

## CHANGES IN FATTY ACID COMPOSITION OF FOODSTUFFS DURING CONVENTIONAL AND MICROWAVE HEAT TREATMENT

### PROMJENE U SASTAVU MASNIH KISELINA HRANE ZA VRIJEME UOBIČAJENOG POSTUPKA I POSTUPKA GRIJANJA U MIKROVALNOJ PEĆNICI

**Rozália Veronika Salamon<sup>1</sup>, Katalin Lóki<sup>2</sup>, Szidónia Salamon<sup>1</sup>, Péter Sára<sup>2</sup>, Beáta Albert<sup>1</sup>, Jánosné Csapó<sup>2</sup>, Anikó Győri<sup>3</sup>, Zoltán Győri<sup>3</sup>, János Csapó<sup>1,2</sup>**

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#### ABSTRACT

In our experiments fatty acid composition was determined of cow's milk with the fat content of 3.6%, Dalia cheese with the fat content of 44%, butter with the fat content of 80% and margarine with the fat content of 24% after a heat treatment performed on a cooking plate and microwave treatment, of different duration. The biggest difference was obtained for oleic acid and elaidic acid since with the exception of margarine, in each case the proportion of the cis-configured oleic acid decreased while that of the trans-configured elaidic acid increased. For all of the other fatty acids in the foodstuffs examined no such differences were obtained regarding change in fatty acid composition, which differences could considerably influence healthy nutrition, therefore we can take it as a fact that neither heat treatment performed on a traditional cooking plate nor microwave treatment affects considerably the composition of food fats.

**Keywords:** milk, cheese, butter, margarine, fatty acid composition, cis-trans isomers, microwave

#### INTRODUCTION

Role of fats and that of fatty acids, they are composed of, in the human nutrition is well-known. Fats contained in foodstuffs provide substantial amount of energy for the human organism, and the essential and semi-essential fatty acids are – as the human organism cannot produce them – indispensable to the human organism. Recently, there have been many discussions on trans fatty acids, some experts considered their harmful effects to be proven, while others could not report on such negative effects. Some were of the opinion that trans fatty acids increased fragility of red blood

cells, changed the aggregation of thrombocytes (Ascherio, 1994; Ascherio et al., 1999; Ascherio, 2002), and evidenced their negative effects on the metabolism of linolenic acid and arachidonic acid (Larque et al., 2000). It was established that they caused lack of essential fatty acids (Kummerow et al., 2004), inhibited synthesis of prostaglandin (Kushi and Giovannucci, 2002) increases the risk of certain cancers. Lately, it has been reported that incorporation of trans fatty acids into the phospholipids of the membranes affected its properties and mainly the activity of enzymes attached to the membrane, in fact, in recent times a positive relation has been established between

MSc Rozália Veronika Salamon, MSc Szidónia Salamon and MSc Beáta Albert - Sapientia Hungarian University of Transylvania, Csíkszereda Campus, Csíkszereda, RO-4100 Szabadság tér 1

MSc Katalin Lóki, MSc Péter Sára, Dr. Jánosné Csapó and Prof. Dr. János Csapó - University of Kaposvár, Faculty of Animal Science, Kaposvár, H-7400 Guba S. u. 40.

MSc Anikó Győri and Prof. Dr. Zoltán Győri University of Debrecen, Centre of Agricultural Sciences, Debrecen, H-4032 Böszörményi út 138.

allergic diseases and trans fatty acid consumption (Kritchevsky, 1997; Stender and Dyerberg, 2004).

Cis-trans transformations can take place due to several technological interventions, as well. The most important operation is partial hydrogenation as a result of which a part of cis-configured bonds transforms into trans configuration. By appropriate choice of technological parameters this transformation can be the slightest and that the products contain trans isomers in minimal quantity. Heating fats can also cause isomerization, cooking in oils can result in appearance of multiple unsaturated trans fatty acids, and heat treatment of fats can produce trans fatty acids and even cyclic fatty acid derivatives (Hansen and Leth, 2000).

It was reported that fatty acids could convert as a result of microwave treatment, as well. With soya bean, after being microwave-treated for 12 min transformation and decomposition of great volume of fatty acids was experienced. Comparing changes in fatty acid composition during food making procedures with the effect of microwave treatment some experts concluded that considerable changes could be expected during such treatment and they suggested other procedure instead of microwave treatment for warming up foods (Sachiko and Hiromi, 2002).

