Personal Report

1. Jarosława Rutkowska

2. Scientific degrees:

2002 - Ph.D. in agricultural sciences; Branch: Food and Nutrition Technology; Specialty: Human Nutrition, Warsaw University of Life Sciences, Faculty of Human Nutrition and Consumer Sciences (WULS – SGGW)

1995 – Master of Science in Food and Nutrition Technology, Faculty of Human Nutrition & Consumer Sciences, Warsaw University of Life Sciences (WULS – SGGW)

3. Positions and honours:

1993 – 1998 **Technologist**, Department of Techniques and Catering Technology, Faculty of Human Nutrition & Consumer Studies, Warsaw University of Life Sciences (WULS – SGGW)

1998 – 2002 **Ph.D. studies** on the Faculty of Human Nutrition & Consumer Studies, Warsaw University of Life Sciences (WULS – SGGW)

2002 – 2003 **Assistant,** Department of Techniques and Catering Technology, Faculty of Human Nutrition & Consumer Studies, Warsaw University of Life Sciences (WULS – SGGW)

From 1.04.2003 **Assistant Professor**, Department of Instrumental Analysis, Chair of Hygiene and Catering Technology, Faculty of Human Nutrition & Consumer Studies, Warsaw University of Life Sciences (WULS – SGGW)

4. Scientific achievements justifying the application:

a) According to Art. 16, Para. 2, Act on the scientific degrees and professorship (14.03.2003; Dz. U. No. 65, It. 595 with amendments: Dz. U. of 2005, No. 164, It. 1365 and Dz. U. of 2011, No. 84, It. 455), the main achievement is presented in a set of reports in the area **"Fatty acids in the milk fat – methodological issues and factors affecting fatty acid profiles"**

b) Published papers contained in the application:

1. **Rutkowska J.,** Adamska A., Białek M. Fatty acid profile of the milk cows reared in the mountain region of Poland **Journal of Dairy Research**, 2012, 79, 469-476. **IF – 1,566.**

Own contribution: originator of the research concept, research planning, preparation of fatty acids methyl esters of milk and forage fats, their qualitative and quantitative analysis by gas chromatography, participation in data analysis and interpretation, formulating conclusions, manuscript preparation (60%).

2. **Rutkowska J.,** Sinkiewicz I., Adamska A. Profile of fatty acids in milk from cows fed on total mixed ration system: **Żywność Nauka Technologia Jakość**, 2012, 5 (84), 135-144. **IF-0,155**

Own contribution: originator of the research concept, research planning, preparation of methyl esters of milk fats and their analysis by gas chromatography, participation in data analysis and interpretation, formulating conclusions, manuscript preparation (50%).

3. **Rutkowska J.**, Sadowska A., Tabaszewska M., Stołyhwo A. Fatty acid composition of hard cheeses from north, eastern and central region of Poland. **Bromatologia i Chemia Toksykologiczna.** 2009, 4, 1104 -1100. - 4 pkt.

Own contribution: originator of the research concept, research planning, gas-chromatographic determination of CLA content, participation in data analysis and interpretation, formulating conclusions, manuscript preparation (50%).

4. **Rutkowska J.**, Adamska A.: Fatty acid composition of butter originating from north-east region of Poland, **Polish Journal of Food and Nutrition Sciences,** 2011. Vol. 61, 3, 197-193 - **8** pkt

Own contribution: originator of the research concept, research planning, gas chromatography of fatty acids and determination of CLA in butter, participation in data analysis and interpretation, formulating conclusions, manuscript preparation (60%).

5. **Rutkowska J.,** Adamska A., Białek M. Comparison of fatty acid composition in mare's and cow's milk fat: **Żywność Nauka Technologia Jakość**, 2011, 1, 28-38. **IF- 0,157**

Own contribution: originator of the research concept, gas chromatography of milk fat, participation in data analysis and interpretation, formulating conclusions, manuscript preparation (60%).

6. Adamska A., **Rutkowska J**. Seasonal changes in triacylglycerols composition in milk fat from Simmental cows in Mazovia region, **Żywność Nauka Technologia Jakość**, 2012, 5 (84), 145-154. **IF-0,155.**

Own contribution: participation in the research concept, improvement of chromatographic separation of triacylglycerols, analysis of samples by gas chromatography, participation in data analysis and interpretation, formulating conclusions, manuscript preparation (50%).

7. **Rutkowska J.**, Adamska A. The content of pro-health fatty acids in butter samples in relation to the manufacture technology. Chapter in a monograph "The consumer of tourist and nutritional services. D.Kołożyn-Krajewska (ed.).Wyd.: WSHiT w Częstochowie, s. 235- 248. – **4pkt**

Own contribution: originator of the research concept, gas chromatography of butter samples and determination of CLA, participation in data analysis and interpretation, formulating conclusions, manuscript preparation (60%).

8. Stołyhwo A., **Rutkowska J**. An improved silver ion HPLC combined with capillary gas chromatography for studying cis/trans fatty acid profiles in alimentary fats and human milk fat. **Food Analytical Methods**, (online 19.06. 2012) **IF – 1,932.**

Own contribution: participation in the research concept, gas chromatography of samples, data interpretation, designing diagrams, formulating conclusions, contribution to manuscript preparation (55%).

9. Stołyhwo A., **Rutkowska J**. Milk fat: structure, composition and pro-health properties. In: Food chemistry – nutritional and health-related properties of food components. Z.E.Sikorski (ed.). Wyd.: WNT Warszawa 2007. str. 37-89. - **4pkt**

Own contribution: 50%, according to the publishing agreement.

Total achievement: 126 pkt. IF: 3,963

c) Presentation of the scientific objective of those publications, and of the potential use of the attained results:

Introduction

Milk fat, the principal energy source of milk, is the easiest digestible animal fat in human diet. Its digestibility is as high as 97 – 99% due to high dispersion and, thus, to its intestinal absorption without preceding hydrolysis. Apart from triacyloglycerols, milk fat contains phospholipids, tocopherols and carotenoids and, in addition, its fatty acid profile is extremely wide including 400 – 500 various fatty acids (FA) containing 2 to 28 carbon atoms. Some of those, like short- and medium-chain saturated FA (SFCA; e.g. vaccenic acid C18:1*11t* or isomers of the conjugated linoleic acid dienes) are of outstanding dietary value. The dominating isomer, C18:2*9c11t* (CLA), amounting to 75 – 90% of all isomers, is considered a potent antiatherogenic agent and, together with butyric acid, an inhibitor of carcinogenesis.

In the European region, bovine milk has been most widely used in human nutrition. However, due to its allergenic properties, milk from other animals (e.g. mares), containing less protein than the bovine one, has attracted attention. It has been shown that 96% of children sensitised to bovine milk tolerate mare milk available through professional processing and distribution channels.

