Sameera and Shamim, J Food Nutr Disor 2017, 6:2
DOI: 10.4172/2324-9323.1000221

Journal of Food & Nutritional Disorders

Research Article

A SCITECHNOL JOURNAL

Effect of Purification on the Antioxidant Activity of Ajowan Extract in Refined Sunflower Oil

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Abstract

Essential fatty acids (EFAs) are integral part of regular diet owing to their vital role to perform normal physiological functions. Unfortunately, they have the tendency to undergo autoxidation decreasing the nutritive value and producing off-flavours during storage. Antioxidants are necessary to be added to the foods containing EFAs. Considering the consumers' demand to utilize natural ingredients, the research has been now fostered on natural antioxidants. These natural antioxidants can be the most trusted substitutes to unsafe synthetic antioxidants. Moreover, with the medicinal properties of the natural antioxidants, the consumers can avail the benefits of them as therapeutic agents. In our study, we have used ajowan as a source of natural antioxidant to stabilize EFAs in sunflower oil. The extraction of ajowan seeds was carried out by ethanol. The crude extract was purified to isolate thymol rich fraction using column chromatography. The crude ajowan extract was found to be more effective as an antioxidant than the purified extract, i.e., thymol rich fraction. Thus, the other constituents present in the extract besides main constituent (i.e., thymol) have remarkable influence on the antioxidant activity of the main constituent.

Keywords

Sunflower oil; Stabilization; Autoxidation; Natural antioxidant; Ajowan extract; Thymol; Purification

Introduction

Essential fatty acids (EFAs) are necessary dietary nutrients as they perform crucial functions for maintaining good health [1]. Their serious drawback is that they cannot be synthesized in human body. Hence, they need to be provided through diet. The vulnerability of EFAs to undergo autoxidation makes their storage a key problem. The oxidation products of these unsaturated EFAs lead to the development of unpleasant flavour in the products containing them [2]. Moreover, the autoxidation negatively affects the nutritional value of foods during storage. Consequently, the addition of antioxidants during their storage becomes an inevitable step.

The synthetic antioxidants like tertiary Butylhydroquinone (TBHQ), Butylhydroxytoluene (BHT) and Butylhydroxyanisole (BHA) are known to be toxic. Besides, they are reported to be carcinogenic in nature [3]. Accordingly, natural antioxidants have received an increased attention and their popularity is growing day by day. The early work is replete with information of spices and herbs that have exhibited antioxidant activity [4].

Ajowan (Carum copticum or Trachyspermum ammi) is an aromatic seed spice, used in certain culinary preparations in India. The antioxidant activity of ajowan extract has been studied earlier [5]. In Ayurvedic medicines, it is used as a medicinal plant for its antispasmodic, stimulant, tonic and carminative properties [6]. The antimicrobial and anti-inflammatory activity of essential oil of ajowan has also been proved [7,8]. It is a protective agent against gastric ulcer [9]. It is known to show a strong germicidal, antispasmodic and antifungal effect [10]. Recently other therapeutic uses of ajowan such as analgesic and antinociceptive effects, antiplatelet, antibacterial and anthelmintic activities, insecticidal assessment, antitussive and bronchodilatory effects have been reviewed [11]. The ajowan extract with other herbs also revealed good synergistic antioxidant [12] as well as antimicrobial activity [13]. It contains about 40-50% thymol. The other major components identified are β-cymene, eugenol, β-pinene, verbenol, β-terpineol, β-myrcene, camphene, D-limone, ocimene, linalool, carveol, apioline and α-thujene. [14-16].

The present work deals with the study of the antioxidant activity of ajowan extract (AE) in sunflower oil. The ajowan extract was purified by column chromatography to isolate thymol rich fraction (TRF). The study was further extended to evaluate the antioxidant activity of TRF in the same oil.

Materials and Methods

Refined sunflower oil (RSFO) was received as a gift sample from M/s Cargill India Pvt. Ltd., New Delhi. Ajowan seeds were procured from local vendor. All other chemical reagents and solvents were obtained from s.d. fiNE-CHEM LiMiTEd, Mumbai.

