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ANTIOXIDANT ACTIVITY OF THE LABIATAE

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I. INTRODUCTION

There are a wide range of valuable products that originate from the plant kingdom, including dyes, flavors, medicines, and cosmetic and food preservatives (Veljkovic and Stankovic, 1993). Although there is no defined distinction between spices and herbs, herbs generally include plants used for their leaves, stems, flowers, and roots while spices include herbs as well as aromatic seeds. During the past 40 years there has been interest in defining the benefits of plant secondary metabolites as food preservatives, that is, antioxidants. However, most of the research conducted on identifi-

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cation, isolation, and testing of active compounds from plants has been conducted within the past 20 years.

There are 31 plant families that have been investigated for active compounds (Veljkovic and Stankovic, 1993); of these, a primary family that has attained a great deal of notarity for active compounds is the Labiatae (Lamiaceae). The Labiatae family consists of approximately 3500 species that are native chiefly to the Mediterranean area, although some have origins in Australia, Southwest Asia, and South America (Veljkovic and Stankovic, 1993). Of these, approximately 500 species have traditionally been used throughout the world for their wide range of medicinal properties (Grieve, 1971; Morton, 1981). Some of the Labiatae species that have been studied are shown in Table I and include balm, basil, hyssop, marjoram, mint, oregano, rosemary, sage, savory, and thyme. Variants can exist within a species, for example, there are 9 variants within oregano (*Origanum vulgare* L.), 17 within rosemary (*Rosmarinus officinalis* L.), 7 within sage (*Salvia officinalis* L.), and 2 within thyme (*Thymus vulgaris* L.) (Veljkovic

TABLE I

LABIATAE SPECIES USED FOR THE ACTIVE COMPOUNDS

Common name	Scientific name
Balm	*Melissa officinalis* L.
Basil	*Ocimum basilicum* L.
Hyssop	*Hyssopus officinalis* L.
Marjoram	
Sweet	*Origanum majorana* L.
Spanish	*Thymus mastichina* L.
Mint	
Spearmint	*Mentha spicata*
Peppermint	*Mentha piperita* L.
Oregano	
Greek	*Origanum vulgare* L.
Turkish	*Origanum onites* L.
Spanish	*Coridothymus capitatus* L.
Rosemary	*Rosmarinus officinalis* L.
Sage	*Salvia officinalis* L.
Savory	*Satureja hortensis* L. or *S. montana* L.
Thyme	*Thymus vulgaris L.* and *T. zygis* L.

Note. Adapted from Veljkovic and Stankovic (1993).

and Stankovic, 1993). Researchers need to be aware that between variants there can be a wide range of concentrations for specific compounds. For instance, Duke (1992) reported that the concentration of rosmarinic acid from rosemary (*R. officinalis*) could range from 3500 to 38,507 ppm.

The driving force for the development of plant-derived compounds for use in the food industry is the increasing concern over the safety of the synthetic antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylated hydroquinone (TBHQ) (Gray *et al.,* 1988; Banias *et al.,* 1992; Wong *et al.,* 1995); this concern impacts consumer acceptance. Consumers have been made very aware of and are sensitive to the use of food additives and respond favorably to the concept of food being "natural."

II. EVOLUTION OF LABIATAE AS ANTIOXIDANT SOURCES

Prior to the 1950s, there were several limited reports on the use of plants (herbs/spices) as antioxidants. The first in-depth study of spices as antioxidants was conducted by Chipault's group. Chipault *et al.* (1952) investigated the antioxidant activity of 32 spices as well as extracts of these spices. Of the spices tested, rosemary and sage had very good antioxidant activity. Petroleum ether extracts of most of the spices showed activity, but they were not as effective as the whole spice. Chipault *et al.* (1956), in a study involving oil-in-water emulsions, found clove to be an outstanding antioxidant. Other spices exhibiting antioxidant activity included allspice, cardamon, cassia, cinnamon, ginger, mace, nutmeg, oregano, black pepper, white pepper, rosemary, sage, thyme, and turmeric.

Since Chipault's work, researchers throughout the world have investigated a wide range of plants for antioxidant activity with the Labiatae family generally being the source of greatest activity. Studies have evaluated: (1) ground plant tissue; (2) essential oil extracts; (3) crude extracts; and (4) isolated, purified, and identified compounds. The following sections will present information relative to the Labiatae family as sources of antioxidant activity with emphasis on rosemary, sage, oregano, and thyme.

III. PLANT TISSUE STUDIES

Palitizsch *et al.* (1969, as cited by Griffiths and McDonald, 1985) showed that whole rosemary, sage, and nutmeg had significant antioxidant activity in commercial lard when added at levels of 0.1 to 0.2%. Further improve-

ments were obtained by adding ascorbic acid (0.1%) or tocopherols (0.05%), which acted synergistically with the whole spices.

Bishov *et al.* (1977) reported that the addition of 2.5% ground oregano, thyme, marjoram, and spearmint to a freeze-dried model system of corn oil and carboxymethyl cellulose held at 65°C showed protection factors (ratio of induction period of control to induction period of treatment samples) of 4, 2, 3, and 2.5, respectively. Gerhardt and Blat (1984) reported that thyme had a protection factor of 4.6 in pork fat, which was equal to those of rosemary and sage, while marjoram had a factor of only 1.7 in the pork fat system.

Pizzocaro *et al.* (1985, as cited by Gray *et al.*, 1988) reported that the addition of ground thyme or oregano to minced sardine muscle stored at 0°C did not show any antioxidant activity while basil had slight activity. Rosemary and sage were tested but no data on their effectiveness were given. Korczak *et al.* (1988) studied the effect of spices (rosemary, sage, and marjoram) in pork-blend meatballs that were deep fat fried and then held frozen (−18°C). They found that rosemary and sage were effective in reducing oxidation; however, marjoram exhibited strong prooxidant activity. Previous work (Korczak *et al.*, 1987, as cited by Korczak *et al.*, 1988) had shown marjoram to have some antioxidant activity at a temperature of 60°C. These authors felt that the antioxidant activity of marjoram was affected by temperature.

Dried leaves of rosemary added to cooked minced pork meatballs retarded the development of warmed over flavor (WOF) during cold storage (Huisman *et al.*, 1994). Rosemary added at a level of 0.05% of the total product weight was found to be acceptable by a sensory panel. Huisman *et al.* (1994) also studied the effect of the combination of rosemary addition and packaging atmospheres on the development of WOF. Cooked meatballs, with or without rosemary addition, were packaged in different atmospheres, including normal air, 5% air/95% N_2, 3% O_2/97% N_2, 1% O_2/99% N_2, and 100% N_2, and stored at 5°C. Samples were monitored for oxidation by 2-thiobarbituric acid-reactive substance (TBARS) values and sensory evaluation. The combination of rosemary and reduced oxygen in the package resulted in significantly lower TBARS values and significantly higher sensory scores.

Hall *et al.* (1962) found that the addition of sage to pork sausages treated with sodium chloride was able to inhibit the oxidative effect of the salt. Sage also helped maintain high flavor (sensory) scores of the pork sausage.

Tsimidou *et al.* (1995), in a series of studies, evaluated the antioxidant activity of ground oregano in an unsaturated lipid system (mackerel oil) compared to ground rosemary, BHA, or TBHQ. All samples were stored at 40°C in the dark. In the first study, oregano at 1% (w/w) was found to

be equivalent to 200 ppm BHA in controlling oxidation of the mackerel oil. In the second study, oregano and rosemary at 0.5% (w/w) inhibited oxidation of the mackerel oil for about 15 days. When oregano was tested at 1% (w/w), it was found to have activity comparable to 200 ppm TBHQ.

