Phenolic Constituents in Rapeseed Flour – the next secondary plant products as important komplex in our biosphere.

Zukalová Helena, Bečka David, Mikšík Vlastimil, Cihlář Pavel Vašák Jan
Czech Agriculture University Prague
Faculty of agrobiology, food and natural resources
Department of Crop production,
165 21 Prague 6 –Suchdol,
Czech Republic

Introduction.

The meal is a high –value by –product used in animal feed due to its high energy content and the high amino acid score of the protein. Feeding value of rapemeal is decrease by antinutritive components- glucosinolates, phenolic compounds and polyphenols. The problem of glucosinolates is solved at the beginning, when the rapeseed happened by source of food industry. Problematique other antinutritive compounds – phenolic a polyphenolic is studied fragmentary.

Phenolic acids and their derivatives are commonly occurring compounds in the plant world. Their presence in seeds causes a deterioration the taste, odor and color of protein concentrates. Besides the fact unfavourable organoleptic changes can oxidize phenolic compounds which are bound with essential amino acids such as lysine or methionine forming complexes unassimilable in the digestive tract of animals and man. In the same way phenolic compounds bind heavy metal into complexes and thus decrease their usability (SHAHIDI et al., 1992) The mentioned taste effects of phenolic compounds have also negative physiological effects on the production of off-flavour or „fishy eggs“ laid by susceptible hens having insufficient trimethylamine oxidase. This phenomenon is restricted to certain strains of hens that lay brown-shelled eggs.

Besides this the negative characters on the other side there are their positive antioxidative characteristics, which inhibit activity lipoxygenazy (YASUMOTO et al., 1970) in rapeseed and restrict oxidative rancidity.The oil stability towards autooxidation is then increased. In consequence of this feature the rapeseed can serve as a raw material for obtaining antioxidants of natural origin.

The content of phenolic compounds in rapeseed is 30 times higher than in other oilseeds and therefore its antioxidative activity and its products are higher.(KOZLOWSKA et al., 1990).

Phenolics are predominantly located in the embryo with smaller amounts found in the hull. The varieties of rapeseed from different origins have similar contents of phenolic compounds (DABROWSKI et al., 1984,).

The seed of rape has wide range of phenolic compounds which come from benzoic and cinnamic acids in the form of:

Structure the main derivatives of phenolic acids:

- Benzoic acid and their derivatives: Salicylic, Gentisic, Protocatechuic, Isovanilic
- Cinnamic acid and their derivatives: Cinnamic, Ferulic, Sinapic, Coffeic

Free phenolic acids in the range of 61,5 - 2 48,0 mg/100g of flour and meal. Sinapic acid content is approxi mately 73 % (KRYGIER et al., 1982) and the present minor phenolic acids are p-hydroxybenzoic, vanillic, gentisic, protocatechuic, syringic, p-coumaric, cis- and trans-ferulic and caffeic acids and trace amounts of chlorogenic acid (KRYGIER et al., 1982).

Esterified phenolic acid in the range of 9 82,0 - 15 38,0 mg/100g meal and flour (SHAHIDI et al., 1992). Sinapin (cholin ester of 3,5-dimethoxy-4-hydroxycinnamic acid) creates approximately 70 - 97 % of phenolic acids derivated from esters and glucosides of rapeseed. Small amounts are the esters from the same free phenolic acids .

Insoluble bound phenolic acids in the range of 96,0 - 1 01,0 mg/100g flour and meal (SHAHIDI et al., 1992). In this part the predominant phenolic compounds are p-coumaric and trans-ferulic .

The aim of this work is focus on quality and quantity phenolic compounds of „00„ rapemeal.This parameters gives basis for improving his nutritive value by breeding or technology process and possibility using of isolate phenolic compounds, as a material for obtaining antioxidants of natural origin, which carry out the requirements on hygienic , healthy harmless and economic modest accessibility, considering surplus of rapemeal.

