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YELLOW PEA (*PISUM SATIVUM*) AND ITS PRODUCTS AS A SOURCE OF NATURAL ANTIOXIDANTS. PART I. MODEL INVESTIGATIONS OF ANTIOXIDANT PROPERTIES OF PEAS

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Key words: yellow pea, natural antioxidants, lard, rapeseed oil.

It was found that yellow pea increased the stability of lard and of low erucic acid rapeseed oil. Fractions obtained from pea by extraction with solvents of various polarity demonstrated a distinct decrease of antioxidant properties. The greatest amounts of substances with antioxidant activity were extracted with ethanol, less by petroleum ether, and the smalest amounts by water.

INTRODUCTION

An important task for the food economy is the obtaining of new protein sources together with a rational utilization of the existing ones. Leguminous plants are receiving increasing attention in this context the world over. The consumption of these plants in Poland is rather low at present (about 1.3 kg per person per year) and its considerable increase is advisable for both nutritional and economic reasons [5].

The most important among leguminous plants in Poland is yellow pea. One of the possible ways of increasing its consumption is its greater utilization in food concentrate production. Recent studies also demonstrated the usefulness of yellow pea in the production of snacks of the potato chips kind [8, 14] and of protein preparations which may well serve as meat substitutes [15]. Food concentrates as well as snacks and meat products contain relatively large proportions of fat, and the changes in fat fraction are the principal factor limiting the shelf life of products of this kind.

Earlier studies have shown that soup concentrates based on pea flour and beef tallow have an exceptionally long shelf life [18], but no detailed studies aimed at explaining this phenomenon have yet been undertaken. It is to be expected that the absence of oxidation changes of the fat fraction in soup concentrates is due to the presence of natural antioxidants in pea, as endogenous inhibitors of fat oxidation have previously been demonstrated in other leguminous plants [1-3, 6, 7, 11-13, 16, 17]. We therefore decided to study the effect of yellow pea on the rate of fat oxidation.

MATERIAL AND METHODS

Three pea varieties were used in the studies: Kaliski, Flawanda (both obtained from the Plant Breeding Station in Wiatrowo) and Wiktoria (obtained from the "Amino" Food Concentrates Plant in Poznań). Prior to experiments the peas were disintegrated in a laboratory grinder. 96% of the obtained material was sifted through a sieve with 0.25 mm square mesh.

The substrates in durabiloty studies were class-I pork lard and refined low erucic acid rapeseed oil obtained from "Janpol" winter rapeseed. The antioxidant properties of yellow pea were examined in relation to lard and rapeseed oil with the Schaall test at 60°C [9] and in relation to lard stored in darkness at 20°C. In all cases the additions were equivalents of 2.5, 5.0, 10.0, 15.0, 20.0 and 30.0% dry mass of pea in fat weight. A 0.01% addition of BHA was used as reference. In all experiments the fat layer thickness was 1.0-1.2 cm. The degree of fat decay was etablished by determining the Lea peroxide number. Experiments were considered over when the Lea number was 10 (in lard stored at 60°C), or 3 (in lard stored at 20°C) or 25 (in oil stored at 60°C). Results of the test were interpreted with the so called antioxidant effectiveness coefficient (Aec) computed as the ratio of time by which the applied addition expanded the period before the fat attains the limit values of the Lea number to the time needed by untreated fat to attain the suitable values of the Lea number.

The natural antioxidants were extracted from yellow pea with petroleum ether (boiling point: 40-60°C), 96% ethanol (pure) and distilled water. Wiktoria pea was subjected to single-stage extraction and to fractional three-stage extractions with the folowing order of solvents: water-ethanol-petroleum ether, and petroleum ether-ethanol-water. Anthioxidant effectiveness of all the obtained fractions (extracts and extraction residues) was studied in relation to lard with Schaal's test. The extracts and extract residues were added to the lard in quantities equivalent to 20.0% dry mass of pea.

Pea extracts were prepared in the following manner: 400 ml of solvent were added to 80.0 g of pea, the solution was mixed and left to steep for 18 h at 12°C. The flask content was then shaken for 3 h. Extracts obtained with organic solvents were filtered through a Whatman 1 paper filter; water extracts were separated by centrifugation and decantation in a centrifuge at 300 r.p.m.

Extraction was performed five or six times and the obtained extracts were combined. The organic solvent extracts were evaporated dry in a rotary vacuum evaporator at 40°C, while the water extracts were preliminarily concentrated in a rotary vacuum exaporator and then transferred to Petri dishes and dried in a vacuum drier at 40°C. After drying the water extracts were scraped off the dishes and ground in a laboratory grinder. The extraction residues were spread on trays and dried at room temperature (residue of organic solvent extraction) or in a vacuum drier at up to 40°C (water extraction residues). After drying the residues were ground in a laboratory grinder. Prior to experiments the extracts and residues were stored at -25°C in air-tight glass vessels.

