

THE FATTY ACIDS CONTENT IN POPULAR BARS

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Abstract

In view of the significance of qualitative fatty acid profile, the aim of this research was to determine and compare the content of fatty acids in two groups popular snacks which were classified into two product groups: healthy or unhealthy. Gas chromatograph was used to analyze the fatty acid profile. The study showed that the products classified as 'unhealthy' generally contained more fatty acids than 'dietary' products. They also had a higher content of fatty acids with atherogenic properties. Approx. 66% of fatty acids in both groups were long-chain fatty acids (LCFAs), followed by *cis* monounsaturated fatty acids (MUFAs) which accounted for 25% of all FAs. In contrast, very long chain fatty acids (VLCFAs) were present in small quantities. We also found quite large amounts of linoleic acid (PUFA), which accounted for 2.6% to 18.5% of the total composition of the snacks and appeared in all samples.

ZAWARTOŚĆ KWASÓW TŁUSZCZOWYCH W POPULARNYCH BATONIKACH

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Słowa kluczowe: batony, kwasy tłuszczowe, MUFA, PUFA, VLCFA.

Abstrakt

Celem pracy była analiza i porównanie zawartości kwasów tłuszczowych w popularnych batonikach, które podzielono na dwie grupy – batoniki „zdrowe” i „niezdrowe”. Analizę przeprowadzono metodą chromatografii gazowej. Wykazano, że produkty oznaczone jako „niezdrowe” ogólnie zawierają znacznie większą ilość kwasów tłuszczowych w porównaniu z produktami oznaczonymi jako „zdrowe”. Dodatkowo zawierają znacznie więcej kwasów tłuszczowych wykazujących właściwości aterogenne. Przeważającą część (ok. 66%) wszystkich kwasów tłuszczowych w obu badanych grupach stanowiły kwasy nasycone długłańcuchowe (LCFA), podczas gdy *cis* jednonienasycone kwasy (MUFA) stanowiły ok. 25% całej puli kwasów. Z kolei długłańcuchowe kwasy tłuszczowe (VLCFA) były obecne w badanych produktach w niewielkich ilościach. W badaniach wykazano, że analizowane produkty zawierały także dość duże ilości kwasu linolowego (PUFA), który stanowił od 2,6% do 18,5% całkowitego składu przekąsek i występował we wszystkich próbkach.

Introduction

The accelerating pace of modern life results in the tendency to maximally shorten the time of food preparation. Yet paradoxically, at the same time dietary preferences of modern consumers reveal a growing interest in healthy lifestyle. Products are now expected to be not only tasty and convenient, but also easy to digest and low in calories. This trend can be seen in the growing popularity of cereal bars, advertized as ideal and dietary snacks whose contents (grains, nuts and fruit) are suggested to be able to replace a complete meal. They are also generally considered a healthier alternative to various chocolate bars and wafers, perceived as unhealthy due to their high sugar and fat content and high calorific value.

Although fats are essential for correct nutrition, they may adversely influence the human body not only through excessive intake in diet, but also through their incorrect profile (WOLAŃSKA and KŁOSIEWICZ-LATOSZEK 2012). For example, the excessive consumption of saturated fatty acids,

especially long chain fatty acids (LCFAs), contributes to an increased plasma cholesterol levels and promotes atherosclerosis (FERNANDEZ and WEST 2005). Artificially derived *trans* fatty acids (TFA) also have a negative impact on health, deteriorating lipid profile parameters and increasing the risk of metabolic syndrome and obesity (KOCHAN et al. 2010). In turn, unsaturated fatty acids (MUFAs and PUFAs) have a generally positive effect on the plasma lipid profile and cardiovascular system, through their anti-atherosclerotic, anti-inflammatory and anti-aggregation actions (HABÁN et al. 2000, KOLANOWSKI 2007).

In view of the significance of qualitative fatty acid profile, the aim of this research was to determine and compare the content of fatty acids in two groups popular snacks: 1) cereal bars popularly regarded as healthy and 2) wafers and bars perceived by most consumers as high-calorie and containing harmful ingredients.

