Evaluation of Antioxidant and Antimicrobial Activities of Ethanol Extracts From 13 Kinds of Spices and Analysis of Meat Patties Manufactured by Selected Spices

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***Abstract*—** This study was carried out to know the antioxidant activity of hot water extracts from 13 kinds of spices commonly used in meat processing products. The superoxide radical scavenging activity of mace extract was highest among the samples. The order of the DPPH scavenging activity was funnel > caraway > termeric > cumin and dill. The hydroxyl radical scavenging activitis of cumin, rosemary, thyme, marjoram, funnel were relatevly higer than the others. Significantly highest total polyphenol contents were found in the order clove > tereric > thyme > mace > majoram. The order of flavonoid content was thyme > rosemary and marjoram > oregano and cumin > and dill. We found that the flavonoid content of a particular spice not only highly correlated with the free radical ($r=0.682$, $p < 0.01$) or superoxide anion radical-scavenging activities ($r = 0.742$ $p < 0.01$), but also correlated with the total polyphenol contents ($r = 0.524$, $p < 0.01$). In additon, total polyphenol contents were shown highly correlation with free radical scavenging activity ($r= 0.875$, $p < 0.01$). In conclusion, used spices had a high antioxidant activities give us a basic data which has implications for further development of processed food products.

***Index Terms* — spices, antioxidant, meat products**

I. INTRODUCTION

Oxidation is one of the major causes of chemical spoilage, resulting in rancidity and/or deterioration of the nutritional quality, colour, flavour, texture and safety of foods (Antolovich et al., 2002). Reactive oxygen species (ROS), such as free radical, superoxide radical (O2-), and hydroxyl radicals (OH), are produced as a part of normal metabolic processes. The oxidative damages caused by ROS on lipids, proteins and nucleic acids may trigger various chronic diseases, such as coronary heart diseases, atherosclerosis, cancer and aging (Madhavi et al., 1996). Medicinal plants and spices are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins (Cai et al., 2004, Kähkönen et al., 1999 and Larson, 1988) are considered to be a major contributor to the antioxidant activity. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Park and Pezzutto, 2002). These antioxidants also possess diverse biological activities, such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities. These activities may be related to their antioxidant activity (Chung et al., 1998). The health-promoting effect of antioxidants from plants and spices is thought to arise from their protective effects by counteracting ROS. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine. The aims of the present study were to measure the antioxidant activity of 13 selected spices using the free radical, hydroxyl radicial and superoxide anion radical-scavenging activity to determine their total phenolic contents and to investigate the relationship between phenolic content and antioxidant activity.

II. MATERIALS AND METHODS

A. Extraction yield of samples

13 kinds of dried spices were purchased from Taewon Food Industry(Seoul, Korea). The samples were dried for 12 hrs to about 4% moisture (dry base) in an air operating at 50℃. The samples were added with 10 volumes (v/w) of 99% ethaol at 4 ℃ for 3 days. The extracts were filtered with filter paper (Whatman No 2), and were completely dried in a freeze drier and stored at -20 ℃ until further use.

C. Radical-scavenging activity on DPPH assay

The free radical scavenging activity of samples (10 mg/ml) was measured using the method of Brand-Williams et al. (1995) with some modification. L-ascorbic acid was used as positive control. The inhibition percentage was calculated from the following equation: Inhibition % = [(absorbance of control-absorbance of sample)/absorbance of control] ×100.

D. Hydroxyl ($OH^{\bullet}$) radical scavenging activity

The scavenging activity of samples on the hydroxyl radical ($OH^{\bullet}$) was measured by the deoxyribose method (Halliwell et al. 1987) with a slight modification. The deoxyribose assay was performed in 10 mM phosphate buffer (pH 7.4) containing 2.5 mM deoxyribose, 1.5 mM H_2O_2, 100 μM $FeCl_3$, 104 μM EDTA, and the test sample **(250 μg/ ml).** There action was started by adding ascorbic acid to a final concentration of 100 μM. There action mixture was incubated for 1 h at 37 °C in a water-bath. After incubation, the color was developed by addition of 0.5% thiobarbituric acid followed by ice-cold 2.8% trichloroacetic acid in 25 mM NaOH and heating for 30 min at 80℃. A control was performed without samples (A1). The sample (A2) was cooled on ice and the absorbance was measured at 532 nm.

