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A rapid and sensitive method for the determination of high-fructose corn syrup (HFCS) in honey

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Abstract. The authors developed a new, sensitive and rapid liquid chromatographic method suitable for the determination of concentrations of high-fructose corn syrup (HFCS) as low as 1% in honey. In this investigation, we determined the oligosaccharides formed in the course of the enzyme hydrolysis of starch, and which cannot occur in honey by means of natural processes. Since the high-performance liquid chromatography (HPLC) method does not require the removal of other sugars present in honey, the procedure is also appropriate for the quantitative determination of such sugars.

Keywords and phrases: high-fructose corn syrup (HFCS), high-performance liquid chromatography (HPLC), adulterated honey, glucose, fructose, disaccharides, oligosaccharides.

1 Introduction

Honey was the first sweetening substance known to man. Due to the extensive production and use of beet and cane sugar, the nutritional importance of honey is now derived mainly from its high biological value. The quantity collected by honey bees (*Apis mellifera*) may vary considerably from year to year, depending upon weather conditions. Honey can only be produced by natural means and in limited quantities – hence its relatively high price. For the purpose of increasing the quantity of honey available, it is often adulterated with alien substances of high sugar content. Since for many years the particular sugar composition of honey could not be imitated, the detection of adulteration was relatively simple. However, by the application of enzyme chemistry on the industrial scale, it is now possible to produce sweetening substances of sugar composition almost identical to that of honey. One of the cheapest and most commonly used of these is the high-fructose corn syrup (HFCS), produced from maize starch by means of enzyme hydrolysis.

In the production of HFCS, the gelatinized and liquidized maize starch is first hydrolysed with amylase enzyme to DP 10-15 dextrins, subsequent to which 94-96% is transformed into glucose by glucoamylase. By the effect of the glucose isomerase enzyme, the so-termed 42HFCS of maximum 47-48% fructose content (in practice, approximately 42%) is formed. By the application of ion exchange enrichment, 90VEFCS (very enriched fructose corn syrup) of fructose content as high as 90% can be produced from this compound. Production of corn syrup of any level of fructose content between 42% and 90% is possible from the above-mentioned products (*Comprehensive Biotechnology*, 1985). Since the dry-matter content and constituency of HFCS are similar to those of honey, while its price is much lower, HFCS can be used effectively in the adulteration of honey. The detection of HFCS is substantially more difficult than that of traditional substances of high sugar content; therefore, the development of a more advanced analytical method has become necessary.

2 Materials and methods

2.1 Examination procedures

In the *Official Methods of Analysis of AOAC International* (1995), two main examination procedures are recommended for the detection of HFCS adulteration of honey. One of these exploits the differences between the stable carbon isotope ratios of honey and HFCS (*Zieglet et al.*, 1979; *White & Robinson*,

1983), while the other one detects oligosaccharides present in HFCS by means of a thin layer chromatographic method (*Kushnir*, 1979; *White et al.*, 1979). Since the determination of the isotope ratio is very costly and substantial development in the level of equipment available for separation technology has been achieved in recent years, liquid and gas chromatographic procedures have gained increasing degrees of prominence. These exploit the fact that – due to the technology involved in the manufacture of HFCS – approximately 1% sugar of higher saccharide number remains in the end-product, which does not occur in unadulterated honey. The presence of such marker compounds (fingerprint oligosaccharides, or FOS) constitutes unambiguous evidence of adulteration. Such procedures are sufficiently sensitive and in other ways suitable for the detection of concentrations as low as a few per cent of HFCS mixed into honey. Nevertheless, the sample preparation required before measurements can be taken with the relevant instruments is not highly suitable for rather lengthy routine quality control procedures. In consequence of this, the development of a rapid, sensitive and non-labour intensive examination procedure is necessary. The HPLC method outlined below, which was developed in the authors' laboratories, is appropriate for the detection of HFCS in concentrations as low as 1% and for the analysis of as many as 70 samples a day. It is also suitable for the quantitative determination of fructose and glucose in honey and the group determination of disaccharides and trisaccharides.

2.2 Experimental materials and equipment

The Supelco oligosaccharide kit (cat. no. 4-7265), disaccharide kit (cat. no. 4-7268) and monosaccharide kit (cat. no. 4-7267) were used for the identification of the fingerprint oligosaccharides (FOS) and for the qualitative and quantitative determination of other sugars occurring in honey. Pure HFCS and honey, guaranteed unadulterated, were applied for the quantitative determination of HFCS and the mapping of the calibration curve.

2.3 Method

Some years ago, the examination procedure outlined below was officially approved by the Deutscher Akkreditierungs Rat, under the designation HERPAI#MEZ.001, for the detection of high-fructose corn syrup adulteration of honey. Statistical analysis of the data obtained from the measurements was performed by means of the BORWIN chromatographic software, version 1.21.60 (JMBS DEVELOPPEMENTS, Grenoble, FRANCE).

2.3.1 Appliance

Pump: Jasco PU-980
Autosampler: Jasco AS-950-02
Column thermostat: Merck 7350
Detector: Merck RI-71

2.3.2 Sample preparation

Sampling and homogenization were performed in accordance with the stipulations of AOAC 920.180. In each case, 1g (accurate to 0.1mg) of the sample to be examined was measured into a 10 ml volumetric flask and dissolved in HPLC water with the assistance of a test-tube agitator; the vessel was then filled up to the mark. The dissolved sample was filtered through a Millex-GV_{13} 0.22µ hydrophilic membrane filter (cat. no. SJGV013NS) and injected from this onto the HPLC. For the purpose of the examination of the shape of the FOS calibration curve, a 50-point calibration range from 1 mg/ml to 100 mg/ml concentration was prepared using pure HFCS. This, taking into account the preparation of the honey samples, corresponds to adulteration levels in the honey, ranging from 1% to 100%.

2.3.3 Chromatographic parameters

Column: Supelcogel Ca $300 * 7.8$ mm, (cat. no. 5-9305-U), with Supelguard Ca $50 * 4$ mm (cat. no. 5-9306-U) guard column
Column temperature: 70 °C
Eluent: water, Milli-q Ultra pure water system
Flow rate: 0.5 ml/min

An Upchurch A-101×2µ in-line filter was positioned after the pump for the in-line filtration of the eluent. In the interest of protecting the analytical column, subsequent to injection, the sample was filtered through an Upchurch A-102×0.5µ in-line filter. This was changed when the pressure of the pump exceeded the initial value of 10 bars. The guard column was changed after every 100 injections.

3 Results and discussion

On the comparison of a chromatogram for guaranteed pure honey and one for HFCS (*Figure 1*), a characteristic difference observed was the peak for FOS, present in the isosugar, at a retention time of 7.76 minutes. The shape of the peak indicates that not only one molecule is eluted at this retention time,

but also several dextrin molecules of similar structure. These molecules are larger than DP7, which can be concluded from the comparison of the retention times of oligosaccharide standards; that is, on the column used for analysis, the larger molecules are eluted first.

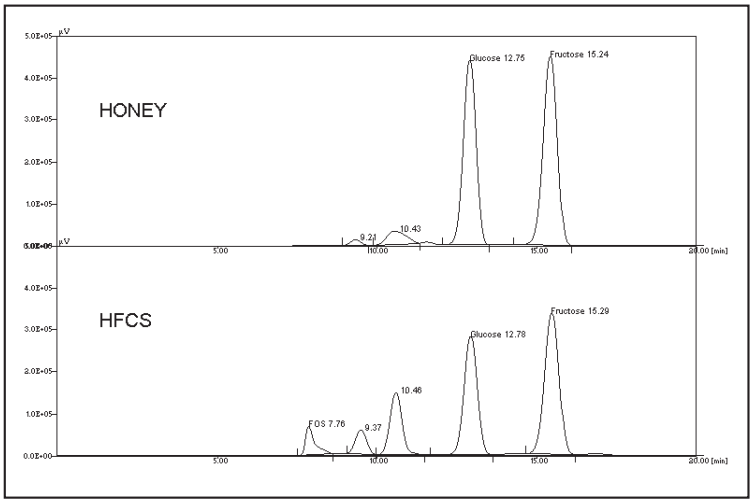

Figure 1: Chromatogram for pure honey and HFCS

The dimensions of the area beneath the FOS peak were taken as the base for the determination of HFCS content. The calibration curve was proved linear for the concentration range examined (*Figure 2*).

