Changes in the Tocopherol and Unsaturated Fatty Acid Constituents of Spices after Pasteurization with Superheated Steam

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Summary The following five spices were pasteurized with superheated steam, as given in parentheses: origanum (1 kg/cm², 132°C, 3.5 sec), laurel (0.5 kg/cm², 118°C, 3.5 sec), turmeric (2 kg/cm², 160°C, 5 sec), coriander (1 kg/cm², 140°C, 5 sec), and aniseed (1 kg/cm², 140°C 5 sec). The detected tocopherols and other constituents were as follows: origanum (α-, β-, γ-, δ-), laurel (α-, β-), turmeric (so little that no comparison could be made), coriander (almost no tocopherols), and aniseed (α-, β-, δ-). The tocopherols and unsaturated fatty acid constituents of origanum, laurel, aniseed and coriander underwent little change after pasteurization with superheated steam, indicating stability, which may be due to the synergistic effect of tocopherols and phenolic compounds present in these spices.

Key Words spices, tocopherol, unsaturated fatty acid, pasteurization with superheated steam, origanum, laurel, turmeric, coriander, aniseed

Spices usually have good keeping qualities, especially those having preservative characteristics such as cinnamon, nutmeg, cloves, allspice and pepper. Evaporation of volatile oils and infestation by insects are the principal causes of their deterioration. It has been established that many of the microorganisms found as contaminants contribute to the spoilage of food products of which such spices are ingredients. The gas-forming thermophilic bacteria, for example, even cause physical distortion of processed foods and/or their containers, in addition to the commonly found development of off-flavored products caused by the biological

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activity of the saprophytic microbes of contaminated spices, and also the discolo-
ration of cured meats and fresh sausages resulting from the action of oxidizing and
hydrogen sulfide-generating bacteria found in natural spices.

It has been determined that prolonged heat treatment of spices causes an
average loss of about 15% in flavor strength, accompanied by a lightening in color
in some cases and a darkening in others. Their treatment with such gases as
formaldehyde, carbon disulfide, carbon tetrachloride, chloropicrin and hydrocyanic
acid, while partially effective in some cases and wholly so in others, cannot be fully
dispensed with, though undesirable tastes and/or odors are often imparted to the
treated spices (1). In addition, it is not certain whether effective penetration of these
gases into large masses of spice materials occurs. Hence, the method of pasteur-
ization of spices with superheated steam may help to solve all these uncertainties and
it was therefore the objective of this study to determine the degree of changes in the
tocopherol and unsaturated fatty acid constituents of spices following the pasteur-
ization process.

MATERIALS AND METHODS

Spices. The countries where the spices were harvested were as follows: Greece
(origanum); India (turmeric); Morocco (coriander) and France (aniseed).

Pasteurization. The spices were pasteurized by means of a machine designed
by Kikkoman Company, Type KP-30 under the conditions given in parentheses:
origanum (1 kg/cm², 130°C, 3.5 sec), laurel (0.5 kg/cm², 120°C, 3.5 sec), turmeric
(2 kg/cm², 160°C, 5 sec), coriander (1 kg/cm², 140°C, 5 sec), and aniseed (1 kg/cm²,
140°C, 5 sec). The spices were placed in glass jars and sealed tightly after treatment,
transported to the laboratory and then kept at 4°C prior to analysis microbial
inspection of the spices was carried out by the ordinary method (2).

Sample preparation. The spices (10 g) were separately homogenized with a
Polytron PT 10/35 Kinematica GmbH homogenizer in a 300 ml mixture of
chloroform–methanol (2 : 1, v/v) following the method of Folch et al. (3). A saline
solution (0.9% NaCl) was added to the homogenized sample and the lower
chloroform layer was separated. The extract was mixed with 1 ml 6% ethanolic
pyrogallol and 0.5 ml 5 N KOH and then saponified at 60–70°C for 30 min. The
tocopherols were extracted with 15 ml hexane by sonic agitation for 5 min. To the
hexane extracts was added an internal standard (2,2,5,7,8-pentamethyl-6-
hydroxychroman), which was kindly supplied by Professor O. Igarashi of
Ochanomizu University, and the mixture was then subjected to HPLC.

