

## BUTTER STABILIZATION BY PLANT PHENOLIC ANTIOXIDANTS

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The aim of the study was to evaluate the effect of natural polyphenolic antioxidants isolated from the roots of skullcap (*Scutellaria baicalensis* Georgi) and procyanidins extracted from the bark of hawthorn (*Crataegus oxyacantha*) on butter during storage.  $\alpha$ -Tocopherol,  $\beta$ -carotene, fatty acids and cholesterol contents were analysed in butter stored for 28 days at a temperature of 4°C and 30°C in darkness as well as at 50°C with light (3600 - 3900 lx). Moreover, an increase in peroxide value was measured using thiocyanate method and the content of secondary oxidation products was analysed during storage with the thiobarbituric reactive substances (TBARS) methods. The highest increase in peroxide value and content of secondary oxidation products was observed for samples exposed to light and temperature. The flavones isolated from skullcap roots were characterized by significantly stronger antioxidant and photoprotection activity than procyanidins extracted from hawthorn bark. In butter samples with skullcap flavones added, the contents of  $\alpha$ -tocopherol and  $\beta$ -carotene, polyunsaturated fatty acids and cholesterol were higher than in control samples, despite the storage conditions. Moreover, ground skullcap roots incorporated in the butter showed antioxidant activity as compared to a highly purified commercial preparation of skullcap.

### INTRODUCTION

Food contains many constituents nutritious to humans which are connected together and dependent on one another. A daily diet provides multiple desirable ingredients but also a number of undesirable often toxic ones. Valuable food components are e.g. vitamins, proteins, polyunsaturated fatty acids (n-3 and n-6). Those constituents can be easily converted to undesirable components, due to, mainly, oxidation processes occurring during food processing and storage. The main factors initiating oxidative changes in food are light, high temperature, radiation and reactive oxygen species (ROS). As a result of the oxidation processes, primary and secondary oxidation products, toxic to consumer, are formed, and vitamins are degraded. One of the best ways to protect food against physical and chemical factors is the incorporation of antioxidants during food processing.

In the last few decades an intensive testing of the safety of synthetic food additives has been carried out and many of them have been found to display some toxic activity [Bandoniene *et al.*, 2000]. As a result, a search for natural substitutes, which in most cases are considered as GRAS (generally recognized as safe) substances, has increased considerably. The number of reports on the isolation and testing of natural, mainly of plant origin, antioxidants has increased during the last decade immensely. These attempts have led to the development of very effective natural antioxidants from rosemary and sage, which are now available commercially. A great number of different spices and aromatic herbs

have been tested for their antioxidant activity, however, there are still many plants that were not examined in this respect or knowledge about their antioxidative properties is very scanty. Skullcap (*Scutellaria baicalensis* Georgi) and hawthorn (*Crataegus oxyacantha*) are among such plants. They have been investigated from some other points of view, mostly in respect of their medicinal properties and flavonoid composition.

Skullcap is one of the most important medicinal herbs widely used for the treatment of various inflammatory and cancer diseases in East Asian countries such as China, Korea and Japan [Lai *et al.*, 2002; Chen *et al.*, 2000; Ishimaru *et al.*, 1995]. They have a variety of interesting activities such as anti-bacterial, anti-viral, anti-allergic, anti-HIV, anti-oxidant and free-radical scavenging [Lamer-Zarawska, 2003; Gao *et al.*, 1999]. Root of this plant has been reported to contain a large number (over 40) of flavonoids (more than 20% in dry weight), frequently found as glucuronides, and other constituents such as phenethyl alcohols, sterols, essential oils and amino acids. Baicalin, a flavone glucuronide, is the most predominant flavonoid whose content varies from 12-19% in the dry root. Other common flavonoids in *Scutellaria baicalensis* is baicalein, wogonin, wogonoside, oroxylin A, skullcapflavone I and II [Loon, 1997].