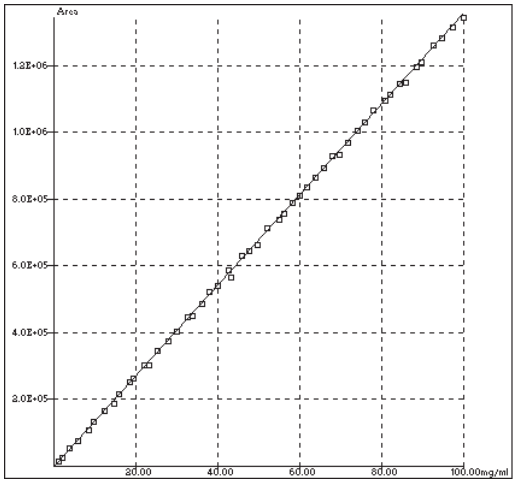

Figure 2: Calibration curve for FOS

Component: FOS, Model: Y = AX Nb of points: 50
A =13518.6233. Correlation = 0.99987
Standard error Vy = 911.6224. Mean % error = S1.134

Statistical analysis of the method is given in *Table 1.*

Table 1: Statistical analysis of fingerprint oligosaccharides (FOS) by means of the Herpai#mez.001 method of measurement

Concentration w%	**n**	**Measured conc. w% mean**	**Recovery %**	**Std. dev. precision**	**%RSD**	**Accuracy**
0 **blank**	9	0.10		0.11	110.87	0.10
1.0	11	0.81	81.0	0.06	7.34	0.19
5.0	11	4.05	81.0	0.09	2.21	0.95
10.0	11	8.32	83.2	0.08	0.96	1.68
30.0	11	26.72	89.1	0.21	0.79	3.28
50.0	11	46.76	93.5	0.23	0.49	4.24
Mean			85.6	0.13		

Limit of detection calculated from calibration curve $(Y = Ax)0.20w\%$
$k * s/A$ where $k = 3$, s = standard error of calibration curve $Vy = 911.6224$
Limit of determination calculated from blank $0.43w\%$
$y + k * s$; where $y = 0.1$ mean concentration of blank, $k = 3, s = 0.11$ Std. dev. of blank
r calculated $r = 2.83\ \sigma_r = 0.36$; where $r = 0.13$
R calculated $R = 2.83\sigma_R = 0.85$; where $\sigma_R = 0.30$

The possibility of the separation of glucose (rt: 12.7 min) and fructose (rt: 15.2 min) on the baseline can be seen clearly; they do not load over the column, and thus there also exists the possibility for the quantitative determination of these. A concentration range of 10 to 50 mg/ml was used for the glucose and fructose calibration curves (Figures 3 and 4), which was proved to be linear. On the HFCS chromatogram, a sharp peak can be seen at a retention time of 10.4 minutes, this being the peak for maltose derived from the incomplete hydrolysis of the maize starch. At the corresponding point on the honey chromatogram, a flat, broader peak is visible, which originates from several molecules. This is resulted from the fact that honey contains a number of different disaccharides and among these a substantial quantity of saccharose. The trisaccharides are eluted from the column prior to these,

at a retention time of 9.3 minutes. It may therefore be concluded that the procedure developed is appropriate for the detection of HFCS concentrations as low as 1% in honey. The advantages of the method are its simple sample preparation procedure and the modest equipment requirement, which allow large quantities of samples to be examined in series. A single chromatographic analysis enables HFCS adulteration to be ascertained and fructose as well as glucose content – important to the quality of honey – to be determined. Although no precise result is provided, conclusions can also be drawn with regard to saccharose adulteration. The disadvantage of the method is that the ion exchange columns required for the measurements are still relatively expensive and a great deal of care is necessary in their application.

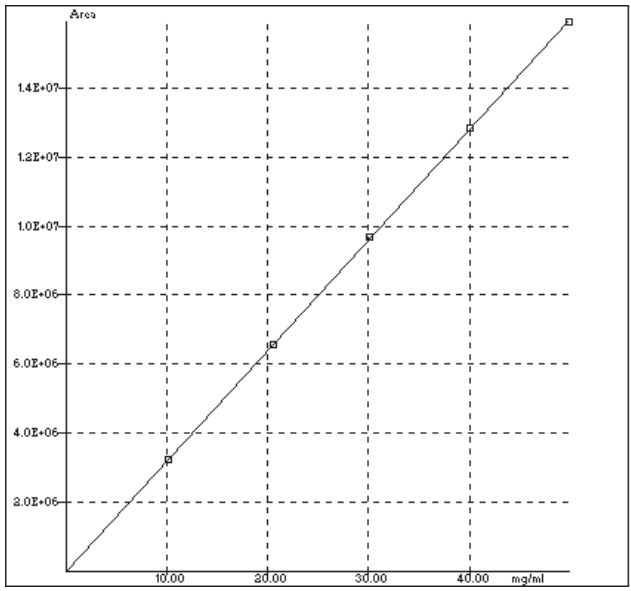

Figure 3: Calibration curve for glucose

Component: Glucose model: $Y = AX$, No. of points: 5. $A = 319844.6318$. Correlation $= 0.99997$. Standard error $Vy = 21336.4215$. Mean % error$= 0.373$.

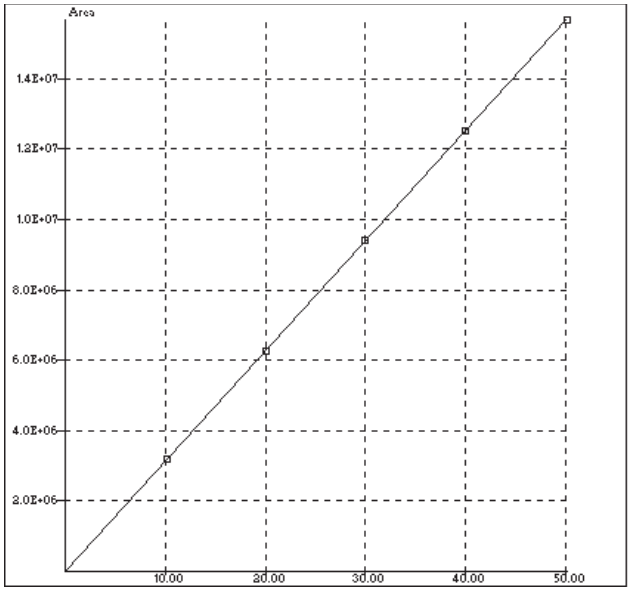

Figure 4: Calibration curve for fructose

Component: Fructose model: $Y = AX$. No. of points: 5. $A = 313119.6087$. Correlation = 0.99998. Standard error $Vy = 17035.8673$. Mean % error= 0.331.

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Estimation of immunoglobulin-G content of colostrum and milk from whey protein content in ruminant animals

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Abstract. After the analysis of the relation between the serum protein and the immunoglobulin-G content of the colostrum and the milk of 141 cattle of various genotypes (pure-bred or cross-bred), 55 goats of various breeds and 171 sheep of various breeds, it was established that there exists a very close correlation between these two components in all three species. R values of 0.9869 for cattle, 0.9723 for goats and 0.9574 for sheep were determined. The R value calculated for the three species evaluated collectively was 0.9667. The very close relation ascertained – by means of linear regression – between serum protein and immunoglobulin-G provides justification for the application of the equations formulated in this study for the estimation of immunoglobulin-G content on the basis of serum protein content.

1 Introduction

In the past decades, there have been substantial changes in the breeds and in the composition of types of the main ruminant species kept in Hungary (cattle, sheep and goats). In the field of cattle breeding, subsequent to the cross-breeding of the Hungarian Simmental (HS) with the Holstein-Friesian (HF)

Keywords and phrases: serum protein, immunoglobulins, colostrum, milk, cow, sheep, goat.

to result in a transformation in the breed, and also following cross-breeding programmes to produce new breeds, production began with new breeds of cattle of unknown colostrum and milk composition (*Csapó* & *Makai*, 1981; *Csapó et al.*, 1981, 1982; *Csapó* & *Csapó-né*, 1983a, b; 1992). In connection with this programme of breed transformation and new breed creation, the author examined the composition of the colostrum and milk of cows of the following types: Hungarian Simmental (30), Holstein-Friesian (30), Hungarian Simmental × Holstein-Friesian F1 (30), Hungarofries B (10, 50% Jersey, 50% Holstein-Friesian gene ratio), created by means of cross-breeding to produce a new breed, and also Holstein-Friesian sired (21, 62.5% HF, 25% J, 12.5% HS) and Jersey sired (20, 62.5% J, 31.2% HF, 6.3% HS) types, produced by alternating (criss-cross) cross-breeding based on the Holstein-Friesian and Jersey (J) breeds (*Szentpéteri et al.*, 1986; *Csapó et al.*, 1991a, b; 1992).

In sheep and goat breeding, in addition to the use of the Hungarian breeds, a number of breeds have been imported from other countries and subjected to trial. In the course of his research work, the author examined the composition of the colostrum and milk of the Hungarian Improved (10), the Hungarian Local Breed (14), the Saanentaler (17) and the Alpine (14) goat breeds (*Csapó et al.*, 1984a, b, c; *Csapó et al.*, 1986, 1987), as well as the Hungarian Combing Wool Merino (58), the Awassi (20), the Langhe (14), the Sarda (22), the Tsigai (16), the Cikta (11), the Black Racka (12), the White Racka (8), the Karakul (4), the Kent (3) and the wild sheep (3) (*Csapó et al.*, 1986). Since it was realized during the investigations that there is a substantial deviation between the first colostrum of mother animals producing twins and that of those giving birth to single progeny, the first colostrum from 32 cows calving twins and 32 giving birth to single calves on two state farms and also from 24 Hungarian Combing Wool Merino ewes dropping twins and 22 dropping single lambs at a production cooperative was collected. The composition of the colostrum and milk samples thus obtained was subjected to comprehensive chemical analysis, particular attention being paid to the immunoglobulin-G (hereafter IgG) and serum protein content of the colostrum (*Csapó* & *Csapó-né*, 1987, 1988, 1991, 1993, 1994; *Csapó et al.*, 1988, 1989, 1991; *Csapó*, 1991, 1995). In possession of the analysis data and in the endeavour to establish the relation between the various components, it was realized that there exists a close correlation between serum protein content and IgG. An account of this correlation is to be covered in this paper. The list of publications produced by the authors, wherefrom the 367 experimental data for serum protein and IgG originate, is contained in the list of the given literature.

2 Materials and methods

Colostrum and milk samples stored in a freezer were warmed in 38-40 °C water, then homogenized, and the casein and serum protein were subsequently separated at pH 4.55. The nitrogen concentration of the serum was determined by means of a Kjel-Foss 16200 type rapid nitrogen analyser.