The composition of milk fat is affected by genetic (breed) and physiological (age, lactation period, health status) factors, as well as by the environmental ones, like nutrition, season, climatic conditions, etc., the nutritional factor being associated with the geographical region. Some authors claim that butter quality may depend on the manufacture technology.

Working hypotheses and aim of studies

The aim of studies consisted of the analysis and assessment of factors affecting the fatty acid profile of milk fat, and of designing chromatographic techniques suitable for determining fatty acid composition, including trans-isomers and triacylglycerols. In view of those objectives, the following **working hypotheses** were formulated:

1. The feeding regimen of cows, and the content and composition of forage fat in particular, is the principal factor determining the fatty acid profile of bovine milk. Pasture grazing is of special importance, as the so-called Total Mixed Ration (TMR), i.e. a monodiet, does not provide a steady fatty acid composition throughout the year.

2. The season and geographical region are the significant factors affecting the fatty acid profile of milk fat due to the dietary needs of dairy cows, for which voluminous fodder is of prime importance. Animal species and its races are the principal non-dietary factors responsible for the fatty acid profile while the butter manufacturing technology does not alter that profile.

3. Chromatographic techniques employed to analyse milk fat need be modified due to a highly diversified fatty acid profile.

The results of studies on the effects of various factors on the fatty acid composition of milk fat were reported in 6 papers and in one chapter; that latter and 3 papers pertained directly to the chromatography of fatty acids including trans-acids and triacylglycerols.

Study results

Effects of dietary factors on the fatty acid composition of milk fat

Papers 1 and 2:

Rutkowska J., Adamska A., Białek M. Fatty acid profile of the milk cows reared in the mountain region of Poland, **Journal of Dairy Research,** 2012, 79, 469-476.
Rutkowska J., Sinkiewicz I., Adamska A. Profile of fatty acids in milk from cows fed on total mixed ration system: **Żywność Nauka Technologia Jakość**. 2012, 5 (84), 135-144.

The first working hypothesis pertained to the effects of forages on the fatty acid profile of bovine milk [Papers 1 and 2]. The effects of seasonal feeding and TMR were studied with respect to the size of Polish farms and, thus, the size of dairy herds. Seasonal feeding dominates in small farms, like e.g. in mountainous regions which, in addition, are of a rather low economic status. On the other hand, that kind of husbandry in mountainous regions has its good points – keeping extensive breeds, seasonal feeding that includes pasture grazing, and pollution-free environment [Paper 1]. On the other hand, high-productive dairy farms usually rely on the TMR regimen based on a steady access to a mixture of voluminous fodder and concentrates [Paper 2].

It was found that in the mountainous region (Beskid Wyspowy), the lipid intake was much higher in the grazing season than in the indoor one, due to the fact that lipid content in fresh grass was twice higher compared with other voluminous fodders. Moreover, mountain grass was found to be extremely rich in polyunsaturated fatty acids (PUFA; 51.8 – 64.1% of total fatty acids) – linoleic and linolenic acids, essential for generating other valuable FA. High PUFA intake by cows resulted in an increased content of CLA (C18:2*9c11t*) and of vaccenic acid (C18:1*11t*). Due to making use of pasture feeding, marked seasonal differences in fatty acid content in milk were found [Paper 1]. On the other hand, in TMR-fed cows, fatty acid content in milk is higher only in the autumn season compared with the rest of the year due to processes taking place in preserved feeds. Those processes decrease FA content in fodders, especially that of α-linolenic acid (C18:3*9c12c15c*). The FA content in fodders is essential for biosynthetic processes in the rumen, thus a reduced FA content in the fodder affected the fatty acid profile of milk by increasing the content of saturated (SCFA and LC-FA) fatty acids and decreasing that of the vaccenic acid, CLA and PUFA throughout the year except the autumn. In that latter season, a lower content of saturated fatty acids and higher of CLA and MUFA, including the vaccenic acid and C18:1 trans-acids was found compared with other seasons [Paper 2].

It was further demonstrated that preserved fodders (corn and hay silages) contain less PUFAs, especially less α-linolenic acid, compared with fresh grass. For that reason, the content of vaccenic acid and of CLA in milk from TMR-fed cows was twice lower than in milk from the mountainous region. On the other hand, milk from TMR-fed cows had a stable and high content of the linoleic acid (an omega-6 acid), higher than milk from the mountainous cows; that latter contained twice as much α-linolenic acid in the grazing period compared with TMR-fed cows [Papers 1 and 2].

The content of stearic acid C18:0, the end product of biohydrogenation of unsaturated FAs, varied seasonally only in the mountainous region making use of grazing. Our studies revealed that fodders are poor in stearic acid, whose intense synthesis in the rumen takes place in the summer, when the fresh grass intake is highest. Thus, highest content of stearic acid in milk was noted in the summer and it was concluded that stearic acid content in milk may serve as one of the markers of biohydrogenation intensity in the rumen in the grazing season [Papers 1 and 2].

Despite the fact that saturated FAs (C4:0 – C16:0) are synthesised *de novo* in the udder, their higher content was noted when cows consumed high amounts of saturated FAs contained in preserved fodders. However, the high content of saturated FAs in winter and spring milk is due mainly to short-chain fatty acids (SCFA; C4:0 – C12:0). When our data were confronted with those from the world literature, it turned out that Polish milk from mountainous regions was more rich in SCFA than that from other countries. It should be emphasised that SCFAs are beneficial for health and that TMR-feeding proved more efficient with respect to SCFA synthesis than other feeding regimens [Papers 1 and 2].

Qualitative and quantitative determination of 19 chromatographic peaks corresponding to odd-numbered and branched fatty acids (OBCFA), rather rarely identified in research reports, represent novel findings [Papers 1 and 2]. Since branched OBCFA were reported to exhibit anticarcinogenic properties both *in vivo* and *in vitro*, the presence of those acids in milk fat is valuable. Bovine milk from Polish mountainous regions contained higher amounts of OBCFAs, especially in the summer and autumn seasons, compared with either TMR-fed cows or with foreign reports. That was probably due to the optimum intake of fibre associated with a high consumption of voluminous fodders by the studied herds.

Another novel finding [Paper 1] was an assessment of the activity of Δ^9-desaturase, an ezyme participating in the transformation of vaccenic acid to CLA in the udder. That finding is of importance since Δ^9-desaturase participates also in the transformation of C18:0, C16:0 and C14:0 acids to the C18:1*9c*, C16:1 and C14:1 acids, respectively. Also, augmented transformations of C14:0 to C14:1 and of C16:0 to C16:1 acids was noted in the grazing season but this was not the case of CLA and C18:1*9c*. Regarding the latter (oleic acid), Δ^9-desaturase contributes to only 40 – 50% of that acid is being generated *de novo* while in case of CLA biohydrogenation in the rumen proved the dominating process, inasmuch other authors claimed that about 60% of CLA was generated in the udder *via* Δ^9-desaturase.

