ESTIMATION OF THE ANTIOXIDATIVE PROPERTIES OF THE NATURAL POLYPHENOLS IN THE OXIDATION PROCESS OF MODEL LIPOSOME MEMBRANES

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The experiment was conducted to proof the new natural plant extracts (Fagopyrum Mill. (buckwheat), Crataegus ex L., (hawthorn), Hypericum L. (St -John's -wort's) and Helichrysum arenarium) and selected flavonoids as reference substances for the *in vitro*. The probing was performed as considering four different parameters: the antioxidant activity, the antiradical activity – DPPH$^{\bullet}$ test, the DPH test and the CF test. The results of antioxidative test of studied extracts show high antioxidant activity against UVC induced peroxidation of phosphatidyl**c**holine (PC) liposomes membranes (except Helichrysum). The main consident parameter for antioxidant activity was IC_{50}^{PC} (**I**nhibition **C**oncentration 50 PC – the concentration of antioxidant which reduces peroxidation intensity of phosphatidylcholine liposomes about 50%). Values of IC_{50}^{PC} were following: buckwheat – hulls (20,8 mg/L) ≥ hawthorn – cortex (21,6 mg/L) > Hypericum L. (25,4 mg/L) > hawthorn – leaves (26,8 mg/L) > buckwheat – stems (29,1 mg/L) >> Helichrysum (121,6 mg/L). The DPPH$^{\bullet}$ free radical test results (IC_{50}^{DPPH} – the concentration of antioxidant which reduces the free radical DPPH$^{\bullet}$ about 50%) show the high antiradical activity of majority extracts (IC_{50}^{DPPH} = 2,7 – 7,4 mg/L), except Helichrysum (IC_{50}^{DPPH} = 34,0 mg/L). The similar relationship between antiradical activity (IC_{50}^{DPPH}) and antioxidant activity (IC_{50}^{PC}) for the Hypericum L., buckwheat – stems and hawthorn – leaves, suggest possible mechanisms of the extracts activity, as the free radical scavengers (scavenger = substance that can deactivates Reactive Oxygen Species or Reactive Nitrous Species). The examination the possibility association of studied extracts to liposomes membrane was one of other aims. His realization was used with the DPH method to assignment the coefficient of constant association ($1/K_D$). The values of $1/K_D$ of the test extracts (ranged 709 – 999 L/mg) with PC liposomes membrane, may suggest their role as substances to protect the membrane from reactive oxygen species. The influence of studied extracts on structure of PC liposome membranes was also verified using CF test. The intensity of leakage from liposomes under presence of investigated flavonoids was examined using the carboxyfluorescein (CF) marker. It shown that compounds in general (except buckwheat stems) do not trigger malicious actions on the PC membrane liposomes. Percentage of carboxifluorescein leakage at various concentration of extract for witch got the IC_{50}^{PC}, didn't cross the 2 % (except buckwheat stems = 10,90 %). The obtained results demonstrate possibility of using them (except Helichrysum) as antioxidants in food and pharmaceutical or cosmetics industry.

Key words: - natural extracts, antioxidants, peroxidation, liposomes, free radicals, flavonoids

The presence of free radicals in organism is natural consequence of oxygenic metabolism. A free radical is an atom, molecule, or ion that contains one or more unpaired electrons and is more reactive than their parent species. Free radicals can realize oxygenation or reduction reactions, that damage the many biological molecule and cause food rancid [Ferrari & Torres, 2002; Kerr et all, 1996; Evans & Halliwell, 1999]. The living organisms adapted to their destructive action, producing many defensive mechanisms. These enzymatic or low molecular mechanisms transform free radicals to less toxic products or deactivate them. The examples of low molecular defense are tocopherol (vitamin E), ascorbate (vitamin C) or carotenes. Defensive systems are not sufficiently effective, and their performance undergoes slightness alongside with age. It is widely agreed, that increasing morbidity on civilization diseases and their complication is relevant with occurrence oxidative stress. Free radicals are stirred up in many pathological processes, especially in arteriosclerosis, Parkinson's syndrome, Alzheimer disease, cataract and tumours. Delivery to organism substances which have antioxidative proprieties may delay the degenerative processes which occur in organism. Biochemical studies prove, that flavownoid components present in plants can be an important preventive factor against cardiovascular diseases which can confirm e.g. the "French paradox" [Miller et al., 2008; Howard et al., 2002]. Flawonoids are classifies to wide group chemical compounds – polyphenols, and they are produced mainly by plants. These substances cover a wide range of functions. There are producing in response to the immoderate UV radiation, ions of transition metals, thermal shock, or as the reaction to fungal infection [Wojcieszyńska & Wilczek, 2006]. The most important sources of flavonoids are drinks such as coffee or tea, fruits and vegetables. Afficacy of many pharmacists is also a result of presence in them a polyphenols extracts (e.g. *Gingko bilobae*). The antioxidant properties of flavonoids are the result of presence in their molecular structure a hydroxyl groups, associated with the benzene ring [Gawlik-

Dziki, 2004]. The most important mechanisms of their action consist: deactivation of active forms of oxygen or nitrogen (known as scavengers), interruptions free radical chain reactions, reduction of reactive oxygen or nitrogen species through giving an electron of the hydrogen atom.

The examples of plant used in the herbalism and in the pharmaceutical industry are among others: a St John's wort (*Hypericum perforatum, Hypericum* L.), a hawthorn (*Crataegus* L.), a helichrysum (*Helichrysum arenarium.*) and a buckwheat (*Fagopyrum Mill.*).