The immunoglobulin-G content of the colostrum and the milk was determined using the simple immunodiffusion method developed by *Mancini et al.* (1965). The cow, goat, sheep and rabbit antiserum and the cow, goat and sheep IgG standard were supplied by the Gödöllő and Budapest units of the Human Vaccine Production and Research Institute.

The relation between the serum protein and the IgG content of colostrum and milk samples taken at various time points subsequent to parturition was calculated by means of linear regression.

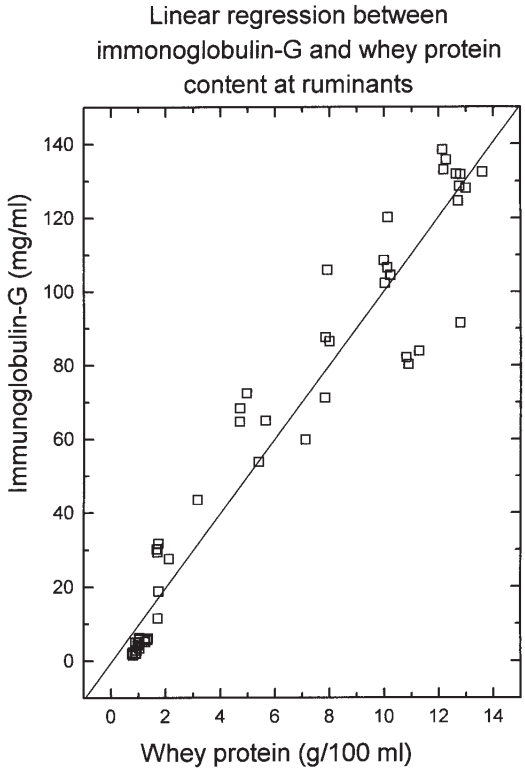
3 Results and discussion

Data relating to the analysis of the colostrum and milk of 141 cows of various breeds, 55 goats of various breeds and 171 ewes cattle of various breeds were included in the evaluation. The equations formulated by means of linear regression are presented in *Table 1*, while *Figure 1* illustrates the correlation between immunoglobulin-G and serum protein content established on evaluation of the data relating to the three species selected as appropriate.

Table 1: Linear regression parameters and statistical characteristics for immunoglobulin-G and whey protein content in cattle, goats, ewes and for the three species collectively ($\mathbf{Y = A + B * X}$)

Parameter, statistical characteristic		**Cattle**	**Goat**	**Ewe**	**Collective**
		whey protein – immunoglobulin-G			
A		-1.52595	-0.82282	-0.86288	-0.6742
	sd	1.8089	2.5700	3.15982	2.78457
B		10.57414	10.16312	10.03298	10.07453
	sd	0.23973	0.34565	0.41946	0.36965
SD		8.34463	118547	14.5959	12.8625
N		141	55	171	54
R		**0.9869**	**0.97228**	**0.95743**	**0.96673**
P		0.0001	0.0001	0.0001	0.0001

The data obtained for each species were evaluated separately by means of linear regression; then, subsequent to random selection of samples such that the immunoglobulin content of the samples should be evenly distributed in the 1-140 mg/ml range, the data relating to the three species were also evaluated collectively. For all three species, a very close correlation between the two components was ascertained; the R value determined for cattle was 0.9869, for goats 0.9723 and that for sheep 0.9574. The R value obtained from the data for the three species evaluated collectively was 0.9667.



The very close relation ascertained – by means of linear regression – between serum protein and immunoglobulin-G provides justification for the application of the equations formulated in this study for the estimation of immunoglobulin-G content on the basis of serum protein content.

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Fatty acid composition and cholesterol content of the fat of pigs of various genotypes

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Abstract. The authors determined the fatty acid composition and the fat cholesterol content of the fat of Mangalica, Hungarian Large White × Hungarian Landrace and Mangalica × Duroc pigs. It was established that no significant difference among the three genotypes could be detected with respect to saturated, unsaturated or the essential fatty acids – not in regard to cholesterol content. The findings of these investigations indicate that in the three pig genotypes studied fat cholesterol content varies between 71 and 109 mg/100 g. Attention is also drawn to the high oleic acid content (relative percentage 43.57–44.81) and linoleic acid content (relative percentage 10.63–11.47) of pig fat.

1 Introduction

The fatty acid composition of the fat content of foodstuffs is of extremely great importance with respect to healthy human nutrition. A number of studies have

Keywords and phrases: fatty acids, cholesterol content, fat, pig, various genotypes of pigs, Mangalica, Hungarian Landrace, Hungarian Large White.

reported on the substantial effect which different ratios of saturated and unsaturated fatty acids may exert on the health of those consuming them. While saturated fatty acids are considered a risk factor for cardiovascular diseases (*Burr et al.*, 1989; *Hrboticky & Weber*, 1993), polyunsaturated fatty acids are regarded as assisting in the prevention of disease (*Simopoulos*, 1991; *Weber et al.*, 1993; *Willett*, 1994). Since it was revealed that the fat contained by foodstuffs of animal origin is very rich in saturated fatty acids, the popularity of pig and cattle meat products for human consumption has recently suffered a decline, while that of poultry, fish and various sea foods – which contain high levels of unsaturated fatty acids – has increased.

The task of improving the fatty acid composition of foods of animal origin constitutes a great challenge both for livestock producers and for those involved in the production of foodstuffs. In the case of monogastric animals, such as the pig, there is a reasonably good possibility for the breeder to influence by varying the composition of the diet, the body composition of the pigs produced and the composition of foodstuffs derived from them (*Bee & Wenk*, 1994; *Klingenberg* et al., 1995; *Overland et al.*, 1996). Despite the fact that the fatty acid composition of the fat of the various regions of the body is relatively constant, when different diets are fed, significant differences are observed between the individual tissues in relation to the fatty acid composition of the diet. In addition, genotype-dependent differences have also been detected in pigs (*Nurnberg et al.*, 1994), in cattle (*May et al.*, 1994) and in poultry (*Reidy et al.*, 1994). According to the findings of *Sather et al.* (1996), there exists a relation of inverse proportionality between the degree of leanness and the hardness of the fat of Lacombe, Landrace and Yorkshire pigs.

In recent years, zoologists and livestock breeders worldwide have joined forces in the interest of saving indigenous and introduced domestic livestock breeds from extermination. The best strategy for preventing the disappearance of such breeds is to strive to maintain genetic diversity, for which it is precisely the indigenous breeds which can prove useful. In 1972, the recommendation made by the Stockholm Conference of the United Nations relating to the safeguarding of gene stocks and the establishment of a gene bank was accepted in Hungary. In 1993, the Rio Conference reaffirmed the importance of the role of gene banks, and drew attention to the fact that the risk of genetic drift is making its effect felt increasingly strongly in the course of genetic breed improvement.

The future of the Mangalica breed, indigenous to Hungary, is largely dependent on how its products can be utilized and how long-term market opportunities for these can be ensured. The Mangalica pig is now enjoying a

renaissance in Hungary: this is due, on the one hand, to endeavours to return to the traditional breeds, and to the new market opportunities presented by the production of Serrano type ham processed by means of specialized Spanish technology, on the other hand. The ham of the Mangalica pig is extremely suitable for the processing of products of this kind, as due to its meat:fat ratio and the distribution of the fat between its muscle fibres the ham does not dry out even during the long-term maturing process. The meat of this breed is of outstanding quality; it has high dry matter content and its red colour corresponds to current requirements. Its palatable flavour is derived from the fat surrounding the muscle tissue.

It is a generally known fact that not only the quantity of fat consumed is of significance from the aspect of nutrition but also the ratio with respect to each other of the various fatty acids within the quantity of the fat consumed. The lipid theory attributes the now widespread incidence of arteriosclerosis, together with high blood pressure or cardiac infarction developing as a consequence of this, to the cholesterol content, on the one hand, and to the lower unsaturated fatty acid content of fats of animal origin, on the other hand. According to this theory, lower cholesterol intake and the consumption of higher quantities of unsaturated fatty acids can lead to a reduction of approximately 10% in the cholesterol level of the blood plasma. A positive relation has been ascertained between the cholesterol level of the blood and arteriosclerosis in the majority of the individuals examined. The cholesterol level of the blood plasma is now regarded as an indicator of arteriosclerosis.

Researchers report that a total of 20% of the daily cholesterol requirement is derived from food, the remaining 80% being produced by the organism. The reason for the proportion of cholesterol ingested with the food being so low is that only about half of the cholesterol consumed with food is absorbed, the remainder being excreted undigested from the body of the organism. A number of factors have been found to possibly influence changes in the cholesterol level of the blood plasma, apart from the cholesterol content of the foodstuffs consumed. High blood plasma cholesterol level may also develop by the effect of the intake of large quantities of saccharose. Other aspects which may be significant in changes in cholesterol level are various hereditary factors, lifestyle, level of food consumption and the state of health of the individual.

A number of authors maintain that the fatty acid composition of dietary fats is ideal where saturated (SAFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) occur in equal proportions. Other authors consider such proportions of PUFA too high due to the risk of lipid peroxidation. They suggest that the proportion of PUFA in dietary fats should not exceed 10-15%,

where MUFA content is between 30% and 35% and SAFA content between 50% and 60%. A composition of similar ratio has also been established for hypothetically ideal fatty acid composition (HIF).