Summing up, the following conclusions could be drawn from the abovementioned studies:

1. Feeding regimen of cows affects the content of fatty acids in milk in the following ways:

– Pasture grazing enhances the synthesis of vaccenic acid (C18:1 1-t), CLA, OBCFA and PUFA. Thus, milk from the grazing season in the mountainous region (Bieszczady) is rich in fatty acids beneficial for health.

– Total Mixed Ration (TMR) feeding enhances the SCFA synthesis, especially in the autumn, when the fatty acid profile of milk fat is the most favourable nutritionally.

2. The quantity and composition of lipids contained in fodders affect the fatty acid profile of milk fat from cows fed seasonally in the extensive husbandry farms in mountainous regions:

– A higher intake of lipids contained in grass, rich in PUFA, in the pasture season results in increasing in milk the content of FAs generated in rumen in the biohydrogenation and other processes (CLA, vaccenic acid, OBCFA).

– In the indoor feeding season, the intake of fodders containing little lipids rich in saturated fatty acids, contributes to a higher content of those acids in milk, including the health-valuable SCFA.

– Milk from the extensive husbandry farms, in which cows are fed own seasonal fodders, is rich in health-valuable fatty acids.

3. Fatty acid profile of milk from high-productive dairy farms was found to depend on changes in fatty acid composition of preserved fodders used in TMR-feeding. Autumn season differs in that respect from other seasons since the contents of many fatty acids decrease with fodder storage time.

Geographical region and season – determinants of fatty acid profile of milk fat and of milk products

Papers 3 and 4

Rutkowska J., Sadowska A., Tabaszewska M., Stołyhwo A. Fatty acid composition of hard cheeses from north, eastern and central region of Poland. **Bromatologia i Chemia Toksykologiczna.** 2009, 4, 1104-1100.

Rutkowska J., Adamska A.: Fatty acid composition of butter originating from north-east region of Poland, **Polish Journal of Food and Nutrition Sciences,** 2011. Vol. 61, 3, 197-193.

The composition of milk fat is variable due to the effects of environmental factors. Climatic conditions, the result of geographical position and annual changes, determine the availability and quality of fodders. Thus, the region of milk production is an essential factor that affects the composition of milk fat, due to nutritional needs of dairy cows, whose diet consists predominantly of voluminous fodders. A verification of that statement was presented in Papers 3 and 4.

In 2007 and 2008 [Paper 3], fatty acid composition of rennet cheese from the Northern, Eastern and Central regions of Poland, produced in the winter/spring (WS) and summer/autumn (SA) seasons was studied. The WS cheese was characterised by a higher content of SFCAs, compared with SA cheese. Since those SFCAs are favourable for health, winter cheese has a good consumer value. Cheese samples, especially WS contained high amounts of long chain saturated acids especially palmitic C16:0 and myristic C14:0 acids. On the other hand, SA cheese had higher content of the less valuable stearic acid C18:0 compared with the WS product but, more importantly, a higher content of unsaturated fatty acids: linoleic C18:2 *9c12c* and linolenic C18:3 *9c12c15c*. The same was true for vaccenic acid (1.5 – 3.2% vs.

0.8 – 1.1%), probably due to a higher intake of fresh grass, which in late summer and early autumn is rich in linoleic and linolenic acids, indispensable for biohydrogenation processes; SA cheese is also extremely rich in CLA. However, geographical regions had also a marked effect on fatty acid contents.

Summing up, Cheese produced in the Eastern and Northern regions of Poland in the winter season is a valuable source of SFCA, and that produced in the summer/autumn season – of vaccenic acid and CLA [Paper 3].

The other study [Paper 4] focused on the evaluation of butter from the North-Eastern region, called "The Green Lungs of Poland". That region is characterised by a high arable area and low industrial density. Again, butter samples from the winter/early spring season had higher content of SFCA than those from summer. Seasonal variations in the long-chain saturated FAs reflected those found for milk fat (see above) and the same was true for oleic C18:1*9c* and vaccenic C18:1*11t* acids. The content of the latter ranged 0.8 – 1.2% and 1.3 – 3.8% in the WS and SA seasons, respectively, highest values being found in samples from the Varmian-Masurian and Podlasie regions, probably due to an exceptionally good access to green forages there. Moreover, an increased content of the C18:1*11t* fatty acid was found in the late spring season; this was associated with an increased supply of its substrates – linoleic and linolenic acids, at the beginning of the grazing season. In addition, lower levels of CLA (C18:2 *9c11t*) were noted from early spring till November than from May to October (about 245 and 580 mg%, respectively).

Summing up, Papers 3 and 4 evidenced the effects of geographical region and of season on the content of fatty acids in rennet cheese and in butter; that latter product manufactured in the North-Eastern region of Poland exhibited annual changes in fatty acid composition:

– Butter samples from the winter/early spring season had higher content of SCFA, palmitic and myristic acids than those from the warm seasons.

– Butter samples from the summer/autumn season had higher content of stearic acid, of mono- and polyunsaturated fatty acids, especially of the particularly valuable ones - vaccenic acid and CLA.

– The high variability of CLA content in butter in the summer/autumn season (490 – 1060 mg%) is indicative of regional differences apart from the seasonal ones.

Fatty acid composition of milk fat from various mammalian species

Paper 5

Rutkowska J., Adamska A., Białek M. Comparison of fatty acid composition in mare's and cow's milk fat. **Żywność Nauka Technologia Jakość**, 2011, 1, 28-38.

Fatty acid compositions of the bovine and mare's milk fat were compared considering the contents of fatty acids essential in human nutrition. The observed differences were due to different anatomy and physiology of the alimentary tract in both species with the resulting dissimilarities of the metabolism of unsaturated fatty acids supplied with fodders. Namely, microbial hydrogenation of unsaturated fatty acids in the mare's stomach is non-existent or only very slight (in its aglandular, "blind sac", part) while in the multicompartmental stomach of ruminants, rumen is the most essential site where biohydrogenation of fodder-supplied unsaturated fatty acids (mainly linoleic and linolenic) takes place resulting in the generation of vaccenic acid and CLA. For that reason, mare's milk is rich in PUFA contrary to bovine milk fat; fatty acid composition of that latter depends on the intensity of microbial hydrogenation processes.

Summing up, mare's milk fat contains less butyric C4:0, caproic C6:0, stearic C18:0 and myristic C14:0 acids and more caprylic C8:0, capric C10:0 and lauric C12:0 acids than the bovine one. Moreover, mare's milk fat is rich in polyunsaturated fatty acids – linoleic C18:2 9c12c and α-linolenic C18:3 9c12c15c, contains only traces of CLA and no vaccenic acid.