IV. LABIATAE ESSENTIAL OILS AS ANTIOXIDANTS

In the plant, essential oils serve as insect attractants for pollination or they can act as a deterrent to microbial and/or insect attack (Tsimidou and Boskou, 1994). The role of essential oils in food preservation is limited due to their strong odor. The chemical composition of essential oils is very complex and they contain on an average 50–100 components; however, some may contain a primary constituent at very high levels (i.e., 80–90%). A wide range of compounds have been isolated and identified from essential oils, including monoterpenes, sesquiterpenes, and their oxygenated derivatives (Tsimidou and Boskou, 1994).

Farag *et al.* (1989) tested the essential oils from spices, including some from the Labiatae family, for antioxidant activity in a linoleic acid emulsion system. In addition they identified and tested the basic component of each oil for activity in the same system. Results showed the antioxidant effectiveness of the essential oils from the spices to be: Clove > thyme > rosemary > cumin > sage > caraway. The activities of the primary components of essential oils (Fig. 1) were concentration dependent, and it was found that thymol (thyme) and eugenol (clove) at 1200 ppm were between 0.6 and 0.7 times as effective as 200 ppm BHT while carvone (caraway), thujone (sage), cumaldehyde (cumin), and borneol (rosemary) had little antioxidant activity. In addition, Farag *et al.* (1989) found that thyme and clove essential oil extracts had antioxidant activity in cottonseed oil. These researchers tested the impact of 50 to 1200 ppm of thyme and clove oil on the sensory (odor) characteristics of cottonseed oil and found no impact.

Thymol and its isomer carvacrol (Fig. 2) are found together but the final flavor impact is affected by their relative concentrations. Thymol is characteristic of thyme while carvacrol is predominant in oregano. The antioxidant activity of the essential oils of oregano has been investigated. Lagouri *et al.* (1993) tested the essential oils from four different oregano plants common to Greece. Results indicated the antioxidant activity of the oils was related to the carvacrol and thymol levels found in the oils. At 1000 ppm the essential oils were equivalent to 200 ppm BHT in lard.

Many spices and herbs that display antioxidant activity also display antimicrobial activity, both activities have been shown to be related to their phenolic structure (Tsimidou and Boskou, 1994). A relationship between

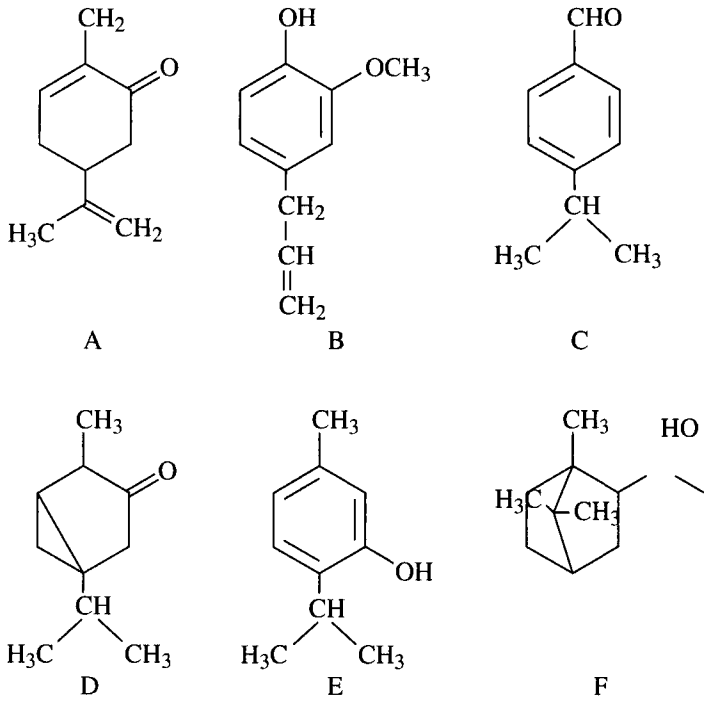

FIG. 1. Structure of monoterpenes from caraway (A; carvone); clove (B; eugenol); cumin (C; cumaldehyde); rosemary (D; borneol); sage (E; thujone); and thyme (F; thymol). From Tsimidou and Boskou (1994).

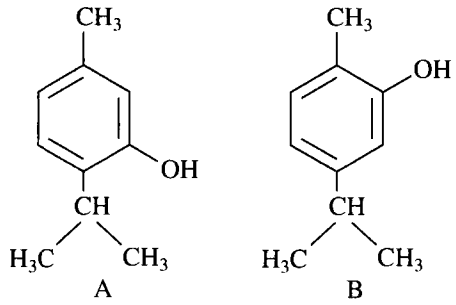

FIG. 2. Structures of thymol (A) and carvacrol (B).

the antioxidant activity and chemical composition was proposed by Farag *et al.* (1989). Structural features required for activity of monoterpenes (Fig. 1) have been shown to include the presence of a phenolic ring containing an electron repelling group. Data indicate that eugenol and thymol have greater antioxidant activity due to the presence of a hydroxyl group on the aromatic ring while carvone, thujone, and borneol lack this structure, explaining their lack of activity (Farag *et al.*, 1989).

While it is accepted that the antioxidant activity of monoterpenes is related to the presence of a hydroxyl group on the aromatic ring there is also evidence that the presence of an ethylidene group, as found in linalool and linayl acetate (Fig. 3), has the ability to interrupt the oxidative free radical chain (Farag *et al.*, 1989). It is believed that the ethylidene side group has the ability to react with lipid free radicals forming a stable allylic tertiary free radical. It has been shown that linalool has a slight prooxidant activity in heated soybean oil while linayl acetate does not display this type of activity (Farag *et al.*, 1989).

The structure–activity of the monoterpene compounds is not dissimilar to those shown to be important for activity in phenolic acids. Cuppett *et al.* (1997) reported that antioxidant activity of phenolic acids was related to the presence of two hydroxy groups *ortho* to each other on the aromatic ring. In addition, cinnamic-based dihydroxyphenolic acids, such as caffeic, ferulic, sinapic, and *p*-coumaric acids, have greater antioxidant activity than dihydroxy derivatives of benzoic acid (Fig. 4) such as *p*-hydroxybenzoic, vanillic, syringic, and 3,4-dihydroxybenzoic acids (Marinova and Yanishieva,1994).

V. ROSEMARY EXTRACTS

Although a wide variety of plant materials and their extracts have been studied for antioxidant activity, rosemary and sage have received the great-

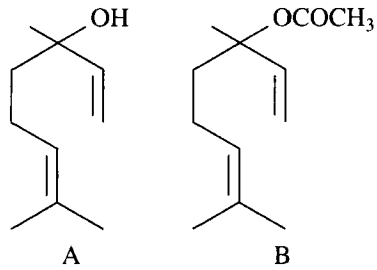

FIG. 3. Structures of linalool (A) and linalyl acetate (B).

