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EFFECT OF INCORPORATION OF *Citrus sinensis* PEEL EXTRACT (CPE) AS NATURAL ANTIOXIDANT ON SHELF STABILITY OF SUNFLOWER OIL IN AN ACCELERATED STORAGE TEST

K. Chavan and V. Yardi
Dept. of Foods, Nutrition and Dietetics, College of Home Science, NirmalaNiketan, Mumbai
Corresponding Author Email :kalyanichavan30@gmail.com

ABSTRACT :
Fats and oils undergo deterioration during storage may be because of their natural structure and other external factors. Due to destruction of natural antioxidant during processing of oil; synthetic antioxidants are added in it, but, its permissible limit is 200ppm as high concentrations are reported to be carcinogenic. Peel is produced as a waste product after recovery of fruit juices. Citrus peels possess antioxidant activity and hence, in the present study, the efficacy of Citrus sinensis peel extract (CPE) as natural antioxidant in Sunflower oil was evaluated. CPE was prepared with methanol, ethanol and ethyl acetate and the best solvent was selected on the basis of yield. The antioxidant effectiveness of CPE was evaluated using Sunflower oil as an oxidative substrate. The oil sample containing Methanolic CPE (1500ppm, 2000ppm and 2500ppm), 150ppm of TBHQ (Tertiary Butyl Hydroxyquinone) and control (without antioxidant) were subjected to accelerated storage (60°C in electric hot air oven, 8hrs heating cycles per day) for the period of 60 days and analyzed after 10 days for the extent of oxidative changes by measurement of peroxide value (PV) and free fatty acid (FFA) value. The results obtained revealed that the oil containing 2000ppm and 2500ppm CPE showed lower PV than oil containing TBHQ. Hence, it can be said that the CPE (2000ppm and 2500ppm) can be used as natural antioxidant in Sunflower oil. But, as the colour of the oil also gets affected, the 2000ppm CPE concentration can be used to obtain best results.Hence, it can be concluded that, Methanolic extract of Citrus sinensis peel possess antioxidant activity($p<0.01$) and best results could be achieved using the CPE concentration of 2000ppm and it could be a waste utilization also.
Keywords : antioxidants, CPE, Peroxide value, Free fatty acid value.

INTRODUCTION :

The oil undergoes quality deterioration such as development of rancidity, lipid oxidation, reversion which lead to development of off flavour and odour; heat and presence of spoilage organisms also lead to quality deterioration of oil. Among all these factors lipid oxidation is major cause of quality loss of oil during processing and storage of oil.

In order to prevent deterioration of oil and to extend the shelf life of the oil some of the synthetic radical scavengers are used as antioxidants in oil such as TBHQ, BHT. But, these synthetic antioxidants have been associated with a number of pathological effects. They are potential carcinogens and may interact negatively with enzymes which have undesirable effects on health and reproduction [(Gower 1998; Sun 1990) cited in Msagati, 2013]. Phenolic antioxidants such as BHA and BHT have been associated with the worsening of diseases such as urticaria [(Goodman et al., 1990) cited in Msagati, 2013]. Propyl Gallate is another phenolic compound which has been listed as a human carcinogen [(van der Heijden et al., 1986) cited in Msagati, 2013]. Hence, the maximum permitted limit of all these synthetic antioxidants in vegetable oils are 200 ppm [(Directive 2002/46/EC) cited in Msagati, 2013]. Hence, many researchers exploited different naturally occurring compounds such as green tea extract, grape seed extract, and pomegranate peel to check their effectiveness as natural antioxidant.

Orange peel also possess antioxidant activity and it have been reported that *C. sinensis* peel contains the highest concentration of phenolics, flavonoids, ascorbic acid, carotenoids and reducing sugars, which certainly contribute to the highest antioxidant potential (Guimaraes et al., 2010) & it can be used as food additive or a pharmaceutical application (Anagnostopoulou, 2005). However, few studies focuses on use of *Citrus sinensis* peel extract as natural antioxidant in oil against lipid peroxidation. One such study includes the evaluation of the activity of citrus peel extract as a natural source of antioxidant in refined corn oil (Zia-ur-Rehman, 2006).

Therefore, in the present study the aim was to evaluate the efficiency of methanolic extract of *Citrus sinensis* peel extract as Natural antioxidant on the basis of calculation of Peroxide value (PV) and Free Fatty Acid value (FFA) in Sunflower oil when subjected to the accelerated storage test.

MATERIAL AND METHODS :

Sample collection :The fruits were selected on the basis of their colour, size, aroma and uniformity of thickness of the peel from Mumbai local market.

Refined Sunflower Oil (SO) samples with antioxidant (150 ppm TBHQ) and without antioxidant were procured from Kamani Oil Industries Pvt. Ltd., Khopoli.

Preparation of sample for extraction :The fruits were washed to remove any dust particles if present, wiped and dried by using clean cotton cloth.Each fruit was cut into the four pieces and the peel was removed carefully.The white portion (albedo) of the fruit was removed with the help of sharp knife and cut into the strips. The peel strips were dehydrated by tray drying method in a dehydrator at 50°C for 4 hrs.These dried peels were ground in a mixer to make a fine powder of it.

