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Citrus peel extract – A natural source of antioxidant

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Abstract

Citrus peel extract as a natural source of antioxidant was evaluated during 6 months storage of refined corn oil at 25 and 45 °C. Extracts of citrus peel were prepared by refluxing the dried ground peel with ethanol, methanol, acetone, hexane, diethyl ether and dichloromethane. Maximum amount of citrus peel extract was obtained with methanol. Antioxidant activity of methanolic extract was assessed by measuring free fatty acid (FFA) content peroxide value (POV) and iodine value (IV) during 6 months storage of refined corn oil at 25 and 45 °C. After 6 months of storage at 45 °C, corn oil containing 1600 and 2000 ppm citrus peel extract, showed lower FFA contents (1.5% and 1.0%), and POVs (8.38 and 7.0 meq kg^{-1}) and higher iodine values (81, 89) than the control sample (FFA 17.0% POV 101 meq kg^{-1} IV 47). Refined corn oil containing 200 ppm of butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) showed FFA contents of 2.0% and 1.8%, POVs 17.0 and 12.7 meq kg^{-1} and IVs 84 and 87, respectively, after 6 months of storage at 45 °C. These results show that methanolic extract of citrus exhibited very strong antioxidant activity, which was almost equal to synthetic antioxidants (BHA and BHA). Therefore, the use of citrus peel extract is recommended as a natural antioxidant to suppress development of rancidity in oils and fats.

Keywords: Antioxidant activity; Citrus peel extract; Corn oil; Phenolic compounds

1. Introduction

Rancidity develops in fats and oils at elevated temperature during storage. Normally, synthetic antioxidants (BHT and BHA) are used to suppress the development of rancidity in fats and oils. These synthetic antioxidants are known to have toxic and carcinogenic effects on human health (Ito et al., 1986; Martin & Gilbert, 1968). Therefore, there is a strong need for effective antioxidants from natural sources as alternatives to prevent deterioration of fats and oils. The literature is replete with reports of extracts obtained from edible and non-edible plant materials, which possess antioxidant compounds (Alexander et al., 1998). Extract of oat showed antioxidant activity in cooking oils due to the presence of phenolic compounds (Emmons & Peterson, 1999). Benavente, Lorcnte, Castillo, Ortno, and Del Rio (2000) also extracted natural antioxidant from olive leaf to prolong the storage life of fats and oils. Antioxidant activity of methanolic extract from peanut hulls has been observed during storage of fried potato chips (Rehman, 2003). Dichloromethane extract of ginger showed antioxidant activity, which was not equal to synthetic antioxidants (Rehman, Salariya, & Habib, 2003). Extracts from herbs and spices were found to be effective for controlling the development of rancidity in fats and oils (Farag, Badel, & El Baraty, 1989; Kohchi, 1995). These extracts have been reported to be more effective, in many instances, than some major synthetic antioxidants (Larson, 1989; Marinova & Yanishlieve, 1997). Citrus peel are a waste material, obtained after extraction of juice from citrus fruit. Methanolic extract of citrus peel is known to have different antioxidative compounds (Alexandra, Marie Elisabeth, Hubert, & Clendette, 1998). However, effect of storage temperature and time on the antioxidant activity of citrus peel extract have not been so far studied. The present study was undertaken to study the antioxidant activity of citrus peel extract in refined corn oil during storage.

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2. Materials and methods

2.1. Material

Refined, bleached and deodorized corn oil was obtained from a local refinery whereas citrus peel were obtained from the fruit processing industry to carry out this study. Synthetic antioxidants, namely butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Company, USA.

2.2. Citrus peel extract preparation (natural antioxidant)

The citrus peel were washed and then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia PA) at 80 °C. The dried peel were ground into a fine powder in a mill (Tecator – Cemotec 1090 samples mill, Hogans, Sweden). The material that passed through an 80-mesh sieve was retained for use. Ten grammes of ground peel were extracted with 100 ml of organic solvents (ethanol, methanol, acetone, hexane, petroleum ether and diethyl ether) overnight in a shaker at room temperature. The extract was filtered through cheese cloth and the residue was re-extracted under the same conditions. The combined filtrate was evaporated in a rotary evaporation (EVF-530-010K-GallenKamp) below 40 °C. The extract obtained after evaporation of organic solvent was used as natural antioxidant.

