In spite of their efficiency, the use of synthetic antioxidants such as tert-butylhydroquinone (TBHQ) has been questioned because of their possible carcinogenic effects. The purpose of this study was to establish a mixture of natural antioxidants that provides the optimum oxidative stability for margarine. Antioxidant treatments included 10 various mixtures (F1-F10) containing 100-500 ppm tocopherol mixture (Toc), 100-200 ppm ascorbyl palmitate (AP), 100-200 ppm rosemary extract (Ros) and 1000 ppm lecithin (Lec) along with a control or F0 (with no antioxidant) and F11 containing 120 ppm TBHQ. The effect of antioxidant mixtures on the stability of margarine samples during an oven test (60±1°C), rancimat test at 110°C and storage at 4°C was evaluated. The final ranking of the natural antioxidant mixtures was as follows: F2, F10>F5, F9>F8>F1, F3, F4>F6, F7. Considering the results of this research and ranking criteria, F2 (200 ppm Ap + 200 ppm Ros) and F10 (200 ppm Ros + 200 ppm Toc + 1000 ppm Lec) were recommended as substitutes for TBHQ to maintain the quality and increase the shelf-life of margarine.

Keywords: margarine, oxidative stability, shelf-life, antioxidant, auisidine

INTRODUCTION

Margarine is a water-in-oil emulsion containing a minimum of 80% fat and a maximum of 16% water and about 4% additives. During storage of oils, fats and foods containing fat, lipid oxidation is one of the principle causes of food quality deterioration. Margarine is no exception to this rule since it has a high fat content. In order to retard or prevent the oxidative deterioration and extend the shelf-life of many food products, addition of antioxidants is necessary (Abdalla and Roozen, 1999; Iranian Standard, 1999; Zandi & Gordon, 1999; Codex Alimentarius Commission, stan 32, 2001; O’Brien, 2004; Robbins and Sewalt, 2005). Synthetic antioxidants such as tert-butyl-hydroquinone (TBHQ), are being used regularly because they are highly efficient in preventing the oxidation of oils and fats. Despite their effectiveness, the use of synthetic antioxidants is restricted in several countries, because of their possible toxicity, metabolism, absorption and accumulation in the body and also their carcinogenic effects (Eskin and Robinson, 2001; Hras et al., 2000). Many studies have evaluated the effects of several natural antioxidants on the oxidative stability of food systems. α, γ and δ-tocopherols (Toc) are the most abundant natural antioxidants among the plant lipids (Hamilton et al., 1998; Chu and Hsu, 1999; Hras et al., 2000).

Amongst the herbs, rosemary (Ros) from the Labiatae family has attracted the greatest attention (Frankel et al., 1994; Zandi and Ahmadi, 2000; Naciye et al., 2008). Since rosemary extract is lipophilic, it is easily incorporated into oils and fats to prevent oxidation (Chu and Hsu, 1999; Hras et al., 2000). Lecithin (Lec) which is generally used as an emulsifier in food processing, can improve the dispersion of active antioxidants in emulsion systems and inhibits the propagation step of free radicals in the autoxidation mechanism by reacting with various free radicals. Lecithin has been widely used as a synergist in combination with phenolic antioxidants (Koga and Terao, 1995; Saito and Ishihara, 1997; Bandarra et al., 1999). Ascorbyl palmitate 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 oil (Hamilton et al., 1998).

The most important commercial natural antioxidants currently being used are: tocopherols, ascorbyl palmitate, rosemary extract and lecithins. As lipid oxidation occurs via multiple mechanisms and no single antioxidant can prevent all the steps in the oxidation process, a mixture of antioxidants must be...
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used to create a synergistic effect (Koga and Terao, 1995; Bandarra et al., 1999; Hras et al., 2000).