Material and Methods

Material to the study

The study included 15 popular products (bars) containing fruits, grains, nuts, chocolate and caramel, available in various discount stores in Poland. The products were classified into two product groups: healthy (dietary) or unhealthy (non-dietary), according to the results of the online survey, in which respondents ($n = 73$) indicated their opinions on the bars. In the survey, respondents could see the photography of the product and the list of its ingredients. They classified bars by answering the question: 'Into which group would you classify snack bar presented below?' The possible answers were: a) healthy, b) unhealthy. The survey revealed a group of 8 supposedly healthy products and 7 products classified as unhealthy.

Preparation of samples

Products were homogenized, and then 1 g of each product was collected as a representative sample. Fatty acids were extracted with the Folch mixture (2:1; chloroform:methanol, v:v), saponified with 2 M potassium base (KOH) and methylated with the solution of BF_3 in methanol by incubation at 70°C. The resulting methyl esters were extracted with hexane. For separation of the phases, saturated solution of NaCl was added. The upper layer of the solution was taken and transferred to glass vials. The obtained fatty acid esters were subjected to qualitative and quantitative analysis by gas chromatography.

Chromatographic analysis of samples

Gas chromatograph (Agilent Technologies 7890 GC System) was used to analyze the fatty acid profile. The study used a capillary column with dimensions of 15 m x 0.10 mm, 0.10 μm (SUPELCOWAX™ 10 Capillary GC Column, Supelco, Bellefonte, PA, USA). Chromatographic conditions were as follows: the initial temperature was 60°C for 0 min, increased at a rate of 40°C/min to 160°C (0 min), increased at a rate of 30°C/min to 190°C (0.5 min) and then increased at a rate of 30°C/min to 230°C for 2.6 min, where it was maintained for 4.9 min. The total analysis was approximately 8 min and the gas flow rate was 0.8 ml/min with hydrogen as the carrier gas. Qualitative analysis was performed by comparing the peak retention time of the identified a substance with the standard peak retention time. The quantitative analysis included a comparison of surface area of the peaks with the standard peak surface area for heneicosanoic acid (C 21:0).

Statistical analysis

Statistical analysis were performed using Stat Soft Statistica 10.0 and Microsoft Excel 2007. The arithmetic means (AM) and standard deviations of the AM (SD) were calculated for each studied group. The distribution of results for individual variables was obtained with the Shapiro-Wilk test. As most of the distributions deviated from the normal Gaussian distribution, non-parametric tests were used for further analyses. To assess the differences between the studied groups, the non-parametric Mann-Whitney test was used. The level of significance was $p \leq 0.05$.

Results

The study showed that both 'healthy' (group I) and 'unhealthy' (group II) contained fats which varied primarily in terms of composition and quality of the fatty acids (Table 1).

Approx. 67% of fatty acids in both groups were long-chain fatty acids (LCFAs), followed by *cis* monounsaturated fatty acids (MUFAs) which accounted for 17% of all FAs. In contrast, very long chain fatty acids (VLCFAs) in the tested products were present in small quantities.

Moreover, for all analyzed products the atherogenic index (AI) and thrombogenic index (TI) has been calculated by using equations gave by Ulbricht and Southgate (ULBRICHT and SOUTHGATE, 1991). The groups showed no statistically significant differences for AI nor TI. The highest values of both indexes were found for product 5 belonging to the 'healthy' group (Table 1).

Table 1
The percentage content of each fatty acid groups, atherogenic index (AI) and thrombogenic index (TI) of tested products

"Healthy" products									
Sum of %	1	2	3	4	5	6	7	8	mean±SD
MCFA	0.22	10.13	0.16	0.20	40.36	7.21	0.18	0.24	7.34±13.00
LCFA	55.32	42.27	72.11	69.55	52.94	59.96	63.14	85.20	62.52±12.33
VLCFA	0.00	5.91	0.46	0.62	0.19	0.71	1.16	1.56	1.33±1.80
MUFA	11.36	32.67	14.17	16.56	3.71	15.69	27.16	1.71	15.38±9.87
PUFA	33.09	9.02	13.10	13.07	2.80	16.43	8.37	11.29	13.40±8.36
Index AI	0.89	3.39	2.50	2.24	19.15	2.19	1.41	5.75	4.69±5.64
Index TI	0.76	4.00	3.14	4.39	15.06	3.21	3.54	12.96	4.88±4.83
"Unhealthy" products									
Sum of %	9	10	11	12	13	14	15		mean±SD
MCFA	0.20	0.21	0.49	3.11	2.46	0.29	3.08		1.41±1.30
LCFA	46.20	81.72	75.94	78.61	75.28	60.66	78.43		70.98±11.91
VLCFA	1.59	0.74	0.66	0.95	1.32	8.71	1.90		2.27±2.66
MUFA	28.73	12.76	19.36	12.60	18.10	25.17	11.94		18.38±6.10
PUFA	23.28	4.56	3.54	4.73	2.85	5.17	4.64		6.97±6.70
Index AI	0.96	3.53	3.10	3.09	1.95	1.14	3.96		2.53±1.10
Index TI	1.23	9.20	6.75	7.71	7.14	3.91	8.91		6.41±2.66

