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Full Length Research Paper

Improving oxidative stability of soya and sunflower oil using *Temnocalyx obovatus* extracts

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Recently an increasing interest in formulations of medicinal plant origin and other plant additives as antioxidants has been observed in the food market and pharmaceutical fields. This is as a result of the realization that synthetic antioxidants can participate as mutagenic and genotoxic agents. In this study *Temnocalyx obovatus* extracts were compared with rosemary *(Rosmarinus officinallis*) and tert- butyl-hydroquinone (TBHQ) as possible antioxidants in sunflower and soya oil. Upon addition of 200 ppm of dried leaf extract, acetone extract yielded protection of the samples against oxidation more efficiently as compared to rosemary, ethanol, methanol and TBHQ extracts added at the same concentration for both peroxide value determinations and Rancimat method. Higher induction periods, 12.45 and 17.17 h were achieved with acetone extracts. Increasing the concentration from 50 to 200 ppm increased the antioxidant effect of the extracts. On analyzing ultra-violet (UV) absorption at 232 nm, acetone extract showed the least absorbance implying greater protection against oil oxidation. Results of the present study are interesting as they may lead to isolation and identification of bioactive compounds present in *T. obovatus* that can be used to prolong shelf lives in oil based foods which are susceptible to rancidification.

Key words: Oxidative stability, *Temnocalyx obovatus*, peroxidation, antioxidant.

INTRODUCTION

Peroxidation of lipids and oil based food products is a major concern in the food processing industry. This is because peroxidation results in food rancidity which eventually affects the food's nutritional and organoleptic qualities Maforimbo (2002). The use of synthetic antioxidants in lipid based foods to maintain oxidative stability is not a first choice anymore because they are considered to be carcinogenic (Bianco and Uccella, 2000; Lafka et al., 2007; Lapornik et al., 2005; Rankovic et al., 2010). Therefore the use of herbs and spices as natural antioxidants has become a worthwhile activity to the food manufacturers for protection against autoxidation (Oktay et al., 2003; Francois et al., 2010; Lis- Balchin and Deans, 1997 in Mugwa et al., 2006). Several plant antioxidants extracts, rosemary, dittany and sage have been reported to be potential antioxidants in processed foods resulting in their application (Dapkevicius et al., 2002; Miliauskas, 2006; Racnicci et al., 2004).

Natural antioxidants prevent autoxidation by donation of hydrogen or an electron to the lipid radical (Benzie and Strain, 1999; Khokhar and Magnusdotti 2002). The benzenoid radical does not participate further in the propagation stages because of resonance stabilization Miliauskas (2006). Several researches have shown that different assays for example, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays may give varying results concerning *in vitro* antioxidant efficiencies. Although, these assays are quick, reproducible and usually correlate well with antioxidant properties, nevertheless they do not reflect real oxidation conditions and cannot be extrapolated to predict activity of extracts in real foods Miliauskas (2006), leading to the conclusion that the final test of a plant- based antioxidant

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Abbreviation: TBHQ, Tert-butyl-hydroquinone.

should always be made in actual food products.

Therefore in this study, we evaluated the efficiency of *Temnocalyx obovatus* as an alternative natural antioxidant on improvement of sunflower and soya oil oxidative stability in comparison to *Rosmarinus officinallis* and tert- butyl-hydroquinone (TBHQ). *T. obovatus* is a herb that grows widely in Africa mainly in clumps on open woodlands or grasslands. It has several traditional uses which include treatment of ailments such as asthma, backache, hypertension coughs and flu, snake bites and boosting the immune system (Mahamadi et al., 2011). This uses indicate the presence of bioactive compounds with antioxidant properties (Maestri et al., 2006; Miliauskas, 2006).

MATERIALS AND METHODS

T. obovatus leaves were collected in April to June, 2011 from St Albert's in Chiweshe Mashonaland central province. The plants were identified by their characteristic leaves and validated by a taxonomist at Harare botanical garden. Voucher specimen (No. 2011/6) was deposited at Bindura University in the Chemistry Department (natural product section), for future reference. Rosemary plants produced by Bindura University were obtained through the Agricultural science department. Leaves were separated from the plants and dried at room temperature and ground in a mortar. The dried material was kept in a freezer at -20°C no longer than two months.