The aqueous layer, after separating the tocopherol, was acidified with 1 N HCl
and extracted with 10 ml hexane by sonic agitation for 5 min for fatty acid analysis.
The hexane extracts were then washed with water and treated with anhydrous
sodium sulfate to remove any trace of water. Heptadecanoic acid was added as an
internal standard. The hexane layer was evaporated under a nitrogen stream. The
samples of fatty acid mixtures were esterified with BF₃-methanol (4) in a boiling
water bath for 10 min. After cooling, hexane and water were added and the upper layer was separated for GLC analysis.

Ten samples were analyzed by HPLC for each spice. The results were compared as to differences between the non-treated and treated samples. In the case of GLC samples, two determinations were made, and the average was taken as the final value.

**HPLC.** The chromatography was performed on a Hitachi 635-A HPLC apparatus with a Zorbax-Sil column (4.6 × 150 mm) with a mobile phase of 0.5% isopropyl alcohol in hexane (HPLC grade) at a flow rate of 2.0 ml/min. Twenty microliter samples were injected for each determination with an automatic sample injector (Atto SJ-1700-ASI). The tocopherols were detected by their absorbance at 294 nm with a UV-visible spectrophotometer, Hitachi Model 200-20. The average of ten determinations was taken as the final value.

**GLC.** A Shimadzu GC 7A with a flame ionization detector was fitted with a 3.1 m × 3.1 mm i.d. glass column containing 15% diethylene glycol succinate on Shimalite 60–80 mesh. The operating conditions were as follows: Column at 195°C for 32 min, followed by linear temperature programming at 2°C/min to 210°C; injector and detector at 250°C; nitrogen (carrier gas) flow, 50 ml/min. The peaks were recorded and determined with a Shimadzu Chromatopak (Model CR-1A) computerized recorder.

**RESULTS**

*Microbial inspection*

The microbial counts of the control and pasteurized spices are shown in Table 1. It can be seen that there is a remarkable decrease in the total plate count and in the coliforms in the treated samples.

*Tocopherol contents*

A comparison between the tocopherol constituents of control and pasteurized samples is shown in Table 2. In origanum, α-, β-, γ- and δ-tocopherols were detected. α-Tocopherol was the major constituent followed by δ-tocopherol. After pasteurization, α-tocopherol was reduced by about 10%, which is significantly different (p < 0.05). There were no appreciable changes in the other tocopherol constituents. In the case of laurel, α- and β-tocopherols were detected. Even after pasteurization, there were almost no changes at all in tocopherol content. There was a large amount of β-tocopherol in aniseed, and α- and δ-tocopherols were also present. Pasteurization with superheated steam caused no changes in the tocopherol constituents.

Turmeric and coriander contained little tocopherol. In both spices, traces of α-, β- and γ-tocopherols were detected, but δ-tocopherol was not detected at all.
Table 1. Microbiological inspection of spices.

| Spice     | Treatment conditions | Moisture content | Total plate count | Thermophile | Coliforms |
|-----------|----------------------|------------------|------------------|-------------|-----------|
|           | Pressure (kg/cm²)    | Temperature (°C) | Time (sec)       | (No/g)      | (No/g)    |
| Origanum  | 1.0                  | 132              | 3.5              | 2.0 × 10³   | 2.0 × 10² |
| Laurel    | 0.5                  | 118              | 3.5              | 2.0 × 10³   | 2.0 × 10² |
| Turmeric  | 2.0                  | 160              | 5                | 2.0 × 10³   | 1.2 × 10² |
| Coriander | 1.0                  | 140              | 5                | 3.0 × 10³   | 1.2 × 10² |

Each value is an average of 10 determinations. * The values are significantly different at p<0.05.

Table 2. Tocopherol constituents of control and puffed spices.

| Spice    | Tocopherol (µg/g sample) |
|----------|--------------------------|
|          | α            | β            | γ            | δ            |
| Origanum, nt | 213.7 ± 7.3* | 33.5 ± 1.0 | 37.3 ± 8.0 | 104.0 ± 2.6 |
| Origanum, t   | 184.3 ± 5.9* | 30.5 ± 6.9 | 35.7 ± 9.8 | 108.2 ± 5.0 |
| Laurel, nt     | 126.3 ± 5.2  | 29.5 ± 0.6  | ND*         | ND          |
| Laurel, t      | 120.4 ± 1.1  | 23.4 ± 4.8  | ND          | ND          |
| Aniseed, nt    | 52.6 ± 8.4   | 237.7 ± 4.8 | trace       | 89.4 ± 5.2  |
| Aniseed, t     | 49.9 ± 0.7   | 254.4 ± 1.8 | trace       | 84.3 ± 2.4  |
| Turmeric, nt   | trace        | trace       | trace       | ND          |
| Turmeric, t    | trace        | trace       | trace       | ND          |
| Coriander, nt  | trace        | trace       | trace       | ND          |
| Coriander, t   | trace        | trace       | trace       | ND          |

Each value is an average of 10 determinations. * nt, non-treated; t, treated; ND, not detected. * The values are significantly different at p<0.05.