A quantity of information has been published recently in connection with the fatty acid composition and cholesterol content of the back fat and other fat of the Mangalica pig. It has been claimed that the fat of the Mangalica pig is softer and more easily digestible than that of the modern pigs. Its softer, granular consistency is attributable to its different and also healthier fatty acid composition. Another view expressed is that the cholesterol content of the fat of the Mangalica pig is substantially lower than that of the fat of the new, intensive genotypes. At present, the validity of this view can be neither corroborated nor refuted, since, as far as the authors are aware, there are no precise relevant experimental data available. The investigations outlined in this paper were performed for the purpose of providing scientific substantiation or disproval of the above assertions; this study included the determination of the fatty acid composition and cholesterol content of the fat of Mangalica, Mangalica × Duroc F_1 and Hungarian Large White × Hungarian Landrace F_1 (MNF × ML) pigs. The MNF × ML genotype is one of the most extensively used crosses in Hungary, and was therefore quite suitable to act as the control.

2 Materials and method

Experimental circumstances

These investigations were performed with the collaboration of the Hungapig Co. Ltd. and the Animal Breeding and Nutrition Research Institute in Herceghalom, at the new performance testing station. The experimental livestock were all housed in the same indoor area, with 6 pigs to a cage and 2.5 m^2 ground area per animal. Throughout the study, both the Mangalica pigs and those of the other genotype constructions were fed ad libitum diets of identical composition, provided from self-feeders. The composition of the diets used and their content are shown in *Table 1.* Diet I was fed in the live weight range of 30–70 kg and diet II when the weight of the pigs exceeded 70 kg.

At live weight between 120 and 130 kg, the pigs were slaughtered and their meat classified at the slaughterhouse of the Animal Breeding and Nutrition Research Institute in Herceghalom. After narcosis and slaughter, hanging to drain off the blood, boiling at 60-64 °C and manual singeing away of the hair, the carcasses were divided into parts. During the routine splitting and cutting into pieces of the carcasses, 100 g back fat samples were taken from the region

of the withers. These samples were stored in a freezer prior to laboratory analysis.

Table 1: Composition of fattening diets I and II

Component	Fattening diet I (%)	Fattening diet II (%)
Barley	15.00	15.00
Maize	59.72	57.00
Soybean meal, CP 46%	13.83	14.10
Full-fat soya (heat-treated)	5.00	-
Sunflower meal, CP 40%	-	3.53
Wheat bran	4.00	8.00
MCP (monocalcium phosphate)	0.29	0.20
Lime meal	0.05	0.06
Salt	0.11	0.11
Complete premix I for fattening pigs 2%	2.00	2.00

Examination of fatty acid composition and cholesterol content

Determination of fatty acid composition

A 1 g quantity of adequately homogenized back fat was measured into a 100 cm^3 Erlenmeyer flask, to which 8 cm^3 concentrated hydrochloric acid was added; the flask was then covered and heated on a steam bath for 60-90 minutes. After cooling, 7 cm^3 of ethanol and 25 cm^3 of ether were added and the flask was shaken for 1 minute. The ether phase was then poured off into a flask, and 25 cm^3 of petrol ether at the boiling point of 40-60 °C was added to the remainder of the sample; this was shaken for 1 minute, and, after separation, the petrol ether phase was poured into the ether phase, followed by homogenization. A quantity of the resultant extract known to contain 150-200 mg fat was then transferred to a round-bottomed flask with a ground glass neck. Subsequent to evaporation, the extract was boiled for 3 minutes with 4 cm^3 of a solution of boron trifluoride in methanol, and, after cooling, mixed with saturated aqueous saline solution. The organic phase was dried on sodium sulphate and then injected into the gas chromatograph. The following simplified procedure was used for the fat: 0.1-0.2 g fat was dissolved in 2 cm^3 of n-heptane, a small quantity of sodium sulphate was added, and 500 µl was measured from the dehydrated organic phase into a vial; 500 µl sodium methylate reagent was added to this, after which the liquid was heated at 60 °C for 1 hour, mixing by shaking it in every 10 minutes. Following cooling, 1 cm^3 of n-heptane and 1 cm^3 of water were added, and after shaking for

1-2 minutes, the fluid was injected from the upper organic phase into the gas chromatograph.

Conditions applied for gas chromatography:

Equipment:	Chrompack P 9000 gas chromatograph
Column:	50 m × 0.25 mm quartz capillary, humidifying phase CP Sil-88 (FAME)
Detector:	FID
Injector:	splitter
Gases:	carrier gas helium, 150 kPa, rate of flow 30 cm^3/min. at the detector: air 250 cm^3/min., hydrogen 30 cm^3/min.
Temperatures:	injector 220 °C, detector 220 °C, column initially 100 °C, then increasing by 6 °C/min. to 210 °C, and subsequently isothermal until the process was completed.
Volume injected:	0.5-2 μl

Determination of the cholesterol

The pure fat contained by 5 g back fat was extracted in Soxhlet extraction equipment with n-hexane; the fatty extract was evaporated, and 10 cm^3 of 60% potassium hydroxide and 40 cm^3 of methanol were added to the residue. The flask was heated in a water bath with a reflux condenser for 30 minutes. After saponification had been completed, the flask was cooled, its contents were washed into a separating funnel with 3×40 cm^3 of water, and the cholesterol was extracted with 3×40 cm^3 of ether. The unified ether phase was evaporated, after which the residue was dissolved in 4 cm^3 of hexane and 0.5 cm^3 of methanol, and then injected into the gas chromatograph.
The conditions applied for the gas chromatography procedure were the following:

Equipment:	Chrompack CP 9000 gas chromatograph
Column:	10 m × 0.25 mm quartz capillary, humidifying phase CP Sil-5 CB
Carrier gas:	helium, pressure 30 kPa
Flow ratio:	50:1
Temperatures:	injector 275 °C, detector 300 °C, column 270 °C
Detector:	flame ionisation detector; hydrogen 30 cm^3/min., air 300 cm^3/min., nitrogen 20 cm^3/min.
Volume injected:	0.5-2 μl

Statistical evaluation of the results

The Student t-test was applied for the statistical evaluation of the experimental data. The analysis of the basic statistics and the correlation analyses were performed by means of the SPSS for Windows (1996) software package, version 7.5.

3 Results and discussion

Table 2 contains the fatty acid composition of the fat of the pigs of different genotypes in terms of relative mass percentages of the fatty acid methyl esters, while *Table 3* shows the cholesterol content of the fat of the pigs of the various breeds.

Table 2: Fatty acid composition of the fat of the pigs of various genotypes (relative percentage of fatty acid methyl esters)

Fatty acid	Genotype		
	Mangalica, $n = 5$	MNF × ML, $n = 5$	Mangalica × Duroc, $n = 5$
	Mean ± SD	Mean ± SD	Mean ± SD
Capric acid	0.071 ± 0.0087	0.080 ± 0.011	0.082 ± 0.0103
Lauric acid	0.090 ± 0.0081	0.084 ± 0.010	0.086 ± 0.0068
Myristic acid	1.640 ± 0.12	1.458 ± 0.116	1.530 ± 0.083
Pentadecanoic acid	0.040 ± 0.0081	0.058 ± 0.012	0.038 ± 0.0062
Palmitic acid	25.97 ± 0.81	25.04 ± 1.01	26.15 ± 0.978
Palmitoleic acid	2.650 ± 0.47	2.270 ± 0.32	2.490 ± 0.424
Margaric acid	0.280 ± 0.034	0.450 ± 0.098	0.262 ± 0.034
Stearic acid	11.56 ± 1.01	13.63 ± 0.698	12.71 ± 1.633
Oleic acid	44.81 ± 1.71	44.34 ± 1.282	43.57 ± 2.155
Nonadecanoic acid	0.059 ± 0.012	0.074 ± 0.019	0.054 ± 0.0049
Linoleic acid	11.47 ± 1.92	10.63 ± 1.609	11.15 ± 0.724
Arachidic acid	0.170 ± 0.017	0.230 ± 0.022	0.200 ± 0.034
Eicosenoic acid	1.020 ± 0.208	0.750 ± 0.095	0.840 ± 0.139
Linolenic acid	0.570 ± 0.042	0.620 ± 0.081	0.630 ± 0.046
Eicosatrienoic acid	0.074 ± 0.0106	0.084 ± 0.022	0.068 ± 0.0091
Arachidonic acid	0.156 ± 0.027	0.196 ± 0.045	0.150 ± 0.021

No significant difference (at $P = 0.05$ level) between the individual genotypes was detected either for unsaturated essential fatty acids or for unsaturated

non-essential fatty acids, with the exception of eicosanoic acid. With respect to saturated fatty acids, with the exception of capric, lauric and palmitic acid, the difference between the genotypes proved significant at $P = 0.05$ level. Of these saturated fatty acids, in the case of stearic, margaric, pentadecanoic and nonadecanoic acid, the MNF × ML genotype contained the higher proportion, only myristic acid being determined in higher quantities in the Mangalica pig. This signifies that the ratio of saturated fatty acids in comparison with the unsaturated fatty acids was the highest in the MNF × ML pigs (41.12:58.88), although the difference was not significant (this ratio proving to be 39.87:60.13 for the Mangalica). The value for the Mangalica × Duroc genotype was found to be closer to that obtained for the MNF × ML group.

Table 3: Cholesterol content of the fat of the pigs of various genotypes

Genotype	Cholesterol content (mg/100 g)
	mean ± SD
Mangalica, $n = 5$	88.40 ± 10.08
Hungarian Large White × Hungarian Landrace, $n = 5$	83.60 ± 11.77
Mangalica × Duroc, $n = 5$	92.00 ± 8.72

The data was obtained from the Student-Newman-Keuls range test, designed for the purpose of distinguishing significantly different genotypes. It can be ascertained from the data that the control group differed non-significantly from the Mangalica pigs for every fatty acid under examination.