Effects of the genetic factor (breed) on fatty acid profile (triacyloglycerol composition) of bovine milk fat

Paper 6

Adamska A., **Rutkowska J**. Seasonal changes in triacylglycerols composition in milk fat from Simmental cows in Mazovia region. **Żywność Nauka Technologia Jakość**, 2012, 5 (84), 145-154.

The dominant component of bovine milk fat are triacylglycerols (TAG) whose content amounts to 97 – 98%. TAGs are generated in the udder in enzymatic esterification processes, fatty acid-specific and glycerol position-stereospecific. The structure of TAGs from milk fat is important not only nutritionally (activities of lipolytic enzymes); that structure affects sensory properties of milk products and some physical properties of milk fat – melting temperature, crystal structure and rheological properties of fat micelles. Two bovine breeds were compared: Simmental and Polish Friesian-Holstein (PFH). Both herds were fed traditionally, i.e. applying pasture and in-barn periods.

By applying chromatographic techniques, 16 TAG classes were detected (CN24 – CN54). In neither herd CN24 class was detected in autumn/winter samples, the dominating ones being CN36, CN38, CN40, as well as CN48, CN50 and CN52. Milk fat from Simmental cows contained most CN38 (12.5%), and least CN26 (0.35%). The CN38 class dominated in all regional samples (12.48%), CN24 class being the lowest (0.24%). The most significant between-breed differences were found for TAG classes CN24 – CN36, CN50 and CN52. Milk fat from Simmental cows contained more short-chain fatty acids and less long-chain FAs compared with the PFH breed. Seasonality had only negligible effect on TAG composition of that latter breed and that effect pertained to 5 classes: CN26, CN28, CN34, CN36 and CN44. Seasonal effects were more pronounced in Simmental cows; spring/summer samples contained more CN28, CN30, CN32, CN34, CN36, CN38, CN48 and CN50 classes, and less CN40, CN42, CN44, CN52 and CN54 classes, compared with the autumn/winter season.

Summing up, milk samples from Simmental and Polish Friesian-Holstein breeds differed in TAG composition. Considering nutritional values, milk fat from Simmental cows contained more SFCAs (TAG classes (CN26 – 34) and less long-chain saturated FAs (TAG classes (CN46 – 54), thus being of higher health/nutritional value compared with PFH cows.

Effects of manufacture technology on fatty acid composition of butter

Paper 7

Rutkowska J., Adamska A. The content of pro-health fatty acids in butter samples in relation to the manufacture technology. Chapter in a monograph "The consumer of tourist and nutritional services. D.Kołożyn-Krajewska (ed.).Wyd.: WSHiT w Częstochowie, s. 235- 248.

It has been reported that the technology of manufacturing butter affects its quality. In Poland, butter is being manufactured by a continuous regimen (Fritz's method) but small-scale producers, especially those engaged in manufacturing "regional or traditional products", traditional, periodic technology is in use. Both technologies apply concentration of fat resulting in generating butter grains, but differ in the aggregation rate; namely, an intense beating lasting only several seconds enables a continuous product output.

In the analysis of fatty acid composition of butter, special attention was put on the pro-health FAs. SFCA content was alike in butter samples obtained by both methods but was higher in spring than in summer samples. However, no significant between-method difference was found for butyric acid whose content, beneficial for human health, was high all year round and exceeded 4.30% of total FA. Spring samples of butter had higher content of palmitic acid C16:0 than samples from the grazing period or those locally manufactured in the mountainous region. The latter ones had, in turn, higher contents of essential FA (EFA) linoleic and α-linolenic acids – substrates for the synthesis of vaccenic acid and CLA. CLA content ranged from 228 to 1164 mg% depending on season, not on the manufacture technique. Summer samples had higher content of CLA than spring samples, especially those from the mountainous region rich in pastures (615 – 1164 mg%), as reported in Paper 1. As to the OBCFAs, their content differed between seasons but not between manufacture techniques.

Application of the principal component analysis (PCA) to fatty acid profiles of butter samples obtained by different technologies enabled identification of two components explaining 57% of total variance. The first principal component (38% of total variance) included C18:0 and C14:0 acids, the second one (19% of total variance) contained mainly vaccenic acid. PCA revealed between-season, but no between-technology differences.

Summing up, manufacture technology had no effect on the fatty acid profile of butter but between-season differences were demonstrated: higher contents of the valuable fatty acids – vaccenic, CLA and OBCFA – were found in samples from the grazing season, while samples from the spring were rich in other pro-health acids like SCFA.

Modifications of chromatographic techniques applied to milk fat analysis

Papers 1, 6, 8, 9

8. Stołyhwo A., **Rutkowska J**. An improved silver ion HPLC combined with capillary gas chromatography for studying cis/trans fatty acid profiles in alimentary fats and human milk fat. **Food Analytical Methods**, (online 19.06. 2012).

9. Stołyhwo A., **Rutkowska J**. Milk fat: structure, composition and pro-health properties. In: Food chemistry – nutritional and health-related properties of food components. Z.E.Sikorski (ed.). Wyd.: WNT Warszawa 2007. str. 37-89.

6. Adamska A., **Rutkowska J**. Seasonal changes in triacylglycerols composition in milk fat from Simmental cows in Mazovia region. **Żywność Nauka Technologia Jakość**. 2012, 5 (84), 145-154.

1. Rutkowska J., Adamska A., Białek M. Fatty acid profile of the milk cows reared in the mountain region of Poland, **Journal of Dairy Research**, 2012, 79, 469-476.

A long-lasting (2004 - 2008), intense collaboration with Professor A. Stołyhwo, an outstanding specialist in lipid chromatography, resulted in designing original or modified methods of lipid analysis in food materials and products and, eventually, in heading a skilled and dedicated research team. One of my prime achievements was methodological improvement that enabled the identification and determination of the positional and geometric trans-isomers of fatty acids. The improved methodology consists of two stages: 1. Chromatographic separation of fatty acid methyl esters (FAME) into classes (SFA, mono-trans, mono-cis, trans-trans, di-unsaturated) by applying silver ion exchange chromatography (HPLC-Ag^+); 2. Gas chromatography of FAME classes. The HPLC-Ag^+ separation was combined with the laser-operated, linear-time line segment detector (LLSD) which, unlike the most popular UV-VIS detector, is capable of detecting practically all non-volatile compounds. The LLSD proved particularly suitable for lipid chromatography, for lipids do not absorb light above 220 nm while light below 220 nm is being absorbed by solvents used as a mobile phase. In order to adapt the LLSD technique to lipid separation, a split-flow device was installed just before the nebulizer that enabled collecting consecutive fractions.