Extraction of ajowan seeds

Ajowan seeds (50 g) were crushed and extracted using ethanol in Soxhlet extractor (400 mL capacity) for 3 hours. The crude AE was obtained after removal of solvent using rotary vacuum evaporator. AE was analyzed with Infrared Spectrometry (Shimadzu 8400S FTIR spectrometer). The presence of thymol in AE was further confirmed by HPTLC on silica gel plates (Merck DC Kieselgel 60 F_{254}) using petroleum ether: ethyl acetate (in volume ratio 9:1) [5] as mobile phase.

Purification of AE to TRF

Previously dried silica gel (60-120 mesh) weighing 180 g was slurried in petroleum ether and filled in a glass column of 3.3 cm internal diameter and 35 cm height. AE weighing 9.0 g was dissolved in 10 mL of petroleum ether and loaded on the silica gel column. Further, the column was continuously eluted with petroleum ether: ethyl acetate (in volume ratio 9:1) at 1 mL min^{-1}. Each 10 mL fraction was separately collected and analyzed by HPTLC. The spots were visualized in UV chamber at 254 nm. Thymol was eluted at 450 to 550 mL fraction. The fractions rich in thymol were combined and the solvent was removed to get TRF. The concentration of thymol in TRF was determined by Gas Chromatography-Mass Spectrometer

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Received: March 13, 2017 **Accepted:** April 07, 2017 **Published:** April 11, 2017

(GC-MS) at Sophisticated Analytical Instrument Facility (SAIF), IIT-Bombay, Mumbai, India.

Study of antioxidant activity

The various blends of oil containing AE (2% and 2.5% w/v) and TRF (1% and 2% w/v) 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 Methods [17] by peroxide value (in meq/kg) (PV, Method Cd 8-53), *p*-anisidine value (*p*-A.V., Method Cd 18-90) and total oxidation (Totox) value (in meq/kg) (Method Cg 3-91).

The antioxidant activities of AE and TRF were compared with control sample and TBHQ (200 ppm) under the same conditions. The relative antioxidant activities were compared using Oxidative Factor with respect to peroxide value (OXF) for each antioxidant, calculated as per the following formula [18].

$$OXF = (PV_{final} - PV_{initial})\ \text{antioxidant} / (PV_{final} - PV_{initial})\ \text{control}$$

Where PVs indicate the mean values of all triplicate determinations of the peroxide value % conjugated dienoic acid (% CDA) was determined according to the AOCS Official Methods [17] (Method Ti 1a-64), on initial and final day of the study. The oxidative factor with respect to % CDA (OXF_{CDA}) was calculated as per the following formula [18].

$$OXF_{CDA} = (\%CDA_{final} - \%CDA_{initial})\ \text{antioxidant} / (\%CDA_{final} - \%CDA_{initial})\ \text{control}$$

Results and Discussion

Analysis of AE and TRF

The FTIR spectra of AE showed the presence of phenolic –OH group (3350 cm^{-1}), alkyl chains (2972 & 2923 cm^{-1}) and aromatic ring (1045 cm^{-1}) that revealed the presence of thymol. The presence of thymol in AE was further confirmed by HPTLC (Figure 1). The thymol content in AE was detected as 31% by HPTLC. The Rf value of the thymol was obtained to be 0.57. The thymol present in AE was purified by column chromatography and analyzed by HPTLC (Figure 2). The purity of thymol in TRF was found to be 94% by Gas Chromatography.

GC-MS of thymol in TRF (Figure 3) showed presence of peak with m/z ratio 150 corresponding to molecular ion. Other peaks were attributed to ions with structures as shown in Figure 3 itself.

Effect of AE on oxidative stability of RSFO

The solubility of AE in RSFO was found to be 2.5% (w/v). The antioxidant activity of AE is shown in Figure 4 and Table 1 along with the antioxidant activity of TBHQ for comparison. AE exhibited marginal antioxidant activity mainly due to presence of thymol. The activity was lower than TBHQ. In the previous studies, Mehta et al. also showed that methanolic extract of ajowan was less effective than BHT [19]. This can be explained on the basis of structural variation between TBHQ and thymol.

