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# Lipids from rapeseed: composition and antilipolytic activity

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*The rapeseeds (Brassica napus var. oleifera) display inhibitory activity against pancreatic lipase. In this paper, we present results on the "Galitsky" cultivar rapeseed. Content of non-polar mono- and triglycerides is exceeds 90 % and these components have no inhibitory effect. It has been found that content of phospholipids in bound lipids is 24,9 % and content of unsaponifiables is 29,5 %. The inhibitory effect of phospholipids was 88 U/g, while other components have no the inhibitory effect against lipase.*

The enzyme inhibitors were until recently considered as antialimentary factors and were eliminated from food substances. However, new principle tendency shows the area of new food supplements elaboration. This tendency consists in inclusion of inhibitors into the food supplements for correction of enzyme activity. It is well known that inhibitors are used in medicine. The essence of many medicine effects consist in various enzymatic systems' inhibition.

Researches' attention is focused on search of compounds influencing enzymes or receptors which functions are affected. The abnormality of these enzymes and receptors result in many lesions including "civilization" diseases such as obesity, diabetes, cardiovascular diseases etc. The origin of these diseases interrelated to carbohydrate and lipid metabolic disorders in organism.

The use of inhibitors is necessary in case of pathological processes of hydrolytic enzymes hyperactivity in organism and inability of natural inhibitors system to provide adequate control of hydrolytic processes. Such pathologies can appear in pancreas, blood, etc. [1, 2, and 3]. Process of hydrolytic digestion of edible fat can be reduced with use of lipolytic enzymes inhibitors.

Plants are the most important source of different hydrolytic enzymes inhibitors including lipase. As well, high activity of species of legumes, cruciferous, papaveracious families against lipases there was detected. It is necessary to note, that inhibitory activity have only seeds of these plants. By their chemical nature, lipase inhibitors can be proteins, lipids, or polyphenolic compounds.

Our previous investigations had shown that lipids of the "Galitsky" cultivar rapeseeds had an inhibitory activity against lipolytic enzymes [4].

The main goal of our researches was the isolation of lipid components, determination of their chemical composition, fractionation and measurement of the antilipolytic activity of individual fractions.

## MATERIALS AND METHODS

As research subject, the rapeseed (Brassica napus var. oleifera) "Galitsky" cultivar was chosen.

Quantitative determination of various lipid groups. Various lipid fractions were exhaustively extracted from rapeseed using a Soxhlet apparatus with several solvents [5]. The free lipids were extracted with light petroleum and bound lipids were extracted from residue with mixture of chloroform and ethanol (2:1, vol/vol). Strongly bound lipids were extracted with light petroleum after acidic or alkaline hydrolysis. After extractions, the solvents were evaporated near to dryness under vacuum at 40 °C. The residues after extractions were weighed. The quantities of lipids were determined by difference between dry substances weight before and after extraction.

The compositions of free and bound lipids were determined using TLC on Sorbfil plates coated with silica gel STH–1A (granulation 5–17 $\mu$m, layer thickness 110 $\mu$m) [6]. The chromatograms were developed applying various systems of mobile phases in a chromatographic chamber (12 x 10 x 8 cm) to the moment when solvent front moved by 8 cm. The free lipids were developed applying mixture light petroleum–diethyl ether–acetic acid (80:20:1, by volume), the bound lipids were developed applying mixtures chloroform–methanol–water (65:25:4, by volume) or chloroform–methanol–acetone–acetic acid–water (50:20:10:10:5, by volume). 2...6 ml of 0,1% sample solution in chloroform was applied on a plate. Fraction compounds' were identified by standard and Rf values of compounds.

Fatty acids composition of lipids were defined using HPLC. The free lipids' fraction was evaporated until dry in a rotor evaporator. The phospholipids were collected from TLC–plates and dissolved in benzene to lipid concentration 10 mg/ml. 1 ml of benzene solution of lipid was dissolved in 10 ml acidified methanol (4 ml HCl in 100 ml methanol) and this solution was scaled up in ampoule. The ampoule content was methylated in a water–bath at 90–95 °C during 1 h. Then ampoule content was evaporated up to dryness and dissolved in 0,5 ml of hexane. The HPLC analysis was performed by the outlined method of M.Kates [6] using a Model 5890 II (Hewlett-Packard) diode array detector. The capillary column has following dimensions: 12,5 m x 0,32 mm, 0.52 µm; autosampler HP 7673. The flow rate was 1 ml/min, the column temperature started from 60 °C with growth 25 °C/min to 240 °C. Gas-carrier was helium, 45 kPa, 60 ml/min. The sample injection volume was 200 $\mu$l. The standard fatty acids were used as standard compounds.

