Stabilization of Emulsion and Butter like Products Containing Essential Fatty Acids Using Kalonji Seeds Extract and Curcuminoids

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Abstract: Owing to the tendency of essential fatty acids (EFAs) to undergo autoxidation, their storage becomes a key problem. Generally, they are stabilized by synthetic antioxidants like TBHQ that are toxic in nature. Recently many studies were reported where these EFAs are stabilized by natural antioxidants. In the present study, curcuminoids and kalonji seeds ethanol extract (KEE) were used to stabilize these EFAs in refined sunflower oil (RSFO), water-in-oil (w/o) emulsion and butter like products (BLPs). In RSFO, though curcuminoids alone exerted pro-oxidant effect, KEE and curcuminoids showed synergistic antioxidant activity that was comparable to TBHQ. KEE exhibited good antioxidant activity in emulsions and BLPs, providing fine physical properties like slipping point, dropping point and spreadability. EFAs increased the nutritional value of BLPs and antioxidants added for their stabilization provided their medicinal benefits.

Key words: sunflower oil, curcuminoids, kalonji seeds extract, antioxidant activity, emulsions, butter like products

1 INTRODUCTION

Traditional medicines in the form of botanical dietary supplements and nutraceuticals have found a place in healthcare of 21st century because of the awareness of consumers about diet related problems. Nutraceuticals are delivered to the consumers in the form of functional food that lowers the risk of disease rather than preventing it. Functional food is not a pill or a capsule, but part of the normal food pattern¹⁾. Thus, it reduces the gap between the food and drug by regulating pertinent physiological functions and valuable biological activities.

Essential fatty acids (EFAs) such as linoleic acid, linolenic acids (α - & γ -) involved in number of crucial functions in the human body. They are the precursors of higher chain EFAs, which are required for eicosanoid synthesis, hormones (such as prostaglandins), regulation of gene expression²⁾ and control many physiological factors. Hence, an attempt was made to formulate BLPs by incorporating these EFAs in the form of sunflower oil. The stability of the products containing these EFAs is an important task to deliver the nutritional benefits to the consumers. Lipid oxidation negatively affects the flavor, odor, color and nutritional value of foods during storage and this may also limit

the utilization of oil in processed and fortified foods as well as nutritional supplements³⁾. Generally, synthetic antioxidants like TBHQ, BHT, BHA used to increase the oxidative stability of these EFAs⁴⁾ are known to have toxic and carcinogenic effect on human health⁵⁾. Now-a-days, consumers are demanding natural ingredients which are expected to be safe and health-promoting. Accordingly, there is a strong need to identify safer and more efficient natural antioxidants for use in the food industry. Spices and some herbs have received increased attention as sources of many effective antioxidants⁶⁾. These antioxidants have been reported to be more effective than some major synthetic antioxidants^{7,8)}.

The seeds of kalonji, an important carminative and spice & rhizome of turmeric, a common household spice contain antioxidants like thymoquinone ($\sim 12\%$), thymol ($\sim 0.8\%$), carvacrol ($\sim 3.7\%$)⁹⁾ and curcuminoids¹⁰⁾ respectively. The seeds of kalonji are known to have number of therapeutic uses¹¹⁾ such as anticarcinogenic, antibacterial, anti-inflammatory, analgesic and antipyretic. Similarly, curcumin, a major component of curcuminoids has medicinal properties against various diseases such as anorexia, coughs, diabetes, hepatic disorders, rheumatism and Al-

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zheimer disease¹²⁾.

In earlier studies, the antioxidant effect¹³⁾ and the pro-oxidant effect of curcuminoids were reported¹⁴⁾. This is the first report on the antioxidant activity of curcuminoids in refined oil rich in EFAs.