John's Wort is a perennial plant occurring in subtropical and temperate climates and on mountainous areas of the tropical zone. Among the polyphenolic components present in *Hypericum perforatum* (Hypericum L.), might mention among others: coffee acid [Škerget et al, 2003], chlorogenic acid, neochlorogenic acid, *p*-coumaric-chinoninic acid, (+)catechin, (-)epicatechin, rutin (Rutosyde), quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-ramnoside, kaempferol-3-glucoside, biapigenin [Oszmiański, 1993-1996], anthocyanins and xanthones [Mulinacci et al, 2007]. The studies Sánchez-Reus'ai et all conducted on rat brains showed, that the antioxidant activity of extracts of St. John's wort may reduce the amount of an oxidative damage, which is one of the causes of degenerative changes in the central nervous system (e.g. dementia, Alzheimer disease, Parkinson disease) [Sánchez-Reus'ai et all, 2007].

Buckwheat (*Fagopyrum esculentum* Möench) is a species of grain that belongs to the family Polygonaceae. It comes from Central Asia gangways. The following polyphenols are found in this plant: rutin, quercetin, quercetin glicosides, apigenin [Oszmiański, 1993-1996], orientin, isoorientin, isovitexin, vitexin [Dolatowski et al, 2006] and catechin [Amarowicz, 2006]. Investigation Dolatowski et al have demonstrated the possibility of using buckwheat as a component of limiting fat rancidity in meat products [2004-2006].

Hawthorn (*Crataegus* L.) is a plant belonging to the Rosaceae family, appearing in areas of the northern hemisphere of America, of Asia and in Europe. Hawthorn extracts contain: vitexin rhamnoside, rutin, quercetin-3-glucoside, spirenin and flavones glicosydes. To the other components belong also: titerpenic acids and trace amounts of essential oils [Urbonavičiūtė et al, 2006]. The study of the properties of components contained in extracts of hawthorn have shown their antisclerosis effect, among others due to lowering the levels of LDL oxidation [Rajendran et al, 1995]. Components such as vitexin-2"-O-rhamnoside and procijanidin oligomers increase the strength of contraction and reducing the excitability of the cardiac muscle [Orhan et al, 2007; Rechciński & Kurpesa, 2003].

Helichrysum is a widespread plant in Europe belonging to the family Asteraceae. In this plant the following biocomponents of flavonoids were identified: kaempferol, kaempferol-3-O-glucoside, apigenin, apigenin-7-O-glucoside, chlorogenic acid [Lemberkovics et al, 2001], neochlorogenic acid, cryptochlorogenic acid, derivatives dicoffeechinon acid and chalcone derivative of isosalipurposide. The chalcone derivatives give a yellow color of flowers helichrysum [Pobłocka-Olech & Krauze-Baranowska, 2007].

The literature reports about the use of PC liposomes as a model biological membranes to verify the antioxidant activity of extracts of these plants are modest. Therefore, the main aim of this investigations was estimation of the antioxidative properties of the natural polyphenols extracts from *Fagopyrum Mill.* (buckwheat, hulls and stems), *Crataegus ex* L., (hawthorn, cortex and leaves), *Hypericum* L. (St -John's -wort's) and *Helichrysum arenarium* and selected flavonoids as reference substances for the *in vitro* oxidation process of model liposome membranes. The studies using free radical DPPH$^{\bullet}$ test and the DPH probe test was aimed to determine the probable mechanism of antioxidant properties of these extracts and their relationship with the ability for incorporation into the membrane.

Material and methods

• **Reagents used to conduct experiments: Bufor „A"** – Phosphate buffer; **CF –** 5(6)-Carboxyfluorescein; **DPH** – l,6-diphenyl-l,3,5-heksatriene; **DPPH$^{\bullet}$** – 1,2- diphenylpicrylhydrazyl; **Etanol**; **Metanol**; **PC –** Phosphatidylcholine lecithin; **Sephadex G-50**; **TBA** – thiobarbituric acid; **TCA** – tricarboxic acid; **TrisHCl** - 2-Amino-2-(hydroxymethyl)-1,3-propanediol;

Antioxidants:

- **Sigma – Aldrich Deisehoffen, Germany:** Rutin hydrate (qercitic-3-rutoside; $C_{27}H_{30}O_{16} \cdot 2H_2O$), Quercitin hydrate (3,3',4',5,7-pentahydroxyflavone, $C_{15}H_{10}O_7 \cdot 2H_2O$), Chlorogenic acid (1,3,4,5tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate, $C_{16}H_{18}O_9$);
- **Carl Roth GmbH x 7685, Karlsruhe:** (-)-Epicatechin ($C_{15}H_{14}O_6$);
- **Department of Fruit and Vegetables Technology [Oszmiański, 1993, 1995]:** extracts from *Fagopyrum esculentum* Möench (buckwheat), Crataegus ex L., (hawthorn), Hypericum L. (St -John's -wort's) and Helichrysum arenarium (helihrysum);

The various extracts were dissolved in methanol, ethanol, TrisHCl buffer (pH 7.4) or phosphate buffer (pH 7.4).

Liposome preparation

Antioxidant activity

The lecithin was dried in atmosphere of nitrogen and incubated under vacuum for 30 min. Tris[(hydroxymethyl)-aminomethane]-HCl buffer (pH 7.4) was added according to the proportion of 1.5 mg PC/ml buffer. Phosphatidylocholin [PC] was then sonicated for 5 min at 0 °C.