E. Superoxide anion ($O_2^{\bullet-}$) radical scavenging activity

Superoxide radicals were generated by a modified method of Liu et al. (1997), with a slight modification. The samples **(250 μg/ ml)** were added to the reaction solution containing 100 ul of 30 mM EDTA (pH 7.4), 10 ul of 30 mM hypoxantine in 50 mM NaOH, and 200 ul of 1.42 mM nitro blue tetrazolium (NBT). After the solution was preincubated at room temperature for 3 min, 100 ul of 0.5 U/ml xanthine oxidase was added to the mixture and the volume was brought up to 3 ml with 50 mM phosphate buffer (pH 7.4). After the solution was incubated at room temperature for 20 min, absorbance was measured at 560 nm. The reaction mixture without xanthine oxidase was used as a blank (A1). The samples (A2) were added to the reaction mixture, in which $O2^{\bullet-}$ was scavenged, thereby inhibiting the NBT reduction. Absorbance was measured and the decrease in $O2^{\bullet-}$ was represented by A2-A1.

F. Determination of total polyphenol content using Folin-Ciocalteu assay

Total phenolic contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965), calibrating against catechin standards and expressing the results as mg catechin equivalents (CE) per 1 ml extract (mg/ml). Data presented are average of six measurements.

G. Measuremant of total flavonoids

The content of total flavonoids was measured by a colorimetric method. Briefly, 0.25 ml of an appropriately diluted sample was added to a tube containing 1 ml of double-distilled water. Next, 0.075 ml of 5% NaNO2, 0.075 ml of 10% AlCl3 and 0.5 ml of 1 M NaOH were added at 0, 5 and 6 min, sequentially. Finally, the volume of the reacting solution was adjusted to 2.5 ml with double-distilled water. The absorbance of the solution at a wavelength of 510 nm was detected using a spectrophotometer (Ultrospec 2100 pro; Amersham Pharmacia Biotech Co., Piscataway, NJ, USA).

III. RESULTS AND DISCUSSION

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. Superoxide anion is an initial free radical formed from mitochondrial electron transport systems. Mitochondria generate energy using a 4-electron chain reactions, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anion. It plays an important role in the formation of other reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems (Lee, Koo, & Min, 2004). Superoxide radicals have been observed to kill cells, inactivate enzymes and degrade DNA, cell membranes and polysaccharides (Fridovich, 1978). The superoxide anion radical scavenging activities of mace were significantly higher than those of others.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used in recent years to quantify antioxidants in complex biological systems. DPPH is a stable nitrogen-centred free radical the colour of which changes from blue/purple to yellow upon reduction by either the process of hydrogen- or electron-donation due to the formation of diphenylpicrylhydrazine (Cai et al., 2006; Sroka and Cisowski, 2003). Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Brand-Williams et al., 1995). This reaction is used as a measure of the ability of the extracts, or any other antioxidant, such as ascorbic acid, to scavenge any free radical. The results from the radical-scavenger assays for water extracts are presented in Fig. 2. The results showed that among 13 tested spices, funnel was the most effective. In addition, a significant and linear relationship existed between the DPPH scavenging activity and phenolic content, indicating that phenolic compounds are major contributors to antioxidant activity. The highly significant correlations obtained in this study support the hypothesis that phenolic compounds contribute significantly to the total antioxidant capacity of medicinal plants ($r = 0.682$, $p > 0.01$). The good correlation between the results from total phenols analysis and the antioxidative assays has been previously reported (Zheng & Wang, 2001).