2.3. Application of citrus peel extract to corn oil

Refined corn oil, free of additives, was used as the substrate for oxidation studies. Corn oil samples containing 1200, 1600 and 2000 ppm methanolic citrus peel extract were separately prepared. Each 100 ml prepared oil sample was placed in a 250 ml brown air-tight glass bottle. Synthetic antioxidants (BHA and BHT) were mixed in with the oil for comparative study at their legal limit of 200 ppm (Duh and Yen, 1997). Control samples of corn oil without antioxidant were also placed under identical conditions. All oil samples of each treatment were prepared in triplicate, which were placed at 25 and 45 °C for 6 months. The oil samples of each treatment were withdrawn periodically after a 2 month intervals to assess the antioxidant activity of citrus extract.

2.4. Antioxidant activity testing

Citrus peel extract, as antioxidant, was tested by the determination of free fatty acids (FFA), peroxide value (POV) and iodine value (IV) during storage of corn oil at 25 and 45 °C. Free fatty acids, as oleic acid percentages in oil samples, were determined using an alkali titration method whereas peroxide value (meq kg^{-1} of oil) was measured by titration with 0.1 N sodium thiosulphate, using starch as indicator (AOAC, 1990). However, iodine value in oil samples was determined by Wij's method, as described in AOAC (1990). All determinations were carried out in triplicate and mean values were calculated. Significant differences ($P < 0.05$) were calculated using Duncan's multiple range test as described by Steel and Torrie (1980).

3. Results and discussion

3.1. General

Table 1 shows the percentage yield of citrus peel extract obtained after refluxing ground and dried citrus peel with six different organic solvents, namely ethanol, methanol, acetone, hexane, petroleum ether and diethyl ether. About 7.88–19.87% of citrus peel extract was obtained with these six organic solvents. However, the maximum amount of citrus peel extract (19.87%) was obtained with methanol, followed by acetone (15.00%) and diethyl ether (12.75%). Alexandra et al. (1998) reported different phenolic antioxidant compounds in the methanolic extract of citrus peel. Therefore, the antioxidant activity of the extract of highest yield (methanol) was tested in refined corn oil at 25 and 45 °C during 6 months of storage. Free fatty acids (FFA), peroxide value (POV) and iodine value (IV) were determined to assess the development of rancidity during storage of corn oil.

3.2. Rancidity development during storage of refined corn oil

Development of rancidity in corn oil was significantly ($P < 0.05$) affected by storage temperature and time. A gradual increase in FFA contents and POV, along with decrease in iodine value was observed during storage of refined corn oil at 25 and 45 °C for 6 months (Table 2). The changes in FFA, POV and IVs were more pronounced at 45 °C than at 25 °C. Initially, the FFA contents, POV and IV of refined corn oil without antioxidant (control) were 0.167%, 0.7 meq kg^{-1} and 104, respectively. After 6 months storage, FFA contents were 13.0% and 17.0%, POVs were 93 and 107 meq kg^{-1} and IVs were 59 and 47 at 25 and 45 °C, respectively. The decrease in iodine value could be attributed to breaking of double bonds of unsaturated fatty acids of lipid during storage of corn oil at elevated temperature, as reported by earlier workers (Noor & Augustin, 1984). Generally, the principal route of the deterioration of fat is through oxidative rancidity, which takes

Table 1
Percentage yield of citrus peel extract with different organic solvents

Organic solvent	Citrus peel extract yield (%)[a]
Ethanol	11.00 ± 0.82
Methanol	19.87 ± 1.00
Acetone	15.00 ± 1.21
Hexane	9.12 ± 1.27
Petroleum ether	7.88 ± 1.42
Diethyl ether	12.75 ± 1.35

[a] g oil/100 g dried citrus peel.