The DPH test

30 µl of Phosphatidylocholin with 11 µl DPH probe was dried in atmosphere of nitrogen and incubated under vacuum for 30 min. After adding 3 ml of phosphate buffer (pH 7.4), lecithin was sonicated for 10 min at 0 °C.

The CF test

50 µl of lecithin was dried in atmosphere of nitrogen and incubated under vacuum for 30 min. To the received lipid film was added 37.5 mg of Carboxyfluorescein (CF) and 0.5 ml of phosphate buffer (pH 7.4).

Antioxidant activity

2.9 ml suspension of lecithin liposomes (PC) were obtained from the solution, and transferred to beakers of 50 ml with magnetic stirring device. The tested extract in an appropriate concentration was added, complementing the buffer to a constant volume of 100 µl. Beaker with a blank sample contained 100 µl TrisHCl buffer to obtain the initial volume of 3 ml. The mixture was incubated for 15 minutes, then collected two 0.5 ml samples to determine the level of oxidation. The remaining amount of liposomal dispersion irradiated UVC radiation for 30 minutes. After this time, collected two consecutive samples (0.5 ml). Sample and test reagents supplemented than with 1 ml TBA and TCA, covered with glass marbles and incubated for 15 min at 90 ÷ 100 °C. After cooling the samples was centrifuged 15 min at a rate of 2500 rpm (centrifuge Multofuge 3L-R). Measuring the absorbance at λ = 535 nm were made in the spectrometer Cary 300 Bio Varian Company, which was resetting in the presence of the blank sample. Percent oxidation of the samples was calculated from the formula:

$$\% \text{ Inhibition} = [\Delta A_0 - \Delta A_A/\Delta A_0] \cdot 100\ \% \qquad [1]$$

Where: ΔA_A - an increase of absorbance at λ = 535 nm, after the time T_{30} in the presence of an antioxidant; ΔA_0 - increase of absorbance at λ = 535 nm, after the time T_{30} without the addition of antioxidant; The IC_{50}^{PC} (Inhibition Concentration 50 PC – the concentration of antioxidant which reduces peroxidation intensity of Phosphatidylcholine liposomes about 50%) parameter was determined using Microsoft Excel 2007. On linear graph section of the percentage of inhibition according the concentration of the extract a trend line equation: y = ax + b was determined. IC_{50}^{PC} was calculated by transforming the equation above and the expression x at which y-value is 50% was accepted as unknown.

The DPPH$^\bullet$ test

2.8 mg of a stable radical DPPH$^\bullet$ was dissolved in 100 ml of methanol. Then the absorbance was read at λ = 516 nm, and examined whether not exceed 1. The sample was a 3 ml solution (DPPH$^\bullet$ + antioxidant). The control sample and containing the extract samples was prepared. Solution of DPPH$^\bullet$ was protect against light. The measurement was performed in a Cary 300 Bio spectrophotometer at λ = 516 nm for a time t_0 and after adding the extract and 15 minutes of incubation to the stirrer. The samples were left under a cover.

$$\% \text{ Reduction} = [A_0 - A_A/A_0] \cdot 100\ \% \qquad [2]$$

Where: A_A - absorbance at λ = 516 nm, 15 minutes after the time in the presence of antioxidant; A_0 - absorbance at λ = 516 nm, after a period of 15 minutes without addition of antioxidant; The IC_{50}^{DPPH} (the concentration of antioxidant which reduces the free radical DPPH$^\bullet$ about 50%) parameter was determined using Microsoft Excel 2007. On linear graph section of the percentage of inhibition according the concentration of the extract a trend line equation: y = ax + b was determined. IC_{50}^{DPPH} was calculated by transforming the equation above and the expression x at which y-value is 50% was accepted as unknown.

The DPH test

A sample with a capacity of 2500 µl was obtained from 300 µl of PC liposomes with DPH probe fluorescencyją (l ,6-diphenyl-l ,3,5-heksatrien) and 2.2 ml of phosphate buffer (buffer A, pH = 7.4). The fluorescence intensity was measured at a wavelength $\lambda_{extinction}$ = 360 nm and $\lambda_{emission}$ = 425 nm in the fluorimeter SFM25 from Kontron Instruments Company. Measurement were made every 60 seconds from the time of reading the value of F_0, and after each measurement by adding a portion of the extract. The fluorescence intensities were read until the required decrease in fluorescence intensity of the test compound. Association constants was calculated using the method of Stern-Volmer, with the Verkman equation [1979]:

$$[1/(F_0/F_X) - 1] = [KD/[PC]] \cdot [1/[s]] + [1/[PC]] \qquad [3]$$

which maps the linear trend of graph of the expression: $[1/(F_0/F_x)-1]$ as a function of the inverse of the concentration of substances to be added, according to the equation: ***y = ax + b***, where: $[F_0/F_x]$ - relative fluorescence; K_D - dissociation constant; [s] - the concentration of antioxidant in the sample;

[PC] - the concentration of Phosphatidylcholine in samples equal to 0.12 mg/ml; Parameter for the association constant (Ka) assumed the inverse of the parameter K_D (dissociation constant), $Ka = 1/K_D$.

The CF test

To the column affixed gel, which was obtained by dissolving 2 g Sephadex ® G-50 in 30 µl of buffer TrisHCl (pH 7.4). Than deposited the solution liposomes with Carboxyfluorescein (CF) and washed TrisHCl buffer. After 10 minutes separated the free Carboxyfluorescein fraction from liposomes (1.5 ml) which contained a CF marker closed. The mixture was diluted 10x with TrisHCl buffer.