Among the reactive oxygen species (ROS), hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic methabolism to initiate cell damage in vivo (Waling, 1975; Chance,

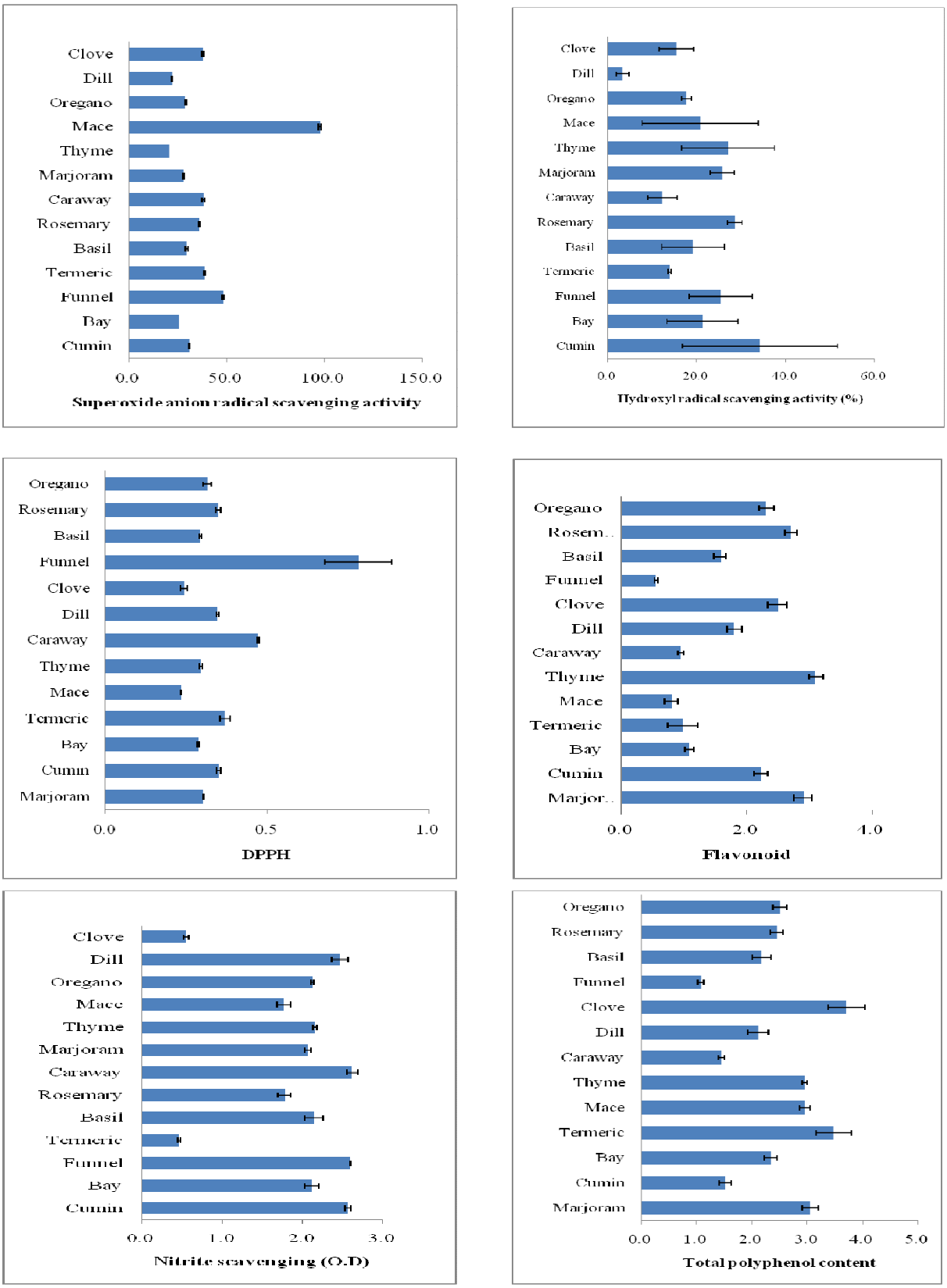

Fig. 1. Superoxide, hydroxyl and DPPH radical scavenging activity and flavonoid, total polyphenol content and nitrite scavenging activity of 13 spices in the concentration of 100 ug/mL. (n=6, error bars represent standard deviation).