Hawthorn is a traditional European medical plant. Dried flowers, leaves and fruits of hawthorn are used as crude drugs. Several studies have shown that aqueous and alcoholic extracts have beneficial effects on the heart and blood circulation, including cardiovascular protective and hypo-

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tensive effects [Rohr *et al.*, 1999]. Oligomeric procyanidins are considered to be the main active constituents, in addition to flavone- and flavonol-type flavonoids. Procyanidins, known as condensed tannins including flavan-3-ol units, epicatechins and/or catechins, can be categorized as oligomeric procyanidins (OPs) consisting of 2-6 flavan-3-ol units, and polymeric procyanidins (PPs) consisting of more than six flavan-3-ol units [Svedström *et al.*, 2002a, b].

The objective of the study was to evaluate the effect of natural phenolic antioxidants from skullcap and hawthorn on butter constituents during storage at different conditions.

## MATERIAL AND METHODS

$\alpha$ -Tocopherol,  $\beta$ -carotene and fatty acid standards were purchased from Sigma, Chemical Co (St. Louis, Mo, USA). HPLC-grade solvents such as hexane, 2-propanol, methanol, acetonitrile, ethyl acetate, and chloroform were purchased from Merck (Darmstadt, Germany). Contents of  $\alpha$ -tocopherol,  $\beta$ -carotene, flavones of skullcap were determined using HPLC Merck-Hitachi L-7455 instrument (diode array detector) with pump L-7100. Procyanidins were analysed using the HPLC Waters system (Milford, MA) (DAD and Scanning Fluorescence detectors).

Butter was made from milk originating from a Jersey cow farm located in the West-Pomeranian Province. Skullcap and hawthorn antioxidants were added at a dose of 500 ppm during butter making. The samples of butter (50 g) were stored at three different conditions, *i.e.* in a refrigerator ( $4\pm 2^\circ\text{C}$ ) and in an incubator ( $30\pm 2^\circ\text{C}$ ) in darkness, and in an incubator ( $50\pm 2^\circ\text{C}$ ) with exposure to light (3600-3900 lx) using lamps (de Luxe TLD 18W/96, Philips) at a distance of about 50 cm. Storage conditions used in the experiment ( $50^\circ\text{C}+\text{L}$ ) were considered as model conditions. Skullcap (*Scutellaria baicalensis* Georgi) (Sb) flavones containing 95% of baicalin were purchased from Wimex Beijing (China). The composition of phenolic compounds of the powder was determined by HPLC. Root of skullcap (DRS) was obtained from Botanical Garden of the Medical Academy of Wrocław (Poland). Dried root was ground to powder in a laboratory mill.

A sample of DRS powder was prepared by dissolving a weighed amount of the product in methanol. Then, it was subjected to 10-min ultrasonic treatment in UNITRA UM-4 ultrasonic washer for accurate dissolution. The solution was centrifuged at 12000 rpm for 10 min. In so-prepared methanol extract, an HPLC analysis of flavone compounds was performed. A Polymer Laboratories PLRP-S 100 Å (5  $\mu\text{m}$ ) column (Shropshire, United Kingdom) was used for the analysis. The solvents used were 80% acetonitrile in 4.5% formic acid (A) and 4.5% formic acid (B), at a flow rate of 1 mL/min. The elution profile was: 0-7 min 0-85% B in A, 7-15 min 85-0% B in A, 15-21 min 0-100% B. Recording was carried out at 280 nm. Powder composition was determined with HPLC and contained: baicalin (12.78%), wogonin 7-glucuronoid (3.94%), baicalein (0.04%) and other compounds (3.24%).

The bark of hawthorn (*Crataegus oxyacantha*) (H) was collected from local Botanical Garden. Procyanidins from hawthorn were prepared with the method of Oszmianski and Bourzeix [1995] and determined with HPLC.

