International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:3, No:1, 2009

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.

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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

Ap +1000ppm Lec; F_{10}: 200ppm Ros +200ppm Toc +1000ppm Lec; F_{11}: 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 millieqivalant oxygen per kilogram($meqO_2/kg$) 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 (N_2): 0.6ml/min [14], [15].

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

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

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 5meq/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 F_2, F_5, F9, F_{10}, and F_{11} had the most antioxidant activity ($p>0.05$). In the fifth day of oven test (Fig. 2), PV of the F_2's oil phase (8.19meq/kg) was the lowest among the samples ($p<0.05$) followed by F_8 and F_{11} (12.1 and 12.4 meq/kg) ($p<0.05$). There was no statistically significant difference between antioxidative activity of F_8 and F_{11} in the fifth day ($p>0.05$).

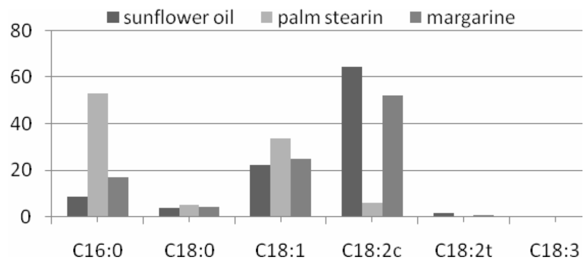

Fig. 1 Fatty acids profile of starting oils and margarine(%)

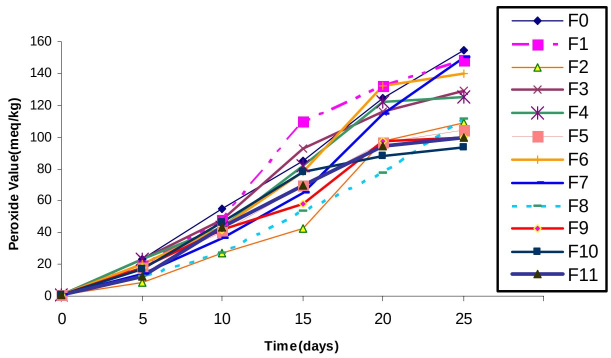

Fig. 2 Peroxide value of margarine samples during storage at 60°C

In days 10 and 15, samples F_2, F_8, and F_9 had significantly lower PV compared to control sample and also other samples ($p<0.05$). In days 20 and 25, F_2, F_5, F_9, F_{10}, and F_{11} 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, F_2 (Ap+Ros), F_8 (Toc+Ap+Lec), and F_{10} (Ros+Toc+Lec) had the highest antioxidant activity ($p<0.05$) and between F_2 and F_8, F_2 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 F_9(Ros+Ap+Lec) , F_{10} (Ros+Toc+Lec) , and F_{11} (TBHQ) had the highest antioxidative activity amongst other treatments ($p<0.05$), and F_{10} 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].

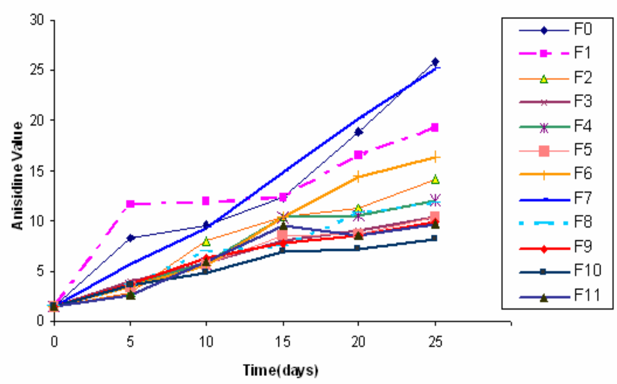

Fig. 3 Anisidine value of margarine samples during storage at 60°C

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 F_2 (Ros+Ap) and F_8 (Toc+Ap+Lec) which their peroxide value reached 20meq/kg after 8 days and were statistically different from F_{11} (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 (F_1, F_3, F_4, F_6, and F_9) had lower activity ($p<0.05$) and F_7 and F_{10} were not significantly different from F_{11} ($p>0.05$).

The results of rancimat test (110°C) were expressed as the stabilization factor (SF) in Table I. SF of samples F_9 (Ros+AP+Lec), F_{10} (Ros+Toc+Lec), and F_{11} had no statistically significant difference with each other ($p>0.05$). F_2 (Ros+AP), F_5 (100ppmToc+Lec), and F_8 (AP+Toc+Lec) had similar SF, so they were ranked as the second group. Other samples including F_1, F_3, F_4, F_6, and F_7 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 F_0, F_1, F_3, F_4, F_6, and F_7 reached the discard point (5meq/kg) after 8 weeks whereas F_2, F_5, F_9, F_{10}, and F_{11} were not rancid (Fig. 4). The shelf-life of the F_2, F_5, F_9, F_{10}, and F_{11} 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 (F_0, F_1, and F_7) and 3 months (F_{10} and F_{11}).