All of the three genotypes included in this study were found to greatly deviate from the hypothetically ideal ratio with respect to fatty acid composition (HIF). Ratios for saturated fatty acids were calculated at only approximately 40% instead of 53-62%, while those for unsaturated fatty acids proved to be around 60% rather than 38-47%. The values for oleic acid (43-44%) were substantially higher than those reported in the literature, while those for linoleic acid (10-11%) and those for linolenic acid (0.5-0.7%) were found to correspond to the literature data.

On the basis of these investigations, it may be established that no substantial difference was ascertained with respect to either the monounsaturated, or the polyunsaturated, or the saturated fatty acids (stearic acid being the exception among the fatty acids present in concentrations above 10%) on the

examination of the fatty acid composition of the fat of these three pig genotypes. In the case of palmitic acid, oleic acid and linoleic acid, which together amount to more than 80% of fatty acid content, the mean values obtained practically concur. Thus, it is possible to draw the conclusion from these investigations that the fatty acid composition of the fat of the Mangalica pig is, practically speaking, totally identical in value to that of the fat of the Hungarian Large White × Hungarian Landrace and the Mangalica × Duroc genotype constructions. There are therefore no grounds for any assumption that the fat of the Mangalica breed has a more favourable fatty acid composition, which would render it more easily digestible and healthier for humans than that of the intensive breeds.

A similar conclusion can be drawn with regard to the cholesterol content of the fat of these genotypes. On the basis of the average for nine animals, the cholesterol content of the fat of the Mangalica was measured at 88.44 mg/100 g, that of the Hungarian Large White × Hungarian Landrace at 83.60 mg/100 g and that of the Mangalica × Duroc F_1 genotype at 92.00 mg/100 g. No significant difference at $P < 0.05$ level was detected between the three genotypes with respect to fat cholesterol content; variance within the genotypes proved greater than between genotypes. Thus, there is no truth in the reports indicating that the fat of the Mangalica pig contains less cholesterol than that of the more generally produced types of fattening pig.

However, on the basis of the findings of these investigations, the authors wish to draw attention to the observation that the fat of all three genotypes examined proved to contain 43-45% oleic acid and 10-12% linoleic acid, and it is thus extremely rich in unsaturated fatty acids and the essential linoleic acid if pigs are kept on a fattening diet based on one of the feed mixes currently in widespread use. The linolenic acid content (0.57-0.63%) and arachidonic acid content (0.15-0.20%) of the fat of the pigs examined proved low, while in comparison with the other fats studied, the stearic acid content was observed to be extremely low (11.56-13.63%).

The measurements made in this study indicate that the cholesterol content of pig fat varies between 71 and 109 mg/100g. This cholesterol content is substantially lower than that of the kidney, liver, egg yolk, bone marrow or cod liver oil.

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The relation between quality and quantity with respect to quality parameters of Hungarian field crops

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Abstract. The crude protein content, amino acid content, crude protein amino acid composition and biological value of various basic materials for livestock feeds (wheat, barley, maize, oat, sorghum seeds, soybean, sunflower meal, rapeseed meal and fish meal) were determined. It was established that the glutamic acid and lysine content of crude protein can be estimated on the basis of crude protein content alone. It was verified by means of regression equations that the quantity of amino acids rose with increase in crude protein content. On the examination of protein amino acid composition in relation to crude protein content, it was ascertained that as crude protein content increased, the quantities of the non-essential amino acids also raised, while those of the essential amino acids decreased.

Keywords and phrases: crude protein content, amino acid composition, essential amino acids, biological value, barley, wheat, corn, soybean.

1 Introduction

For the monogastric animals, the fodder stuff mix is to be prepared on the basis of precise calculation based on amino acid analysis, and to be supplemented with synthetic amino acids. Nowadays, the amino acid analysis is rather expensive; it costs 60-70 euros depending on laboratories. In the case of feeding stuff mixes, the amino acid analysis is essential for the purpose of quality control or interventions during production. What is the situation with the raw materials? Is there any way to estimate the amino acid composition of the different feeding stuff raw materials, the amount and proportions of the essential and limiting amino acids; is it possible to omit the long-lasting and expensive amino acid analysis? Is it possible to estimate the amino acid composition of the feeding stuff raw material on the basis of its crude protein content, and if so, with what certainty? Is there any relationship between the crude protein content and amino acid composition of the feeding stuff raw material, as well as between the crude protein content of the feeding stuff raw material and amino acid composition of the feeding stuff protein? In order to find an answer to these questions, we collected the crude protein content and amino acid composition of various feeding stuff raw materials as well as amino acid composition of feeding stuff protein for several years retrospectively at the Department of Chemistry and Biochemistry of the Faculty of Animal Science, Kaposvár University, and tried to find out by computer evaluation whether it is possible to estimate the amino acid composition on the basis of the crude protein content, and if so, with what certainty.

According to investigations of *Michael et al.*, (1961); *Schiller & Oslage*, (1970); and *Schipper*, (1975), with increasing crude protein content in the corn seeds, the proportion of the different proteins changes, which is ultimately the base of the change of the amino acid composition. The change in protein composition with increasing crude protein content is especially striking in the case of wheat and barley as well as of rye, while oats and maize respond to increasing nitrogen fertilizer portions much less (*Schiller*, 1971; *Hoffmann et al.*, 1975; *Jahn-Deesbach*, 1981). *Győri & Bocz*, (1982) and *Bocz & Pepó* (1984) established that in the case of the wheat variety Jubilejnaja-50 the protein content of the wheat grain increases and changes the protein composition due to fertilizing. The amount of the albumin increases, that of the globulin decreases and the high-molecular gluten proteins accumulate.

According to *Kiss et al.* (1985), nitrogen fertilizer applied late increases mainly the reserve proteins of the grain but also changes the amount and proportion of the individual protein fractions. Experiments of *Sonntag* & *Michael*

(1973) proved that all the factors that considerably increase the amount of the crude protein in the grain will result in a relative decrease of the biologically valuable components. On the basis of his experiments with barley, *Eppendorfer* (1975) established that within the crude protein content the decrease of the sulphur amino acids is considerably lower in the case of enhanced nitrogen fertilizing if the fertilizer is supplemented with sulphur.

Vincze & Szüts (1978) examined the change of the protein content and amino acid composition of wheat grain, and found the biological value of the maize protein in the case of nitrogen fertilizer portion of 150-200 kg/ha to be the highest.

Jahn-Deesbach & Schipper (1982) established that the crude protein content of barley can reach 16-18%, in extreme cases even 20%. According to their examinations, with increasing nitrogen fertilizer portions, the proline, glutamic acid and phenylalanine content of the barley protein increase, the tyrosine, cystine, methionine and isoleucine content do not change significantly, while all the other amino acids decrease.

Németh (1983) established that the crude protein content of maize was increased by the nitrogen to a smaller extent than that of the wheat. Wheat protein contains few lysine and methionine that further increase due to nitrogen fertilizing.

Whitacre (1985), *Jensen* (1986), *Monsoto* (1986) and *Ivery* (1986) elaborated a computer programme for composing feeding stuff portions on the basis of amino acid composition. They established that although the determination of the composition of the critical amino acid cannot replace the regression equations, this method still gives a better result than if amino acid results were taken only from the tables.

Most of the authors agree that due to increasing nitrogen fertilizer portions the crude protein content of the cereal grains increases, the amount and proportions of the protein fractions in the grain changes, which finally results in differences in the amino acid composition. It appears that this change in the case of wheat, barley and oats is much more expressed than in the case of maize. It appears to be proven that the increase of the crude protein content results in a decrease of the more valuable components, thus, the degradation of the biological value of the protein; this is, however, unambiguously proven if other requirements of the plant are not satisfied adequately when fertilizing with increased portion of nitrogen fertilizer.

2 Materials and methods

Experimental materials. During our experiments, we examined the crude protein content and amino acid composition of 154 wheats, 172 barleys, 210 maizes, 42 oats, 41 seed sorghums, 118 soya, 102 sunflower grits and 21 rape grits. For the examined materials, the crude protein content varied between the following extreme values: wheat: 10.5-16.2%, maize: 7.9-11.0%, seed sorghum: 10.1-13.5%, sunflower grits: 35.8-45.5%, barley: 7.9-13.7%, oats: 9.8-13.7%, soya: 39.1-51.5% and rape grits: 32.1-39.1%.

Chemical examination of the samples. Determination of dry-matter and crude protein content of samples was carried out according to the feeding stuff examination standard No. MSZ 6830, whereas amino acid composition according to as described in the works of *Csapó* & *Csapóné* (1985) as well as *Csapó et al.*, (1986).

Mathematical analysis of the results. We tried to establish relationships between crude protein and amino acid composition as well as the crude protein and amino acid composition of the feeding stuff protein by bivariate regression relationship; thus, we expressed the relationship between the crude protein and amino acid composition by the regression equation $Y = a + bx$.

3 Results and discussion

Results of the experiments. Parameters of the bivariate linear regression equation between the amino acid composition and crude protein content of barley and barley protein are shown in *Table 1*; the change in amino acid composition as a function of the crude protein content is shown in *Table 2*. For the rest of the examined feeding stuffs, the numerical results cannot be presented due to lack of space.

