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INFLUENCE OF THERMAL TREATMENT ON TANNIN CONTENT AND ANTIOXIDATION EFFECT OF OAK ACORN *QUERCUS CERRIS* EXTRACT

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Abstract: The results of investigation of the tannin content in the oak acorn kernel *Quercus cerris*, qualitative analysis of tannin and antioxidation effect of ethanol extracts of acorn on porcine lipid (prime steam lard) as a substrate are presented in this paper.

Experiments were carried out on kernel samples of the domestic oak acorn, from the location Zaglavak, near by the town of Bajina Bašta. Tannin content was determined by spectrophotometric procedure using phosphorus Wolframic acid on wave length of 715 nm. Qualitative analysis of tannin included sediment and stain responses as well as tannoform test with formalaldehyde and HCl. Antioxidant effect of ethanol extracts was investigated on fat samples treated at the temperature of 60^0C in the dark (Schaaloven test). The rate of oxidation was determined by measuring Peroxide value (Pb) and TBA value . The investigated extracts were obtained based on drying of acorn kernel and extracts based on thermal treatment – dry frying of acorn kernel. The obtained results show that dried acorn kernel contains 11.69 % of tannin and thermally treated acorn kernel 8.55%. Qualitative analysis confirmed the presence of gallic acid (pyrogallic, hydrolysing) tannins based on positive general sediment and stain responses on tannins. Ethanol extracts demonstrate antioxidation traits on porcine lipids in trial conditions. Synergistic effect of citric acid with primary anti oxidant was not proved. Thermal treatment of acorn kernel does not reduce the antioxidation activity of extracts.

Key words: oak acorn, tannins, ethanol extract, antioxidation action, porcine lipids, TBA, peroxide value.

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Introduction

Plant materials are very complex with regard to their composition. Some of them contain substances that demonstrate protective, antioxidation abilities. The best-known and very frequently the most efficient antioxidants in food are phenol and poly phenol substances (C h i p a u l t, 1962 ; B i s h o v and H e n i c k, 1977.). Different spice plants, oil plant seeds etc. were used as a source of natural antioxidants. Some of them were used over centuries because of their antioxidation activity, whether or not it was known at that time. Based on scientific achievements, demands of a modern consumer and from the standpoint of food producer, some natural antioxidants have been commercialised and used in nutrition, such as rosemary extracts (*Rosmarinus officinalis)*, E vitamin, β-carotene, etc. (H u d s o n, 1985; J u r g L o l i g e r, 1991). Good selection of dissolution and extraction procedure result in the concentration of substances with antioxidation effect, whereby the protection effect is considerably achieved.

Fruit of oak tree has been traditionally used as food component. On the basis of dry fried and ground oak acorn kernel (*Quercus semen tostum)*, food and beverages are prepared that are used in medicine as astringents and antidiarrhoeals (T u c a k o v, 1996). There are data available in literature about antioxidation action of certain acorn components, first of all tannin (C h i o u, 1989; M i – H y u n , 1992 ; R a k i ć, 2000 and 2001).

Tannins are complex, poly-phenol, nitrogen free, amorphous and non-toxic compounds of acrid aroma that are present in plants as defence mechanisms against the action of parasites. Tannins are compounds soluble in water and of molecular mass of 500 (1000)-3000. The application of tannins in medicine is based on their astringent as well as anti bacterial and fungicidal action. Effects of tannins on animal and human cells and tissues are related to their ability to chemically react with albumen/proteins and build insoluble complexes, which is manifested by precipitation of proteins in superficial layers (astringent effect). Antiseptic effect of tannins is conditioned by their poly-phenol character. The role of tannins in plants is multiple: they prevent the decay of plant tissue, they are used as reserve substances for seed in germination, they are transporters of carbohydrates through the plant as tannin glycosides, etc. Recent researches relating to the action and use of tannin indicate that tannins possess anti herpes and cytotoxic effect *in vitro* on carcinoma cells of uterus and nasal pharynx. They also have antioxidation effect (K o v a č e v i ć , 2000; G o r u n o v i ć, 2001). Tannins are present in bark and fruits of various oak types. In chemical sense, tannins are known as tannin acid, gallous tannin and gallous tannin acid, which is very complex, and they can be divided into two groups: a) Condensed tannins, derivatives of flavone, they can be 4.8 or 2.8 C-C dimers or 3.3-ethereouslly connected catechin dimers and related compounds. Condensed tannins are sometimes considered as polymer flavonoids; b) Hydrolysing tannins, the most

important group, are sugar esters, usually glucose, with mono or poly trihydroxyl benzene carboxyl acid, including gallous tannins and elagitannins, more exactly polymers of gallic acid and elagic acid (J o s l y n and M a c K i n n e y, 1938 ; J o n e s et al., 1963; M e r c k , 1976; F r a n c i s, 1985).

