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Stabilization of canola oil with flavonoids

Udaya N. Wanasundara & Fereidoon Shahidi*

Department of Biochemistry, Memorial University of Newfoundland, St. John's, NF, Canada, A1B 3X9

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The antioxidant activity of a number of flavonoids in refined-bleached (RB) canola (double-zero rapeseed) oil is compared with that of commonly used synthetic antioxidants, namely, butylated hydroxyanisole, BHA, and butylated hydroxytoluene, BHT. The study was carried out over a thirteen-day period at 65°C and progression of oxidation was followed by monitoring weight gain and peroxide and 2-thiobarbituric acid (TBA) values. Among the flavonoids tested, myricetin, (-)epicatechin, naringin, rutin, morin, and quercetin were superior to BHA and BHT in inhibiting oil oxidation. The addition of myricetin, the most active flavonoid tested, delayed the induction period of lipid oxidation by up to fifteen days and also inhibited the formation of oxidation products by 69% during this period. Natural flavonoids may therefore have potential application for the stabilization of canola oil.

INTRODUCTION

Autoxidation is considered to be the main route of spoilage of edible oils, and its progression leads to oxidative rancidity via a free-radical-chain mechanism (Sherwin, 1976). Vegetable oils containing substantial amounts of linolenic or other unsaturated fatty acids undergo rapid oxidation and produce off-flavours and off-odours (Min *et al.*, 1985). Antioxidants are therefore added to fats and oils to retard oxidation of unsaturated fatty acids and to decrease the development of rancidity (Sherwin, 1976). Phenolic antioxidants inhibit oxidation reactions by generally acting as a hydrogen donor and afford relatively stable free radicals and/or non-radical products (Shahidi & Wanasundara, 1992). Commonly used antioxidants for fats and oils are synthetic phenolic compounds, such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), and TBHQ (tert-butylhydroquinone) (Sherwin & Luckadoo, 1970). However, synthetic food additives are under rigorous investigation by government agencies and there is also an increasing pressure from consumer groups to reduce the amount of synthetic compounds in foods (Marshall, 1974).

Natural products that possess antioxidant activity have been consumed for centuries and are assumed to be safe for human use (Pokorny, 1991). Sources of natural antioxidants are primarily plant phenolics, which may occur in all parts of the plants (Pratt & Hudson, 1990). They can be found in fruits, vegetables, nuts, seeds, leaves, flowers, and bark (Wollenweber & Dietz, 1981). The daily intake of these natural products in human diet is approximately 1 g through the consumption of plant foods (Kühnau, 1976).

* To whom correspondence should be addressed.

Flavonoids occurring naturally in plants are recognized as important compounds in conferring stability against autoxidation to vegetable oils (Mehta & Seshadri, 1958; Herrmann, 1976; Hudson & Mahgoub, 1980; Das & Pereira, 1990). The relationship between the chemical structure of flavonoids and their antioxidant activity has been thoroughly investigated (Hudson & Lewis, 1983). The effectiveness of flavonoids in retarding lipid oxidation in fat-containing foods is related to their ability to act as free-radical acceptors (Das & Pereira, 1990; Pratt & Hudson, 1990; Shahidi *et al.*, 1993), or as chelators of metal ions. Metal chelation by flavanoids is due to the ortho-dihydroxy (3',4'-dihydroxy) grouping on the B-ring and to the ketol structure in the C-ring in their chemical structure (Shahidi *et al.*, 1991). The lack of at least one of these groups may reduce or even delete the chelating ability of flavonoids.

The objective of this study was to examine the effect of different flavonoids on the oxidative stability of refined-bleached canola oil. Weight gain and peroxide and 2-thiobarbituric acid values were used to monitor the development of oxidation in the oil.