The following fats, converted to FAMEs, were analysed using the above procedure: virgin rapeseed oil catalytically hardened, hard and soft spread margarines, bovine and human milk fats. The HPLC-Ag^+ column proved highly selective with respect to the number of double bonds, their positions in molecules and geometric isomers (cis-trans); this enabled separation of FAME classes according to the number of double bonds. The two-stage procedure thus enabled a complete separation of all trans-isomers and fully eliminated co-elution of peaks. It was, for example, demonstrated that trans-isomers detected in human milk fat originated from dietary fats catalytically hydrogenated. Since trans-isomers of fatty acids contained in alimentary products may have a negative impact on health, their detection and identification in human milk offers a serious benefit [Paper 8].

Another significant improvement of milk fat analysis was application of gas chromatography to determine the composition of triacylglycerols (TAG), a highly complex task because of the enormous range of fatty acids present in TAGs in diverse combinations; the first report on it appeared in Paper 9. The procedure enabled elution of individual TAG peaks along their boiling temperature gradient, reflecting the mounting numbers of carbon atoms and of double bonds. That methodology may be particularly useful to detect fat additives, e.g. palm oil or its fractions; having a specific TAG profile of milk fat, any unspecified alien fat additives may be easily detected and identified in e.g. butter, cheese or cream. It found use in comparing milk fat composition of Simmental and PFH cows and in surveying milk products available on the market.

Summing up, my original contribution to advancing chromatography techniques applied to milk fat analysis includes:

- Improvement of a full chromatographic separation and identification of positional and geometric trans-isomers of fatty acids. The presented methodology proved suitable both for milk fat and for catalytically hydrogenated fats and enabled detection of trans-isomers of hardened fats in human milk and in other products;

- Designing a unique gas-chromatographic methodology for determining the composition of triacylglycerols (TAG) that enabled a full identification of TAGs which contain an enormous range of combinations of fatty acids present in them;
- Improvement of the chromatographic analysis of milk fat composition that resulted in a full identification and quantitation of 47 fatty acids, including 19 odd-number or branched ones (OBCFA; cf. Paper 1).

Summary of publications in the area of "Fatty acids in the milk fat – methodological issues and factors affecting fatty acid profiles""

It was demonstrated that fatty acid profile of the milk fat depends on the feeding mode, the content and composition of lipids in the fodders playing the dominant role; the season and geographical region of milk production were also of importance, as well as the genetic factors – mammal species and breed. The improvements introduced to the chromatographic methodology of qualitative and quantitative analysis of milk fat proved highly efficient and broadened the range of its practical applications. By and large, the previously formulated presumptions had been verified as reported in publications. All this enabled formulating the following conclusions:

1. Fatty acid composition of milk and milk products from regions rich in pastures and of low industrial density (the mountainous Beskidy region and the North-Eastern region of Poland), particularly valuable nutritionally, calls for expanding the dairy cow husbandry in those regions;
2. The storage of preserved fodders ought to meet technological requirements necessary to minimise qualitative and quantitative losses of lipid substances and, eventually, to maintain a high quality of milk products throughout the year;
3. Mare's milk may serve as a good source of short- and medium-chain saturated fatty acids and, thus, as a valuable substitute of bovine milk;
4. Increasing the population of Simmental cattle in Poland is recommendable due to the nutritionally valuable composition of triacylglycerols in the milk of that breed;
5. A detailed analysis of fatty acid profiles, including the trans-isomers and triacylglycerol composition, may be a valuable tool to detect unspecified alien fat additives of plant origin to the milk fat.

5. Other research achievements

After having graduated from the Faculty of Human Nutrition and Household in November 1993 I was employed as a food technologist and in 1995 I presented master's thesis ("Comparative analysis of selected physical properties of vegetables prepared in diverse kitchenware" under the supervision of Prof. A. Neryng), received my M.S. degree and started research work on the functional properties of shortenings. In 1998 I became a research assistant and started my postgraduate (doctoral) studies at the Warsaw University of Life Sciences, Faculty of Human Nutrition and Consumption. In October 2002 I presented my thesis ("Analysis of rheological and chemical properties of raw materials and semi-finished products on the quality of sponge dough", prepared under the supervision of Prof. A. Neryng), awarded by the Rector. In 2003 I was appointed Assistant Professor at the Department of Techniques and Catering Technology, then at the Department of Food Analysis and Quality Assessment, and since 2009 I am heading the Department of Instrumental Analysis.

Training courses completed

- Training course: "Nutrition Code of the European Union", Natural Resources Institute (U.K.); 1995;
- Training course: "Quality Guarantees in the Food Industry according to ISO 9000", Natural Resources Institute (U.K.); 1995;
- Training course: "HACCP System – Analysis of Hazards and the Critical Point of Control" (1999);
- Training course: "Principles and Methods of Total Quality Management in Food Industry", Gent University and Warsaw University of Life Sciences (2001);
- In-service Pedagogical Course (2003);
- Training course: "Thin-layer Chromatography – Trends and Applications" (2010);
- Training course: "Modern Methods of Food Analysis", Sigma-Aldrich (2012).

In addition to the issues of milk fat discussed above, I am engaged in other research projects pertaining to lipids:

5.1. **Effects of fatty acid composition, rheological properties of fats and semi-finished products on the quality of cakes and quality assessment of shortenings and cakes (with special attention to the trans-isomers);**

5.2. **Functional properties of liquid margarines used in manufacturing shortbread products (safety, dietary and quality aspects);**

5.3. **The use of additives improving the nutritional value of cakes, that have documented pro-health effects;**

5.4. **Application of classical and of modern chemical and sensory methods to the detection of changes in fats in the course of frying;**

5.5. **Adapting diverse techniques of acquiring valuable bioactive compounds from plants.**

***Ad 5.1*:** Rheological properties of semi-finished products were found to determine the texture and sensory quality of shortbreads. Thick layer and, thus, high mass of the dough reduced its elasticity and volume resulting in decreasing texture quality and in increasing the results of shear test of the final product. Dynamic viscosity of the semi-finished product was significantly correlated with texture variables [Paper 1]. That project included research that resulted in preparing my doctoral thesis.

Solid fat content (SFC), closely related to plasticity, reflects the percent content of solid fats at various temperatures: at 20°C, SPC equal to 17.0 – 24.5% in shortenings was correlated with their dynamic rheological properties and the latter were affected also by PUFA and trans-isomer contents [Paper 2]. It was demonstrated [Paper 5] that SFC reflects the usefulness of shortenings in manufacturing shortbreads, as well as in determining their nutritional quality: high SFC is indicative of low quality, while low one – of a high cis-isomer content (including EUFA). When SFC ranges 3 – 39% at 20°C, the manufacturing process of shortbreads is easy and the end product has an appropriate hardness.

The best way to assess the texture of shortbreads was found to consist of two instrumental approaches: 1. Texture profile analysis (TPA) that enables measuring elasticity, hardness and brittleness of cakes, 2. Puncture test to assess the hardness and brittleness of the cake, or shear test, when only the hardness is of importance [Paper 3]. Mechanical features of shortbreads determined instrumentally may reflect their hardness, while shear test enabled establishing maximum cutting force, at which the product had adequate sensory quality, brittleness and hardness [Paper 4].