The quantitative and qualitative analysis of phospholipids were conducted using one-dimensional and two-dimensional TLC–chromatography on Sorbfil plates coated with silica gel STH–1A (granulation 5–17 $\mu$m, layer thickness 110 $\mu$m) [6]. The chromatograms were developed applying various system of mobile phases in a chromatographic chamber (12 x 10 x 8 cm) to the moment when solvent front moved by 8 cm. The phospholipids were developed applying mixtures chloroform–methanol–water (65:25:4, by volume) and chloroform–methanol–acetone–acetic acid–water (50:20:10:10:5, by volume). 2...6 ml of 0,1% sample solution of chloroform was applied on a plate. The fraction compounds' were identified by the standard and Rf values of compounds. The quantitative analysis was performed by Resental method [8].

Antilipolytic activity of seeds elements was determined by the ability of analyzed components to inhibit the lipase activity [9]. There were added 8–15 mg of sample in 1 ml of water, 0,1 cm3 of pancreatine (0,1%), 0,4 cm3 of tris–HCl buffer (0,2M, pH 8,0). The temperature was controlled during 5 min at 37 °C. Then 1,2 cm3 of olive oil emulsion was added. Precisely after one hour, reaction was interrupted by addition of 1 cm3 of ethanol (96 %). In controlled samples the olive oil emulsion was added after the ethanol addition. The components were titrated by 0,05 M NaOH up to light blue colour. Thymolphthalein was used as indicator. Lipase activity was expressed in standard units by the difference of

NaOH volume for titration components and control samples spent after the olive oil emulsion hydrolysis.

## RESULTS AND DISCUSSION

Conception 'lipids' unites different in chemical sense substances. Following are the most important groups of substances fats, phosphatides, waxes, sterols, carotenoids, carbohydrates, fatty acids, lipid-soluble vitamins, dyestuffs, cerebrosides. Therefore, there are many different substances among lipids and because of that the classification of them is difficult. Usually lipids are classified as free, bound and strongly bound [6, 10].

*These groups of lipids were isolated from rapeseed. Theirs quantity and antilipolytic* activity are presented in table 1.

Table 1

Antilipolytic activity of lipid groups of 'Galitsky' rapeseed

| Lipid class | Content, % solid | Antilipolytic activity (ALA), U/g |
|---|---|---|
| Free | 45,60 | 60 |
| Bound | 4,30 | 65 |
| Strongly bound | 0,86 | – |
| Total | 50,76 | |

By our results we displayed that antienzyme activity concerning lipase have free (41 %) and bound (44 %) lipids. Strongly bound lipids did not inhibit lipase and were excluded from our subsequent analysis. In addition, analysis displayed that the main part of lipids were free lipids, i.e. mono–, bi–, triglycerides, sterols, carbohydrates. Inasmuch as free and bound lipids inhibit lipase then appropriately determined free and bound lipids compositions for an inhibitory component uncovering. They were fractionated using TLC–method.

The results are presented in table 2.

Table 2

Fractional composition of free and bound lipids of rapeseed

| Components of fractions | Content, % of free lipids | Content, % of bound lipids |
|---|---|---|
| Monoglycerides | 1,2 | 0,6 |
| Diglycerides | — | — |
| Chlorophyll | 4,5 | 10,6 |
| Carotenoids | — | 0,06 |
| Free fatty acids | 0,14 | 17,2 |
| Triglycerides | 86,90 | 14,03 |
| Phospholipids | 0,48 | 24,88 |
| Polar lipids | 1,4 | 3,1 |
| Unsaponifiables* | 2,63 | 29,53 |
| Unidentified | 2,75 | — |

* — sterols, carbohydrates, triterpene alcohols, aliphatic alcohols, squalen.

The free lipids were mainly presented by triglycerides. Minor part of polar lipids and phospholipids belonged to free lipids too. The bound lipids contain large amount of phospholipids and unsaponifiables. Therefore, predominant fractions' constituent compositions in free and bound lipids were determined.

Inasmuch as the main component of free lipids were triglycerides and minor content of free fatty acids and monoglycerides, fatty acid content was determined using HPLC–method (table 3).