TBHQ and thymol both are phenolic hydroxyl compounds (Figure 5). The presence of bulky alkyl groups at ortho or para positions increases the electron density on the hydroxyl group by an inductive effect and thus, increases hydrogen donation ability [20]. It implies that a phenolic compound acts as an antioxidant if an electron donating group is present at ortho or para position to phenolic hydroxyl group. TBHQ contains a bulky tertiary butyl group at ortho position and a hydroxyl group at para position to phenolic hydroxyl group. On the other hand, thymol contains an isopropyl group at ortho position to phenolic hydroxyl group. Thus, the two activating groups at ortho and para positions to phenolic hydroxyl group makes TBHQ to have more potential to scavenge the radical than thymol which contains only one activating group at ortho position to phenolic hydroxyl group.

Thymol after hydrogen radical donation itself undergoes oxidation. It has been proved that the oxidation products of thymol, i.e., thymoquinone and thymohydroquinone have considerable antioxidant activity [21]. Especially, thymohydroquinone showed very high antioxidant activity due to its structural resemblance to TBHQ. Hence, the activity of thymol is attributed to the formation of its degradation products as well. AE showed much lower totox values (Table 1) and lower OXF_{CDA} (Table 2). It can be concluded that AE has potential to stabilize peroxy radical thereby reducing the formation of secondary degradation products like carbonyl compounds and also to inhibit the isomerisation.

Effect of TRF on oxidative stability of RSFO

The effect of purification on the antioxidant activity of AE was evaluated by using TRF in RSFO (Table 1). The TRF exhibited lower antioxidant activity than AE itself despite of the fact that 1% and 2% TRF contain higher amount of thymol (0.94% and 1.88% respectively) than 2% and 2.5% AE (containing 0.61% and 0.77% of thymol respectively). The same trend was also observed in totox values and OXF_{CDA}. It has clearly indicated that the other constituents present in AE had definitely showed certain synergistic activity with thymol [22]. A similar type of naturally evolved synergism is present in case of curcuminoids that showed higher activity than the main constituent, i.e., curcumin itself [23]. Thus, the antioxidant activity of natural extracts is due to the synergism of the main constituent with the other constituents present in the extract. Since the crude AE itself showed better antioxidant activity than the purified extract (i.e., TRF), AE as such should be used as an antioxidant.

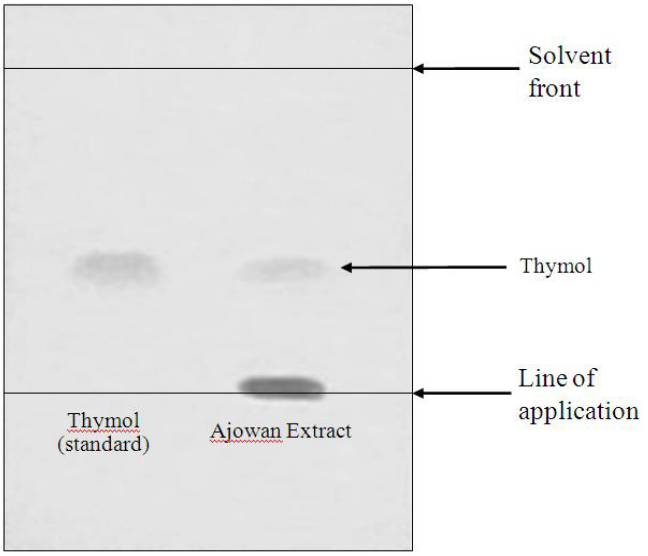

Figure 1: Thin layer chromatography of standard thymol and ajowan extract.