The table includes data on the content of following fatty acids:

MCFA: caprylic acid C8:0, lauric acid C12:0

LCFA: myristic acid C14:0, pentadecanoic acid C15:0, palmitic acid C16:0, heptadecanoic acid C17:0, stearic acid C18:0

VLCFA: behenic acid C22:0, cerotic acid C26:0

MUFAs: myristoleic acid C14:1, palmitoleic acid C16:1, oleic acid C18:1 n9

PUFAs: linoleic acid C18:2 n6, γ -linolenic acid C18:3n6, α -linolenic acid C18:3n3

Saturated Fatty Acids (SFAs)

Among long chain saturated fatty acids, the groups showed statistically significant differences in the levels of palmitic and stearic acids (Figure 1). Their content in 'unhealthy' bars usually exceeded 10 000 $\mu\text{g g}^{-1}$, although similar values were also found in the healthy bars, in particular in samples 2 and 3, especially regarding palmitic acid. The product 1 contained negligible amounts of both acids. Statistically significant difference was also demonstrated for pentadecanoic acid and heptadecanoic acids (Figure 1), present in greater amounts in 'unhealthy' snacks, especially in

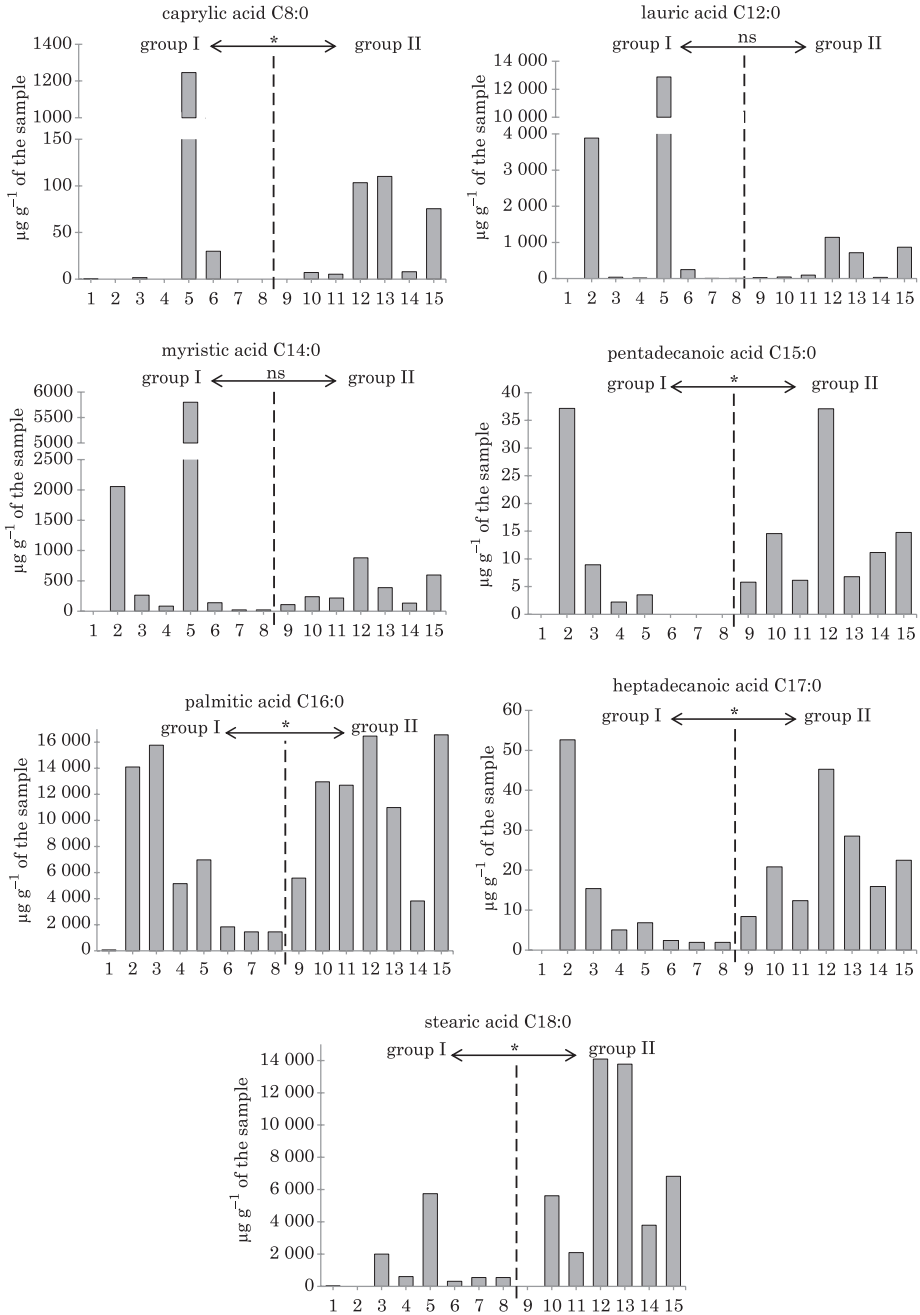


Fig. 1. The contents of medium and long chain saturated fatty acids (MCFAs: caprylic and lauric acid; LCFAs: pentadecanoic, palmitic, heptadecanoic and stearic acid) in various bars in group I („healthy, dietary”) and group II („unhealthy, nondietary”). * – statistical significance between the two groups, $p \leq 0.05$