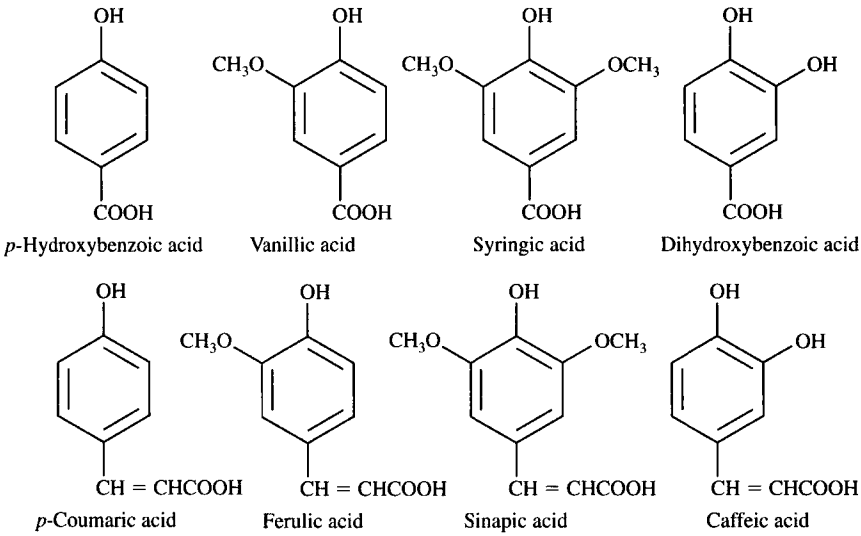

FIG. 4. Structures of cinnamic acid based phenolic acids (upper row) and dihydroxy derivatives of benzoic acid (lower row).

est level of attention. The first use of an extract of rosemary leaves as an antioxidant was reported by Rac and Ostric-Matijasevic in 1955 (as cited by Chen *et al.*, 1992). Berner and Jacobson (as cited by Chen *et al.*, 1992) obtained a patent in 1973 for the production of an antioxidant extract from rosemary using oil as the solvent. Chang *et al.* (1977) reported a process for the extraction of rosemary and sage, followed by vacuum steam distillation in an edible oil or fat to obtain a colorless, odorless natural antioxidant. Today rosemary is being commercially exploited and sage is being developed as a source of antioxidants.

At present, rosemary extracts are available as the original colored and flavored extract that can be produced in either a lipid-soluble or a water-soluble form (Duxbury, 1989). In addition a decolorized, deodorized product is available (Tsimidou and Boskou, 1994). The process for production of an alcoholic extract of rosemary was described by Loliger (1989). The process involves an initial steam distillation of the rosemary leaves to remove the essential oils. The stripped leaves are then extracted with food grade alcohol. Bracco *et al.* (1982) described the production of an extraction process using peanut oil as the solvent. This process involved extracting the ground rosemary leaves with oil followed by micronization, heat treatment, and molecular distillation. The condensate from the molecular distillation contains the antioxidants. This process produced an odorless and colorless system.

More recently another technique, supercritical carbon dioxide extraction, has been used to produce extracts of rosemary and sage. This process has been reported to extract more than 60% of the phenolic diterpenes from rosemary and sage. Carnosic acid is the major (77%) component within this extract (Gerard *et al.,* 1995). Supercritical carbon dioxide extraction has the advantage of not using excessive heat (Djarmati *et al.,* 1991), which affects the production of artifacts in the rosemary and sage extracts (Hall and Cuppett, 1997).

The development of commercialized rosemary extracts was based on a great deal of research beginning with work by Chang *et al.* (1977) who extracted rosemary and sage with a series of solvents: hexane, benzene, ethyl ether, chloroform, ethylene dichloride, dioxane, and methanol. Resultant extracts were then tested, at a 0.02% level, in a prime steam lard system held at 60°C in the dark. These researchers found that the greatest antioxidant activity was located in the methanol extract of rosemary. The methanol extract was further purified and the resultant fraction was tested for activity in potato chips fried in sunflower oil and held at 60°C in the dark for 60 days. Chang *et al.* (1977) found the purified fraction from the methanol extract of rosemary had outstanding antioxidant activity; however, they did not identify the active compound(s) at that time.

Research has been conducted to evaluate the effectiveness of rosemary extracts on the stability of a variety of food systems. MacNeil *et al.* (1973) found that a rosemary extract at a 0.01% level was as effective as a polyphosphate (0.5%) treatment; but a 0.05% level of the extract was needed to be equivalent to a mixture (0.075%) of BHT and citric acid in a cooked mechanically deboned poultry meat system stored at 3°C for 11 days.

Barbut *et al.* (1985) studied the effectiveness of a rosemary oleoresin (RO) in turkey breakfast sausages composed of 25% mechanically deboned meat. These reseachers found that RO was as effective as the combination of BHA/BHT/citric acid in suppressing oxidative rancidity. In addition, it was found that rosemary-treated samples did not produce the same profile of volatile compounds.

Lai *et al.* (1991) studied the effectiveness of an oleoresin of rosemary (OR) alone or in combination with sodium tripolyphosphate (STPP) vs STPP plus TBHQ in controlling lipid oxidation in restructured chicken nuggets during refrigerated and frozen storage. Lipid oxidation was monitored by TBARS, sensory evaluation, and chromatographic analysis. TBARS and sensory data showed that STPP/OR was comparable to STPP/TBHQ in preventing WOF. STPP or OR alone was less effective than the STPP/OR combination in both studies. STPP/OR and STPP/TBHQ prevented oxidation of polyunsaturated fatty acids during frozen storage.

Stoick *et al.* (1991) studied the oxidative stability of restructured beef steaks processed with OR, TBHQ, and STPP; they found that the addition of the OR gave no benefit over STPP. The OR/STPP combination was equivalent to the TBHQ/STPP treatment in preventing oxidation.

Liu *et al.* (1992) studied the effectiveness of both a water-soluble rosemary extract and an oil-soluble OR in combination with STPP vs TBHQ and STPP in controlling lipid oxidation in restructured pork steaks. They found OR, either water-soluble or oil-soluble in combination with STPP, did not increase lipid stability over STPP alone.

Boyd *et al.* (1993) studied the effectiveness of TBHQ, ascorbic acid, and rosemary extract in inhibiting oxidation in cooked frozen fish flakes. These researchers found that the combination of TBHQ with ascorbic acid, was the most effective followed by a mixture of TBHQ, ascorbic acid, and rosemary extract; rosemary extract alone was third in activity.

VI. ISOLATION AND IDENTIFICATION OF ROSEMARY COMPOUNDS

Concurrent with the evaluation of rosemary extracts as antioxidants to inhibit lipid oxidation in food systems, research was also focused on isolating, identifying, and testing the active compounds contained in the extracts. Wu *et al.* (1982) reported the fractionation and identification of urosolic acid and carnosol from a methanol extract of rosemary. They found that urosolic acid was not an effective antioxidant in prime steam lard, but carnosol was more effective than BHT. Note that carnosol had been previously isolated and identified by Breiskorn *et al.* (1964).