With regard to the role of edible oils in human health, the vulnerability of oils and fats to oxidative deterioration, the possible undesirable effect of synthetic antioxidants on consumers' health, and awareness of harmful effects of synthetic compounds must all be taken into account during the production of margarine. As margarine is a relatively new product in Iran, the potential difficulties mentioned above must be overcome during its production so that it can be introduced to the market as a safe substitute for butter. The purpose of this work was to test mixtures of natural antioxidants in margarine and select the combination that is most efficient in preserving its quality and which can be used as a substitute for TBHQ, which is currently being used as a synthetic antioxidant.

MATERIALS AND METHODS

Materials

Refined, bleached and deodorized sunflower oil with no added antioxidant was purchased from Kesht-o-Sana’at-e-Shomal, Iran. Refined, bleached, deodorized and antioxidant-free palm stearine was 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, soy lecithin was obtained from ADM (Archer Daniels Midland), citric acid and potassium sorbate and other analytical grade chemicals and solvents were purchased from Merck Co. β-Caroten (dissolved in edible oils) was obtained from Roche (Switzerland), vitamin A and D₃ were purchased from BASF (Germany), sodium casinate, low fat milk powder and diacetyl were obtained from Iran Caseinate, Moghan Co. (Iran) and Roberte (Iran).

Sample Preparations

Lipid Phase Preparation

The lipid phase was prepared by heating the margarine base (20% palm stearin and 80% sunflower oil) to a temperature of at least 5.6°C above the melting point (melting point of palm stearin: 45°C) before adding the oil soluble ingredients. These ingredients included the emulsifier (0.5%), vitamins A and D₃ (0.01%), β-caroten (0.003%), flavor (0.02%) and antioxidants. The antioxidant mixtures were added to the oil phase as follows: F₀: control (with no added antioxidant); F₁: 500 ppm Toc + 100 ppm Ap; F₂: 200 ppm Ros + 200 ppm Ap; F₃: 200 ppm Toc + 200 ppm Ros; F₄: 200 ppm Ap + 200 ppm Toc + 100 ppm Ros; F₅: 100 ppm Toc + 1000 ppm Lec; F₆: 250 ppm Toc + 1000 ppm Lec; F₇: 1000 ppm Ap + 500 ppm Toc + 1000 ppm Lec; F₈: 200 ppm Ros + 100 ppm Ap + 1000 ppm Lec; F₉: 200 ppm Ros + 200 ppm Toc + 1000 ppm Lec; F₁₀: 200 ppm Ros + 200 ppm Toc + 1000 ppm Lec; F₁₁: 120 ppm TBHQ.

Aqueous Phase Preparation

The aqueous phase was prepared separately. The skim milk powder (1%) was reconstituted, pasteurized and cooled to 45°C. The water soluble ingredients were added which included salt (0.5%), potassium sorbate as preservative (0.02%), sodium caseinate (1%) and citric acid (0.06%).

Emulsion Preparation

The oil and aqueous phases were blended together with high-shear agitation at 45°C to form a water-in-oil emulsion. The water phase was added to the oil phase in order to promote the formation of a water-in-oil emulsion. After preparation, the emulsion was transferred to an agitated holding tank that supplied the chilling units in which the emulsion was agitated 7 minutes.

Chilling and Crystallization

After preparation of emulsion, in order to achieve desirable consistency, it was transferred into a laboratory scale worker unit. The shaft in the worker unit revolves at about 35rpm with a residence time of approximately 3 minutes, it continued until large crystals disappeared. The temperature of margarine adjusted at 9-11°C in the worker unit.

Filling and Final Crystallization

The crystallized margarine emulsion was filled in 250 g poly-ethylene cups and stored in freezer (-18°C) for 48 hours to allow complete crystallization.

Chemical Analysis

Acid value, iodine value and melting point of oils and margarine samples were determined as described previously (AOCS Methods, 1997).