The determination of procyanidin content was performed by high performance liquid chromatography with Waters™ 486 Tunable Absorbance Detector, equipped in Waters 600 E Multisolvant C-R6A (WATERS) reagent mixing system and computer data acquisition system Star Chromatography Workstation (Varian). Procyanidins were determined on RP-18 column Chromolith RP-18e 100-4.6 (Merck). The solvents used were 2.5% formic acid (A), and 80% acetonitrile in 2.5% formic acid (B), at a flow rate of 2 mL/min. The elution profile was: 0-10 min 0-10% B in A, 10-30 min 10-20% B in A, 30-35 min 20-40% B in A, 35-40 min 40-100% B, 40-45 min 100-0% B. The analysis was performed by recording a chromatogram on the absorption detector at 280 nm wavelength. Procyanidins from the bark of hawthorn contained (-)-epicatechin (10.1%) and their polymers: B2 (24.2%), B4 (14.0%) and other compounds B4+B5 (10.5%).

Polyphenols were extracted from the butter samples (2 g) with 5 mL of methanol followed by 10-min sonification in UNITRA UM-4 ultrasonic washer for accurate dissolution. Samples were then centrifuged at 12 000 rpm for 10-min (MPW 350 R, Poland). In so-prepared methanol extracts, an HPLC analysis of flavone and procyanidins compounds was performed.

Peroxide value (PV) was determined with the procedures described by Haraguchi *et al.* [1992]. The sample (1 g of butter) was mixed with 0.05 mol/L of TRIS:HCl buffer (pH 7.54) and emulsified with 3% Tween-20. The mixture was then homogenized by Heidolph DIAX 900 (Merck, Germany) for 1 min. The 0.1 mL butter emulsion was diluted with 9.7 mL of 75% EtOH, which was followed by the addition of 0.1 mL of 30% ammonium thiocyanate. Three minutes after the addition of 0.1 mL of 20 nmol/L ferrous chlorides in 3.5% hydrochloric acid to the reaction mixture, the absorbance at 500 nm was measured using a PC 2401 UV-VIS spectrophotometer (Shimadzu, Tokyo, Japan).

Thiobarbituric acid reactive substances (TBARS) were analysed using a modified method of Mei *et al.* [1998]. A TBA solution was prepared by mixing 15 g of trichloroacetic acid with 0.375 g of TBA, 2 mL of HCl, and 82.9 mL of distilled water. TBA solution (2 mL) was mixed with 5 mL of butter-emulsion and 3 mL of distilled water. The mixture was heated in boiling water bath for 20 min, cooled to room temperature using tap water, and centrifuged at 10 000 rpm (MPW 211, Poland) for 5 min. Absorbance was measured at 532 nm. A calibration curve was prepared with pure malondialdehyde (MDA) standard, and results were expressed as nmol/L MDA equivalents per g of products.

$\alpha$ -Tocopherol,  $\beta$ -carotene and cholesterol contents were determined with the procedures described by Katsanidis & Addis [1999]. Two grams of butter were placed in a 50 mL plastic tube and 8 mL of absolute EtOH were added, then the mixture was homogenized for 30 s in a Heidolph DIAX 900 (Merck) homogeniser. After 10 mL of distilled water has been added to the sample, the tube was homogenized for 15 s. Finally, 8 mL of hexane were added and the sample was homogenized for 15 s. The tubes were capped and centrifuged at 6000 rpm for 10 min (MPW 350 R, Poland). The upper layer was collected and centrifuged (MPW 211, Poland) then analysed with HPLC. Quantification of  $\alpha$ -tocopherol

and  $\beta$ -carotene in samples was performed with HPLC using a LiChrosper™ Si 60, 5 $\mu$ m column (250 x 4.6 mm (Phenomenex, USA)) and diode array detection using an external standard for quantification. The mobile phase consisted of hexane:2-propanol (99:1,v/v) at a flow rate of 1 mL/min. The wavelength was programmed at 295 nm for  $\alpha$ -tocopherol, at 450 nm for  $\beta$ -carotene and at 202 nm for cholesterol.

Fatty acid profile was determined with GC after lipid extraction by Soxhlet method (Büchi extraction system B-811), on a Philips apparatus equipped with a RTX-2330 (105 m) column and a flame ionization detector. The injector temperature was 230°C. The following temperature program was used: 160°C – 30 min, 180°C – 17 min, and 210°C – 45 min. Fatty acid methyl esters (FAME) were prepared according to Zadernowski & Sosulski [1978] using a mixture of chloroform:methanol:sulphuric acid (100:100:1, v/v/v). The

retention times were compared with standards (Sigma, St. Louis, Mo, USA).