Table 3

Content of fatty acids in rapeseed lipids

| Name of acid | Formula | Content, % |
|---|---|---|
| Palmitic | $C_{16:0=}$ | 6,22 |
| Palmitooleinic | $C_{16:1=}$ | traces |
| Stearic | $C_{18:0=}$ | 0,52 |
| Oleinic | $C_{18:1=}$ | 51,34 |
| Linoleic | $C_{18:2=}$ | 28,43 |
| Linolenic | $C_{18:3=}$ | 13,46 |
| Behenic | $C_{22:0=}$ | traces |
| Erucic | $C_{22:1=}$ | 0,00 |
| Arachidonic | $C_{20:4=}$ | traces |

In analyzed compounds were identified and quantitatively determined line of fatty acids. The main part of rapeseed glycerides consisted of oleinic, linoleic, linolenic acids. The content of saturated acids was palmitic and stearic acids (contained in negligible amounts). Others were found as signs. Erucic acid that is hazardous to human health was not detected.

Antilipolytic activity was analyzed among individual lipid fractions too. It was specified that the inhibitory activity concerning lipase have phospholipids only. The inhibitory activity of phospholipids formed 88 U/g. Other components have not the inhibitory effect on lipase. The results of phospholipids fractioning from free and bound lipids have shown their compositions identity.

The composition and antilipolytic activity of phospholipids are presented in table 4.

Table 4

Composition and antilipolytic activity of phospholipids

| Fractions | Content, % | Antilipolytic activity, U/g |
|---|---|---|
| Phosphatidylcholine | 29,8 | 105 |
| Phosphatidylethanolamine | 18,2 | 53 |
| Phosphatidylinositol | 19,1 | 42 |
| Lysophosphatidylcholine | 3,1 | 33 |
| Lysohosphatidylethanolamine | 1,6 | 12 |
| Phosphatidic acids | 2,0 | – |
| Phytoglycolipids | 9,0 | – |
| Mono- and digalactosylglycerides | 6,3 | – |
| Unidentified | 10,9 | – |

Thus, the phosphatidylcholine (the most active), the phosphatidylethanolamine, their lysoforms and the phosphatidylinositol inhibited lipase. Obtained data agreed with published ones. Inasmuch as content of phospholipids in free lipids was the minimum they have not significant influence on the antilipolytic activity of the complex of free lipids. The main component of the free lipids that were triglycerides which did not have the inhibitory activity concerning lipase. It is possible that antilipolytic activity was conditioned by mutual synergetic influence of individual fractions. This influence predetermined such behaviour for the free lipids fraction.

Therefore, phospholipids were the crucial component in the inhibitory activity of rapeseed lipids. Moreover, phospholipids were the main source of unsaturated and polyunsaturated fatty acids [11]. Totally, fatty acid composition of the rapeseed phospholipids as well as fatty acid composition of the individual fractions were analyzed by the HPLC method (tables 5, 6 respectively).

Table 5

The fatty–acid composition of the rapeseed phospholipids

| Fatty acid | Content, % |
|---|---|
| Palmitic | 9.7 |
| Stearic | 3.8 |
| Total saturated acids | 13.5 |
| Oleinic | 12.8 |
| Linoleic | 51.0 |
| Linolenic | 22.7 |
| Total unsaturated acids | 86.5 |

Table 6

The fatty–acid composition of the individual fractions of phospholipids

| Fraction | $C_{16:0}$ | $C_{18:0}$ | $\sum S$ | $C_{16:1}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{18:3}$ | $\sum US$ |
|---|---|---|---|---|---|---|---|---|
| Phosphatidylcholine | 9.38 | 5.30 | 14.68 | 1.17 | 16.36 | 62.75 | 5.04 | 85.32 |
| Phosphatidylinositol | 19.46 | 6.40 | 25.86 | 1.60 | 15.35 | 52.19 | 5.00 | 74.14 |
| Phosphatidylethanolamine | 8.56 | 5.18 | 13.74 | 0.25 | 15.12 | 61.87 | 9.02 | 86.26 |
| Phosphatidic acids | 14.92 | 5.41 | 20.33 | 1.85 | 18.09 | 53.59 | 6.14 | 79.67 |

The receiving data achieved that in the rapeseed phospholipids composition the content of unsaturated oleinic, linoleic, linolenic fatty acids was quite high. Thus, they could not only be inhibitors but could used also as the source of essential fatty acids.

SUMMARY

Our researches had shown that rapeseeds lipids are characterized by considerable antilipolytic activity. They could be consider as prospective components of food supplements using for food correction of states that need fats hydrolysis process inhibition in human gastrointestinal duct.

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