Sies, & Boveris, 1979; Rollet-Labelle et al., 1998). We examined the inhibitory action of tested samples on deoxyribose degradation which gives an indication of hydroxyl radical scavenging action and iron chelating activity (Lopes et al., 1999; Halliwell et al., 1987). When hydroxyl radical, generated by the Fenton reaction,

attacks deoxyribose it degrades into fragments that react with TBA to form a pink color. Hydroxyl radicals attack deoxyribose starting a set of reactions, which eventually results in TBARS formation. When a molecule scavenges hydroxyl radicals the TBARS formation is decreased. Hydroxyl radical scavenging activity of 13 spices in the concentration of 1 ug/mL were shown in Fig. 1. The result in decreasing order of hydroxyl radical scavenging activity was cumin > .thyme, majoram, rosmary, and funnel.

Phenolic substances have been shown to be responsible for the antioxidant activity of plant materials (Rice-Evans et al., 1996). Therefore, the amount of total phenols in the extracts was investigated by the Folin-Ciocalteu method. Significantly highest results were found in the order clove > turmeric > thyme, mace, and majoram > oregano and rosemary.

Generally, antioxidant activity depends on the number and positions of hydroxyl groups and other substituents, and glycosylation of flavonoid molecules. Presence of certain hydroxyl groups on the flavonoid nucleus enhances antioxidant activity (Cai et al., 2006). There have been a few studies on the structure-antioxidant activity relationships of certain natural coumarins, lignans, tannins, and quinones and some synthesized curcuminoids and stilbenes (Foti et al., 1996; Sreejayan and Rao, 1996; Yokozawa et al., 1998; Cassidy et al., 2000; Lu et al., 2002; Cai et al., 2004b).

The antioxidant properties of phenolic acids and flavonoids are due to their redox properties, ability to chelate metals and quenching of singlet oxygen (Rice-Evans, Miller, & Paganga, 1996). Flavonoids, which are partly responsible for the pigmentation of flowers, fruits and leaves, are subdivided into flavanols, flavonols, flavones, flavanones and anthocyanins based on the saturation of the flavan ring and also their hydroxylation. They occur mostly as glycosylated derivatives, sometimes conjugated with sulphate or organic acids (Youdim, Spencer, Schroeter, & Rice-Evans, 2002). The flavonoid content of the aqueous spice extracts is shown in Table 2. The flavonoid content of the spice extracts varied greatly. Thyme had the highest flavonoid content.

Table 2. Changes of color values in meat patties characteristics added spices at day 7 of storage

	CIE L*	CIE a*	CIE b*	Chroma	Hue
Control	65.00	6.65	11.05	12.85	59.00
ascorbic acid	65.45	7.40	10.60	12.95	55.05
clove	57.95	6.10	13.85	15.15	66.50
Thyme	64.85	7.85	12.20	14.95	51.40
Savory	64.20	10.00	10.75	14.70	47.10
Rosemary	65.65	6.65	11.05	12.90	58.80
Oregano	65.35	8.45	12.20	14.85	55.10

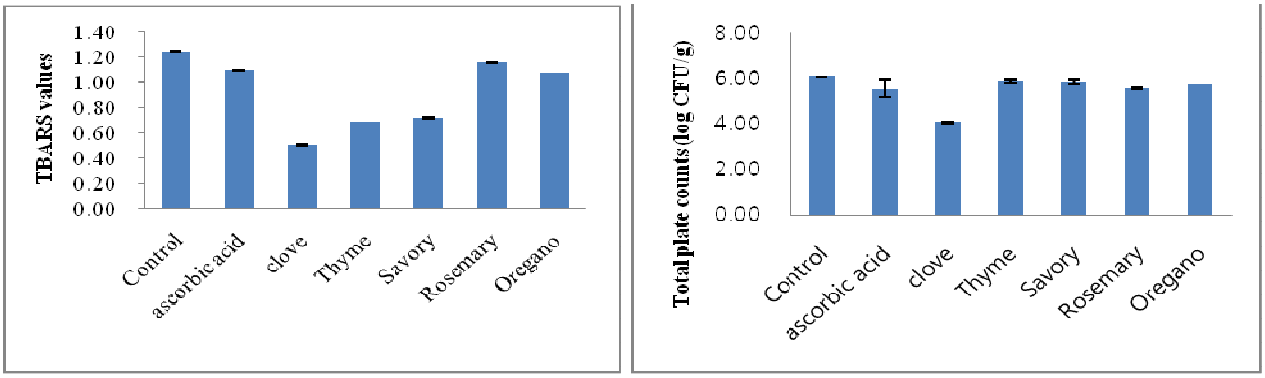

Table 2. Changes of TBARS and total plate counts in meat patties characteristics added spices at day 7 of storage

IV. CONCLUSION

Resuts suggest that relatively high antioxidant potential spices give a good characterististics in meat products.

