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PHENOLIC ACIDS IN RAPESEED*

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A b s t r a c t. Phenolic acids especially their structure, forms of occurrence, their contents in rapeseed are characterised. Moreover, the biological activity of this group of compounds is presented.

K e y w o r d s: phenolic acids, rapeseed, biological activity

INTRODUCTION

Phenolic compounds, especially phenolic acids, beside phytins and glucosinolates are the most important among other biologically active compounds occurring in rapeseed [15, 21,24].

The aim of the presented paper is to characterise phenolic acids, regarding both their chemical structure and occurrence in rapeseeds. The other aim is to present the data showing their biological activity.

CHEMICAL STRUCTURE AND SYNTHESIS OF PHENOLIC ACIDS IN PLANT

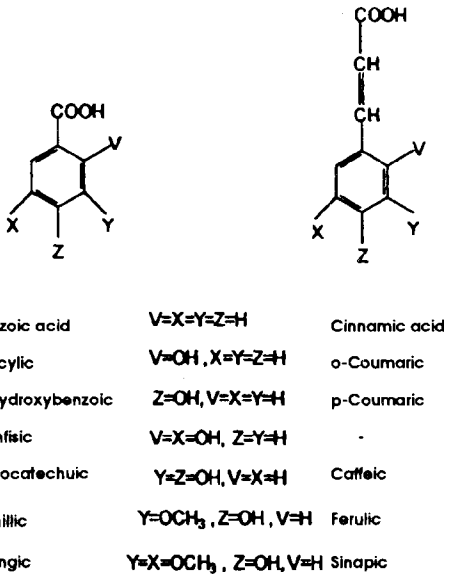

Fig. 1. Chemical structure of phenolic acids.

Phenolic acids occurring in rapeseed are derivatives of two phenolic compounds - benzoic and cinnamic acids. The chemical structure of the main phenolic acids is presented in Fig. 1. The basic pathway of synthesis of phenolic acids in plants leads from sugars through aromatic amino acids - phenylalanine, rarely tyrosine (Fig. 2). Deamination of amino acids to the appropriate phenolic acids occurs as follows: *trans*-cinnamic acid from phenylalanine and p-hydroxycinnamic acid (p-coumaric) from tyrosine are catalyzed by phenylalanine ammonia lyase (PAL). Derivatives of cinnamic acid are generated during the successive

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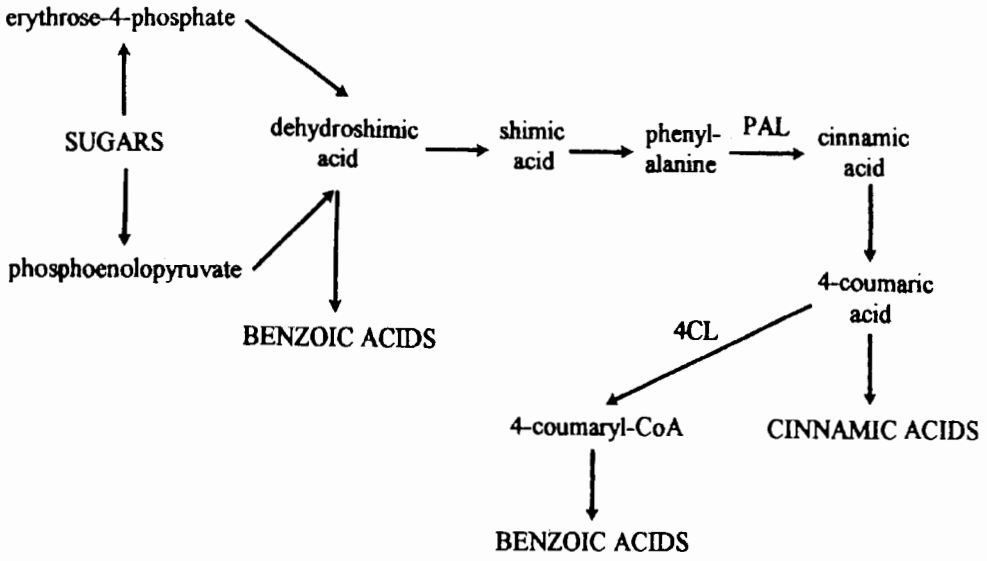

Fig. 2. Scheme of metabolic tracts of phenolic acids in plant - adapted from Stobiecki [28].

methylation and hydroxylation of cinnamic acid catalyzed by cinnamic acid 4-hydroxylase (C4H).