Preparation of methyl esters and GC analysis of samples In order to prepare fatty acids methyl esters, the oil phase of samples was separated from aqueous phase and heated to above its melting point. 1 g of the oil phase was added to 10 ml heptane in a test tube, and then the mixture was converted to fatty acid methyl esters by treatment with 0.5 ml KOH in methanol. It was shaked for 30 seconds, the upper layer which
contained methyl esters was separated and analyzed using GC system.

ISO methods (ISO Methods, 2002 a,b) of analysis were used in these determinations. The GC system (Varian 3800/Autosampler) was equipped with a FID detector and capillary column (Cp splitter-88 Fame, 100 m 25 mm 25 m) and was operated under the following conditions: FID: 270°C, oven: constant temperature at 175°C, injector: 250°C and constant flow rate of carrier gas (N₂): 0.6 ml/min.

**Oven Test**
The samples were transferred in beakers to an oven maintained at 60±1°C. Oxidative stability was determined by measuring peroxide and anisidine values every 5 days over a 25-day period. Primary oxidation products were determined by peroxide measurements (AOCS,1997). Formation of secondary oxidation products was measured using the p-anisidine value (AOCS,1997). The induction period was considered to be the number of days required for the peroxide value of the samples to reach 20 meqO₂/kg of fat (Economou et al., 1991). This is in agreement with the general acceptance that oils become rancid at peroxide values greater than 20 (Economou et al., 1991; Hras et al., 2000; Pokorny et al., 2001).

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

\[ SF = \frac{IP_{inh}}{IP_0} \]

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 (Hras et al., 2000; Yanishlieva and Marinova, 1996).

**Shelf-life evaluation**
The shelf-life of samples stored in the refrigerator (4±1°C) was measured by determination of peroxide values in the fresh sample and in samples stored for 2, 4, 8, 12, and 14 weeks after production. The induction period in the refrigerator was considered to be the number of days required for the peroxide value of the samples to reach 5meq/kg of fat (Economou et al., 1991; ISIRI,1999).

**Ranking of antioxidant mixtures**
The antioxidant mixtures were ranked on the basis of several factors including the number of the days needed for the peroxide levels in the samples to reach 20 at 60°C (Economou et al., 1991; Hras et al., 2000; Pokorny et al., 2001) and to reach a PV of 5 at 4°C (ISIRI,1999), the stabilization factor (at 110°C) and also the economic aspects of using natural antioxidants.

**Statistical analysis**
All determinations were carried out in triplicate. The results obtained for peroxide and anisidine values were subjected to analysis of student's t-test with a significance level of \( p<0.05 \). Results of the induction period data, stabilization factor and ranking points were analyzed using the Mann Whitney test. \( P \) value less than 0.05 were considered statistically significant.

**RESULTS**
The peroxide, anisidine and iodine values together with the melting point of the oils and also the data for the control sample (\( F_0 \)) are presented in Table 1. The fatty acid composition of the oil samples was within the normal range i.e.: 16:0 (8.46%), 18:0 (3.68%), 18:1 (22.16%), 18:2c (63.10%), 18:2t (1.38%), 18:3 (0.2%), 20:0 (0.11%), 20:1 (0.35%) and 0.56% of other fatty acids for sunflower oil; the fatty acid composition of palm stearine was as follows: 16:0 (53.36%), 18:0 (5.40%); 18:1 (33.70%), 18:2 (6.24%), 18:3 (0.43%) and 0.87% of other fatty acids (Fig.1). In the case of margarine, the corresponding values were as follows: 16:0 (171.15%), 18:0 (4.34% ), 18:1 (24.98%); 18:2c (51.26%), 18:2t (0.89%), 18:3 (0.26%), 20:0 (0.09%), 20:1 (0.23%) and 0.80% of other fatty acids.

