

Effects of Some Natural Antioxidants Mixtures on Margarine Stability

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Abstract—Application of synthetic antioxidants such as tert-butylhydroquinon (TBHQ), in spite of their efficiency, is questioned because of their possible carcinogenic effect. The purpose of this study was application of mixtures of natural antioxidants that provide the best oxidative stability for margarine. Antioxidant treatments included 10 various mixtures (F_1 - F_{10}) containing 100-500ppm tocopherol mixture (Toc), 100-200ppm ascorbyl palmitate (AP), 100-200ppm rosemary extract (Ros) and 1000ppm lecithin(Lec) along with a control or F_0 (with no antioxidant) and F_{11} with 120ppm TBHQ. The effect of antioxidant mixtures on the stability of margarine samples during oven test (60°C), rancimat test at 110°C and storage at 4°C was evaluated. Final ranking of natural antioxidant mixtures was as follows: $F_2, F_{10} > F_5, F_9 > F_8 > F_1, F_3, F_4 > F_6, F_7$. Considering the results of this research and ranking criteria, F_2 (200ppmAp + 200ppmRos) and F_{10} (200ppmRos + 200ppmToc + 1000ppmLec) were recommended as substitutes for TBHQ to maintain the quality and increase the shelf-life of margarine.

Keywords—Margarine, Natural antioxidant, Oxidative stability, Shelf-life.

I. INTRODUCTION

MARGARINE is a water-in-oil emulsion containing minimum of 80% fat and maximum of 16% water and about 4% additives. During storage of oils, fats and fatty foods, lipid oxidation is one of the principle causes of quality deterioration. In order to retard or prevent the oxidative deterioration and extend the shelf-life of fatty food, adding antioxidants is necessary [1],[2]. In spite of high effectiveness of synthetic antioxidants such as tert-butylhydroquinon (TBHQ), their application is restricted in several countries because of their possible toxicity and carcinogenic effects [3]. The effects of several natural antioxidants on oxidative stability of food systems have been studied for several decades. Tocopherols (Toc) are the most abundant natural antioxidants in plant lipids [4], [5], [6]. Among the herbs, some research has been focused on rosemary (Ros) from Labiatae family [7], [8]. Since rosemary extract is lipophylic, it is used easily in oils and fats to prevent oxidation [4],[6]. Lecithin (Lec) which is generally used as emulsifier in food processing, can improve dispersion of active antioxidants in emulsion systems and inhibits the propagation step of free radicals in autoxidation

mechanism by reacting with various free radicals. Lecithin has been widely used as synergist in combination with phenolic antioxidants [9], [10]. Ascorbyl palmitate (AP) is usually used in food products to prevent or retard the oxidation of oils and fats, and it is preferred to ascorbic acid because of its greater solubility in oils [5]. Technologically, the most important natural antioxidants are tocopherols, ascorbyl palmitate, rosemary extract and lecithin. As lipid oxidation proceeds by different multiple mechanisms and also no single antioxidant can prevent all stages of oxidation, so mixtures of antioxidants must be used to create a synergistic effect [6], [9], [11].

The aim of this study was to *apply mixtures of natural antioxidants to margarine and select the most efficient combination of natural antioxidants which could be good substitutes for TBHQ*, the most potent and commonly used synthetic antioxidant.

II. MATERIALS AND METHODS

A. Materials

The refined, bleached and deodorized sunflower oil with no added antioxidant was purchased from a local producer in Iran. The refined, bleached, deodorized and antioxidant-free palm stearine was kindly donated by PORIM (Palm Oil Research Institute of Malaysia).

Tocopherol mixture (α , γ and δ), rosemary extract, TBHQ and emulsifier (mono and di-glycerides) were purchased from Danisco (Denmark), soy lecithin from ADM (Netherlands), citric acid, potassium sorbate and other chemicals from Merck Co., β -carotene from Roche (Switzerland), vitamin A and D_3 from BASF (Germany), Sodium casienate from Iran Caseinate, low fat milk powder from Moghan Co. (Iran) and diacetyl from Robert (Iran).