In certain cases, the increase in the persistence of substrate is achieved when, in addition to antioxidant, adequate synergist is used. Different acids (organic and non-organic) and their derivatives can act as synergist in combination with primary antioxidant. Citric acid is most often used as synergist. Synergism is reflected in the increase of anti oxidation action of the primary antioxidant (C o p e n, 1983; N i k e, et al., 1984).

The aim of this investigation was to determine the tannin content in oak acorn kernel, also a type of tannin, to evaluate the anti oxidation value of ethanol extracts from the samples of native oak acorn kernel, and to investigate the synergy effect of the primary antioxidant with citric acid. Special attention is directed towards the pre-treatment of initial material in order to improve the application of this raw material, which would introduce to the food the components of biologically active effect as well as nutritious substances.

Material and Methods

In carrying out this trial the oak acorn of cultivar *Quercus cerris* belonging to the family *Fagaceae* was used. Fruits were collected from the ground during October, and only good fruits, without mechanical or any other damages, from the location Zaglavak–Rastik, near the town of Bajina Bašta. The total of 10 kg of acorn was collected. Considering high water content (over 35%) samples were dried in a dryer with increased ventilation at 70^0C during 12 hours.

Thermal treatment was carried out using procedure of "dry frying" at the temperature of 200^oC during 15 minutes, after the sample had been ground in mortar. Preparation of ethanol extracts was carried out on dried and ground as well as thermally treated sample previously ground in a laboratory mill MLU 202 (Buhler – Switzerland), size of particles was 0.2 mm.

Tannin content was determined by spectrophotometric procedure with phosphorus wolframic acid according to the method described by Yugoslav Farmacopy (Books I and III, 2000.). Reading of values was done with spectrophotometer JENWAY B6105 UV/Vis on wave length of 715 nm.

Considering the complexity and significance of tannin, especially of anti oxidation traits attributed to them, qualitative analysis was also carried out, or more exactly, proving responses of tannin deriving from oak acorn. Responses were sediment (with water gelatine solution), stain (tannin analysis with $FeCl_3$) as well as tannoform test (formaldehyde – HCl) done according to methods of G o r u n o v i ć and L u k i ć (1995).

Thiobarbiturate test (TBA) is a sensitive method adequate for the evaluation of the intensity of secondary reactions of self-oxidation. Oxidation products of

unsaturated fatty acids, mainly of linolenic acid, give stained responses with thiobarbituric acid. TBA number was determined by reading the extintion value $\left(E_{1cm}^{1\%}\right)$ on 532 nm (P o k o r n y, et al.,1987). The same apparatus was used in this case as for determining of tannin content.

Ethanol extract was prepared on the basis of dried and ground kernel of oak acorn *Quercus cerris.* Firstly, the extraction according to Soxchlet in petrol-ether was caried out, and subsequently the extraction with ethanol. Based on mass ratio of active component in extract, trials with porcine lipids at the temperature of 60°C were designed. In certain time intervals TBA extintion value and peroxide value were determined. Determination of oxidation stability of lipids at 60°C was carried out by Schaal-Oven test for monitoring of peroxide formation as oxidation measure at moderate temperatures. Monitoring of the peroxide progress was done according to the method by Wheeler, modified by Hadorn and associates (S h e r w i n, 1985). Extract concentrations from 0.02 to 0.06% were investigated, since these are the values common for commercial antioxidants addition.

Lipid samples of 10 g were measured in glass containers - diameter of 45 mm and 55 mm of height, covered with one side of Petri dish and exposed to the temperature of 60°C in darkness, followed by determination of TBA extintion value and peroxide value on treated sample in certain time intervals.

Porcine lipid was used as a substrate and a control sample was prepared according to moist procedure (without additives). Procedure for the preparation of extracts was adopted from previously published papers (R a k i ć, et al. 1996; M i –H y u n L e e, et al., 1992) .