The present study focuses the effect of curcuminoids as such and in synergism with kalonji seeds ethanol extract (KEE) in refined sunflower oil (RSFO), water-in-oil (w/o) emulsions and butter like products (BLPs). Both EFAs and antioxidants affected the physical properties like slipping point, dropping point and spreadability. Consequently, the study of oxidative stability and physical properties was carried out.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

RSFO was received as gift sample from M/s Cargill India Pvt. Ltd., New Delhi. The oil contained 428 ppm residual tocopherol and did not contain any synthetic antioxidants. The hydrogenated fat (K-care; melting point 38.8°C and Iodine Value 47.5% I₂/g) was procured from Kamani Oil Industries Pvt. Ltd., India. Polyglycerol Polyricinoleate (PGPR) and Glycerolmonostearate (GMS) were procured from Fine Organics, India. Tween 80 was procured from Croda Chemicals India Pvt. Ltd. Curcuminoids powder was obtained from Kancor India Ltd., Angamaly South, India. Kalonji seeds were procured from local vendor. The purity of curcuminoids was 96.0% with three components namely curcumin (59.3%), demethoxycurcumin (20.0%) and bis-demethoxycurcumin (16.7%) as analyzed by HPTLC (Desaga Sarstedt Gruppe, Applicator AS 30 & Densitometer CD 60) as per the previously mentioned method¹⁵⁾. All other chemical reagents and solvents were obtained from s.d. fine-CHEM LiMiTEd, Mumbai.

2.2 Extraction of kalonji seeds

Kalonji seeds were crushed and extracted using ethanol in Soxhlet extractor with minimum 14 siphons. The crude KEE was obtained after removal of solvent using rotary vacuum evaporator.

2.3 Preparation of emulsions and BLPs

Water in oil emulsions were prepared by dropwise addition of 15 mL water phase containing 1% (w/v) Tween 80 to 85 g RSFO containing 18% (w/v) PGPR. Emulsification was done using homogenizer (Komal scientific, India) at 3000 rpm for 30 min. Curcuminoids and KEE were added to oil phase (RSFO) in appropriate proportion to study their effect on the oxidative stability of emulsion.

The fat base (85 g, containing RSFO:hydrogenated fat, 40:45 w/w) and emulsifier (1.5% w/v GMS) were gradually heated to melt at 50 ± 5°C on water bath, continuously

stirred with a three bladed stirrer at 200 rpm to dissolve emulsifiers and further 15 mL of warm water containing 1 g of common salt was added drop wise over 5 min with stirring continued for the next 20 min. The mixture was cooled for about 5 min using cold water bath at 15°C. The antioxidants were added to RSFO prior to preparation of BLPs during the study.

2.4 Study of antioxidant activity

The various blends of oil, emulsions and BLPs containing curcuminoids (50 ppm), KEE (2%) and their combination (50 ppm curcuminoids + 2% KEE) were prepared. The oxidative stability of oil blends was checked at 60°C for 30 days at regular interval of 5 days according to the AOCS Official Methods¹⁶⁾ by peroxide value (PV, Method Cd 8-53), p-anisidine value (p-A.V., Method Cd 18-90) and total oxidation (Totox) value (Method Cg 3-91). The oxidative stability of emulsions was checked at 35°C for 10 days at regular interval of 2 days by PV and induction period (IP) at 110°C was measured using Rancimat (Rancimat 679, Metrohm Ltd., Switzerland) with air flow at 20 L/h. The oxidative stability of BLPs was checked after 30 days at 10°C by PV. All the experiments were carried out in triplicate and the values were expressed as arithmetic mean of the experiments along with standard deviation.

The antioxidant activities of natural antioxidants were compared with control samples and TBHQ (200 ppm) under the same conditions. The relative antioxidant activities were compared using Oxidative Factor (OXF) for antioxidants based on mean peroxide value of triplicate experiments using following formula¹⁷⁾,

$$\text{OXF} = \frac{(\text{PV}_{\text{final}} - \text{PV}_{\text{initial}})_{\text{antioxidant}}}{(\text{PV}_{\text{final}} - \text{PV}_{\text{initial}})_{\text{control}}}$$

Protection coefficient (Pc) was calculated as the ratio of IP with antioxidant to control sample¹⁸⁾.

$$\text{Pc} = \frac{(\text{IP})_{\text{antioxidant}}}{(\text{IP})_{\text{control}}}$$

2.5 Physical properties of BLPs

The physical properties of BLPs (slipping point, dropping point and spreadability) were determined after 24 hours of preparation as per the following procedures.