B. Methods

Sample Preparation: Samples of margarine were produced in the laboratory scale (3kg) containing approximately 16% water and 80% oil. The oil phase was consisted of sunflower oil and palm stearine (80:20), emulsifier (0.5%), β -carotene solution (0.003%), diacetyl (0.02%), vitamins (A and D_3 , 0.01%) and antioxidants. The antioxidant mixtures were added to the oil phase as follows:

F_0 : control (with no antioxidant); F_1 : 500part per million (ppm) Toc+100ppmAp; F_2 : 200ppmRos+200ppmAp; F_3 : 200ppm Toc+200ppm Ros; F_4 : 200ppm Ap+200ppm Toc +100ppm Ros; F_5 : 100ppm Toc +1000ppm Lec; F_6 : 250ppm Toc +1000ppm Lec; F_7 : 500ppm Toc +1000ppm Lec; F_8 : 100ppm Ap+500ppm Toc +1000ppm Lec; F_9 : 200 ppm Ros + 100ppm

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Ap +1000ppm Lec; F₁₀: 200ppm Ros +200ppm Toc +1000ppm Lec; F₁₁: 120ppm TBHQ.

The aqueous phase consisted of water, NaCl (99%), milk powder (1%), potassium sorbate (0.02%), citric acid (0.06%) and sodium caseinate (1%). The oil and aqueous phases were mixed at 40-45°C, and then the emulsion was cooled and mixed to obtain proper texture. Samples were packed in 250g polyethylene cups and stored in the freezer (-18°C) for 48 hours in order to complete crystallization.

Oven Test: The samples were transferred in beakers to the oven (60±1°C). Oxidative stability was determined by measuring peroxide value (PV) and anisidine values (AV) in 5-days intervals up to 25 days. The amount of primary oxidation products was determined through measuring of PV. Formation of secondary oxidation products was measured as p-anisidine value [12]. The induction period was considered as the number of days needed for PV of the samples to reach 20 milliequivalent oxygen per kilogram (meqO₂/kg) of fat [13]. This is in agreement with general consideration that oils become rancid at PV higher than 20 [6], [13].

GC analysis of samples: To determine the fatty acids profile of the margarine samples, a GC system (Varian 3800/ Auto sampler) equipped with FID detector and capillary column (Cp splitter-88 Fame, 100m×0.25mm×0.25µm) was applied. It was operated in the following condition: FID: 270°C, oven: constant temperature at 175°C, injector: 250°C and constant flow rate of carrier gas (N₂): 0.6ml/min [14], [15].

Rancimat Test: The induction period of the oil phase of the samples was determined by rancimat at 110±1°C by Metrohm679. The effectiveness of antioxidants mixtures was expressed as the stabilization factor (SF):

$$SF = \frac{IP_{inh}}{IP_0}$$

where IP_{inh} is the induction period in the presence of an inhibitor and IP_0 is the induction period of the non-inhibited system [6], [16].

Shelf-life evaluation: Shelf-life of samples stored in the refrigerator (4±1°C) was determined through measuring the PV of the fresh sample and also after 2, 4, 8, 12, and 14 weeks. The induction period in the refrigerator was considered as the number of days needed for the PV of the samples to reach 5 meq/kg [13], [17].

Ranking of antioxidant mixtures: Ranking of antioxidant mixtures was carried out on the basis of several factors including the number of the days needed for PV of samples to reach 20 at 60°C [3], [6], [13] and to reach PV of 5 at 4°C [17], stabilization factor (at 110°C) and also economical aspects of applying natural antioxidants.

Statistical analysis: All measurements were carried out three times. The results obtained for peroxide and anisidine values were statistically analyzed with the student's *t*-test using a significance level of $p < 0.05$. The results of induction period,

stabilization factor and ranking points were analyzed with the Mann Whitney test using a significance level of $p < 0.05$.

III. RESULTS AND DISCUSSION

Results showed that the fatty acids profile of margarine samples (Fig. 1) includes 21.58% saturated fatty acids (S), 24.98% mono-unsaturated fatty acids (M), 52.64% poly-unsaturated fatty acids (P), 0.89% trans fatty acid (T), with high nutritional value ($P/S+T=2.34$) and acceptable physical and chemical characteristics in accordance with Codex and also Iranian standards [17], [18]. The peroxide and anisidine values of the samples at 60°C showed that F₂, F₅, F₉, F₁₀, and F₁₁ had the most antioxidant activity ($p > 0.05$). In the fifth day of oven test (Fig. 2), PV of the F₂'s oil phase (8.19 meq/kg) was the lowest among the samples ($p < 0.05$) followed by F₈ and F₁₁ (12.1 and 12.4 meq/kg) ($p < 0.05$). There was no statistically significant difference between antioxidant activity of F₈ and F₁₁ in the fifth day ($p > 0.05$).