Material and Methods

Composition and content of phenolic compounds was observed by „00“ varieties Lirajet (DSV Lippstadt, SRN), Falcon(Lembke, SRN), Idol(Semences Cargill, Francie), Olymp (Lembke, SRN), Stela(SEMPRA,a.s.,CZ) in comparison with „0“ variety Solida (SEMPRA, a.s., CZ). Assortment of choice varieties ensures spectrum of varieties from early (Falcon, Idol, Stela) as late (Lirajet and Olymp).

Changes of phenolic compounds content in the last phase of maturing were observed up to the present time growers survey stable variety Lirajet on five trial stations of Central Institute for Supervising and one trial was on station of Czech University of Agriculture, Praque.

Charakteristic of trials site.

[Location]	*[Production region]*	*[Altitude (m)]*	*[Temperatura (°C)]*	*[Rainfall (mm)]*	*[Code of soil]*
Jaroměřice n.R.	Cereal region	425	8,0	481	HMm^1-jh^4
Staňkov	Cereal region	370	8,1	537	HMm^1-h^5
Hradec nad Svitavou	Cereal region	465	7,4	616	KMm^2-ph^6
Kujavy	Cereal region	260	8,2	604	LMg^3-jh^4
Vysoká	Cereal region	370	8,1	537	HMm^1-h^5
Červený Újezd	Sugar beet region	398	7,7	549	HMm^1-jh^4

Type of soil: 1)HMm – Orthic Luvisol, 2)KMm – Eutric Cambisol, 3)LMg – Albo-gleyic Cambisol
Code of soil: 4)Clayey-loam (heavy), 5)Loamy soil (medium), 6)Sandy-loam (medium)

Free phenolic acids and phenolic acids released by means of hydrolysis from the esters forms are determined by gas liquid chromatography as their silylderivates (ZADERNOSKI et al., 1981).

Gas chromatography of trimethylsilyl derivatives phenolic compounds

Isolation of phenolic compounds was prepared according to KRYGIER et al., (1982b).

Gas chromatography trimethylsilyl derivatives of phenolic acid.

An aliquot part of each methanolic fraction was evaporated to dryness under nitrogen.
The dryness residue was silylated in sealed vials with mixture BSA (N,O – Bis (trimethylsilyl)acetamid) with the catalytic action of TMCS (N- (Trimethylsilyl)acetamid at 105 ^{0}C for 24 h.
Gas chromatography condition: Apparatus- Hewlett - Packard HP 5890A, column WCOT Fused Silica with stationary phase CP - SIL - 8CB (Chrompact), length of column 50 m, inner diameter 0,25 nm, detector FID - 300 ^{o}C ,carrier gas N_2-flow rate 2 ml/min, temperature programm 100 ^{o}C is 5 min and than increase 6 ^{o}C/min up to 230 ^{o}C.

TMS derivatives of phenolic acids were identified by comparison of the retention time of the TMS derivatives with the values for retention time of TMS derivatives of standard acids and their content was determined according calibration this component.

Spectrophotometric method of sinapine.

The spectrophotometric procedure is based on the absorption of phenolic compounds in UV range (EVRARD, 1985) at 334 nm .

Through these methods the changes in sinapin content during maturation and the influence of varieties and locality were determined

RESULTS AND DISCUSSION.

Contents of phenolic compounds is charecterized by the varieties. Solida - „0“ variety of winter rapeseed showed a higher content in comparison with „00“ varieties. It is in harmony with previous experiments (KRYGIER et al., 1982, KOZLOWSKA et al., 1990, DABROWSKIet al., 1984). Their total content is 1,6 –2,8%. The highest content has „0“ Czech variety „Solida“ (2,80%). Long time of vegetation partly influenced their content. Early varieties – Falcon, Idol and Stela (1,60 –2,20%)

showed lowest content of phenolic compounds against late varieties – Lirajet (2,4%) and Olymp (2,5%) .

