

## Antioxidant Properties of Selected Spices Used in Iranian Cuisine and Their Efficacy in Preventing Lipid Peroxidation in Meat Sausages

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### ABSTRACT

This study involved analyzing antioxidant properties of spices, namely clove (*Syzygium aromaticum* Linn.), cinnamon (*Cinnamomum verum*, syn *C. zeylanicum* Blume) and sumac (*Rhus coriaria* L.) and exploring their efficacy in a food system. In the first part of the study, the antioxidant activity of water extract of spices treated at different temperatures were measured by three different methods namely, DPPH, reducing power and phosphomolybdenum complex assay and the results were compared to control unheated sample. In the second part, these three spices were incorporated in home-made sausages separately and stored for a month under refrigeration during which Free Fatty Acids (FFA) and Peroxide Value (PV) were measured every 10th day. The results were compared with control sample (without spices). Results showed that, extracts of heat treated spices had higher antioxidant activity in comparison with the control sample and sausages with added spices showed lower levels of FFA and PV in comparison with control samples. It can be concluded that spices possessed antioxidant properties and heat treatment had a positive effect on antioxidant activity and they were effective in delaying oxidation in sausages, hence they can be used as sources of natural antioxidants.

**Keywords:** Cinnamon, Clove, Free fatty acids, Peroxide value, Sumac.

### INTRODUCTION

In food systems, lipid and protein oxidation are the most important chemical changes causing deterioration in meat quality during cooking and storage leading to rancidity. Lipid oxidation causes development of off-odours and off-flavours whereas muscle pigment oxidation has been reported to negatively affect colour, appearance and acceptability of meat (Kolakowska, 2003). Application of antioxidants to fresh and processed meat prevents oxidative rancidity, delays development of off-flavours and improves colour stability in fresh and processed meat (Shahidi, 2000).

Antioxidants are essential in retarding oxidation processes in fats and oils (Diaz-

Reinoso *et al.*, 2006). Natural antioxidants, with potential nutritional and therapeutic value, can be used for increasing the stability of foods by preventing lipid peroxidation (Zia-ur-Rehman, 2006). Interest in natural sources of antioxidants like herbs and spices which are used as flavoring agents in many dishes has increased of late, and they can be used as natural food preservatives (Benkeblia, 2007). Spices and aromatic herbs exhibit strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants and hence this interests both industry and research scientists alike. Some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. are the substances that impart these properties to

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spices and function as antioxidant components and preservative agents in foods (Calucci *et al.*, 2003).

Many studies have reported that several plants or their phenolic extracts such as rosemary, potato peel, tea catechins, sage, cloudberry, beetroot, willow herb, rapeseed and pine bark (Karpinska *et al.*, 2000; McCarthy *et al.*, 2001; Kanatt *et al.*, 2005; He and Shahidi, 1997; Tang *et al.*, 2001; El-Alim *et al.*, 1999; Rey *et al.*, 2005; Vuorela *et al.*, 2005) are capable of being used as meat lipid antioxidants. Spices are good sources of natural antioxidants. They are mainly good sources of polyphenols which have high antioxidant activities. Increasing trend to use healthy ingredients and natural ways of preventing diseases are contributing to the increased use of spices.

The use of spices for culinary purposes is a universal practice. While some spices are common and associated with different ethnic cultures all over the world, others are specific to certain geographical locations; for example, cloves (*Syzygium aromaticum* Linn.), the dried flower buds of a tree of myrtle family (*Myrtaceae*), are aromatic, stimulant and carminative and used for gastric irritation and dyspepsia. Clove has a strong intensive fragrance and a fiery burning taste. Cloves are also known to promote enzymatic flow and boost digestive functioning (Selvan, 2003). Sesquiterpenes, found in clove, are said to be potential anticarcinogenic agents (Zheng and Kenney, 1992). The antioxidant activity in clove is reported to be due to the presence of components such as  $\beta$ -caryophyllene, eugenol and acetyleugenol (Lee and Shibamoto, 2001).

Cinnamon (*Cinnamomum zeylanicum* Blume) from the family of *Lauraceae* is taken from the inner bark of cinnamon trees. It prevents nervous tension, improves complexion and memory. It is also a stimulant, relieves flatulence and is a diuretic. It is said to be an effective remedy for common cold (Selvan, 2003). Sumac (*Rhus coriaria* L.) a member of the *Anacardiaceae* family, grows in the region

extending from the Canary Islands over the Mediterranean coast line to Iran and Afghanistan. Sumac has strong antioxidant and antibacterial activity owing to the presence of tannins (hydrolysable gallatannins), flavonoids (fustin, fisetin, sulfuretin, butein, quercetin and mycetin) and essential oil (Lauk *et al.*, 1998; Nimri *et al.*, 1999; Digrak *et al.*, 2001; Sađdic and Özcan, 2003; Zalacain *et al.*, 2003; Nasar-Abbas and Halkman, 2004; Son *et al.*, 2005). Medicinal uses of the spice have been indicated for digestion and bowel problems and it is said to have diuretic and antipyretic properties.

Since spices can be used for their antioxidant function in foods, the present study was planned to investigate the antioxidant properties of heat treated spices and their efficacy in delaying lipid peroxidation in stored meat sausages.

## MATERIALS AND METHODS

### Materials

Whole spices namely clove (*Syzygium aromaticum* Linn.) and cinnamon (*Cinnamomum verum*, *syn C. zeylanicum* Blume) were purchased from a supermarket in Mysore, India in a clean and packed form. Sumac (*Rhus coriaria* L.) was purchased from a supermarket in Iran. All the chemicals purchased were of AR grade from Sd Fine chemicals, and Qualigens Ltd., Mumbai, India. DPPH (1,1-Diphenyl-2-Picrylhydrazyl) was procured from Sigma Chemical Co., USA. Glass double distilled water was used for all analyses.