Table 1: Parameters of the bivariate linear regression equation between the amino acid composition and crude protein content of barley and barley protein ($n = 62$; examined crude protein range: 7.9-13.7%, dispersion of the crude protein = 1.8564)

1/1 Crude protein and amino acid content of the barley									
Parameters	Amino acid								
	Thr	Cys	Val	Met	Ile	Tyr	Lys	Glu	Pro
Regression coefficient	0.0244	0.0149	0.0378	0.0080	0.0194	0.0259	0.0205	0.3145	0.1717
Regression constant	0.1176	-0.0084	0.0480	0.0453	0.1511	0.1787	0.1780	-0.6441	-0.5896
Correlation coefficient	0.5907	0.2895	0.4849	0.0970	0.4541	0.6747	0.2835	0.8432	0.7376
Probability level(P)	0.1%	5%	0.1%	-	0.1%	0.1%	5%	0.1%	0.1%
1/2 Crude protein and amino acid composition of the barley protein									
Regression coefficient	-0.0993	0.0044	-0.0514	-0.0182	-0.1068	-0.0517	-0.1410	0.7209	0.5096
Regression constant	4.7560	1.4173	4.9828	1.4431	4.5821	3.9513	5.3374	17.7746	6.3033
Correlation coefficient	0.2158	0.00054	0.0632	0.0067	0.2681	0.1165	0.2276	0.3906	0.2510
Probability level(P)	10%		-	-	5%	-	10%	1%	5%

In the case of the barley for Thr, Val, Ile, Tyr, Glu and Pro at $P = 0.1\%$ level, for Cys, Lys at $P = 5\%$ level, whereas for Met even at $P = 10\%$ level, there is no positive correlation between the crude protein content and amino acid composition. Between the crude protein content of the barley and the amino acid composition of the barley protein for Glu at $P = 1\%$, for Pro at $P = 5\%$ level, there is a positive correlation, while for Ile at $P = 5\%$, for Lys and Thr at $P = 10\%$ level, a negative correlation. On the basis of the regression coefficient value, it appears that with increasing crude protein content – in a significantly not provable way – beyond the above, decreases Val, Met and Tyr content of the barley protein.

Table 2: Amino acid composition of the barley as a function of the crude protein content (gram amino acid/100 gram barley)

Crude	Amino acid								
protein %	Thr	Cys	Val	Met	Ile	Tyr	Lys	Glu	Pro
7.0	0.289	0.097	0.313	0.102	0.287	0.260	0.324	1.557	0.612
7.5	0.301	0.105	0.332	0.106	0.297	0.273	0.334	1.715	0.698
8.0	0.313	0.112	0.351	0.110	0.307	0.286	0.344	1.872	0.784
8.5	0.325	0.120	0.370	0.114	0.316	0.299	0.355	2.029	0.780
9.0	0.337	0.127	0.388	0.118	0.326	0.312	0.365	2.186	0.956
9.5	0.350	0.135	0.407	0.122	0.336	0.325	0.375	2.344	1.041
10.0	0.362	0.142	0.426	0.126	0.345	0.338	0.385	2.501	1.127
10.5	0.374	0.150	0.445	0.130	0.355	0.351	0.395	2.658	1.213
11.0	0.386	0.157	0.464	0.134	0.365	0.364	0.406	2.815	1.299
11.5	0.398	0.165	0.483	0.138	0.375	0.377	0.416	2.973	1.385
12.0	0.411	0.172	0502	0.142	0.384	0.390	0.426	3.130	1.471
12.5	0.423	0.179	0.521	0.146	0.394	0.402	0.436	3.287	1.557
13.0	0.435	0.187	0.540	0.150	0.404	0.415	0.446	3.444	1.642
13.5	0.447	0.194	0.559	0.154	0.413	0.428	0.457	3.601	1.728
14.0	0.460	0.202	0.578	0.158	0.423	0.441	0.467	3.759	1.814
14.5	0.472	0.209	0.597	0.162	0.433	0.454	0.477	3.916	1.900
15.0	0.484	0.217	0.615	0.166	0.442	0.467	0.487	4.073	1.986
15.5	0.496	0.224	0.634	0.170	0.452	0.480	0.498	4.231	2.071
16.0	0.508	0.232	0.653	0.174	0.462	0.493	0.508	4.388	2.157

Summarizing the results obtained for the other examined materials, it can be said that the percentage amino acid composition of the feeding stuff, that is, the amount of amino acids present in 100 g feeding stuff raw material, can be estimated on the basis of the crude protein content with appropriate accuracy for most of the examined amino acids. Reliability of the estimation is the highest for glutamic acid, for tyrosine (at $P = 0.1\%$ reliability level),

followed by valine, threonine and lysine ($P = 1\%$), and cystine ($P = 5\%$), isoleucine ($P = 10\%$), and finally by methionine, where no estimation can be carried out even at a $P = 10\%$ level. It can be clearly stated that, on the basis of crude protein content, in the case of most of the feeding stuff raw materials, all amino acids, which are important regarding feeding – with the exception of methionine –, can be estimated with an accuracy necessary for the production of feeding stuff mixes.

The above statement is not true to amino acids present in the feeding stuff protein. On the basis of the crude protein content, only glutamic acid and lysine content of the feeding stuff protein can be estimated at a $P = 10\%$ probability level; the amount of the rest of the amino acids cannot be estimated.

Based on the regression coefficient, we established that in the feeding stuff each examined amino acid increases along with the crude protein content. Examining the amount of the amino acids in the percentage of the protein, it can be established that glutamic acid and proline increased in the function of the crude protein content in the case of all the other feeding stuff raw materials, while the other amino acids – in most cases – decreased. It can be concluded that with increasing crude protein content the amount of non-essential amino acids increases and that of essential amino acids decreases in the feeding stuff protein.

Evaluating our results in the light of the literature, we come to the following conclusions: similarly to the results of *Vincze* & *Szüts* (1978), we experienced the increase of the Glu and Pro content of the maize protein and the decrease of its Lys content with increasing crude protein content. In the case of wheat, we established the increase of all the amino acids with increasing crude protein content in contrast with the above authors, who obtained maximums and minimums due to different nitrogen doses.

Similarly to the conclusion of *Jahn-Deesbach* & *Schipper* (1982), we obtained a rapid increase of Glu and Pro in the case of the barley with increasing crude protein content. Similar results were obtained regarding the change of the amino acid composition of the barley protein. Glu and Pro increased, while the other amino acids did not change considerably, or decreased due to increasing crude protein content in the barley protein.

Németh (1983) observed the decrease of Lys and Met content of the wheat protein, as well as the amount of most of the amino acids of the maize protein due to a nitrogen dose. In our experiments, the Lys content of the maize protein decreased, while the Met content increased with increasing crude protein content.

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Determination of seleno-amino acids by ion-exchange column chromatography and high-performance liquid chromatography (Preliminary study)

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Abstract. The biological role of selenium was discovered in the second half of the last century. It was established that selenium was a part of the antioxidant system of the organism, and it protected the organism from the attack of the harmful free radicals. The researches made it clear that the organically bound selenium, like selenomethionine (SeMet) and selenocysteine (SeCys), had an especially important role. It is very important for experts dealing with both food science and feeding stuffs to determine the concentration of these two substances in foods and feeding stuffs. We have no information on whether the enantiomers of these two seleno-amino acids have ever been examined before by anyone else; therefore, we have elaborated new methods and amended old ones, respectively, for the exact determination of these compounds by

Keywords and phrases: selenomethionine, selenocysteine, determination of seleno-amino acids, ion-exchange column chromatography, IEC, high-performance liquid chromatography, HPLC.

ion-exchange column chromatography (IEC) and high-performance liquid chromatography (HPLC). In the first step, we carried out the separation of the seleno-amino acids along with protein-components amino acids by ion-exchange column chromatography. In the next step, we attempted the separation of the oxidized derivatives of these amino acids by IEC, utilizing our experiences gained during the determination of methionine (Met) and cysteine (Cys) in oxidized form. Afterwards, we tried to increase the sensibility by applying HPLC after precolumn derivatization with OPA/2-mercaptoethanol and OPA/2-mercaptoethane sulphonic acid, respectively, as the seleno-amino acids are contained in the biological samples, in a several orders of magnitude lower concentration as compared to the protein-component amino acids. For the separation of the seleno-amino acid enantiomers, the OPA/TATG derivatization method was used, which we applied for amino acid enantiomers beforehand, but derivatization with 1-(9-fluorenyl)-ethyl chloroformate (FLEC) was also attempted.

1 Introduction

About 1930, selenium was still considered a toxic element; however, by 1943, it had already been proven that its presence in the living organism is essential and it reduces the number of cases of malignant tumours (*Nelson et al.*, 1943; *Clayton* & *Bauman*, 1949; *Schwarz* & *Foltz*, 1957), while in 1966 the anticarcinogenic effect of selenium was reported (*Shamberger* & *Rudolp*h, 1966), but this time the total selenium content of the foodstuff was mentioned. In recent times, however, with the improved sensibility of the analytical methods, its important physiological role was established, that is, as an antioxidant along with the tocopherols, selenium participated in the metabolism, helped with the cure of certain cancers, in fact, even with the prevention, and helped maintain the healthy condition of the cell membranes. The selenium-containing glutathione peroxidase protects the unsaturated lipids by catalysing the peroxide decomposing reaction.

Foodstuffs produced in Scandinavia and in some other countries of Europe are extremely deficient in selenium. The amount of selenium getting into the human organism during daily meals (0.05-0.1 mg) is not considerable. The Hungarian soils are also extremely poor in selenium; therefore, it is not possible to satisfy the selenium needs of the organism with foodstuffs of vegetable origin. The selenium supplementation of foodstuffs is almost indispensable according to modern nutrition science. The researchers have also discovered that it is not enough to know only the total selenium content of the foodstuffs,

but it would be also necessary to know in what chemical form selenium is present in the foodstuffs, since the different chemical forms substantially differ from each other in toxicity, absorption ability and utilization in the human organism. Despite this, there is only a few number of studies in which the different selenium forms were examined.