Other active compounds have been identified from rosemary. Nakatani and Inatani (1981) identified rosmanol and carnosol (Fig. 5) and showed that both were more effective than α-tocopherol, BHT, and BHA using an active oxygen method (AOM). They also reported that rosmanol (Fig. 5) had greater antioxidant activity than carnosol. Inatani *et al.* (1982) reported a "new" antioxidant phenolic diterpene from rosemary that in reality was a rediscovery of rosmanol and carnosol. Nakatani and Inatani (1983) reported the isolation of rosmadial (Fig. 5) from rosemary. Inatani *et al.* (1983) reported the antioxidant effectiveness of rosmanol, rosmadial, carnosol, and their derivatives, including diacetylcarnosol, four methyl derivatives of carnosol, triacetylrosmanol, and two monomethyl derivatives of rosmanol. Inatani *et al.* (1983) also studied two flavones, 5-hydroxy-7,4′-dimethyl flavone and 5,4′-dihydroxy-7-methyl flavone. Three tests were used for antioxidant activity: ferric thiocyanate (FeCN), TBARS, and AOM. When tested by the AOM, carnosol and rosmanol (0.02% each) had greater activity

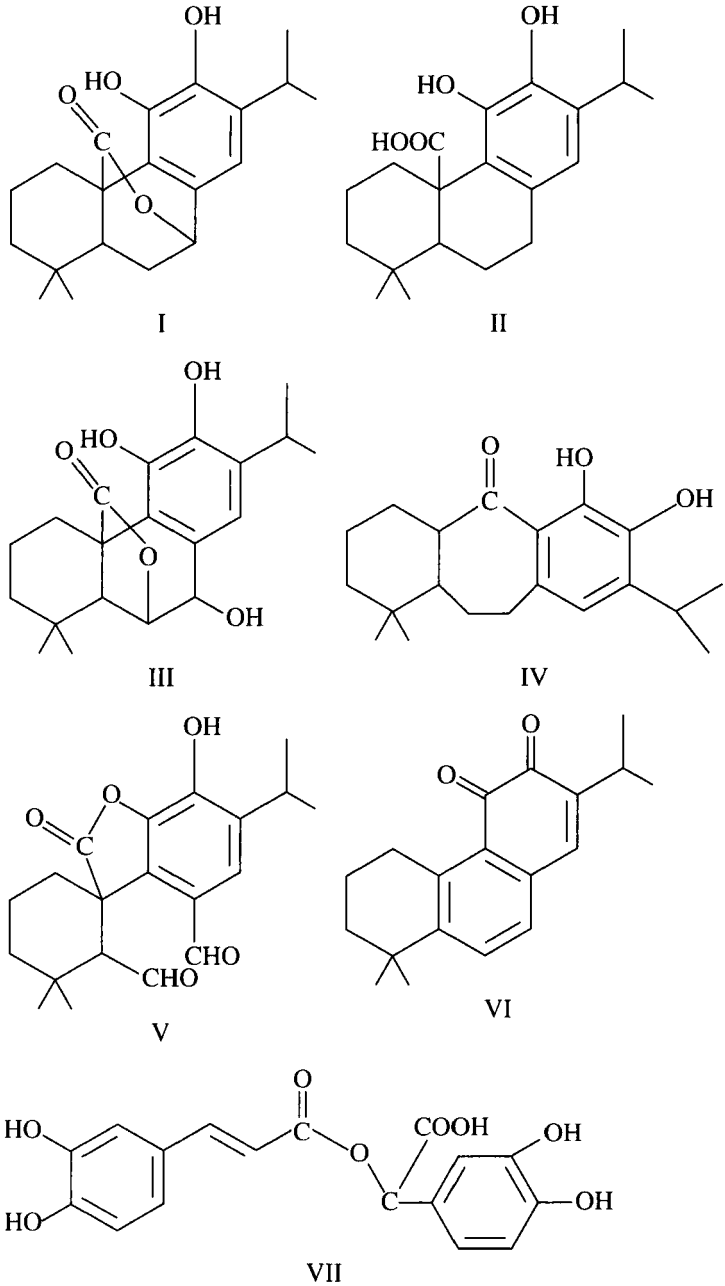

FIG. 5. Antioxidant compounds identified from rosemary and sage: carnosol (I), carnosic acid (II), rosmanol (III), rosmaridiphenol (IV), rosmadial (V), rosmariquinone (VI), and rosmarinic acid (VII).

than α-tocopherol, BHA, and BHT, and at 0.01% rosmanol was more active than carnosol (0.01%) while carnosol was comparable to BHA. In the FeCN test, carnosol and rosmanol were equivalent but were slightly less active than BHT, and rosmadial was moderately active. In the TBARS test rosmanol and carnosol were equivalent to BHT and slightly better than tocopherol, and rosmadial was moderately active. The derivatives, triacetyl- and dimethylrosmanol, showed weak activity in FeCN and TBARS tests, but monomethylated derivatives were as strong as rosmanol at a 0.02%.

Rosmarinic acid (RA) (Fig. 5) was reported by Gerhardt and Schroter (1983) to be the second most frequently occurring caffeic acid ester, following chlorogenic acid, and to have antioxidant activity equivalent to that of caffeic acid. Gerhardt and Schroter (1983) detected RA in balm, rosemary, sage, thyme, oregano, marjoram, savory, peppermint, and for the first time in basil, and the levels detected ranged from 0.07 to 0.84%. However, no correlation between antioxidant activity of the herb and its RA content was found. Houlihan *et al.* (1984) reported the isolation and identification of rosmaridiphenol (Fig. 5), a unique compound having a seven carbon ring in its structure. They also found rosmaridphenol to be more active than BHA in lard and only equivalent to BHT in this test system. Shahidi and Naczk (1995) reported that rosmaridiphenol (0.02%) had activity equivalent to BHT (0.02%) in steamed lard. Nakatani and Inatani (1984) isolated and identified isorosmanol and epirosmanol from rosemary. When tested, using AOM and FeCN methodologies, both compounds showed high activity in both lard and linoleic acid; in lard they were four times more active than BHA and BHT. Houlihan *et al.* (1985) isolated and identified rosmariquinone (Fig. 5), which was found to be superior to BHA and equivalent to BHT in controlling the oxidation of a lard system. Rosmariquinone had also been isolated from *Salvia militorrhiza* Bunge by Hayashi *et al.* (1970). These researchers named the compound militrone.

VII. COMPOUND ACTIVITIES

Brieskorn and Domling (1969) first reported on the antioxidant activity of carnosic acid and carnosol compared to BHT in inhibiting O_2 uptake of methyllinolenate using a Barcroft-Warburg apparatus. Data showed that carnosic acid and carnosol were as effective as BHT and that their effectiveness was concentration dependent.

Chen *et al.* (1992) investigated methanol, acetone, and hexane extracts of rosemary as well as a bleached methanol rosemary extract for their ability to inhibit lipid oxidation (Rancimat) and lipoxygenase. The extracts were analyzed and found to contain carnosol, carnosic acid, and urosolic

acid. The hexane extract (4.2% yield) contained 100.3 mg/g carnosic acid, 16.4 mg/g carnosol, and 13.7 mg/g urosolic acid; the methanol extract (26% yield) contained only a trace of carnosic acid, 24.1 mg/g carnosol, and 76.6 mg/g urosolic acid; and the acetone extract (13.8% yield) contained 58.4 mg/g carnosic acid, 36.5 mg/g carnosol, and 88.5 mg/g urosolic acid. Bleached methanol extract (5% yield) contained trace amounts of carnosic acid, 42.7 mg/g carnosol, and 180 mg/g urosolic acid. When tested in lard using a Rancimat system it was found that 0.02% carnosol and carnosic acid were superior to BHA and BHT; urosolic acid had minimal activity. The hexane and acetone extracts had better activity than BHA and BHT, but the two methanol extracts were equivalent to BHA and BHT. When tested for inhibition of lipoxygenase, urosolic acid was an effective inhibitor followed by carnosol and carnosic acid. Within the extracts tested, the bleached methanol was best at inhibiting lipoxygenase followed by methanol, acetone, and hexane extracts.

