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Trans fatty acids in margarines and shortenings in the food supply in Slovenia

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Highlights

- We investigated *trans* fatty acids (TFAs) in margarines/shortenings in Slovenia.
- The majority of margarines on the market contained less than 0.8% TFAs.
- In 25% of shortenings used by food manufacturers, over 2% TFAs were found.
- Samples labelled as containing partially hydrogenated fats contained up to 11% TFAs.
- Efficient regulation and control are needed to assure lower exposure to TFAs.

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Abstract

Trans fatty acid (TFA) content in foods is not regulated in most European countries. Fatty acid (FA) composition, total TFA levels, and TFA isomers were determined for 43 margarines available on the Slovenian market and 33 samples of shortenings used by food manufacturers. In most margarines, the TFA content (as a percentage of total FA) was low (0.1–0.8%), although two different margarines contained 3.1% and 6.4% TFA, respectively. In shortenings, a wider variation in TFA content (from 0.1% to 11.2%) was observed. In samples with high total TFA, *trans*-C18:1 was present in higher quantities than *trans*-C18:2 and *trans*-C18:3. In almost all samples, the predominant TFA isomers were elaidic acid (*t*9-C18:1) and *t*10-C18:1, followed by *t*11-C18:1 and (*t*6-*t*8)-C18:1. Isomers *t*12-C18:1 and (*t*13-*t*14)-C18:1 were also detected. Among *trans*-C18:2 isomers he *t*/*c*-C18:2 predominated over *c*/*t*-C18:2 and *t*/*t*-C18:2. Above 2% TFA was only found in samples labelled to contain partially hydrogenated fats. Results show a high variability in the FA composition of hard vegetable fats in the food supply, and indicate that more efficient regulation and control on the market are needed to minimize the exposure of the population to TFAs.

Keywords: *trans* fatty acids, polyunsaturated *trans* fatty acids, isomers, conjugated linoleic acid, food composition, partially hydrogenated vegetable oil, margarines, shortenings, gas chromatography, atherogenicity and thrombogenicity indices

1. Introduction

Trans fatty acid (TFA) isomers of monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs, respectively) are not synthesised by the human body, nor are they essential to the human diet. Moreover, dietary intake of most TFAs is well-recognised as an independent risk factor for the development of cardiovascular diseases (CVDs) (Mozaffarian, 2016) and is also associated with mortality from all causes (Kiage et al., 2013). While some TFAs are naturally produced by the bacteria in the rumen and as such found in the meat, milk and dairy products of ruminants (MacGibbon and Taylor, 2006), their contribution to overall TFA consumption is minimal (Allison et al., 1999). Industrially produced TFAs are considered a major source of TFAs in the human diets in many countries.

In the middle of the last century the objective of replacing hard fats rich in saturated fatty acids (SFAs) with preparations with similar physical, sensorial, and technological properties but lower in SFAs led to the excessive production of hardened vegetable oils. These newly-produced fats had a solid consistency and a prolonged shelf life as compared to non-hydrogenated highly unsaturated vegetable oils (Menaa et al., 2013). TFAs, due to their molecular structure, possess the potential for closer packing and alignment of their acyl chains, which results in decreased molecular mobility and reduced fluidity. For these reasons, and also because of a consumer preference for vegetable fats (in comparison to animal fats), hardened vegetable fats have become widely used. Although TFAs can be also formed endogenously via oxidative stress and by free radicals (Hung et al., 2016), and are commonly formed during preparation of food, particularly during frying (Cui et al., 2017), industrially-produced partly hydrogenated fats (PHFs), which are used for the production of margarines and shortenings in baking, are considered a major source of industrial TFAs. A number of different isomers of TFAs are present in PHFs (Ratnayake and Cruz-Hernandez, 2012). Considering that vegetable oils in general contain mainly C18 unsaturated fatty acids (FAs),

67 the PHFs being prepared from these oils predictably consist of mainly *trans*-C18 isomers
68 (Albers et al., 2008; Alves et al., 2008). Among *trans*-18:1 isomers, the most prevalent is
69 elaidic acid (*t*9-C18:1), followed by *t*10-18:1 which is also found in ruminants.

70 The first safety concerns related to the TFAs present in PHFs emerged in last quarter of
71 last century, as an increased risk of coronary heart disease was suggested (Mensink and
72 Katan, 1990; Thomas et al., 1981). Also considering that heart disease is the leading cause of
73 death in most countries, several competent organisations, including the World Health
74 Organisation (World Health Organization, 2003) as well as the European Food Safety
75 Authority (EFSA Panel on Dietetic Products, 2010) have recommended that intakes of TFA
76 should be as low as possible within the context of a nutritionally adequate diet. Considering
77 this, responsible food manufacturers began to reformulate food products and stopped using
78 PHFs, while policymakers started to regulate this area. Different policy approaches are being
79 used in different countries to limit the dietary intake of industrial TFAs, for example
80 mandatory labelling of the presence of PHFs (currently in use in the European Union,
81 including Slovenia), labelling of the content of TFAs (currently in use in the USA and
82 Canada), and the most restrictive policy—regulatory limits with respect to either use of PHFs
83 or TFA content in foods (Pravst, 2015). An approach to limit TFA levels to up to 2% of the
84 total fat content was first introduced in Denmark in 2003 (a similar approach was latter also
85 accepted in some other countries) (L'Abbé et al., 2009). Quite different approach was used in
86 the USA, where in June 2015, the US Food and Drug Administration (FDA) finalised the
87 decision to completely ban PHFs, as a major dietary source of artificial TFAs, by June 2018
88 (Food and Drug Administration, 2015). A question remains, which of those approaches is
89 better, considering the protection of consumers, the effect of the food producers, and
90 complexity of the control by food authorities (Pravst, 2015).

Extensive analyses of dietary intake of TFAs in Western European countries were performed at the end of the last century within the TRANSFAIR Study (Hulshof et al., 1999). More recently, a review regarding TFAs in foods and in the overall diet of the EU population was prepared by the European Commission (2015), showing lack of available data on this issue—particularly in Central and Eastern European countries. For Slovenia, no data is available on the exposure of the population to TFAs, and scarce data is available on TFA levels in foods in the food supply, which limits the implementation of efficient public health interventions in this area. Quite unexpectedly, Stender and co-workers revealed a considerable increase in the TFAs in some popular foods in Slovenia and some other countries in Central Eastern Europe (CEE) (Stender et al., 2014, 2016). For example, in the Slovenian food supply, the availability of biscuits/cakes/wafers with added PHFs increased by almost 300% from 2012 to 2015, as did the TFA levels in those foods (Stender et al., 2016). At a similar time, reports showing that TFAs were still present in modest quantities in margarines in some European counties also became available (Vučić et al., 2015). Considering this, the research project "Trans Fats in Foods in Slovenia" (TFFS) was funded in Slovenia with the objective of investigating the exposure of the population to TFAs and informing policymakers for making evidence-based policy decisions (Zupanič et al. 2018). The project is also addressing the conclusions of the European Commission's Report on *trans* fats in foods (European Commission, 2015), which highlighted that additional data on the content of TFAs in foods (particularly in the Central Eastern Europe) is needed for harmonized regulatory intervention on the EU level.

The aim of this study, conducted as part of the larger TFFS project, was to determine total levels of TFAs and levels of specific TFA isomers in margarines and shortenings in the food supply in Slovenia. Margarines were sampled from the shops of all major retailers in the country, while shortenings originated from food business operators dealing with the

116 production of non-prepacked foods. To provide insights for better regulation of this topic on
117 the global level, a secondary objective was to investigate, if notable amounts of TFAs (>2%)
118 are also found on samples not labelled to contain PHFs. Additionally, to provide more
119 accurate information for recommendations to produce healthier food products, the atherogenic
120 and thrombogenic potential of sampled fats was also evaluated.
121
122 **2. Materials and Methods**
123 **2.1. Sampling plan**
124 Margarines (*n*=43) were sampled in food stores in Slovenia, while shortening samples
125 (*n*=33) were sampled at Slovenian food business operator (FBO) sites dealing with the
126 production of non-prepacked foods. For each sampled product, information recorded from the
127 product label included product name, package size, list of ingredients, quantities of total and
128 saturated fat. All collected information were then entered into a spreadsheet, while samples
129 were labelled with an identification code, appropriately stored and analysed before expiry
130 date. Sampling of margarines was carried out in March 2016 in markets of all retailers
131 operating nationwide (Mercator, Spar, Shower, Eurospin, Hofer, Lidl, and Jager). We
132 sampled all different products which were available on shelves of the selected stores at the
133 time of purchase/sampling. The products sampled from the stores met the following
134 conditions: (1) labelled as margarine, (2) packaged at a weight of at least 100 g; (3) not
135 combined with animal fats; (4) in the case of the same product in different quantities, the
136 product sampled was the one with smaller quantity; and (5) in the case of the same product
137 brand but with different labelled ingredients, both products were sampled. The sampling
138 included 43 different samples of margarines. On the sampling site, the samples were stored in
139 thermoisolated portable box cooled with reusable freeze boards, delivered to the laboratory,
140 and stored below 6 °C until the analyses.