RESULTS AND DISCUSSION

The effect of additions of the studied pea varieties on the rate of lard and low erucic acid rapeseed oil oxidation is presented in Table 1. Under Schall test conditions al pea varieties significantly retarded lard oxidation and their antioxidant activity increased with the increase of their proportion in the fat. The inversion of antioxidant properties characteristic for some antioxidants was not observed in the studied range of concentrations. It was found, however, that the pea's ability to retard lard autooxidation is a variety-specific property. The effectivity of the studied pea varieties decreased in the following order: Wiktoria, Kaliski, Flawanda. The very strong antioxidant properties of pea are indicated by the fact that 10% additions of all the varieties were already as effective as BHA (Aec = 6.16) and at maximum additions to the fat Wiktoria, Kaliski and Flawanda peas displayed activities 7, 5 and 4 times higher, respectively, than that of the synthetic antioxidant.

The applied pea additions also prolonged lard autooxidation in natural storage conditions. The antioxidant properties of the investigated pea varieties increased with the increase of their concentration in fat similarity as at 60°C, and in one case only, when the addition was increased from 20 to 30%, the increase of their antioxidant activity was smaller than in the Schaal test. This may be attributed to the action of the enzyme lipoxygenase, previously found to occur in pea [4, 10], which was inactivated in conditions of the Schaal test. Comparing the

Table 1. Antioxidant properties of tested pea varieties

Variety	Concentration (% d.m.)	Antioxidant effectiveness coefficient (Aec); tests in:		
		lard (60°C)	lard (20°C)	rapeseed oil (60°C)
Kaliski	2.5	1.08	0.31	0.17
	5.0	2.20	0.68	0.43
	10.0	5.45	1.51	0.56
	15.0	11.14	3.10	1.03
	20.0	15.99	4.63	1.23
	30.0	32.88	7.80	2.51
Flawanda	2.5	0.94	0.27	0.16
	5.0	2.12	0.44	0.34
	10.0	4.53	1.19	0.54
	15.0	9.42	2.54	0.71
	20.0	16.64	4.27	1.03
	30.0	27.03	7.45	2.32
Wiktoria	2.5	1.98	0.28	0.45
	5.0	3.36	0.99	0.56
	10.0	7.50	1.97	0.88
	15.0	12.20	3.59	1.03
	20.0	25.43	6.28	1.24
	30.0	42.40	8.30	2.79
BHA	0.01	6.16	2.05	0.01

Aec of pea and BHA tested at 20 and 60°C, we see that although their values in conditions of natural storage were over three times lower, they displayed a tendency similar to that obtained in the Schaal test.

All the pea varieties in all the applied concentrations also retarded the oxidation of low erucid acid rapeseed oil, unlike the sythetic antioxidant BHA which had no effect on the oil decay rate. Comparing the mean Aec values for all concentrations we find that the highest antioxidant capabilities were exhibited by Wiktoria pea, with Flawanda variety being the least effective in this respect. The antioxidant activity of pea with respect to oil was much lower than in the case of lard.

The antioxidant properties of fractions extracted from pea with solvents of various polarity by single-stage and fractional extraction are illustrated in Fig.

Of all the pea extracts obtained by single-stage extraction, the ethanol one (Aec = 8.7) inhibited lard oxidation most effectively, but its antioxidant capabilities were smaller than those of pea before extraction by over 300%. The antioxidant effectivity of ethanol extracts was about three times higher than that of other extracts and nine times higher

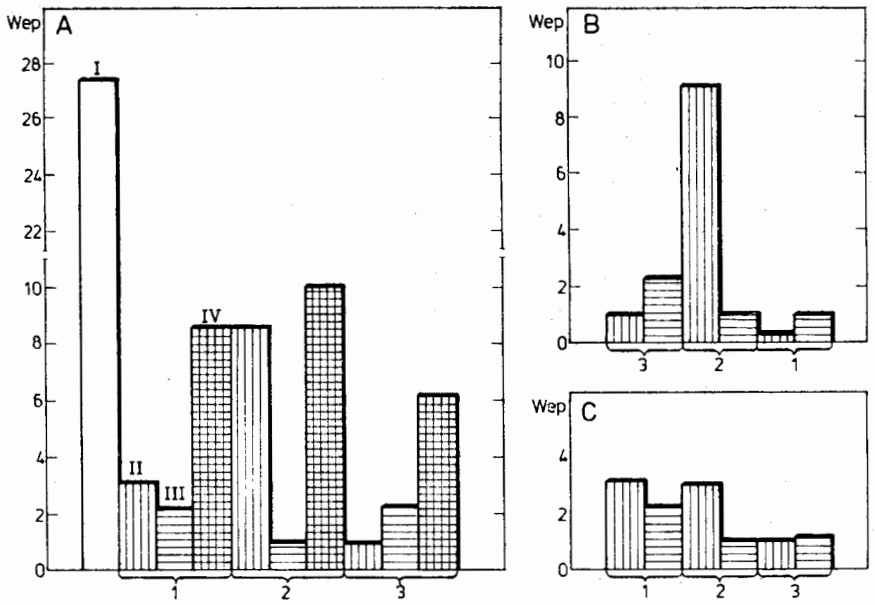

Fig. Antioxidant efectiveness of pea and its fractions, obtained by extraction with petroleum ether (1), ethanol (2) and water (3); A — single-stage extraction; B — fractional extraction, sucessively with water, ethanol and petroleum ether; C — fractional extraction, successively with petroleum ether, ethanol and water; I — pea, II — extract, III — residue after extraction, IV — mixture of extract and residue after extraction

that the effectiveness of water extracts. No great differences were found in antioxidant properties of residues after extractions with the applied solvents. The Aec values for the residues were low, ranging from 1 to 2. The fact that extractions and residues after ethanol, and especialy after ether and water extraction retarded oxidation processes in lard to a far lesser degree than pea suggests that there may occur synergism between fractions.