The sample consisted 50 ml of dilute solution of liposomes with Carboxyfluorescein and a known quantity of antioxidant. Then supplemented TrisHCl buffer to a volume of 2.5. Blank sample contained a mixture of liposomes with Carboxyfluorescein and TrisHCl. The fluorescence intensity was measured at a wavelength $\lambda_{extinction} = 490$ nm and $\lambda_{emission} = 520$ nm in the fluorimeter SFM25 Kontron Instruments Company by 10 minutes. The remaining amount of Carboxyfluorescein was released by the breakdown of liposomes using a 10% solution of Triton X-100 (20 µl). Relative increase in fluorescence Carboxyfluorescein was calculated by the formula:

$$\text{Carboxyfluorescein release [\%] CF} = [Ft - F_0/F\infty] \cdot 100\ \% \qquad [4]$$

Where: F_0 - initial fluorescence tests; F_t fluorescence after incubation time t; $F\infty$ - final fluorescence after addition of Triton.

Results and discussion

TEST COMPOUNDS	Values of parameter IC_{50}^{PC} [mg/L]	Values of parameter IC_{50}^{DPPH} [mg/L]	Values of parameter $1/K_D$ [L/mg]	Estimated value of CF leakage for parameter IC_{50}^{PC} [%]
***Hypericum* L.**	25,4	3,5	709	1,72
buckwheat – hulls	20,8	7,4	981	1,95
buckwheat – stems	29,1	6,9	729	10,90
hawthorn – cortex	21,6	3,0	896	0,20
hawthorn – leaves	26,8	2,7	868	0,24
helichrysum	121,6	34	999	1,50

Tab. 1. Summary results of the test extracts, for the antioxidative test, antiradical test, DPH test and CF test.

Antioxidant activity

The inhibitory effects of buckwheat, hawthorn, *Hypericum* L. and helichrysum on the rate of UVC irradiation peroxidation are illustrated in Fig. 1 – 6. The extracts of *Hypericum* L. (Fig. 1), buckwheat hulls (Fig. 2), buckwheat stems (Fig. 3), showed higher antioxidant activity in relation to rutin (reference substance). Similar effect was obtained for hawthorn (cortex and leaves, Fig. 4 – 5) in relation to epicatechin. The obtained results may suggest the possibility of a synergistic action of the different fractions of extracts, due to their diverse structures and related properties [Foti i wsp. 1996, Sokół-Łętowska et al, 2002-2004]. Another effect was observed in an extract from the helichrysum (Fig. 6), which showed significantly lower activity than quercetin and chlorogenic acid, and the lowest activity among all tested compounds. The efficacies of these compounds as antioxidants were evaluated as the IC_{50}^{PC} (PC = Phosphatidylcholine liposomes) parameter. The IC_{50}^{PC} parameter (Inhibition Concentration 50 % PC) indicates the concentration of antioxidant which reduces peroxidation intensity of Phosphatidylcholine liposomes about 50% [mg/L]. The results are shown in Fig. 7 and in Table 1. The values of IC_{50}^{PC} are following: buckwheat – hulls (20,8 mg/L) ≥ hawthorn – cortex (21,6 mg/L) > *Hypericum* L. (25,4 mg/L) > hawthorn – leaves (26,8 mg/L) > buckwheat – stems (29,1 mg/L) >> helichrysum (121,6 mg/L).

The DPPH$^{\bullet}$ test

The DPPH$^{\bullet}$ (1,1-diphenyl-2-pircylhydrazyl) free radical test is usually used in measurements of antiradical activity. Figures 8 - 13 show the percentage reduction of DPPH$^{\bullet}$ radical on depending to the concentration of the obtained extracts. The antiradical properties of these compounds were evaluated as the IC_{50}^{DPPH} - the concentration of antioxidant which reduces the free radical DPPH$^{\bullet}$ about 50% (Fig. 14). The extracts of buckwheat (hulls and stems, Fig. 9 – 10) and hawthorn (cortex and leaves, Fig. 11 – 12) obtained a lower ability of reducing free radicals in relation to their standards (rutin, epicatechin). These results do not confirm a similar sequence of antioxidant activity obtained for PC liposomes peroxidation. The activity of both extracts of buckwheat were almost the same. A similar ability of scavenging free radicals in comparison to the antioxidant activity demonstrated the extracts of *Hypericum* L. (Fig. 8) and helichrysum (Fig. 13). The sequence of antiradical activity of test substances is as follows: hawthorn – leaves (2,7 mg/L) > hawthorn – cortex (3,0 mg/L) > *Hypericum* L. (3,5 mg/L) > buckwheat – stems (6,9 mg/L) > buckwheat – hulls (7,4 mg/L) > helichrysum (34 mg/L). These values for the majority of extracts are within the range 2,7 mg/L – 7,4 mg/L. Only the helichrysum extract received a significantly worse result (34 mg/L,), which was more than 12,5 times higher than the extract of hawthorn.