141 Shortening sampling was carried out by the Administration of the Republic of Slovenia
142 for Food Safety, Veterinary Sector and Plant Protection. Collection was done during the
143 official control of Slovenian food business operators (FBO) producing non-prepacked foods
144 like bread, croissants, and other bakery products. All hardened vegetable oils/fats (not
145 combined with animal fats) were sampled. In the case of smaller packaging (weight up to
146 250 g), the individual package was gathered as a sample, while in large packaging about
147 150 g of sample was collected. The samples were delivered to laboratory in thermoisolated
148 portable box, cooled with reusable freeze boards and stored below 6 °C until analysis.
149 Shortenings were sampled at 34 different food producers in different Slovenian regions;
150 altogether 56 shortenings were sampled during the official control. Of those, 33 samples were
151 considered as different products and selected for laboratory analyses.
152
153 **2.2. Fatty acid analysis**
154 The FA composition of the samples was determined by gas–liquid chromatography
155 (GLC) by applying *in-situ* trans-esterification (Park and Goins, 1994). Two different GLC
156 methods were used for the analysis of fatty acid methyl esters (FAMEs). In the first method,
157 an Agilent Technologies 6890 GC with a flame ionisation detector and a capillary column
158 (BPX 70, 120 m × 250 μm × 0.25 μm) was used. Separation and detection were performed
159 under the following conditions: temperature programme starting at 130 °C (0 min), with an
160 increase of 1 °C/min to 220 °C (hold 10 min); injector temperature: 240 °C; detector
161 temperature: 280 °C; injector: split:splitless, 1:50; volume: 1 μL; carrier gas: He at 24 cm/s;
162 make-up gas: N_2 at 45 mL/min; detector gases: H_2 at 40 mL/min; and synthetic air (21% O_2)
163 at 450 mL/min.
164 The first method provided total *trans*-C18:1 isomers and for the identification of
165 individual *trans*-C18:1 isomers the second method was used, whereby, FAMEs were analysed

166 using a Thermo Trace GC with a flame ionisation detector and a capillary column SP2560
167 (100 m × 250 µm × 0.25 µm). Separation (see chromatogram on Fig. 1) and detection were
168 performed under the following conditions: temperature programme starting at 120 °C (1 min),
169 increase of 20 °C/min to 175 °C (hold 30 min), increase of 5 °C/min to 220 °C (hold for 10
170 min); injector temperature: 250 °C; detector temperature: 250 °C; injector: split, 1:100;
171 volume: 1 µL; and carrier gas: H_2 at 1.5 mL/min constant flow. For additional identification
172 of the *cis*/*trans* isomers and for checking the chromatographic resolution between *cis* and
173 *trans* isomers, a preparative separation was performed with Ag^+ Thin Layer Chromatography
174 (Buchgraber and Ulberth, 2001). Ag^+ TLC plates were prepared from commercial TLC plates
175 (Silicagel G 60 10x20 cm; Merck, Darmstadt) by submersing the plates into a 1% solution of
176 $AgNO_3$ in acetonitrile for 30 min. After drying the plates at 120 °C for 10 min in the dark,
177 approximately 5 mg of sample was applied to the plate in a line of cca 5 cm using a Hamilton
178 syringe. Separation was performed in a mobile phase made of *tert*-butyl methyl ether/ hexane
179 (60:40). After that the bands were visualized with 2% dichlorofluorescein in methanol applied
180 by a nebulizer. Bands representing saturated and *trans*-monoene were scrapped off separately
181 from bands representing *cis*-monoenes and polyenes. The FAMES were eluted from the silica
182 with hexane/ diethyl ether (1:1) and injected onto the GC.
183
184 **Figure 1 about here**
185
186 **2.3. Identification and calculation of fatty acid methyl esters**
187 The FAMEs were determined through their retention times in comparison to a standard
188 mixture (Supelco FAME mix of 37 components (Cat. No. 18919-1AMP)) and individual
189 standards of *trans*-C18:1isomers *t*6 (U-45-M), *t*9 (U-47-M) and *t*11 (U-49-M) by Nu-Check.
190 Calculation of *trans* FA amount was performed according to Mossoba and Kramer (2009) by

applying theoretical response factors (Rfi) after confirming their validity as described in AOCS method Ce 1b-89 (AOCS method Ce 1b-89). Additionally, as required by Mossoba and Kramer (2009), separation of *cis*/*trans* isomers was optimised by fine-tuning the instrument until a maximum resolution was achieved between the *t*13-C18:1 and the *c*9-C18:1isomers. This approach was confirmed to produce valid results not only for *trans* FA (Tyburczy et al., 2009), but also for PUFA (Schreiner, 2005). The identification of the *trans*-C18:1 isomers (*t*8, *t*9, *t*10, *t*11, *t*12, *t*13) is based on elution order, which matches the order presented in the AOCS Official Methods for the Determination of *Trans* Fat (Mossoba and Kramer, 2009) and Reporter (2012), or order published in Ratnayake et al. (2006), Delmonte et al. (2009) and Tyburczy et al. (2012). Mixture of individual *trans*-C18:1 isomers is not commercially available as a reference material. As seen from representative chromatogram (Fig. 1), chromatographic separation of *trans*-C18:1 positional isomers in this study achieved with the SP-2560 column showed improvement as compared to separation on the same column carried out by Tyburczy et al. (2012). The identification of the *trans*-C18:2 isomers was carried out by comparing retention times to Supelco Linoleic Acid Methyl Ester *cis*/*trans* Isomer Mix (Cat. No. 47791). Using the Rfi and the factor of transformation of FA content from FAME content the weight portion of each FA in the sample was calculated and expressed as a percentage of total FA (g/100 g).

2.4. Atherogenicity and thrombogenicity indices

The atherogenicity and thrombogenicity indices (AI and TI, respectively) were calculated by the equations proposed by Ulbricht and Southgate (1991) and modified by Vučić et al. (2015) who included TFA content in the sum of SFA, using the data as a percentage of total FAs for lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), total SFAs, total MUFAs, total PUFAs, *n*-3 FAs, and *n*-6 FAs:

$$AI = [C12:0 + 4 \times (C14:0) + C16:0 + TFA] / (MUFA + PUFA) \quad (1)$$

$$TI = (C14:0 + C16:0 + C18:0 + TFA) / [0.5 \times MUFA + 0.5 \times (n\text{-}6) + 3 \times (n\text{-}3) + (n\text{-}3 / n\text{-}6)] \quad (2)$$

2.5. Data processing and statistical analyses

Reported values are mean of three replicates ± standard deviation (sd). The data were processed and evaluated (means, sd and coefficient of variation (*CV*)) using Microsoft Excel 2016 (Redmond, Washington, USA). Further statistical analyses were performed using R commander software 3.3.3 version. Due to the fact that variances were heterogeneous, non-parametric Wilcoxon test was performed.