2.5.1 Slipping point

The aluminium tube (6 mm i.d. X 7 mm with 2 mm thickness) open from both ends was filled with BLP and suspended in cold water using a thin wire. The temperature was slowly raised while stirring the water. Slipping point was noted as the temperature at which the column slips from the tube.

2.5.2 Dropping point

The glass tube (7 mm i.d. with 1 mm thickness) open from both ends was filled with BLP and suspended in a beaker with lid. The beaker was kept in water-bath and the temperature is slowly raised while stirring the water. Drop-

ping point was noted as the temperature at which the column drops from the tube.

2.5.3 Spreadability

Sample weighing 1 g was spread uniformly in the circle of 2 cm diameter on a glass plate. Another glass plate (weighing 244.26 g) was placed on the circle and the increase of area in 60 seconds was measured as spreadability of BLPs.

3 RESULTS AND DISCUSSION

The polyunsaturated linoleic acid and linolenic acid are respectively 10 and 25 times more unstable as compared to monounsaturated oleic acid¹⁹⁾. The plant oils contain natural antioxidants like tocopherol that were substantially removed during processing of oils like bleaching and deodorization²⁰⁾. The residual level of tocopherol (400–500 ppm) is insufficient to provide antioxidant activity to the processed oils. Hence, a suitable antioxidant has to be used to prevent autoxidation and to increase the shelf life of sunflower oil. The antioxidants are phenolic compounds substituted by electron donating groups at *ortho* and/or *para* positions. They act by inactivating free radicals generated during autoxidation process by proton donation mechanisms²¹⁾. The use of synthetic antioxidants can be avoided as there are various easily available natural products that contain antioxidants. The natural antioxidants like curcuminoids and KEE are non-toxic and moreover they retain their antioxidant activity in functional foods like emulsions and BLPs as water affects the stability of EFAs negatively^{22, 23)}.

3.1 The antioxidant activity of curcuminoids and KEE in RSFO

The peroxides are primary oxidation product that can further undergo degradation to low molecular weight aldehydes and ketones. The radicals are prone to peroxidation and follow the chain reactions typical of radicals¹⁹⁾. The

Fig. 1 Effect of KEE and curcuminoids on oxidative stability (peroxide formation) of RSFO at 60°C.

PVs, OXF and tottox value of RSFO with TBHQ, KEE, curcuminoids and KEE + curcuminoids are shown in Fig. 1 and Table 1.

The curcuminoids showed pro-oxidant effect, indicated by higher amount of peroxide formation as compared to control (RSFO). The pro-oxidant effect was also confirmed by higher tottox values of RSFO containing curcuminoids as compared to RSFO (Table 1). KEE showed antioxidant activity that was enhanced further in presence of curcuminoids. The synergistic effect resulted in the antioxidant activity that was comparable to synthetic antioxidant, 200 ppm TBHQ (Fig. 1).

Kalonji seeds contain components like thymoquinone, thymol, carvacrol⁹⁾ that act as conventional antioxidants by scavenging radicals. Curcuminoids exhibit keto-enol tautomerism (Fig. 2) in solutions, with up to 95% curcuminoids in the enol form depending on the solvent²⁴⁾. The keto-enol-enolate equilibrium of the heptadienone moiety of curcumin determines its physicochemical and antioxidant properties²⁵⁾. It is a common prejudice that a phenolic part of any molecule is always responsible for the antioxidant