The next step consisted of relating the quality of cakes to fatty acid composition of shortenings. The catalytically hydrogenated fats are the principal source of nutritionally disadvantageous trans-isomers (TFA) whose advantage, however, is in their low melting point and high plasticity – very desirable features in manufacturing shortbreads and puff pastries. It was found experimentally that a high TFA content improved the texture and general quality of puff pastries while high SFA content decreased their quality; the lowest acceptable TFA content amounted to 16% [Paper 6]. It was further demonstrated that no significant oxidative and hydrolytic changes took place in baking puff pastries over a wide range of TFA content in shortenings (4.1 – 54.2%); no changes were also found in the contents of basic classes of fatty acids. Gas-chromatographic determination of fatty acid composition was found extremely helpful in assessing the technological usefulness of shortenings and their quality. Shortenings having high contents of both SFA and TFA were resistant to oxidation thus being presumably appropriate to baking at high temperatures [Papers 7 I 8].

Considering the adverse health effects of TFA, I conducted an overview of fat products (oils, shortenings, soft margarines and fats present in pastries) available on the market in 2005. Most shortenings (analysed in 2005 year) contained high amounts of TFA (15.1 – 56.6%) and were probably manufactured from hydrogenation of various plant oils; when palm oil was the raw product, the TFA content was rather low [Paper 9].

An analysis of fatty acid composition of soft (and other) margarines conducted in 2008 enabled monitoring of meeting by manufacturers the recommendations pertaining to the limitation of health-adverse trans-acids. The TFA content in all samples did not exceed 10.9% in “hard” (kitchen) margarines and 7.9% in soft margarines and in 7 out of 12 samples of the latter TFA content was below 0.7%. Those results were markedly lower than in previous years – 3 times lower than in 1998. Moreover, soft margarines contained markedly more cis-MUFA and PUFA (with linoleic acid dominating) than the hard ones. The latter, in turn, contained more SFA (palmitic acid dominating) than soft margarines; in 6 samples also lauric and myristic acids were in relatively high quantities which were indicative of using coconut oil in the manufacture process [Paper 10].

Paper 11 presented the chemical quality of the refined and cold-pressed plant oils, manufactured by diverse technologies and available on the market in 2006. In most cases, the manufacturers did not adulterate the products by additions of cheaper or qualitatively inferior fats. However, the content of primary oxidation products was above the recommended limit in 40% of refined oils and in 50% of extra virgin oils. Small amounts of TFA (0.11 – 1.17%) were probably the result of deodorisation -induced isomerisation of unsaturated fatty acids at long exposure to high temperatures. The excessive oxidation of plant oils was probably due to inadequate storage conditions. Since the consumption of fats undergoing gradual oxidation may lead to civilisation-related diseases, monitoring the oxidation products in edible oils and in those that would undergo thermal processing is of utmost importance.

The last report in this area [Paper 12] pertained to fatty acid composition of fats in cookies offered for children; 12 products of 4 manufacturers were inspected in 2009. All those products had low fat content (below 15%) – a valuable feature due to the mounting incidence of obesity in children. The SFA and MUFA dominated and the high content of palmitic and oleic acids was indicative of using palm oil as the raw material. A high (about 20%) PUFA content and marked amounts of α-linolenic acid in 5 products indicated rapeseed oil, and high content of SCFA in 5 samples – milk fat or coconut oil. The TFA content was low (0.03 – 2.0 g /100g product), lower than cookies for general use in other countries, and met the requirements introduced in Denmark in 2004 banning the sale of food products containing more than 2% TFA in the fat.

Annex 3B - List of published 12 papers-: II D-15; II D-6; II D-14; II D-2; II A-5; II D-20; II D-17; II D-3; II D-8; II A-4; II D-7; II A-6

***Ad 5.2*:** The solid fats, usually the hard margarines, are traditionally in use in manufacturing pastries but despite technological advantages they are the principal source of harmful TFAs. In a study of functional properties of liquid margarines used in sponge cakes manufacture, the superiority of those margarines in the instrumental texture measures of cakes was demonstrated as opposed to cakes containing solid fats. The softness and delicacy of cakes prepared with liquid fats were confirmed by the shear test, relatively low density and a high sensory rating of the texture. Liquid margarines may thus replace the traditionally used solid fats; among other benefits, they save time of preparing the semi-finished product and that, under industrial conditions, saves energy and reduces the manufacturing costs [Paper 13].

The unquestioned advantage of liquid margarines is their fatty acid composition – high content of PUFAs (about 25%) and low of TFAs. Thus, an increased contribution of liquid fats to an industrial manufacture of sponge cakes over the existing 25% might reduce the consumption of the highly controversial TFAs. The index of nutritional quality (INQ) of those pastries with regard to EUFA was 3 – 6-fold higher than in case of pastries prepared with solid fats [Papers 13, 14].

The forthcoming studies on the use of liquid margarines in sponge cakes manufacture focused on the sensory quality and consumer preferences. Regarding the appearance, texture and colour, no differences were noted between pastries based on liquid (LFP) or on solid (SFP) fats. Regarding the taste and odour, LFPs were somewhat lower rated than those based on butter; this was due to significant quantities of PUFAs in liquid margarines; PUFAs are known to be particularly sensitive to oxidation of the α-linolenic acid. In as much young consumers preferred pastries based on butter to those based on liquid margarines, as many as 65% of them rated the latter as "very likeable" or "likeable", and only 5% as "decidedly dislikeable". Statistical analysis revealed that the hedonistic rating of cakes was positively correlated with SFA content and negatively with PUFA content [Paper 15].

Unlike solid fats, liquid margarines are practically devoid of TFAs but, on the other hand, contain high quantities of UFAs; the latter are unstable under the conditions of technological processes and may undergo unwanted changes like hydrolysis of triacylglycerols, oxidation, ring formation and polymerisation of unsaturated fatty acids. It was noted that baking sponge cakes and storage them in freezing conditions at -24°C for 8 months did not significantly increase the quantities of primary oxidation products. However, an increase in the secondary oxidation products in stored pastries was noted in cases of using fats with higher contents of linolenic acid. It was demonstrated that the complexity of the oxidation process of unsaturated fatty acids increases with the number of double bonds. The hydroperoxide products of linolenic acid oxidation were found to undergo degradation faster than those of oleic or linoleic acids as evidenced by an increased anisidine index in bakery products containing higher amounts of the C18:3*9c12c15c* acid [Paper 16.].

