Journal of International Scientific Publication:
Materials, Methods & Technologies, Volume 4, Part 1

INVESTIGATION OF POSSIBILITY FOR STABILIZATION OF DIFFERENT NATURAL LIPIDS AND OILS THROUGH THE ADDITION OF PLANT EXTRACT FROM SOPHORA JAPONICA

Slava T. Bahchevanska[1], Dasha Sp. Mihaylova[2]

Department of Biotechnology, University of Food Technologies, Plovdiv, Bulgaria, Mariza 26, 4002

[1] E-mail: sbahchevanska@gmail.com

[2] E-mail: mihailova@uft-bio.com

Abstract

The present work explored the possibility of improving the quality of natural lipids and oils by studying their oxidative stability to the addition of one raw flavonoid powder concentrate extracted from flowers of Sophora japonica.

The antioxidative effect of the flavonoid concentrate was compared to those of the pure natural flavonol- rutin and synthetic antioxidant BHT through the Rancimat method.

Results for the oxidative stability of the samples analyzed show that the rutin-containing flavonoid concentrate (RFC) have similar antioxidative effect compared to the synthetic antioxidant BHT and to pure rutin.

It was also carried out a characterization of the obtained rutin- containing concentrate that shows high content of rutin.

Key words: *Rancimat method, Sophora japonica, lard, goose fat, sunflower oil*

1.INTRODUCTION

Nowadays people are particularly concerned about the quality and the safety of their food. Only selected additives are added to the food to protect it from off- flavor. Antioxidants are often used in oils and fatty foods to retard their autooxidation [7,9,10]. Lipid peroxidation is one of the major factors that cause deterioration during the storage and processing of foods [1]. Oxidized polyunsaturated fatty acids may induce aging and carcinogenesis. Many spices possess potent antioxidant activity. Herbs are also found to be potent sources of natural antioxidants. Some of them have been used for thousands of years in China and their clinical and pharmacological effects have been extensively studied from various viewpoints. Apparently, some of the clinical effects of herbs are closely related to their antioxidant activities [4]. For that reason scientists have focused on natural antioxidants.

Antioxidants play very important role in food industry. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxyytoluene (BHT) and terbutylhydroquinone (TBHQ), are widely used in the food industry, but BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis. Therefore, the development and utilization of more effective antioxidants of natural origin are desired [13]. Marinova and Yanishlieva screened 6 species of the family Lamiaceae on sunflower oil and 4 of them were found to be more active than BHT[8].

The animal lipids are protoned to peroxidation by molecular air oxygen and thus are perishable during manufacturing and storage [11]. The oxidation process greatly reduces the natural value of lipids. The colour alters as the pigments oxidize and the flavour and aroma also change as result of the accumulation of secondary volatiles [3].

The flavonoids possibility of fast oxidation and their ability to form chelates with metal ions show great promise. They are able to help solvening the problem of modern science for nutrition and food engineering - to ensure protection of the products from some bad influence of the environment. Flavonoids are primary antioxidants. They cut off the free-radical chain reaction by giving hydrogen or electrons to free radicals and this makes them stabile products.

2. MATERIALS AND METHODS

2.1.Materials

The flowers of *Sophora japonica* was collected in earlier phase of growth at Plovdiv, Bulgaria, air dried by dark and then ground.

2.2.Chemicals

The chemical standards used were all of analytical grade. BHT was obtained from Merck (Germany), Quercetin, Rutin, BHA and α- tocopherol were obtained from Sigma- Aldrich Chemie (Steinheim, Germany). Citric acid and C_2H_5OH were obtained locally.

2.3.Preparation of plant extracts

In this study was investigated flavonoid concentrate from flowers of *Sophora japonica*: rutin-containing flavonoid concentrate (RFC).

Extraction was carried out with dry and ground sample three times with 80 % C_2H_5OH on a water bath for 30 min at 70°C. The solution was then filtered, purified with coal (15% from the plant mass) for 15 min and than the solvent was removed by a rotary evaporator until 1/3 from the previously volume. The extract was diluted with hot water to the original volume and storaged in refrigerator without any preservatives. The obtained product after filtration and drying was RFC- powder [2].