the sample 12. However, their levels were the highest in the sample 2 from the 'healthy' group. In contrast, product no. 1 did not contain any of these acids.

Among very long chain fatty acids (VLCFAs) special attention should be paid to cerotic and behenic acids (Figure 2). Statistically significant difference was observed for both of these acids and they were the only ones that were found in each 'unhealthy' product, while in the 'healthy' group their large amounts were found only in sample no. 2, where the level of behenic acid was the highest (2224 $\mu\text{g g}^{-1}$) and much higher than in the other 'healthy' products.

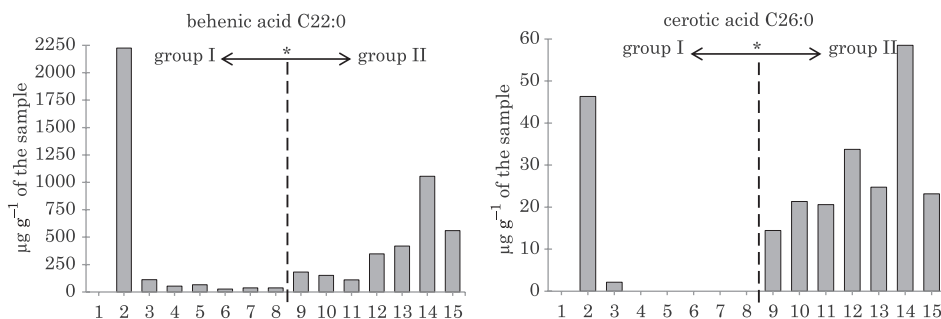


Fig. 2. The content of very long-chain saturated fatty acids (VLCFA: behenic and cerotic acid) in various bars in group I („healthy, dietary”) and group II („unhealthy, nondietary”). * – statistical significance between the two groups, $p \leq 0.05$

Statistically significant differences in saturated fatty acids between the 'healthy' and 'unhealthy' products were also found for caprylic acid belonging to the medium chain fatty acids (MCFAs), but its amounts in the majority of the samples were very low or undetectable. The only exception was the product 5 where its level was above 1200 $\mu\text{g g}^{-1}$ (Figure 1).

The analyzed products also contained SFAs which showed no difference between the groups I and II. These were lauric and myristic acids. It should be noted, however, that in individual samples they were present at levels greater than 1000 $\mu\text{g g}^{-1}$ (Figure 1).