However, the study of antioxidant effectiveness of mixtures of extracts and extraction residues did not confirm this suggestion. The mixtures of extracts and residues from extractions with all the solvents were decidedly less active than pea it self, and weak synergism was observed only between fractions extracted with water and petroleum ether.

During fractional extraction of pea by solvents of decreasing polarity, the first fractions — water extract and residue from water extraction — displayed low activity in lard oxidation inhibition. However, and additional treatment of the water extraction residue with ethanol made it possible to isolate a group of compounds with very strong antioxidant properties; the residues from extraction with this solvent demonstrated a weak, albeit significant activity. A subsequent extraction with ether

did not produce further antioxidants from pea. On the other hand, in extraction with solvents of increasing polarity the highest antioxidant activity was exhibited by the ether extraction. The ethanol extract was slightly less active but was more effective than the residue after ether etraction, i.e. the fraction from which it was isolated. The ethanol residue and the subsequent fractions — water extract and residue from water extraction — also demonstrated a weak but significant antioxidant effect.

The obtained results indicate that the low activity of residues after single-stage extraction with water and petroleum ether was due to changes brough about in the raw material structure by the solvents. It may be that these changes reduced the antioxidant components' ability to pervade the fat, and it was only subsequent extraction which facilitated their contact with the fat. Also, one cannot preclude the possibility that the action of the solvents leads to the appearance of compounds antagonistic towards some of the antioxidants.

Table 2. Substances extracted from pea by solvents with various polarity (% dry substance)

Kind of extraction	Solvent		
	petroleum ether	ethanol	water
Single-stage extraction	1.40	3.09	28.72
Fractional extraction successively with petroleum ether, ethanol and water	1.40	1.82	20.01
Fractional extraction successively with water, ethanol and petroleum ether	0.11	2.84	28.72

The masses of substances eluted from pea by the various solvents and the obtained Aec values given in Table 2 additionally indicate that a large part of comopuds in the active ethanol extracts obtained by single-stage extraction are substances that are also soluble in petroleum ether.

CONCLUSIONS

1. Pea exhibits strong antioxidant properties towards lard and low erucic acid rapeseed oil which increase with the increase of the pea addition to the fat.

2. Antioxidant effectiveness of pea is a variety-specific property. In the studied pea varieties it increased in the following order: Flawanda, Kaliski, Wiktoria.

3. During pea extraction by slovents of various polarity the vast majority of antioxidants becomes inactivated. The greatest amounts of

substances with antioxidant properties dissolve in ethanol, less substances in petroleum ether, and the smallest amounts dissolve in water.

4. A large propertion of compounds in active ethanol extracts obtained by single-stage extraction are substances also soluble in petroleum ether.

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GROCH (*PISUM SATIVUM*) I PRODUKTY JEGO PRZEROBU JAKO ŹRÓDŁO NATURALNYCH PRZECIWUTLENIACZY. I.
BADANIA MODELOWE WŁAŚCIWOŚCI PRZECIWUTLENIAJĄCYCH GROCHU

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Streszczenie

Badano wpływ nasion 3 odmian grochu: Kaliski, Flawanda i Wiktoria na szybkość utleniania smalcu oleju rzepakowego niskoerukowego w temp. 60°C oraz smalcu w temp. 20°C. W celu wyodrębnienia naturalnych przeciwutleniaczy z grochu użyto: eteru naftowego 96% etanolu i wody destylowanej. Zastosowano ekstrakcję prostą jednostopniową, ekstrakcję trzystopniową frakcjonowaną kolejno

rozpuszczalnikami o rosnącej polarności oraz kolejno rozpuszczalnikami o malejącej polarności.

Badano efektywność przeciwutleniającą uzyskanych wyciągów i pozostałości po ekstrakcji w stosunku do smalcu w temp. 60°C. Stwierdzono, że groch wykazuje silne właściwości przeciwutleniające w stosunku do smalcu i oleju rzepakowego niskoerukowego, które rosną wraz ze zwiększeniem ilościowego dodatku do tłuszczu, a jego efektywność przeciwutleniająca jest cechą odmianową (tab. 1). W czasie ekstrakcji rozpuszczalnikami o różnej polarności zdecydowaną większość antyoksydantów grochu ulega unieczynnieniu (rys.). Największe ilości substancji wykazujących właściwości przeciwutleniające rozpuszczają się w etanolu, mniejsze w eterze naftowym, a najmniejsze w wodzie. Wśród związków aktywnych wyciągów etanolowych otrzymanych metodą ekstrakcji prostej znaczną część stanowią również substancje rozpuszczalne w eterze naftowym.