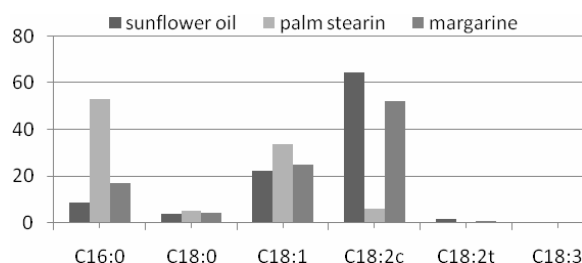


Fig. 1 Fatty acids profile of starting oils and margarine (%)

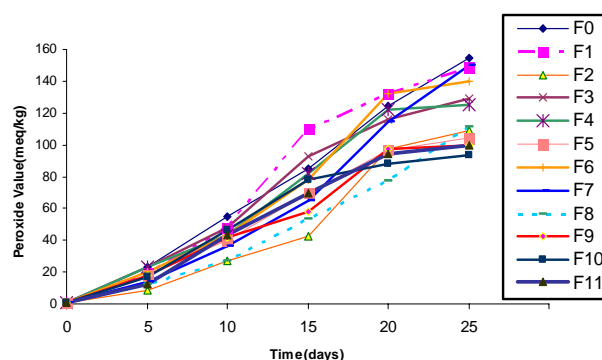


Fig. 2 Peroxide value of margarine samples during storage at 60°C

In days 10 and 15, samples F₂, F₈, and F₉ had significantly lower PV compared to control sample and also other samples ($p < 0.05$). In days 20 and 25, F₂, F₅, F₉, F₁₀, and F₁₁ had the highest antioxidative activity compared to the other antioxidant mixtures ($p < 0.05$). On the whole, considering the PV changes of samples during 25 days of oven test, F₂ (Ap+Ros), F₈ (Toc+Ap+Lec), and F₁₀ (Ros+Toc+Lec) had the highest antioxidant activity ($p < 0.05$) and between F₂ and F₈, F₂ was significantly more effective ($p < 0.05$). This is in agreement with findings of Chu *et al.* [4] and Hras *et al.* [6] who found that AP+Ros, Toc+AP+Lec, and Ros+Toc+Lec had higher

antioxidative activity than other natural antioxidant mixtures in their studies.

Changes of AV in the oven test (Fig. 3) showed that F₉(Ros+Ap+Lec), F₁₀ (Ros+Toc+Lec), and F₁₁ (TBHQ) had the highest antioxidative activity amongst other treatments ($p<0.05$), and F₁₀ with statistically significant difference had lower level of secondary products than the control and other samples. This result is in agreement with that reported by Chu *et al.* [4] and Hras *et al.* [6].

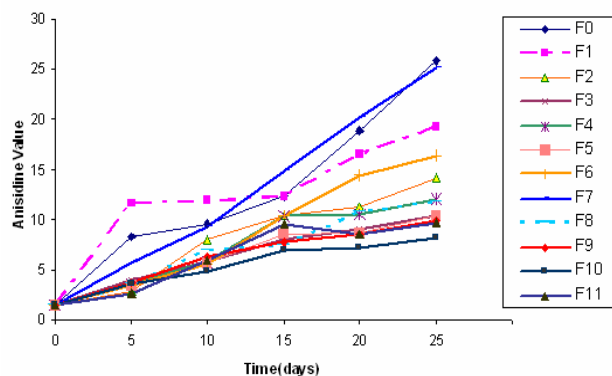


Fig. 3 Anisidine value of margarine samples during storage at 60°C

The induction period of the samples to reach PV=20 meq/kg at 60°C (Table I) had been used to evaluate antioxidative activity [3], [6], [13]. In this regard, the highest antioxidative activity in the oven test belonged to F₂ (Ros+Ap) and F₈ (Toc+Ap+Lec) which their peroxide value reached 20meq/kg after 8 days and were statistically different from F₁₁ (TBHQ). According to the findings of Hras *et al.* [6], the mixture of rosemary extract and tocopherols extended the induction period in sunflower oil in the test condition which showed a strong synergism between them. On the other hand, lecithin had synergistic effect on tocopherol [4]. Other antioxidants mixtures (F₁, F₃, F₄, F₆, and F₉) had lower activity ($p<0.05$) and F₇ and F₁₀ were not significantly different from F₁₁ ($p>0.05$).

The results of rancimat test (110°C) were expressed as the stabilization factor (SF) in Table I. SF of samples F₉ (Ros+AP+Lec), F₁₀ (Ros+Toc+Lec), and F₁₁ had no statistically significant difference with each other ($p>0.05$). F₂ (Ros+AP), F₅ (100ppmToc+Lec), and F₈ (AP+Toc+Lec) had similar SF, so they were ranked as the second group. Other samples including F₁, F₃, F₄, F₆, and F₇ did not show considerable antioxidative activity in this test. The results were in agreement with findings of some other works [4], [6], [19].