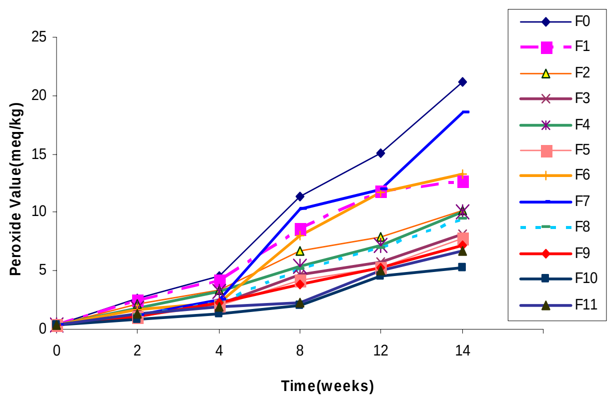

Fig. 4 Peroxide value of margarine samples during storage at 4°C

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 F_1, F_3, F_4, F_6, 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
RANKING[1] 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, F2 (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.

ACKNOWLEDGMENT

The authors are grateful to Mr. H. Safafar and Mr. I. Gaeeni for their helpful advices and Mrs. Z. Akhavan Attar for her valuable help in laboratory.

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REFERENCES

[1] R. D. O' Brien, *Fats and oils: Formulation and processing for application*. 2nd ed., London & New York: CRC Press, 2004, pp. 235–474W.-K. Chen, *Linear Networks and Systems* (Book style). Belmont, CA: Wadsworth, 1993, pp. 123–135.
[2] N. A .M. Eskin, , and D. S. Robinson, *Food shelflife stability*. London: CRC Press, 2001, pp.178-182.B. Smith, "An approach to graphs of linear forms (Unpublished work style)," unpublished.
[3] J. Pokorny, N. V. Yanishlieva, and M.H. Gordon, *Antioxidants in food*, Boca Raton: CRC press, 2001, pp. 324–344,360.
[4] Y. H. Chu, and H. F. Hsu, "Effects of antioxidants on peanut oil stability," *Food Chemistry J.,* vol. 66, pp. 29-34, 1999.
[5] R. J. Hamilton, C. Kula, G. P. McNeill, F. B. Padley, and J. H. Pierce, "Effects of tocopherols, ascorbyl palmitate, and lecithin on autoxidation of fish oil, " *AOCS J.* , vol. 75, no. 7, pp. 813-823,1998.
[6] A. R. Hras, M. Hadolin, Z. Knez, and D. Bauman, "Comparison of antioxidative and synergistic effects of rosemary extract with alfa-tocopherol, ascorbyl palmitate, and citric acid in sunflower oil," *Food Chemistry J*., vol. 71, pp. 229-233, 2000.
[7] E. N. Frankel, S. W. Huang, J. Kanner, and B. German, "Interfacial phenomena in the evaluation of antioxidants: Bulk oils vs. emulsions," *Agriculture & Food Chemistry J*., vol. 42, no. 5, pp.1054-1059, 1994.
[8] P. Zandi, and L. Ahmadi, "Antioxidant effect of plant extracts of Labiatae family," *Food Science & Technology J.* , vol. 37, no. 4, pp. 436-439,2000.
[9] T. Koga, and J. Terao, "Phospholipids increase radical-scavenging activity of vitamin E in a bulk model system, " *Agriculture & Food Chemistry J.* , vol. 43, no. 6, pp. 1450-1454, 1995.
[10] H. Saito, and K. Ishihara, "Antioxidant activity and active sites of phospholipids as antioxidants," *AOCS J.*, vol. 74, no. 12 , pp. 1531-1536,1997.
[11] N. M. Bandarra, R. M. Campos, I. Batista, M. L. Nunes, and J. M. Empi, "Antioxidant synergy of α-tocopherol and phospholipids," AOCS J., vol. 76, no.8, pp.905-913, 1999.
[12] *Official methods and recommended practices of the American Oil Chemists' Society Method* Cd8-53, Cd 18-90, Cd 3d-63, Cd 1d-92, Cd 12d-92.(5th ed.). Champaign: *American Oil Chemists' Society*.1997.
[13] K. D. Economou, V. Oreopoulou, and C. D. Thomopoulos, "Antioxidant activity of some plant extracts of the family Labiatae, *AOCS* J., vol. 68, no. 2, pp.109-113, 1991.
[14] Animal and vegetable fats and oils-Preparation of methyl esters of fatty acids, ISO 5509, 2002.
[15] Animal and vegetable fats and oils- Analysis by gas chromatography of methyl esters of fatty acids, ISO 5508, 2002.
[16] N. V. Yanishlieva, and E. M. Marinova, "Antioxidant effectiveness of some natural antioxidants in sunflower oil, " *Zeitschrift fur Lebensmittel-Untersuchung und Forschung*, vol. 203, pp. 220-223,1996.
[17] Standard for margarine, *Institute of Standard & Industrial Research of Iran*, no. 143, 2nd. ed., 1999.
[18] *Codex Alimentarius Commission*, Codex Standard for margarine, Codex stan32, Second Edition, 2001.
[19] A. Judde, P. Villeneuve, A. R. Castera, and A. L. Guillou, "Antioxidant effects of soy lecithins on vegetable oil oxidative stability and their synergism with tocopherols," *AOCS J.* , vol.80, no.12, pp.1209-1215, 2003.