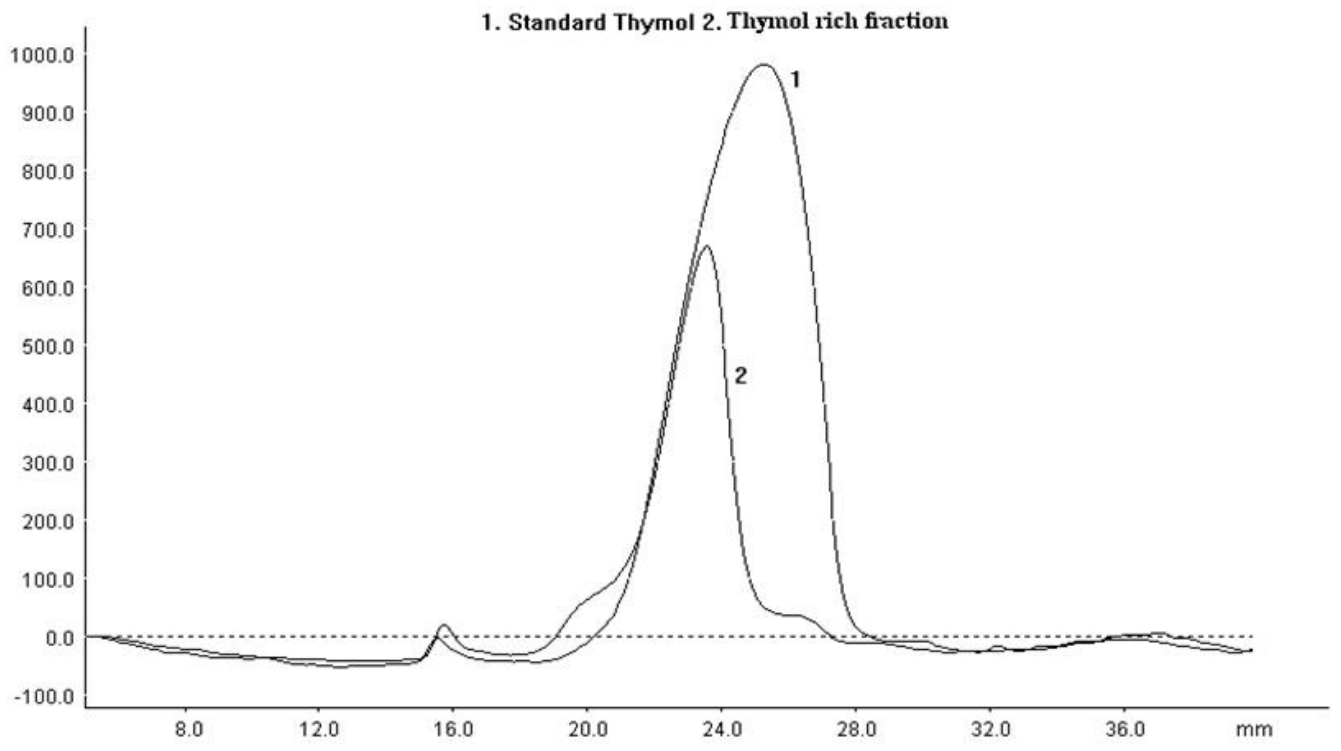

Figure 2: Overlay of HPTLC chromatogram of standard thymol and thymol rich fraction.

