42. Griffiths, N.M., D.G. Land and A. Hobson-Frohock, Br. Poult. Sci. 20:555 (1979).
43. Overfield, N.D., and H.A. Elson, Ibid. 16:213 (1975).
44. Bolton, W., T.G.Carter and R.M. Jones, Ibid. 17:313 (1976).
45. Hobson-Frohock, A., G.R. Fenwick, R.K. Heaney, D.G. Land and R.F. Curtis, Ibid. 18:539 (1977).
46. Mueller, M.M., R.N. Coleman and D.R. Clandinin, 5th International Rapeseed Conference, Malmo, Vol. 2, 1978, p. 303.
47. Fenwick, R.G., and S.A. Hoggan, Br. Poult. Sci. 17:59 (1976).
48. Mueller, M.M., E.B. Ryl, T. Fenton and D.R. Clandinin, Can. J. Anim. Sci. 58:579 (1978).
49. Goh, Y.K., D.R. Clandinin, A.R. Robblee and K. Darlington, Ibid. 59:313 (1979).
50. Pearson, A.W., E.J. Butler, R.F. Curtis, G.R. Fenwick, A. Hobson-Frohock and D.G. Land. J. Sci. Food Agric. 30:799 (1979).
51. Pearson, A.W., E.J. Butler, R.F. Curtis, G.R. Fenwick, A. Hobson-Frohock, D.G. Land and S.A. Hall, Res. Vet. Sci. 25:307 (1978).
52. Pearson, A.W., E.J. Butler and G.R. Fenwick, Vet. Rec. 104: 168 (1979).
53. Leeson, S., and J.D. Summers, Poult. Sci. 57:314 (1978).
54. March, B.E., and C. MacMillan, Ibid. 58:93 (1979).
55. Goh, Y.K., M.M. Mueller, D.R. Clandinin and A.R. Robblee, Can. J. Anim. Sci. 59:545 (1979).
56. Fenwick, G.R., A. Hobson-Frohock, D.G. Land and R.F. Curtis, Br. Poult. Sci. 20:323 (1979).
57. Jackson, N., J. Sci. Food Agric. 20:734 (1969).
58. Hall, S.A., Vet. Rec. 91:495 (1972).
59. Hall, S.A., Ibid. 94:42 (1974).
60. Yamashiro, S., M.K. Bhatnagar, J.R. Scott and S.J. Slinger, Res. Vet. Sci. 19:312 (1975).
61. March, B.E., J. Biely and R. Soong, Poult. Sci. 54:1875 (1975).
62. Olomu, J.M., A.R. Robblee, D.R. Clandinin and R.T. Hardin, Can. J. Anim. Sci. 55:71 (1975).
63. Clandinin, D.R., Z. Hawrysh, J. Howell, J.A. Hanson, R.G. Christian and G. Milne, 4th International Rapeseed Conference, Giessen, 1974, p. 463.
64. Campbell, L.D., Br. Poult. Sci. 20:239 (1979).
65. Slinger, S.J., 4th Progress Report, Rapeseed Association of Canada Pub. No. 40, 1976, p. 84.
66. Grandhi, R.R., S.J. Slinger and J.D. Summers, Poult. Sci. 56:1904 (1977).
67. Papas, A., L.D. Campbell and P.E. Cansfield, Can. J. Anim. Sci. 59:133 (1979).
68. Castell, A.G., and T.M. Mallard, Ibid. 54:443 (1974).
69. Bowland, J.P., and J.A. Newell, Ibid. 54:455 (1974).
70. Woodly, A., J.D. Summers and W.K. Bilanski, Ibid. 52:189 (1972).
71. Olomu, J.M., A.R. Robblee and D.R. Clandinin, Poult. Sci. 53:175 (1974).
72. Olomu, J.M., A.R. Robblee, D.R. Clandinin and R.T. Hardin, Can. J. Anim. Sci. 55:219 (1975).
73. Leeson, S.,S.J. Slinger andJ.D. Summers, Ibid. 58:55 (1978).
74. Slinger, S.J., Rapeseed Association of Canada Pub. No. 45, 1977, p. 93.
75. Josefsson, E., and B. Uppstrom, J. Sci. Food Agric. 27:433 (1976).

[Received November 10, 1980]

# Production and Use of Natural Antioxidants[1]

**U. BRACCO, J. LÖLIGER and J.-L. VIRET,** Nestle Products Technical Assistance Co. Ltd., PO Box 88, CH-1814 La Tour De Peilz, Switzerland

## ABSTRACT

We have developed a new industrial process for obtaining natural antioxidants from spices and other vegetables by primarily adapting mechanical and physical treatments. Rosemary, sage, paprika, nutmeg and cocoa shells have been submitted to a mechanical treatment (micronization), and the finely powdered material was extracted with an edible vegetable oil, i.e., groundnut. The antioxidant dissolved in the lipid phase was collected by two-stage falling film molecular distillation to separate the lipid phase to be recycled) from the active, low molecular weight fraction. Antioxidant activity was measured for fats, oils and fat-containing foods by oxygen absorption, head-space analysis (i.e., pentane) extent of secondary degradation products and organoleptic evaluations. Results obtained indicate that molecular distillates from spices, i.e., rosemary derivatives, effectively protect foods against oxidative rancidity.