**Table 1. Physical and chemical characteristics of the samples of oils and margarine**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Peroxide value (meq/kg)</th>
<th>Anisidine value (meq/kg)</th>
<th>Iodine value (g/100g)</th>
<th>Acid value (mg/g)</th>
<th>Melting point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower Oil</td>
<td>0.2±0.035</td>
<td>1.24±0.002</td>
<td>131.87±0.78</td>
<td>0.04±0.0030</td>
<td>-</td>
</tr>
<tr>
<td>Palm Stearine</td>
<td>0.75±0.054</td>
<td>2.04±0.006</td>
<td>38.65±0.19</td>
<td>0.01±0.0004</td>
<td>49.0±0.22</td>
</tr>
<tr>
<td>Margarine</td>
<td>0.4±0.040</td>
<td>1.50±0.003</td>
<td>111.5±0.45</td>
<td>0.03±0.0025</td>
<td>36.5±0.16</td>
</tr>
</tbody>
</table>
Effects of some natural antioxidant mixtures on margarine stability

The peroxide and anisidine values of the samples treated at 60°C showed that F2, F6, F9, F10 and F11 had the greatest antioxidant activity (p>0.05). On the fifth day of the oven test (Fig. 2), the peroxide value of the oil phase of F2 (8.19 meq/kg) was the lowest among all the samples (p<0.05). F8 and F11 (12.1 and 12.4 meq/kg) showed the lowest peroxide value (p<0.05). There was no statistically significant difference between the antioxidant activity of F8 and F11 on the fifth day (p>0.05).

By days 10 and 15, F2, F8 and F9 contained a significantly lower level of primary oxidation products compared to the control sample and also the other samples (p<0.05). By days 20 and 25, the antioxidant mixtures in F2, F5, F8, F9, F10 and F11 showed the greatest antioxidant activity compared to the other antioxidant mixtures (p<0.05).

Anisidine value of control (F0) and F1 by day 25 was 25.75 and 19.28 meq/kg (Fig. 3) which had statistically significant difference with F11 (TBHQ) (p<0.05). By day 25, anisidine value of F2 (9.82), F5 (10.30) and F9 (10.35) had no statistically significant difference with F11 (9.77) (p>0.05). This is in agreement with findings of Hras et al. (2000) and Chu and Hsu (1999). On the same day anisidine value of F3, F4 and F6 reached to 14.20, 12.17 and 11.82 meq/kg, respectively. The results of statistical analysis showed that anisidine value of margarine samples containing these antioxidant mixtures had statistically significant difference with F11 (p<0.05). By increasing the concentration of Toc, but not Lec, in F5, F6 (100 ppm Toc+1000 ppm Lec), F8 (250 ppm Toc+500 ppm Lec) and F7 (500 ppm Toc+1000 ppm Lec) at the end of the incubation period at 60°C (day 25), the anisidine value reached to 10.30, 16.32 and 25.09 meq/kg, respectively. F6 and F7 had statistically significant difference with F11 (p<0.05). It seems that by increasing the concentration of Toc from 100 ppm to 500 ppm, Toc shows prooxidative activity. The anisidine value of F10 (Ros+Toc+Lec) by day 25 reached to 8.12 and had statistically higher antioxidant activity compared to F11 (p<0.05). Totally, changes in anisidine value in the oven test (Fig. 3) showed that F8 (Ros+Ap+Lec), F10 (Ros+Toc+Lec) and F11 (TBHQ) had the highest antioxidant activity compared to the other treatments (p<0.05).

The induction period of the samples (Table 2) was used as an appropriate criterion for evaluating antioxidant activity (Economou et al., 1991; Hras et al., 2000; Pokorny et al., 2001). In this regard, the highest antioxidant activity in the oven test was associated with F2 (Ros+Ap) and F4 (Toc+Ap+Lec) in which the peroxide value reached 20 meq/kg after 8 days and was statistically different from that achieved with F11 (TBHQ). Other antioxidant mixtures (F1, F3, F4, F6 and F9) demonstrated lower activity (p<0.05) and the activity of F7 and F10 was not significantly different from that of F11 (p>0.05).
Effects of some natural antioxidant mixtures on margarine stability