Because of the above, in the present research we aimed at analyzing changes of fatty acid composition of milk and foodstuffs with high fat contents (cheese, butter, margarine) as an effect of traditional heat treatment and microwave treatment. We paid special attention to the cis-configured oleic acid and trans-configured elaidic acid which forms from the former by isomerization.

## MATERIALS AND METHODS

### Samples examined

Milk sample with the fat content of 3.6% was obtained from a seven years old Simmental cow, feed mainly hay and minimal feed supplementation, in the 2nd month of lactation. Sample was taken from the mixed milk of the completely milked-out udder. Other components of

the milk corresponded to the values characteristic for normal cow's milk in every respect.

The cheese examined was commercially obtainable under the trade name of Dalia, which was semi-hard, coagulated with mixed coagulated rennet, pressed, formed in brine of 10%, then matured over 2 weeks at 13–14 °C. Its dry matter content was 55% and the fat content referring to dry matter content was 44%. The other cheese used in our experiment was commercially obtainable under the trade name of Telemea, a type of feta cheese, coagulated with *Lactobacillus acidophilus* pure culture, with enzymatic rennet, pressed, sliced and matured over 2 days. Dry matter contents of this cheese was 55% and its fat content referring to dry matter content was 44%.

The butter used was a commercially obtainable butter of trade name Alpenbutter. The margarine examined was of a Holland-24 type, commercially obtainable margarine.

### Heat treatment and microwave treatment

Heat treatment was carried out on a laboratory hot plate, for 2 and 8 min, respectively, after the boiling point was reached. Microwave treatment was applied for 1, 2, 4, and 8 min using a normal Electrolux EMN 2015 microwave oven at electrical power of 450 W. Subsequent to the heat treatment the samples were cooled down immediately and stored at –25 °C until the preparation for analysis.

### Determination of fatty acid composition

#### Sample preparation

A sample quantity containing approx. 0.5–1.0 g fat was destructed with 8–20 cm<sup>3</sup> of hydrochloric acid (37%) for 1 hour in a hot water bath. After having cooled down, 7 cm<sup>3</sup> of ethanol was added. Lipids were extracted with 15 cm<sup>3</sup> diethyl ether and 15 cm<sup>3</sup> benzine (b.p.<60 °C), and the organic layers were combined. From a portion of this solution, containing approx. 150–200 mg fat, the solvents were removed under reduced pressure (a complete evaporation not necessary). To the 4 cm<sup>3</sup> residue of 0.5 M sodium hydroxide methanol solution was added and boiled until all the fat

drops disappeared (approx. 5 min), then 4 cm<sup>3</sup> of 14% boron trifluoride methanol solution was added, boiled for 3 min, finally 4 cm<sup>3</sup> of hexane, dried on water-free sodium sulphate, was added and boiled for 1 min, and the mixture was allowed to cool down. Saturated aqueous sodium chloride solution was added and after having separated the organic layer was collected into a 4 cm<sup>3</sup> vial containing water-free sodium sulphate and was directly examined by gas chromatography.

Conditions of the gas chromatographic analysis

Instrument: Chrompack CP 9000 gas chromatograph. Column: 100 m·0.25 mm id, CS-Sil 88 (FAME) phase. Detector: FID 270 °C. Injector:

splitter, 270 °C. Gas carrier: He, 235 kPa. Temperature program: 140 °C for 10 min; at 10 °C/min up to 235 °C; isotherm for 26 min. Injected volume: 0.5–2 µl.

## RESULTS AND CONCLUSIONS

Change in the fatty acid composition of milk as an effect of traditional heat treatment and microwave heat treatment

Change in the fatty acid composition of raw milk in the control sample and in the samples heat treated for 2 and 8 min, on a cooking plate and in a microwave oven, respectively, is shown in Table 1.

Table 1. Change in the fatty acid composition\* as an effect of heat treatment performed on a cooking plate and of microwave treatment, respectively

Tablica 1. Promjena u sastavu masnih kiselina kao djelovanje toplinskog postupka na ploči za kuhanje odnosno u mikrovalnoj pećnici

Fatty acid / Masna kiselina	Control / Kontrola	Microwave treatment / Mikrovalni postupak		Cooking plate / Ploča za kuhanje	
		2 min	8 min	2 min	8 min
Miristic acid, C14:0 Miristinska kiselina	11.45	11.62	11.35	11.05	10.94
Palmitic acid, C16:0 Palmitinska kiselina	42.23	42.17	43.21	42.95	42.27
Stearic acid, C18:0 Stearinska kiselina	10.07	11.58	11.80	11.27	11.42
Linoleic acid, C18:2 Linolna kiselina	1.38	1.32	1.31	1.36	1.30
Linolenic acid, C18:3 Linolenska kiselina	1.17	1.19	1.15	1.09	1.12

\*In relative weight% of fatty acid methyl esters. Only fatty acids with concentrations higher than 9–10%, as well as linoleic acid and linolenic acid are shown. Oleic acid and elaidic acid are shown in Table 3.

