# TRANS FATTY ACIDS IN FREQUENTLY CONSUMED PRODUCTS FROM SERBIAN AND SLOVENIAN MARKET

Danijela Ristić-Medić<sup>1</sup>, Snježana Petrović<sup>1</sup>, Tomaž Polak<sup>2</sup>, Jasna Bertoncelj<sup>2</sup>, Aleksandra Arsić<sup>1</sup>, Marija Takić<sup>1</sup>, Vesna Vučić<sup>1</sup>, Mirjana Gurinović<sup>1</sup>, Mojca Korošec<sup>2</sup>

<sup>1</sup>Centre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research, National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia

<sup>2</sup>Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

#### **SUMMARY**

Objectives: Industrially produced trans fatty acid (iTFA) have adverse health effects and thus their consumption should be limited. The aim of this study was to determine and compare the iTFA content in frequently consumed food products by young adults from the Serbian and Slovenian market with supposedly elevated iTFA content in 2015. At the time of this study, there was no recommended limit of iTFA in both countries, and reduction of iTFA in foods was on voluntary basis.

Methods: We determined iTFA content in food products, 19 from the Serbian and 22 from the Slovenian market, blinded and analysed in the same analytical run. Contents of fatty acids (FA) methyl esters were analysed by capillary gas chromatography with a flame ionisation detector. Heptadecanoic acid was used as internal standard. Individual FA along with TFA were expressed as percentages of total measured FA. The amount of each FA in the sample was then calculated from the response factor and the transformation factor of the FA from the FA methyl ester content.

Results: Elaidic acid (C18:1t) was found as the most abundant TFA in analysed products, ranging from 0.52 g/100 g of total FA in chocolate candy up to 60.4 g/100 g in a salami from Serbian market. In Slovenian products, the values for elaidic acid were lower, 0.04–3.95 g/100 g of total FA, except in one type of wafers (24.3 g/100 g).

Conclusions: The majority of analysed products from the Serbian and three from Slovenian market exceeded the recommended WHO and EU limit of 2% iTFA of total fat in foods. Samples of frequently consumed salami, wafers, tea biscuits, and snacks were identified as products with potentially higher burden of iTFA in diets of young adults in Serbia.

Key words: trans fatty acids, confectionary products, snacks, fast food, Serbia, Slovenia

Address for correspondence: M. Korošec, Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia. E-mail: mojca.korosec@bf.uni-lj.si

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#### INTRODUCTION

Trans fatty acids (TFA) are unsaturated fatty acids that contain at least one double bond in the trans configuration. Trans fats present in the food products can be naturally occurring (ruminant rTFA) or industrially produced (iTFA). The majority of dietary iTFA comes from partially hydrogenated vegetable oils (1) and products that contain these oils, including some commercial baked, pre-packaged, processed, and fast food. Industrial TFA are found to have adverse health effects, such as increased total and LDL-cholesterol, and decreased cardio protective HDLcholesterol levels. Available literature strongly connects iTFA intake with cardiovascular diseases (CVD) (2-4). The risk associated with rTFA is still unclear but effect, if any, is probably small considering their low intake levels (5). By far the most important health effect of TFA consumption is the detrimental effect on heart disease. The declines in CVD deaths after legislative limits for iTFA in food in Denmark, also recently dealt with by Slovenian researchers (7), showed that TFA policy's effects are specific to the CVD death rate and obesity rates. According to recent national reports and the World Health Organization (WHO), in Serbia 54% of deaths were due to CVD, while in Slovenia CVD are responsible for 40% of all death causes (8).