Derivatives of benzoic acid can be generated from dehydroshimic acid or p-coumaric acid through p-coumaryl-CoA. Synthesis of phenolic acids tioesters occurs with the aid of ligase: p-coumaric acid: CoA (4CL).

Sinapic acid, which is a dominant phenolic acid of rapeseeds usually has a form *trans*. The form *cis* of this acid is a result of an isomerization that can be caused by light and UV radiation [23].

FORMS OF PHENOLIC ACIDS OCCURRENCE

Phenolic acids in rapeseed can occur as free acids, esters, glucosides and insoluble-bond phenolic acids [35].

Esters are the dominant form of phenolic acids occurrence. The results of many analytical examinations [8,16,35,36] confirm this phenomenon. The main ester - sinapine is a compound of choline and cinnamic acid (Fig. 3) [5,6,10,37]. Choline esters with ferulic and

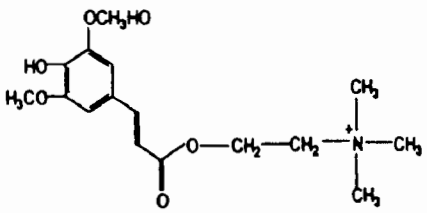

Fig. 3. Chemical structure of sinapine.

coumaric acids are also known from the literature [17]. Aromatic choline esterase catalyzed forming of sinapine [7].

Tanatavy *et al.* [29] mentioned revealed the occurrence of sinapic acid and flavonoids in rapeseed. Zadernowski [38] identified the presence of a methyl ester of sinapic acid in the phenolic extract obtained from rapeseed. Amarowicz *et al.* [4] noticed methyl ester of ferulic acid (Fig. 4). According to examinations carried out by Zadernowski [38], it was showed that mutual ester bounding between two molecules of sinapic acids so-called depside may occur.

Free acids have significantly lower participance

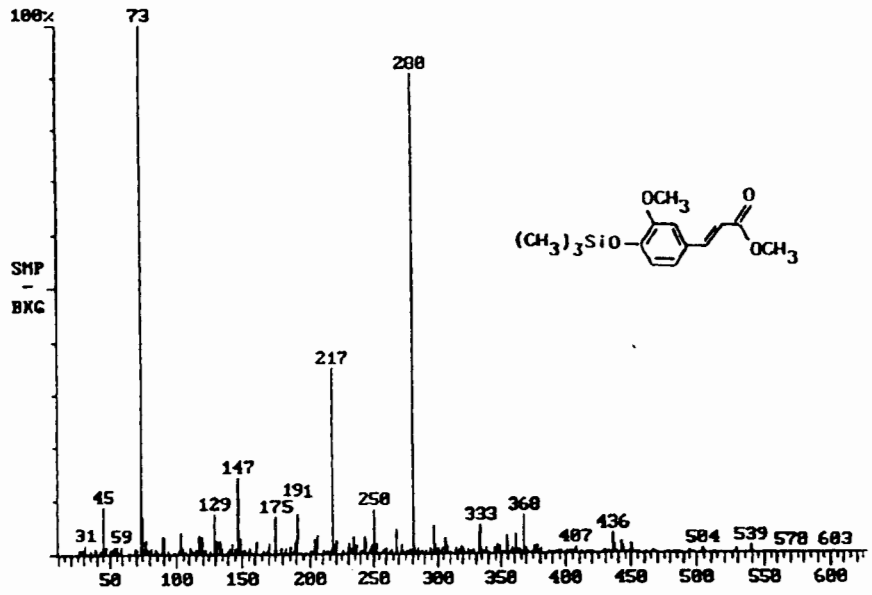

Fig. 4. Mass spectra of TMS derivative of methyl ester of ferulic acid.