As it can be seen from the data in Table 1, milk contains more fatty acids than the microwave-treated milk, no significant differences, however, could be found between the microwave treatments. Similarly, no significant differences could be found in the palmitic acid and stearic acid content, the fatty acids are mounting the major part of the total fatty acid content. In the raw milk the milk fat contained 16.26% oleic acid and 1.53% elaidic

acid; the cis configuration was 91.36% and the trans configuration was 8.64% of the total C18:1 fatty acids. After cooking for 2 min the proportion of the cis-configuration reduced by 2%, and after cooking for 8 min it reduced by 4%; the proportion of the trans configuration increased as an effect of cooking (for 2–8 min) by around 15–20%. Similar changes could be observed due to microwave treatment: after a treatment of 2 min the proportion

of the cis configuration reduced by 2%, and after 8 min by nearly 10%, whereas the proportion of the trans configuration increased by 10–15% after a treatment of 2 min, and by 40–50% after a treatment of 8 min. Hence, the conclusion can be drawn that heat treatment carried out for 2 and 8 min at 100 °C, and microwave treatment performed for 2 and 8 min at 450 W reduces the proportion of the cis-configured oleic acid and increases the

proportion of the trans-configured elaidic acid to a significant extent.

In the case of the cheeses Dalia and Telemea changes occurred due to microwave treatment and are summarized in Table 2, changes of the oleic acid and elaidic acid content of examined dairy products as an effect of traditional and microwave heating are summarized in Table 3.

**Table 2. Changes in the fatty acid composition\* of cheeses Dalia and Telemea due to microwave treatment**

**Tablica 2. Promjena u sastavu masnih kiselina u siru Dalia i Telemea nakon mikrovalnog postupka**

Fatty acid / Masne kiseline	Dalia			Telemea		
	Control	2 min	8 min	Control	2 min	8 min
Miristic acid, C14:0	9.83	9.39	9.27	10.23	10.87	10.11
Palmitic acid, C16:0	29.24	28.99	30.33	30.79	31.77	32.14
Stearic acid, C18:0	14.41	14.66	15.45	13.13	13.42	13.85
Linoleic acid, C18:2	1.80	1.79	1.72	1.89	1.85	1.83
Linolenic acid, C18:3	1.50	1.42	1.40	1.08	1.06	1.04

\*In relative weight% of the fatty acid methyl esters.

In the cheese of Dalia-type with 44% fat content in the control sample not microwave-treated the proportion of oleic acid was 83.84% that of elaidic acid was 16.16% in the total of C18:1 fatty acids. As an effect of the 2 min microwave treatment the proportion of cis configuration decreased by 1.5%, after treatment of 8 min it decreased by 2%. In 2 min treatment the proportion of trans configuration increased by 8% and after 8 min by 9–10%. For all of the other fatty acids no significant changes were experienced in the microwave treatment, and the composition of treated samples was practically identical with that of the control sample. In the two cheese samples examined fatty acids behaved in the same way as in the microwave treatment.

The oleic acid content of butter with 80% fat content was found to be 23.37%, the elaidic acid content was 3.62%, of total fatty acids. Within C18:1 fatty acids in untreated butter oleic acid was

86.58%, elaidic acid 13.42%. These proportions changed slightly in samples treated both on cooking plate at 215 °C and in microwave oven at 450 W. Increasing from 2 min to 8 min the duration of the treatment with a cooking plate at 215 °C the proportion of oleic acid decreased by 1%, and similar results were obtained when time of microwave treatment was increased from 2 to 8 min. Looking at the proportions in both treatment durations and both heat treatment methods the decrease of oleic acid corresponds within the limits of error to the increase of elaidic acid. From our experiments we can draw the conclusion that with increasing duration of the heating in both experiments performed on the cooking plate and that performed in the microwave oven the quantity of cis-configured oleic acid reduces whereas that of trans-configured elaidic acid grows.