The DPH test

Determination the possibility of association studied extracts to liposomes membrane was made using the DPH (l,6-diphenyl-l,3,5-hexatriene) method. The efficacies of studied extracts were evaluated as the degree of inhibition of the fluorescence intensity on the probe DPH. Association constants was calculated using the method of Stern-Volmer. It is expected that higher values of association constants (1/KD) may correlate with better protection of membranes against free radical attack, penetrating the membrane. The values of $1/K_D$ of the

test extracts are ranged in 709 – 999 L/mg. Analysis of the results for extracts from buckwheat (hulls and stems) show a weak correlation between their antioxidant activity and the intensity of binding to the membranes of liposomes PC. It should be noted, that it is difficult to discuss the differences between the values $1/K_D$ for the studied extracts, because they are of the same order. By contrast, *Hypericum* L. despite the relative good antioxidant activity in relation to the oxidation of lipid membranes (25.4 mg / L) and very good activity against radical $DPPH^{\bullet}$ (3.4 mg / L), relatively less tied to the surface of liposomes (709 L / mg). This observation suggests, that the protection of the liposomal membranes against UVC irradiation is mainly the result of scavenging a hydroxyl radical, which arises under the influence of UV light. Also, the literature subject points often to the ability of scavenging reactive oxygen species by flavonoids and polyphenols extracts. Scavenging mechanism were demonstrated by Benedí et al. [2004] on the example of reduction oxidative damage by *Hypericum* L. polyphenols extract in the brains of rats. The results for hawthorn leaves extract has shown repeat sequences results from the antioxidant activity and complexation constant value, but remained in negative correlation with the $DPPH^{\bullet}$ test results. For the cortex of hawthorn, the sequence of activities in the experiments was very similar. This suggests the existence of many different factors affecting the mechanism of antioxidant activity of extracts, which are a mixture of different substances. In summary, all of the tested extracts except helichrysum have value of IC_{50}^{DPPH} parameter in the same order of magnitude. This suggests one of the possibility mechanisms of antioxidant action the most of extracts (except helichrysum) as scavengers of free radicals.

The CF test

The last stage of this study was to determine the negative impact of present the extracts on the lipid phase of membranes. This objective were realized by using Carboxyfluorescein (CF), they used as marker for the percentage leakage of the content in the presence various concentration of extracts. The results shown in Table 1 and Fig. 15 presents the percentage leakage of Carboxyfluorescein from liposomes obtained for the concentration of antioxidants for witch got the values of parameter IC_{50}^{PC}. The results show, that percentage leakage of Carboxifluorescein didn't cross the 2 % for the most extracts. Only for extract from buckwheat stems reached the value 10,90 % and exceeded more than 54 times the value obtained for the leaves of hawthorn. The order of the results is as follows: hawthorn – leaves (0,20 %) > hawthorn – cortex (0,24 %) > helichrysum (1,50 %) > *Hypericum* L. (1,72 %) > buckwheat – hulls (1,95 %) >> buckwheat – stems (10,90 %). The results show, that compounds in general (except buckwheat stems) do not trigger malicious actions on the PC membrane liposomes.

Summary

The tested polyphenols extracts from *Hypericum* L., buckwheat, and hawthorn were similar, slightly different from each other, the high antioxidant activity compared to the liposomal membranes, which oxidized with UV radiation. The extract of helichrysum was over five times weaker antioxidant. Determination of antioxidant activity of flavonoids as a reference substance for the extracts, as compared to liposomes showed that in general (except for quercetin) their lower capacity to protect membranes against peroxidation than extracts. High antiradical properties (in relation to free radical $DPPH^{\bullet}$) tested extracts (except helichrysum), and a similar sequence of activity as the antioxidant properties of *Hypericum* L., buckwheat (stems) and hawthorn (leaves), suggests one possible mechanism of action, as scavengers of free radicals. The designated values of the constant association of extracts with the liposomal membrane were in the same order value for all extracts and may suggest a role protect the membrane from RFT. The degree of leakage of Carboxyfluorescein from liposome membranes under the influence of tested polyphenols are generally not exceed 2% (except for extract of buckwheat stems) do not trigger malicious actions on the PC membrane liposomes. The obtained results demonstrate possibility of using them (except Helichrysum) as antioxidants in food and pharmaceutical or cosmetics industry.

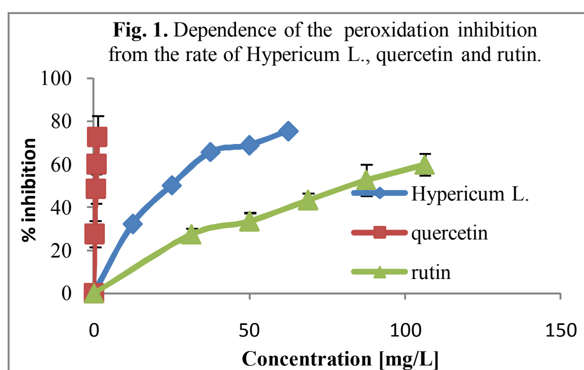

Fig. 1. Dependence of the peroxidation inhibition from the rate of Hypericum L., quercetin and rutin.

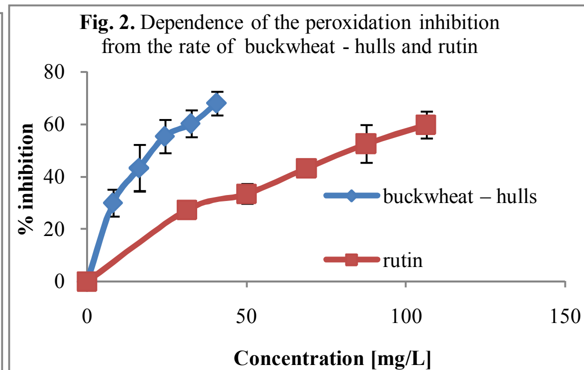

Fig. 2. Dependence of the peroxidation inhibition from the rate of buckwheat - hulls and rutin