### Antioxidant Activity of Spices

Fifty grams of moisture free whole spices were powdered in a grinder to pass through a 40-mesh sieve and stored in airtight PET (PolyEthylene Terephthalate) bottles under refrigeration at 4°C until further use.

Preparation of Water Extract : One gram of powdered spice sample was suspended in 100 ml distilled water and shaken at a constant rotating speed of 150 rpm min<sup>-1</sup> in a water bath shaker at different temperatures (room temperature, 40, 60, 80 and 100°C) for 30 minutes. It was centrifuged for 10min at 4,000×g and filtered through Whatman No. 1 filter paper to get a clear extract. Fresh extract was used for each experiment. Antioxidant activity of all extracts were measured by standard techniques such as reducing power, DPPH, and phosphomolybdenum complex assay as given below. All extracts were made in duplicate and all analyses were conducted in triplicate. Hence, the results represent average and standard deviations of six determinations for all samples. Since ascorbic acid is water soluble antioxidant, it was used as a standard for comparison.

Determination of Antioxidant Activity by Reducing Power: The Reducing Power (RP) was determined by the method of Oyaizu (1986). One ml of extract was mixed with 2.5 ml of phosphate buffer (2 mol l<sup>-1</sup>, pH 6.6) and 2.5 ml of potassium ferricyanide (K<sub>3</sub>Fe(CN)<sub>6</sub>; 1%), and incubated at 50°C for 20 minutes. Thereafter, 2.5 ml of 10% trichloro acetic acid was added to the mixture, which was then centrifuged at 4,000×g for 10 minutes. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl<sub>3</sub> (0.1%) and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increasing reducing power. Water extract without reagents was used as negative control.

Determination of Antioxidant Activity by DPPH: Free Radical Scavenging Activity (FRSA) was measured using the method of Shimada *et al.* (1992). A solution of 0.1 mmol of DPPH in methanol was prepared, 1 ml of this solution was mixed with 3 ml of extract, and after 30 minutes of incubation the absorbance was measured at 517 nm. Water extract without reagent was used as negative control. The DPPH concentration

in the reaction medium was calculated from the following formula:

Percent free radical scavenging activity= (Control Optical density (OD) - sample OD/Control OD) × 100.

Determination of Antioxidants by Total Antioxidant Capacity: This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex in acidic pH (Prieto *et al.*, 1999). An aliquot of 0.1 ml of sample solution was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 minutes. After the samples had cooled to room temperature; the absorbance was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the sample, and it was incubated under the same conditions as the rest of the samples. The antioxidant activity of extracts was expressed as equivalents of ascorbic acid.

Effect of Adding Spices on Lipid Peroxidation in Stored Homemade Sausages: The effect of clove, cinnamon and sumac was investigated on lipid peroxidation in stored homemade sausages. Three sets of products were made using a combination of spices. The first set was based on nitrite, which is used as a curing salt. While nitrites themselves have preserving and antioxidant functions, the effect of addition of spices on nitrite based products was studied. The second set of product was based on garlic as it is a very common ingredient used in meat sausages. For the third set of products both nitrite and garlic along with other spices were used. Each set of variable had its own control in addition to a control with an antioxidant additive namely, Butylated Hydroxyl Anisole (BHA). All products were stored under refrigeration and analyzed for Free Fatty Acids (FFA) and Peroxide Value (PV) on 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> days of storage.

**Table 1.** Effect of heating on antioxidant activity of water extracts of spices determined using reducing power assay. <sup>a</sup>

| Temperature (°C) | Absorbance at 700 nm |             |             |                          | ANOVA (F ratio)           |            |
|------------------|----------------------|-------------|-------------|--------------------------|---------------------------|------------|
| Clove            | Concentration (mg)   |             |             |                          | -                         |            |
|                  | 0.2                  | 0.4         | 0.6         | 0.8                      | -                         |            |
|                  | Control              | 0.323±0.010 | 0.504±0.045 | 0.701±0.007              | 0.844±0.042 <sup>a</sup>  | 163.913*** |
|                  | 40                   | 0.334±0.002 | 0.549±0.004 | 0.738±0.037              | 0.900±0.011 <sup>b</sup>  |            |
|                  | 60                   | 0.344±0.003 | 0.559±0.020 | 0.758±0.029              | 0.949±0.006 <sup>c</sup>  |            |
| 80               | 0.389±0.019          | 0.637±0.009 | 0.847±0.007 | 1.060±0.545 <sup>d</sup> |                           |            |
| 100              | 0.410±0.011          | 0.714±0.049 | 1.014±0.022 | 1.304±0.029 <sup>e</sup> |                           |            |
| Cinnamon         | Concentration (mg)   |             |             |                          |                           |            |
|                  | 2.0                  | 4.0         | 6.0         | 8.0                      |                           |            |
|                  | Control              | 0.655±0.003 | 1.105±0.021 | 1.548±0.077              | 2.019±0.003 <sup>a</sup>  | 137.340*** |
|                  | 40                   | 0.672±0.012 | 1.159±0.022 | 1.625±0.047              | 2.076±0.070 <sup>bc</sup> |            |
|                  | 60                   | 0.71 ±0.017 | 1.198±0.027 | 1.65±0.032               | 2.097±0.017 <sup>c</sup>  |            |
| 80               | 0.826±0.029          | 1.386±0.064 | 1.904±0.036 | 2.41±0.061 <sup>d</sup>  |                           |            |
| 100              | 0.885±0.011          | 1.441±0.014 | 1.951±0.007 | 2.453±0.016 <sup>d</sup> |                           |            |
| Sumac            | Concentration (mg)   |             |             |                          |                           |            |
|                  | 1.0                  | 2.0         | 3.0         | 4.0                      |                           |            |
|                  | Control              | 0.799±0.039 | 1.304±0.082 | 1.788±0.041              | 2.312±0.046 <sup>ab</sup> | 146.874*** |
|                  | 40                   | 0.825±0.049 | 1.309±0.082 | 1.792±0.047              | 2.335±0.075 <sup>b</sup>  |            |
|                  | 60                   | 0.883±0.046 | 1.426±0.023 | 1.940±0.012              | 2.453±0.037 <sup>c</sup>  |            |
| 80               | 0.939±0.014          | 1.523±0.047 | 2.185±0.107 | 2.820±0.058 <sup>d</sup> |                           |            |
| 100              | 0.996±0.058          | 1.674±0.017 | 2.253±0.095 | 2.875±0.049 <sup>d</sup> |                           |            |