**Table 3. Changes of the oleic acid and elaidic acid content\* of various dairy products due to conventional and microwave heat treatment**

**Tablica 3. Promjene sadržaja oleinske i elaidne kiseline u raznim mliječnim proizvodima nakon uobičajenog i mikrovalnog toplinskog postupka**

A vizsgált minta	C18:1 fatty acid ratio / omjer masnih kiselina	
	Oleic acid / Oleinska kiselina	Elaidic acid / Eladinska kiselina
Milk control / Kontrola mlijeka	91.36	8.64
heat treatment / toplinski postupak 2 min	89.64	10.36
8 min	87.18	12.82
microwave treatment / mikrovalni postupak 2 min	90.08	9.92
8 min	84.11	15.89
Dalia control / Kontrola Dalia	83.84	16.16
heat treatment / toplinski postupak 2 min	82.46	17.54
8 min	82.21	17.79
Telemea control / Telemea	84.73	15.27
microwave treatment / mikrovalni postupak 2 min	82.19	17.81
8 min	81.47	18.53
Butter control / Kontrola maslaca	93.63	6.37
heat treatment / toplinski postupak 2 min	90.86	9.14
8 min	90.49	9.51
microwave treatment / mikrovalni postupak 2 min	91.23	8.77
8 min	90.63	9.37
Margarine control / Kontrola margarina	14.36	85.64
heat treatment / toplinski postupak 2 min	14.23	85.77
8 min	14.40	85.60
microwave treatment / mikrovalni postupak 2 min	14.29	85.71
8 min	14.73	85.27

\*Percentages of C18:1 fatty acids (total C18:1 fatty acid contents=100%).

In margarine with 25% fat content it appears that the heat treatment and microwave treatment, respectively, do not affect concentration of cis and trans fatty acids. This contradicts our previous examinations, our experiences until now, and the literature as well, since the cis configuration is substantially less stable than trans, so after a heat treatment in principle the quantity of fatty acids cis configuration should decrease and that of fatty acids with trans configuration should be increase. As an effect of heat treatment concentration of unsaturated fatty acids should be reduced due to various oxidative reactions and chain-cracking at the double bonds.

Summarizing the effect of heat treatment and microwave treatment in our experiments it can be

said that with the exception of oleic and elaidic acids, the differences in fatty acid composition are so slight that they do not indicate any harmful effect of either heat treatment or microwave treatment. For these fatty acids there is no difference in the effect of the duration of heat treatment and microwave treatment, either, since no difference could be evidenced between the treatments with duration of 2 min and 8 min, respectively. We can take it as a fact therefore, that for the examined foodstuffs time and energy combination used does not result in considerable deviation in the fatty acid composition, thus, there is no need to be afraid that during microwave treatment any artificial product harmful to human beings will form or that biological value and

utilization in the human organism of fat in foodstuffs treated this way will reduce significantly.

In the case of oleic acid and elaidic acid it was established that by both heat treatments performed on a cooking plate and in a microwave the proportion of the cis-configured oleic acid decreased and that of the trans-configured elaidic acid increased. This decrease and increase, do not reach, however, the extent, which could affect the healthy nutrition.

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## SAŽETAK

U našim smo pokusima odredili sastav masnih kiselina u kravljem mlijeku sa sadržajem masnoće od 3,6%, u siru Dalia sa sadržajem masnoće od 44%, u maslacu s 8% i u margarinu s 24% nakon postupka na ploči za kuhanje i u mikrovalnoj pećnici, različitog trajanja. Najveća je razlika dobivena za oleinsku kiselinu i eladičnu kiselinu jer, s iznimkom margarina, u svakom se slučaju omjer cis-konfiguracije oleinske kiseline smanjio dok je trans-figuracija eladične kiseline porasla. Za sve druge masne kiseline u ispitanoj hrani nisu dobivene takve razlike u vezi s promjenom sastava masnih kiselina, što bi mogle u znatnoj mjeri djelovati na zdravu hranidbu, pa možemo uzeti kao činjenicu da niti mikrovalni postupak izveden na tradicionalnoj ploči za kuhanje niti mikrovalni postupak ne djeluju na sastav masnoća u hrani u znatnoj mjeri.

Ključne riječi: mlijeko, sir, maslac, margarin, sastav masnih kiselina, cis-trans izomeri, mikroval