MATERIALS AND METHODS

Fresh, refined-bleached (RB) canola oil containing no antioxidants was obtained from CanAmera Foods, Saskatoon, Saskatchewan. Flavonoids shown in Fig 1, namely, apigenin, chrysin, quercetin, myricetin, morin, kaempferol, rutin, naringenin, naringin, taxifolin, and (-)epicatechin, and other synthetic antioxidants (BHA and BHT) were obtained from either Sigma (St. Louis, MO, USA) or Aldrich (Milawaukee, WI, USA)

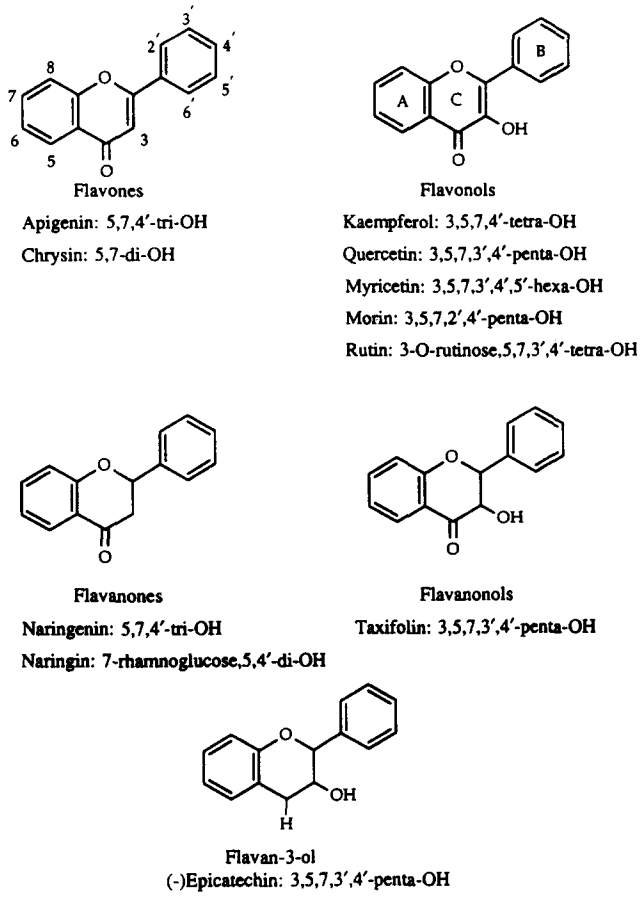

Fig. 1. Chemical structure of different classes of flavonoids.

Chemical Company. All other chemicals used in this study were ACS grade.

The antioxidant activity of flavonoids and synthetic antioxidants in RB canola oil was tested at a 200-ppm-addition level. These compounds were dissolved in a minimum amount of absolute ethanol in an ultrasonic water bath and subsequently added to the oil (200 g) and mixed for 10 min. The control sample contained only the same amount of absolute ethanol as was used to dissolve additives. To follow the weight gain during oxidation, a 2-g mass of each sample (in triplicate), prepared as mentioned above, was placed in glass Petri dishes (of 60-mm diameter and 15-mm height), which were kept in a vacuum oven overnight at 35°C to remove any traces of moisture. The sample was reweighed and stored in a forced-air oven at 65°C. It is generally accepted that each day of storage in a Schaal-oven test at 65°C is equivalent to one month of storage at ambient temperatures (Evans *et al.*, 1973). The rate of oxidation in terms of weight increase was recorded at 24 h intervals. The time required for a 0·5% weight increase for oil was taken as the index of stability. A 25-ml volume of each sample was stored separately under the same conditions in small, open glass containers (of 30-mm diameter and 60-mm height) for performing other chemical analyses. Samples of each treatment were removed on days 0, 2, 5, 9, and 13, flushed with nitrogen, covered with aluminium foil and stored at −20°C until further analyses were carried out.

Chemical analysis of oils, subjected to accelerated oxidation, included determination of peroxide value (AOCS, 1990) and the percentage inhibition of TBARS formation by using the classical 2-thiobarbituric acid (TBA) test (AOCS, 1990) as given in the following equation.

$$\% \text{ inhibition of TBARS formation} = \left(1 - \frac{\text{TBARS content of treated sample}}{\text{TBARS content of control}}\right) \times 100$$

Statistical analysis of data was performed by analysis of variance (ANOVA) and Tukey's test by using a statistical-analysis system (SAS, 1990).