<sup>a</sup> Values represent average±standard deviation of six determinations. Figures in a column with different superscript are significantly different from each other as determined by Duncan's Multiple Range Test. \*\*\*:  $P \leq 0.001$ .

The details of products made are provided below:

Ingredients: The ingredients used were: meat, 7,000 g (beef); soya granules, 875 g; bread crumbs, 875 g; refined wheat flour, 200 g; black pepper, 30 g; salt, 200 g and water, 500 g.

### Procedure

Meat was washed and minced in a meat grinder thoroughly. Mincing was continued with the addition of soy granules, bread crumbs, wheat flour and salt sequentially until it was a homogenized mass. The dough was divided into three parts and each part had its own control. Each part was divided in four batches: control, control with 0.5%

clove, control with 0.5% cinnamon and control with 0.5% sumac. Part one contained 0.01% nitrite, part two contained 3% garlic and part three included 3% garlic and 0.01% nitrite.

0.02% of BHA was added to the dough which had garlic without nitrite. Each part was mixed thoroughly and homogenized. It was then passed to the casing through the special machine for preparing homemade sausages, boiled for 45 minutes, then dried, after that the samples were stored in the refrigerator at 4°C for 1 month. All samples were analyzed for FFA and PV by AOCS methods initially and every 10<sup>th</sup> day up to 30 days (AOCS, 2000).

For determination of free fatty acids and peroxide value, as indicative of keeping quality, the oil from the fried products was

extracted in petroleum ether (boiling point, 60- 80°C) and subjected to analysis using following procedures.

**Free Fatty Acids:** The number of milligrams of potassium hydroxide required to neutralize the free acid present in one gram of the oil or fat under prescribed conditions is indicative of free fatty acids. A known weight of sample was mixed with 50ml of neutralized alcohol with phenolphthalein indicator and shaken well. It was heated to 50°C and then titrated against standardized 0.1N NaOH to pink color as the end point. The titre value was noted and expressed as percent free fatty acids.

**Peroxide Value:** The peroxide value of an oil or fat is the amount of peroxides present and expressed as milliequivalents of peroxides per kg of oil. The sample is dissolved in the solvent, treated with potassium iodide and the iodine liberated by the peroxides present in rancid fat or oil is titrated with sodium thiosulphate solution to obtain the peroxide value.

### Statistical Analysis

The results of the study were subjected to statistical analysis to determine the significant difference between the control and thermally treated samples using one way analysis of variance (for the data obtained for the highest concentration of sample). Further, a post test, Duncan's Multiple Range Test was used to determine the significance between individual samples with the probability level fixed to  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Antioxidant Activity of Spices

The antioxidant activity of spices determined using Reducing Power assay (RP) is presented in Table 1. In all three spices the antioxidant activity was dose-dependent and exhibited an increase with

increasing concentration. Among them, clove exhibited the highest antioxidant capacity, wherein the RP could be measured at a very low concentration of spice, i.e., 0.2-0.8 mg. Untreated spice (0.2-0.8 mg) exhibited an absorbance of (0.323-0.844) at 700 nm. On application of heat treatment to water extract of clove at 40, 60, 80 and 100°C, the absorbances were 0.334-0.900, 0.344-0.949, 0.389-1.06 and 0.41-1.304 respectively. Application of ANOVA revealed highly significant differences among all samples. The increase in antioxidant activity was significant at 40°C in comparison with the control and increased with a rise in temperature. There were significant differences between the control and samples heated at higher temperatures at all levels as evident by Duncan's multiple range test. In cinnamon also, it was dose dependent, and increased with increasing concentration (Table 1). An absorbance of 0.655 to 2.019 was exhibited by untreated spice (2.0-8.0 mg) at 700nm which increased gradually with heating to 0.672-2.076; 0.71-2.097; 0.826-2.410 and 0.885-2.453, respectively. ANOVA revealed significant differences among all samples whereas individual comparisons indicated that heat treated samples had significantly higher antioxidant activity in comparison with the control. Between degrees of heat treatment, samples heated at 40 and 60°C, and 80 and 100°C were similar in antioxidant activity, though all were significantly higher than the unheated sample.