Monounsaturated Fatty Acids (MUFAs)

Analysis of monounsaturated fatty acids, i.e. MUFAs, showed oleic acid was the most plentiful among *cis* acids; it was noted in all products except 1 and 8. In the 'unhealthy' group, its level was more or less stable, exceeding 2000 $\mu\text{g g}^{-1}$, while in the 'healthy' group differences were much higher – ranging from 4100 $\mu\text{g g}^{-1}$ in the product 2 to 570 $\mu\text{g g}^{-1}$ in the

product 6 (Figure 3). The significance of the difference in the oleic acid level between the groups was 0.009. Significant differences between the study groups were also observed for palmitoleic acid, but it was presented only in very small quantities. Its highest values were found in samples 2 and 12. MUFAs also include myristoleic acid, but its selective occurrence in the investigated samples showed no statistically significant differences between the groups.

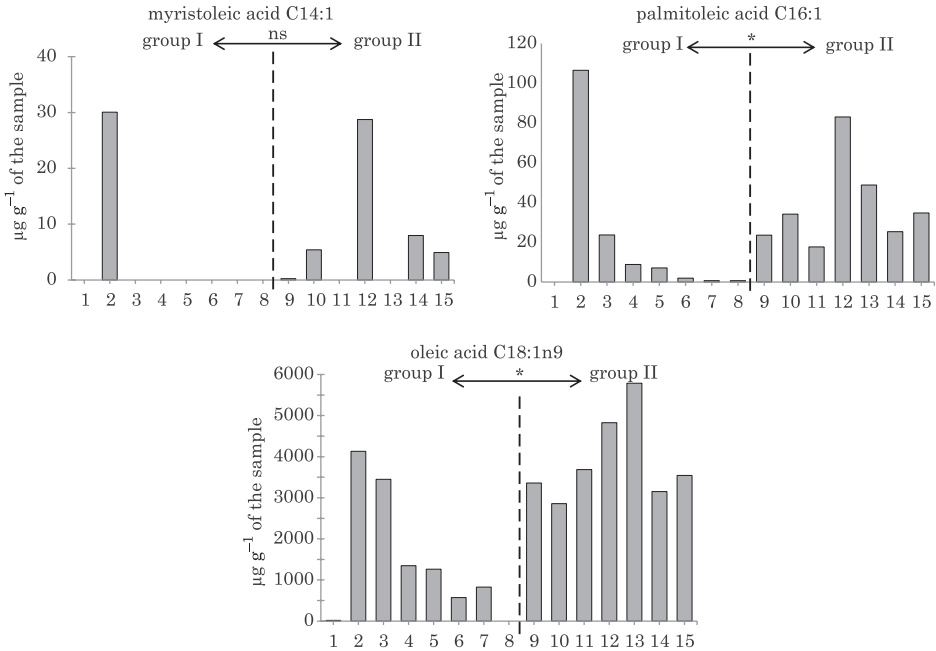


Fig. 3. The content of cis monounsaturated fatty acids (cis-MUFA: myristoleic, palmitoleic and oleic acid) in various bars in group I („healthy, dietary”) and group II („unhealthy, nondietary”).

* – statistical significance between the two groups, $p \leq 0.05$

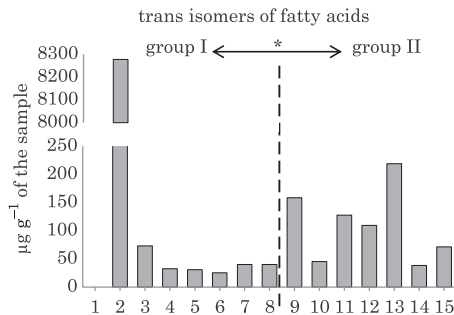


Fig. 4. The content of *trans* monounsaturated fatty acids (*trans*-MUFA) in various bars in group I („healthy, dietary”) and group II („unhealthy, nondietary”). * – statistical significance between the two groups, $p \leq 0.05$

Trans MUFAs were equally important in our analysis. These acids were present in both groups of products, but not exceeding $200 \mu\text{g g}^{-1}$ with the exception of the ‘dietary’ bar 2 which once again revealed extremely high level in comparison to other products ($8277 \mu\text{g g}^{-1}$) sample. In terms of TFA, only product 1 proved to be safe – it contained no such fatty acids. At the same, much larger amounts of TFAs were found in ‘unhealthy’ (Figure 4).