All experiments were carried out in duplicate and analyses were performed in triplicate. Significant differences between the samples were calculated by an analysis of variance at a significance level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

Butter samples with added polyphenols stored under different conditions were characterised by a high content of vitamins, which are considered antioxidative.  $\alpha$ -tocopherol content amounted to 5.0 mg/100 g of product and  $\beta$ -carotene – to 1.2 mg/100 g of products. Moreover, vitamin A was detected at a level of 0.13 mg/100 g. The butter analysed contained 87% of lipids that consisted of 76.96% of saturated

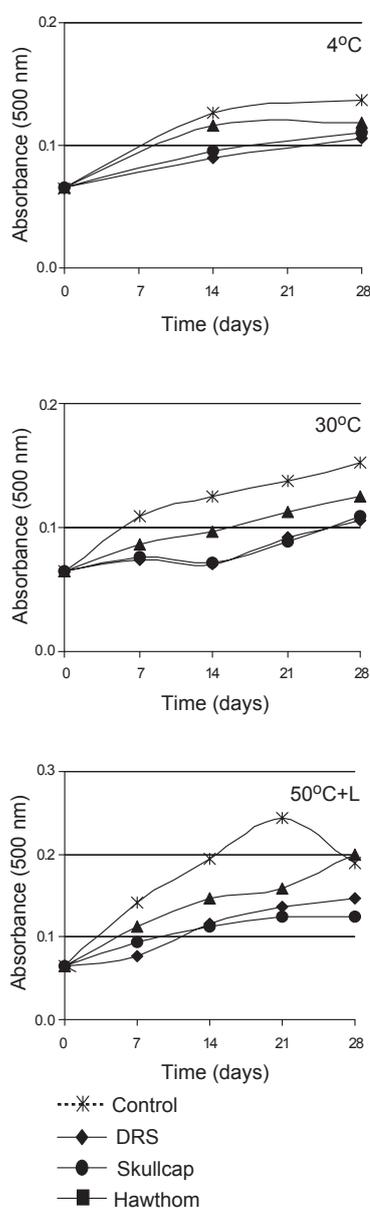


FIGURE 1. Stability of butter with the addition of polyphenols – changes in peroxide values.

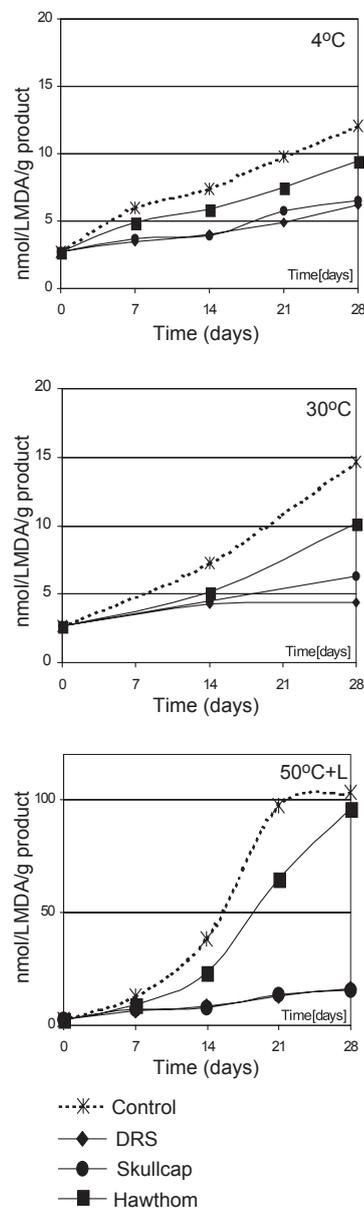


FIGURE 2. Stability of butter with the addition of polyphenols – changes in TBARS.

fatty acids, 24.45% of unsaturated fatty acids and 3.47% of polyunsaturated fatty acids. Cholesterol content accounted for 77.31 mg/100 g of product.