The antioxidant activity of untreated sumac with RP presented in Table 1 showed an absorbance of 0.799-2.312 for concentrations ranging from 1.0-4.0 mg at 700 nm. The water extracted sample treated at varying temperatures showed a range of the antioxidant activity as follows: 0.825-2.335 (40°C); 0.885-2.453 (60°C); 0.939-2.820 (80°C), and 0.996-2.875 (100°C), respectively. The sample treated at 40°C showed similar result as the control, however, the antioxidant activity of samples which were extracted in higher temperatures

**Table 2.** Effect of thermal treatment on free radical scavenging activity of spices (%).<sup>a</sup>

| Temperature (°C)        | Concentration (mg) |             |             |                          | ANOVA (F ratio) |
|-------------------------|--------------------|-------------|-------------|--------------------------|-----------------|
| Ascorbic acid (Control) | 0.01               | 0.02        | 0.03        | 0.04                     | -               |
|                         | 27.7±0.043         | 61.0± 0.008 | 82.6± 0.003 | 97.2± 0.001              | -               |
| Clove                   | Concentration (mg) |             |             |                          |                 |
|                         | 0.05               | 0.1         | 0.15        | 0.2                      |                 |
| Control                 | 31.0±3.730         | 50.2±2.670  | 65.5±2.730  | 77.0±2.843 <sup>a</sup>  | 29.086***       |
| 40                      | 36.0±3.130         | 53.9±1.186  | 68.8±0.792  | 80.8±0.837 <sup>a</sup>  |                 |
| 60                      | 38.2±3.950         | 55.5±3.400  | 69.5±1.332  | 80.9±0.354 <sup>a</sup>  |                 |
| 80                      | 41.8±1.041         | 57.2±0.746  | 70.0±0.606  | 83.0±0.769 <sup>b</sup>  |                 |
| 100                     | 42.2±0.712         | 57.6±2.319  | 71.8±0.602  | 85.5±0.581 <sup>c</sup>  |                 |
| Cinnamon                | Concentration (mg) |             |             |                          |                 |
|                         | 0.05               | 0.1         | 0.15        | 0.2                      |                 |
| Control                 | 11.0±0.462         | 20.6±0.910  | 29.7±0.806  | 39.0±0.691 <sup>a</sup>  | 163.218***      |
| 40                      | 12.0±0.737         | 25.7±0.845  | 39.7±1.268  | 53.8±0.708 <sup>b</sup>  |                 |
| 60                      | 21.5 ±3.606        | 34.7±2.304  | 50.6±2.010  | 64.0±4.597 <sup>c</sup>  |                 |
| 80                      | 23.0±2.457         | 37.0±1.109  | 51.5±3.450  | 66.0±2.931 <sup>c</sup>  |                 |
| 100                     | 24.0±2.140         | 38.0±2.015  | 53.4±0.141  | 70.8±0.383 <sup>d</sup>  |                 |
| Sumac                   | Concentration (mg) |             |             |                          |                 |
|                         | 0.1                | 0.2         | 0.3         | 0.4                      |                 |
| Control                 | 40.4±0.763         | 57.8±1.261  | 72.2±2.422  | 83.8±0.554 <sup>a</sup>  | 292.411***      |
| 40                      | 40.9±0.806         | 58.7±0.513  | 73.0±0.998  | 84.7±0.893 <sup>bc</sup> |                 |
| 60                      | 43.4±0.691         | 60.0±0.476  | 74.4±0.836  | 85.0±1.127 <sup>c</sup>  |                 |
| 80                      | 45.3±0.662         | 65.0±0.321  | 80.0±1.110  | 93.3±0.508 <sup>d</sup>  |                 |
| 100                     | 52.0±0.897         | 70.3±0.437  | 84.6±1.153  | 94.0±0.344 <sup>d</sup>  |                 |

<sup>a</sup> Values represent average±standard deviation of six determinations. Figures in a column with different superscript are significantly different from each other as determined by Duncan's Multiple Range Test. \*\*\*:  $P \leq 0.001$ .

were significantly higher than the control sample. Similar activities were observed at 80 and 100°C.

The antioxidant activity of clove determined using DPPH assay (Table 2) could be measured at low concentration of 0.05–0.2 mg indicating that clove is a powerful antioxidant. As the concentration increased, a higher antioxidant activity was seen. Unheated spice used as control sample showed 31–77% Free Radical Scavenging Activity (FRSA). The water extract of spice treated at different temperatures showed higher antioxidant activity in comparison with the control. It was 36–80.8% at 40°C ( $P \leq 0.05$ ) and 38.2–80.9, 41.8–83% ( $P \leq 0.01$ ) and 42.2–85.5% ( $P \leq 0.001$ ) at 60, 80 and 100°C respectively. The wet heat treated sample exhibited significantly higher

antioxidant activity at all temperatures in relation to the control as indicated by both ANOVA and Duncan's multiple range test.

The FRSA of cinnamon was 11% at 0.05 mg and increased to 39.0% at 0.2 mg concentration. Heat treated samples exhibited significantly higher activities than unheated samples as revealed by ANOVA. A further post test showed three levels of difference at: (i) 40°C, (ii) 60 and 80°C, and (iii) 100°C. The FRSA of sumac, which showed antioxidant activity at concentration of 0.1–0.4 mg ranged between 40.4–83.8% for the control sample. For heat treated samples, though showing higher FRSA, significant differences were observed at two levels of: (i) 40 and 60°C, and (ii) 80 and 100°C as seen from the application of the post test.