in a phenolic quota. Examinations of rapeseed flour revealed that 13-24 % of phenolic acids occurred in this form [15]. Whereas in case of flour obtained from canola occurred approximately 15 % of phenolic acids [15]. Krygier *et al.* [16] observed merely trace amounts of free phenolic acids in the flour obtained from rapeseed of the Yellow Sarson variety. However, in examinations of phenolic acids carried out by Dąbrowski and Sosulski [8] no free phenolic acids were found.

Phenolic acids bound with sugars and compounds occurring as insoluble residue make only a slight part of a phenolic compound quota of rapeseed (Table 1). Special attention should be paid to a sinapoyl-glucose (Fig. 5) of strong antioxidant properties [33]. Also it can act as a precursor of growth inhibitors [29].

Table 1. Free, liberated from esters, glucosides and insoluble residue phenolic acids in rapeseed flours (μmol/g flour) acc. to Zademowski [38]

Phenolic acids	Range
Free phenolic acids	0.73-15.33
Liberated from esters	38.86-64.18
Liberated from glucosides	0.16-0.65
Liberated from insoluble residue	0.46-0.79
Total	40.21-75.28

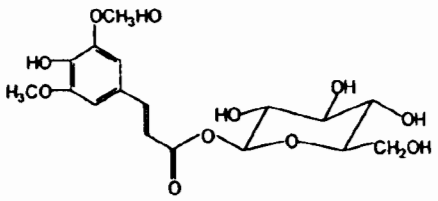

Fig. 5. Chemical structure of sinapoyl-glucose [1].

CONTENT OF PHENOLIC COMPOUNDS IN RAPESEED

The total content of phenolic acids in rapeseed flours and meals occurs in wide ranges. The rapeseed meal of the Tower, Regent and Altex varieties had the following total content of phenolic acids respectively: 1542, 1837 and 1807 mg/100 g [15]. The total content of phenolic acids in rapeseed flour of the Tower, Candle, Górczański and Start was 1067, 1281, 639, and 777 mg/100 g, respectively [14,16]. Examinations of Zademowski [38] showed that the total content of phenolic acids in flours obtained from selected varieties was found to be from between 40.21 to 75.28 μmol/g.

The values quoted above significantly exceed the levels of phenolic acids in seeeds of other oil plants. Kozłowska *et al.* [15] state that the total content of phenolic acids in flour obtained from soya, cotton and peanuts was 23.4, 35.7, and 63.9 mg/100 g, respectively.

Applying the appropriate methods of extraction, separation, hydrolysis and instrumental analysis (GLC, HPLC) enables precise measurements to be made of the particular phenolic acids content in rapeseed [16,18,35, 36,38]. It was found that the highest content of sinapic acid occurs in all forms of phenolic acids (Table 2). The content of isomer *trans* of this acid was significantly higher than the level of isomer *cis*. The rest of phenolic acids occur in rapeseed in the amounts of two grades of quantities. The ferulic acid dominates in this group.

BIOLOGICAL ACTIVITY OF PHENOLIC ACIDS

It was found in many laboratories that a symptom of biological activity of phenolic acids is their ability to bind with proteins. Complexation of blood plasma albumin (BSA) by phenolic acids depends on pK_a of the individual acids [32]. According to spectrofluorimetric examinations BSA forms complexes with phenolic acids much easier in neutral and alkaline pH than in acid one [26]. Phenolic acids can react with proteins by the ε - NH_2 and CH_3S methionine group [18]. The presence of phenolic acids was found in rapeseed albumins fractions obtained on a column with Sephadex G-25 using spectrometric analysis (absorption and fluorescence) and thin-layer chromatography on cellulose and silica gel. Depsime of sinapic acid was probably the main compound forming the complexes [27].