To reduce the incidence of coronary heart disease, the WHO has recommended that the daily intake of total TFA should not exceed 1% of total energy intake, corresponding to approximately 2.5 g of TFA per day per adult (9, 10). Recent public health recommendations indicated that TFA dietary intake should be less than 0.5% of total daily energy intake for patients with CVD (11). The elimination of iTFA from food is in line with WHO priority targets for 2019–2023 (10, 12). Thus, the highest content of iTFA in foodstuffs of total fat was set in EU countries with limiting legislation (13). There are worldwide efforts to meet these goals. The European Commission (EC) recognized two possible approaches to limiting iTFA in foodstuffs: legislative actions and voluntary restriction. Legislative limit of iTFA content in food was confirmed as the most effective measure aimed to protect public health. Up to now, more than 40 countries have acted to

initiate or advance TFA policy (12). In Slovenia, after several years of intended voluntary reduction and gathering evidence the rule imposing maximum 2% iTFA of total fat came fully in effect by April 2019 (14). Regional regulations, covering 34 countries, will contribute immensely to Europe's success in iTFA elimination. However, there are still products on the South-Eastern European food market with high levels of iTFA (6). Despite increased extent of voluntary reductions by the food producers, the intake of iTFA remains high in countries, which have no legislative limitations on iTFA levels, such as Serbia. In support of this, a study by Stender et al. (15) revealed high levels of iTFA in many biscuits, cakes and wafer products from national markets in this region in 2012 and 2014. This study covered all former Yugoslavian countries and reported similar situation in all these countries. On the other hand, Slovenia is an EU member from 2004, and it could be expected that TFA content in food is reduced on voluntary basis, following other EU countries and objectives of the National Programme on Nutrition and Health for 2015–2025 (16), in spite of absence of legislation on dietary TFA at the time of this study. Information on iTFA intake, considering that Slovenia is a member of the EU and Serbia not, is essential for estimation whether these countries are meeting the population goal intake set by EC nutrition experts.

The aim of this study was to compare the TFA content in frequently consumed food products from the Serbian and Slovenian market among young adults with supposedly elevated TFA content. Pre-packaged biscuits, wafers and chocolate spreads, as well as some chesses, pastry and processed meat products were chosen as frequently consumed and easily accessible in both countries. Another goal was to identify foods with high content of iTFA that could be potentially associated with risk for CVD.

# MATERIALS AND METHODS

## **Samples**

The priority list of samples was compiled on the basis of previously identified food categories high in TFA content, by checking the lists of ingredients for the term "partially hydrogenated fat" (15) and country of production, together with available food consumption data from studies on dietary habits in both countries (17, 18). Nineteen frequently consumed food products from the Serbian and 22 from the Slovenian market were selected for the analysis. Samples were purchased from retail stores in Serbia and Slovenia during March and April 2015. Sampling included confectionary products (chocolate, chocolate spread, chocolate-coated candies, biscuits, wafers), salty snack (chips), dairy products (cheese spread, kajmak – a traditional creamy dairy product similar to clotted cream), processed meat products (hot dog, salami, pate), fast food (puff pastry, pizza, burek – a traditional filo pastry pie filled with meat), margarine and dressing (mayonnaise). All samples from both countries were blinded and analysed in the same analytical run.

# **Fatty-acid Analysis**

After homogenization of samples in a blender, the direct boron trifluoride-methanol in situ trans esterification of fatty acids (19) in samples was applied as modified by Polak et al. (20). Contents

of fatty acids (FA) along with TFA methyl esters were analysed by capillary gas chromatography on GC Agilent Technologies 6890 with a flame ionization detector and HP-88 capillary column 100 m × 0.25 mm × 0.20 μm (Agilent Technologies, Santa Clara, California, USA). Heptadecanoic acid was used as internal standard and the following temperature conditions were used for separation and detection: 150 °C (hold for 10 min), rate of 1.5 °C/ min to 180 °C (hold for 40 min), and rate of 3 °C/min to a final temperature of 240 °C. Total analysis time was 95 min. The injector and detector temperatures were 250 °C and 280 °C, respectively. Detected retention times were compared with standard mixtures NuChek GLC-68D and GLC-85 (Nu-Chek Prep, Elysian, Minnesota, USA) in order to identify TFA methyl esters. Individual FA and TFA were expressed as percentages of total measured FA. A sum of the three individual TFA detected in analysed samples, trans-palmitoleic acid (C16:1t), trans-heptadecenoic acid (C17:1t), and elaidic acid (C18:1t) represents the total TFA. Further, the amount of each FA in the sample was determined using the response factor and the transformation factor of the FA content from the FA methyl ester content, and expressed as g/100 g FA in food. Sum of saturated fatty acids (g/100 g of food) was calculated for additional nutritional information. Data for the total fat content in food samples were adopted from the labelling.