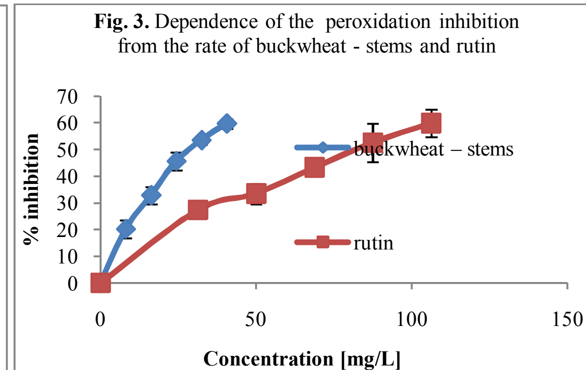

Fig. 3. Dependence of the peroxidation inhibition from the rate of buckwheat - stems and rutin

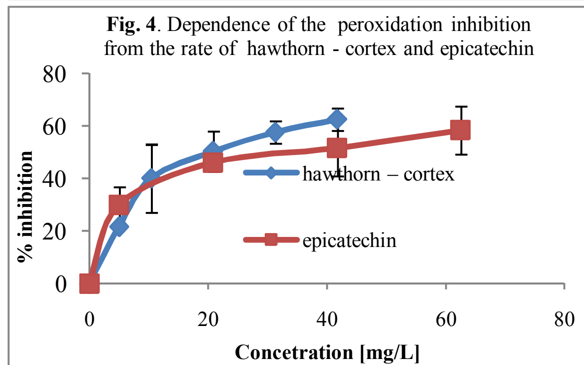

Fig. 4. Dependence of the peroxidation inhibition from the rate of hawthorn - cortex and epicatechin

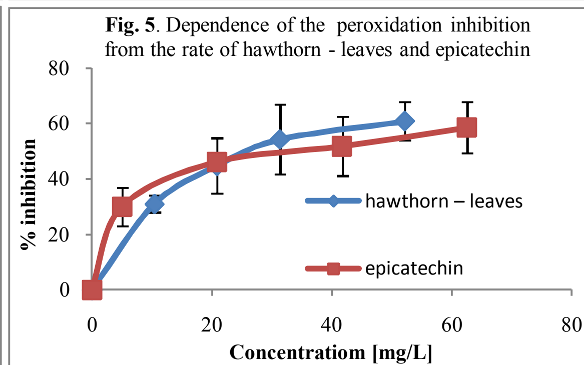

Fig. 5. Dependence of the peroxidation inhibition from the rate of hawthorn - leaves and epicatechin

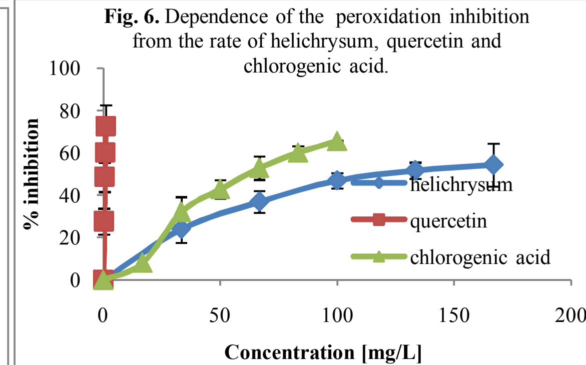

Fig. 6. Dependence of the peroxidation inhibition from the rate of helichrysum, quercetin and chlorogenic acid.

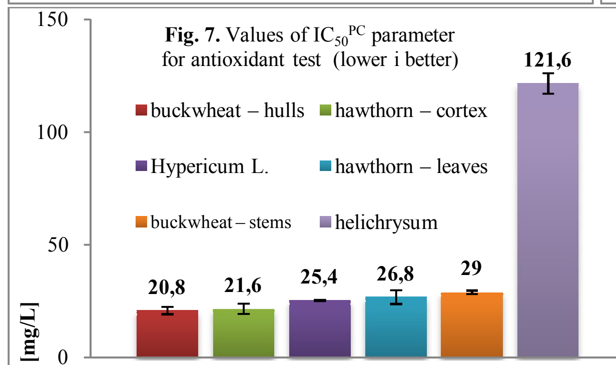

Fig. 7. Values of IC_{50}^{PC} parameter for antioxidant test (lower i better)

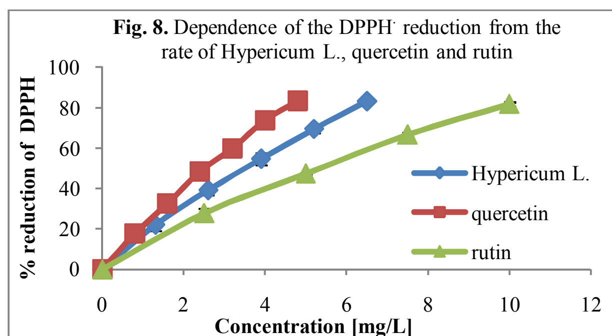

Fig. 8. Dependence of the DPPH· reduction from the rate of Hypericum L., quercetin and rutin

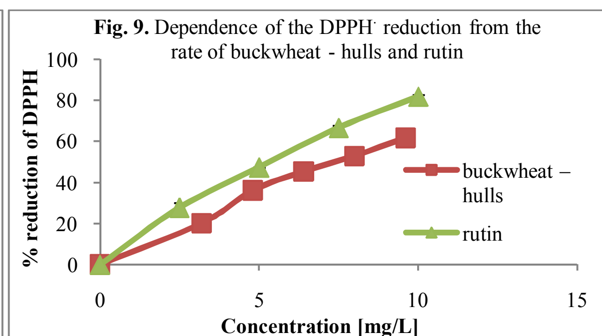

Fig. 9. Dependence of the DPPH· reduction from the rate of buckwheat - hulls and rutin