2.4.Determination of oxidative stability of lipids and oils

Oxidative stability is an important parameter for the quality assessment of animal and vegetable fats and oils. Antioxidation is affected by atmosphere oxygen; the oxidation process is initiated by radical reactions involving unsaturated fatty acids. The oxidative stability of lipids and oils was investigated according to the Rancimat method [6]. The principle of the method consists in condumetric measurement of solution permeability at increasing by time [5].

It was 617 Rancimat (METROHM AG, CH-91000, Switzerland) used. Parameters of the process are: T=100ºC and air flow rate 20l/h. The antioxidant effect of the invested antioxidant is interpreted as protection factor (Pf): Pf = (Induction period for lipid + antioxidant) / Induction period for lipid.

The antioxidative effectiveness of the extracts was estimated on the basis of the induction period (IP, h), which was determined by the method of the tangents to the two parts of the kinetic curve [12]. The period of time between the initiation and propagation is called the induction period. The longer the IP, the greater is the oxidative stability of the oil. IP correlates closely with the outset of rapid absorption and notable deterioration on flavon.

2.5. HPLC

The qualitative and quantitive composition of the RFC was determined with HPLC "Perken Elmer" 4, USA under the following conditions: 6 µl of each sample was injected onto PE Analytical (C_{18}), 250x4,6mm,10µm column. The HPLC flow rate was 1ml/min. The compounds were detected at 365nm by LC-85. The eluent was consisted of CH_3OH and 2% CH_3COON=50:50.

3.RESULTS AND DISCUSSION

3.1Characteristic of obtained rutin- containing flavonoid concentrate

The presence of rutin and quercetin in RFC was proved with HPLC analysis and the peaks were identified by their retention times (fig. 1). The investigated extract was light yellow powder. It was estimated that the obtained extract (RFC) consists mainly rutin – 94% and only 2% quercetin (fig. 2).