Pearson correlation coefficient (R) was calculated to explore linear relations between TFA, saturated fatty acids, and total fat content using IBM SPSS Statistics 21 (IBM, Armonk, New York, USA). The significance level was taken as p < 0.05.

#### RESULTS

Frequently used food products from Serbian and Slovenian market were analysed for total FA and individual FA and TFA content, respectively. Obtained results were expressed as contents of FA in total FA (Table 1). In different samples various amounts of sum of TFA were detected, in a range of 0.67–60.4 g/100 g, with an average of 12.9 g/100 g TFA of total FA analysed in Serbian samples. The range of TFA regarding total FA analysed in Slovenian samples was 0.08–24.3 g/100 g, with an average of 1.93 g/100 g (Table 1). The total TFA content consists of three individual TFA detected, trans-palmitoleic acid (C16:1t), transheptadecenoic acid (C17:1t), and elaidic acid (C18:1t). Elaidic acid was the most abundant TFA in the majority of food samples, with 0.53–60.4 g/100 g of total FA in Serbian foods, and 0.04–24.3 g/100 g in foods from Slovenian market (Table 1). Proportions of trans-palmitoleic acid were less than 0.7 g/100 g and 0.4 g/100 g, in products from Serbian and Slovenian market, respectively. Trans-heptadecenoic acid was quantified in four Serbian samples (0.016-0.052 g/100 g total FA) and in a sample of pate from Slovenian market (0.077 g/100 g total FA).

Between food groups, high variability of TFA amounts per 100 g of product and total fat, respectively, was observed (Table 2). Percentage of TFA in total fat was the highest in salami from Serbian market (60.5%), followed by wafers 4 of non-EU origin purchased in Slovenia (19.4%), and wafers 2 and tea biscuits 1 from Serbian market (17.6% and 16.1%, respectively). The majority of TFA derived from partially hydrogenated fats (PHO) commonly used in confectionery industry in Serbia, while we detected less than 1% TFA in total fat in most of Slovenian con-

 Table 1. Trans fatty acid content in analysed fatty acids of food products from Serbian and Slovenian market