The selenium content of the plants is determined mainly by the soil (*Terry et al.*, 2000), more exactly by the selenium content uptakeable from the soil, but not by the total selenium content. Selenide and elemental selenium (Se^{2-}, Se) can hardly be taken up, while the absorption of selenite and selenate (Se^{4-}, Se^{6-}) is significantly better. Practically, selenate can be fully absorbed, but a great portion of it empties with the urine before the incorporation into the protein, while only around 50% of the selenite can be absorbed, but the absorbed amount is better utilized. Beside the inorganic selenium compounds, there are seleno-amino acids or their derivatives present in the plants in substantial amounts. Foodstuffs of vegetable origin contain selenomethionine, whereas those of animal origin contain selenomethionine and selenocysteine. Selenomethionine is formed in the plants from the selenium content of the soil, and the animals can convert it to selenocystine. Selenomethionine can transform into the active form in the organism in around 90%, which is almost 100% (*Food and Nutrition Board*, 2000; *Dumont et al.*, 2004). In foodstuffs for human consumption, selenium is present mainly in the form of selenite and selenomethionine.

2 Materials and methods

Materials used:

DL-SeMet and L-SeCys2 seleno-amino acid standards, DL-cysteic acid standard, *o*-phthaldialdehyde (OPA), 1-thio-β-D-glucose tetra acetate (TATG), 2-mercaptoethanol, 2-mercapto-ethane sulphonic acid, p-toluene sulphonic acid, acetonitrile, sodium acetate, acetonitrile, methanol and ethanol was purchased from Sigma Aldrich Company.

Examinations performed:

I. Determination of seleno-amino acids by ion-exchange column chromatography (IEC)

The following experiments were carried out using an Ingos AAA 400 amino acid analyser:

Separation of SeMet and SeCys2 (selenocystine) standards.

Examination of the oxidation of SeMet and SeCys2.

Examination of the effect of hydrolysis performed with various hydrolysis acids.

Examination of selenic yeast nutritional supplement products.

II. Determination of seleno-amino acids by high-performance liquid chromatography

Determination after precolumn derivatization with OPA/2-mercaptoethanol.

Determination of SeCys2 in oxidized form after performic acid oxidation by high-performance liquid chromatography.

The derivatization and analysis were carried out with a MERCK-Hitachi HPLC containing the following modules: L-7250 programmable autosampler, L-7100 pump, L-7350 column thermostat, L-7480 fluorescence detector, AIA data conversion utility for D-7000 HPLC system manager (MERCK, Darmstadt, Germany). The separation was performed with a Superspher 60 RP-8e column or with a Purospher RP-18e 125 × 4 mm column; the temperature of the oven was 40 °C. The derivatives were detected with a fluorescence detector (ex.: 325 nm, em.: 420 nm).

3 Results and discussion

I. Determination of seleno-amino acids by ion-exchange column chromatography

1. Separation of SeMet and SeCys2 (selenocystine)

During the chromatographic run of the seleno-amino acid standards, using the normal chromatographic programme for protein hydrolysates, SeCys2 eluted between Val and Met, and SeMet eluted between Ile and Leu. We also attained a better separation of SeMet from Leu, which interfered under the normal conditions.

2. Examination of the oxidation of SeMet and SeCys2

As known, Met and Cys are determined in their stable oxidized forms: methionine-sulphone and cysteic acid. Similarly, we wanted to investigate

the behaviour of SeMet and SeCys2 against oxidizing effects. Prior to the chromatographic run, SeMet and SeCys2 were oxidized by performic acid using different temperature/duration combinations (50 °C/15 min; 30 °C/5 min and 0 °C/2 hrs). Results show that the seleno-amino acids are rather sensitive to oxidative effects, with SeCys2 found to be more sensitive than with SeMet. This latter underwent partial decomposition under the condition of 50 °C/15 min, giving – beside the peak of the rest of the starting material – two other peaks and a higher amount of ammonia on the chromatogram. Oxidation under milder conditions (30 °C/5 min and 0 °C/2 hrs, respectively) led to only a little deterioration of SeMet. Rigorous oxidation of SeCys2 (50 °C/15 min) resulted in complete decomposition with the appearance of only an ammonia peak on the chromatogram. Due to oxidation of SeCys2 under milder conditions (30 °C/5 min and 0 °C/2 hrs, respectively), a definite peak appeared at the beginning of the chromatogram (practically where normally cysteic acid elutes), which was presumably selenocysteic acid, the selenium analogue of cysteic acid.

3. Examination of the effect of hydrolysis performed with various hydrolysis acids

As biological samples are usually subjected to hydrolysis before an analysis, we attempted to find out how different hydrolysis agents affected the seleno-amino acids. Three hydrolysis acids were applied to the seleno-amino acid standards.

Hydrolysis with *hydrochloric acid*: this, for the proteins normally used hydrolysis method (6 M HCl, at 110 °C for 24 hrs), led to complete deterioration of both seleno-amino acids.

Hydrolysis performed with 2-*mercaptoethane sulphonic acid* (3M aqueous solution, at 110 °C for 24 hrs): this method resulted in the appearance of a peak of the hydrolysis acid at the beginning of the chromatogram in both cases, with complete deterioration of SeCys2, while SeMet remained intact.

Hydrolysis performed with *p-toluene sulphonic acid* (3M aqueous solution, at 110 °C for 24 hrs): both SeMet and SeCys2 remained practically intact, only the latter deteriorated a little.

4. Examination of selenic yeast nutritional supplement products

Two selenic yeast products – both containing selenium in the form of SeMet – were analysed subsequent to a hydrolysis with *p-toluene sulphonic acid*. It was experienced, however, that beside the big peaks of the amino acids present

in the samples, the presence of SeMet in small concentration could not be easily evaluated. Separation has to be further enhanced.

II. Determination of seleno-amino acids by high-performance liquid chromatography

The following examinations have been carried out so far:

1. *Determination after precolumn derivatization with OPA/2-mercapto ethanol*

Chromatographic run of the SeMet and SeCys2 standards was carried out after derivatization with OPA/2-mercaptoethanol. The measurement was performed on a 250×4-mm-sized C18 column, with a three-component eluent mixture (sodium acetate buffer/acetonitrile/methanol). Only SeMet appeared on the chromatogram, while SeCys2 did not form a measurable derivative with the reagents which can be measured by fluorescence detector. Detection in the UV-range was not possible due to the small sensibility.

2. *Determination of SeCys2 in oxidized form after performic acid oxidation by high-performance liquid chromatography*

Chromatographic run of the oxidized product of L-SeCys2 (30 °C/5 min and 0 °C/2 hrs) – which is presumably selenocysteic acid – was carried out along with DL-cysteic acid standard, after derivatization with OPA/TATG. L-selenocysteic acid appeared at the beginning of the chromatogram, having eluted after 4 min. The enantiomers of cysteic acid were separated and eluted later, after 17-18 min. Further investigations by HPLC are in process, which can be reported at a later stage.

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The impact of different fatty acids and their thermal preparation method on atherosclerosis. A review

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Abstract. Atherosclerosis is a multifactorial disease where both oxidative and inflammatory processes play a key role in its development. Lipoproteins facilitate the entering of the lipids into the blood stream and carry them to their destination in the body. Because of the finite number of lipoprotein receptors, the excess amount of lipids enters into the circulatory system; they may become subject to oxidative processes. Fatty acids influence not only the lipoprotein secretion but even the cholesterol efflux and its homeostasis. Compared to the food production processes used in the food industry, domestic preparation methods of lipids do not significantly influence the properties of fatty acids, they do not contribute to the extra formation of trans-fatty acids, but they expose the fatty acids to oxidation. Characterizing the fatty acids according to their beneficial effect on health, it can be concluded that poly-unsaturated fatty acids have proven to be the most beneficial, followed by mono-unsaturated and saturated fatty acids, while the consumption of trans-fatty acids should be avoided as much as possible in order to eliminate a possible source of the development of atherosclerosis.

Keywords and phrases: atherosclerosis, lipoproteins, fatty acids, PUFA, trans-fatty acids.

1 Atherosclerosis

Atherosclerosis is considered to be the inflammation of the arteries. The inflammation is initiated by the entrance of lipids into the vascular system (*Toh et al.*, 2014).

The American Heart Association defines this disease as follows: fatty substances, cholesterol, cellular waste products, calcium and fibrin form an interior lining in the arteries. The accumulation of these substances is called arterial plaque (http://www.heart.org/HEARTORG/Conditions/Cholesterol/WhyCholesterolMatters/Atherosclerosis_UCM_305564_Article.jsp#).

The accumulation of lipids in the lesions of blood vessels eventually leads to the occlusion of these vessels. Both the oxidative and inflammatory processes play a role in the development of this multifactorial disease. Environmental factors, such as nutrition as well as genetic factors, play an important role in the modulation of inflammatory and oxidative state of the components that influence the development of the disease. Although some risk factors are already known to researchers, the molecular characteristics of the development and progression of this disease are still undiscovered (*Ortega et al.*, 2012). However, it is already known that lipids have an important role both in the development and in the progression of atherosclerosis.

2 Lipids

The term lipid is the generic name of a group of hydrophobic molecules, which includes fats, waxes, sterols (e.g. cholesterol), fat-soluble vitamins (such as vitamin A, D, E and K), mono-glycerides, di-glycerides, tri-glycerides or phospholipids. Lipids are not only a rich source of energy, but they also have an important role in intracellular signalling, regulating hormones, in the crossing of membranes and blood clotting (*Saba* & *Oridupa*, 2012).