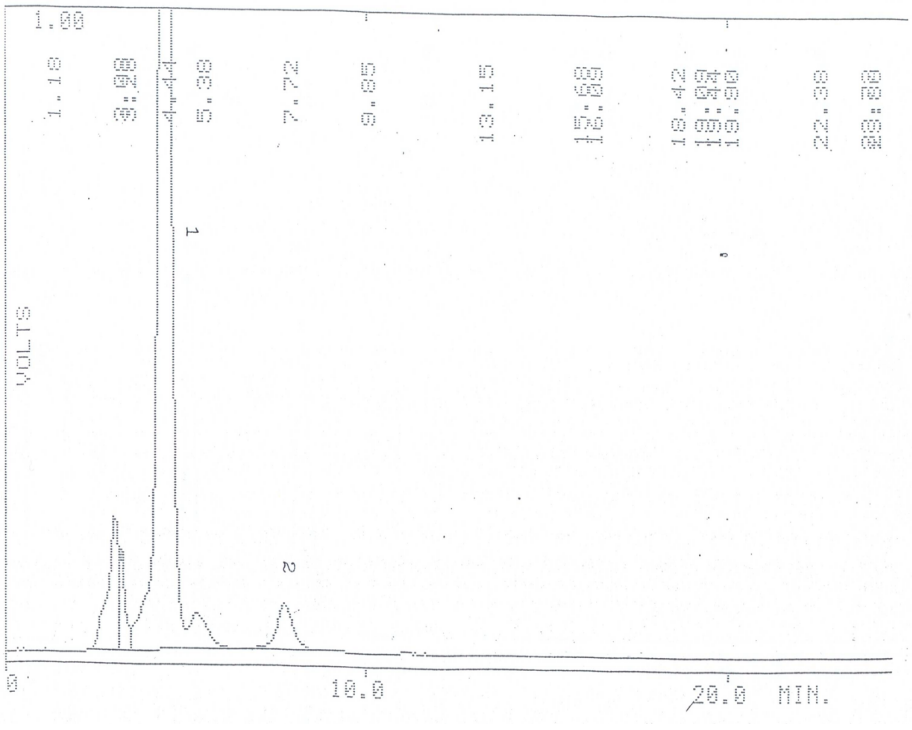


Figure 1. HPLC of 80% ethanol extract from *Sophora japonica*- RFC

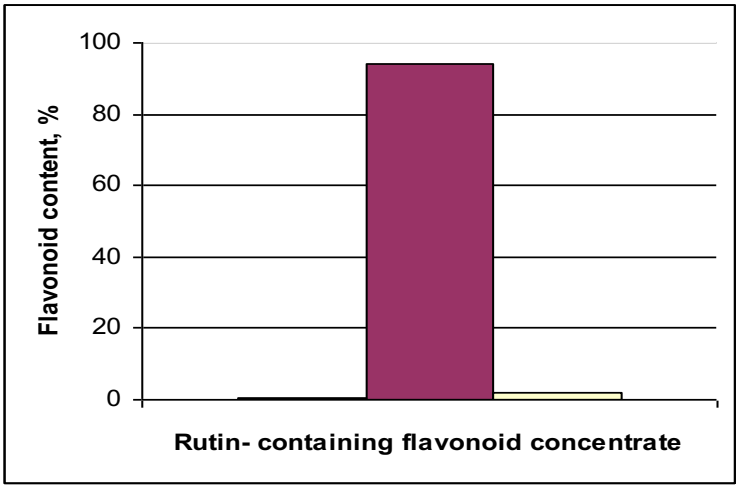


Figure 2. Flavonoid content of the RFC from *Sophora japonica*

3.2 Investigation of sunflower oil

Sunflower oil is widely used in nutrition and is highly appreciated as a source of the essential linoleic (9-cis, 12-cis-octadecadienoic) acid. A comparative study of the antioxidative action of flavonoid products and BHT was carried out with sunflower oil. It was used sunflower oil with peroxide value = 3,7 meq O_2/kg.

Table 1. The effect of different concentration of rutin and 0,02% BHT on sunflower oil

Sample	**Blank (sunflower oil)**	**Rutin- 0,016%**	**Rutin - 0,033%**	**Rutin - 0,066%**	**BHT- 0,02%**
IP,h	9,3	9,80	11,10	11,00	11,0
Pf	-	1,05	1,19	1,18	1,18

In table 1 and 2 are represented the results of oxidative stability of sunflower oil by addition of different antioxidants- the synthetic BHT 0,02% and pure rutin in different concentration. It was estimated that the control sample have low induction period- 9,3 h. By addition of 0,033% rutin IP increased to 11,1h and the protection factor is 1,19, which is similar to the IP of 0,02% BHT and Pf of BHT is 1,18. By addition of RFC (table 2) the oxidative stability of sunflower oil increased to 1,33 by 0,033% and the induction period increase to 13,45 h. By comparison of the results from tables 1 and 2 was estimated that by identical concentrations of the added antioxidants (pure rutin and RFC) higher antioxidant effect was observed by the natural extract (RFC). This is probably due to synergistic influence of concomitants in RFC (quercetin, kempherol and others).

Table 2. The effect of RFC in different concentration on sunflower oil

Sample	Blank (lard)	RFC- 0,016%	RFC - 0,033%	RFC - 0,066%
IP,h	10,10	10,50	13,45	13,00
Pf	-	1,04	1,33	1,29

In order to discover natural mixture, which ensure preservation of oxidative stability of sunflower oil maximum in time, it was also of interest for us to investigate the synergistic effect of pure rutin by addition of one of the most used synergist- citric acid (0,05%). The results are shown in table 3.

Table 3. Stabilization of sunflower oil from rutin and rutin 0,033% + citric acid 0,05%

Sample	Rutin (0,033%)+ 0,05% citric acid	Rutin (0,033%)
IP,h	12,8	11,10
Pf	1,38	1,19

By addition of 0,05% citric acid, it was developed synergistic effect, the protective factor is higher by mixture of rutin (0,033%) + citric acid (Pf= 1,38) compared to pure rutin (Pf= 1,19).

3.3 Investigation of lard

In order to investigate the oxidative stability of lard by the addition of antioxidant were used the fallowed: BHT, quercetin, rutin and rutin- containing flavonoid concentrate (RFC) from *S. japonica*. It was also analyzed the synergistic effect of mixtures: rutin + citric acid (0,05%). The results for lard are represented in table 4. It was used fresh obtained lard with peroxide value=1,2 meq O_2/kg.

