Grasas y Aceites
Vol. 51. Fasc. 4 (2000), 251-253

Use of propolis extract as a natural antioxidant for plant oils

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RESUMEN

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

Se ha ensayado las actividades antioxidantes de extractos metanólicos 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 0,06 y 0,08% 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 bálsamo 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 0.06 and 0.08% 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 ketons 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.

Autooxidation 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 CH_3COOH:$CHCl_3$ (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).

$$I = (M-L) / M \times 100$$

I: Inhibition (%),
M: peroxide value of control at the same time (meq/Kg),
L: peroxide value of tested oil at the same time (meq/Kg).

2.4. Statistical analysis

The data from experiment were subjected to ANOVA using randomized complete block design Düzgünes *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
Effect of BHA, BHT and propolis extract at different concentrations on peroxide values* of olive oil at 60°C

Storage period (Days)	Oil Control	BHA (0.01%)	BHT (0.01%)	Extract (0.02%)	Extract (0.04%)	Extract (0.06%)	Extract (0.08%)
7	16.073 ± 0.169E**	13.017 ± 0.179A	13.155 ± 0.200C	12 502 ± 0.041B	11.610 ± 0.149b***	10.622 ± 0 207A	9 648 ± 0 090A
14	25.055 ± 0.830D	12.132 ± 0.580B	12.868 ± 0.108D	14.163 ± 0.131A	12.295 ± 0.219a	10.530 ± 0.179A	8.940 ± 0 053B
21	34.627 ± 0.360C	11.938 ± 0.082B	13.722 ± 0.067B	13.820 ± 0.165A	10.470 ± 0.149d	9.122 ± 0.051B	7 408 ± 0.197C
28	48.932 ± 0.255B	11.085 ± 0.160C	13.962 ± 0.161B	11.783 ± 0.226C	10.913 ± 0 075c	7.455 ± 0.185C	7.395 ± 0.133C
35	67.098 ± 0.146A	12.235 ± 0.302B	14.330 ± 0.301A	11.900 ± 0.488C	9.298 ± 0.157e	6.700 ± 0.221D	6.678 ± 0.123D

* 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

Table II
The inhibition rate of autooxidation by BHA,BHT and extracts at different concentrations of propolis (%), (according to control test)

(Days)	BHA (0.01%)	BHT (0.01%)	Extract (0.02%)	Extract (0.04%)	Extract (0.06%)	Extract (0.08%)
7	19.013	18.154	22.217	27.767	33.914	39.974
14	51.578	48.641	43.472	50.928	57.972	64.318
21	65.524	60.372	60.089	69.763	73.656	78.606
28	77.346	71.467	75.920	77.698	84.765	84.887
35	81.765	78.643	82.265	86.143	90.015	90.047

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.

ACKNOWLEDGMENTS

Thanks to Dr. SS Parlat, Dr. Iskender Yildirim and Dr. Alp Önder Yildiz for help with statistical analyses.

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Recibido: Mayo 1999
Aceptado: Septiembre 1999