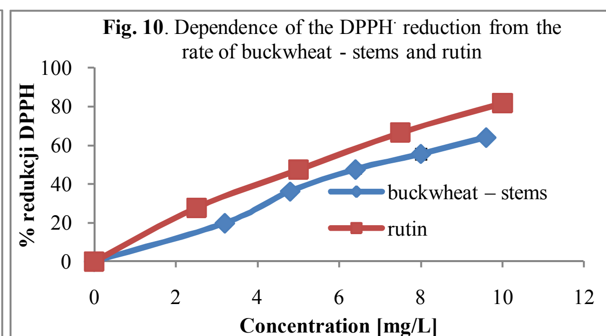

Fig. 10. Dependence of the DPPH· reduction from the rate of buckwheat - stems and rutin

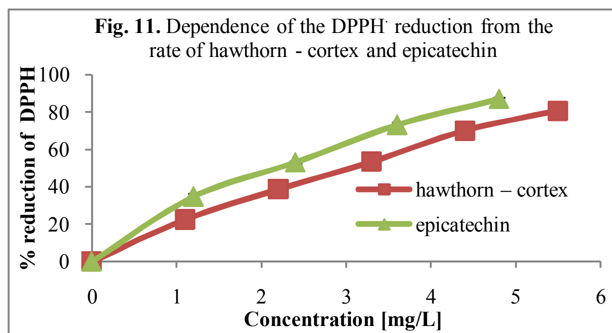

Fig. 11. Dependence of the DPPH· reduction from the rate of hawthorn - cortex and epicatechin

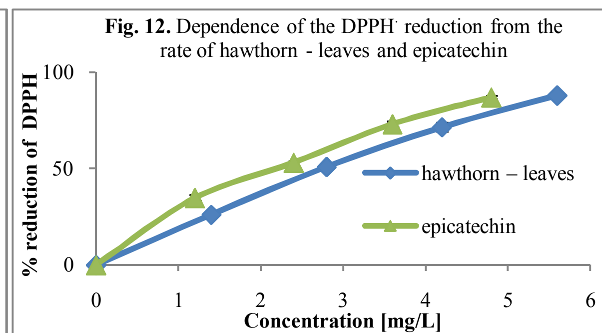

Fig. 12. Dependence of the DPPH· reduction from the rate of hawthorn - leaves and epicatechin

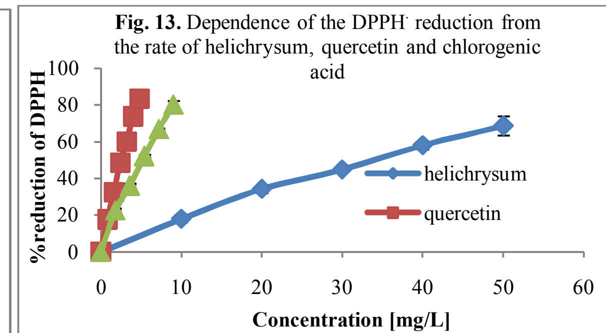

Fig. 13. Dependence of the DPPH· reduction from the rate of helichrysum, quercetin and chlorogenic acid

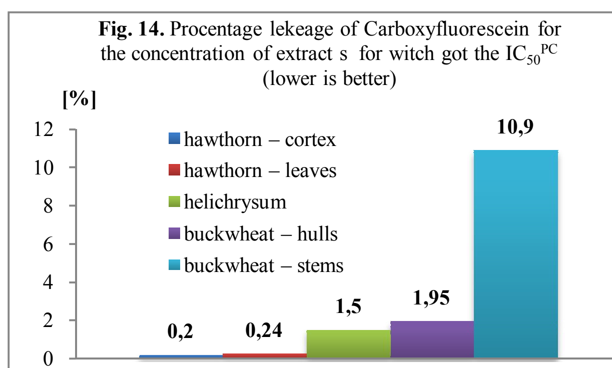

Fig. 14. Procentage lekeage of Carboxyfluorescein for the concentration of extract s for witch got the IC_{50}^{PC} (lower is better)

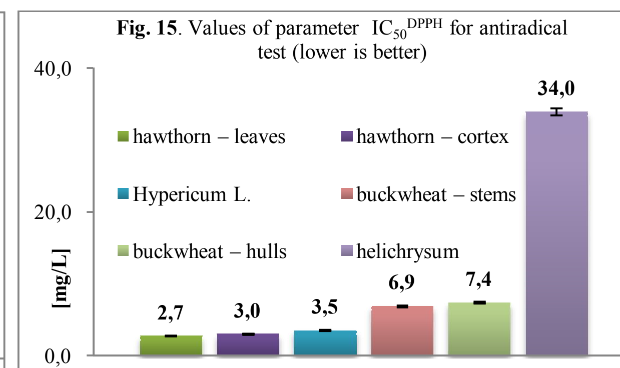

Fig. 15. Values of parameter IC_{50}^{DPPH} for antiradical test (lower is better)

Benedí J., Arroyo., Romero C., Martín-Aragón S., Villar A. M.; 2004; Antioxidant properties and protective effects of a standardized extract of Hypericum perforatum on hydrogen peroxide-induced oxidative damage in PC12 cells, Life Sciences 75; 1263:1276