RESULTS AND DISCUSSION

Weight-gain data of canola oil treated with antioxidants are presented in Fig. 2(A–C). All antioxidant-treated oil samples showed a delayed induction period as compared with that of the control. The time required to achieve a 0·5% weight increase for samples was 3·5 days for apigenin, kaempferol, chrysin, and naringenin, 4·0 days for taxifolin and BHA, 4·7 days for BHT, 5·5 days for naringin and morin, 5·8 days for quercetin, 6·0 days for rutin, 7·0 days for (-)epicatechin, and 15 days for myricetin. The corresponding time for the control sample was only 3·2 days. Furthermore, the extension of the induction period by (-)epicatechin and

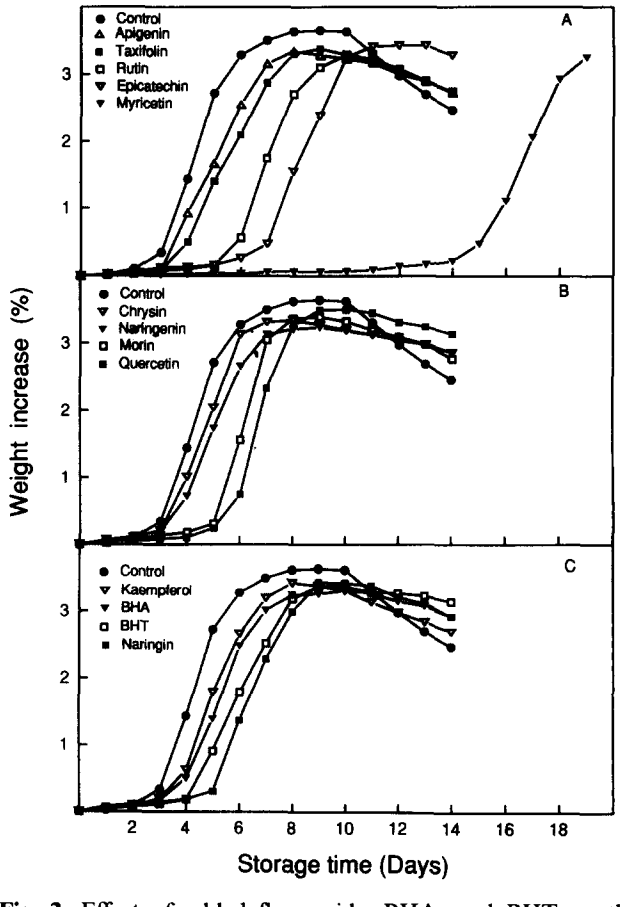

Fig. 2. Effect of added flavonoids, BHA, and BHT on the weight gain of canola oil stored at 65°C.

Table 1. Effect of flavonoids, BHA, and BHT on peroxide value (meq/kg oil) of refined-bleached canola oil stored at 65°C[1]

