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Extraction of total carotenoids from calendula officinalis and their effects on the oxidation stability of mustard oil

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Abstract: *Extraction of carotenoids was carried out from Calendula officinalis (English marigold) flowers and their effects on the oxidation stability of mustard oil under light storage was determined. Four solvents viz., hexane, acetone, ethanol and tolune were used for extraction of total caroteinoids.The carotenoid extract of the calendula flowers obtained was incorporated in mustard oil at 10, 25 and 50 ppm concentrations to prevent oxidation in oil samples placed in 20w illuminating closed chamber.The antioxidant effectiveness of carotenoid extract towards mustard oil was dependent upon concentrations. Samples were observed to determine peroxide and TBA values after storage period of 0,1,3, 5 and 7 days. Samples incubated with carotenoid extracts of calendula flowers exhibited lower values of Peroxide and TBA values as compared to control samples. Minimum values of peroxide and TBA values were observed in case of oil samples incubated with 50 ppm concentrations as that of 25 and 10 ppm concentrations.*

Keywords: *Calendula, oxidation stability Carotenoids, solvent mixtures*

I. Introduction

Calendula officinalis L., also known as English marigold or pot marigold, belong to the Asteraceae (Compositae) family; it is an annual with bright or yellow orange daisy-like flower which are used for therapeutic or culinary purposes[1].*C. officinalis* can be largely useful as an antiseptic, anti-inflammatory and cicatrizing[2]and also as a light antibacterial [3]and antiviral [4]agent. *C. officinalis* can be used as a colorant because it principally contains two classes of pigments, the flavonoids and carotenoids, which can be used as yellow and orange natural colors, respectively. Natural colors are gaining significant attention since several synthetic colorants have given rise to allergic, toxic and carcinogenic effects [5]. Flavonoids possess antioxidant activities which play an important role in food preservation and human health by combating damage caused by oxidizing agents [6].The yellow pigment, carotenoids are very essential to humans and other animals as precursors of vitamin A and retinoids.

Carotenoids are natural pigments that give the natural yellow, orange, and red colours of fruits, vegetables, plants, flowers, birds, and marine animals. These colours are due to the occurrence of conjugated double bonds, also providing carotenoids with antioxidant property. In addition, carotenoids are well attributed with important health-promoting functions or activities, such as provitamin A activity, enhancement of the immune system and reduce risk of degenerative diseases, as cancer and cardiovascular diseases [7-9].

The majority methods of extraction of carotenoids from plant sources make use of organic solvents such as hexane, ethanol, acetone, methanol, tetrahydrofuran, benzene, and petroleum ether [10-12]. Furthermore, mixtures of hexane with acetone, ethanol or methanol are frequently used [13] because other compounds, such as diethyl ether and tetrahydrofuran, might contain peroxides that react with carotenoids. It has been observed that the stability of carotenoid extracts obtained with hexane/acetone or hexane/ethanol was higher than that of extracts obtained with other organic solvents, such as chloroform, methanol or dichloromethane[14]. Also, supercritical fluids are appropriate for the extraction of compounds that can simply become degraded by light, oxygen and high temperatures like carotenoids, however the solubility of these substances is still somewhat low compared to their solubility in organic solvents, and high pressures must be used to obtain practical extraction yields [15].Therefore, solvent extraction method has been always the primary option as far as industrial point is concerned due to its simplicity and low costs.

It has been reported that β-carotene reduced lipid oxidation of soybean oil [16]. The effects of lutein, zeaxanthin, lycopene, isozeaxanthin and astaxanthin on the photooxidation of soybean oil was also studied and has been found that the antioxidant effectiveness of carotenoids increased as the number of the conjugated double bonds of carotenoids increased[17]. Evidently the antioxidant capacity of carotene and xanthophyll is because of the fact that they have similar numbers of conjugated carbon-carbon double bonds.

II. Materials And Methods

2.1.Materials

Fresh flowers that were free from decay were collected from the horticulture department, GJUST, Hisar, Haryana, India. The flowers were washed with distilled water to remove any foreign particles. Mustard oil was purchased from a local market. All the chemicals used in the investigation were of A. R. grade from Qualigens Standard solution (Mumbai), Spectrochem (Mumbai), E.Merck (Mumbai), Lobachemie (Mumbai), NICE chemicals (Cochin) and Central Drug House (New Delhi).

2.2. Carotenoid Extraction

Carotenoids were extracted using different organic solvent mixtures by the method described by Chen &Yang with slight modification [18]. The polar solvents used were acetone and ethanol, whilst the non-polar solvent used were hexane and toluene.15 grams of calendula flower petals were dissolved in 225 ml extractant (hexane-acetone-ethanol-toluene)and 15 ml methanolic KOH (40%) in 1000 ml volumetric flask. The mixture was left standing in the dark at ambient temperature for 16 h to allow extraction and saponification to proceed simultaneously. Then 225 ml hexane was added to the flask, which was swirled gently for 1 min. The flask was then diluted to volume 750 ml with 10% Na_2so_4 (285ml).The solution was shaken vigorously for 1 min and kept in the dark for 1 h until two phases were separated. The upper phase containing Carotenoids was collected, evaporated under vacuum (Rota evaporator) and stored for further analysis.