| Food product         |                          |        | Fatty acids of total fat (g/100 g) |         |         |       |  |
|----------------------|--------------------------|--------|------------------------------------|---------|---------|-------|--|
|                      |                          | Market | TFA C16:1t C17:1t C18:1t           |         |         |       |  |
|                      | Chocolate 1              | RS     | 0.992                              | 0.100   | < 0.005 | 0.892 |  |
| Confectionary        | Chocolate 1              | SI     | 0.451                              | 0.088   | < 0.005 | 0.342 |  |
|                      | Chocolate 2              | SI     | 0.656                              | 0.125   | < 0.005 | 0.502 |  |
|                      | Chocolate 3              | SI     | 0.562                              | 0.038   | < 0.005 | 0.494 |  |
|                      | Chocolate spread 1       | RS     | 12.7                               | 0.034   | < 0.005 | 12.7  |  |
|                      | Chocolate spread 2       | RS     | 9.85                               | 0.029   | < 0.005 | 9.82  |  |
|                      | Chocolate spread 1       | SI     | 0.081                              | 0.041   | < 0.005 | 0.040 |  |
|                      | Chocolate spread 2       | SI     | 0.087                              | 0.035   | < 0.005 | 0.052 |  |
|                      | Chocolate spread 3       | SI     | 0.088                              | 0.034   | < 0.005 | 0.054 |  |
|                      | Chocolate spread 4       | SI     | 0.530                              | 0.028   | < 0.005 | 0.502 |  |
|                      | Chocolate coated candy 1 | RS     | 3.18                               | 0.018   | < 0.005 | 3.17  |  |
|                      | Chocolate coated candy 2 | RS     | 26.7                               | 0.041   | < 0.005 | 26.7  |  |
|                      | Chocolate coated candy 3 | RS     | 0.667                              | 0.142   | < 0.005 | 0.525 |  |
|                      | Wafers 1                 | RS     | 4.95                               | 0.013   | < 0.005 | 4.94  |  |
|                      | Wafers 2                 | RS     | 52.1                               | < 0.005 | < 0.005 | 52.1  |  |
|                      | Wafers 1                 | SI     | 0.090                              | 0.016   | < 0.005 | 0.057 |  |
|                      | Wafers 2                 | SI     | 0.361                              | 0.078   | < 0.005 | 0.260 |  |
|                      | Wafers 3                 | SI     | 0.238                              | 0.025   | < 0.005 | 0.214 |  |
|                      | Wafers 4                 | SI     | 24.3                               | 0.038   | < 0.005 | 24.3  |  |
|                      | Wafers 5                 | SI     | 0.231                              | 0.053   | < 0.005 | 0.143 |  |
|                      | Tea biscuits 1           | RS     | 10.1                               | 0.024   | < 0.005 | 10.1  |  |
|                      | Tea biscuits 1           | SI     | 2.29                               | 0.094   | < 0.005 | 2.07  |  |
|                      | Tea biscuits 2           | SI     | 0.760                              | 0.034   | < 0.005 | 0.717 |  |
|                      | Tea biscuits 3           | SI     | 3.97                               | 0.028   | < 0.005 | 3.95  |  |
|                      | Tea biscuits 4           | SI     | 3.84                               | < 0.005 | < 0.005 | 3.84  |  |
|                      | Tea biscuits 5           | SI     | 0.163                              | 0.040   | < 0.005 | 0.117 |  |
|                      | Tea biscuits 6           | SI     | 0.110                              | 0.034   | < 0.005 | 0.076 |  |
|                      | Tea biscuits 7           | SI     | 0.220                              | 0.023   | < 0.005 | 0.180 |  |
| Fast food and snacks | Chips 1                  | RS     | 8.35                               | 0.030   | < 0.005 | 8.32  |  |
|                      | Puff pastry 1            | RS     | 12.7                               | 0.020   | < 0.005 | 12.7  |  |
|                      | Burek 1                  | SI     | 0.391                              | 0.143   | < 0.005 | 0.248 |  |
|                      | Pizza 1                  | SI     | 1.82                               | 0.367   | < 0.005 | 1.38  |  |
| Dairy products       | Cream cheese 1           | RS     | 2.58                               | 0.603   | 0.002   | 1.96  |  |
|                      | Melted cheese 1          | RS     | 8.46                               | 0.693   | < 0.005 | 7.77  |  |
|                      | Kajmak 1                 | RS     | 10.0                               | 0.589   | < 0.005 | 9.43  |  |
| Processed meat       | Hot dog 1                | RS     | 3.07                               | 0.305   | 0.052   | 2.72  |  |
|                      | Salami 1                 | RS     | 60.4                               | 0.008   | < 0.005 | 60.4  |  |
|                      | Pate 1                   | RS     | 7.67                               | 0.158   | 0.017   | 7.50  |  |
|                      | Pate 1                   | SI     | 1.07                               | 0.392   | 0.077   | 0.555 |  |
| Oily products        | Mayonnaise 1             | RS     | 5.78                               | 0.033   | < 0.005 | 5.75  |  |
|                      | Margarine 1              | RS     | 5.26                               | 0.192   | 0.016   | 5.05  |  |