Treatment \ Storage period (days)	0	2	5	9	13
Control	0·37 ± 0·01^{a}	22·5 ± 0·7^{a}	83·6 ± 0·5^{a}	125·0 ± 4·0^{a}	159·0 ± 3·0^{a}
Flavones:					
Apigenin	0·37 ± 0·01^{a}	20·1 ± 0·1^{b}	63·4 ± 0·1^{b}	107·0 ± 1·0^{b}	153·0 ± 1·0^{b}
Chrysin	0·35 ± 0·01^{a}	22·2 ± 0·2^{a}	65·8 ± 0·3^{b}	93·1 ± 2·3^{d}	145·0 ± 2·0^{d}
Flavonols:					
Quercetin	0·34 ± 0·03^{a}	7·4 ± 0·2^{h}	31·3 ± 0·3^{i}	54·7 ± 0·8^{hi}	100·0 ± 1·0^{hi}
Myricetin	0·36 ± 0·01^{a}	2·6 ± 0·0^{j}	10·2 ± 0·4^{j}	12·0 ± 0·3^{k}	27·8 ± 0·1^{i}
Morin	0·36 ± 0·04^{a}	9·4 ± 0·5^{g}	38·4 ± 1·4^{h}	66·4 ± 0·7^{f}	130·0 ± 2·0^{e}
Kaempferol	0·37 ± 0·01^{a}	12·0 ± 0·4^{f}	47·1 ± 1·4^{ef}	96·9 ± 1·2^{c}	145·0 ± 1·0^{d}
Rutin	0·37 ± 0·00^{a}	4·6 ± 0·1^{i}	45·2 ± 0·6^{f}	64·2 ± 1·1^{fg}	133·0 ± 1·0^{e}
Flavanones:					
Naringenin	0·35 ± 0·01^{a}	14·6 ± 0·1^{d}	40·4 ± 1·2^{gh}	52·5 ± 0·7^{i}	85·5 ± 1·0^{h}
Naringin	0·36 ± 0·01^{a}	13·6 ± 0·3^{e}	48·0 ± 0·4^{e}	57·5 ± 0·1^{h}	85·9 ± 0·3^{h}
Flavanonols:					
Taxifolin	0·38 ± 0·02^{a}	21·9 ± 0·2^{a}	55·5 ± 0·4^{c}	76·9 ± 0·7^{e}	149·0 ± 1·0^{c}
Flavan-3-ols:					
(-)Epicatechin	0·36 ± 0·01^{a}	8·0 ± 0·0^{h}	41·1 ± 0·3^{g}	46·9 ± 0·5^{j}	90·9 ± 1·3^{g}
BHA	0·37 ± 0·00^{a}	21·7 ± 0·1^{a}	51·2 ± 0·5^{d}	63·5 ± 0·5^{fg}	103·0 ± 1·0^{f}
BHA	0·36 ± 0·00^{a}	16·0 ± 0·1^{c}	46·0 ± 1·2^{ef}	61·6 ± 0·5^{g}	90·7 ± 0·8^{g}

[1] Values in the same column bearing different superscripts are significantly ($p < 0·05$) different.

myricetin was two and five times that of the control, respectively. All other flavonoids were more effective than BHA and BHT in delaying the induction period except for apigenin, kaempferol, chrysin, naringenin, and taxifolin.

The changes in peroxide value (PV) of treated oils as a function of time are shown in Table 1. Canola oil treated with quercetin, myricetin, morin, rutin, and (-)epicatechin exhibited lower PV (<50%) for up to nine days as compared with the control sample. However, samples treated with commercial antioxidants (BHA and BHT) showed higher peroxide values than some of the flavonoids tested in this work. Oil samples treated with apigenin and taxifolin had peroxide values similar to that of the control for up to two days of storage. Among the flavonoids tested, myricetin, quercetin, and rutin served best in lowering peroxide formation. Myricetin was superior to all other flavonoids and gave peroxide values of 2·6, 10·2, 12·0, and 27·8 (meq/kg oil) on days 2, 5, 9, and 13, respectively; corresponding values of the control samples were 22·5, 83·6, 125·0, and 159·0 (meq/kg oil). These data also indicate that antioxidant activity of most of the flavonoids as well as BHA and BHT decreased with the length of storage time.

The main purpose of using antioxidants in lipids is to delay a significant accumulation of free radicals and thus to improve oxidative stability. Flavonoids with free hydroxyl groups may act as free-radical acceptors and could delay the formation of free-radicals. Myricetin, (-)epicatechin, quercetin, and naringin with multiple hydroxyl groups in their structures possessed good antioxidant activity and extended the induction period and decreased the formation of peroxides in canola oil more effectively than BHA and BHT. Previous findings (Vaisey-Genser & Ylimaki, 1985; Tokarska, *et al.*, 1986; Hawrysh, *et al.*, 1988) have also concluded that BHA and BHT are not beneficial for improving the storage stability of canola oil. It is further found that percentage weight gain during oxidation parallels the formation of hydroperoxides as monitored by PV measurements during the initial stages of oxidation. However, during the later stages of oxidation, owing to the breakdown of hydroperoxides to secondary products, this relationship was changed. Measurement of the induction period by monitoring changes in weight gain is therefore possible, and it is also theoretically accepted that the addition of oxygen to form hydroperoxide is quantitative during the initial stages of autoxidation (Farmer, *et al.*, 1943; Privett & Nickell, 1956). Olcott and Einset (1958) have reported that the weight-gain technique is very useful for comparing the effect of antioxidants on the oxidative stability of edible oils.