Table 2. Content of individual phenolic acids in rapeseed flours (μmol/g flour) acc. to Zadernowski [38]

Individual acids	Free acids	Estrified acids	Glucosides	Insoluble residue
Salicylic	0.01-0.11	0.1-0.20	0.01-0.03	0.01-0.03
Cinamic	0.01-0.07	-	0.01-0.03	0.00-0.02
p-Hydroxybenozoic	0.00-0.11	0.00-0.22	0.00-0.08	0.00-0.02
Vanillic	0.00-0.05	0.04-0.16	0.01-0.03	0.00-0.02
Gentisic	0.01-0.05	0.00-0.06	0.00-0.03	0.00-0.01
o-Coumaric	0.02-0.05	-	0.00-0.02	0.01-0.06
Protocatechuic	0.03-0.05	0.00-0.05	0.00-0.05	0.00-0.02
Syringic	0.01-0.09	0.01-0.05	0.01-0.05	0.01-0.03
p-Coumaric	0.00-0.05	0.00-0.02	0.00-0.02	0.01-0.06
cis-Caffeic	-	0.00-0.02	0.02-0.02	0.00-0.03
cis-Sinapic	0.01-0.43	0.07-0.11	0.07-0.11	0.03-0.06
trans-Ferulic	0.01-0.16	0.00-0.04	0.00-0.04	0.04-0.08
trans-Caffeic	0.00-0.05	0.00-0.06	0.00-0.06	-
trans-Sinapic	0.44-3.41	36.48-60.26	0.02-0.21	0.30-0.50
X	0.00-12.20	-	-	-

The content of sinapine in rapeseeds of different degree of ripening was 0.29-2.10 % in the case of the Jantar variety. The content of the same compound in the Start variety was 0.06-1.41 % [22], whereas in the flours of the selected rapeseed varieties - 35.88-57.20 μmol/g [38]. The content of sinapine in the rapeseeds cultivated in the Czech Republic was 50.12-80.15 μmol/g [31].

Reaction of phenolic acids with enzymes can influence their activity. Zadernowski and Nowak-Polakowska [40] confirmed it in their investigations. Phenolic compounds obtained from rapeseed inhibited the activity of mouldy lipase in a model system. The degree of hydrolysis of triacylglycerols was lower from 48 to 29.4 % by increasing the addition of a lipophylic fraction of rapeseed phenolic compounds. A better effect was achived using a

hydrophylic fraction. Phenolic acids also inhibited the activity of lipoxygenase. Oxidation of linoleic acid decreased in the model system containing this fatty acid and lipoxygenase with increased addition of phenolic compounds from rapeseed. More significant inhibitive effect occurred at the addition of a hydrophylic fraction.

The influence of phenolic acids on animal organisms is not yet widely known. It can occur in a direct way or through the interaction with tissues proteins.

According to Larsen *et al.* [17], sinapine decomposition occurs in the alimentary tract of rats. An increase of animal weight does not depend on the presence of phenolic acids in the diet.

Although the addition of rapeseed flour decreased the nutritive value of chicken feed, the reasons for this could be either phenolic acids or tannins [31].

Sinapine, except rapeseed flour, as a component of chicken feed can cause fish and a crab smell and taste in eggs. Trimethylamine, a derivative of choline, can be a reason for this [20]. The compound accumulates as a result of a metabolic block. A hepatic trimethylamine oxidase is probably inhibited by sinapine [11] and degraded products of glucosinolates occur in rapeseed [9,12].