3. Results and Discussion

3.1. Fatty acid composition

The FA composition in margarines generally depends on the used vegetable oil. Considering the *CV* for SFAs, MUFAs, *n*-6, and *n*-3 it can be seen in Table 1 that the FA composition of the investigated margarines appreciably differs. In general, in the investigated margarines, unsaturated FAs (UFAs) predominated over the saturated FAs. The MUFA content exceeds PUFA levels in more than half of margarines. The *n*-6 FAs are present in higher amounts than *n*-3 FAs. In almost two-thirds of analysed margarines we observed an *n*-6/*n*-3 ratio over 5, which is considered as unfavourable (Simopoulos, 2008). However, this is not unusual for margarines since it was also reported by Vučić et al. (2015) and Kuhnt et al. (2011). In shortenings, the SFA content is higher than in case of margarines; high variability in FA composition among samples was again observed (Table 2). This is in line with the

observed variability of the constituents used in the production of these products (i.e. canola, soybean, rapeseed, sunflower, corn, coconut, and palm kernel). In approximately 50% of investigated shortening samples, the SFA content exceeded that of UFAs. In all shortenings, the MUFA content notably exceeded that of PUFAs and in more than three-quarters of analysed samples the *n*-6/*n*-3 ratio was higher than 5. The most abundant FAs in majority of margarines were linoleic acid (C18:2*n*-6) and oleic acid (C18:1*n*-9), and in most samples the latter exceeded the former. In shortenings, C16:0 and C18:1*n*-9 were predominant. The high content of C18:1*n*-9 in these samples could originate from the partial hydrogenation of C18:2*n*-6. Some samples of shortenings (SH18, SH19) were rich in C12:0 and C14:0. The content of mentioned FAs in these samples is appreciably higher than the content of C16:0, confirming the labelled use of coconut oil.

Table 1 and Table 2 about here

3.2. *Trans* fatty acid content

The results show a high variation in TFA content (Fig. 2) within shortenings (CV_{TFA} = 1.60), while within margarines the variation of TFA content was notably lower (with the exclusion of samples MA40 and MA42 the CV_{TFA} amounted to 0.56). Such high variation within the shortenings reflects the heterogeneity of processing parameters. Considering TFAs in margarines, their amounts, with the exception of mentioned samples, were low (ranging from 0.11% to 0.77% of total FAs), while in samples MA40 and MA42 we found appreciably higher amounts of TFAs (6.37% and 3.13%, respectively). It should be mentioned that these two margarines were the only margarine samples where the presence of PHF was declared on the label. For a comparison, while in our study 5% of the margarines exceeded 2% TFAs (with average 0.55% TFAs of total FAs), ten years ago almost 70% of the

265 margarines in the Slovenian market contained more than 2% TFAs, with average TFA content
266 2.2% (Cencič-Kodba, 2007).

267 **Figure 2 about here**

268

269 Considerably higher TFA levels were determined in shortenings, reaching up to 11.16%
270 in sample SH1. It should be stressed that the TFA content in all investigated margarines, with
271 the exception of two mentioned samples (MA40 and MA42), and in approximately three-
272 quarters of shortening samples, was less than 2% of total FAs. In all margarines and
273 shortenings with more than 2% TFAs, the presence of PHFs was declared on the labels
274 (ingredient lists). This indicates that PHF as an ingredient was a very reliable indicator of
275 increased TFA content in the product.

276 The reported results are somewhat similar to those obtained by Vučić et al. (2015).
277 They reported TFA levels ranging from 0.17% to 1.89% for soft margarines obtained on the
278 Serbian market, however much higher levels were also observed in some samples of hard
279 margarines (up to 28.84%). In Portugal, Costa et al. (2016) observed no statistical differences
280 between total TFAs in margarines and shortenings, with average levels of around 0.85%.
281 Kuhnt et al. (2011) investigated the composition of 27 margarines on the German market and
282 reported TFA levels ranging from 0.11 to 4.28%, while in shortening/cooking fats (n=30)
283 TFA levels reached 14.48%. Due to high variability of TFA levels in both subgroups, the
284 differences between margarines and shortening/cooking fats in Kuhnt's investigation were not
285 statistically significant. Similarly, despite the fact that in our study notable (>2%) TFA levels
286 were observed in over 24% of shortenings and in less than 5% of margarines, the difference in
287 TFA contents between investigated subgroups is not significant (p = 0.18). The average TFA
288 content in margarines amounted to 0.55% ± 1.02% and in shortenings 1.72% ± 2.74%.

289 The amount of total *trans*-C18:1 isomers in analysed margarines represented less than
290 0.6% of the total FAs, with the exception of the previously mentioned samples MA40 and
291 MA42. In these two samples high amounts of total *trans*-C18:1 were determined, with values
292 representing almost 5.52% and 2.77% of total FAs, respectively. Considering shortenings, in
293 the sample with the highest TFA amount (SH1) the content of total *trans*-C18:1 isomers
294 represented 10.52% of total FAs. There are only few samples (SH18, SH19) where none of
295 TFA isomers belonging to C18:1 group were determined. It should be mentioned that in those
296 margarines and shortenings where high total amounts of TFAs were determined (more than
297 2% of total FA) the total *trans*-C18:1 isomers were present in much higher quantities than
298 total *trans*-C18:2 isomers, while in the rest of analysed samples the difference between *trans*-
299 C18:1 and *trans*-C18:2 isomers was far less established. It could be observed that the amount
300 of *trans*-C18:1 was greater than that of *trans*-C18:2 in samples with over 0.5% TFAs. The
301 amount of *trans*-C18:2 in margarine samples did not exceed a value of 0.85% of total FAs
302 (for MA40), whereas in the rest of the analysed margarines *trans*-C18:2 was present in much
303 lower quantities, on average amounting to 0.24%. The amounts of total *trans*-C18:2 isomers
304 in shortenings are in general comparable to those in margarines. Among *trans*-C18:2 isomers
305 *t*/*c*-C18:2 predominated over *c*/*t*-C18:2 levels, while the presence of the *t*/*t*-C18:2 was
306 confirmed mostly in samples with high TFA content (Table 3). In this respect it should be
307 noted that *t*/*t*-C18:2 was not detected in samples which contained less than 0.5% of TFAs. It
308 has been argued that different TFA isomers have different contributions to the development of
309 chronic diseases. Some evidence exists that *trans*-C18:2 and *trans*-C18:3 might cause a
310 higher risk of developing cardiovascular disease (CVD) than *trans*-C18:1 isomers (Baylin et
311 al., 2003; Lemaitre et al., 2006). The presence of TFA isomers belonging to the C18:3 group
312 has not been found in investigated margarines, while in some analysed shortenings amounts

313 of almost 0.3% of total FAs were detected. The values are in agreement with those of (Costa
314 et al., 2016).

315 **Table 3 about here**

316 In margarines and shortenings in which the content of TFAs achieved a value higher
317 than 2% of total FAs, among isomers belonging to *trans*-C18:1 group we have additionally
318 analysed the amounts of the individual *trans*-C18:1 isomers using capillary column SP2560
319 (Table 3). *t*9-C18:1 and *t*10-C18:1 in these samples were present at the highest levels,
320 followed by vaccenic acid (*t*11-C18:1) and (*t*6-*t*8)-C18:1. Isomers *t*12-C18:1 and (*t*13-*t*14)-
321 C18:1 were present in appreciably lower quantities. The sum of *t*9-C18:1 and *t*10-C18:1 was
322 approximately 50% of the total *trans*-C18:1 isomers with *t*9-C18:1, representing in general
323 over 30% of the total *trans*-C18:1. This is in accordance with previous findings that *t*9-C18:1
324 is the major *trans*-C18:1 isomer identified in industrially hydrogenated fats (Mozaffarian et
325 al., 2006). It should be stressed that the generally accepted opinion is that *t*10-C18:1 is one the
326 major *trans*-C18:1 isomers in PHFs, which correlates with CVD risk (Aldai et al., 2013).

327 The contents of *trans*-C18:1, *trans*-C18:2 or *trans*-C18:3 depend on the FA profile of
328 the oils used for partial hydrogenation, the extent of hydrogenation, and the deodorization
329 temperature during the oil refining process (Aldai et al., 2013). The contents of *trans*-C18:2
330 or *trans*-C18:3 are related to whether the oil being used consists mainly of linoleic or
331 linolenic acid, respectively. At a moderate stage of partial hydrogenation, mainly the mono-
332 *trans* isomers from the naturally present *cis*-C18:1 counterparts are formed. Heating of oils at
333 elevated temperatures during the deodorization step leads to mono-*trans* (*c*/*t, t*/*c* or *c*/*c*/*t*, *c*/*t*/*c*,
334 *t*/*c*/*c*) and minor amounts of di-*trans* isomers of PUFAs, with several times greater
335 isomerization of linolenic acid than linoleic acid. Accordingly, for the samples with the
336 content of TFAs higher than 2% of total FAs, the ratio of *trans*-C18:1/(*trans*-C18:2 + *trans*-

337 C18:3) was calculated and the amounts were appreciably higher than 1 (ranging from 5 to 40)
338 which could indicate the use of PHFs (Tyburczy et al., 2012).