Dolatowski Z. J., Dudek M., Budoran M.; 2004; Nasiona gryki w produkcji dietetycznych wyrobów miesnych, Annales UMCS, Sec. E, 59; 1607:1612;

Dolatowski Z. J., Dudek M.; 2006; Trwałość barwy modelowego wyrobu mięsnego z częściową wymianą tłuszczu nasionami gryki, Acta Agrophysica, 8(1), 35:42;

Dolatowski Z. J., Dudek M.; 2006; Właściwości fizyczne i funkcjonalne wybranych preparatów nasion gryki jako dodatku do wyrobów mięsnych, Inżynieria Rolnicza 7/06; 101:109;

Evans P., Halliwell B.; 1999; Free Radicals and Hearing, Cause, Consequence, and Criteria, Annals New York Academy of Sciences; 19:40;

Ferrari C.K.B, Torres E.A.F.S.; 2002; Biochemical pharmacology of functional foods and prevention of chronic diseases of aging; Biomedicine & Pharmacotherapy; 251:258;

Foti M., Pitatelli M., Baratta M. T., Ruberto G.; 1998; Flavonoids, Coumarins, and Cinnamic Acids a Antioxidants im a Micellar System. Structure-Activity of Different Phenolic Fractions Separated from an Italian Red Wine, J. Argic. Food Chem, 46; 361:376;

Gawlik-Dziki U.; 2004; Fenolokwasy jako bioaktywne składniki żywności, Żywność. Nauka. Technologia. Jakość, 4(41)S; 29:40;

Howard A., Chopra M.,. Thurnham D. I,. Strain John J, Fuhrman B., Aviram M.; 2002; Red wine consumption and inhibition of LDL oxidation: what are the important components?, Medical Hypotheses 59(1); 101:104,

Kerr M. E., RN, Bender C. M., RN, Monti E. J.; 1996; "An introduction to oxygen free radicals"; Heart & Lung Vol.: 25, No. 3; 200:208;

Lemberkovics É., Czinner E., Szentmihályi K., Balázs A., Szöke É.; 2002; Comparative evaluation of Helichrysi flos herbal extracts as diet ary sources of plant polyphenols, and macro- and microelements, Food Chemistry 78; 119:127;

Miller E., Malinowska Katarzyna, Gałęcka E., Mrowicka M., Kędziora J.; 2008; Rola flawonoidów jako przeciwutleniaczy w organizmie człowieka; Pol. Merk. Lek., XXIV; 144, 556;

Mulinacci N., Giaccherini C., Santamaria A. R., Caniato R., Ferrari F., Valletta A., Vincieri F. F., Pasqua G.; 2008; Anthocyanins and xanthones in the calli and regenerated shoots of Hypericum perforatum var. angustifolium (sin. Fröhlich) Borkh; Plant Physiology and Biochemistry 46; 414:420;

Orhan B., Kartal M., Őzdeveci B., Duman H.; 2007; HPLC Quantification of Vitexine-2"-O-rhamnoside and Hyperoside in Three Crataegus Species and Their Antimicrobial and Antiviral Activities, Chromatographia Supplement Vol. 66; 153:157;

Pobłocka-Olech L., Krauze-Baranowska M.; 2007; Aktywność farmakologiczna chalkonów, Postępy Fitoterapii 4/07; 194:201;

Rajendran S., Deepalakshmi P. D., Parasakthy K., Devaraj H., Devaraj N. S.,; 1996; Effect of tincture of Crataegus on the LDL-receptor activity of hepatic plasma membrane of rats fed an atherogenic diet, Atherosclerosis 123; 235:241;

Rechciński T., Kurpesa M.; 2003; Przydatność preparatów głogu w leczeniu niewydolności serca, Forum Kardiologów; 8, 1, 27:28;

Sánchez-Reus M. I. , Gómez del Rio M. A., Iglesias I., Elorza M., Slowing K., Benedí J.; 2007; Standardized Hypericum perforatum reduces oxidative stress and increases gene expression of antioxidant enzymes on rotenone-exposed rats, Neuropharmacology 52; 606:616;

Škerget M., Kotnik P., Hadolin M., Rižner Hraš A., Simonic M., Knez Željko; 2005; Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities, Food Chemistry 89; 191:198;

Sokół-Łętowska A., Jarosławska A., Oszmiański J., Graszkiewicz M.; 2002; Aktywność przeciwrodnikowa preparatów polifenolowych i ich mieszanin, Flawonoidy i ich zastosowanie, IV Konferencja, Rzeszów 2002; 159:165;

Sokół-Łętowska A., Jarosławska A., Oszmiański J.; 2004; Aktywność przeciwrodnikowa polifenoli głogu i tarczycy oraz ich frakcji w układach modelowych; Flawonoidy i ich zastosowanie, V Konferencja, Rzeszów 2004; 287:298;

Urbonavičiūtė A., Jakštas V., Kornyšova O., Janulis V., Maruška A; 2006; Capillary electrophoretic analysis of flavonoids in single-styled hawthorn (Crataegus monogyna Jacq.) ethanolic extracts, Journal of Chromatography A 1112; 339:344;

Verkman A. S.; 1979; The Quenching of an intermembrane fluorescent probe phloreti through bilayers, Biochimica et Biophysica Acta, 599; 370:379;

Wojcieszyńska D., Wilczek A.; 2006; Związki fenolowe pochodzenia naturalnego, Nauka i technika; 6:12;