Extraction of antioxidants from *T. obovatus* was performed using 100 g in ethanol, methanol and acetone (1 L) (absolute) for 5 h on a shaker at room temperature followed by filtration through Whatman no. 1 filter paper. The residues were reextracted under the same treatment and the filtrates combined. The extracts were then centrifuged at 1536 ×g for 20 min at 20°C. The supernatants were taken into 100 ml flasks and then evaporated in a Bucchi rotary evaporator at 40°C. Commercial virgin soya and sunflower oil was purchased locally at the Bindura farmers' market place (Zimbabwe) and frozen until used. Ethanol, methanol, acetone, chloroform, potassium iodide, sodium thiosulphate, acetic acid and TBHQ were of analytical grades purchased from Sigma Chemical Co. (St, Louis, USA)

Determination of oxidation stability (accelerated oxidation test)

Determining oxidative stability of oil is complex as this phenomenon is influenced by many factors. The structural indices that can be used to assess the resistance of oil to rancidity are, the fraction in fatty acids with a low unsaturation degree, acidity, number of peroxides, specific absorbance, organoleptic qualities and the amount of antioxidative compounds (Vassilis et al., 2008).

Determination of peroxide value

Determination of the peroxide value was performed as described by Lafka et al. (2007) with slight modifications. Ethanol, methanol and acetone extracts were added at different concentrations (50, 100 and 200 ppm) to commercial virgin soya and sunflower oil. Then, all the samples were put in an oven at 85°C where thermal oxidation took place. Every 24 h, for a period of five days, the samples were analyzed for peroxide value in order to monitor the oxidation process. The peroxide value was determined according to the European Commission (EEC) method (EEC Regulation No. 2568/91, L-248/05-09-1991). In a stoppard conical flask, 2, 00 g of sample was weighed and 10 ml chloroform, 15 ml acetic acid and 1 ml potassium iodide 10% were added. The flask was shaken for 1 min and left in the dark for 5 min. Then, titration took place with solution of sodium thiosulphate 0.01 M and 1% starch solution as indicator. Simultaneously, a blank run was carried out. The peroxide value expressed as mmoles of active oxygen per kg of sample was calculated by the following formula:

$$PV\ (mM/kg) = [(V - V_o) \times C \times 1000]/m$$

Where, V is the volume (ml) of sodium thiosulphate solution for the sample, V_o is the volume (ml) of sodium thiosulphate solution for the blank, C is the concentration of sodium thiosulphate solution and, m is the sample weight (g). All the assays were performed five times.

The Rancimat method

The Rancimat method involves passing a flow of air through the oil sample contained in a sealed and heated container. This method produces peroxides during the initial oxidation phase and organic acids with a low molecular mass, aldehydes and ketones with a typical rancid odor during the secondary oxidation phase (Vassilis et al., 2008). The compounds are directed by the air flow into a second reservoir containing distilled water that is continuously monitored for conductivity. The sudden variation of conductivity shows the presence of organic acids. The time that elapses between the beginning of the process and the appearance of the secondary reaction products is oxidation induction period or oil stability index (OSI) Vassilis et al. (2008).

In this study freeze-dried ethanol, methanol and acetone extracts of *T. obovatus* were added to commercial virgin sunflower and soya oil without any added antioxidant at concentrations ranging from 50 to 200 ppm. The antioxidant potential of these extracts was investigated and compared to the antioxidant potential of samples of commercial virgin sunflower and soya oil containing synthetic TBHQ and rosemary extracts. The measurements were repeated five times at an air flow rate of 20 L/h and temperature of 100°C

Statistical analyses

The data is presented as mean ± standard deviation (SD) of five determinations and was subjected to Student's *t*- test. Differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using SPSS Version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Determination of peroxide value

Increasing heating time for the sunflower samples resulted in an increase in peroxide value of all samples. Higher inhibition of peroxidation was achieved in samples containing acetone extracts. Even on the fifth day oil oxidation was inhibited greatly for acetone extracts (Table 1). At a concentration of 200 ppm, acetone extract was a stronger antioxidant than TBHQ, student `s t-test p = 0.05. Also for soya oil TBHQ and rosemary were inferior antioxidants as compared to acetone extracts (Table 2).

Table 1. Peroxide value of sunflower oil enriched or not with extracts (T = 85°C).