Richheimer *et al.* (1996) studied the activity of carnosol, carnosic acid, 12-methoxycarnosic acid, 7-methoxyrosmanol, rosmanol, and 7-epimethoxyrosmanol vs BHT, BHA, and TBHQ in soybean oil. These researchers also quantitated the level(s) of these compounds in rosemary leaves. Carnosic acid was found to be more potent than BHT and BHA but less potent than TBHQ. Rosemary leaves contained between 2 and 3% carnosic acid and small amounts of 12-methoxycarnosic acid and carnosol.

Relative antioxidant activity levels were reported as follows (Richheimer *et al.,* 1996): With carnosic acid assigned a level of 1, carnosol was at 0.44, 7-epimethoxyrosmanol was at 0.42, and 12-methoxycarnosic acid was at 0.1. These values were in agreement with those of Chen *et al.* (1992) who reported that when added to lard at the 200 ppm level, carnosic acid was about 1.2 times more active than carnosol in the Rancimat test. Relative to synthetic controls, carnosic acid had approximately 7 times the activity of BHT and BHA and a little less than half the activity of TBHQ (Chen *et al.,* 1992). The low level of activity of 12-methoxycarnosic acid found by Richheimer *et al.* (1996) was thought to be due to its lack of the two *ortho* phenolic groups adjacent to the isopropyl group. Brieskorn and Domling (1969) had noted that the activity of carnosol and carnosic acid was due to the cooperation of their two *ortho* phenolic groups with their isopropyl group.

Cuvelier *et al.* (1994) isolated six major antioxidant compounds from a oleoresin of sage (*S. officinalis*): rosmanol, epirosmanol, carnosol, rosmadial, carnosic acid, and methylcarnosate. The researchers questioned the source of methylcarnosate since it was not found in all extracts. To determine the source of the methylcarnosate, the researchers tested the stability of carnosic acid and showed that when carnosic acid in a methanol solution

was monitored over time (temperature not given) there was a gradual loss of carnosic acid and increases in carnosol, methylcarnosate, and rosmadial, and in that order of concentration.

Frankel *et al.* (1996) studied carnosol, carnosic acid, and rosmarinic acid for their ability to inhibit hydroperoxide decomposition in tocopherol-stripped corn (bulk) oil and in a corresponding corn oil-in-water emulsion. In the bulk oil system carnosic acid and rosmarinic acid were more active than carnosol; however, in the emulsion (pH 4.8–5.0) system, carnosol and carnosic acid were more active than rosmarinic acid. When the effect of pH (4.0, 5.0, and 7.0) of the emulsion system on activity of the three compounds was investigated, it was found that carnosol and carnosic acid were more active at pH 4–5 than at pH 7. The difference in activity at different pHs was attributed to the concept that at the lower pHs the compounds may be more stable and/or may have better reducing capacity while at the higher pH they may be partitioning in such a way as to be lost from the phase interface. The effect of pH on the activity of rosmarinic acid was that it had minimal activity at pH 5 and no activity at pH 4 or 7.

The antioxidant activity of rosemary and sage extracts has been shown to be due to the presence of carnosic acid and carnosol, with carnosic acid being the more potent of the two compounds (Chen *et al.*, 1992; Schwarz and Ternes, 1992; Cuvelier *et al.*, 1994; Richheimer *et al.*, 1996). However, carnosic acid has also been reported to be unstable and artifact compounds can be produced during extract production or during isolation and analysis of the active compounds from rosemary and sage (Hall and Cuppett, 1997). Conversion of carnosic acid to carnosol has been shown to occur via the formation of a semiquinone–quinone intermediate (Fig. 6). However, the conversion of carnosic acid to rosmanol is not a direct conversion, as proposed by Wenkert *et al.* (1965) (Fig. 7), but requires carnosol as an intermediate (Fig. 8), which then converts to rosmanol (Gonzalez *et al.*, 1992).

VIII. ROSEMARY SYNERGISM(S) AND HEAT STABILITIES

The potential for rosemary extracts to have synergism with other known antioxidants, such as tocopherol, has been investigated, and conflicting results have been reported. Wada and Fang (1992) evaluated the antioxidant effectiveness of α-tocopherol (AT) (0.05%) and rosemary extract (RE) (0.02%), alone or in combination vs BHA, in a sardine oil model system and in frozen-crushed fish meat. The AT–RE mixture had the strongest activity [as measured by TBARS and peroxide value (PV)]; it delayed the onset of rancidity 5 days longer than the individual compounds and this

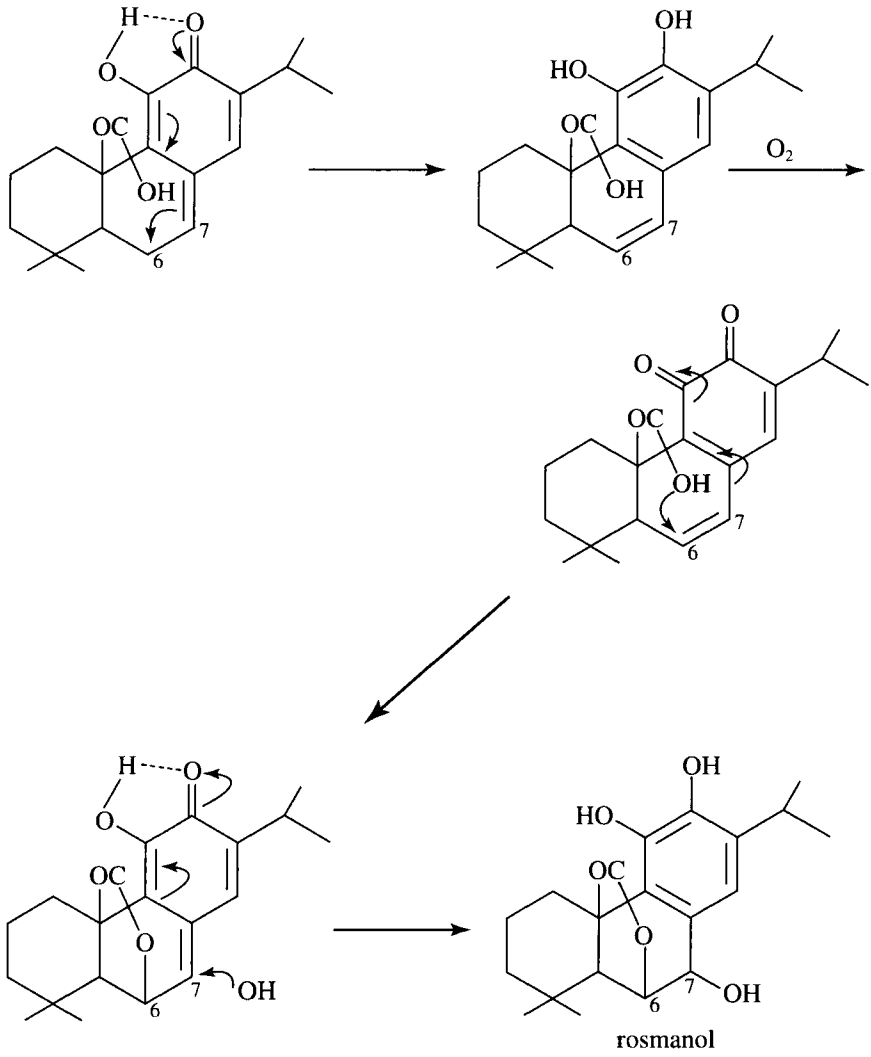

FIG. 6. Oxidation mechanism involved in conversion of carnosic acid to rosmanol as proposed by Wenkert *et al.* (1965).

level of activity was comparable to that of BHA. The AT content remained 5 days longer when used in combination with RE than when used alone.