The total antioxidant activity of clove was measured using phospho-molybdenum assay. The water extracts of spice were heated to different degrees of temperatures and analyzed for total antioxidant activity. As can be seen from Table 3, the antioxidant activity increased slowly with the rise in temperature; a small increase was seen at 40°C and a large increase was seen between 60 and 100°C, the values being 266.55 to 287.08 mg ascorbic acid g<sup>-1</sup> of sample. Similarly, cinnamon extracted in water at different temperatures showed that the total antioxidant activity increased with an increase in temperature and the difference was highly significant between the heat treated samples and control ( $P \leq 0.001$ ). The control sample exhibited total antioxidant activity of 120.65 mmol of ascorbic acid g<sup>-1</sup> of sample, and this value increased to 218.24 mmol of ascorbic acid g<sup>-1</sup> for the sample heated to 100°C. Water extracted sumac treated to different temperatures also showed that thermal treatments increased the activity as seen for other spices. The control sample had a value of 61.50 mmol ascorbic acid g<sup>-1</sup> of samples, when the samples were heated to 40 and 60°C, 20 and 27.7% increase in activity was obtained, respectively. On further heating of the sample to 80 and 100°C, a remarkable increase of 47.5 and 54.3% was seen. Heat treated samples showed highly significant differences in comparison with the control ( $P \leq 0.001$ ) at all temperatures. For clove and sumac, similar levels of increase were seen in samples

heated to 80 and 100°C. An increase in total antioxidant capacity of pepper and cumin on wet heat treatment has also been shown in a related study (Nikousaleh and Prakash, 2009).

The thermal treatments of clove increased antioxidant capacity as observed in different wet heat treated samples analyzed by the three different methods. While the effect was lesser at 40°C, at higher temperatures a larger significance was observed ( $P < 0.01$  and  $P < 0.001$ ). Also in comparison with other spices, clove showed a higher antioxidant activity as evident by a lower concentration of samples used. For cinnamon, the results were similar to those seen in clove. With all three methods, wet heat treated samples showed significantly higher antioxidant activity in comparison with control unheated sample ( $P < 0.001$ ) with the exception of samples heated to 40°C using RP assay. Our earlier work with these spices had shown that dry heat treatment also increased their antioxidant activity (Nikousaleh and Prakash, 2008; 2014). According to Murica *et al.* (2004) the high antioxidant activity of cinnamon is due to components such as cinnamaldehyde, eugenol, and  $\beta$ -caryophyllene. Tomaino *et al.* (2005) investigated the influence of heating on antioxidant activity of selected spice essential oils. All spice essential oils exhibited good radical scavenging activity as determined by DPPH assay. The antioxidant activity of essential oils of clove and cinnamon as reported by them at room

**Table 3.** Total antioxidant capacity of dry heat-treated spices ( $\mu$ moles of ascorbic acid g<sup>-1</sup>).<sup>a</sup>

| Spice    | Temperature (°C)              |                               |                               |                               |                               | ANOVA<br>F Ratio |
|----------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|------------------|
|          | Control                       | 40                            | 60                            | 80                            | 100                           |                  |
| Clove    | 150.29<br>± 5.38 <sup>a</sup> | 177.75<br>± 7.36 <sup>b</sup> | 266.55<br>± 8.34 <sup>c</sup> | 281.31<br>± 8.56 <sup>d</sup> | 287.08<br>± 3.95 <sup>d</sup> | 505.494***       |
| Cinnamon | 120.65<br>± 4.66 <sup>a</sup> | 134.92<br>± 2.22 <sup>b</sup> | 181.45<br>± 5.66 <sup>c</sup> | 201.60<br>± 3.95 <sup>d</sup> | 218.24<br>± 8.71 <sup>c</sup> | 355.438***       |
| Sumac    | 61.50<br>± 0.97 <sup>a</sup>  | 73.86<br>± 3.08 <sup>b</sup>  | 78.53<br>± 1.48 <sup>c</sup>  | 90.70<br>± 3.42 <sup>d</sup>  | 94.89<br>± 2.73 <sup>d</sup>  | 186.335***       |

<sup>a</sup> Values represent average ± standard deviation of six determinations. Figures in a row with different superscript are significantly different from each other as determined by Duncan's Multiple Range Test. Comparative value for ascorbic acid: 134216/0.01 mg. \*\*\*:  $P \leq 0.001$ .



temperature was 34.8 (0.026  $\mu\text{l ml}^{-1}$ ) and 55.3% (0.065  $\mu\text{l ml}^{-1}$ ), respectively. Khatun *et al.* (2006) studied the free radical scavenging activity of several spices such as clove, allspice, cinnamon, nutmeg, mustard, cumin, ginger, fennel, fenugreek, black pepper, red pepper, mace, coriander, turmeric, cardamom and white pepper for different heating times (1, 3 and 6 hours) at 100°C. The highest activity was exhibited by clove followed by allspice and cinnamon. Results indicated that, after heating, DPPH and peroxy radical-scavenging activities as well as the total phenol content increased in most of the spices. Some spices such as black pepper, red pepper and turmeric showed a distinct increase in the activities.

Heat treated samples of sumac also showed higher activity in comparison with the control. With RP method, this was marginally higher than the control sample ( $P < 0.05$ ). Use of Sumac as a natural antioxidant has been recommended by Koşar *et al.* (2007) who investigated the antioxidant activity of fractionated methanolic extract of sumac. They found that the fractions of extract rich in anthocyanins and hydrolysable tannins had high antioxidant activities. It can be

concluded from the overall results of the study that all three samples exhibited a higher antioxidant potential with all the three assays in comparison with the control sample.

### Effect of Adding Spices on Lipid Peroxidation in Stored Meat Sausages

The lipid peroxidation in stored sausages was monitored by periodical estimation of FFA and PV and the results presented in Table 4 show that on addition of BHA, the FFA at the end of one month storage was 0.35%. In the sample with nitrite, it increased to 0.304% after one month from initial value of 0.135%. However, in spice added products in this category, the increase ranged from 0.263 to 0.267, which was lower than the control value. With garlic also, the control product showed a higher value of 0.347% and it was lesser in spice added products. Similar trends were observed in products with both garlic and nitrite, wherein spice added products had a lesser level of FFA formation at the end of one month of storage.