Table 2. Total fat, and trans and saturated fatty acids content in food products from Serbian and Slovenian market

|                         |                          | Market | Origin               | Conter                          | TEA ::: 4-4-1 f-4 |                  |                      |
|-------------------------|--------------------------|--------|----------------------|---------------------------------|-------------------|------------------|----------------------|
| Food product            |                          |        |                      | Labelled total<br>fat (g/100 g) | TFA<br>(g/100 g)  | SFA<br>(g/100 g) | TFA in total fat (%) |
|                         | Chocolate 1              | RS     | Serbia               | 30.0                            | 0.27              | 16.9             | 0.9                  |
|                         | Chocolate 3              | SI     | Germany              | 35.0                            | 0.21              | 24.3             | 0.6                  |
|                         | Chocolate 2              | SI     | Poland               | 34.5                            | 0.18              | 17.6             | 0.5                  |
| Confectionary           | Chocolate 1              | SI     | Austria              | 32.1                            | 0.13              | 19.7             | 0.4                  |
|                         | Chocolate coated candy 2 | RS     | Serbia               | 16.9                            | 1.0               | 1.2              | 5.7                  |
|                         | Chocolate coated candy 1 | RS     | Serbia               | 7.6                             | 0.18              | 3.6              | 2.4                  |
|                         | Chocolate coated candy 3 | RS     | Serbia               | 9.0                             | < 0.05            | 3.2              | 0.5                  |
|                         | Chocolate spread 1       | RS     | Serbia               | 32.0                            | 4.0               | 10.3             | 12.5                 |
|                         | Chocolate spread 2       | RS     | Poland               | 31.0                            | 2.6               | 11.7             | 8.3                  |
|                         | Chocolate spread 4       | SI     | Macedonia            | 23.0                            | 0.22              | 11.0             | 1.0                  |
|                         | Chocolate spread 3       | SI     | Germany              | 31.0                            | < 0.05            | 11.8             | 0.1                  |
|                         | Chocolate spread 2       | SI     | Poland               | 31.0                            | < 0.05            | 8.5              | 0.1                  |
|                         | Chocolate spread 1       | SI     | Germany              | 35.7                            | < 0.05            | 8.6              | 0.1                  |
|                         | Tea biscuits 1           | RS     | Bulgaria             | 15.0                            | 2.4               | 12.4             | 16.1                 |
| products                | Tea biscuits 3           | SI     | Croatia              | 21.0                            | 1.0               | 12.0             | 4.6                  |
|                         | Tea biscuits 4           | SI     | Serbia               | 5.6                             | 0.22              | 2.8              | 3.9                  |
|                         | Tea biscuits 1           | SI     | Slovenia             | 32.1                            | 0.35              | 14.8             | 1.1                  |
|                         | Tea biscuits 2           | SI     | Slovenia             | 18.3                            | 0.19              | 14.8             | 1.1                  |
|                         | Tea biscuits 7           | SI     | Serbia               | 24.5                            | 0.06              | 16.5             | 0.2                  |
|                         | Tea biscuits 5           | SI     | Slovenia             | 25.0                            | 0.05              | 16.0             | 0.2                  |
|                         | Tea biscuits 6           | SI     | Germany              | 30.4                            | < 0.05            | 15.6             | 0.1                  |
|                         | Wafers 4                 | SI     | Bosnia & Herzegovina | 33.0                            | 6.4               | 11.5             | 19.4                 |
|                         | Wafers 2                 | RS     | Serbia               | 32.1                            | 5.6               | 2.7              | 17.6                 |
|                         | Wafers 1                 | RS     | Serbia               | 31.0                            | 1.3               | 14.2             | 4.1                  |
|                         | Wafers 2                 | SI     | Germany              | 35.7                            | 0.10              | 17.1             | 0.3                  |
|                         | Wafers 3                 | SI     | Bosnia & Herzegovina | 32.3                            | 0.09              | 18.0             | 0.3                  |
|                         | Wafers 5                 | SI     | Slovenia             | 27.0                            | 0.06              | 19.3             | 0.2                  |
|                         | Wafers 1                 | SI     | Austria              | 33.5                            | < 0.05            | 16.6             | 0.1                  |
| Fast food<br>and snacks | Puff pastry 1            | RS     | Serbia               | 22.6                            | 2.4               | 8.7              | 10.5                 |
|                         | Chips 1                  | RS     | Serbia               | 34.6                            | 2.4               | 12.9             | 6.8                  |
|                         | Pizza 1                  | SI     | Slovenia             | 10.0                            | 0.18              | 4.3              | 1.8                  |
|                         | Burek 1                  | SI     | Slovenia             | 12.9                            | < 0.05            | 3.3              | 0.3                  |
| Dairy products          | Melted cheese 1          | RS     | Serbia               | 11.0                            | 0.83              | 6.2              | 7.5                  |
|                         | Kajmak 1                 | RS     | Serbia               | 17.0                            | 1.0               | 6.6              | 6.1                  |
|                         | Cream cheese 1           | RS     | Serbia               | 23.5                            | 0.31              | 7.2              | 1.3                  |
| Processed<br>meat       | Salami 1                 | RS     | Serbia               | 41.0                            | 24.8              | 12.5             | 60.5                 |
|                         | Pate 1                   | RS     | Serbia               | 17.0                            | 1.3               | 3.1              | 7.6                  |
|                         | Hot dog 1                | RS     | Serbia               | 19.5                            | 0.38              | 4.7              | 1.9                  |
|                         | Pate 1                   | SI     | Slovenia             | 13.6                            | 0.10              | 2.5              | 0.7                  |
| Oily products           | Margarine 1              | RS     | Serbia               | 70.0                            | 5.1               | 20.4             | 7.3                  |
|                         | Mayonnaise 1             | RS     | Serbia               | 79.0                            | 4.0               | 8.1              | 5.1                  |