Polyunsaturated Fatty Acids (PUFAs)

When it comes to polyunsaturated fatty acids (PUFAs), the tested products showed the presence of linoleic acid and α -linolenic acid (Figure 5). Although there were no statistically significant differences in the content of these acids between the two groups, it should be noted linoleic acid levels in most products were as high as about $1000 \mu\text{g g}^{-1}$ sample or more.

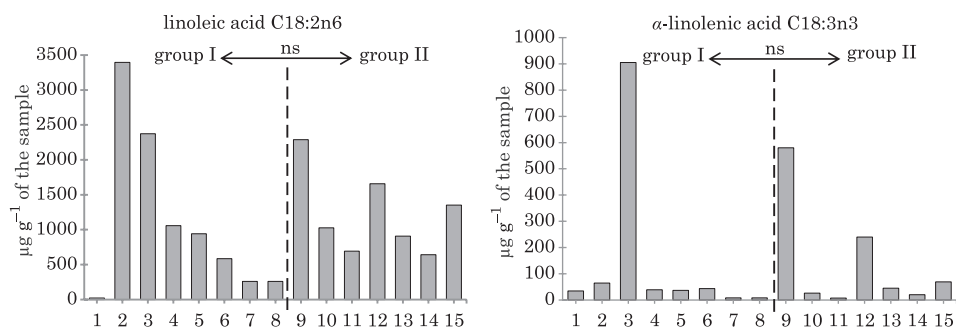


Fig. 5. The content of polyunsaturated fatty acids (PUFA: linoleic and α -linolenic acid) in various bars in group I („healthy, dietary”) and group II („unhealthy, nondietary”).

* – statistical significance between the two groups, $p \leq 0.05$

Discussion

Many studies confirm that the excessive consumption of saturated fatty acids, especially long chain fatty acids (LCFAs), contributes to the incidence of many metabolic disorders resulting from an increase in plasma cholesterol and promotes atherosclerotic lesions. At the same time, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), may reduce the level of cholesterol, although not proportionately. According to WHO recommendations for healthy people, 25 to 30% of energy the diet should come from fat. Saturated fatty acid should not provide more than 10% of energy per day, while omega-6 polyunsaturated

fatty acids from 4 to 8% of energy. Omega-3 fatty acids should be consumed at an amount of 2 g per day (α -linolenic acid) and 200 mg per day for omega-3 long chain eicosapentaenoic and docosahexaenoic acids. The remaining portion of energy should come from monounsaturated fatty acids (CONNOR 1999, ACHREMOWICZ and SZARY-SWORST 2005).

Among saturated fatty acids, most atherogenic properties are shown by lauric C 12:0, myristic C 14:0 and palmitic C 16:0 acids, able to regulate the expression of the LDL receptor gene and inhibit its activity. This results in increased LDL cholesterol and total cholesterol (FERNANDEZ and WEST 2005). The study by WOLAŃSKA et al. (2012) shows a significantly higher consumption of these atherogenic acids in men, who also had a higher total cholesterol than women. Tests carried out by WOLLETT et al. (1992) indicate that a diet rich in palmitic, myristic and lauric acids increases LDL-C by lowering LDL apoB/E receptor activity. This results in an increased production of this cholesterol fraction. Stearic acid has a different effect; although both palmitic and stearic acids belong to the group of saturated fatty acids, stearic acid C 18:0 shows no effect on total cholesterol and LDL-C (EMKEN, 1994).

Our results showed that palmitic acid dominated in the candy bars belonging to the group perceived as 'unhealthy' or 'non-dietary'. Unfortunately, it was also present in bars considered healthy. Particularly worrying is the fact that most of 'healthy' products had high levels of this atherogenic acid. Moreover, two products belonging to the mentioned group had the highest values of atherogenic index and thrombogenic index among all analyzed bars. Therefore, it is very disquieting that respondents basing on the product ingredients could qualified them as 'healthy'. On the other hand, lauric and myristic acids occurred only in some samples, with a statistically insignificantly higher content in 'healthy' snacks.

Very long chain fatty acids (VLCFA) are saturated, unbranched fatty acids whose chains are composed of at least 24 carbon atoms. Many studies show that VLCFAs accumulate in the human body, mainly in the white matter of the central nervous system, adrenal cortex and male gonads (ZGORZALEWICZ-STACHOWIAK et al. 2006).