Table 2
Effect of storage conditions on free fatty acids (FFA), peroxide value (POV) and iodine value (IV) of corn oil without antioxidant[A]

Storage time (months)	25 °C			45 °C		
	FFA (%) Oleic acid	POV (meq kg^{-1})	IV	FFA (%) Oleic acid	POV (meq kg^{-1})	IV
0	$0.167^{a} \pm 0.02$	$0.70^{a} \pm 0.17$	$104^{a} \pm 1.37$	$0.167^{a} \pm 0.03$	$0.70^{a} \pm 0.20$	$104^{a} \pm 1.37$
2	$3.5^{b} \pm 0.04$	$13.5^{b} \pm 0.18$	$91^{b} \pm 1.11$	$5.0^{b} \pm 0.04$	$17.0^{b} \pm 0.20$	$83^{b} \pm 1.29$
4	$7.0^{c} \pm 0.05$	$57.0^{c} \pm 0.11$	$74^{c} \pm 1.23$	$11.0^{c} \pm 0.07$	$72.0^{c} \pm 0.15$	$69^{c} \pm 1.19$
6	$13.0^{d} \pm 0.02$	$93.0^{d} \pm 0.21$	$59^{d} \pm 1.20$	$17.0^{d} \pm 0.04$	$101.0^{d} \pm 0.16$	$47^{d} \pm 1.22$

Values within a column with different superscripts are significantly different at $P < 0.05$.
[A] Values are averages of triplicate determinations with standard deviation.

place at the double bond in the triglyceride molecule (Akhtar, Asghar, & Sheikh, 1985). In fat deterioration, the first initiating step is the formation of free fatty acids, which are susceptible to oxygen attack in the presence of light, resulting in the formation of many organic compounds and free fatty acids which are responsible for development of rancidity and off-flavours in fatty food materials (Sattar & Demen, 1973). Production of free fatty acids and increase in peroxide values are the best predictors of fat deterioration, which could be used to monitor the extent of fat spoilage. It is well known that decrease in iodine value is another factor by which deterioration of fat can also be examined.

3.3. Effect of synthetic antioxidants on development of rancidity during storage of corn oil

The changes in FFA, POV and IVs during storage of refined corn oil at 25 and 45 °C after the addition of BHA and BHT are listed in Table 3. It is apparent from Table 3 data, that addition of BHA and BHT significantly ($P < 0.05$) retarded the development of rancidity in corn oil but BHT showed better results than BHA. The FFA values were reduced from 17.0% (control) to 2.0 and 1.8%, POVs decreased from 101 meq kg^{-1} to 17.0 and 12.7 meq kg^{-1}, respectively, after 6 months of storage at 45 °C after the addition of BHA and BHT in corn oil. At 25 °C, the addition of BHA and BHT caused reduction in FFA from 13.0% (control) to 1.7% and 1.4%, POVs decreased from 93 meq kg^{-1} (control) to 14.0 and 11.0 meq kg^{-1}, respectively, after 6 months of storage. Besides increase in free fatty acids and peroxide values, a marked decrease in iodine value was observed during storage of corn oil at 25 and 45 °C (Table 3). In fact, a decreasing trend in iodine value indicates the development of rancidity due to formation of secondary oxidation products during storage of fats and oils. Addition of synthetic antioxidants retarded the decreasing trend of iodine value during storage of corn oil. Addition of BHA and BHT to corn oil, showed iodine values of 86 and 89 at 25 °C and 84 and 87 at 45 °C, respectively, during 6 months of storage, whereas iodine values of the fresh corn oil were 59 at 25 °C and 47 at 45 °C on storage for 6 months. Therefore, iodine values of stored corn oil were significantly ($P < 0.05$) higher than control samples of corn oil. The findings of Rehman (2003) revealed that addition of BHA and BHT to cooking oil retarded the development of rancidity in fried potato chips during storage. Kathy, Randel, Peter, and George (1994) suggested that addition of BHA, along with another antioxidant, inhibited food deterioration during storage at high and ambient temperature. Our results are also consistent with the finding of other workers who reported that lipid peroxides were significantly reduced by the addition of antioxidants in fats and oil (Kiyomi & Yasuko, 1995; Yanping, Mourning, Yuhang, & Zhying, 1999). Statistical analysis of the data