The PV for BHA added sample was higher

**Table 4.** Effect of adding spices on free fatty acid (%) and peroxide value ( $\text{meq kg}^{-1}$  of fat) of stored meat sausages.

| Variations              | Free fatty acid |       |       |       | Peroxide value |      |      |      |
|-------------------------|-----------------|-------|-------|-------|----------------|------|------|------|
|                         | Days of storage |       |       |       |                |      |      |      |
|                         | 0               | 10    | 20    | 30    | 0              | 10   | 20   | 30   |
| + BHA                   | 0.129           | 0.213 | 0.294 | 0.350 | 3.3            | 3.3  | 4.0  | 5.0  |
| With nitrite            |                 |       |       |       |                |      |      |      |
| Control                 | 0.135           | 0.219 | 0.292 | 0.304 | 7.0            | 12.5 | 13.0 | 13.5 |
| + Cinnamon              | 0.136           | 0.213 | 0.258 | 0.267 | 6.8            | 12.5 | 14.3 | 14.7 |
| + Clove                 | 0.141           | 0.244 | 0.252 | 0.263 | 4.8            | 5.75 | 7.25 | 11.0 |
| + Sumac                 | 0.134           | 0.235 | 0.238 | 0.263 | 7.5            | 9.25 | 13.5 | 14.0 |
| With garlic             |                 |       |       |       |                |      |      |      |
| Control                 | 0.129           | 0.235 | 0.280 | 0.347 | 11.6           | 13.3 | 14.0 | 15.8 |
| + Cinnamon              | 0.130           | 0.235 | 0.235 | 0.252 | 6.4            | 6.5  | 11.0 | 13.0 |
| + Clove                 | 0.121           | 0.230 | 0.252 | 0.308 | 7.2            | 7.6  | 9.9  | 12.7 |
| + Sumac                 | 0.121           | 0.274 | 0.277 | 0.280 | 7.5            | 9.3  | 10.4 | 11.0 |
| With garlic and nitrite |                 |       |       |       |                |      |      |      |
| Control                 | 0.128           | 0.241 | 0.269 | 0.314 | 9.3            | 11.8 | 15.8 | 23.0 |
| + Cinnamon              | 0.125           | 0.258 | 0.274 | 0.280 | 6.0            | 10.3 | 12.5 | 18.8 |
| + Clove                 | 0.118           | 0.269 | 0.291 | 0.302 | 4.0            | 5.9  | 7.5  | 10.8 |
| + Sumac                 | 0.129           | 0.244 | 0.263 | 0.269 | 7.0            | 10.1 | 11.4 | 12.5 |



at the end of one month, the value being 5.0 meq kg<sup>-1</sup> of fat from the initial value of 3.3 meq kg<sup>-1</sup>. All other experimental products had a higher initial and final value for PV on day 30. In samples with nitrite the values ranged from 11.0 to 14.7 meq kg<sup>-1</sup> of fat. In products with garlic, the control had higher PV and addition of spice reduced the level of PV on storage. Similar trends were seen in products with both garlic and nitrite. Overall, it was seen that addition of spice was able to delay the lipid peroxidation in meat sausages.

Many literature studies report the use of spices for preventing the lipid peroxidation in meat products. Sindhu and Emilia (2006) in their study on the antioxidant activities of cinnamon bark extracts, through various in vitro models showed that cinnamon had a very good antioxidant activity. A study on the effect of paprika, garlic and salt on rancidity in dry sausages was carried out by Aguirrezábal *et al.* (2000). As per total FFA content and PV during the ripening period, antioxidant and prooxidant properties were exhibited by paprika and salt respectively. Moreover, the role of paprika and garlic in inhibiting lipid oxidation was as effective as the mixture of nitrate, nitrite and ascorbic acid. Juntachote *et al.* (2007) carried out a study on the antioxidative effect of dried holy basil and its ethanolic extracts on inhibition of lipid oxidation of cooked ground pork, during 14 days of refrigerated storage (5°C). Results showed that the addition of dried holy basil powder to cooked ground pork inhibited the lipid oxidation in a dose-dependent manner (0.35, 0.18 and 0.07%), however, its inhibition effect as dry powder was more than its ethanolic extract. Jayathilakan *et al.* (2007) in their study on the effect of natural antioxidants on the lipid stability of fluidised bed-dried mutton indicated that as an alternative to synthetic antioxidants, natural antioxidants, such as Maillard reaction products, spices and ascorbic acid could be used for controlling the lipid oxidation, and as a result could increase the stability of products.

Spices are used as flavoring agents in many ethnic cuisines all over the world. Therefore, this study aimed at investigating the antioxidant properties of selected spices used in Iranian cuisine and their efficacy in preventing lipid peroxidation in meat sausages. It can be concluded from the results of the present study that all spices investigated, namely, clove, cinnamon and sumac exhibited very good antioxidant activities which increased upon application of heat. The effect was more pronounced on application of temperature higher than 60°C. The overall assessment of the role of spices in preventing free fatty acids or peroxide formation in prepared sausages indicated that spices were able to exert a positive effect on the keeping quality of sausages. Among different sets namely, nitrite and garlic+ nitrite, clove added samples exhibited the lowest PV followed by sumac and cinnamon added samples on the 30<sup>th</sup> day of storage. For the set of products with garlic alone, sumac exhibited a low value. These results indicated that spices could prevent oxidation of fat in stored meat sausages to varied extents and they can be used as natural preservatives.

