Use of propolis extract as a natural antioxidant for plant oils

By Musa Özcan
Department of Food Engineering, Faculty of Agriculture, Selçuk University, 42031 Konya, Turkey

RESUMEN

Uso de extracto de propóleos como antioxidante natural para aceites vegetales.

Se ha ensayado las actividades antioxidantes de extractos metanolícos de propóleos en aceite de oliva almacenado a 60°C. La concentración de extractos en aceite de oliva varió desde 0,02 a 0,08%. Los extractos con concentraciones del 0,08 y 0,06% tuvieron una mayor actividad antioxidante comparando con el hidroxianisol butilado (BHA) y el hidroxitolueno butilado (BHT) a concentraciones del 0,01%. Las mayores actividades antioxidantes se encontraron en extractos de balsamo de propóleos a niveles del 0,08%. Puede decirse que la actividad antioxidante de propóleos aumenta con la concentración, constituyendo una nueva fuente de antioxidantes naturales.

PALABRAS-CLAVE: Aceite de oliva – Antioxidante – Propóleos.

SUMMARY

Use of propolis extract as a natural antioxidant for plant oils.

The antioxidant activities of methanol extracts of propolis were tested in natural olive oil stored at 60°C. The concentration of extracts in olive oil varied from 0.02 to 0.08%. Extracts at 0.08 and 0.06% concentrations had better antioxidant activity as compared to butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) at 0.01% levels. The greatest antioxidant activities were exhibited by propolis balsam extract at 0.08% levels. It can be said that the antioxidant activity of propolis increases with concentration. This product is considered as a new source of natural antioxidants.

KEY-WORDS: Antioxidant - Olive oil - Propolis.

1. INTRODUCTION

Propolis is a resinous substance collected by honey bees, and used by them to wax and disinfect their nests. It is well known for its antiseptic, antimycotic, bacteriostatic, and antioxidant activity (Dobrowolski et al., 1991, Yamauchi et al., 1992, Scheller et al., 1994). Its chemical composition is a complex mixture of compounds. Propolis contains a variety of chemical compounds, including various phenolic compounds such as flavonoid aglycones, various volatile aldehydes and ketones and sesquiterpens (Maciejewics et al., 1983, Krol et al., 1990, Volpert and Elstner 1993).

Flavonoids are the most abundant and most effective antioxidants in propolis balsam (Scheller et al., 1990, Yamauchi et al., 1992). Flavonoids were shown to possess antioxidative properties, due to their ability to scavenge free radicals (Krol et al., 1994). Yamauchi et al., (1992) reported that propolis resinous, an alcohol extract of propolis, is known to have antioxidative properties. Antioxidative activity of propolis balsam was measured by its inhibition of methyl linoleate autoxidation (Yamauchi et al., 1992). The same, researchers showed that benzyl caffeate found into propolis also had strong antioxidative activity.

Autoxidation and lipolysis are responsible for off-flavors in lipid-containing food products. Antioxidants such as BHA and BHT are widely used in many foods to prevent fat rancidity. In recent years, there has been some discussion about the possible toxicity of use of these chemicals used as antioxidants. Consequently, there is a need for other types of antioxidants and to render food products safer for mankind. Nowadays, the interest for this complex resin as a harmless medicine is increasing again. The antioxidative effect of ethanol-soluble extract of propolis has been illustrated so far only indirectly, by its protection of food products and by its radical scavenging ability (Krol et al., 1994).

The purpose of this study is to establish, the antioxidant effect of propolis extracts at different concentrations on olive oil as compared to other antioxidants such as BHA and BHT.

2. MATERIALS AND METHODS

2.1. Materials

Propolis sample was collected from hive on Selçuk University high collage of Taskent's farm. Cristalline BHA and BHT were obtained from Sigma Chemical Company (St. Louis, MO). Olive oil without added antioxidant was purchased from a local merchant. Its peroxide number was 4.3 meq/Kg.

2.2. Preparation of extract

Propolis was extracted with pure methanol (E. Merck, Darmstadt, Germany) in a Soxhlet apparatus.
The crude extracts was filtered and evaporated in rotary evaporator completely. Residue was kept in a hermetically-closed glass vessel at 4°C until using. The methanol extract of propolis was prepared according to (Yamauchi et al., 1992).