Table 3
Effect of synthetic antioxidants on free fatty acids (FFA), peroxide value (POV) and iodine value (IV) during storage of corn oil[A]

Storage time (months)	25 °C			45 °C		
	FFA (%)	POV (meq kg^{-1})	IV	FFA (%)	POV (meq kg^{-1})	IV
BHA – 200 ppm						
0	$0.167^{a} \pm 0.02$	$0.70^{a} \pm 0.17$	$104^{a} \pm 1.37$	$0.167^{a} \pm 0.02$	$0.7^{a} \pm 0.17$	$104^{a} \pm 1.37$
2	$0.9^{b} \pm 0.07$	$6.4^{b} \pm 0.16$	$100^{a} \pm 1.22$	$0.9^{b} \pm 0.03$	$7.0^{b} \pm 0.16$	$97^{b} \pm 1.29$
4	$1.2^{b} \pm 0.18$	$10.5^{c} \pm 0.21$	$94^{b} \pm 1.09$	$1.7^{c} \pm 0.13$	$13.0^{b} \pm 0.21$	$92^{b} \pm 1.44$
6	$1.7^{a} \pm 0.16$	$14.0^{d} \pm 0.22$	$86^{c} \pm 1.17$	$2.0^{c} \pm 0.09$	$17.0^{c} \pm 0.27$	$84^{c} \pm 1.05$
BHT – 200 ppm						
2	$0.2^{a} \pm 0.04$	$3.9^{b} \pm 0.11$	$102^{a} \pm 1.29$	$0.7^{b} \pm 0.05$	$4.3^{b} \pm 0.19$	$100^{a} \pm 1.38$
4	$0.8^{b} \pm 0.17$	$7.7^{c} \pm 0.23$	$97^{b} \pm 1.44$	$1.8^{c} \pm 0.13$	$8.9^{b} \pm 0.13$	$94^{b} \pm 1.40$
6	$1.4^{c} \pm 0.19$	$11.0^{d} \pm 0.16$	$89^{c} \pm 1.51$	$1.8^{c} \pm 0.09$	$12.7^{c} \pm 0.14$	$87^{c} \pm 1.49$

After 6 months' storage of corn oil (without antioxidant) at 25 and 45 °C, FFA = 13.0 and 17.0%, POV = 93.0 and 101.0 meq kg^{-1} and IV = 59 and 57, respectively.
Values within a column with different superscripts are significantly different at $P < 0.05$.
[A] Values are averages of triplicate determinations with standard deviation.

Table 4
Effect of citrus peel extract on free fatty acid (FFA), peroxide value (POV) and iodine value (IV) during storage of corn oil[A]