The obtained experimental data were processed by the method of descriptive statistics (H a dž i v u k o v i ć, 1991).

Results and Discussion

Results obtained by qualitative analysis of tannin from oak acorn *Quercus cerris* are presented in Table 1. Based on these results, the presence of gallous (pyrogallous, hydrolysing) tannins on the basis of positive general sediment and stain responses to tannins was determined.

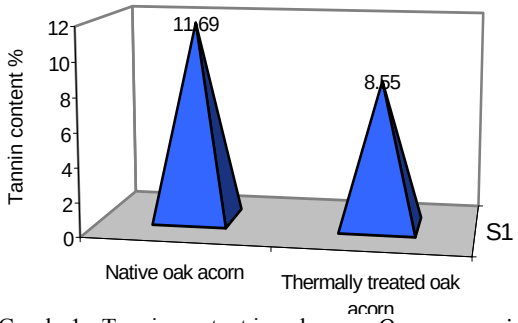
T a b. 1. - Presence of tannin in oak corn *Quercus cerris*

	Tannins	
Reactives	Native oak corn	Thermally treated oak corn
Gelatine	+*	+*
$FeCl_3$	Blue stain	Blue stain
Tannoform test (formaldehid-HCl)	-	+

+* - presence of stirring - sediment

Tannins as phenol, or poly-phenol, derivatives, and due to the presence of phenol groups, provide stain response with iron salts. The analysis of tannin with $FeCl_3$ developed blue stain in all samples thus indicating the presence of gallous tannins. The presence of tannins in oak acorn was confirmed based on positive general sediment responses. The reaction with water gelatine solution created stirring. The result of tannoform test is negative in case of non-thermally treated samples. In case of thermally treated samples, slight brown sediment appeared. This sediment is most probably the product of condensation of gallic acid and formaldehyde, or more exactly, gallic acid was released by hydrolysis during thermal treatment. Sediments of tannoform were filtered and with further analysis procedure purple-blue ring was formed confirming the presence of gallous (pyrogallous, hydrolyzing) tannins.

Considering the role and importance of tannin, the objective of our research was to determine its content in oak acorn kernel and define the potential effect on antioxidation traits. In graph 1 the results relating to tannin content in investigated oak acorn samples *Quercus cerris* are presented.



Graph. 1.- Tannin content in oak acorn *Quercus cerris*

The obtained results show that tannin content in native oak acorn was 11.69%, whereas in thermally treated acorn content was lower by 3.14%, the value determined was 8.55%. During the thermal treatment procedure of plant material a complex group of influences is present and new compounds are formed, hydrolysis of substances where the mentioned compounds were linked also occurs, etc. The most important reactions are hydrolysis, oxidation, polymerisation, interaction of composition and reactions of thermal decomposition (D j o r d j e v i ć, 1995) due to which registering of reduced tannin content is possible. The results regarding tannin content in native oak acorn obtained by M i-H y u n-L e e, 1992, of 6%, in relation to tannin content value in oak acorn of domestic origin is lower, which is explained as a consequence of the origin and type of analysed samples.

Based on determined tannin content in oak acorn kernel and results of qualitative analysis of tannin, we assume that carriers of anti oxidation effect are exactly tannins. The investigation of antioxidation action of ethanol extracts of kernel (EtJ) and ethanol extract of thermally treated kernel (EtTtJ) of oak acorn is presented in Tables 2 and 3. Changes of TBA extintion value $\left(E_{1cm}^{1\%}\right)$ on 532 nm and peroxide value in function of time were monitored on lipid samples (initial TBA extintion value and Pv was 0) into which 0.02% of dry matter of ethanol extracts (EtJ i EtTtJ) of oak acorn was introduced.