Sample	Peroxide value (mM/kg) Mean values ± SD n = 3				
	24 (h)	48 (h)	72 (h)	96 (h)	120 (h)
Sunflower oil	13.50 ± 0.12	16.50 ± 0.09	17.77 ± 0.09	22.01 ± 0.09	28.21 ± 0.41
Sunflower oil + ethanol extract 50 ppm	9.06 ± 0.09	10.87 ± 0.08	11.12 ± 0.42	14.83 ± 0.09	21.07 ± 0.21
Sunflower oil + ethanol extract 100 ppm	7.17 ± 0.31	9.91 ± 0.14	10.55 ± 0.09	13.44 ± 0.16	18.03 ± 0.09
Sunflower oil + ethanol extract 200 ppm	6.55 ± 0.09	8.63 ± 0.14	10.21 ± 0.12	12.22 ± 0.08	17.14 ± 0.43
Sunflower oil + methanol extract 50 ppm	8.07 ± 0.14	9.12 ± 0.09	10.13 ± 0.14	12.00 ± 0.21	16.02 ± 0.22
Sunflower oil + methanol extract 100 ppm	5.95 ± 0.09	8.66 ± 0.08	9.98 ± 0.14	10.97 ± 0.09	15.51 ± 0.09
Sunflower oil + methanol extract 200 ppm	5.87 ± 0.14	8.17 ± 0.14	9.41 ± 0.09	10.23 ± 0.21	14.07 ± 0.21
Sunflower oil + acetone extract 50 ppm	7.43 ± 0.12	8.44 ± 0.16	9.01 ± 0.09	10.89 ± 0.16	10.91 ± 0.09
Sunflower oil + acetone extract 100 ppm	7.41 ± 0.14	8.11 ± 0.09	8.89 ± 0.42	10.22 ± 0.09	10.24 ± 0.09
Sunflower oil + acetone extract 200 ppm	5.12 ± 0.21	7.44 ± 0.12	8.71 ± 0.21	9.13 ± 0.08	9.15 ± 0.22
TBHQ 200 ppm	6.01 ± 0.21	7.97 ± 0.03	8.76 ± 0.03	9.89 ± 0.08	9.91 ± 0.21
Rosemary 200 ppm	5.29 ± 0.42	7.47 ± 0.09	8.83 ± 0.41	9.17 ± 0.21	9.18 ± 0.21

Table 2. Peroxide value of soya oil enriched or not with extracts (T = 85°C).

Sample	Peroxide value (mmoles/kg) Mean values ± SD n = 3				
	24 (h)	48 (h)	72 (h)	96 (h)	120 (h)
Soya oil	17.50 ± 0.14	22.80 ± 0.21	24.62 ± 0.18	27.30 ± 0.43	27.88 ± 0.43
Soya oil + ethanol extract 50 ppm	10.50 ± 0.14	12.33 ± 0.19	13.99 ± 0.21	15.22 ± 0.09	17.01 ± 0.21
Soya oil + ethanol extract 100 ppm	10.65 ± 0.16	11.94 ± 0.08	13.21 ± 0.09	14.91 ± 0.14	16.00 ± 0.08
Soya oil + ethanol extract 200 ppm	8.51 ± 0.18	9.37 ± 0.17	10.97 ± 0.14	11.99 ± 0.14	14.13 ± 0.12
Soya oil + methanol extract 50 ppm	17.21 ± 0.08	21.20 ± 0.17	22.52 ± 0.21	24.50 ± 0.21	26.08 ± 0.14
Soya oil + methanol extract 100 ppm	7.50 ± 0.14	8.90 ± 0.09	9.99 ± 0.14	11.21 ± 0.08	12.03 ± 0.23
Soya oil + methanol extract 200 ppm	7.21 ± 0.14	8.30 ± 0.14	9.62 ±0.16	10.30 ± 0.08	11.17 ± 0.03
Soya oil + acetone extract 50 ppm	10.00 ± 0.16	12.05 ± 0.08	13.90 ± 0.14	14.60 ± 0.21	15.21 ± 0.21
Soya oil + acetone extract 100 ppm	7.10 ± 0.16	8.99 ± 0.14	9.55 ± 0.21	10.40 ± 0.09	11.18 ± 0.03
Soya oil + acetone extract 200 ppm	5.50 ± 0.14	6.20 ± 0.21	6.97 ± 0.09	7.73 ± 0.21	8.81 ± 0.08
TBHQ 200 ppm	7.80 ± 0.16	8.20 ± 0.08	9.10 ± 0.21	9.88 ± 0.18	10.23 ± 0.14
Rosemary 200 ppm	5.61 ± 0.16	6.30 ± 0.17	7.10 ± 0.14	9.22 ± 0.21	10.55 ± 0.08

The Rancimat method

The induction periods for both sunflower and soya oil subjected to accelerated oxidation conditions without or with added antioxidant are presented in Tables 3 and 4. Acetone extract, 200 ppm was the best protector against oil oxidation with induction period of 12.45 and 17.17 h respectively. Ethanol extracts increased the induction period of sunflower oil from 6.48 to 11.07 h. So, ethanol extracts also exerted good protection against oxidation but less efficiently as compared to acetone extracts. Their relatively strong protective effect in oily systems could be explained in terms of amphiphilic properties of phenolic constituents. It is generally believed that hydrogen donor ability and inhibition of oxidation are enhanced by increasing the number of hydroxyl groups in the phenol. This may imply the presence of polyphenols in extracts (Benavente-Garcia et al., 2000). A protection factor expresses the ratio of induction times for the stabilized and unstabilised oil; therefore a protection factor greater than 1 indicates inhibition of peroxidation. The higher the protection factor the most efficient the extract is (Tsaknis, 1996). For all the samples, the protection factor was greater than one with acetone extracts exhibiting the greatest for sunflower and soya oil, 1, 92 and 2, 76 respectively.