TABLE I
CRITERIA USED IN RANKING ANTIOXIDANT MIXTURES

Samples	Time(days) to reach PV=20 at 60°C	SF at 110°C	Time(days) to reach PV=5 at 4°C	Cost of antioxidant mixtures(\$)
F ₀	4 ^a	1 ^a	29 ^a	0
F ₁	4.98 ^a	1.41 ^a	34 ^a	0.07
F ₂	8 ^b	2.74 ^b	71 ^b	0.05
F ₃	4.3 ^a	1.69 ^a	45 ^a	0.05
F ₄	4.1 ^a	1.70 ^a	50 ^a	0.06
F ₅	5 ^a	2.77 ^b	84 ^c	0.07
F ₆	4.95 ^a	1.48 ^a	44 ^a	0.09
F ₇	6.1 ^c	1.35 ^a	38 ^a	0.12
F ₈	8 ^b	2.28 ^b	56 ^b	0.13
F ₉	5.1 ^a	2.96 ^c	84 ^c	0.1
F ₁₀	6 ^c	2.63 ^c	99 ^c	0.11
F ₁₁	6.2 ^c	2.23 ^c	90 ^c	0.05

^{a-d} Values that are significantly different in each column according to the student *t*-test and Mann-Whitney test ($p<0.05$).

At 4°C, the peroxide value of F₀, F₁, F₃, F₄, F₆, and F₇ reached the discard point (5meq/kg) after 8 weeks whereas F₂, F₅, F₉, F₁₀, and F₁₁ were not rancid (Fig. 4). The shelf-life of the F₂, F₅, F₉, F₁₀, and F₁₁ had no statistically significant difference with each other ($p>0.05$), and their shelf-life (Table I) was longer than other samples ($p<0.05$). The shelf-life of margarine samples at 4°C was variable between one month (F₀, F₁, and F₇) and 3 months (F₁₀ and F₁₁).

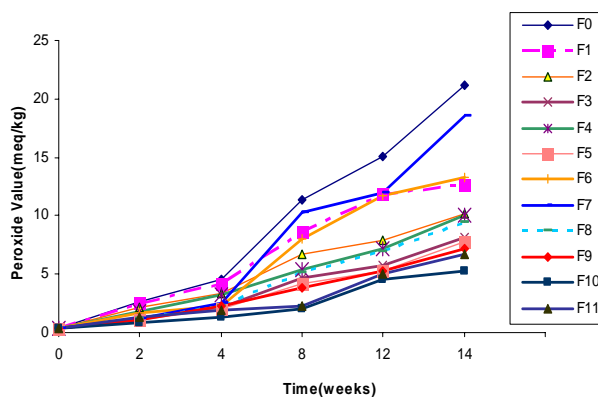


Fig. 4 Peroxide value of margarine samples during storage at 4°C

The results of this study showed that some natural antioxidants mixtures could be used as substitutes for TBHQ, which can be harmful to the body, improving the shelf-life of margarine. The results of the tests showed that F₁, F₃, F₄, F₆,

and F_7 had lower antioxidant activity compared to others, and the shelf-life of these samples was less than 2 months. They reached the discard point before the other samples; also they had the lowest SF in rancimat test. F_5 extended the shelf-life of the samples up to 12 weeks and showed high antioxidative activity through other tests. The final ranking of natural antioxidant mixtures was as follows: $F_2 > F_{10} > F_5$, $F_9 > F_8 > F_1$, F_3 , $F_4 > F_6$, and F_7 (Table II).

TABLE II
RANKING¹ OF ANTIOXIDANT TREATMENTS

Samples	oven test	SF	Shelf- life	Economical value	Final Ranking
F_0 (control)	3	3	3	-	9
F_1	3	3	3	1	10
F_2	1	2	2	1	6
F_3	3	3	3	1	10
F_4	3	3	3	1	10
F_5	3	2	1	1	7
F_6	3	3	3	2	11
F_7	2	3	3	3	11
F_8	1	2	2	3	8
F_9	3	1	1	2	7
F_{10}	2	1	1	2	6
F_{11}	2	1	1	1	5

1."1" is considered as the best in each column.

IV. CONCLUSION

Considering the adverse effects of synthetic antioxidants on consumers' health and in regard to the results of this work and also economical aspects, F_2 (200ppmRos+200ppmAp) and F_{10} (200ppmRos+200ppmToc+1000ppmLec) are recommended as substitutes for TBHQ to maintain the quality and improve the shelf-life of the margarine and to continue the research on larger scale.

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