2.3. Determination of oxidation stability of soybean oil

Fifty ml mustard oil containing total carotenoid extract was added to a 100 ml volumetric flask with a rubber cap sealing firmly on the top. Carotenoid extract was incorporated at concentrations of 10, 25 and 50 ppm. The flasks were placed in a chamber with two 20W fluorescent tubes (General Electric) illuminating for 1, 3, 5 and 7 days. All samples were analyzed in triplicates. The oxidation stability of mustard oil during light storage was determined by measuring its Peroxide and TBA values according to the AOAC method [19].

2.4. Statistical analysis

The results were analysed statistically using SPSS 16.0 software. Data were subjected to one way ANOVA and Duncan's test to find significant difference in treatments. A value of $p \leq 0.05$ was used to indicate significant difference.

III. Results And Discussion

3.1. Extraction of carotenoids

Carotenoids were extracted from calendula petals with a mixture of solvents to yield a maximum recovery. The combination of solvents improved the total carotenoid yield compared with that obtained by any of the individual solvents. The combination of polar solvents with the non-polar hexane seems to improve the solubilisation of the non-polar carotenoids while individual polar solvents (ethanol and acetone) enhance the solubilisation of the polar lutein. This is probably related to the relative solubility of lutein in ethanol and acetone which is 15–40 folds higher than the respective one in hexane. Similarly, the relative solubility of β-carotene in ethanol is 20-fold less than in hexane [20].

3.2. Effect of total carotenoid extract on the oxidation stability of mustard oil

The oxidation stability of mustard oil was evaluated by the incorporation of total carotenoid extract prepared by combination of four solvents, at 10, 25 and 50 ppm concentrations. Experiment was carried out in a chamber under the effect of 20w fluorescent tube. Oil samles were taken after an interval of 0,1,3,5 and 7 days for estimation of peroxide and thiobarbituric acid (TBA) values. It was found that at concentrations of 50 ppm resulted in the lowest peroxide and TBA values followed by 25 and 10 ppm.This was due to the antioxidant activity of carotenoid extract. It was found that with increase in illumination period the peroxide and TBA values were increased. This is due to the degradation of extract after prolonged exposure of light. The control group showed the highest peroxide and TBA values at the end of experiment design. The result indicated that at 10 and 25 ppm concentrations, peroxide and TBA values were higher as compared to 50 ppm concentrations, indicating that a higher concentration of extract (50 ppm) was necessary to achieve good oxidation stability of mustard oil during illumination. **Table 1.** shows the effect of TC extract on peroxide value at 10, 25 and 50 ppm concentrations.

From the above discussions, it can be found that the carotenoid extract which was investigated possessed antioxidant ability, and the ability increased with increasing concentration. Under the same illumination time it was found that the highest concentration (50 ppm) resulted in the lowest peroxide and TBA values followed by 25 and 10 ppm. These results were similar to that reported by Fakourelis *et al.* [21],who

found that β-carotene minimized lipid oxidation of olive oil under light storage by its light-filtering effect. Effect of TC extract on TBA value at 10, 25 and 50 ppm concentrations is shown in **Table 2.**

Table 1. Effect of total carotenoid extract on the peroxide value of mustard oil under light storage , n=3, mean ± SD

	Days				
Sample	**0**	**1**	**3**	**5**	**7**
Control	5.26^{a} ± 0.86	66.26^{a} ± 0.97	158.13^{a} ± 1.0	271.10^{a} ± 0.95	283.60^{a} ± 0.79
10 ppm	5.26^{a} ± 0.86	64.96^{a} ± 0.83	107.36^{b} ± 0.53	126.21^{b} ± 0.91	134.31^{b} ± 0.92
25 ppm	5.26^{a} ± 0.86	48.49^{b} ± 0.88	76.48^{c} ± 0.97	98.30^{c} ± 0.95	104.37^{c} ± 0.85
50 ppm	5.26^{a} ± 0.86	27.33^{c} ± 0.85	63.59^{d} ± 0.95	72.22^{d} ± 0.95	76.50^{d} ± 0.85

Means with different superscripts within a column for a particular treatment differ significantly ($p \leq 0.05$).

Table 2. Effect of total extract carotenoid on the TBA values of mustard oil under light storage, n=3, mean ± SD

	Days				
Sample	**0**	**1**	**3**	**5**	**7**
Control	3.93^{a}±0.66	60.10^{a}±0.69	140.43^{a}±0.60	255.83^{a}±0.63	394.44^{a}±0.99
10 ppm	3.93^{a}±0.66	40.13^{b}±0.90	74.33^{b}±0.95	118.43^{b}±0.90	134.33^{b}±0.95
25 ppm	3.93^{a}±0.66	26.23^{c}±0.95	60.30^{c}±0.95	104.30^{c}±0.85	121.23^{c}±0.98
50 ppm	3.93^{a}±0.66	17.14^{d}±0.80	49.53^{d}±0.89	85.45^{d}±0.95	104.57^{d}±0.97

Means with different superscripts within a column for a particular treatment differ significantly ($p \leq 0.05$).

IV. Conclusion

The use of mixture of polar and non-polar solvents for the extraction of carotenoids from calendula officinalis flower petals resulted in the highest yield compared to the individual solvents. Samples of mustard oil incubated with carotenoid extracts of calendula flowers exhibited lower values of peroxide and TBA values as compared to control samples. Minimum values of peroxide and TBA values were observed in case of oil samples incubated with 50 ppm concentrations compared to 25 and 10 ppm concentrations.

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