From the point of view of atherosclerosis, the most important components to consider are the fats and the sterols.

2.1 Fats

As a subgroup of lipids, fats are not soluble in water. We distinguish between two types of fats, the liquid fats and the solid fats. Liquid fats are called oils, and the term fat remains the name of the solid fats. The difference between the physical state of these fats is due to the differences of the constituent components of the fat, the fatty acids. Each type of fatty acid has a different melting point.

2.2 Fatty Acids

Fatty acids are hydrocarbon structures, formed of at least three methyl groups attached to a carboxylic acid. Their physical and chemical properties (e.g. solubility in non-polar solvents, melting point) change according to the number of methyl groups (the number of carbon atoms) in the molecule. The greater the number of carbon atoms in the fatty acid chain, the higher the melting temperature will be. Depending on the number of carbon atoms, we further distinguish among short-chain fatty acids (from C4 to C10), medium-chain fatty acids (C12 to C14), long-chain fatty acids (C16 and C18), and very-long-chain fatty acids (C20 or more). Carbon atoms can be linked through a single bond; in this case, we are talking about saturated bond or double bonds, which are the unsaturated bonds. Fatty acids containing no double bonds are called saturated fatty acids, and those containing at least one double bond (and up to 6 double bonds) are called unsaturated fatty acids. Depending on the number of double bonds in the chain, we may talk about mono-unsaturated fatty acids (MUFA) containing a single double bond and poly-unsaturated fatty acids (PUFA) containing more than one double bond. The melting temperature is also influenced by the number of double bonds in the chain, and this way the unsaturated fatty acids have a lower melting point as saturated fatty acids, though containing the same number of carbon atoms. There are two essential fatty acids in the human body. These are the linoleic acid (C18:2ω-6) and the α-linolenic acid (C18:3ω-3). Another important fatty acid, the arachidonic acid (fulfilling important roles in cell signalling), becomes also essential in the absence of linoleic acid. Depending on the structure of unsaturated fatty acids, we distinguish between cis and trans isoforms. In the cis isoform, the hydrogen atoms linked by the carbon atoms involved in the double bond are positioned on the same side of the linkage, while in the case of the trans isomers, the hydrogen atoms are situated opposite one another. The main difference between these isomers is to be found in their melting temperatures. Thus, not only the biochemical behaviour of the fatty acid changes, but also its value as a nutrient (*Valenzuela* & *Valenzuela*, 2012).

3 Atherosclerosis and lipoproteins

The initial step in the development of atherosclerosis is the accumulation of lipoproteins containing apolipoprotein B in the endothelium. ApoB lipoproteins are composed from cholesterol esters and triglycerides (tri-acyl-glycerides), surrounded by a layer of phospholipids and proteins (*Ortega et al.*, 2012).

Lipoproteins are formed to facilitate the circulation of lipids (in the form of triglycerides) in the blood (thus becoming soluble in aqueous solutions). The proteins that bind to the lipids carry them to their destination in the human body. Lipoproteins are characterized based on their density. There are high-density lipoproteins (HDL), low-density lipoproteins (LDL), intermediate-density lipoproteins (IDL), very-low-density lipoproteins (VLDL) and chylomicrons (CM). Each group has different functions, but only the high-density lipoproteins are beneficial from the point of view of preventing the development of atherosclerosis (*Saba* & *Oridupa*, 2012).

High LDL level decreases, while low HDL level raises the risk of the development of cardiovascular diseases. In addition to various factors, such as smoking, hypertension, high serum cholesterol level or diabetes, high LDL cholesterol and low HDL cholesterol have an important role in the development of atherosclerosis. Another function of lipoproteins is the transport of cholesterol. Cholesterol enters the cell via receptor-mediated endocytosis. This receptor recognizes the LDL molecule, which transports the cholesterol. The number of LDL receptors is a finite number. In the case of a deregulation of cholesterol synthesis in the body, the automatically increased formation of lipoproteins that will carry the formed cholesterol will cause an excess in circulating lipoproteins, which cannot be recognized by the already blocked LDL receptors. This excess LDL is prone to oxidization and will be recognized as waste by the macrophages, which will soon form the so-called foam cells, which remain blocked in the arteries, leading to inflammation (*Saba* & *Oridupa*, 2012).

4 Fatty acids regulate the lipoprotein secretion

The risk of developing atherosclerosis is highly influenced by the lipoproteins secreted by the body. Various factors influence the rate of secreted lipoproteins (e.g. disturbances in the production of cholesterol).

4.1 Poly-unsaturated fatty acids

Carluccio et al. demonstrated that the balanced rate of mono-unsaturated fatty acid and poly-unsaturated fatty acids may have a regulatory effect on lipoprotein secretion, increasing HDL cholesterol and lowering LDL cholesterol (*Carluccio et al.*, 2007).

Another study effectuated by Machado et al. also demonstrated the role of poly-unsaturated fatty acids (PUFA) in preventing the development of

atherosclerosis. Mice, on which the study was carried out, had the genes responsible for the production of LDL receptors deleted. Because of the excess LDL cholesterol formed, they had to develop atherosclerosis. When feeding them a diet rich in PUFA (ω-6), the concentration of the total cholesterol in the aortic root and that of the total triglycerides was lower than when the mice were fed trans-fatty acids or saturated fatty acids. After measuring other factors involved in the development of atherosclerosis, the research team ultimately could conclude that a diet rich in PUFA prevented the development of atherosclerosis in the case of the mice analysed, even in pro-inflammatory conditions (*Machado et al.*, 2012).

4.2 Trans-fatty acids

Trans-fatty acids are the isomers of unsaturated fatty acids with cis geometry. The trans isomer form does rarely occur in nature, but it is formed under different conditions during the processing of food in the food industry. The only known natural source of trans-fatty acids are the conjugated acids. They are formed by bacteria in the rumen of the ruminants. The acids formed enter into the meat through the circulation, making it the only natural source of trans-unsaturated fatty acids.

The main industrial procedure by which trans-fatty acids are formed is the hydrogenation of unsaturated fatty acids. This procedure is used to produce saturated or partially saturated fatty acids from unsaturated fatty acids, thus obtaining a fat with a melting temperature higher than the initial one. In this way, spreadable fats, such as margarine or others made to replace butter, can be obtained from vegetable oils.

During the process of hydrogenation of unsaturated fatty acids, unsaturated trans-fatty acids are secondary – also referred to as contaminant – products brought into the product by the side reactions of the isomerization catalyst.

It is believed that elevated temperatures, such as temperatures reached while toasting or baking (200 °C), could also result in the formation of trans-fatty acids. But recent studies show that even temperatures reached during cooking in the kitchen, or even higher temperatures, do not lead to significant amount of isomerization of unsaturated fatty acids into trans-fatty acids. A more important problem to consider in this case would be the oxidation of unsaturated fatty acids (*Przybylski* & *Aladedunye*, 2012).

Epidemiological evidence associates trans-fatty acids (TFA) with heart disease. It is believed that the trans-fatty acids stimulate the development of atherosclerosis but have no additional effect when consuming high amounts of

dietary cholesterol, which is considered to be even more atherogenic (*Chantal*, 2009).

A recent study demonstrates similar detrimental effect of unsaturated trans-fatty acids on health as well, analysing it on the molecular level. In addition to the fact that unsaturated fatty acids (UFA) have the ability to prevent the harmful effects of diet consisting only of saturated fatty acids, which have as a result a reduced cholesterol efflux, it was demonstrated that unsaturated trans-fatty acids increase the level of cholesterol in macrophages, reducing the expression of the gene responsible for the production of the particular protein which regulates the cholesterol efflux and its homeostasis (ABCA1 gene protein CERP) (*Milessa da Silva et al.*, 2013).

Kummerow et al. also showed that the consumption of unsaturated trans-fatty acids disturbed the metabolism of essential fatty acids of 30 pigs analysed, thereby leading to a change of aortic fatty acid composition, which is considered to be the most vulnerable tissue in terms of the development of atherosclerosis (*Kummerow et al.*, 2007).

Mozaffarian et al. describe the unsaturated trans-fatty acids to be harmful because of the following facts: the consumption of only unsaturated trans-fatty acids in place of unsaturated fats, or even instead of saturated fats, increases the level of LDL cholesterol and decreases the level of HDL cholesterol, while it also increases the level of triglycerides in the blood, promotes inflammation (increasing the activity of the tumour necrosis factor system, TNF) and leads to endothelial dysfunction (*Mozaffarian et al.*, 2006).

5 Conclusions

In addition to their role as an energy source, fats have an increased importance in the health of the human body. Depending on their physico-chemical structure, the components of fats, such as fatty acids, can have both beneficial and harmful effects. Categorizing fatty acids according to their harmful effects on health and their impact on atherosclerosis, it can be concluded that unsaturated trans-fatty acids is a category of fatty acids which needs to be avoided as much as possible. Being derived as by-products of industrial processes, the largest amount of consumed trans-fats is not part of a natural diet. On the other hand, we do not know exactly if even the naturally occurring conjugated fatty acids do have a beneficial effect on the body, although many studies try to answer this question. The second category indicated to be consumed in reduced quantities is the category of saturated fatty acids. Because

these fatty acids occur naturally in many of our foods too, they should not be avoided completely, but they should be consumed in moderate amounts to avoid the potential adverse consequences from the point of view of the development of atherosclerosis. On the other hand, poly-unsaturated fatty acids show interesting and beneficial effects in preventing atherosclerosis.

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