With evaluated of phenolic compounds content in individual fraction follow:

- Free phenolic acids are in the range of 563,0 -921,0 mg/100g. Content of free phenolic acid is higher in compare with publicate date ((KRYGIER et al., 1982, KOZLOWSKA et al., 1990). Free phenolic compounds are from 85,5 – 91,30% created by sinapic acid derived from cinnamic acid. From this are derived also o- coumaric and ferulic, which are in minorite content. From acids derived from benzoic acid are salicylic and protocatechuic acid. The second important component this fraction is syringic acid (5,50 – 8,40%) which content is 5670 – 8868 mg /kg rapemeal and are higher in compare with publicate date.
- Esterified phenolic acid are in the same way as in previous fraction created esters of sinapic acid (88,66 – 93,9 %). The later prevail acid is ferulic acid (3,70 – 9,60%), which is in similar content as syringic acid in fraction free phenolic acid whole content of this fraktion is 11709 – 20688 mg/kg.
- The other phenolic acid, their esters and insoluble bound phenolic acids are in rapeseed in minorite content and in our case were not detection.

By spectrofotometric method was observed behavior and dynamics of phenolic compouds in last 14 days before harvest.

Phenolic compounds content was stable in the last phase of maturing. Their content is in range 1,18 –1,65%. Their decrease appeared in the samples which were desiccated with desiccant Reglone four days before the expect harvest.(Tab.1.). This was probably caused by the unequal maturity of desiccated sample.

Tab.1: Dynamics of phenolic compounds production during winter rape seed ripening (the Lirajet variety) on six sites (%)

Site	14 days before harvest	7 days before harvest	harvest	7 days after harvest	desiccated[1]
Jaroměřice	1,54	1,48	1,56	1,68	1,29
Kujavy	1,08	1,15	1,18	1,12	0,98
Hradec n. Svit.	1,62	1,59	1,65	1,65	1,35
Vysoká	1,40	1,50	1,55	1,59	1,42
Staňkov	1,15	1,37	1,45	1,19	1,06
Červ.Újezd	1,35	1,40	1,48	1,45	1,36

1) stand desiccated 4 days before pedicted harvest

The statistically significant are also differences among places , which are caused by agroecological influences.

Lower content of phenolic compounds were on place Kujavy, with the lowest altitude and by significant different code of soil and type

Biosynthesis of phenolic compounds during maturing rapeseed after statistical evaluation by method ANOVA, showed that content of sinapin is statistical significant as among places as also in individual phases of maturing.

Between place Kujavy and Staňkov with the lowest altitude was the lowest content of sinapine. The others places are compare results of Červený Újezd, Vysoké and Jaroměřice, which are similar by altitude and average rainfal. In Hradec n. Svitavou are results compare with Vysokou and Jaroměřice.

Conclusion.

Contents of phenolic compounds is charecterized by the varieties. Solida - „0“ variety of winter rapeseed showed a higher content in comparison with „00“ varieties. Their total content is 1,5 –3,0%. The highest content has „0“ Czech variety „Solida“ (2,80%). Long time of vegetation partly influenced their content. Early varieties – Falcon, Idol and Stela (1,60 –2,20%) showed lowest content of phenolic compounds against late varieties – Lirajet and Olymp (2,5%)

Phenolic compounds in rapeseed are in form

- Free phenolic acid have content 5670 – 8868 mg /kg and is created by sinapic acid from 85,5 – 91,30%.
- Esterified phenolic acid have content 11709 – 20688 mg/kg and also is created by sinapic acid from 88,66 – 93,9 %.
- Other phenolic acid, their esters and insoluble bound phenolic acids are in rapeseed in minorite content and in our case were not detection.

On the other side phenolic compounds have positive antioxidative characteristics, which inhibit activity lipoxygenazy and can serve as a raw material for obtaining antioxidants of natural origin, which carry out the requirements on hygienic, healthy harmless and economic modest accessiblement.

By means of oxidative tests was founded out that addition 500 – 1000ppm is more effective than synthetic antoxidante of type BHA (terc. butylhydroxyanisol), BHT (di-terc. butylhydroxy toluen), BHA/BHT/MGC (monoglycerid citrát) and less effective than TBHQ (derivát kys. hydroquajaretové).

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The statistically significant are also differences among places , which are caused by agroecological influences.

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