Annex 3B - List of published 4 paper - II D-5; II D-16; II A-3; II D-13

Ad 5.3: Pastries represent a significant dietary source of simple sugars and fats (SFA and TFA); their excessive consumption leads to civilisation-related diseases (diabetes, atherosclerosis, hypertension) but they are liked by consumers, therefore efforts to improve the nutritional value of pastries are fully justified. Papers published in 2008 – 2011 presented the results of using diverse pro-health additives in manufacturing pastries and bakery products. One of such additives was inulin added to pastries in order to reduce the fat content. The effects of inulin are associated with its prebiotic properties, resulting from the presence of β-glycoside bonds, resistant to enzymatic hydrolysis in the intestine. In an experiment [Paper 17], 50% of fat in cookies was replaced by inulin on the assumption that 1 g of inulin may substitute 4 g of fat. The inulin-spiked cookies were perceived as less sweet, with reduced butter aroma, and proved both sensory and instrumentally harder. Yet, butter taste, that is decisive for consumers' preferences, remained unaffected.

Another additive suitable for pastries is amaranth – flour, flakes or expanded seeds ("popping"). Amaranth contains much protein rich in lysine, methionine and cysteine, thus being highly valuable, complementary to wheat flour. Cookies containing 6% of amaranth popping had a high sensory rating and were resistant to oxidation, and their storage decreased somewhat their brittleness. Such pastries may thus serve as a source of valuable protein, pro-health SFCA and of fiber [Paper 18].

In another study [Paper 19] the use of oat flakes to the manufacture of cookies was evaluated. Oat flakes are rich in fiber (14%) and highest content of the soluble fraction (8%) among all cereals, the principal component being β-glucanes, potent compounds in reducing the cholesterol level in serum, in fighting inflammatory processes in the intestine and stomach mucosa and in relieving the effects of gastric ulcer disease. The content of hydrolysis products in such cookies was low independently of the amount of oat flakes added or of the storage time. Highest content of fat oxidation products was noted in the first 3 weeks of storage, especially in pastries with a high content of oat flakes (30%). Thus, considering the high nutritive value of oat flakes and the safety of their products, it was recommended that the addition of oat flakes to cookies be high, the storage time not exceeding 3 weeks. The consumption of such pastries would reduce the risk of diabetes and of atherosclerosis due to the effects of β-glucanes on reducing the postprandial glycaemia by forming gel layer in the alimentary tract.

Considering the indispensability of PUFAs and their insufficient consumption in Poland, an attempt was made at an enrichment of rye bread with PUFA [Paper 20]. A collaborating bakery prepared rye bread with an addition of cod-liver oil; 100 g of that bread contained 104.6 mg of EPA + DHA. According to the recommendations of ISSFAL and of FAO/WHO, 250 g of such bread consumed daily might supply 40% of the demand on omega-3 FAs. An important issue was the assessment of fat oxidation products in that bread as both EPA and DHA are polyunsaturated acids. In fresh bread, the peroxide content was low (4 mEq O/kg) but close to the limit, while following storage for 5 days that content increased to 12.80 mEq O/kg, that was reflected in a lower sensory rating. Thus, consuming only fresh bread enriched with cod-fish oil was recommended. It was concluded that products of that kind ought to be examined by using both conventional and modern techniques, e.g. GC/MS, in order to analyse the secondary oxidation products. Summing up, the results presented in Papers 17 – 20 may have a significant impact on designing functional food products.

Annex 3B - List of published 4 papers: II D-1; II D-9; II A-2; II D-10.

Ad 5.4: The reports on changes taking place in oils during frying were based on conventional and on modern chemical and sensory methods. The HPLC-RP technique combined with UV-Vis detectors was applied to refined oils in order to determine their chemical stability under frying conditions in relation to the sensory quality frying product (French-fries) [Paper 21]. All TAGs were recorded at 215 – 218 nm, and oxidation products (hydro-peroxides, aldehydes, ketones) at 240 nm. Rapeseed oil proved sensitive to high temperature and oxygen due to the high content α-linolenic acid as reflected by *K*-coefficients (K_2: about 125%, K_3: about 450%), i.e. the percent ratio of oxidation products in the post- vs. pre-frying material, determined for the frying oil or in the fat extracted from the French-fries. On the other hand, French-fries fried in the rapeseed oil were rated as most tasteful and crisp. Lowest oxidation-induced changes were noted in case of olive oil ($K_2 = 76\%$) but the sensory rating was lower from the former oil. This was associated with a negative impact of the oleic acid on the tastiness of the product and, besides, it was demonstrated that some amount of oxidation products is indispensable for a good taste of fried products. Summing up, it was found the usefulness of RP HPLC-UV-Vis and sensory evaluation for the detection of changes in fat oxidation during frying.

Another report [Paper 22] pertained to a detailed qualitative analysis of frying oils and of fats extracted from French-fries (samples taken from fast-food bars). The following determinations were conducted: peroxide and anisidine values, oxidation degree of TAGs, TAG and fatty acid composition, and tocopherol content. Marked amounts of superoxides and of secondary oxidation products (anisidine-reactive compounds) were detected together with other products of TAG oxidation (by HPLC/UVis). The temperature-altered fats had a decreased content of tocopherols. Moreover, as shown by chromatographic determination of fatty acid composition, the fat from French-fries did not meet the recommendations as to not exceeding 20% of SFA and TFA combined – the assayed value amounted to 33.5%. The same analysis revealed that 100 g of French-fries may supply about 2.3 g of SFA and 3.15 g of TFA. A detailed analysis of fatty acid and TAG composition of fats enabled also identifying the sources of frying oils.

Annex 3B - List of published 2 papers: II D-4; II D-11

Ad 5.5: A very promising project initiated in 2006 focused on extracting various important lipophilic and hydrophilic compounds from plants, e.g. carotenoids, tocopherols, tocotrienols and squalen, as well as flavonoids and glucosynolanes and their degradation products. The bioavailability of those compounds is often reduced by the presence of e.g. cellulose or antinutritional products and, besides, may be negatively affected by organic solvents used in the extraction. Further, conventional techniques used to extract odorants, e.g. steam distillation, result in losing the most volatile, most intense odorants. All those drawbacks can be avoided by using extraction with carbon dioxide in subcritical state (LCO_2). The so obtained extracts retain their original properties for the process is conducted at low temperatures with the use of a neutral gas. Moreover, the LCO_2 is non-toxic and non-flammable, and its use in extraction produces no waste and may thus be considered fully safe for food industry. Interestingly, the polarity of LCO_2 increases with its density and this enables extracting compounds along the polarity gradient by gradual decreasing the temperature. However, extraction combined with fractionation of products requires knowledge of solvation characteristics of liquid CO_2 for various classes of compounds.

The LCO_2 technique was applied to obtaining amaranth seed oil, and to determining product composition and solvation properties of individual compounds. That technique was found extremely efficacious. Determining the solvation properties of LCO_2 for squalene enabled its refinement in the extract. Squalene, a potent antioxidant, neutralises reactive oxygen species (ROS), arrests the propagation of lipid oxidation and eliminates all kinds of xenobiotics from the body. Those findings are of scientific and practical value as the necessity to protect the environment makes the acquisition of squalene indispensable; until recently, the only source of squalene was the liver of abyssal sharks, a vanishing species, thus strictly protected, therefore a reliable plant source of this compound is a precious alternative. The respective results were presented at a conference (Chromatography and related techniques in human health) in 2006.