T a b. 2. - Change of TBA extintion value $\left(E_{1cm}^{1\%}\right)$ of porcine lipid using extracts of oak acorn *Quercus cerris* on 60°C

	Value of TBA extintion $\left(E_{1cm}^{1\%}\right)$ on 532 nm									
	Days of thermal treatment on 60°C									
	0	1	2	3	4	6	7	8	9	10
Type of sample										
Control	0	0.131	0.463	0.805	1.364	-	-	-	-	-
0.02% EtJ	0	-	-	-	-	0.74	1.08	-	-	-
0.02**%** EtTtJ	0	-	-	-	-	-	0.075	0.266	0.594	1.284

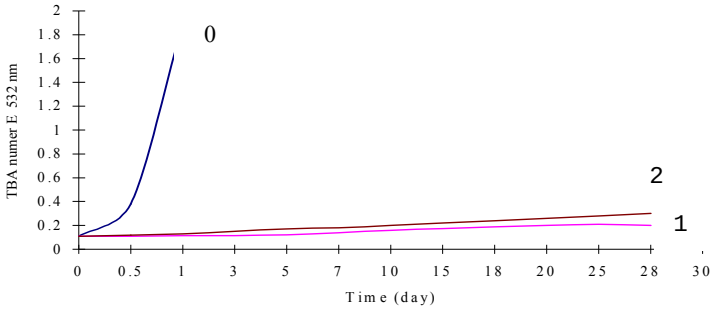
T a b.3. - Change of peroxide value of porcine lipid using extracts of oak acorn *Quercus cerris* on 60°C

	Peroxide value mmol/kg									
	Days of thermal treatment on 60°C									
	0	1	2	3	9	10	11	12	14	15
Type of sample										
Control	0	1.2	3.1	11	-	-	-	-	-	-
0.02% EtJ	0	-	-	-	1.3	2.1	3	8.1	32	-
0.02% EtTtJ	0	-	-	-	-	-	1.8	2.0	3.5	3.8

Based on the results of determination of TBA extintion value $\left(E_{1cm}^{1\%}\right)$ on 532 nm and peroxide value, the maintenance of lipids depends on the addition and type of ethanol extract of oak acorn. Samples of lipid with added 0.02% of ethanol extract of oak acorn kernel EtJ increase the maintenance of lipids compared to control sample. Extract from thermally treated kernel EtTtJ added in

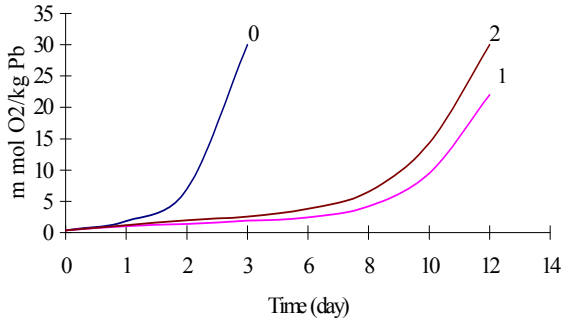
concentration of 0.02% demonstrated higher activity than EtJ, which indicated the positive effect of thermally treated oak acorn sample. In his investigations related to this topic M i – H y u n - L e e, 1992, discovered gallic acid as ingredient of the extract from oak acorn within tannin fraction whose protective traits are investigated and antioxidation effect confirmed.

In the second part of the trial the persistence of porcine lipid samples with addition of ethanol extract of thermally treated (EtTtJ) oak acorn kernel and ethanol extract of oak acorn kernel (EtJ) was investigated, and citric acid as a potential synergist. The effect of synergist is reflected in the increase of antioxidation action of primary antioxidant. Oxidation progress of samples of porcine lipid as a substrate with and without citric acid was monitored by determination of peroxide value and TBA extintion value $\left(E_{1cm}^{1\%}\right)$ on 532 nm. The results are presented in Graphs 2 and 3.



0 –Control sample 1 -Lipid+0.06% EtTtJ 2 - Lipid+0.04% EtTtJ +0.015 L.K.

Graph. 2.- Effect of EtTtJ of oak acorn *Quercus cerris* and combination with citric acid on persistence of porcine lipid according to TBA test on 60°C



0 - Control sample 1 - Lipid+0.06% EtJ 2 - Lipid+0.04% EtJ +0.015 L.K.

Graph. 3.- Effect of EtTtJ of oak acorn *Quercus cerris* and combination with citric acid on persistence of porcine lipid according to Pv on 60°C

Based on the results obtained by TBA extintion value test $\left(E_{1cm}^{1\%}\right)$ on 532 nm (Graph 2), it can be concluded that maintenance of investigated samples depended on added ethanol extract from thermally treated oak acorn kernel EtTtJ and that these changes of TBA value did not occur after 15 days. The results regarding determination of peroxide value (Pv) on substrate with ethanol extract of oak acorn kernel EtJ (Graph 3) have also confirmed the dependence of its addition on the maintenance of the substrate and that the significant change of Pv did not occurred after 7 days.