Extraction Procedure : 20 g of orange peel powder was extracted with 300 ml of solvents (Methanol, Ethanol and Ethyl acetate) separately by using Soxhlet apparatus for 6 hrs at 80°C. The residual solvent was evaporated. The extracts were cooled and filtered through the filter paper (Whatman No. 42). The yield of extract was calculated and the extracts were stored in air tight container at 4°C till its use for further analysis (Source: Kumar et al., 2007, cited in Kadam et al., Beverage and food world, Jun 2012).

Addition of CPE into SO sample : CPE was added to preheated (50°C) SO sample (500 ml) to make concentrations of 1500 ppm (SOCPE-1), 2000 ppm (SOCPE-2) and 2500 ppm (SOCPE-3) separately. The oil samples were stirred for 30 mins for uniform dispersion. These oil samples were placed in 50 ml dark brown glass bottles with narrow neck. The stabilize oil sample (containing TBHQ-150 ppm) i.e. SOSA and the control oil samples i.e. SONA were also placed in dark brown coloured bottles with narrow necks and the bottles were capped. All these oil samples were subjected to accelerated storage in an electric hot air oven at 60°C for 8 hrs heating cycles per day for the period of 60 days. The effect of extract on the lipid peroxidation of Sunflower oil samples was evaluated by measurement of Peroxide Value (PV) and free fatty acid (FFA) value of oil during storage period of 60 days at the interval of 10 days (Source: Kadam et al., 2012).

Peroxide Value : 5 g of sample was weighed into 250 ml stoppered flask. 30 ml of Acetic acid – Chloroform solvent mixture (3:2 v/v) was added and swirled to dissolve. With occasional shaking 0.5 ml of KI was added and shake for 1 min. 30 ml of D/W was added. The liberated iodine was titrated with 0.01 N Sodium Thiosulphate, using 0.5 ml starch solution as indicator. End point was from blue to colourless. Estimations were conducted in triplicates and an average was used.

PV expressed as mili equivalent of Peroxide O_2 per kg sample (meq/kg) :

$$PV = \frac{B.R. \times N \times 1000}{\text{weight of sample (g)}}$$

Where,

B.R. = Burette reading,

N = Normality of Sodium Thiosulphate (0.01)

(Source: Kamani Oil Industries, Quality Manual, Issued on: 15-3-2002, pp 139).

Free Fatty Acid Value : 1 g of oil sample was weighed. It was dissolved in 50 ml of 95% ethanol, heat to dissolve the fat. Titrated with 0.1 M KOH solution using Phenolphthalein indicator (5 drops). End point was development of pink colour persisting atleast for 10 secs. Estimations were conducted in triplicates and an average was used.

FFA value was calculated as:

$$\% \text{ FFA (as oleic)} = \frac{B.R. \times 0.1 \times 28.2 \text{ mg}}{\text{sample wt}}$$

Where, B.R. = Burette reading

[(n.d.). Retrieved on 19-2-14 from google.com: http://www.lrrd.org/lrrd19/7/olur19089.htm]

RESULTS AND DISCUSSION :

Yield of extracts were as given in Table-1:

Table-1: The yield of different extracts

Sr. No.	Solvents	Yield of extract (%)
1.	**Methanol**	**19.65**
2.	Ethanol	11.76
3.	Ethyl acetate	4.24

It was observed that methanolic extract possessed highest yield and hence it was planned to incorporate Methanolic CPE into Sunflower oil.

Table-2: The FFA values

Days	CPE concentration			TBHQ	Control
	1500 ppm	2000 ppm	2500 ppm		
0	0.05	0.05	0.05	0.05	0.05
10	0.06	0.06	0.06	0.06	0.07
20	0.06	0.06	0.06	0.06	0.09
30	0.07	0.07	0.07	0.07	0.10
40	0.09	0.09	0.09	0.09	0.7
50	0.20	0.20	0.20	0.20	1.0
60	0.92	0.90	0.98	0.94	1.86

Table-3: The Peroxide Values

Days	CPE concentration			TBHQ	Control
	1500 ppm	2000 ppm	2500 ppm		
0	1.06	1.06	1.06	0.91	1.06
10	3.24	2.06	1.50	1.05	5.84
20	7.09	5.26	4.08	1.80	9.06
30	10.86	6.73	5.02	2.94	12.90
40	12.08	6.89	6.52	3.94	14.04
50	17.48	7.69	7.24	6.42	19.84
60	19.24	8.05	8.12	9.81	23.46

The values of yield of different extracts; FFA values and Peroxide Values of different concentrations of Methanolic extract, oil containing TBHQ as well as Control oil samples are given in Table-1, Table-2, Table-3 respectively.The PV of TBHQ was higher as compare to the PV of 2000 ppm and 2500 ppm of CPE after storage period of 60 days. Therefore, it can be said that CPE concentration of 2000 ppm and 2500 ppm were effective in delaying the lipid peroxidation in turn release of peroxides and hydroperoxides. It must be noted that values attained with the different concentrations of CPE are way below the values obtained for control. Thus, showing positive effect of CPE.

CONCLUSION :

It can be concluded that, the methanolic extract of *Citrus sinensis* peel possess antioxidant activity and the best antioxidant results could be achieved using the CPE concentration of 2000 ppm & 2500 ppm.But, as the colour of the oil also gets affected, the 2000ppm CPE concentration can be used to obtain best results. Also, as citrus peel is a waste product of fruit juice industries, therefore, it can be waste utilization also.

However, longer duration of study period with varying proportions of synthetic and natural antioxidant may show varying results. More studies are required to draw a concrete conclusion.

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