	20.0±3.0	2.2±0.6	6.9±0.7	15.6±2.7	35.1±4.3	5.3±1.2
Hard margarine	1.1±0.4	22.5±2.0	3.0±0.5	6.2±0.8	21.2±2.8	27.6±3.8	4.7±1.1

Table 3
Average fatty acid compositions of different margarine samples consumed daily in Turkey (wt% of total acid as mean and ±S.D. for three separate determinations)

Fatty acid	Soft margarine	Breakfast margarine	Hard margarine	Paste and cake margarine
12:0	0.05	0.06	0.12	0.12
14:0	0.08	0.10	0.74	0.68
15:0	0.18±0.04	0.20±0.05	0.22±0.06	0.18±0.05
16:0	5.86±0.82	6.34±0.64	25.9±1.62	24.6±2.14
9c-16:1	0.16±0.05	0.72±0.08	0.76±0.12	0.74±0.11
9t-16:1	0.08	0.14	0.22±0.05	0.20±0.04
17:0	0.28±0.07	0.24±0.06	0.30±0.08	0.32±0.08
17:1	0.09	0.08	0.10	0.10
18:0	4.12±1.14	3.86±0.96	2.34±0.78	2.68±0.86
9c-18:1	18.1±4.42	19.1±4.34	42.7±8.76	45.2±7.92
9t-18:1	2.54±0.56	3.16±0.68	14.6±2.92	18.6±3.24
9c,12c-18:2	62.5±6.38	61.3±7.40	9.60±1.56	9.38±2.12
9c,12t-18:2	0.58±0.32	0.68±0.12	0.12	0.08
9t,12t-18:2	1.38±0.06	1.52±0.28	0.05	0.05
9t,12c-18:2	0.46±0.52	0.48±0.16	0.14	0.10
9c,12c,15c-18:3	0.56±0.14	0.50±0.08	0.64±0.10	0.48±0.08
9t,12c,15t-18:3	0.16±0.04	0.12	0.14	0.11
19:2	0.46±0.12	0.38±0.08	0.12	0.14
20:0	0.32±0.08	0.34±0.05	0.46±0.14	0.52±0.10
22:0	0.05	0.05	0.08	0.08
24:0	0.04	0.05	0.06	0.06
Total *cis* acids	81.3	81.7	53.7	55.8
Total *trans* acids	5.20	6.10	15.2	19.2
Total *trans* mono-	2.62	3.30	14.8	18.8
Total *trans* di-	2.42	2.68	0.31	0.23
Total *trans* tri	0.16	0.12	0.14	0.11

Table 4
Membrane fatty acid composition of human erythrocytes from margarine users and non-users (wt% of total acid as mean and ±S.D. for three separate determinations)

Fatty acid	Soft margarine user	Hard margarine user	Non-margarine user
12:0	0.05	0.12	0.14
14:0	0.78	0.94	1.36±0.32
15:0	0.18±0.04	0.22±0.06	0.34±0.05
16:0	5.16±0.82	23.9±1.62	24.2±2.08
9c-16:1	0.16±0.05	0.76±0.12	2.94±0.62
9t-16:1	0.08	0.22±0.05	0.28±0.06
17:0	0.28±0.07	0.30±0.08	0.56±0.12
17:1	0.09	0.10	0.14
18:0	3.92±1.14	2.34±0.78	7.52±1.12
9c-18:1	20.1±4.42	46.7±8.76	17.9±2.64
9t-18:1	2.86±0.56	11.5±3.02	8.40±1.24
9c,12c-18:2	57.5±6.38	12.2±1.56	32.1±3.82
9c,12t-18:2	0.40±0.10	0.12	0.38±0.12
9t,12t-18:2	1.38±0.36	0.24±0.06	1.18±0.26
9t,12c-18:2	0.40±0.08	0.14	0.28±0.06
9c,12c,15c-18:3	0.56±0.14	0.64±0.10	0.52±0.12
9t,12c,15t-18:3	0.16±0.04	0.14	0.16±0.04
19:2	0.46±0.12	0.12	0.08
20:0	0.32±0.08	0.46±0.14	0.18±0.04
20:4	5.46±1.08	4.28±0.66	5.26±1.00
22:0	0.05	0.08	0.08
24:0	0.04	0.06	0.06
Total *cis* acids	78.3	60.3	53.4
Total *trans* acids	5.28	12.4	10.7
Total *trans* mono-	2.94	11.7	8.68
Total trans di-	2.18	0.50	1.84
Total trans tri-	0.16	0.14	0.16

TFA concentrations ranging from 15.2 to 19.2% (average 17.2%). Average *trans* monoenoic-acid concentrations in soft- and hard-type margarines were 3.0 and 16.8%, respectively. Average *trans* dienoic-acid concentrations in soft- and hard-type margarines were 2.55 and 0.27%, respectively. Manufacturer's information on the soft margarines indicated that they were composed of 82% sunflower and 18% of modified safflower, corn, and soybean oils. The hard margarines contained cottonseed and peanut oils. The hard and soft margarines contained one-half and one-third as much saturated fatty acids as butter-olive and butter-sun and butter, respectively (Wood, Kubena, O'Brien, Tseng & Martin, 1993).