Protection factor representing the quotient of substrate maintenance with and without the addition of antioxidant for sample 1 from Graph 3 is 6, and for sample 2 5.7, which means that analyzed sample of lipids containing EtJ has by 6 and sample EtJ+LK by 5.7 times higher maintenance compared to control sample. Protection factor for Graph 2 could not be determined for samples 1 and 2 because after the period of 15 days no change in TBA value was registered indicating high antioxidation effect of extracter in given conditions. Synergy effect of combination of primary antioxidant (EtTtJ i EtJ) and synergist (citric acid) could not be determined. The results of this experiment indicate that synergy effect of citric acid in combination with EtTtJ and EtJ does not exist.

Conclusion

The results obtained show that oak acorn *Quercus cerris* contains 11.69% of tannin. Thermal treatment affects the tannin content and causes reduction of its content to 8.55%, loss being by 3.14%. Based on qualitative analysis of tannin from oak acorn, the presence of gallous (pyrogallous, hydrolysing) tannins was confirmed. The results of tannoforme test for the sample that was thermally treated indicate that sediment is probably the product of condensation of gallic acid and formaldehyde, or more exactly, gallic acid was released by hydrolysis during thermal treatment. Ethanol extract obtained based on thermally treated kernel of oak acorn by the procedure of "dry frying" at the temperature of 200^{o}C during 15 minutes, (EtTtJ) added in concentration of 0.02% to porcine lipids as analysed substrate demonstrates higher activity than EtJ in the same concentration, which further indicates the positive effect of thermal treatment of oak acorn samples. Based on induction period (IP) and calculated protection factor (PF) for analysed sample of lipids containing 0.06% EtJ has by 6 and sample EtJ+LK by 5.7 times higher maintenance compared to control sample. It was established that citric acid does not increase the antioxidation effect of extracts and has no synergy effect. Thermal treatment affects the content of tannin in such a way that it reduces it, however, it does not effect the decrease of antioxidation activity of extracts, which imposes the conclusion that antioxidation abilities of oak acorn are not lost during thermal treatment.

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UTICAJ TOPLOTNOG TRETMANA NA SADRŽAJ TANINA I ANTIOKSIDATIVNI EFEKAT EKSTRAKTA HRASTOVOG ŽIRA *QUERCUS CERRIS*

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R e z i m e

U radu su prikazani rezultati ispitivanja sadržaja tanina u jezgru hrastovog žira *Quercus cerris*, kvalitativna analiza tanina i antioksidaciono dejstvo etanolnih ekstrakata žira na svinjsku mast (pripremljenu vlažnim postupkom) kao supstratu.

Ogledi su vršeni na uzorcima jezgra hrastovog žira domaćeg porekla, sa lokaliteta Zaglavak, okolina Bajine Bašte. Sadržaj tanina određivan je spektrofotometrijskim postupkom sa fosforvolframovom kiselinom na talasnoj dužini od 715 nm. Kvalitativna analiza tanina obuhvatila je taložne i bojene reakcije kao i tanoformsku probu sa formaldehidom i HCl. Zaštitni efekat etanolnih ekstrakata ispitivan je na uzorcima masti koji su temperirani na 60^0C u mraku. Tok oksidacije masti praćen je određivanjem TBA $\left(E_{1cm}^{1\%}\right)$ vrednosti

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ekstincije na 532 nm i peroksidnog broja. Ispitivani ekstrakti su dobijeni na bazi osušenog jezgra žira i ekstrakata na bazi termički tretiranog - suvo prženog žira. Dobijeni rezultati pokazuju da osušeno jezgro hrastovog žira sadrži 11,69% tanina a termički tretirano 8,55%. Kvalitativnim analizama dokazano je prisustvo galnih (pirogalnih, hidrolizujućih) tanina na osnovu pozitivnih opštih taložnih i bojenih reakcija na tanine. Etanolni ekstrakti ispoljavaju antioksidaciona svojstva na svinjsku mast pri uslovima ogleda, sinergistički efekat limunske kiseline sa primarnim antioksidantom nije dokazan, termički tretman jezgra hrastovog žira ne smanjuje antioksidacionu aktivnost ekstrakata.

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