339 The analysis of individual TFA isomers of the C18:1 group is also important for
340 assigning the origin of TFA in foods. In almost all analysed samples we determined an
341 appreciably higher amount of *t*9-C18:1 than of *t*11-C18:1 which is mostly associated with
342 PHFs. In one sample (MA40) *t*10-C18:1 and *t*11-C18:1 predominated notably with respect to
343 *t*9-C18:1. These TFAs are characteristic of ruminant fats, yet the ingredient list of sample
344 MA40 did not contain ingredients of animal origin. It should be mentioned that current data
345 do not conclusively indicate that moderate consumption of ruminant TFAs (*t*11-C18:1 and
346 rumenic acid (*c*9,*t*11-C18:2)) has adverse effects of on human health (De Souza et al., 2015).
347 Recent research has identified some potential benefits of these ruminant TFAs, such as the
348 lowering of blood cholesterol content as well as anti-carcinogenic, anti-diabetic and
349 immunomodulation effects, which confirms that studies should not only focus on measuring
350 total TFAs in foods and diets, but also on the identification of specific TFA isomers (Ganguly
351 and Pierce, 2015; Mills et al., 2011). On the other hand, as stated by Stender (2015) adverse
352 effects with respect to the LDL (low density lipoprotein) cholesterol of the major ruminant
353 TFAs are similar to those of industrially produced TFAs. However, the latter are easier to
354 remove from human diet. It should be also stressed that the majority of studies reported
355 increased CVD outcomes with increased level specifically of *trans*-C18:2 isomers, which
356 showed a higher risk than *trans*-C18:1. In particular, *t*/*t*-C18:2 (and possibly also the *t*/*c*-
357 C18:2 isomer) may be more potent than *c*/*t*-C18:2 (Chowdhury et al., 2014). By contrast, the
358 evidence regarding an association of *trans*-C18:1 to CVD risk is somewhat mixed.
359

360 **3.3. Atherogenicity and thrombogenicity indices**

361 The potential health effects of the investigated samples were assessed by calculation of
362 the AI and TI. High AI and TI values indicate unfavourable effects in context of risks for
363 development of cardiovascular diseases (Ulbricht and Southgate, 1991). Values of AI below
364 0.5 are considered nutritionally optimal, with fish as an example of a food with lower AI and
365 TI indices due to high content of unsaturated fatty acids (Garaffo et al., 2011). Relatively low
366 AI/TI values are also observed for most samples in our study. In most of the investigated
367 margarines, the AI and TI levels (Table 1) could be considered as low (AI levels between 0.20
368 and 1.40 and TI levels between 0.25 and 2.05), while shortenings (Table 2) have notably
369 higher values of both AI and TI, with values ranging from 0.29 to 26.82 and from 0.48 to
370 13.27, respectively. This is to be expected since the most pro-atherogenic and pro-
371 thrombogenetic FAs are present in much higher quantities in shortenings than in margarines.
372 In the calculations of AI, a coefficient of 4 has been assigned to C14:0, due to its higher
373 atherogenicity in comparison to C12:0 and C16:0 (Ulbricht and Southgate, 1991). In the
374 calculations of TI, MUFAs and *n*-6 FAs coefficients of 0.5 were assigned because they are
375 less antithrombogenic than *n*-3 FAs, which were assigned a coefficient of 3. Considering that,
376 in addition to SFA, TFAs are also responsible for those negative effects, it was suggested that
377 TFA levels be considered in the calculation of AI and TI (Vučić et al., 2015). In this respect
378 we have confirmed that in samples with notable TFA amounts (more than 2% of total FAs)
379 the consideration of TFAs notably contributes to AI and TI values. AI and TI levels calculated
380 with consideration of TFAs were up to 20% higher (Equations (1) and (2)).
381 However, for sample SH18 which had the highest amount of SFAs (94.7% of total FAs)
382 and relatively low TFA content (0.07%), high AI and TI values (26.82 and 11.92,
383 respectively) were calculated, while on the other hand, in samples with lower SFA levels and
384 relatively high TFA content, AI and TI values were considerably lower. For example, for
385 SH5, with relatively high TFA content (4.37%) but low SFA content (41.3%) as compared to

386 other samples, the AI and TI indices are correspondingly lower despite TFA content being
387 taken into consideration in calculations (values of 0.78 and 1.14, respectively).
388
389 **3.4. Regulatory implications**
390 A considerable lowering of TFA levels in erythrocytes in the populations of some
391 Western European countries has been shown recently, leading to suggestions that further
392 actions against TFAs are not needed (von Schacky et al., 2017). Significant reductions in TFA
393 intakes were also observed in the USA (Restrepo, 2017), but it was reported recently that
394 even with very low TFA intakes, plasma TFA concentrations remained significantly
395 associated with serum lipid and lipoprotein concentrations (Yang et al., 2017). This is another
396 indication that the consumption of industrially produced TFAs in foods should be as low as
397 possible, not only in Western countries, but globally. The reported results provide evidence
398 that there are only a few margarines on the market in Slovenia where TFAs can be found in a
399 notable quantity, but this is not the case for shortenings used for production of non-prepacked
400 foods. About one-third (32%) of the selected food business operators used at least one type of
401 shortening with TFA levels above 2%. This indicates the need for more efficient regulation of
402 this area. Various EU member states are currently taking action at a national level, but a
403 harmonised approach would be most efficient and will assure equal protection of EU
404 population, including those in countries where data on TFAs in foods is lacking. Considering
405 that in our study a notable content of TFAs was found particularly in fats used for production
406 of non-prepacked foods, and the fact that food labelling is mandatory only in pre-packed
407 foods, regulatory approaches other than food labelling are needed. While voluntary
408 approaches were found to be very successful in some countries (Arcand et al., 2014), this is
409 obviously not the case in Central Eastern Europe. The optimal decision would be to therefore
410 to limit TFA levels in foods using international regulations.

411 A major strength of the study is the use of extremely robust sample collection and
412 analyses. In case of margarines, all samples which were found on the market were purchased
413 and analysed. In case of shortenings, samples were collected by the food authorities directly
414 from food business operator sites, and these operators were not informed that the inspection
415 would be conducted. Furthermore, chemical determination of TFA was replicated with a
416 secondary method in all samples with notable TFA levels. On the other hand, we should also
417 note some limitations. We were not able to obtain standards for all the reported TFA isomers;
418 approaches described in the literature were used for their quantification. Additionally, we
419 were not in position to analyse certified reference materials, which are now already
420 commercially available. It would be very valuable for further studies, if appropriate
421 commercially available certified reference materials were analysed and results reported
422 together with new/revised analytical methods. Another limitation of the study is also that the
423 AI and TI were calculated considering previously reported coefficients for different types of
424 fatty acids, but additional clinical research would be needed to evaluate if those actually
425 corresponded well with the risk factors for the development of cardiovascular disease.
426
427 **4. Conclusions**
428 In conclusion, the TFA content in the majority of investigated margarines was found to
429 represent less than 0.8% of total FAs. With regard to shortenings, in approximately three-
430 quarters of samples the TFA content was less than 2% of total FAs, however, TFAs were
431 found to represent 11% of FA content in one sample. Notable TFA levels were only observed
432 in samples labelled as containing PHFs. This indicates that a regulatory ban of the use of
433 PHFs in foods (as accepted in the USA) would limit TFAs in margarines and shortenings as
434 efficiently as regulatory limit of the TFA level in foods to 2% (as in use in some EU
435 countries), noting that regulatory limit of the TFAs present a higher burden for both food

authorities and food manufacturers due to the need of regular chemical analyses of foods. We also determined that in samples with more than 2% TFAs, *trans*-C18:1 was a highly predominant TFA while in the samples with less than 0.5% TFAs, *trans*-C18:2 was present in greater amounts than *trans*-C18:1. However, the differences among the isomers are expressed less strongly. In analysed samples besides *t*9-C18:1, which is the major *trans*-C18:1 isomer, (*t*6-*t*8)-C18:1, *t*10-C18:1, *t*11-C18:1, *t*12-C18:1 and (*t*13-*t*14)-C18:1 were detected. In all analysed samples *t*/*c*-C18:2 and *c*/*t*-C18:2 were determined, while *t*/*t*-C18:2 was detected only in the samples with the highest amounts of TFAs. The presence of *trans*-C18:3 was analysed in margarines and shortenings with more than 2% TFAs and was confirmed in five of eight analysed shortening samples and in none of analysed margarines. Consideration of TFAs in the calculation of AI and TI indices was particularly important in samples with TFA levels above 2%. The reported results present for the first time analytical data on the content of total TFA as well as of specific TFA isomers in margarines and shortenings in the food supply in Slovenia, gathered with the aim of minimizing dietary intake. The results are important for controlling the quality and safety of margarines and shortenings in the food supply, as well as for international comparisons on this topic. Furthermore, the reported results are important for informing further policy decisions for reducing TFA intake, both nationally and globally.