TFA – trans fatty acids; SFA – saturated fatty acids; RS – product from Serbian market; SI – product from Slovenian market

fectionary products. Similarly, values for TFA in total fat in a pate from Slovenia were far lower (0.7 g/100 g total fat) than in similar Serbian product (7.6 g/100 g total fat). All Serbian products, but four, contained more than 2.0 g/100 g TFA of total fat, while this limit was exceeded in 3 out of 22 samples purchased in the Slovenian market (Table 2). Data in Table 2 also suggest that the highest intake of TFA with 100 g of food would be with salami (24.8 g/100 g) from Serbian market, followed by wafers 4 (6.4 g/100 g) from Slovenian market and wafers 2 (5.6 g/100 g) produced and purchased in Serbia. The values for TFA and total fat per 100g of food products were found to be in a weak positive correlation (R=0.335, p=0.032), while the relationship between the content of saturated and total fat proved to be moderate (R=0.497, p=0.001).

# DISCUSSION

Strategies to reduce dietary intake of iTFA, and thereby prevent CVD, include reduction of iTFA amounts in foods. Recommendations on daily intake from relevant authorities were defined below 1% TFA of total energy (e.g. approximately 2.5 g/day) according to the WHO (9), and as low as possible, according to EFSA (21). From 2003, Denmark legally restricted iTFA content in foodstuffs to 2% of the total fat. The legislation of EU Commission adopted in 2019 limit the iTFA content to the same level of 2% and was fully implemented in 2021 (13). At the same time, the WHO announced an action plan called REPLACE with goal to free the world from iTFA by 2023 (22). Based on results from studies carried out in Slovenia (15, 23) in 2015 and again in 2017, in biscuits, the percentage of PHO dropped from 17 to 8%; however, in cakes, muffins and pastries the PHO percentage increased from 7 to 10%. Thus, it was concluded that voluntary guidelines and public communication on the cardiovascular risks related to iTFA in that period did not result in sufficient removal of PHO from foods in Slovenia. In Serbia, there is still no national study nor legislative for iTFA food content. As the primary aim of our study was to compare frequently consumed food with supposedly elevated iTFA content in young adults in these two countries, our results indicate that selected Serbian pre-packed products contained markedly higher amounts of iTFA in 2015 than Slovenian products.