In contrast to the findings of Wada and Fang (1992), Wong *et al.* (1995) evaluated the antioxidant effectiveness of α-tocopherol, a rosemary extract, and a sage extract, alone and in combination, in a cooked beef homogenate.

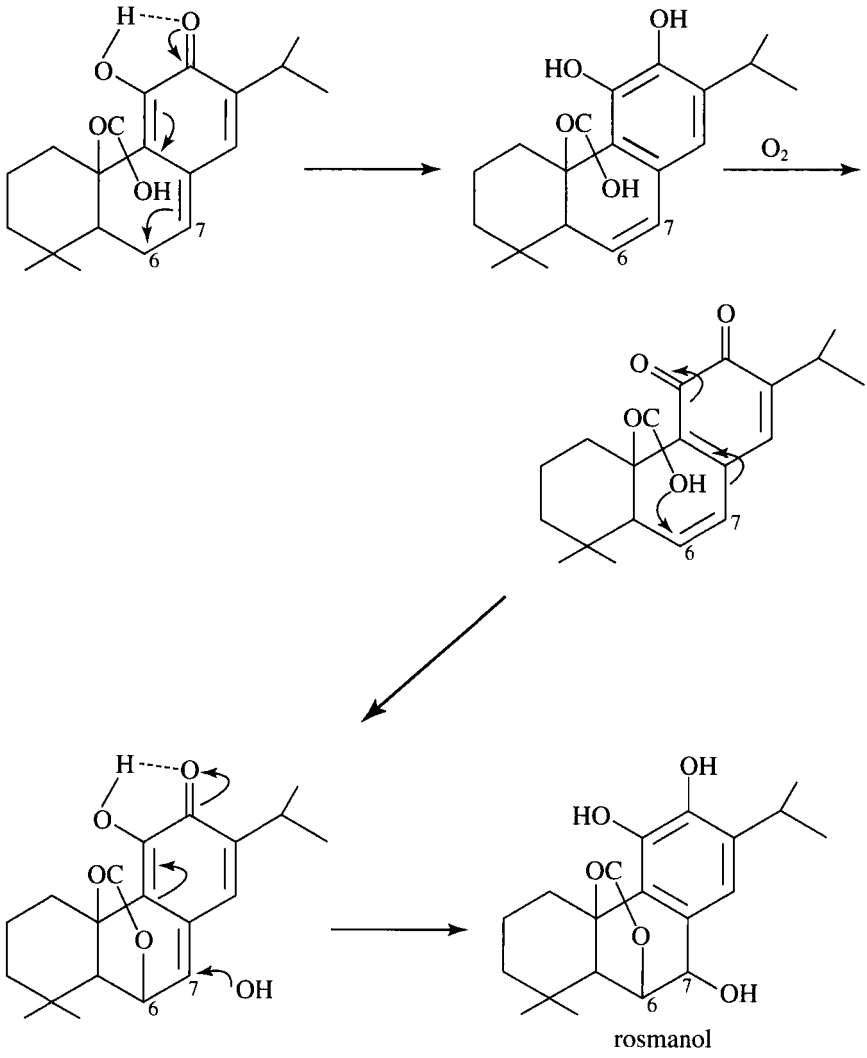

FIG. 7. Proposed mechanism for conversion of carnosol to rosmanol (Gonzalez *et al.*, 1992).

Their results showed that the combination of the herb extracts with tocopherol were comparable to the activity exhibited by the tocopherol alone, indicating that no synergism was occurring.

In another study Fang and Wada (1993) evaluated the antioxidant activity of AT and RE, alone or in combination, in Fe^{2+}/ascorbic acid and hemoprotein catalyzed oxidation in a sardine oil or bonito dark muscle model system. Results showed that the mixture of AT and RE extended the induction

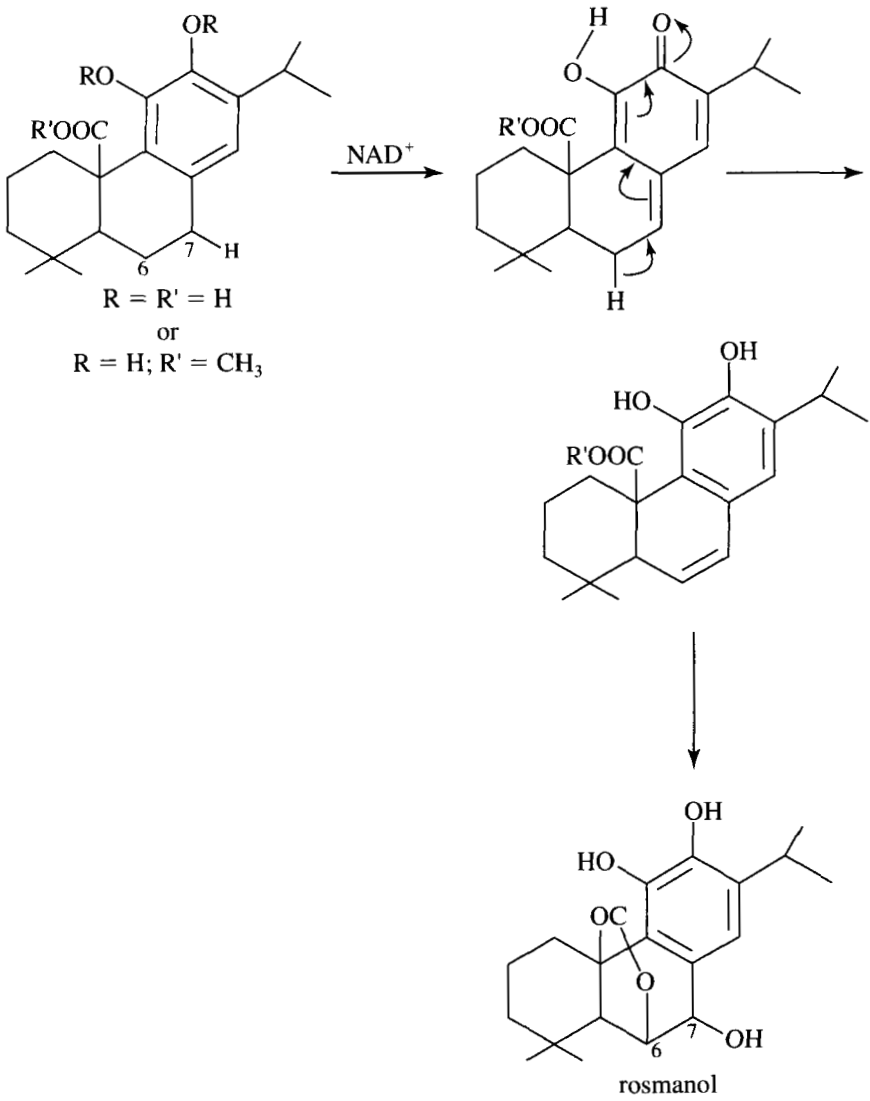

FIG. 8. Proposed mechanism for conversion of carnosic acid to rosmanol via 6,7-dehydrocarnosic acid (Gonzalez *et al.*, 1992).

period of the sardine oil oxidation catalyzed by Fe^{2+}/ascorbic acid for 10 and 16 days longer than AT or RE alone, respectively. In addition the AT lifetime in the mixture treatment in the oil system was 10 days longer than when used alone. In the dark muscle system, again the mixture of AT and RE showed greater activity than AT or RE alone.