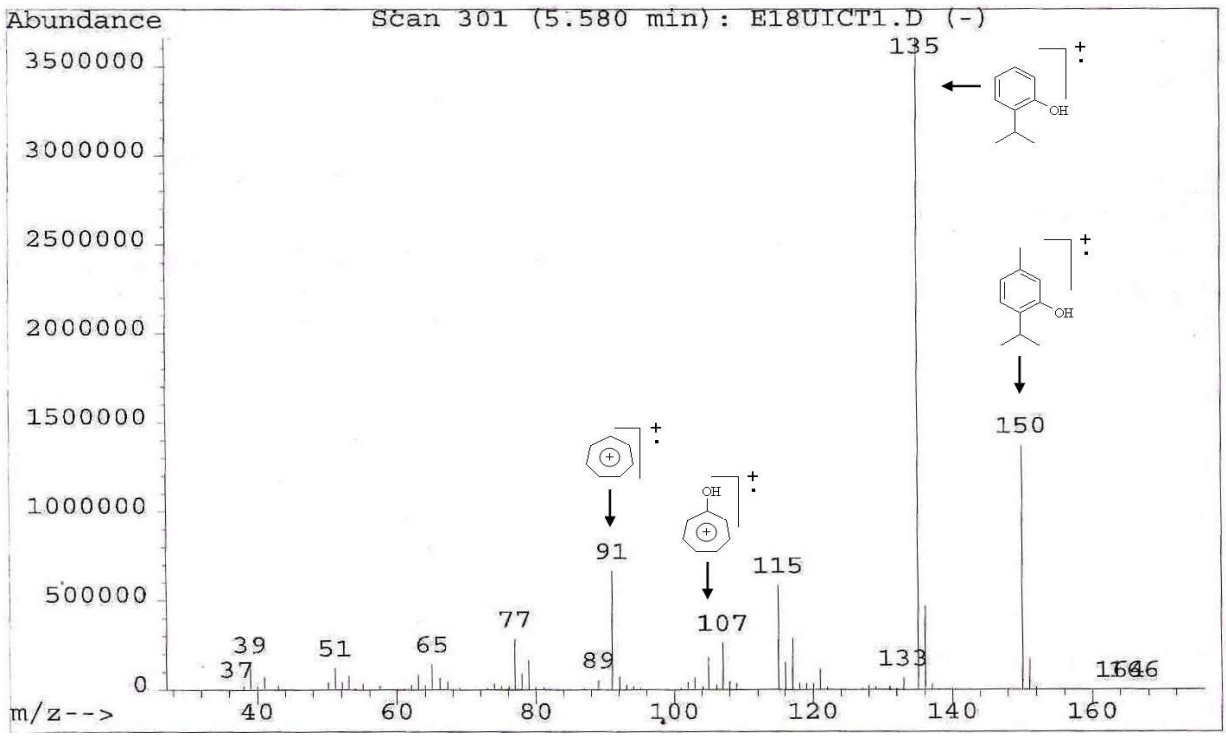

Figure 3: GC-MS of thymol in thymol rich fraction.

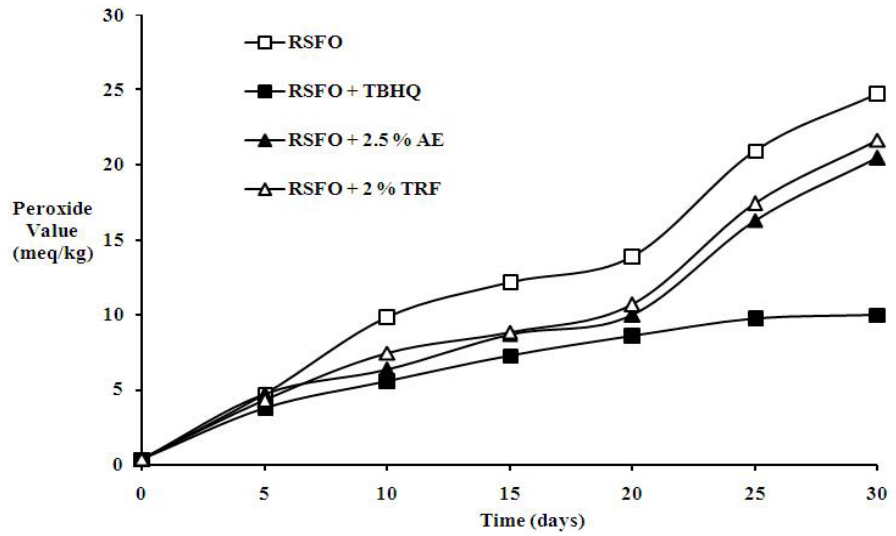

Figure 4: Effect of ajowan extract and thymol rich fraction on oxidative stability (peroxide formation) of RSFO at 60°C.