Previous studies on iTFA content in our region have identified biscuits as a critical food category (6, 15, 23–25). High amounts of more than 2% iTFA of total fat was observed in many products of prepacked biscuits, cakes and wafers with at least 15 g fat/100 g of product and PHO or a similar term on list of ingredients in Serbian (n=35) and Slovenian markets (n=18) in 2012 (15). Two years later mean content of iTFA in products went down in Serbia from 33.5% in 2012 to 20.0% in 2014 and slightly increased in Slovenia from 15.5% in 2012 to 17.9% in 2014 (15). Moreover, high content of more than 2% of iTFA in total fat was observed in 243 of 434 analysed samples in six former Yugoslavian countries. Based on these results the authors concluded that voluntary reduction of iTFA is an ineffective strategy and that alternative strategies are needed.

Our study included pre-packed wafers and tea biscuits (n=15), produced in 2015. The content of TFA in total fat for wafers was from 0.1-19.5% (>2% of total fat in 42.9% of analysed sam-

ples) and for tea biscuits 0.1–16.1% (>2% of total fat in 37.5% of analysed samples). When we compared our data with regard to the country of product production (EU countries with those from non-EU countries), we found high TFA content in total fat in 37.5% products from EU compared to 75% from non-EU countries, confirming variability in TFA between countries. In 2016, Kušar et al. (25) determined iTFA content in 282 selected food purchased in Slovenia, including 39 biscuits. The authors reported that considerable variability was observed in TFA content in biscuits, ranging up to 4.88 g/100 g of food within the PHO-containing samples. They also found presence of over 2 g TFA per 100 g of fat in 65% of biscuits with declared PHO on label, while in biscuit samples without declared PHO levels of TFA were within EU regulatory limit. However, a recent study estimating total amount of TFA in biscuits of the former Yugoslavian countries Serbia, Croatia and Slovenia showed that there was a decline in presence of iTFA from 2014 to 2018 (by 47.4% and 63.4% in Serbia and Slovenia, respectively), but that high amounts are still present in this critical food group (6). Still, higher amounts of iTFA in food products were found in Serbia compared to Slovenia in 2014 and also in 2018. In contrast to some other countries, in Slovenia and Serbia the amount of TFA was not labelled on food packaging.

Intake values for iTFA were published in systematic analyses of 266 countries (26), but there is still no data for direct measurement of TFA dietary intake for Slovenia and Serbia. Only few databases have data for content of TFA in various foodstuffs. To provide new reliable data for Serbian and Slovenian database, we analysed a selection of frequently consumed industrial products from several food groups, and found high levels of TFA in some samples of meat products, confectionary and snacks. We also assumed that high content of TFA could be found in nonprepacked foods, since margarine, being PHO, is often used for bakery products and homemade products in both countries. As expected, TFA were very high in puffed pastry from Serbia with about 10% of total fat. Meanwhile two studies on TFA content in margarines in Serbia (27) and in Slovenia (28) showed that TFA were present in significantly higher amount in Serbian margarines. The results of a recent study showed that potential iTFA intake from 100 g of analysed salty snack products in Serbia was in range 0.1–4.9 g (29). Fatty acid analysis of table creams sold in Slovenia indicated low contents of TFA in cream replacements made of plant oils in 2011 (30).

Our results suggest that voluntary reduction of iTFA in foodstuff is less effective. To reduce iTFA intake through the diet, the most effective way at population level is reduction of iTFA by their replacement with other type of fats, preferably polyunsaturated fats (9) in foods. A growing number of countries worldwide make great efforts in this direction, with some form of mandatory iTFA limits – either best-practice TFA policies or less restrictive TFA limits (12). The comparison of Serbian and Slovenian products has shown that Slovenian products contained less amounts of iTFA even in 2015, when both countries had no legislation on iTFA. However, we analysed different products from the two markets, since dietary intakes are different in the two countries and thus different foodstuffs contribute to the intake. Although the number of analysed foodstuffs is relatively small, we covered the most frequently consumed products among young adults from both countries. The product with highest percentage TFA in total fat in

Serbia is salami (60.5 g/100 g total fat), however, the ingredients list of the product suggests on rTFA. Regarding dairy products, Serbian melted cheese and kajmak sample contained 7.5% and 6.1%, respectively, while a cream cheese sample contained 1.3% TFA in total fat. Dairy products contain natural ruminant TFA, which seems not to increase CVD risk in the low amounts usually consumed (31, 32), although their high intake appears to have the same effects on blood lipids as the iTFA (33), but there is no consensus (5).