The natural antioxidative properties of phenolic acids obtained from rapeseed (Fig. 6) are significantly important from the nutritive and technological point of view. Examiantion of these properties was a subject of many publications and scientific reports. It was found according to Nawak *et al.* [19], that the highest antioxidative activity revealed free phenolic acids isolated from rapeseed. Lower activity was found for sinapic acid released from sinapine. Sinapine itself only slightly inhibited the oxidative processes in a model emulsion. Zadernowski *et al.* [39] examined an extract of phenolic compounds obtained from rapeseed. They found that both hydrophylic and lipophylic fractions of rapeseed phenolic compounds are carriers of the antioxidative activity. Activities of three hydrophylic subfractions isolated with the aid of preparative thin-layer chromatography were approximately the same. Antioxidative activity of nine lipophylic subfractions - isolated in the same way - was distinctly different. Wanasundara *et al.* [33] found very high antioxidative activity for a phenolic fraction, isolated by column chromatography with Sephadex LH-20, containing sinapoyl-glucose. Shahidi *et al.* [25] reported that an addition of 0.5-5.0 % of flour from canola to a model meat system inhibited oxidation of fat (measured by TBA test) in the range of 73-97 %. The authors observed a less significant protective effect using an equivalent quantity of phenolic extracts from flour obtained from canola. Moreover, they found that the phenolic extract had good antioxidative properties when used to stabilise canola oil. An addition of this extract (200 mg/kg) inhibited oxidation of canola oil more effectively than the same amount of butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT). The examinations did not reveal any influence of the added extract on colour, taste and smell of oil. Amarowicz *et al.* [3] carried out experiments that showed the strong antioxidative activity of phenolic extract obtained from rapeseed oil cake. The parameters of the pressing process did not have any effect on the activity of the extract. Analysis did not reveal the effect of storage on the antioxidative activity of extracts [2].

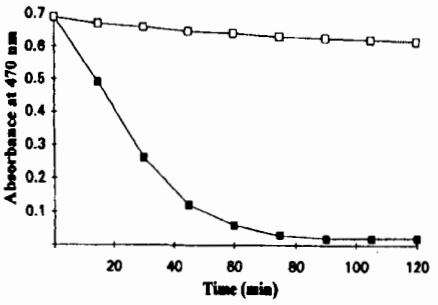

Fig. 6. Antioxidative properties of phenolic acids. Extract of phenolic acids added to model emulsion of linoleic acid with addition of β-carotene (□) prevents acid from oxidation (colour derived from β-carotene maintains during incubation of a sample at 50 °C). Process of linoleic acid oxidation occurs in a control sample (■) without addition of an extract. Products of acid oxidation decolour β-carotene - an orange colour of emulsion fades during heating.

The bactericidal properties of phenolic acids are especially valuable [19]. The complete growth inhibition of *Escherichia coli, Enterobacter aerogenes, Pseudomonas fluorescens, Bacillus subtilis, Bacillus cereus, Streptococcus cremoris* and *Streptococcus lactis* was observed after 48 h of incubation on a solid foundation with the addition of a 0.3 % sinapic acid fraction released from sinapine (SA). In the case of liquid cultures the addition of fraction SA (0.6 %) caused, after 48 h of incubation, the decrease of live cells by 99.7-99.1 %. For *Bacillus cereus, Streptococcus lactis* and *Pseudomonas fluorescens* it caused a total inhibition of growth. Similar antibacterial activity towards the investigated strains was performed by fraction of free phenolic acids (FPA). Introducing of 0.3 % of FPA into a solid foundation decreased after 48 h of incubation, the amount of *Escherichia coli* cells by 18.8 % and completely inhibited development of *Pseudomonas fluorescens*. An addition of 0.6 % of FPA into liquid cultures reduced live cells by 70-96.5 %.

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FENOLOKWASY W NASIONACH RZEAPKU

W artykule omówiono fenolokwasy pod względem ich budowy chemicznej, formy występowania, ich zawartości w nasionach rzepaku. Przedstawiono aktywność biologiczną tej grupy związków fenolowych.

S ł o w a k l u c z o w e: fenolokwasy, rzepak, aktywność biologiczna.