Table 4. The effect of different concentration of rutin on lard

Sample	Blank (lard)	Rutin- 0,016%	Rutin- 0,033%	Rutin- 0,066%
IP,h	6,92	8,53	9,97	9,32
Pf	-	1,23	1,44	1,35

It was found that the oxidation stability of the lard- IP_0 during its oxidation at 100ºC was low. By addition of different concentration of the pure antioxidant rutin (table 4) IP increase to 9,97 h (0, 033% rutin). 0,02% of BHT was used for comparison as an approved synthetic antioxidant in food processing industry. The protective effect of BHT (0,02%) is 1,56 (table 5) and it is quite similar to the effect of 0,066% RFC and 0,033% pure rutin.

Table 5. The effect of different concentration of RFC and 0,02% BHT on lard

Sample	Blank (lard)	RFC- 0,016%	RFC - 0,033%	RFC - 0,066%	BHT- 0,02%
IP,h	7,8	8,50	8,30	10,30	12,2
Pf	-	1,09	1,06	1,32	1,56

The obtained results for RFC in table 5 show similar antioxidative effect to rutin for lard by addition of 0,066%- Pf= 1,32 (for RFC) and Pf = 1,35 (for rutin). The pure antioxidant has a little superiority over the investigated flavonoid- containing concentrate by addition of 0,016% and 0,033%. Because of high content of rutin in RFC its display sufficient antioxidant effect- Pf = 1,32 in concentration of 0,066%.

In order to discover natural mixture, which ensure preservation of oxidative stability of lard maximum in time, it was of interest for us to investigate the synergistic effect of pure rutin (0,033%) by addition of one of the most used synergist- citric acid (0,05%). The results are shown in table 6.

Table 6. Stabilization of lard from rutin and rutin 0,033% + citric acid 0,05%

Sample	Rutin (0,033%)+ 0,05% citric acid	Rutin (0,033%)
IP,h	2,14	1,79
Pf	1,27	1,07

By addition of 0,05% citric acid, it was developed synergistic effect, and the protective effect is higher by mixture of rutin + citric acid (Pf= 1,27) compared to pure rutin (Pf= 1,07).

The obtained results demonstrate that from practical point of view the extracts from flowers of *Sophora japonica* should be recommended as suitable antioxidant for stabilization of lard.

3.4 Investigation of goose fat

It was investigated also the oxidative stability of goose fat by the addition of RFC in different amounts, BHT, α- tocopherol, BHA. The results for the antioxidant capacity of the used rutin-containing flavonoid concentrate are represented in table 7.

Table 7. The effect of different concentration of RFC on goose fat

Sample	Blank (goose fat)	RFC- 0,005%	RFC - 0,01%	RFC - 0,02%	RFC - 0,05%	RFC - 0,1%
IP,h	6,0	11,00	16,80	32,50	58,0	>90,0
Pf	-	1,83	2,80	5,42	9,67	>15

From table 7 is obvious that the antioxidant effect of the RFC is high and the oxidative stability of goose fat increase vastly to > 90h by addition of 0,1% RFC, which is 15 times higher than those of

goose fat itself. With the increase of the concentration from 0,05 to 0,1% this effect increase 1,83 times for the low concentration and up to 15 times for 0,1% content of RFC in the sample. Thus goose fat, which is easy oxidative lipid (the control sample oxidize for 6,0 hours only by the apply oxidative conditions), become considerably stable and induction period increase up to 90,0 hours.

Table 8. The antioxidant effect of RFC, BHA, BHT and α- tocopherol (0,02%) on goose fat

Sample	**Blank (goose fat)**	**BHT- 0,02%**	**BHA - 0,02%**	**α- tocopherol 0,02%**	**RFC - 0,02%**
IP,h	6,0	12,60	29,0	38,0	29,5
Pf	-	2,1	4,83	6,33	4,92

In table 8 are represented the results of comparative study for oxidative stability of goose fat by addition of different antioxidants- the synthetic BHT, BHA, Vitamin E (α- tocopherol) and RFC in concentration 0,02%. The highest activity possess 0,02% α- tocopherol, which stabilized the goose fat > 6,33 times and the end of the oxidative process is after 38 hours. On the other hand the induction period for the control sample (pure goose fat) is 6,0 hours. In this study RFC shows ability to stabilize goose fat (IP= 29,5 h) in similar ratio to BHA- IP= 29,0 h, while the effect of the most widely used synthetic antioxidant- BHT is only 12,60 h, i.e. Pf = 2,1 in comparison with Pf= 4,92 for RFC.