It is likely that the excessive accumulation of VLCFAs in the nervous system gives rise to an immune reaction involving macrophages and astrocytes, triggered by the impairment of the acylation of gangliosides and phospholipids. The result is the development of inflammation and progressive demyelination of white matter of the brain and – less frequently – in the peripheral nervous system (ZGORZALEWICZ-STACHOWIAK et al. 2006). The excessive accumulation of VLCFAs in the adrenal cortex leads to the disruption of ACTH-induced cortisol secretion. This is due to the formation

of structures in the cytoplasm of the cells in the zona fasciculata and reticularis of the adrenal cortex that include esterified cholesterol of VLCFAs which cannot be used for the synthesis of steroid hormones. Moreover, the high concentrations of VLCFAs increase the viscosity of cell membranes and impede access to ACTH receptors (FICHNA et al. 2004). Our study showed that the analyzed VLCFAs (cerotic acid C 26, behenic acid C 22, tricosanoic acid C 24) can be found in very large quantities in the groups of 'unhealthy' products. However, the presence of these acids at similar levels were also recorded in 'healthy' products, advertised as wholesome snacks.

Monounsaturated fatty acids (MUFAs) are acids which contain one double bond, e.g. oleic acid (C 18:1) commonly found in olive oil. Various studies have shown that MUFAs reduce blood triglycerides and also lower the level of total cholesterol and LDL-cholesterol, while increase HDL-cholesterol (ABIA et al. 2003, HABÁN et al. 2000, WOLAŃSKA and KŁOSIEWICZ-LATOSZEK 2012). Furthermore, LDL enriched with oleic acid reduces the concentration of polyunsaturated fatty acids and their pro-inflammatory properties. It was also found that the content of oleic acid in the LDL inversely correlates with monocyte chemotaxis. CARLUCCIO et al. (1999) found that the incorporation of oleic acid into cultured endothelial cells stimulated with cytokines results in a reduction of leukocyte adhesion to endothelial cells (CARLUCCIO et al. 1999). The activation of factor VII and factor VII antigen concentrations were significantly lower as a result of a diet rich in monounsaturated fatty acids (HABÁN et al. 2000). Enriching a low-calorie diet with MUFAs enhances the beneficial effect of weight loss on the improvement of parameters which constitute risk factors for cardiovascular diseases in obese patients with type 2 diabetes (HABÁN et al. 2000).

Almost all of the naturally occurring unsaturated fatty acids have double *cis* bonds. Some unsaturated fatty acids have a double trans bond and acids containing at least one such bond are known as trans fatty acids (TFAs) (KOCHAN et al. 2010). These acids may occur as solids, which is of great biological importance because it is the type of fatty acids in the phospholipids of cell membranes influences the flexibility of these membranes. TFAs oxidize slower and can be used for repeated frying (CRAIG-SCHMIDT 2006); when incorporated into cell membranes, they alter their permeability and activity, and the number of receptors and enzymes associated with these membranes. This results in a deterioration of vital functions of cells. TFAs are produced by the hydrogenation of saturated fats and the human body they are metabolized similar to saturated fats, causing adverse effects, especially atherosclerotic lesions in blood vessels (ACHREMOWICZ and SZARY-SWORST 2005, KOZŁOWSKA-WOJCIECHOWSKA