2.3. Assessment of antioxidant activity

Extract was indirectly melted at 50°C in a water bath and added to natural olive oil in 10x100 mm open beakers at 0.02, 0.04, 0.06 and 0.08% (w/w). BHA and BHT-containing and control sample (without adding any antioxidant) were also prepared under the same conditions. The mixtures were stirred. All samples of 20 g each were stored at 60°C in the dark. The antioxidant activities of substances were evaluated by determining peroxide values at definite time intervals according to the Method Cd 8-53 of the American Oil Chemists' Society. For the peroxide numbers, a known weight of olive oil was dissolved in a mixture of CH₃COOH:CHCl₃ (3:2,v/v), and saturated solution of KI (1 ml) was then added. The liberated iodine was titrated with sodium thiosulfate solution (0.01 N) in the presence of starch as an indicator (AOCS, 1992). The inhibition rate of autooxidation (cfr. Tab. II) was performed using the following formula (Krol et al., 1994).

\[ I = \frac{(M - L)}{M} \times 100 \]

2.4. Statistical analysis

The data from experiment were subjected to ANOVA using randomized complete block design (Düğünes et al., Minitab, 1991). Differences among means were Partitioned by the Waller-Duncan test (Mstat C 1980).

3. RESULTS AND DISCUSSION

Antioxidant effects and inhibition rates of propolis extracts at different concentrations, BHA and BHT are given in Table I and II respectively.

After 7 days of storing, BHA, BHT and all tested extracts of propolis were active in varying degrees on preventing autoxidation of olive oil compared with the control test. The propolis extract at 0.02% level was only slightly active. Extracts at 0.04% (except for 14 days), 0.06% and 0.08% levels had better antioxidant activity in comparison with BHA and BHT. In addition, BHA shows higher antioxidant effect than BHT.

### Table I

<table>
<thead>
<tr>
<th>Storage period (Days)</th>
<th>Oil Control</th>
<th>BHA (0.01%)</th>
<th>BHT (0.01%)</th>
<th>Extract (0.02%)</th>
<th>Extract (0.04%)</th>
<th>Extract (0.06%)</th>
<th>Extract (0.08%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>16.073 ± 0.169E**</td>
<td>13.017 ± 0.179A</td>
<td>13.155 ± 0.200C</td>
<td>12.502 ± 0.041B</td>
<td>11.610 ± 0.149E***</td>
<td>10.622 ± 0.207A</td>
<td>9.548 ± 0.090A</td>
</tr>
<tr>
<td>14</td>
<td>25.055 ± 0.830D</td>
<td>12.132 ± 0.580B</td>
<td>12.868 ± 0.108B</td>
<td>14.162 ± 0.131A</td>
<td>12.295 ± 0.216a</td>
<td>10.530 ± 0.179A</td>
<td>8.940 ± 0.053B</td>
</tr>
<tr>
<td>21</td>
<td>34.627 ± 0.360C</td>
<td>11.938 ± 0.082B</td>
<td>13.722 ± 0.067B</td>
<td>13.820 ± 0.165A</td>
<td>10.470 ± 0.149d</td>
<td>9.122 ± 0.051B</td>
<td>7.408 ± 0.197C</td>
</tr>
<tr>
<td>28</td>
<td>48.932 ± 0.255B</td>
<td>11.085 ± 0.160C</td>
<td>13.962 ± 0.161B</td>
<td>11.763 ± 0.226C</td>
<td>10.913 ± 0.075c</td>
<td>7.465 ± 0.185C</td>
<td>7.395 ± 0.133C</td>
</tr>
<tr>
<td>35</td>
<td>67.098 ± 0.145A</td>
<td>12.235 ± 0.302B</td>
<td>14.330 ± 0.301A</td>
<td>11.900 ± 0.488C</td>
<td>9.298 ± 0.157e</td>
<td>6.700 ± 0.221D</td>
<td>6.678 ± 0.123D</td>
</tr>
</tbody>
</table>

* Initial (zero day) peroxide value of the oil was 4.3 meq/Kg.
** Differences among means indicated with majuscules are significant in p<0.05
*** Differences among means indicated with minuscules are significant in p<0.01
Notes: n=4, average values ± standard errors.

Inhibition rate on autoxidation of extracts increased with concentrations (Table II). The most remarkable antioxidative effect was established in the sample with addition of extract at 0.08% level (Table II). While antioxidant activities of BHA, BHT and extracts at 0.02 and 0.04% levels were closely similar, the activities of those extracts at 0.06 and 0.08% levels markedly increased (p<0.05). The
potent antioxidant activity of propolis extract is probably due to the presence of high amounts of flavonoids (Krol et al., 1990, Scheller et al., 1990, Yamauchi et al., 1992).

Little is known about the extent to which propolis extract may contribute to the antioxidant activity, although this substance has been extensively examined for their medicinal and antimicrobial properties (Krol et al., 1990, Volpert and Elstner 1996). Our results indicate that high concentrations may enhance the potency of the antioxidant activity of propolis. The results obtained can support and direct the formulation of propolis to optimize antioxidant potency in relation to application in cosmetics.

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REFERENCES


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