The induction period of 0,02% RFC was 29,5 h and is better in comparison with the same amount of BHT (12,6 h) and BHA (29,0 h), but lower than α- tocopherol- 38,0 h. The best antioxidative effect in goose fat has been achieved using 0,1% RFC- Pf > 15.

4. CONCLUSION

The obtained results demonstrate that the investigated extract from flowers of *Sophora japonica*- RFC should be recommended as suitable antioxidant for stabilization of the examined lipids and oil- lard, goose fat and sunflower oil because RFC`s supplement increases their oxidative stability. In case of sunflower oil RFC has higher protection factor in comparison with rutin and the synthetic BHT. RFC added to goose fat has similar effect to those of BHA and higher than BHT, which are widely used synthetic antioxidants. Otherwise RFC added to lard have higher antioxidant effect compared to pure rutin, but lower in comparison with the synthetic BHT.

In general from the obtained results appear that BHT compared to flovonolic antioxidants has lower stabilized effect. This facts and also the information for toxicity of the synthetic antioxidants give us reason to consider the natural concentrates such RFC from *Sophora japonica* and also the pure flovonolics as the alternative sources for antioxidant additives in food processing industry, which are a substitute for the synthetic one.

REFERENSES

1.Angelo A. J., Lipid oxidation in food. CRIT. *Rev. Food Sci. Nutrition* 1996; 36, 175-224

2. Bahchevanska S., Mihaylova D., Kosturkova P. Influence of hydrolytically enzymes over the process of isolation of bioflavonoids from Sophora japonica. *Food science, engineering and technologies* 2009; 1: 287-292

3. Balev D., Vulkova T., Dragoev St., Zlatanov M., Bahchevanska S. A comparative study on the effect of some antioxidants on the lipid and pigment oxidation in dry-fermented sausages. *International Journal of Food Science and Technology* 2005; 40: 1-7

4. Gu L., Weng X. Antioxidant activity and components of Salvia plebeia R. Br. – a Chinese herb. *Food Chemistry* 2001;73: 299-305

5. Lachman J., Orsak M., Pivec V. Antioxidant content and composition in same fruits and their role in human nutrition. *Zahradnictvi- Hort. Sci*. 2000; 27: 3, 103-117

6. Laubli M. W., Bruttel P. Determination of oxidative stability of fats and oils, Comparison between the active oxigen method and Rancimat method. *JAOCS* 1986; vol. 63, 6, 792-795

7. Lu Y., Yeap F. L. Antioxidant activities of polyphenols from sage (salvia officinalis). *Food Chemistry* 2001; 75: 197-202

8. Marinova E. M., Yanishlieva N. V. Antioxidative activity of extracts from selected species of thefamily Lamiaceae in sunflower oil. *Food Chemistry* 1997, Vol. 58, 3, 254-248

9. Milovanovic M., Picur-Jovanovic K. Vucelic-Radovic B., Vrbaski Z. Antioxidant effects of flavonoids of Anthricus sylvestris in lard. *AOCS* 1996; 73: 6, 773-776

10. Miyake T., Shibamoto T. Antioxidative activities of natural compounds found in plants. *J. Agric. Food Chem*. 1997; 45: 1819-1822

11.Speiner A. M., Angelo St.& Shaffer G. P. Response of beef flavor to oxygen depletion and an antioxidant chelator mixture. *Journal of Agriculture and Food Chemistry* 1992; 40, 1656-1662

12.Yanishlieva N., Popov A. Über einige Eigentümlichkeiten in der Kinetik zu Beginn der Autoxidation von Estern ungesättiger Fettsäuren. Inhibierte Autoxidation*, Nahrung*. 1971; 15, 671-681

13. Wang W., Weng X., Cheng D. Antioxidant activities of natural phenolic components from Dalbergia odorifera T. Chen. *Food Chemistry* 2000; 71: 45-49