Abbreviations

AI, atherogenicity indices; CVD, cardiovascular disease; FA fatty acids; FAMEs, fatty acid methyl esters; GLC, gas–liquid chromatography; MA, margarine; MUFA, monounsaturated fatty acids; PHF Partly hydrogenated fats; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SH, shortening; TFA *trans* fatty acids; TFFS, Trans Fats in Foods in Slovenia; TI, thrombogenicity indices; UFA, unsaturated fatty acids

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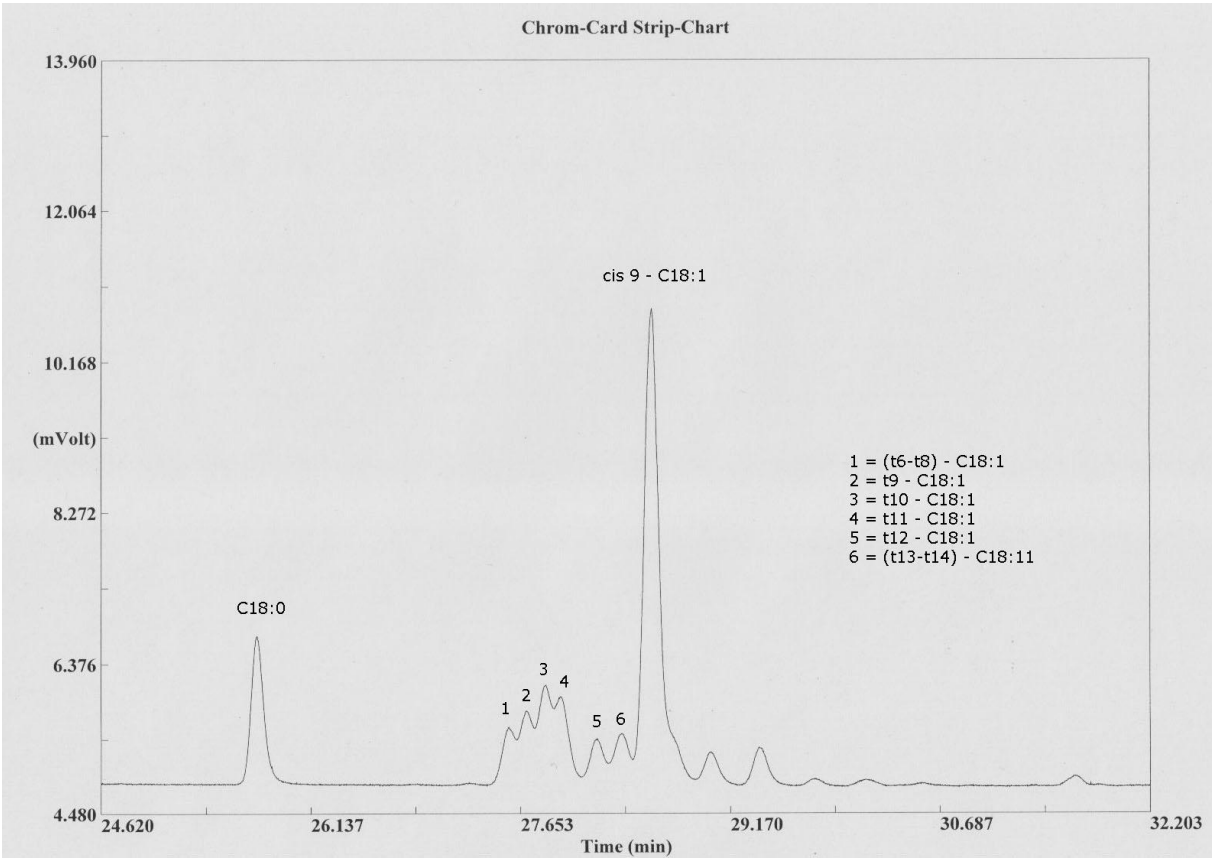

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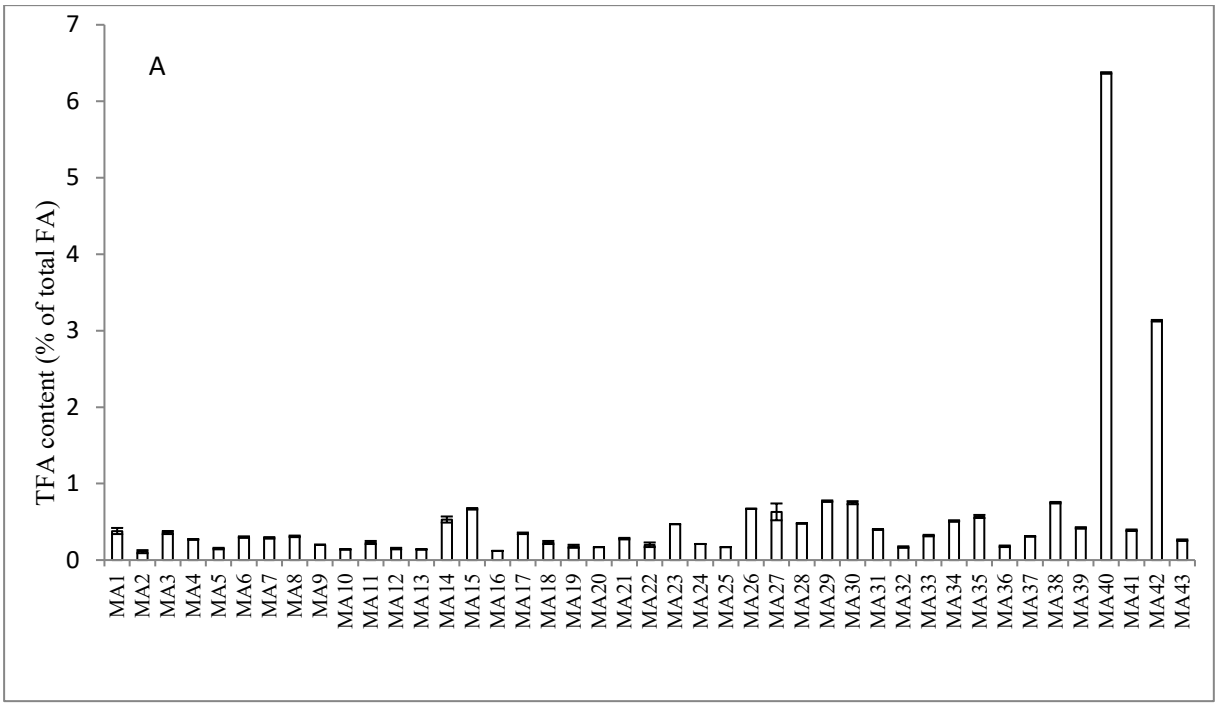
639 Figure 1: Representative chromatogram of individual *trans*-C18:1 isomers analysed on