## REFERENCES

1. American Oil Chemists' Society (AOCS). 2000. Free Fatty Acids and Peroxide Value: Approved Methods of the American Association of Cereal Chemists, *J. Am. Oil Chem. Soc.*, **2**: 1-3.
2. Aguirrezábal, M. M., Mateo, J., Domínguez, M. C. and Zumalacárregui, J. M. 2000. The Effect of Paprika, Garlic and Salt on Rancidity in Dry Sausages. *Meat Sci.*, **54**: 77-81.
3. Benkeblia, N. 2007. Natural Biological Compounds and Food Preservation. *Food*, **1**: 111-245.
4. Calucci, L., Pinzono, C., Zandomenighi, M., Capocchi, A. 2003. Effects of Gamma-Irradiation on the Free Radical and Antioxidant Contents in Nine



- Aromatic Herbs and Spices. *J. Agric. Food. Chem.*, **51**: 927–934.
5. Diaz-Reinoso, B. Moure, A. Dominguez, H. and Parajo, J.C. 2006. Supercritical CO<sub>2</sub> Extraction and Purification of Compounds with Antioxidant Activity. *J. Agric. Food. Chem.*, **54**: 2441–2469.
  6. Digrak, M., Alma, M. H. and Ilcim, A. 2001. Antibacterial and Antifungal Activities of Turkish Medicinal Plants. *Pharm. Biol.*, **39**: 346-350.
  7. El-Alim, S.S.L.A., Lugasi, A. Hovari, J. and Dworschak, E. 1999. Culinary Herbs Inhibit Lipid Oxidation in Raw and Cooked Minced Meat Patties during Storage. *J. Sci. Food Agric.*, **79**: 277-285.
  8. He, Y. and Shahidi, F. 1997. Antioxidant Activity of Green Tea and Its Catechins in a Fish Meat Model System. *J. Agric. Food. Chem.*, **45**(11): 4262–4266.
  9. Jayathilakan, K., Sharma, G. K., Radhakrishna, K. and Bawa, A. S. 2007. Effect of Natural Antioxidants on the Lipid Stability of Fluidised Bed-dried Mutton. *Food Chem.*, **100**: 662–668.
  10. Juntachote, T., Berghofer, E., Siebenhandl, S. F. and Bauer, F. 2007. Antioxidative Effect of Added Dried Holy Basil and Its Ethanolic Extracts on Susceptibility of Cooked Ground Pork to Lipid Oxidation. *Food Chem.*, **100**(1): 129-135.
  11. Kanatt, S. R., Chander, R., Radhakrishna, P. and Sharma A. 2005. Potato Peel Extracts: A Natural Antioxidant for Retarding Lipid Peroxidation in Radiation Processed Lamb Meat. *J. Agric. Food. Chem.*, **53**(5): 1499-1504.
  12. Karpinska, M., Borowski, J. and Danowska-Oziewicz, M. 2000. Antioxidative Activity of Rosemary Extract in Lipid Fraction of Minced Meat Balls during Storage in a Freezer. *Nahrung*, **44**(1): 38-41.
  13. Khatun, M., Eguchi, S., Yamaguchi, T., Takamura, H. and Matoba, T. 2006. Effect of Thermal Treatment on Radical-scavenging Activity of Some Spices. *Food Sci. Technol.*, **12**(3): 178-185.
  14. Kolakowska, A. 2003. Lipid Oxidation in Food Systems. In: “*Chemical and Functional Properties of Food Lipids*”. CRC Press LLC, London.
  15. Kosar, M., Bozan, B., Temelli, F. and Baser, K. H. C. 2007. Antioxidant Activity and Phenolic Composition of Sumac (*Rhus coriaria* L.) Extracts. *Food Chem.*, **103**(3): 952-959.
  16. Lauk, L., Caccamo, F., Speciale, A. M., Tempera, G., Ragusa, S. and Pante, G. 1998. Antimicrobial Activity of *Rhus coriaria* L. Leaf Extracts. *Phytother. Res.*, **12**: 152-153.
  17. Lee, K. G. and Shibamoto, T. 2001. Antioxidant Property of Aroma Extract Isolated from Clove Buds [*Syzygium aromaticum* (L.) Merr. Et Perry]. *Food Chem.*, **74**: 443-448.
  18. McCarthy, T. L., Kerry, J. P., Kerry, J. F., Lynch, P. B. and Buckley, D. J. 2001. Assessment of the Antioxidant Potential of Natural Food and Plant Extracts in Fresh and Previously Frozen Pork Patties. *Meat Sci.*, **57**(2): 177-184.
  19. Murcia, M. A., Egea, I., Romojaro, F., Parras, P., Jimenez, A. M. and Martinez-Tome, M. 2004. Antioxidant Evaluation in Dessert Spices Compared with Common Food Additives: Influence of Irradiation Procedure. *J. Agric. Food Chem.*, **52**: 1872-1881.
  20. Nasar-Abbas, S. M. and Halkman, A. K. 2004. Inhibition of Some Foodborne Bacteria by Alcohol Extract of Sumac (*Rhus coriaria* L.). *J. Food Saf.*, **24**: 257-267.
  21. Nikousaleh A. and Prakash J. 2014. Effect of Microwave Heating and Roasting on the Antioxidant Properties of Cinnamon. *Adv. Food Sci.*, **36**(4): 177-183.
  22. Nikousaleh A. and Prakash J. 2008. Effect of Dry Heat Treatment of Six Spices on Antioxidant Activities of