These researchers (Fang and Wada, 1993) theorized that the mechansim for the synergism between RE and AT was that AT acts as a radical scavenger, combining with free radicals and stabilizing them. RE acts to regenerate the AT by H donation. When the RE was depleted, that is, totally oxidized, then the AT began to oxidize and was lost from the system.

Synergism between rosmariquinone (RQ) and mixed tocopherols in both auotoxidation and photo-sensitized oxidation of stripped soybean oil was investigated by Hall (1996). Data showed that the mixture of RQ (200 ppm) with tocopherols (825 ppm) extended the induction period to reach a PV of 20 meq/kg in a stripped soybean oil undergoing autoxidation to over 200 hr as opposed to RQ alone (75 hr) or tocopherols alone (50 hr), indicating a strong synergism. It was found that during autoxidation RQ acted to spare the tocopherol content of the oxidized oil. In photo-sensitized oxidation, the combination of RQ and tocopherols did not show synergism in controlling oxidation. The differences in activity of the mixture of RQ and tocopherols was attributed to the fact that in autoxidation RQ had the ability to spare the loss of tocopherol, probably by donation of hydrogens to regenerate the tocopherol. However, no such regenerating effect was found in the photo-sensitized system, indicating that tocopherol was being oxidized by singlet oxygen to the tocopheryl quinone, which could not be regenerated (Hall, 1996).

The mechanisms/structure–activities of the active compounds that have been identified in rosemary and sage have been reviewed by Hall and Cuppett (1997) and evidence is that carnososic acid, carnosol, rosmanol, rosmarinic acid, and rosmariquinone act primarily as hydrogen donors. Aruoma *et al.* (1992) reported that carnosic acid and carnosol showed the ability to chelate iron and were effective radical scavengers of peroxy radicals.

The heat stability of rosemary extracts has been studied. Wada and Fang (1992) reported that when AT and RE were tested, alone or in combination, at a higher temperature (60 vs 30°C) in the same system there was an increase in the rate of oxidation and RE showed no activity, having the same induction period as the control at the higher temperature. These resarchers indicated that this lack of activity could be due to instability of RE at the higher temperature or to an increased interaction between the free radicals and the antioxidant compounds of RE. In addition there was a weakened synergism between AT and RE at the higher temperature.

Gray *et al.* (1988) reported on the antioxidant activity of a rosemary extract that had been heated. When a RO was heated under vacuum to either 204° or 260°C, the samples at 204°C maintained activity for 18 hr of heating and the samples at 260°C maintained activity at the end of 1 hr of heating. However, when the RO was heated in open vials at 204°C, there

was a gradual loss of activity; after 4 hr of heating only 40% of the original level of activity remained.

Schwarz *et al.* (1992) evaluated the heat stability of diterpenes found in rosemary extracts under three sets of conditions: heating in lard at (1) 100°C over time (up to 90 hr); or (2) at temperatures between 100 and 170°C for 10 hr; and (3) exposed to steam at 200°C and a pressure of <1 mbar for 2 hr. Compounds that were monitored included rosmanol, epirosmanol, 7-methylepirosmanol, and carnosol. In the constant 100°C system there was a continual degradation of the four compounds and an appearance of four unidentified substances that were described as *de novo* synthesis products of the degradation process. During the 90-hr test period at 100°C, rosmanol was reduced from 11.4% to 1.0% of the diphenolic compounds; epirosmanol was reduced from 4% of the compounds to not being present at 90 hr; 7-methylepirosmanol was also absent at 90 hr after starting at 8% in the initial sample; and carnosol went from 78% of the initial content to 5% after 90 hr of heating at 100°C.

In samples heated for 10 hr at temperatures between 100 and 170°C, the four identified compounds, rosmanol, epirosmanol, 7-methylepirosmanol, and carnosol, were monitored as a group and it was found that as the level of heat increased there was a concomitant decrease in the concentration of these compounds. The greatest level of degradation occurred at 170°C where there was a decrease from accounting for 67% of the active components at Time 0 to accounting for only 2% at 10 hr. Similarly, in the steam-heated system, the four compounds were studied as a group and it was found that after 4 hr of heating there was a reduction from 66 to 18% of the diphenolic compound content in the test samples. Even with this reduction of diphenolic compound content, the test sample still exhibited antioxidant activity, but at a much reduced level.

Richheimer *et al.* (1996) noted that most commercial rosemary extracts are deodorized and bleached; both of these processes can involve heating, which converts carnosic acid to carnosol. In addition, bleaching involves the use of activated charcoal, which can convert carnosic acid to carnosol. Richheimer *et al.* (1996) found that when carnosic acid or carnosol was heated in methanol, 7-methylrosmanol and 7-methylepirosmanol were formed with 7-methylrosmanol being the primary product.

IX. HEALTH IMPLICATIONS

Beckstrom-Sternberg and Duke (1994) summarized the number of active compounds in 27 spices/herbs and the diseases they have been reported to impact. Rather than reproducing this listing, the goal here is to show the

wide range of activities of four of the plants receiving attention for use in food systems: rosemary, sage, thyme, and oregano. The following indicates the potential health benefit of spices and herbs and the number of active compounds (in parentheses) that had been identified in each at the time of publication. *Cancer prevention*: oregano (38), rosemary (26), sage (13), and thyme (5). *Antiseptic*: rosemary (14), oregano (11), sage (5), and thyme (3). *Antiinflammatory*: oregano (21), rosemary (15), and sage (6). *Fungicide*: oregano (10), rosemary (8), and sage (4). *Bactericide*: oregano (19), rosemary (19), sage (6), and thyme (5). *Spasmolytic*: oregano (16), rosemary (14), sage (5), and thyme (5). *Antiviral*: oregano (11), rosemary (10), and thyme (3). *Choleretic*: oregano (11). *Sedative*: rosemary (6). *Anesthetic*: rosemary (6). *Antiaggregant*: thyme (2). *Antitumor*: rosemary (9) and sage (7). *Hypocholesterolemic*: sage (4). *Hepatoprotective*: rosemary (6). *Hypotensive*: oregano (7). *Analgesic*: rosemary (7), oregano (6), and thyme (2). *Antidepressant*: thyme (2). *Antiasthmatic*: sage (4). *Antihistaminic*: oregano (6). *Diuretic*: oregano (6) and rosemary (6). *Acetylcholinesterase* and *Antilipoperoxidant*: rosemary (5 and 3, respectively). *Antihepatotoxic*: sage (5) and rosemary (3). *Anti-Crohn's*: thyme (2). *Antiacne, Antiatherosclerotic, Anticold, Antidandruff, Anti-insomniac,* and *Antiosteoporotic*: thyme (2–3). *Antioxidant*: oregano (14), rosemary (12), sage (7), and thyme (4).