640 column Supelco 2560 for sample containing trans fatty acids

641



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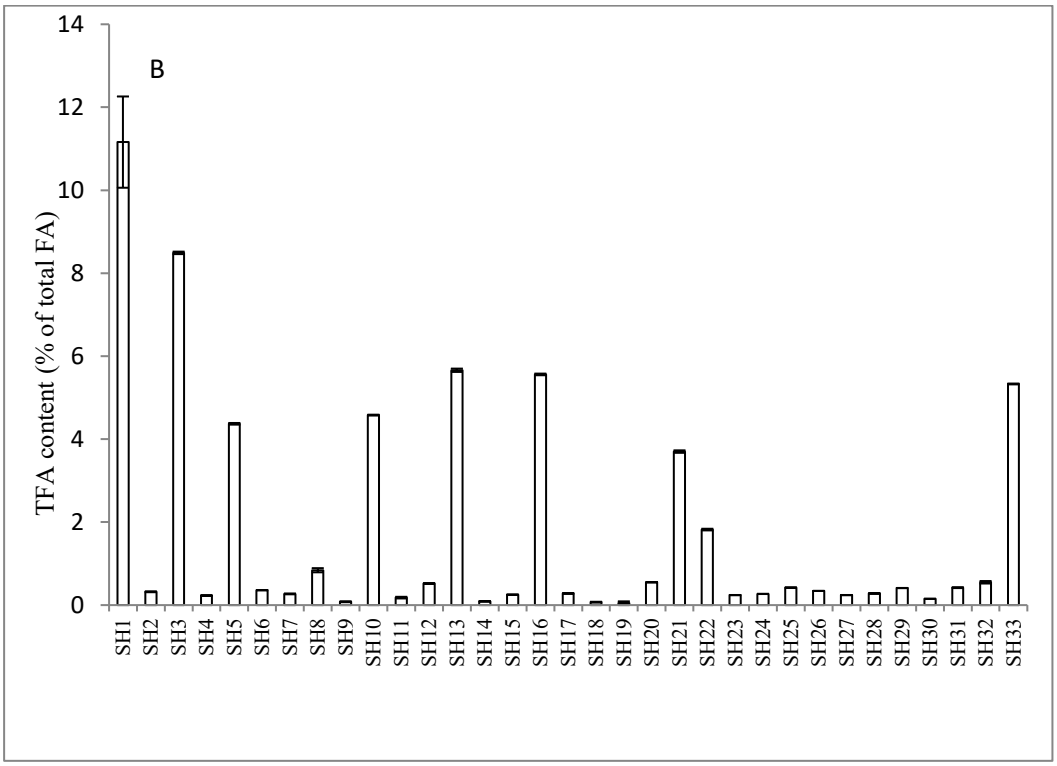

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644 Figure 2: Amount of *trans* fatty acids in margarines (A) and shortenings (B) in Slovenian

645 food supply (N=76)

646

647 Table 1. The content of total SFA, MUFA, *n*-6, *n*-3, TFA, *trans*-C18:1 and *trans*-C18:2 (% of total FA), and atherogenicity and thrombogenicity

648 indices (AI and TI) in margarines.

	FA composition (% of total FA)[a]																		labelled
sample	SFA		MUFA		*n*-3		*n*-6		**TFA**		*trans* -C18:1		*trans* -C18:2		AI		TI		PHF
MA1	35.9	0.1	27.9	0.0	3.61	0.01	32.27	0.10	**0.38**	0.04	0.10	0.05	0.28	0.01	0.56	0.00	0.74	0.01	/
MA2	29.4	0.3	49.8	0.2	5.79	0.03	14.88	0.05	**0.11**	0.02	0.01	0.01	0.10	0.00	0.44	0.01	0.45	0.01	/
MA3	47.7	0.5	36.0	0.3	0.82	0.02	15.16	0.23	**0.36**	0.02	0.14	0.02	0.21	0.00	0.97	0.02	1.45	0.03	/
MA4	50.9	0.4	33.2	0.2	0.15	0.00	15.53	0.18	**0.27**	0.00	0.08	0.00	0.19	0.00	1.10	0.02	1.76	0.03	/
MA5	46.6	0.4	38.2	0.2	3.50	0.03	11.56	0.09	**0.15**	0.01	0.04	0.01	0.11	0.00	0.99	0.01	1.00	0.02	/
MA6	48.1	0.8	34.4	0.4	0.14	0.00	17.11	0.40	**0.30**	0.01	0.08	0.01	0.22	0.00	0.97	0.03	1.62	0.06	/
MA7	55.7	0.1	31.8	0.1	0.86	0.00	11.38	0.03	**0.29**	0.01	0.08	0.02	0.20	0.01	1.40	0.00	1.83	0.01	/
MA8	46.7	0.4	26.9	0.1	0.16	0.00	26.01	0.26	**0.31**	0.01	0.15	0.01	0.16	0.00	0.96	0.02	1.38	0.02	/
MA9	46.0	0.2	39.3	0.1	3.05	0.02	11.42	0.05	**0.20**	0.00	0.04	0.00	0.15	0.00	0.94	0.01	1.04	0.01	/
MA10	22.6	0.2	29.4	0.0	11.61	0.03	36.23	0.09	**0.14**	0.00	0.00	0.00	0.14	0.00	0.27	0.00	0.28	0.00	/
MA11	36.5	0.2	47.0	0.1	3.63	0.01	12.71	0.05	**0.23**	0.02	0.01	0.02	0.22	0.00	0.55	0.00	0.68	0.01	/
MA12	29.3	0.3	42.3	0.1	6.77	0.03	21.44	0.10	**0.15**	0.01	0.03	0.01	0.12	0.00	0.43	0.01	0.44	0.01	/
MA13	26.4	0.2	36.2	0.1	7.96	0.03	29.35	0.08	**0.14**	0.00	0.00	0.00	0.14	0.00	0.36	0.00	0.37	0.00	/
MA14	33.9	0.4	26.1	0.1	4.01	0.04	35.55	0.27	**0.53**	0.04	0.25	0.04	0.28	0.01	0.51	0.01	0.67	0.01	/
MA15	29.0	0.2	30.7	0.1	0.79	0.00	38.84	0.12	**0.67**	0.01	0.52	0.01	0.15	0.00	0.42	0.00	0.64	0.01	/
MA16	27.7	0.6	41.0	0.3	6.71	0.12	24.46	0.28	**0.12**	0.00	0.00	0.00	0.12	0.00	0.39	0.01	0.41	0.01	/
MA17	23.2	2.2	28.4	1.0	7.76	0.25	40.32	1.07	**0.35**	0.01	0.00	0.00	0.35	0.01	0.25	0.00	0.31	0.00	/
MA18	24.7	0.2	28.7	0.2	10.95	0.10	35.48	0.31	**0.23**	0.02	0.04	0.02	0.19	0.00	0.31	0.00	0.32	0.00	/
MA19	35.6	0.1	27.8	0.0	0.14	0.01	36.30	0.05	**0.18**	0.02	0.02	0.02	0.16	0.00	0.67	0.00	0.65	0.00	/
MA20	29.8	0.2	40.0	0.0	0.25	0.01	29.79	0.13	**0.17**	0.00	0.02	0.00	0.15	0.00	0.40	0.00	0.71	0.01	/
MA21	25.5	0.2	43.4	0.1	4.06	0.01	26.72	0.08	**0.28**	0.01	0.11	0.00	0.17	0.01	0.32	0.00	0.46	0.00	/
MA22	31.6	0.1	27.4	0.1	0.64	0.00	40.19	0.02	**0.20**	0.03	0.04	0.03	0.17	0.01	0.47	0.00	0.71	0.00	/
MA23	25.7	0.2	27.2	0.1	0.13	0.00	46.51	0.13	**0.47**	0.00	0.05	0.00	0.43	0.00	0.30	0.00	0.67	0.01	/
MA24	52.4	0.0	36.4	0.0	1.54	0.00	9.42	0.02	**0.21**	0.00	0.04	0.00	0.16	0.00	1.25	0.00	1.45	0.00	/
MA25	20.7	0.2	27.9	0.8	12.34	0.14	38.97	0.44	**0.17**	0.00	0.00	0.00	0.17	0.00	0.24	0.00	0.25	0.00	/
MA26	24.8	0.3	53.4	0.2	4.76	0.02	16.40	0.06	**0.67**	0.00	0.53	0.01	0.14	0.00	0.34	0.00	0.42	0.01	/
MA27	25.6	0.1	48.1	0.1	4.72	0.01	20.93	0.01	**0.63**	0.11	0.46	0.11	0.17	0.00	0.35	0.00	0.45	0.00	/
MA28	28.6	0.5	29.6	0.2	0.20	0.02	41.11	0.32	**0.48**	0.00	0.00	0.00	0.48	0.00	0.41	0.01	0.65	0.02	/