Table 1 Effect of KEE and curcuminoids on oxidative stability of RSFO at 60°C

Time (days)	RSFO		RSFO + TBHQ		RSFO + 50 ppm curcuminoids		RSFO + 2% KEE		RSFO + 2% KEE + 50 ppm curcuminoids	
	Totox [†]	OXF	Totox [†]	OXF	Totox [†]	OXF	Totox [†]	OXF	Totox [†]	OXF
5	17.5 ± 0.7	0.36	8.0 ± 0.2	2.88	28.3 ± 2.2	0.70	13.0 ± 0.9	0.80	14.5 ± 0.5	0.80
10	24.2 ± 0.5	0.43	12.2 ± 0.4	2.06	41.9 ± 1.9	0.86	21.2 ± 0.7	0.59	15.8 ± 0.6	0.59
15	31.0 ± 1.0	0.57	19.4 ± 1.1	1.87	52.1 ± 1.3	0.78	26.5 ± 1.0	0.54	19.5 ± 1.0	0.54
20	47.3 ± 1.2	0.50	25.6 ± 1.2	1.30	63.4 ± 1.5	0.66	33.6 ± 1.8	0.56	30.9 ± 1.2	0.56
25	57.3 ± 1.9	0.46	28.9 ± 1.6	1.20	68.2 ± 1.7	0.64	39.1 ± 1.6	0.52	34.0 ± 1.4	0.52
30	77.3 ± 2.2	0.38	32.7 ± 2.1	1.10	76.7 ± 3.5	0.62	50.2 ± 2.2	0.52	44.8 ± 1.8	0.52

[†] The values given are means of three consecutive experiments ± standard deviations

When, R1 = R2 = -OCH₃; curcumin

R1 = -H, R2 = -OCH₃; demethoxycurcumin

R1 = R2 = -H; bisdemethoxycurcumin

Fig. 2 Keto and enol forms of curcuminoids.

activity. Based on the one electron reduction potential of the half curcumin that lacks active methylene group; Jovanovic *et al.*²⁵⁾ have shown that the antioxidant activity in case of curcumin (for pH ≤ 7) is due to the active methylene group. In the enol form of curcumin, the active methylene group disappears, which is clear from the structures (keto and enol forms) of curcumin. In alkaline conditions, they rapidly decompose to ferulic acid and feruloylmethane²⁶⁾ that further undergo hydrolysis to form vanillin and acetone. Masuda *et al.*²⁷⁾ also supported autooxidation of curcuminoids. In neutral and acidic solutions (from pH 3 to 7), the keto form predominates that acts as H-atom donor. Thus it is crucial to maintain these curcuminoids in the keto form by employing acidic conditions to utilize their antioxidant activity (Fig. 2).

The presence of free fatty acids from KEE⁹⁾ inhibited the degradation of curcuminoids thereby retaining the radical scavenging activity of curcuminoids. This resulted in syn-

ergistic antioxidant activity of KEE and curcuminoids.

3.2 The antioxidant activity of curcuminoids and KEE in emulsions

In emulsions, curcuminoids showed unexpectedly antioxidant activity (Table 2 & Fig. 3). KEE showed better antioxidant activity that was better than the combination of KEE and curcuminoids.

In emulsions, the setting in of chemical reactions that occur in the droplet interior, interfacial region or continuous phase may change the properties of the system during storage. The oxidation of the lipidic fraction of an emulsion system is one of the causes of emulsion quality deterioration. Some lipid oxidation products are fairly surface active and therefore, they can modify the composition of the interface²⁸⁾. Antioxidants are able to delay and/or prevent lipid oxidation in fatty systems by different mechanisms^{28–30)}. The presence of the aqueous phase generally decreases the activity of antioxidants because hydrogen bonded complexes are ineffective hydrogen donors to lipid radicals³¹⁾. The interaction of antioxidant with emulsifiers at oil/water interface also influences its activity³²⁾ by affecting permeability (rate of transfer) of oxygen across the interface. The

Fig. 3 Effect of KEE and curcuminoids on oxidative stability (peroxide formation) of emulsions at 35 °C.