Table 1: Effect of ajowan extract and thymol rich fraction on oxidative stability of RSFO at 60°C.

Time (days)	RSFO	RSFO+TBHQ		RSFO+2% AE		RSFO+2.5% AE		RSFO+1% TRF		RSFO+2% TRF	
	Totox†	OXF	Totox†	OXF	Totox†	OXF	Toto†	OXF	Toto†	OXF	Totox†
5	27.1 ± 1.1	0.77	25.2 ± 1.0	0.95	21.2 ± 1.0	1.00	18.4 ± 0.7	1.01	19.3 ± 0.8	0.90	17.3 ± 0.7
10	33.0 ± 1.6	0.55	27.5 ± 1.2	0.70	25.8 ± 0.8	0.63	22.7 ± 0.9	0.75	24.6 ± 0.8	0.79	22.5 ± 0.7
15	40.9 ± 2.6	0.59	29.4 ± 1.1	0.75	32.3 ± 1.9	0.71	29.3 ± 0.7	0.69	32.1 ± 1.0	0.75	30.0 ± 0.4
20	46.2 ± 2.4	0.61	33.0 ± 1.2	0.78	35.7 ± 0.6	0.71	31.9 ± 2.4	0.81	38.3 ± 1.5	0.79	35.6 ± 0.7
25	62.3 ± 2.9	0.45	36.1 ± 1.9	0.82	48.3 ± 2.2	0.77	44.9 ± 1.9	0.84	51.2 ± 3.6	0.83	50.3 ± 1.8
30	70.9 ± 2.3	0.40	37.9 ± 2.2	0.85	56.3 ± 2.6	0.82	53.0 ± 2.3	0.91	65.5 ± 3.4	0.87	63.5 ± 1.9

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

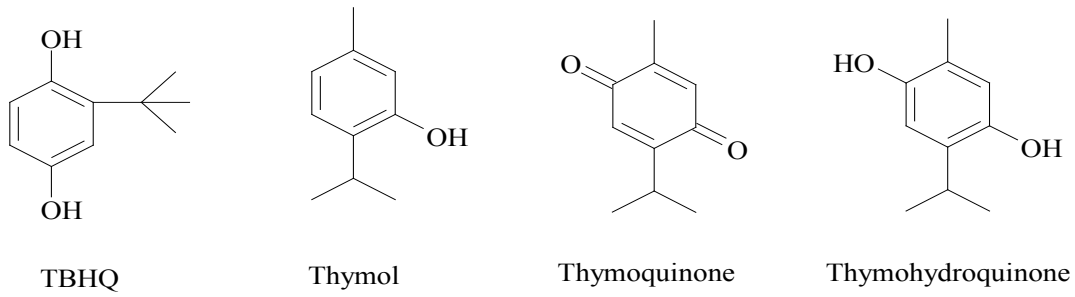

Figure 5: Chemical structures of antioxidants.

Table 2: Effect of ajowan extract and thymol rich fraction on conjugation measured at λ=233 nm.

Blends	% CDA		OXF_{CDA}
	Initial	Final	
RSFO	0.1682	0.3860	-
RSFO + TBHQ	0.1903	0.3731	0.8393
RSFO + 2% AE	0.2568	0.3860	0.5932
RSFO + 2.5% AE	0.2937	0.3891	0.4380
RSFO + 1% TRF	0.2623	0.3851	0.5638
RSFO + 2% TRF	0.3001	0.4221	0.5601

Conclusion

Ajowan being a natural product is safe as against synthetic and toxic TBHQ. Hence, even though its extract demonstrated lower antioxidant activity than TBHQ, it can be preferred to the synthetic antioxidant. In addition, ajowan exhibits its own therapeutic actions and the flavour is well-known to the consumers. Ajowan extract showed better antioxidant activity than the purified extract concluding that the crude extract should be directly used as an antioxidant without purifying it. This will reduce the additional cost of purification.

Acknowledgement

This study was financially supported by Technical Education Quality Improvement Programme (TEQIP), Government of India and World Bank.

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