	FA composition (% of total FA)[a]																		labelled
sample	SFA		MUFA		*n*-3		*n*-6		**TFA**		*trans* -C18:1		*trans* -C18:2		AI		TI		PHF
MA29	27.0	0.4	27.6	0.1	0.09	0.00	44.59	0.25	**0.77**	0.01	0.41	0.00	0.36	0.01	0.38	0.01	0.60	0.01	/
MA30	26.9	0.1	27.8	0.1	0.10	0.00	44.44	0.14	**0.75**	0.02	0.39	0.02	0.36	0.00	0.38	0.00	0.60	0.00	/
MA31	39.3	0.2	28.5	0.1	0.09	0.00	31.74	0.09	**0.40**	0.00	0.06	0.00	0.34	0.00	0.67	0.00	1.12	0.01	/
MA32	24.1	0.4	46.2	0.2	4.56	0.03	24.98	0.17	**0.17**	0.01	0.03	0.01	0.13	0.00	0.33	0.01	0.36	0.01	/
MA33	45.1	1.3	42.5	0.8	1.76	0.10	10.34	0.37	**0.32**	0.01	0.16	0.01	0.15	0.00	0.79	0.04	1.39	0.08	/
MA34	30.2	0.3	23.9	0.1	0.21	0.00	45.14	0.16	**0.51**	0.01	0.10	0.00	0.40	0.00	0.20	0.00	0.75	0.01	/
MA35	33.0	0.0	47.7	0.0	5.00	0.01	13.76	0.01	**0.57**	0.02	0.38	0.00	0.19	0.01	0.55	0.00	0.54	0.00	/
MA36	53.7	0.5	35.7	0.3	1.57	0.03	8.83	0.11	**0.18**	0.01	0.03	0.01	0.15	0.00	1.30	0.03	1.56	0.04	/
MA37	46.4	0.1	40.6	0.1	2.15	0.01	10.56	0.03	**0.31**	0.00	0.14	0.00	0.16	0.00	0.87	0.00	1.33	0.00	/
MA38	30.7	0.1	29.4	0.0	3.45	0.02	35.69	0.14	**0.75**	0.01	0.14	0.01	0.61	0.00	0.40	0.00	0.70	0.00	/
MA39	53.7	0.2	33.4	0.1	0.12	0.00	12.33	0.12	**0.42**	0.01	0.07	0.00	0.35	0.00	1.22	0.01	2.05	0.02	/
MA40	45.0	0.2	32.9	0.1	1.09	0.01	14.70	0.05	**6.37**	0.01	5.52	0.01	0.85	0.00	1.00	0.01	1.75	0.02	YES
MA41	51.1	0.1	33.9	0.0	1.10	0.01	13.52	0.04	**0.39**	0.01	0.19	0.01	0.20	0.00	1.12	0.00	1.61	0.01	/
MA42	23.6	0.0	33.2	0.0	0.23	0.01	39.86	0.07	**3.13**	0.01	2.77	0.01	0.37	0.01	0.30	0.00	0.69	0.00	YES
MA43	27.4	0.4	51.1	0.2	3.84	0.05	17.40	0.19	**0.26**	0.01	0.02	0.02	0.25	0.00	0.41	0.01	0.44	0.01	/
average	35.3		35.4		3.08		25.7		0.55		0.31		0.24		0.62		0.87		
sd	10.7		7.9		3.32		12.3		1.02		0.92		0.15		0.34		0.51		
CV	0.30		0.22		1.08		0.48		1.85		2.98		0.61		0.56		0.59		

Notes: FA, fatty acids; TFA, *trans* FA; SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; sd, standard deviation; *CV*, coefficient of variation; PHF, partly hydrogenated fats; MA, margarine.

[a] values are mean of three replicates ± standard deviation

[b] PHFs were labelled in the ingredient list

654 Table 2. The content of total SFA, MUFA, *n*-6, *n*-3, TFA, *trans*-C18:1, *trans*-C18:2 and *trans*-C18:3 (% of total FA), and atherogenicity and

655 thrombogenicity indices (AI and TI) in shortenings.

	FA composition (% of total FA)[a]																				Labelled
sample	SFA		MUFA		*n*-3		*n*-6		TFA		*trans*-C18:1		*trans* -C18:2		*trans* -C18:3		AI		TI		PHF[b]
SH1	51.9	0.5	23.9	1.3	1.01	0.04	12.12	0.42	**11.16**	1.10	10.52	1.29	0.40	0.03	0.23	0.01	1.33	0.08	2.55	0.17	YES
SH2	49.8	0.3	34.9	0.2	0.18	0.00	14.81	0.13	**0.32**	0.01	0.11	0.02	0.22	0.00	n.d.	-	1.03	0.01	1.74	0.02	/
SH3	49.8	0.1	24.7	0.1	0.96	0.01	15.99	0.09	**8.49**	0.03	7.77	0.02	0.48	0.01	0.24	0.01	1.17	0.01	2.32	0.01	YES
SH4	61.0	0.8	29.9	0.6	1.23	0.03	7.67	0.16	**0.23**	0.01	0.12	0.01	0.11	0.00	n.d.	-	1.83	0.06	1.79	0.06	/
SH5	41.3	0.1	41.6	0.0	3.24	0.03	9.50	0.11	**4.37**	0.02	4.23	0.01	0.14	0.01	n.d.	-	0.78	0.01	1.14	0.01	YES
SH6	43.6	0.1	44.4	0.1	0.97	0.01	10.61	0.05	**0.36**	0.00	0.07	0.00	0.29	0.00	n.d.	-	0.74	0.00	1.41	0.01	/
SH7	49.6	0.6	39.0	0.4	1.18	0.03	9.89	0.18	**0.27**	0.01	0.05	0.00	0.22	0.00	n.d.	-	0.96	0.02	1.75	0.05	/
SH8	28.6	0.1	51.3	0.1	5.10	0.02	14.19	0.06	**0.84**	0.05	0.61	0.05	0.23	0.01	n.d.	-	0.43	0.00	0.49	0.00	/
SH9	45.1	0.1	41.6	0.1	2.26	0.01	10.93	0.03	**0.08**	0.01	0.01	0.01	0.07	0.00	n.d.	-	0.85	0.00	1.21	0.01	/
SH10	41.5	0.2	41.2	0.1	2.91	0.01	9.82	0.08	**4.58**	0.01	4.24	0.01	0.199	0.00	0.14	0.00	0.78	0.01	1.19	0.01	YES
SH11	46.3	0.2	41.5	0.2	0.88	0.07	11.08	0.43	**0.18**	0.02	0.04	0.00	0.14	0.02	n.d.	-	0.83	0.00	1.57	0.00	/
SH12	42.0	0.5	44.0	0.3	0.28	0.00	13.21	0.12	**0.52**	0.00	0.07	0.00	0.45	0.00	n.d.	-	0.72	0.01	1.41	0.03	/
SH13	54.1	0.1	24.8	0.1	1.10	0.01	14.36	0.02	**5.66**	0.04	4.94	0.03	0.44	0.01	0.28	0.02	1.55	0.00	2.00	0.01	YES
SH14	74.1	0.2	19.1	0.2	1.28	0.01	5.47	0.05	**0.09**	0.00	0.03	0.01	0.06	0.00	n.d.	-	3.76	0.05	2.07	0.03	/
SH15	93.2	0.0	5.4	0.0	0.03	0.00	1.12	0.01	**0.25**	0.00	0.22	0.00	0.03	0.00	n.d.	-	18.29	0.13	13.27	0.04	/
SH16	51.8	0.1	31.5	0.0	0.12	0.00	11.00	0.05	**5.56**	0.02	5.56	0.01	0.01	0.01	n.d.	-	1.23	0.00	2.58	0.01	YES
SH17	52.1	0.0	32.5	0.0	0.15	0.00	14.93	0.01	**0.28**	0.00	0.07	0.00	0.21	0.00	n.d.	-	1.16	0.00	1.86	0.00	/
SH18	94.7	0.1	4.4	0.1	0.00	0.00	0.77	0.02	**0.07**	0.01	0.00	0.00	0.07	0.01	n.d.	-	26.82	0.46	11.92	0.24	/
SH19	90.6	0.1	7.5	0.0	0.00	0.00	1.84	0.01	**0.05**	0.04	0.00	0.00	0.05	0.04	n.d.	-	13.84	0.10	6.69	0.04	/
SH20	62.0	0.2	28.3	0.1	0.43	0.00	8.77	0.07	**0.55**	0.00	0.31	0.01	0.24	0.01	n.d.	-	1.98	0.02	2.17	0.02	/
SH21	22.6	0.2	53.0	0.2	5.75	0.00	15.03	0.03	**3.70**	0.03	3.29	0.02	0.11	0.01	0.30	0.01	0.29	0.00	0.48	0.00	YES
SH22	43.3	0.2	42.7	0.1	1.88	0.01	10.33	0.05	**1.82**	0.02	1.65	0.01	0.17	0.01	n.d.	-	0.75	0.01	1.35	0.01	YES
SH23	46.7	0.5	41.7	0.3	1.06	0.02	10.27	0.12	**0.24**	0.00	0.09	0.00	0.15	0.00	n.d.	-	0.85	0.02	1.56	0.03	/
SH24	50.6	0.1	39.1	0.1	0.74	0.01	9.33	0.05	**0.27**	0.00	0.06	0.00	0.21	0.00	n.d.	-	0.99	0.01	1.89	0.01	/
SH25	48.3	0.3	41.6	0.2	0.35	0.00	9.24	0.07	**0.42**	0.00	0.07	0.00	0.35	0.00	n.d.	-	0.90	0.01	1.80	0.02	/
SH26	46.9	0.3	42.3	0.2	0.90	0.01	9.59	0.08	**0.34**	0.00	0.10	0.00	0.23	0.00	n.d.	-	0.85	0.01	1.61	0.02	/
SH27	55.5	0.2	34.3	0.1	0.17	0.00	9.74	0.07	**0.24**	0.00	0.04	0.00	0.20	0.00	n.d.	-	1.21	0.01	2.44	0.02	/