Analysing individual TFA in our samples, trans-C18:1, elaidic acid, commonly found in PHO (30), was found to be the most prevalent. Considering well documented association between elaidic acid, systemic inflammation, endothelial dysfunction, obesity-associated insulin resistance and higher risk of clinical outcomes, the decline of this iTFA is particularly desirable to protect cardiovascular health (34, 35). The improved hydrogenation process accompanied with reduction of hydrogenated fats would contribute to a decrease of total TFA, as well as elaidic acid in final products (36). Trans-palmitoleic acid C16:1 was also found in almost all samples, but in amounts that were significantly lower comparing to elaidic acid. Although relatively specific for dairy products, trans-palmitoleic acid can also be derived by partial hydrogenation of plant oils (32). Possible deleterious effects of this TFA will be of no concern due to very low content detected in our study. Minor quantities of trans-heptadecenoic acid C17:1 detected in only three samples (Serbian dairy and processed meat products) are also not expected to be of physiological significance.

It should be noted that the majority of Serbian samples originated from domestic producers (16/18), while Slovenian samples were produced in Slovenia and EU (17/22), and in non-EU countries (5/22). As reported in the last column in Table 2, nearly 85% of Serbian products had percentage of iTFA in total fat above 2% in comparison to less than 15% of samples from Slovenian market (3 out of 22). When we grouped Slovenian samples by the origin, the domestic products and those made in the EU had markedly lower average iTFA percentage in total fat (0.7%) than those made in the non-EU countries (5%). Such a difference in favour of lower iTFA levels in EU products is in accordance with considerably decreased iTFA intake within the EU, as well as with high trans fats content still present in some European diets (15). As both Serbia and Slovenia had not introduced legal limits on TFA at the time of this study, the significantly lower TFA content in Slovenian food could be due to other TFA-related interventions, such as reformulation of food products and voluntary reduction in TFA content by food industries, which was observed also by Zupanič et al. (23). In contrast, alarmingly high levels of iTFA found in Serbian food indicate the lack of TFA-related activities.

Given that processed foods are widely consumed in Serbia, legislative changes and improved consumer information are urgently needed.

The main strength of this study is that all samples were analysed in the same laboratory that ensured comparability of the results; iTFA content was examined in food products, which are frequently used, and therefore significantly contribute to overall exposure to iTFA. The results of this study will be used to fulfil the missing data on TFA content in the Serbian and Slovenian food composition databases (37, 38), which are needed for evaluation of the TFA dietary intake in population. It will be incorporated into recently developed Serbian and Slovenian software tools for diet

planning in clinical and public health settings. The limitation of this study is that individual food items from Serbia and Slovenia are not the same, although they belong to the same food groups. In addition, total number of samples is rather small.

#### **CONCLUSIONS**

In conclusion, the average iTFA content in Serbian frequently consumed food by young adults is very high, while it is mostly acceptable in foodstuffs from Slovenia. Most of the Serbian products, but distinct minority of Slovenian samples had more than 2% TFA in total fat. Due to high exposure, Serbian population is potentially at TFA-associated cardiovascular risk, while this risk seems to be relatively low in Slovenia. In a view of consumers' health, it is necessary to reduce amount of iTFA in Serbian food. TFA-related activities such as those widely adopted in the EU, e.g., consumer awareness, reformulation of food products, and appropriate labelling of TFA content are urgently needed. In Slovenia, more attention should be paid to iTFA control of imported products, especially those of non-EU origin. The introduction of legislation limiting the content of TFA in foodstuffs would contribute to overall public health in both countries.

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# **Conflict of Interests**

None declared

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