Initial stages in butter oxidation were monitored by the analysis of lipid peroxides. The influence of temperature and light on the development in the PV of the lipid extracted from butter samples was found to be complex (4°C and 30°C), as shown in Figure 1. The most intensive production of peroxides was observed in samples exposed to light at a temperature of 50°C ( $p < 0.05$ ). The addition of polyphenols, especially flavones from skullcap, significantly decreased oxidation processes in butter ( $p < 0.05$ ). At a temperature of 30°C and 4°C the formation of peroxides was slower and spread in time.

Thiobarbituric reactive substances (TBARS), which are secondary lipid oxidation products, were quantified using the thiobarbituric acid method. The development of the TBARS in butter samples is presented in Figure 2. An increase in TBARS in butter sample was noticeable after approximately 7 days. The highest value was reported for butter samples stored at 50°C and exposed to light. During the storage period, the level of TBARS was noted to increase after 28 days for the sample stored at 30°C (data not shown), while in the samples stored at 4°C their level was rather steady. In control samples after 28 days of storage at 50°C with light, the content of secondary oxidation products was 6 times higher than in the samples stored at temperatures of 30°C and 4°C. The amount of MDA formed in the samples stored for 28 days without light was comparable to that of the samples stored at 50°C for 7

days. Added antioxidants were effective in inhibiting the increase of secondary oxidation products, which was observed in the samples stored at 50°C. The addition of ground roots of skullcap (DRS) was as much effective, as an antioxidant, as a commercial baicalin preparation (95%). Procyanidins isolated from hawthorn's bark showed the antioxidative effect on butter samples but only at the first days of storage. Significant differences in the antioxidative activity of procyanidins and flavones were observed in the samples stored at 50°C ( $p < 0.05$ ).

$\alpha$ -Tocopherol and  $\beta$ -carotene were found to be degraded, especially upon light exposure (Figure 3). Complete degradation could be observed after 28 days of storage at 50°C + L in control samples and those with hawthorn ( $p < 0.05$ ). The samples stored under the other conditions (4°C and 30°C) were characterized by a slower dynamics of vitamin and provitamin degradation. Butter stored at 4°C had the highest amount of vitamin and provitamin despite polyphenols addition. There were no significant differences in vitamin and provitamin contents of butter samples with polyphenols stored at 30°C ( $p < 0.05$ ). Skullcap flavones, either as a DRS or commercial preparation, had the strongest protective properties towards  $\alpha$ -tocopherol and  $\beta$ -carotene. Under model conditions, only a little amount of vitamin and provitamin was saved in samples supplemented with flavones.

Fatty acids analyses showed that butter consisted mainly of saturated and monounsaturated fatty acids, whereas the content of polyunsaturated fatty acids was low (to 4 %). The results of the experiment proved that the oxidation processes of fatty acids were closely dependent on storage conditions of the butter samples ( $p < 0.05$ ). The greatest changes within fatty acids profile were noticed for control butter and samples with procyanidins. In the samples with flavones, 78% of fatty acids were still saved after the storage, whereas in the control samples and in butter with hawthorn - only 15% and 22%, respectively. Far less degradation of fatty acids, mainly PUFA (n-3 and n-6), was observed in samples stored in darkness ( $p < 0.05$ ). Changes in the contents of n-3 and n-6 fatty acids in butter stored under different conditions are shown in Figure 4. Fatty acids n-3 and n-6 appeared to be very sensitive under model conditions (light and high temperature) and were degraded very rapidly. Those unfavourable processes were blocked

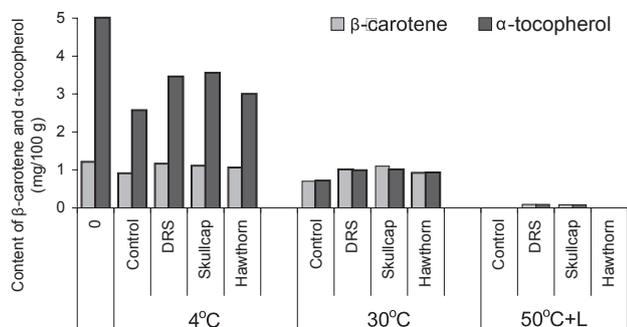


FIGURE 3. Changes in  $\alpha$ -tocopherol and  $\beta$ -carotene contents of butter after 28 days of storage under different conditions.