	FA composition (% of total FA)[a]																				Labelled
sample	SFA		MUFA		*n*-3		*n*-6		TFA		*trans*-C18:1		*trans* -C18:2		*trans* -C18:3		AI		TI		PHF[b]
SH28	46.1	0.2	27.0	0.1	0.10	0.00	26.48	0.13	**0.28**	0.00	0.10	0.00	0.18	0.00	n.d.	-	0.94	0.01	1.35	0.01	/
SH29	50.7	0.2	39.6	0.1	0.14	0.00	9.12	0.05	**0.41**	0.00	0.06	0.00	0.35	0.00	n.d.	-	1.01	0.01	2.03	0.01	/
SH30	50.2	0.2	40.2	0.2	0.23	0.00	9.20	0.07	**0.15**	0.00	0.04	0.00	0.11	0.00	n.d.	-	0.98	0.01	1.94	0.02	/
SH31	53.7	0.2	31.5	0.0	0.14	0.00	14.20	0.17	**0.42**	0.01	0.23	0.01	0.19	0.00	n.d.	-	1.25	0.01	1.95	0.02	/
SH32	53.4	1.3	32.1	0.7	0.17	0.01	13.71	0.57	**0.55**	0.03	0.28	0.03	0.28	0.00	n.d.	-	1.13	0.06	2.24	0.12	/
SH33	54.5	0.2	28.9	0.2	0.09	0.00	11.20	0.05	**5.33**	0.01	5.20	0.02	0.13	0.01	n.d.	-	1.35	0.01	2.80	0.03	YES
average	52.8		33.5		1.06		10.9		1.72		1.48		0.20		0.24		2.75		2.54		
sd	15.4		11.7		1.37		4.69		2.74		2.64		0.12		0.06		5.61		2.74		
CV	0.30		0.30		1.30		0.43		1.43		1.78		0.61		0.26		2.04		1.08		

Notes: FA, fatty acids; TFA, *trans* FA; SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA, n.d., not determined; sd, standard deviation; *CV*, coefficient of variation; PHF, partly hydrogenated fats; SH, shortening.

[a] values are mean of three replicates ± standard deviation

[b] PHFs were labelled in the ingredient list

662 Table 3. The content of individual *trans*-C18:1 isomers (% of total *trans*-C18:1) and individual *trans*-C18:2 isomers (% of total *trans*-C18:2) in

663 margarines and shortenings with the content of TFAs higher than 2% of total FA.

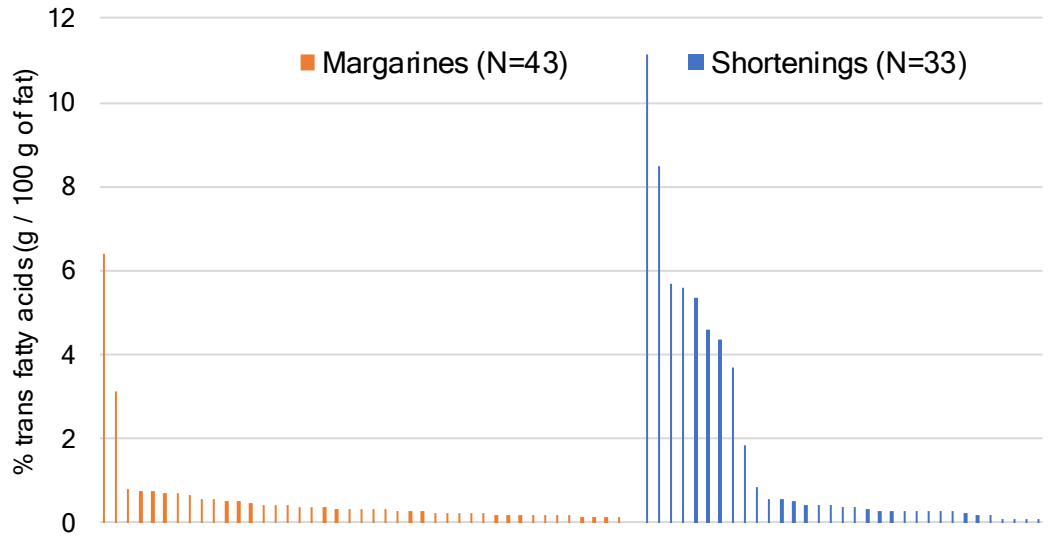
Sample ID:	**MA40**	**MA42**	**SH1**	**SH3**	**SH5**	**SH10**	**SH13**	**SH16**	**SH21**	**SH33**
TFA:										
(*t*6-*t*8)-C18:1[a]	15.5	19.7	19.4	19.4	21.2	20.9	19.8	21.1	19.4	16.7
*t*9-C18:1[a]	19.8	39.1	37.7	36.6	34.0	32.6	34.9	32.2	36.8	42.6
*t*10-C18:1[a]	31.3	22.3	23.5	33.0	24.6	29.0	24.1	25.8	24.8	23.9
*t*11-C18:1[a]	20.0	10.9	9.4	5.5	12.9	9.2	10.0	10.3	10.6	8.6
*t*12-C18:1[a]	7.6	3.9	5.4	5.5	4.3	5.0	5.3	4.7	5.1	4.2
(*t*13-*t*14)-C18:1[a]	5.8	4.0	4.7	n.d.	3.0	3.3	5.9	5.9	3.3	3.9
c/*t*-C18:2[b]	40.5	41.0	35.1	38.8	29.0	36.2	38.6	34.3	23.6	34.9
t/*c*-C18:2[b]	48.2	53.0	39.1	43.4	40.3	43.4	43.9	41.5	40.4	41.0
t/*t*-C18:2[b]	11.3	6.0	25.8	17.8	30.7	20.4	17.5	24.2	36.0	24.1

664 Notes: [a] as % of total *trans*-C18:1; *trans*-C18:1 isomers were determined by Supelco 2560 column; [b] as % of total *trans*-C18:2, *trans*-C18:2 isomers were determined by BPX

665 70 column; MA, margarine; SH, shortening.

666

Graphical Abstract



Amount of *trans* fatty acids in margarines and shortenings in Slovenian food supply (N=76)