Rosemary has been suggested as a potential treatment for Alzheimer's disease because of its anticholinesterase activity; Alzheimer's may result from the degradation of acetylcholine, a necessary neurotransmitter. Rosemary has been shown to contain five compounds capable of inhibiting acetylcholinesterase: carvacrol, fenchone, limoene, thymol, and 1,8-cineole (Beckstrom-Sternberg and Duke, 1994).

The value of rosemary, sage, thyme, and oregano in the medical arena is just beginning to be defined by researchers. Information on the biological activity of rosemary and some of its active compounds has begun to appear in the literature over the past 5 years.

Huang and Ferraro (1992) reported strong inhibitory activity of rosemary extract against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation, ornithine decarboxylase (ODC) activity, and tumor promotion. The major component of this extract, carnosol, demonstrated activity similar to the rosemary extract.

Ho *et al.* (1994) reported that topical application of a methanol extract of rosemary, prepared using the procedure of Wu *et al.* (1982), 5 min prior to treatment with [^{3}H]BP (benzopyrene) to the backs of CD-1 mice inhibited the formation of [^{3}H]BP–metabolite–DNA adduct in the mouse epidermis. There was a dose response to the level of rosemary extract used. Rosemary was shown to inhibit skin tumor initiation by BP in CD-1 mice. Rosemary inhibited skin tumor initiation by 7,12-dimethylbenz[*a*]anthra-

cene (DMBA) by up to 92%. In addition, it was also shown that rosemary extract was not a tumor initiator on mouse skin. Topical application to the backs of mice of rosemary extract in combination with 5 nmol TPA inhibited the TPA induction of ODC; this occurred in a dose-dependent manner. Inhibition ranged from 66 to 100%. Rosemary also inhibited TPA-induced skin inflammation and hyperplasia.

Huang *et al.* (1994) reported that a methanol extract of rosemary was studied for its effects on mouse skin tumor initiation and promotion by benzo[*a*]pyrene (BAP) and DMBA topically applied to mouse skin. The rosemary extract was found to inhibit the covalent binding of BAP and inhibited tumor promotion by BAP, TPA, and DMBA. There was also an inhibition of ODC, TPA-induced inflammmation, arachidonic acid-induced inflammation, TPA-induced hyperplasia, and TPA-induced tumor promotion. Topical application of carnosol or ursolic acid inhibited TPA-induced ear inflammation, ODC activity, and tumor promotion. They also showed activity toward inhibiting DMBA-intiated tumorigenesis.

Orally administered rosemary extract (1%) was found to reduce the incidence of DMBA-induced mammary cancer (Singletary and Nelshoppen, 1991); these authors also reported that rosemary extract inhibited *in vivo* binding of DMBA to mammary epitheila cell DNA and the formation of DNA adducts. Ho *et al.* (1994) found that dietary (2%) rosemary extract inhibited BP-induced forestomach and lung tumors in A/J mice as well as azoxymethane-induced colon tumors in Sencar treated interperitoneally with DMBA.

Munnunni *et al.* (1992) reported that rosemary extract that had carnosol and carnosic acid as its primary ingredients was shown to inhibit *tert*-butyl hydroperoxide and H_2O_2 induced mutagenesis of Ames tester strain TA102.

Offord *et al.* (1995) reported that in animal model systems, a rosemary extract was found to inhibit the initiation and tumor promotion phases of carcinogensis. This study focused on the mechanisms of rosemary components for blocking caricogenesis induced by the pro-carcinogen BAP in human bronchial cells (BEAS-2B) by monitoring the inhibition of cytochrome P450 (CYP) 1A1 activity and CYP 1A1 mRNA expression and DNA adduct formation. Results showed that when rosemary and its components, carnosol and carnosic acid, at 6 μg/ml or equivalent levels of pure compounds,respectively, were incubated with human bronchial cells in the presence of 1.5 μm BAP there was an 80% reduction in DNA adduct formation, a 50% reduction in CYP 1A1 mRNA expression and a 70–90% inhibition of CYP 1A1 activity. It was conjectured that the observed reduction in DNA adduct formation by the rosemary/rosemary components was due to the inhibition of BAP conversion to its ultimate active metabolites. Carnosol was found to affect the expression of the phase II enzyme

glutathione-*S*-transferase (GST), which is known to detoxify the proximate carcinogen BAP metabolite. When BEAS-2B cells were treated with 1 mg/ml carnosol for 24 hr there was a three- to fourfold induction of GST π mRNA; carnosol also induced the expression of another important phase II enzyme, NAD(P)H:quinone reductase. Offord *et al.* (1995) concluded that rosemary and carnosol show promise in chemoprevention due to their ability to decrease activation and increase detoxification of BAP, an important human carcinogen.

Carnosol and urosolic acid inhibited TPA-induced ear edema, ODC activity, and tumor promotion in mouse skin. Topical application of carnosol together with TPA (5 nmol) to backs of mice previously initiated with DMBA reduced the formation of skin tumors. Urosolic acid has also been shown to reduce TPA-induced skin tumors on mice skin (Ho *et al.*, 1994).

Chan *et al.* (1995) found that carnosol inhibited lipopolysaccharide and interferon-γ induced nitrite production by mouse peritoneal calls by greater than 50%. Paris *et al.* (1993) studied carnosol, carnosic acid, and rosmanol, and their semisynthetic derivatives (7-*O*-methylrosmanol, 7-*O*-ethylrosmanol, and 11,12-*o*-*o*-dimethylcarnosol) for their potential to inhibit HIV protease. Carnosic acid showed the strongest activity against the HIV-1 protease; carnosic acid also showed potential for inhibiting the HIV-1 replication.

Cuppett *et al.* (1996) showed that RQ was found to inhibit the mutagenicity of *N*-methyl-*N*-nitrosourea and *N*-nitrobis(2-oxopropyl)amine in V79 cells. Complete inhibition was obtained with a dose level of 250 n*M*, indicating that RQ has power as a chemopreventative agent. RQ also inhibited ODC activity in mouse skin treated with TPA.

Haragguchi *et al.* (1995) showed that carnosol, carnosic acid, rosmanol, and epirosmanol inhibited superoxide anion production in an xanthine/xanthine oxidase system. Carnosic acid was the most effective inhibitor of superoxide anion generation by xanthine oxidase, reducing production by 70% at 9 μM concentration.

These compounds were also tested for their ability to reduce microsomal and mitochondrial lipid peroxidation induced by NADH or NADPH oxidation (Haragguchi *et al.*, 1995). Rosmanol and epirosmanol were the most effective in reducing microsomal oxidation, producing complete inhibition of oxidation at the 3 μM level. Carnosol and carnosic acid reduced oxidation by 88 and 53%, respectively. Carnosic acid and carnosol achieved full inhibition at 9 μM while rosmanol and epirosmanol required a concentration of 30 μM to achieve complete inhibition of mitochondrial oxidation. Carnosic acid was effective in protecting human erythrocytes against oxidative hemolysis (Haragguchi *et al.*, 1995).

Rutherford *et al.* (1992) found that ethanolic extracts of sage inhibited *t*-butylcyclophosphoro[^{35}S]thionate binding to the chloride channel of rat cerebrocortical membranes. Active compounds were shown to be carnosol and carnosic acid.

Although much data have been and are being collected on the potential of compounds from the Labiatae plant family, additional data are needed to fully evaluate the range of antioxidant activities, including possible synergisms with each other and other established natural antioxidants, for instance, tocopherols and ascorbic acid.

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