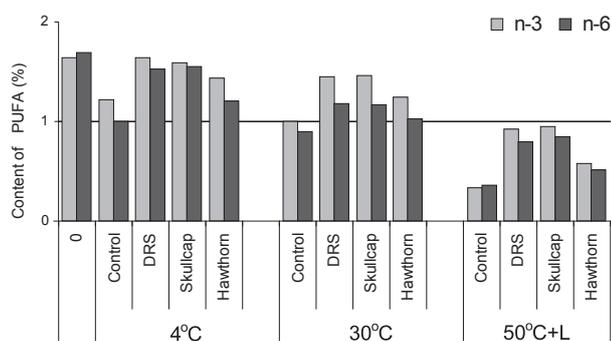


FIGURE 4. Influence of polyphenols addition on PUFA content of butter samples.

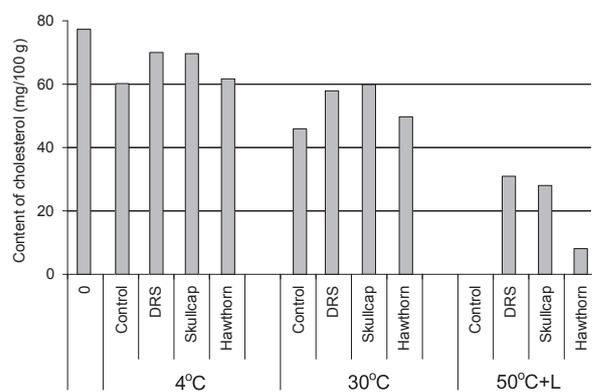


FIGURE 5. Change in cholesterol content of butter after 28 days of storage under different conditions.

TABLE. 1. Change of polyphenol content of butter samples stored under different conditions.

Polyphenolic compounds isolated from:	Content of polyphenols (mg/100 g)			
	before storage	after 28 days of storage		
		4°C	30°C	50°C+L
DRS	28.42	19.66	12.91	6.02
Skullcap	23.32	15.71	11.88	3.45
Hawthorn	26.48	18.43	7.63	0.00

by the addition of antioxidants, mainly flavones. The antioxidative activity of procyanidins was much lower, only in the samples stored at the temperature of 4°C and 30°C was the activity of procyanidins comparable to flavones.

During storage of butter, qualitative and quantitative changes of cholesterol were also noticed. The most extended degradation of cholesterol after 28 days of storage was observed in the samples treated with 50°C and light (Figure 5). Butter stored under the other experimental conditions did not show any significant differences ( $p > 0.05$ ) in cholesterol content. In control butter, *i.e.* without polyphenols, exposed to light cholesterol was completely degraded, whereas in the samples containing flavones from *Sb* cholesterol degradation was significantly lower ( $p < 0.05$ ). Cholesterol degradation is closely connected with the formation of cholesterol oxidation products (COPs). The results of the study revealed that cholesterol present in butter was oxidised to 7 $\alpha$  and  $\beta$ -hydroxycholesterol, cholesterol- $\alpha$ ,  $\beta$ -epoxide and 7-ketocholesterol.

In the experiment, the degradation of added polyphenols in butter samples was also analysed. The highest disintegration of polyphenols was observed in butter stored with light. Procyanidins from the bark of hawthorn were entirely degraded under those storage conditions (Table 1). Flavones isolated from *Sb* were much more active as antioxidants and more resistant to storage conditions. The incorporation of ground roots of *Sb*, containing flavones, without any additional purification procedure, did not have any significant effect on lowering their antioxidative and photoprotective activities.