The next step in the research pertained to determining solvation properties of LCO_2 and of the parameters of an efficient and selective extraction of carotenoids from red peppers (*Capsicum annum L).* Carotenoids, e.g. lycopene, capsanthin or β-carotene, are known to have antioxidative properties which makes them potentially efficacious in preventing cancer and cardiovascular diseases. Their extraction from natural sources so as to preserve their structure and, thus, their biological activities, is thus of great importance. Solvation properties of LCO_2 for carotenes and xanthophylls were determined together with the possibility of separating those compounds by fractional extraction from red peppers. As to yellow carotenoids, mainly β- and α-carotenes, colourless ones – phytoene and phytofluene, as well as to principal triacylglycerols of red peppers, the best solvation properties of LCO_2 were noted at +6°C. At -6°C, xanthophyll esters were extracted together with carotenoids, and strongest solvation properties for the extremely valuable red xanthophylls (capsanthin and capsorubin) occurred at -16°C. In contrast to the conventional hexane extraction, the LCO_2 extraction enabled both obtaining and fractionating red pepper carotenoids along their polarity gradient [Paper 23].

Another study [Paper 24] included conventional and LCO_2 extractions of valuable compounds from broccoli at various temperatures (-16 – +5°C), and qualitative and quantitative analyses of extracts using HPLC and GC/MS techniques. The results are under preparation for publication in *Food Chemistry*. So far, the GC/MS results of volatile compounds from the above-surface fraction of the LCO_2 broccoli extract obtained at -6°C. In that extract, 29 compounds were identified, most of which were aromatic compounds responsible for the characteristic broccoli odour: metanethiol, heptanal, hexanic acid, 2,4-heptadienal stereoisomers, and lynalool and nonanal, giving broccoli the citrus and flower scents. Besides, the dimethyl sulfoxide (DMSO), an analgesic, antiflogistic and antioxidant, probably a product of glucosynolane metabolism, was identified. Another identified compound was dimethyl sulfone, structurally related to DMSO and its metabolite. Summing up, a GC/MS analysis of broccoli extracts revealed the presence of typical, volatile odorants, as well as glucosynolane metabolites, typical of the *Brassica oleracea L. var. Italica*.

Another project initiated by our team focused on compounds present in the wild rose (*Rosa Rugosa*) fruits, rich in valuable substances [Paper 25]. The composition and antioxidative properties of extracts from conventionally dried and lyophilised fruits were determined. A higher content of PUFAs, especially of the α-linolenic acid containing 3 double bonds and, thus, sensitive to oxidation, was found in the lyophylised material, the conventionally dried one containing over 16% less of the C18:3 *9c12c15c*. Both kinds of extracts contained many carotenoids (lycopene, β- and ζ-carotene, phytoene) and xanthophylls (luteine, zeaxanthin, rubixanthin, β-cryptoxanthin) but more lycopene and rubixanthin was found in the lyophilisate than in the conventionally dried product. Those findings evidenced the superiority of lyophilisation to the conventional drying with respect to antioxidants, carotenoids and other compounds containing conjugated double bonds. The content of polyphenols in wild rose fruits was about threefold higher in *Rosa Rugosa* than in other species. Another source of bioactive compounds studied by our team are black chokeberry (*Aronia Melanocarpa*); a literature review of that subject was presented in Paper 26.

Annex 3B - List of published 4 papers II A-1; II D-12; II A-7; II A-8.

Research projects and grants

1. Developing a new, efficient instrumental analysis for detecting foreign fat additives to butter (especially the *extra* brand) based on the presence and composition of tocopherols, phytosterols, composition of fatty acids and of triacylglycerols (2007; project deputy head; project supported by the National Association of Dairying Co-operatives).
2. Implementation of own studies on the composition and nutritional value of lipids and of vitamins contained in amaranth seeds and products, aimed at defining technical requirements of raw materials for baking bread ("Bread for the heart"; the Polish Artificial Heart project, 2006; project participant).
3. Equipment and methodology of extracting valuable compounds from natural sources with carbon dioxide in subcritical status (2007; project head; supported by Rector's grant No. 50410050010).
4. Designing and implementing a training programme for the interdisciplinary laboratory personnel of the National Centre of Cell and Tissue Banking (included in the project PL2006/018-180.03.04 "Improvement of the Potential of the National Centre of Cell and Tissue Banking for the Safety and Quality of Human Cells and Tissues Used in Transplantations"; 2009; project head). ***(Annex 3B: papers from II D-21 to II D-26)***
5. Fatty acid composition of fats from milk and from selected milk products manufactured in various regions of Poland (2010 – 2011; faculty grant; project head).

Summing up, the following original, postdoctoral achievements, not mentioned in my application for professorship are presented:

1. Developing a technology of manufacturing functional products with the use of liquid margarines for sponge cakes and of adjusting the recipes of cookies so as to reduce the content of controversial components by replacing them by those having documented pro-health properties.
2. Application of modern chromatographic techniques in developing new indicators for detecting frying-induced changes in fats: tocopherol content, increased content of oxidation products, changes in triacylglycerols determined by liquid chromatography combined with UV-VIS detection.
3. Developing methodology of extracting from amaranth squalene, until now obtained from the liver of the strictly protected abyssal sharks, by using extraction with carbon dioxide in subcritical status (LCO_2).
4. Developing methodology of LCO_2 extracting carotenoid compounds from red peppers (*Capsicum annum L*) enabling their fractionation according to the polarity gradient, an alternative to the conventional extraction with organic solvents.
5. Assessment of the composition and properties of wild rose (*Rosa Rugosa*) fruits. Documenting a higher content of bioactive compounds and a higher antioxidant potential of lyophilised fruits compared with the conventionally dried ones.
6. Initiation of studies on the fatty acid composition of fats from milk and from milk products manufactured in various regions of Poland (in close collaboration with the manufacturers and associated with certifying the products from given region).

7. Designing and implementing methodology and a training programme for the personnel of the National Centre of Cell and Tissue Banking, pertaining to the use of chromatographic techniques in standardising the radiation-sterilised cells and tissues for transplants. Detailed tasks:
   - GC/MS methodology of a qualitative and quantitative evaluation of the degradation products of bone marrow lipids, generated in transplants by sterilising radiation;
   - Liquid chromatographic (UPLC, with fluorescence detector) methodology of a qualitative and quantitative determination of non-enzymatic (pentozidine) and enzymatic substances cross-linking collagen in the radiation-sterilised tissue transplants of spongy bone.

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