UV absorbance at 232 nm

An increase in UV absorbance at 232 nm due to conjugated diene formation from the unsaturated fats is an indicator of progressive staling of oil St. Angelo and Allen (1992). The control oil consisting of no stabilization

Table 3. Induction period at 100°C of virgin sunflower oil without or with the addition of TBHQ or rosemary extracts.

Sample	Induction period (h)	Protection factor
Sunflower oil	6.48 ± 0.07	1.00
Sunflower oil + TBHQ	10.26 ± 0.13	1.58
Sunflower oil + Rosemary	10.92 ± 0.03	1.69
Sunflower oil + methanol extract 50 ppm	10.33 ± 0.07	1.59
Sunflower oil + methanol extract 100 ppm	10.34 ± 0.04	1.60
Sunflower oil + methanol extract 200 ppm	10.97 ± 0.13	1.69
Sunflower oil + ethanol extract 50 ppm	9.93 ± 0.03	1.53
Sunflower oil + ethanol extract 100 ppm	10.90 ± 0.03	1.68
Sunflower oil + ethanol extract 200 ppm	11.07 ± 0.07	1.71
Sunflower oil + acetone extract 50 ppm	10.12 ± 0.03	1.56
Sunflower oil + acetone extract 100 ppm	10.95 ± 0.06	1.69
Sunflower oil + acetone extract 200 ppm	12.45 ± 0.03	1.92

Table 4. Induction period at 100°C of virgin soya oil without or with the addition of TBHQ or rosemary extracts.

Sample	Induction period (h)	Protection factor
Soya oil	6.21 ± 0.03	1.00
Soya oil + TBHQ 200 ppm	9.97 ± 0.07	1.61
Soya oil + Rosemary 200 ppm	10.08 ± 0.17	1.62
Soya oil + methanol extract 50 ppm	9.82 ± 0.08	1.58
Soya oil + methanol extract 100 ppm	10.14 ± 0.03	1.63
Soya oil + methanol extract 200 ppm	10.88 ± 0.13	1.75
Soya oil + ethanol extract 50 ppm	9.88 ± 0.04	1.59
Soya oil + ethanol extract 100 ppm	10.22 ± 0.03	1.65
Soya oil + ethanol extract 200 ppm	10.93 ± 0.07	1.76
Soya oil + acetone extract 50 ppm	10.07 ± 0.13	1.62
Soya oil + acetone extract 100 ppm	10.46 ± 0.04	1.68
Soya oil + acetone extract 200 ppm	17.17 ± 0.07	2.76

Table 5. UV absorbance at 232 nm.

Sample	Absorbance at 232 nm
Soya oil	1.417 ± 0.002
Soya oil + TBHQ 200 ppm	0.824 ± 0.001
Soya oil + Rosemary 200 ppm	0.645 ± 0.007
Soya oil + methanol extract 50 ppm	0.684 ± 0.003
Soya oil + methanol extract 100 ppm	0.621 ± 0.003
Soya oil + methanol extract 200 ppm	0.586 ± 0.001
Soya oil + ethanol extract 50 ppm	0.668 ± 0.002
Soya oil + ethanol extract 100 ppm	0.633 ± 0.007
Soya oil + ethanol extract 200 ppm	0.577 ± 0.001
Soya oil + acetone extract 50 ppm	0.645 ± 0.001
Soya oil + acetone extract 100 ppm	0.522 ± 0.001
Soya oil + acetone extract 200 ppm	0.294 ± 0.003

exhibited the highest UV absorbance (Table 5), and the least was acetone extract at a concentration of 200 ppm showing the greatest protection factor. The extracts showed better protection against oil oxidation than TBHQ

and Rosemary. In similar studies, plant extracts showed better antioxidant activities against oil oxidation than synthetic antioxidants (Maforimbo, 2002; Lafka et al., 2007)

Mahamadi et al. (2011) extracted phenolic compounds from *T. obovatus*. Phenolic compounds act as antioxidants for a number of potential factors. The most important is by free radical scavenging in which the phenol can break the free radical chain reaction. The presence of different substituents in the phenol backbone structures is responsible for their different antioxidant properties, in particular their hydrogen-donating capacities.

Conclusion

The possibility of using natural antioxidants from *T. obovatus* extracts as potent antiperoxidants for sunflower and soya oil was confirmed. This may encourage prospects of their application in food industry as natural antioxidants to increase the shelf life by preventing lipid oxidation.

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