2003). MOJSKA (2006) reports that TFA consumption in Poland ranges from 2.8 to 6.9 g/day, which greatly exceeds the nutritional recommendations for the maximum content of *trans* fatty acids in the daily ration. TFA intake should not exceed 1% of energy per day, which is approx. 2 g in the diet of 2000 kcal (ACHREMOWICZ and SZARY-SWORST 2005, MOJSKA 2006). PASZCZYK et al. (2007) analyzed the content of *trans* fatty acids in products bought in Polish stores (biscuits, biscuits and wafers). Of the 32 different cakes, 17 contained TFA at more than 2 g/100 g of the product, and in one case the TFA content was as high as 12 g/100 g. In some popular products in this category the amount of TFAs exceeds the recommended daily ration (PASZCZYK et al. 2007). Our study also showed the presence of *trans* fatty acids in both 'dietary' and 'non-dietary' products. An alarmingly high content of TFAs was observed in one of the dietary products, where it considerably exceeded the levels found in the 'unhealthy' group. Numerous studies have shown that artificially produced *trans* fatty acids impair the blood lipid profile, lowering HDL cholesterol (HDL-C) and increasing the levels of LDL cholesterol (LDL-C) compared to a diet with the predominance of oleic acid (*cis* fatty acid) (ACHREMOWICZ and SZARY-SWORST 2005, MENSINK and KATAN 1990). In addition, TFAs increase the ratio of total cholesterol to HDL (TC/HDL-C), a lipid predictor of coronary artery disease (ACHREMOWICZ and SZARY-SWORST 2005). They increase the levels of atherogenic lipoprotein, decrease plasma apoAI levels and increase the concentration of apoB in the blood, especially compared to MUFAs and PUFAs. TFAs also contribute to an increase in the concentration of triacylglycerols in the blood (ACHREMOWICZ and SZARY-SWORST 2005, LICHTENSTEIN et al. 1999). *Trans* fatty acids increase the risk of coronary heart disease, arrhythmias and sudden cardiac arrest. This is confirmed by numerous studies which show that even small amounts of TFA consumed in the diet (1.6% of energy) have a negative effect on the cardiovascular system. In addition, they contribute to increased weight and body fat, which contributes to the formation of abdominal obesity. This results in a weakening of the sensitivity of cells to insulin, resulting in changes in the structure and function of cell membranes, which can be a risk factor for type 2 diabetes (ACHREMOWICZ and SZARY-SWORST 2005). At the same time, as shown by HU et al. (1999), the replacement of a diet with 2% TFA-derived energy by a diet rich in unsaturated fatty acids (MUFA and PUFA) reduces the risk of cardiovascular disease by 53%.

However, *trans* fatty acids also occur naturally in the milk and meat of ruminants and as such may have a health-promoting effect. They are formed in the stomach of ruminants from α -linolenic acid and linoleic acid by the enzymes of anaerobic bacteria found in the rumen. Intermediate

metabolites are vaccenic acid (C 18:1) and CLA (*cis*-9, *trans*-11 C18:2). It is believed that the CLAs prevent atherosclerotic changes resulting from improper diet, and have antimutagenic and anticancerogenic effects probably due to their antioxidant properties (STACHOWSKA et al. 2004). The results of BARTNIKOWSKA (2000) indicate that CLAs protect cell membranes against free radicals, while their anticancerogenic abilities may consist in the inhibition of the production of eicosanoids, stimulators of cell growth (BARTNIKOWSKA, 2000). Research carried out in 2003 by IP et al. (2003) demonstrated that conjugated linoleic acids (CLAs) are incorporated into lipids in fat cells (where they are accumulated) and indirectly may influence the development of carcinogenesis by inhibiting cell differentiation (BIAŁEK and TOKARZ 2013, IP et al. 2003). In recent years, many publications have shown the contribution of CLAs in inhibiting tumors of the colon and breast. It has been shown that under this acid inhibits the proliferation of colon cancer cells dependent on the activation of PPAR γ receptor. In addition, *trans*-10, *cis*-12 CLA is most likely to inhibit the expression of HER2 protein by interfering with the NF- κ B signaling pathway (BIAŁEK and TOKARZ 2013).

Conclusions

A comparison of the two groups of products was difficult because of the very large differences in the quantity of individual fatty acids between individual products. Nevertheless, the products classified as 'unhealthy' generally contained more fatty acids than 'dietary' products. They also had a higher content of fatty acids with atherogenic properties. Although, there were no statistically significant differences between the groups for atherogenic and thrombogenic indexes, it is worth to mention that several bars had very high levels of AI and TI.

The majority of the total fatty acids in all the products were long-chain saturated C12–18 fatty acids (LCFAs), constituting 41.7–85.1% of the total fatty acids. These were mostly palmitic acid and stearic acid, showing very large differences in their contents between individual products, regardless of the group to which the products were assigned ('healthy' vs 'unhealthy'). Among VLCFAs, statistically significant differences between the groups were found in the levels of behenic and cerotic acids, but their quantities were usually low. In general, VLCFAs usually represented less than 2% of the total fatty acid content in individual samples (with the exception of the product 2–5.9% and product 14–8.7%).

Declaration of interest statement

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