Oxidation is a very complex process, especially when it proceeds in food emulsions (type o/w or w/o). The results of the study showed that it is impossible to maintain all nutritional constituents of butter at their initial levels by using antioxidants. Unfavourable oxidative changes could be minimised only by the selection of proper antioxidants. The highest oxidizing changes of the product were found to be strongly dependent on storage conditions. Light and temperature are important for the product stability as these factors induce the formation lipid peroxides which, however, have been found to provide little information on the oxidative processes during storage of butter. The formed peroxides may accordingly be assigned to monounsaturated fatty acid. Lipid peroxides are thermally cleaved to yield secondary lipid oxidation products. The formation of secondary lipid oxidation products was strongly dependent on temperature (Figure 2), fatty acids content, especially PUFA, but also on conjugated dienes, aldehydes and al-

kanes which were able to react with TBA and formed red complexes. Moreover, partial oxygen pressure, water activity and product composition have to be taken under consideration when analysing the oxidation processes of dairy products [Bergamo *et al.*, 1998]. The results of the study clearly showed that light and higher temperature (30°C and 50°C+L) accelerated oxidative degradation of butter constituents. Storage of the product at 4°C significantly minimised the oxidation processes in butter.

Vitamins and PUFA are very sensitive substances and they could be degraded easily in foodstuffs during high-temperature culinary processes, long-lasting storage under inappropriate conditions and exposure to light [Heinonen *et al.*, 1997].  $\alpha$ -Tocopherol and  $\beta$ -carotene present in a lipid fraction of milk and dairy products [Salo-Väänänen *et al.*, 2000; Subagio & Morita, 2001] serve an important function as antioxidants [Heinonen *et al.*, 1997]. Moreover,  $\alpha$ -tocopherol shows a synergistic effect with polyphenols, which strengthens their antioxidative activity [Kulas & Ackman, 2001; Jaroslawska *et al.*, 2002]. Carotenoids, which are sensitive to light, are degraded very readily, especially in rancid butter [Burri, 1997]. Andersson & Lingnert [1998] reported that  $\beta$ -carotene oxidation in butter occurs much more rapidly than in other dairy products exposed to light. The results obtained in our study for samples stored at 50°C + L confirmed those observations. In addition,  $\beta$ -carotene as a photosensitiser, was very active in the protection of product constituents against photooxidation [Hala & Heinonen, 1994].

Fatty acids composition in butter is determined by animal breed, feeding regime and seasons [Žegarska *et al.*, 2001]. Lipids from dairy products are easily and quickly assimilated by humans due to the content of short-chain fatty acids. Every oxidative change in unsaturated fatty acids profiles causes deterioration of food products and lowers their nutritional value.

There is still a lack of research on food products stabilisation by natural polyphenols and on their degradation upon storage. The results of this study indicated that polyphenol preparations used in butter were characterised by different oxidative activity. The antioxidative activity of polyphenols depends on a number of factors. 3',4'-dihydroxy structure present at ring B is considered to be the most important. Also, the double bond in position 2, 3 in connection with 4-oxo group at ring C as well as the presence of hydroxyl group at position 3 of ring A has been reported to enhance the antioxidative activity. The compounds used in this study considerably differed from one another in the structure of the

phenolic ring. Procyanidins isolated from the bark of hawthorn are a class of condensed tannins consisting of flavan 3-ols units, primarily oligomers (-)epicatechins (2-10 units). In their specific structure there are OH groups at ring B. The dimeric (B2, B4, B5) and trimeric (C1) structure of compounds making up this preparation causes the total number of hydroxyl groups to increase, which indicates their higher antioxidative activity. However, flavones isolated from *Scutellaria baicalensis* demonstrate a different structure of the phenolic ring. They do not possess any hydroxyl groups at ring B but do have them at ring A. Baicalin, the main flavonoid isolated from root of *Sb*, was shown to have an 5,6,7-trihydroxyl group on the A ring, whereas baicalein to have an *o*-trihydroxyl group on the A ring. Yang *et al.* [2001] also proved that baicalin (a pyrogallol-containing flavonoids) was the most powerful antioxidant among the flavonoids investigated, also compared to (+/-)- (epi)catechins. Moreover, there is a double bond at ring C in the primary structures of skullcap's flavones, *i.e.* baicalin and baicalein. This bond is responsible for the photoprotective activity of polyphenols.