Fatty acid constituents

Table 3 shows the fatty acid composition of the five types of spices. Attention is concentrated on linoleic and linolenic acids which are labile to autoxidation, origanum, laurel, aniseed and coriander samples hardly changed at all even after the pasteurization process. On the other hand, degradation of turmeric was noted, probably due to the lack of tocopherol constituents.
Table 3. Fatty acid constituents of spices.

| Spice          | Myristic | Palmitic | Stearic | Linoleic | Linolenic | Arachidic |
|----------------|----------|----------|---------|----------|-----------|-----------|
| Origanum, nt   | 0.073    | 1.77     | 0.492   | 0.659    | 2.32      | 0.107     |
| Origanum, t    | 0.061    | 1.33     | 0.410   | 0.571    | 2.14      | 0.066     |
| Laurel, nt     | 0.062    | 0.744    | 0.263   | 0.408    | 0.528     | 0.056     |
| Laurel, t      | 0.051    | 0.737    | 0.266   | 0.300    | 0.518     | 0.043     |
| Aniseed, nt    | 0.048    | 1.52     | trace   | 5.80     | 0.367     | 0.157     |
| Aniseed, t     | 0.044    | 1.20     | trace   | 5.49     | 0.306     | 0.103     |
| Turmeric, nt   | 0.065    | 0.492    | 0.039   | 0.723    | 0.191     | 0.020     |
| Turmeric, t    | 0.037    | 0.188    | 0.034   | 0.209    | 0.050     | 0.004     |
| Coriander, nt  | 0.188    | 3.64     | trace   | 9.20     | 0.233     | 0.329     |
| Coriander, t   | 0.162    | 3.48     | trace   | 9.22     | 0.226     | 0.267     |

Each value is an average of two determinations. *nt, non-treated (control); t, treated (pasteurization with superheated stem).

DISCUSSION

The presence of tocopherols in origanum, laurel and aniseed may be responsible for their antioxidative activities, as was reported by Cort (5). He found that by their direct addition, origanum exhibited antioxidant activity. He further found that origanum had an antioxidant activity equivalent to 6% that of BHT. Herrmann (6) reported that the known antioxidant action of origanum was due in part to labiatic acid and α-hydroxyhydrocaffeic acid.

Laurel contained 47.6 mg% total tocopherol, as reported by Saito et al. (7), that is, higher levels than those for origanum and aniseed. Laurel showed an antioxidant effect equivalent to that of tocopherols at a concentration of 0.1%, as reported by Saito and Asari (7). This may reflect the high tocopherol content of laurel as determined in this study.

Saito and Asari (7) also reported that aniseed, origanum and coriander, were found to be antioxidative as a result of petroleum ether-soluble fractions. Ramaswamy and Banerjee (8) found that the phenolic pigment, curcumin, present in turmeric, was partly responsible for the antioxidant properties of this spice.

The finding in this study that turmeric and coriander had the lowest tocopherol content does not coincide with the data of Chipault et al. (9) who examined about 36 spices for their antioxidative properties, and reported that coriander was found to have the lowest antioxidant index (1.3), against laurel (2.1), origanum (3.5),
turmeric (2.4) and aniseed (1.9).

In the case of turmeric, there seemed to be changes in the linoleic and linolenic acid values, indicating instability. This is probably due to the low tocopherol content. The findings in this study are in accordance with those of Chipault et al. (10) who reported that turmeric was found to be emulsion-unstable.

From the above findings, it was concluded that in the spices which contained large amounts of tocopherol, the unsaturated fatty acids were unaffected, but that those which had small amounts of tocopherols, both linoleic and linolenic acids, showed degradation, and hence they are susceptible to oxidation. The amounts of tocopherols and unsaturated fatty acid varied with different spices, but on the whole, there were little differences between control and pasteurized samples, suggesting the effectiveness of the pasteurization method.

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