Storage time (months)	25°C			45°C		
	FFA %	POV (meq kg^{-1})	IV	FFA (%)	POV (meq kg^{-1})	IV
Citrus Peel Extract 1200 ppm						
0	$0.167^{a} \pm 0.02$	$0.70^{a} \pm 0.17$	$104^{a} \pm 1.37$	$0.167^{a} \pm 0.02$	$0.70^{a} \pm 0.17$	$104^{a} \pm 1.37$
2	$1.2^{b} \pm 0.01$	$4.8^{b} \pm 0.17$	$100^{a} \pm 1.44$	$1.5^{b} \pm 0.02$	$5.0^{b} \pm 0.17$	$97^{b} \pm 1.32$
4	$1.6^{b} \pm 0.04$	$7.5^{b} \pm 0.19$	$98^{b} \pm 1.29$	$2.0^{c} \pm 0.03$	$8.3^{c} \pm 0.21$	$95^{b} \pm 1.21$
6	$2.0^{c} \pm 0.05$	$10.0^{c} \pm 0.14$	$87^{c} \pm 1.27$	2.2c ± 0.04	$10.8^{c} \pm 0.12$	$84^{c} \pm 1.29$
Citrus Peel Extract 1600 ppm						
2	$0.8^{b} \pm 0.03$	$3.1^{b} \pm 0.11$	$98^{b} \pm 1.31$	$1.0^{b} \pm 0.04$	$3.5^{b} \pm 0.22$	$96^{b} \pm 1.33$
4	$1.0^{b} \pm 0.05$	$5.0^{b} \pm 0.13$	$94^{b} \pm 1.04$	$1.2^{b} \pm 0.05$	$5.8^{c} \pm 0.39$	$92^{b} \pm 1.40$
6	$1.2^{c} \pm 0.05$	$7.4^{c} \pm 0.14$	$83^{c} \pm 1.41$	$1.5^{c} \pm 0.06$	$8.3^{d} \pm 0.40$	$81^{a} \pm 1.09$
Citrus Peel Extract 2000 ppm						
2	$0.7^{b} \pm 0.02$	$2.5^{b} \pm 0.21$	$96^{b} \pm 1.30$	$0.9^{b} \pm 0.04$	$3.0^{b} \pm 0.21$	$98^{b} \pm 1.22$
4	$0.8^{b} \pm 0.04$	$4.0^{b} \pm 0.29$	$92^{b} \pm 1.40$	$1.0^{b} \pm 0.05$	$4.4^{b} \pm 0.40$	$95^{b} \pm 1.11$
6	$1.0^{c} \pm 0.07$	$6.3^{c} \pm 0.41$	$81^{c} \pm 1.12$	$1.0^{b} \pm 0.03$	$7.0^{c} \pm 0.45$	$89^{c} \pm 1.11$

After 6 months' storage of corn oil (without antioxidant) at 25 and 45 °C, FFA = 13.0% and 17.0%, POV = 93.0 and 101 meq kg^{-1} and IV = 59 and 47, respectively.
Values within a column with different superscripts are significantly different at $P < 0.05$.
[A] Values are averages of triplicate determinations with standard deviation.

revealed that the developments of rancidity of corn oil were significantly ($P < 0.05$) reduced by addition of BHA and BHT.

3.4. Effect of citrus peel extract on development of rancidity during storage of corn oil

Addition of citrus peel extract caused significant changes in FFA, POV and IVs of refined corn oil during 6 months of storage at 25 and 45 °C. It is evident from these results that, as the concentration of citrus peel extract increased, inhibitory effects on FFA, POV and IVs also increased considerably (Table 4). After 6 months of storage at 45 °C, FFA values of corn oil treated with 1200, 1600 and 2000 ppm of citrus peel extract were 2.2%, 1.5% and 1.0%, POVs were 10.8, 8.3 and 7.0 meq kg^{-1} and IVs values were 84, 81 and 80, respectively. Significant differences ($P < 0.05$) in free fatty acids, peroxide value and iodine values were observed between the control and the corn oil treated with different concentrations of citrus peel extracts. The decrease in FFA and POVs and increase in iodine values clearly indicate that autoxidation of corn oil was greatly inhibited in the presence of citrus peel extract at concentrations of 1600 and 2000 ppm. However, there was no distinct difference between synthetic antioxidants (200 ppm) and citrus peel extract (1600 and 2000 ppm) in inhibition of corn oil peroxidation. These results confirm the findings of earlier workers, who identified phenolic and flavonoid antioxidative compounds in the non-volatile fraction of methanolic extract of citrus peel (Alexandra et al., 1998). Many other workers found that antioxidant activity in the extract of edible and non-edible plant materials due to the presence of phenolic compounds (Kaehkoenen et al., 1999). The findings of John (2004) also revealed that the antioxidant property was observed in orange peel ultra-filtered molasses due to the presence of phenols, including numerous flavanones, flavone glycosides, polymethoxylated flavones, hydroxy cinnamates and other miscellaneous phenolic glycosides and amines. In this study, addition of methanolic extract of citrus peel showed strong antioxidant activity during storage of refined corn oil, which could be attributed to the presence of different phenolic compounds in orange peel. This study revealed that the level of citrus peel extract was 8–10 times higher than that of synthetic antioxidant to control the development of rancidity in corn oil. However, natural antioxidant extract of citrus peel would be preferred over synthetic antioxidants to minimize the adverse health effects.

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