Table 2 Effect of KEE and curcuminoids on oxidative stability of emulsions at 35°C

Antioxidant used	IP [†] (h)	Pc	OXF				
			Day 2	Day 4	Day 6	Day 8	Day 10
Control	2.40 ± 0.06	—	—	—	—	—	—
TBHQ	3.79 ± 0.09	1.58	0.65	0.43	0.45	0.49	0.54
50 ppm curcuminoids	3.64 ± 0.11	1.52	0.29	0.73	0.67	0.73	0.84
2% KEE	3.96 ± 0.17	1.65	0.65	0.67	0.59	0.60	0.64
2% KEE + 50 ppm curcuminoids	4.07 ± 0.16	1.69	0.65	0.72	0.65	0.66	0.69

[†] The values given are means of three consecutive experiments ± standard deviations

Table 3 Effect of curcuminoids and KEE on oxidative stability (at 10°C) and physical properties of BLPs

Antioxidant added	OXF	Dropping Point [†] (°C)	Slipping Point [†] (°C)	Spreadability [†] (cm ²)
Control	—	41.1 ± 0.3	40.0 ± 0.3	6.76 ± 0.25
TBHQ	0.65	42.2 ± 0.1	41.5 ± 0.2	5.94 ± 0.19
50 ppm curcuminoids	2.01	41.3 ± 0.1	40.2 ± 0.2	5.94 ± 0.22
2% KEE	0.37	39.9 ± 0.2	39.1 ± 0.3	9.59 ± 0.46
2% KEE + 50 ppm curcuminoids	0.57	40.1 ± 0.3	38.1 ± 0.1	7.61 ± 0.44

[†]The values given are means of three consecutive experiments ± standard deviations

stabilization of curcuminoids may also be because of slightly acidic nature of tween 80 and PGPR.

The OSI (Table 2) of emulsion containing KEE was higher than that of TBHQ. The improvement of the OSI of the emulsion after addition of curcuminoids to KEE was negligible which were in consistent with the PV results (Fig. 3).

3.3 The antioxidant activity of curcuminoids and KEE in BLPs

Curcuminoids act as pro-oxidant in BLP in contrast to their antioxidant activity in emulsions (Table 3). The pro-oxidant activity of curcuminoids in BLPs was due to absence of acidic ingredients that were present in KEE as tween 80 and PGPR. The oxidative stability of BLPs was superior to that of emulsions because of lesser content of unsaturation and due to oxygen transfer taking place only by diffusions in BLPs and not by convection as in case of emulsions. The pro-oxidant effect of curcuminoids indicated that it disturbed the close packing of the emulsifier molecules at interface and increases the permeability of oxygen. The KEE reduced the diffusion of oxygen thereby showing better antioxidant activity (Table 3) in BLP also. Addition of curcuminoids to KEE reduced the stability of BLP.

The melting and spreading behaviour of BLPs is primarily important and it is expected that butter should melt at around human body temperature. The plain BLP containing RSFO had higher melting temperature. Presence of TBHQ and curcuminoids did not improve dropping point, slipping point and spreadability (Table 3). KEE improved the dropping point, slipping point (up to 39°C) and spreadability as against no change in case of TBHQ and curcuminoids.

Thus, addition of KEE had resulted in better stability of BLP as well as better physical properties. Moreover, the incorporation of RSFO along with KEE in BLPs will improve its nutritive value.

4 CONCLUSION

The synergistic antioxidant activity of KEE and curcuminoids comparable to synthetic antioxidant in RSFO was observed. The study showed the antioxidant potential of natural products (KEE and curcuminoids) to be used as an alternative for synthetic antioxidant. Even KEE was used to stabilize emulsions containing sunflower oil. The sunflower oil was incorporated in BLPs along with KEE that increased the oxidative stability and also improved the physical properties like slipping point, dropping point and spreadability. By means of such functional foods like oil based emulsions and BLPs, the consumers will avail the benefits of EFAs as well as natural antioxidants. It has opened new avenues and there is scope to check the antioxidant activity of various other natural products.

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