The 2-thiobarbituric acid (TBA) values of treated canola oils are presented in Fig. 3. The order of potency of flavonoids to inhibit the formation of TBARS was myricetin > (-)epicatechin naringin > naringenin > quercetin > rutin > BHT > morin > kaempferol > BHA > taxifolin > apigenin > chrysin. Myricetin, (-)epicatechin, naringin, naringenin, quercetin, and rutin exhibited >40% inhibition of TBARS formation. Corresponding values for BHA and BHT were 27% and 36%, respectively. Compared with all flavonoids used in this study, myricetin showed the strongest (69% TBARS inhibition) antioxidant activity. Ramanathan and Das (1992) reported that the addition of myricetin at 200 ppm into ground fish considerably reduced the formation of TBARS during storage.

Among the flavonoids tested, flavonols (myricetin,

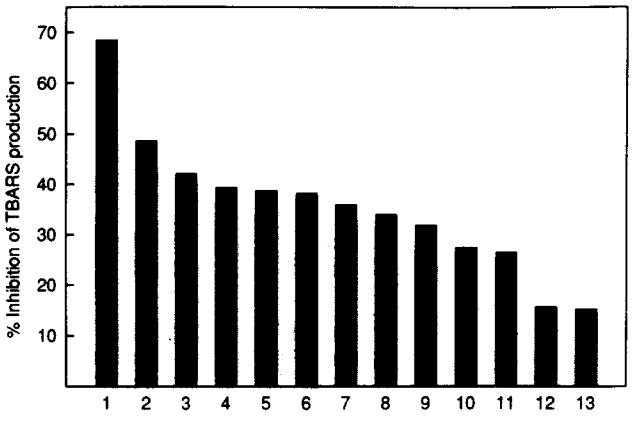

Fig. 3. Percentage inhibition of TBARS formation by different flavonoids and synthetic antioxidants (200 ppm) during thermal oxidation of refined-bleached canola oil: 1, myricetin; 2, (-)-epicatechin; 3, naringin; 4, naringenin; 5, quercetin; 6, rutin; 7, BHT; 8, morin; 9, kaemferol; 10, BHA; 11, taxifolin; 12, apigenin; 13, chrysin.

morin, quercetin) and flavan-3-ols ([-]epicatechin) were found to be the best antioxidants. Furthermore, it is evident that the antioxidant activity of flavonoids is generally governed by their chemical structures. Flavonoids may act as primary antioxidants by donating a hydrogen atom and act as free-radical acceptors or chain breakers and may also act as metal chelators. Extensive hydroxylation therefore enhanced the antioxidative effect of flavonoids. Shahidi *et al.* (1991) have illustrated that the presence of ortho-hydroxyl groups at the 3' and 4' positions of ring-B contributes to the enhancement of flavonoids' antioxidant activity. Myricetin with the highest number of hydroxy groups in ring-B was the most active flavonoid tested in this study. The relationship between the structure and antioxidant activity of flavonoids has been well documented (Hudson & Lewis, 1983; Pratt & Hudson, 1990; Shahidi & Wanasundara, 1992).

CONCLUSIONS

The present study indicated that myricetin, (-)epicatechin, naringin, rutin, morin, and quercetin were more effective than BHA and BHT in retarding the formation of primary and secondary oxidation products of canola oil. These flavonoids occur naturally in many plant products and act as free-radical terminators and chelating agents. Owing to their widespread consumption in foods, the potential application of these natural antioxidants to stabilize canola oil may be considered.

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