- Their Water Extracts. *Food.*, **2(2)**: 139-144.
23. Nikousaleh A. and Prakash J. 2009. Antioxidant Activity of Water Extracts from Thermally Treated Black Pepper (*Piper nigrum*, Linn) and Cumin (*Cuminumcuminum*Linn.). *Indian J. Nutr. Diet.*, **46(9)**: 364-371.
24. Nimri, L.F., Meqdam, M. M. and Alkofahi, A. 1999. Antibacterial Activity of Jordanian Medicinal Plants. *Pharmacol. Biol.*, **37(3)**: 196-201.
25. Oyaizu, M. 1986. Studies on Product of Browning Reaction Produced from Glucose Amine. *Jpn. J. Nutr.*, **44**: 307-315.
26. Prieto, P., Pineda, M. and Aguilar, M.1999. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem.*, **269**: 337-341.
27. Rey, A.I., Hopia, A., Kivikari, R. and Kahkonen, M. 2005. Use of Natural Food/Plant Extracts: Cloudberry (*Rubus chamaemorus*), Beetroot (*Beta vulgaris* "Vulgaris") or Willow Herb (*Epilobium angustifolium*) to Reduce Lipid Oxidation of Cooked Pork Patties. *LWT Food Sci. Technol.*, **38**: 363-370.
28. Sađdic, O. and Özcan, M. 2003. Antibacterial Activity of Turkish Spices Hydrolysis. *Food Control*, **14**: 141-143
29. Selvan, M. T. 2003. Role of Spices in Medicine. *Indian J. Arecanut Spice. Medicinal Plant.*, **5**: 129-135.
30. Shahidi, F. 2000. Antioxidants in Food and Food Antioxidants. *Nahrung*, **44(3)**: 158-163.
31. Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative Properties of Xanthine on Autoxidation of Soybean Oil in Cyclodextrin Emulsion. *J. Agric. Food. Chem.*, **40**: 945-948.
32. Sindhu, M. and T. Emilia, A. 2006. Studies on the Antioxidant Activities of Cinnamon (*Cinnamo mumverum*) Bark Extracts, through Various *In vitro* Models. *Food Chem.*, **94**: 520-528.
33. Son, Y. O., Lee, K.Y., Lee, J. C., Jang, H.S., Kim, J. G., Jeon, Y. M. and Jang, Y. S. 2005. Selective Antiproliferative and Apoptotic Effects of Flavonoids Purified from *Rhusverniciflua* Stokes on Normal Versus Transformed Hepatic Cell Lines. *Toxicol. Lett.*, **155**: 115-125.
34. Tang, S., Sheehan, D., Buckley, D. J., Morrissey P. A. and Kerry J. P. 2001. Antioxidant Activity of Added Tea Catechins on Lipid Oxidation of Raw Minced Red Meat, Poultry and Fish Muscle. *Int. J. Food Sci. Technol.*, **36**: 685-692.
35. Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., Pasquale, A. De. and Saija, A. 2005. Influence of Heating on Antioxidant Activity and the Chemical Composition of Some Spice Essential Oils. *Food Chem.*, **89**: 549-554.
36. Vuorela, S., Salminen, H., Makela, M., Kivikari, R., Karonen, M. and Heinonen, M. 2005. Effect of Plant Phenolics on Protein and Lipid Oxidation in Cooked Pork Meat Patties. *J. Agric. Food. Chem.*, **53(22)**: 8492-8497.
37. Zalacain, A., Prodanov, M., Carmona, M. and Alonso, G. L. 2003. Optimization of Extraction and Identification of Gallotannins from Sumac Leaves. *Biosys. Eng.*, **84** (2): 211-216.
38. Zheng, G. Q. and Kenney, P. M. 1992. Sesquiterpenes from Clove (*Eugenia caryophyllata*) as Potential Anti Carcinogenic Agents. *J. Nat. Prod.*, **55**: 999-1003.
39. Zia-ur-Rehman, Z. U. 2006. Citrus Peel Extract: A Natural Source of Antioxidant. *Food Chem.*, **99(3)**: 450-454.



## خاصیت آنتی اکسیدانی ادویه جات انتخاب شده مورد مصرف در آشپزی ایرانی و تأثیر آنها در جلوگیری از پراکسیداسیون چربی در سوسیس گوشت

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### چکیده

این تحقیق شامل بررسی خواص آنتی اکسیدانی ادویه جاتی از قبیل میخک (*Syzygium arimaticum* Linn)، دارچین (*Cinnamomum verum*, syn *C. zeylanicum* Blume) و سماق (*Rhus coriaria* L.) و یافتن تأثیر آنها در سیستم غذایی می باشد. در مرحله نخست این تحقیق، فعالیت آنتی اکسیدانی عصاره آب ادویه جات که دردهای مختلف گرفته شده بود از طریق سه روش Reducing power, DPPH Phosphomolybdenum complex، اندازه گیری شد و نتایج با نمونه شاهد که تحت حرارت قرار نگرفته بود مقایسه گردید. در مرحله دوم، این سه ادویه به طور جداگانه به سوسیس خانگی اضافه شدند و نمونه ها به مدت یک ماه در یخچال نگهداری شدند. در طول این یک ماه هر ده روز یک بار Free fatty acids (FFA) and Peroxide value (PV) اندازه گیری شدند و نتایج با نمونه کنترل که فاقد این سه ادویه بودند مقایسه گردیدند. نتایج نشانگر فعالیت آنتی اکسیدانی بیشتر عصاره ادویه جات حرارت دیده در مقایسه با نمونه شاهد بوده و همچنین میزان FFA and PV نمونه سوسیس همراه ادویه کمتر از نمونه کنترل بود. می توان نتیجه گیری نمود که ادویه جات مورد آزمایش دارای خاصیت آنتی اکسیدانی بوده و حرارت تأثیر مثبتی بر روی فعالیت آنتی اکسیدانی آنها داشته است و در ضمن این ادویه جات موجب به تاخیر افتادن اکسیداسیون در سوسیس گشتند. بنابراین می توان از این ادویه جات به عنوان منبعی از آنتی اکسیدان طبیعی استفاده نمود.