Trans fatty acid composition of four important margarines available in Bulgaria has been investigated (Tsanev, Russeva, Rizov & Dontcheva, 1998). The *trans* oleic acid content varied from 1.9 to 8.0%, while *trans* linoleic acid concentrations were 0.4 to 1.4% in the imported margarine studied. Total TFA values were $<3\%$ in all samples from Portugal and Belgium and also for Netherlands margarines with the exception of one containing only 17.1% TFA (Matsuzaki, Ota, Kinoshita, Maruyama, Niiya & Sugano, 1998). Japanese hard-type margarines had TFA concentrations of 15–28%, while Japanese soft-type margarines contained $<3\%$ TFA. UK hard-type margarines had TFA concentrations of 3–17%, while UK in soft-type margarines ranged from 1–18%. Average TFA concentration in US margarines were 24.8 and 14.2% for hard- and soft-type samples, respectively (Matsuzaki, Ota, Kinoshita, Maruyama, Niiya & Sugano, 1998).

References

Aktaş, M., Keha, E., Yılmaz, N., & Demirbaş, A. (1999). Phospholipids from erytrocyte membranes of margarine users and non-users. *Energy Education Science & Technology, 2*, 91–96.

Angeligo, F., Amodeo, P., Borgogelli, C., Catafora, A., Montali, A., & Ricci, G. (1980). Red blood cell fatty acid composition in a sample of Italian middle-aged men on free diet. *Nutr. Metab., 24*, 148–153.

Bonanome, A., & Grundy, S. M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *New England Journal of Medicine, 318*, 1244–1248.

Christie, W. W. (1973). *Lipid analysis*. New York: Pergamon Press.

Dutton, H. J. (1971). Hydrogenation of fats. In R.T. Holman, *Progress in Chemistry of Fats and Other Lipids 9*, 351–370.

Dutton, H. J. (1979). Hydrogenation of fats and its significance. In E. A. Emken, & H. J. Dutton, *Geometrical and positional fatty acid isomers* (pp. 1–16). Champaign, IL: Am Oil Chem Soc.

Dougherty, R. M., Galli, C., Ferro-Luzzi, A., & Iacono, J. M. (1987). Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. *American Journal of Clinical Nutrition, 45*, 443–455.

Glatz, J. F. C., Sofferz, A. E. M. F., & Katan, M. B. (1989). Fatty acid composition of serum cholesteryl esters and erythrocyte membranes as indicators of linoleic acid intake in man. *American Journal of Clinical Nutrition, 49*, 269–276.

Grundy, S. M., & Denke, M. A. (1990). Dietary influences on serum lipids and lipoproteins. *Journal of Lipid Research, 31*, 1149–1172.

Hanahan, D. J., Watts, R. M., & Pappajohn, D. (1960). Some chemical chracteristics of the lipids of human and bovine erythrocytes and plasma. *Journal of Lipid Research, 1*, 421–432.

Kinsella, J. E., Bruckner, G., Mai, J., & Shimp, J. (1981). Metabolism of trans fatty acids with emphasis on the effects of *trans*, *trans*-octadecadienoate on lipid composition, essential fatty acid, and prostaglandins: an overview. *American Journal of Clinical Nutrition, 34*, 2307–2318.

Manku, M. S., Horrobin, D. F., Huang, Y.-S., & Mors, N. (1983). Fatty acids in plasma and red cell membranes in normal humans. *Lipids, 18*, 906–908.

Matsuzaki, H. C., Ota, C., Kinoshita, Y., Maruyama, T., Niiya, I., & Sugano, M. (1998). Trans-fatty acid content of margarines in Portugal, Belgium, Netherlands, United Kingdom, United States, and Japan. *Journal of Japan Oil Chemists' Society, 47*, 195–199.

Nordoy, A. (1974). Plasma and platelet lipids in man. *Haemostatis, 2*, 103–117.

Phillips, G. B., & Dodge, J. T. (1967). Composition of phospholipids and of phospholipid fatty acids of human plasma. *Journal of Lipid Research, 8*, 676–681.

Pop-Snijders, C., Schouten, J. A., van Blitterswijk, W. J., & van der Veen, E. A. (1986). Changes in menbrane lipid composition of human erythrocytes after dietary supplementation of (*n*-3) polyunsaturated fatty acids. Maintenance of membrane fluidity. *Biochimica et Biophisica Acta, 854*, 273–287.

Renaud, S., Morazain, R., & Godsey, F. (1981). Platelet functions in relation to diet and serum lipids in British farmers. *British Heart Journal, 46*, 562–570.

Schelenk, H., & Gellerman, J. L. (1960). Esterification of fatty acids with diazomethane on a small scale. *Analytical Chemistry, 32*, 229–231.

Tsanev, R., Russeva, A., Rizov, T., & Dontcheva, I. (1998). Content of *trans*-fatty acids in edible margarines. *Journal of American Oil Chemists Society, 75*, 143–145.

Vergroesen, A. J. (1972). Dietary fat and cardiovascular disease: possible modes of action of linoleic acid. *Proc Nutr Soc., 31*, 323–329.

Weihrauch, J. L., Brignoli, C. A., Reeves, J. B. III, & Iverxson, J. L. (1977). Fatty acid composition of margarines, processed fats, and oils. *Food Technology, 31*, 80–85.

Wood, R., Kubena, K., O'Brien, B., Tseng, S., & Martin, G. (1993). Effect of butter, mono- and polyunsaturated fatty acid-enriched butter, *trans* fatty acid margarine, and zero *trans* fatty acid margarine on serum lipids and lipoproteins in healthy men. *Journal of Lipid Research, 34*, 1–11.