In addition, Frankel & Huang [1996] have paid attention to the so-called *paradox of polarity Porter*. Polarity of these compounds has been examined in respect of their individual lipophilic and hydrophilic nature in relation to the environment in which they will actively function as antioxidants [Yang *et al.*, 2001]. Polar compounds – flavones in this case [Lai *et al.*, 2002] – are more active in the lipophilic environment, and the non-polar ones – procyanidins – in the hydrophilic environment. According to this, it is expected that the activity of polyphenols depends primarily on the nature of compound and the type of environment, and it should not be connected only with the presence of hydroxyl groups and their number at ring B.

Therefore, there is a need to find antioxidative compounds that have not only antioxidative properties but photoprotective as well. In order to prevent this antioxidant compounds may be used that possess not only antioxidative properties but photoprotective as well. It is crucial to find proper natural antioxidative compounds, as, according to many studies, synthetic antioxidants do not display such properties. Furthermore, due to the toxic activity of those compounds, their use is very often restricted. However, as it has been demonstrated, not all polyphenols may serve the function of active antioxidants in butter environment. The causes of such behaviour should be sought in the structure and orientation of compounds in this environment. The tested flavones of Skullcap offer such a possibility in the form of a refined commercial preparation as well as in the form of powdered roots.

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## POLIFENOLE ROŚLINNE JAKO STABILIZATORY PRZEMIAN OKSYDACYJNYCH WYBRANYCH SKŁADNIKÓW MASŁA

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Celem niniejszej pracy była ocena działania naturalnych przeciwutleniaczy fenolowych izolowanych z korzenia tarczycy bajkalskiej (*Scutellaria baicalensis* Georgi) i procyanidyn z kory głogu (*Crataegus oxyacantha*) na wybrane składniki masła podczas przechowywania. Oceny dokonano w oparciu o zmiany zachodzące w zawartości  $\alpha$ -tokoferolu i  $\beta$ -karotenu, kwasów tłuszczowych oraz cholesterolu w trakcie 28 dniowego przechowywania masła w lodówce (4°C), cieplarnie (30°C) bez dostępu światła i cieplarnie z dostępem światła (50°C, 3600 - 3900 lx). Analizę zmiany składu witamin i cholesterolu dokonano w oparciu o metody chromatografii cieczowej, natomiast kwasów tłuszczowych – chromatografii gazowej. W trakcie całego okresu przechowywania oznaczono przyrost nadtlenków metodą tiocyjanianową oraz wtórnych produktów utleniania metodą TBARS.

Największy przyrost nadtlenków oraz wtórnych produktów utleniania zmierzono w próbach poddanych działaniu światła oraz podwyższonej temperatury. Spośród zastosowanych związków polifenolowych silniejszymi właściwościami przeciwutleniającymi, ale i fotoprotekcyjnymi wykazały się flawony wyizolowane z korzeni tarczycy bajkalskiej, niż procyanidyny z kory głogu. W próbach z dodatkiem flawonów tarczycy bajkalskiej, niezależnie od warunków przechowywania, zostały zachowane większe ilości  $\alpha$ -tokoferolu,  $\beta$ -karotenu, polienowych kwasów tłuszczowych oraz cholesterolu, niż w próbkach z dodatkiem procyanidyn. Największe zmiany stwierdzono w próbkach kontrolnych i z dodatkiem procyanidyn, głównie wystawionych na działanie światła. Po przechowywaniu 78% z całej puli kwasów tłuszczowych (PUFA) zostało zachowane w próbach z dodatkiem flawonów, podczas gdy w próbie kontrolnej i z dodatkiem procyanidyn z głogu tylko 15% i 22%. Otrzymane wyniki wskazują, że zastosowanie flawonów w postaci zmielonych korzeni, skutkuje wysoką aktywnością przeciwutleniającą porównywalną do aktywności wysoko oczyszczonego preparatu handlowego tarczycy bajkalskiej.