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REFERENCES

Amro, B., Aburjai, T., & Al-Khalil, S. (2002). Antioxidative and radical scavenging effects of olive cake extract. *Fitoterapia,* 73, 456-461.

Aneta, W., Jan, O., & Renata, C. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105, 940-949.

Babu, B. H., Shylesh, B. S., & Padikkala, J. (2001). Antioxidant and hepatoprotective effect of Alanthus icicifocus. *Fitoterapia*, 72, 272–277.

Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuno, A., & Del Rio, J. A. (2000). Antioxidant activity of phenolics extracted from Olea europaea L. leaves. *Food Chemistry*, 68, 457-462.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel- Wissenschaft und Technologie*, 28, 25-30.

Briante, R., Febbraio, F., & Nucci, R. (2003). Antioxidant properties of low molecular weight phenols present in the Mediterranean diet. *Journal of Agricultural and Food Chemistry*, 51, 6975-6981.

Cai, Y. Z., Sun, M., Xing, J., Luo, Q., & Corke, H. (2006). Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Science*, 78, 2872–2888.

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food Drug Analysis*. 10, 178–182.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Carlo, M. D., Gallina-Toschi, T., Lercker, G., Compagnone, D., & Fernandez-Gutierrez, A. (2005). Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. *Journal of Agricultural and Food Chemistry*, 53, 8918-8925.

Fang, Y. Z., Yang, S., & Wu, G. (2002). Free radicals, antioxidants, and nutrition. *Nutrition*, 18, 872-879.

Halliwell, B., Gutteridge, J. M. C., & Arurma, O. I. (1987). The deoxyribose method: a simple "test-tube" ssay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry*, 165, 215–219.

Hatano, T., Edamatsu, R., Mori, A. (1989). Effects of interaction of tannins with co-existing substances. *Chemical & Pharmaceutical Bulletin*, 37, 2016-2021.

Hochestein, P., & Atallah, A. S. (1988). The nature of oxidant and antioxidant systems in the inhibition of mutation and cancer. *Mutation Research*, 202, 363–375.

Huang, D., Ou, B., & Priop, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53, 1841-1856.

Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry,* 47, 3954–3962.

Kang, D., & Hamasaki, N. (2003). Mitochondrial oxidative stress and mitochondrial DNA. *Clinical Chemistry and Laboratory Medicine*, 41, 1281–1288.

Komissarenko, N. F., Derkach, A. I., Kovalyov, I. P., & Bublik, N. P. (1994). Diterpene glycosides and phenylpropanoids of Stevia rebaudiana Bertoni. *Rast. Research*, 1(2), 53–64.

Liu, F., Ooi, V. E. C., & Chang, S. T. (1997). Free radical scavenging activity of mushroom polysaccharide extracts. *Life Science*, 60, 763–771.

Manian, R., Anusuya, N., Siddhuraju, P., & Manian, S. (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of Camellia sinensis (L.) O. Kuntz, Ficus bengalensis L. and Ficus racemosa L. *Food Chemistry*, 107, 1000–1007.

Park, Y. K., Lee, W. Y., Park, S. Y., Ahn, J. K., & Han, M. S. (2005). Antioxidant activity and total phenolic content of callistemon citrinus extracts. *Food Science and Biotechnology*, 14, 212-215.

Perez-Bonilla, M., Salido, S., Beek, T. A., Linares-Palomio, P. J., Altarejos, J., Nogueras, M., & Sanchez, A. (2006). Isolation and identification of radical scavengers in olive tree (Olea europaea) wood. *Journal of Chromatography A*, 1112, 311-318.

Sahin, K., Kucuk, O., Sahin, N., & Sari, M. (2002). Effects of vitamin C and vitamin E on lipid peroxidation status, some serum hormone, metabolite, and mineral concentrations of Japanese quails reared under heat stress (34°C). *International Journal for Vitamin and Nutrition Research*, 72, 91–100.