Table 2. Criterion used in ranking antioxidant mixtures

<table>
<thead>
<tr>
<th>Samples</th>
<th>Time (days) to reach PV=20 at 60°C</th>
<th>SF at 110°C</th>
<th>Time (days) to reach PV=5 at 4°C</th>
<th>Cost of antioxidant mixtures ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0 (control)</td>
<td>4.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
<tr>
<td>F1</td>
<td>4.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07</td>
</tr>
<tr>
<td>F2</td>
<td>8.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05</td>
</tr>
<tr>
<td>F3</td>
<td>4.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05</td>
</tr>
<tr>
<td>F4</td>
<td>4.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06</td>
</tr>
<tr>
<td>F5</td>
<td>5.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.07</td>
</tr>
<tr>
<td>F6</td>
<td>4.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09</td>
</tr>
<tr>
<td>F7</td>
<td>6.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12</td>
</tr>
<tr>
<td>F8</td>
<td>8.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13</td>
</tr>
<tr>
<td>F9</td>
<td>5.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.10</td>
</tr>
<tr>
<td>F10</td>
<td>6.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.63&lt;sup&gt;c&lt;/sup&gt;</td>
<td>99&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.11</td>
</tr>
<tr>
<td>F11</td>
<td>6.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05</td>
</tr>
</tbody>
</table>

<sup>a-d</sup> Values that are significantly different in each column according to the student t-test and Mann-Whitney test (<0.05).

The results of the Rancimat test (110°C) were expressed as the stabilization factor (SF) in Table 2. The SF of samples F<sub>9</sub> (Ros+Ap+Lec), F<sub>10</sub> (Ros+Toc+Lec) and F<sub>11</sub> were not statistically significantly different from one another (p>0.05). Samples F<sub>2</sub> (Ros+Ap), F<sub>5</sub> (100 ppm Toc+Lec) and F<sub>8</sub> (Ap+Toc+Lec) had similar SFs and ranked as the second most effective group. The other samples including F<sub>1</sub>, F<sub>3</sub>, F<sub>4</sub>, F<sub>6</sub> and F<sub>7</sub> did not show substantial antioxidant activity in this test (rancimat test).

![Fig.4. Peroxide value of margarine samples during storage at 4°C](image)

At 4°C, the peroxide value of the control and samples F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>, F<sub>6</sub> and F<sub>7</sub> reached the point of rancidity (5meq/kg) after 8 weeks while samples F<sub>5</sub>, F<sub>9</sub>, F<sub>10</sub> and F<sub>11</sub> did not become rancid after this period (Fig. 4). The shelf-life of samples F<sub>5</sub>, F<sub>9</sub>, F<sub>10</sub> and F<sub>11</sub> was not statistically significantly different (p>0.05) but their shelf-life was longer than that of the other samples (p<0.05) (Table2). The shelf-life of margarine samples stored at 4°C was variable and ranged between 1 month (control, F<sub>1</sub> and F<sub>7</sub>) and 3 months (F<sub>10</sub> and F<sub>11</sub>).

DISCUSSION

Margarine samples contained less than 1% trans fatty acids (0.89%) and were of high nutritional value (PUFA/SFA+TFA=2.34). According to the changes in peroxide values in the samples over the 25 days of the oven test, it appeared that F<sub>2</sub> (Ap+Ros), F<sub>5</sub> (100 ppm Toc+Lec) and F<sub>8</sub> (Ap+Toc+Lec) had the highest antioxidant activity (p<0.05) and comparing F<sub>2</sub> and F<sub>8</sub>, the effect of F<sub>2</sub> was significantly (p<0.05) higher compared to F<sub>8</sub>. This is in agreement with the findings of Hras <i>et al.</i> (2000), Chu <i>et al.</i> (1999) and Haak <i>et al.</i> (2008) who reported that Ap+Ros, Toc+Ap+Lec and Ros+Toc+Lec had higher antioxidant activity than the other natural antioxidant mixtures tested in their studies. Also, the results of another study (Djenene <i>et al.</i>, 2002) demonstrated that combination of ascorbic acid with rosemary and the combination of tocopherol and ascorbic acid extended the shelflife of the product. In anisidine test, F<sub>10</sub> showed statistically significant lower level of secondary oxidation products compared to the control and other samples. This result is in agreement with the results reported by Chu <i>et al.</i> (1999), Hras <i>et al.</i> (2000) and also with the work of Beddows <i>et al.</i>(2002) who found that the optimum effect against racidity in sunflower oil was with a mixture of ascorbyl palmitate and rosemary. Also, the results of another study (Fang and Wada, 1993) demonstrated that a mixture of tocopherol and rosemary extract had a significantly stronger antioxidant effect rather than tocopherol alone and rosemary alone. According to the findings of Hras <i>et al.</i> (2000) the mixture of rosemary extract and ascorbyl...
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palmitate extends the induction period required to reach a peroxide value of 20 meq/kg in sunflower oil under test conditions, which indicates a strong synergism between these two natural antioxidants (Hras et al., 2000). On the other hand, lecithin had a synergistic effect with tocopherol (Chu and Hsu, 1999). The rancimat test indicated that the antioxidant combination of tocopherol and rosemery decreased lipid oxidation (Haak et al., 2008; Govaris et al., 2008) and also in agreement with the findings of Chu et al. (1999), Erkan et al. (2008), Frutos and Hernandez-Herrero (2005), Hras et al. (2000) and Judde et al. (2003) in which rosemary extract was found to have a higher phenolic content than other natural antioxidants.

The results of this research have shown that certain natural antioxidant mixtures can be used as substitutes for TBHQ to improve the shelf-life of margarine thus eliminating the need to use synthetic antioxidants as preservatives in food products which can accumulate in body organs and cause cancers and tumors. The results of the tests on margarine samples containing added natural antioxidants were in accordance with one another and indicated that F₁, F₃, F₄, F₅ and F₇ had lower antioxidant activity compared to the other samples. The shelf-life of these samples was less than 2 months which caused them to become rancid more rapidly than the other samples, they also showed the lowest SFs in the Rancimat test. F₅ extended the shelf-life of the samples to 12 months and showed high antioxidant activity in all other tests. The final ranking of the natural antioxidant mixtures was as follows: F₂ > F₁₀ > F₅, F₆ > F₈ > F₁, F₃, F₄ > F₆ and F₇ (Table 3).

With regard to the adverse effects of synthetic antioxidants on consumers' health and the economic aspects of the results of this research, F₂ (200 ppm Ros+200 ppm Ap) and F₁₀ (200 ppm Ros + 200 ppm Toc+ 1000 ppm Lec) are recommended as substitutes for TBHQ to maintain the quality and increase the shelf-life of the margarine. It is also recommended that further research on a larger scale should be carried out on this topic.

ACKNOWLEDGEMENT

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REFERENCES


<table>
<thead>
<tr>
<th>Samples</th>
<th>Ranking in oven test (60°C)</th>
<th>Ranking in SF(110°C)</th>
<th>Ranking in Shelf-life (4°C)</th>
<th>Economical value</th>
<th>Final Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₀(control)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>F₁</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>10</td>
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<tr>
<td>F₂</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
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<td>3</td>
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<td>3</td>
<td>1</td>
<td>10</td>
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<td>F₄</td>
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<td>3</td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
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<td>1</td>
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<td>3</td>
<td>3</td>
<td>2</td>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
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<td>8</td>
</tr>
<tr>
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<td>1</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>F₁₀</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>F₁₁</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

1."1" is considered as the best in each column.
Effects of some natural antioxidant mixtures on margarine stability