## INTRODUCTION

It is known that crude lipid extracts from selected leafy materials are stable to autoxidation despite their high linolenic acid contents. This resistance to oxidation appears to be due to the presence of active naturally occurring antioxidants, probably of the phenolic and polyphenolic class. Extracts of many plants have been shown to have varying degrees of antioxidant activity in oils, fats and fat-containing foods. For example, cocoa shells (1-3), roasted coffee powder and coffee constituents (4,5) (caffeic acid and quinic acid), and cereals such as oats, barley, malt and rice bran (6-8) have all been described as bearing antioxidant materials.

The antioxidant properties of herbs and spices are also well known: red chili, cinnamon leaf, turmeric, clove, black pepper, nutmeg, dry ginger, rosemary, sage and paprika are reported to retard the development of rancidity in oils. Chipault (9) reported a study of 32 common spices used as antioxidants in lard, and showed that only rosemary and sage are effective as antioxidants. The use of rosemary extracts as an antioxidant in foods has already been reported by Rac and Ostric (10), Berner and Jacobson (11) and Chang (12,13). The antioxidant activity of spices is dependent on the recovery technique, i.e., the type and polarity of solvent, particle size of the antioxidant-bearing material and extraction parameters, used for their isolation. In order to clarify the fat-stabilizing constituents of spice extracts, Palitch (14) compared the activity of whole extracts, residues and extract fractions, and found significant differences in the antioxidant activity. The problems related to the recovery of natural antioxidants concern mostly the proper choice of starting material, an effective extraction procedure capable of giving active, odorless, colorless antioxidants and the use of methods to screen and evaluate their activity.

We describe here a new technique to recover natural antioxidants from spices and vegetable material (i.e., rosemary and cocoa shells) based on the following steps: micronization of antioxidant-bearing material in an edible oil to obtain a mechanical transfer of the antioxidant to the lipid phase; cleaning the lipid phase by filtering or centrifuging; and molecular distillation on falling film or centrifugal systems to collect the low molecular weight, active components, which deodorizes and partially bleaches them.

Variations in processing factors such as lipid phase composition, use of codistillants and molecular distillation parameters have also been investigated so that we can define a standard process for obtaining natural antioxidants

[1] Presented at the ISF-AOCS.

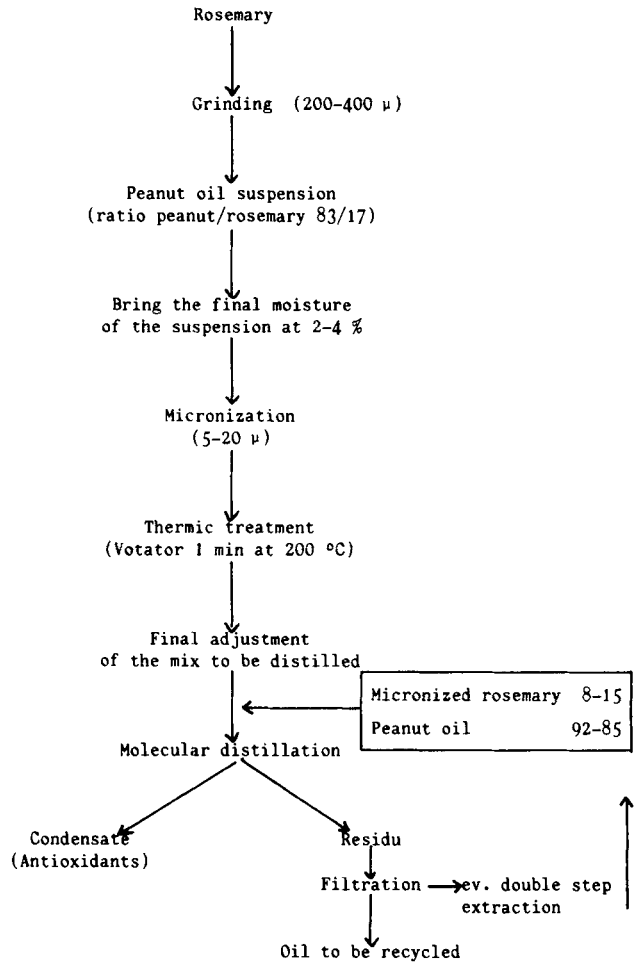



FIG. 1. Recovery of antioxidant from rosemary.

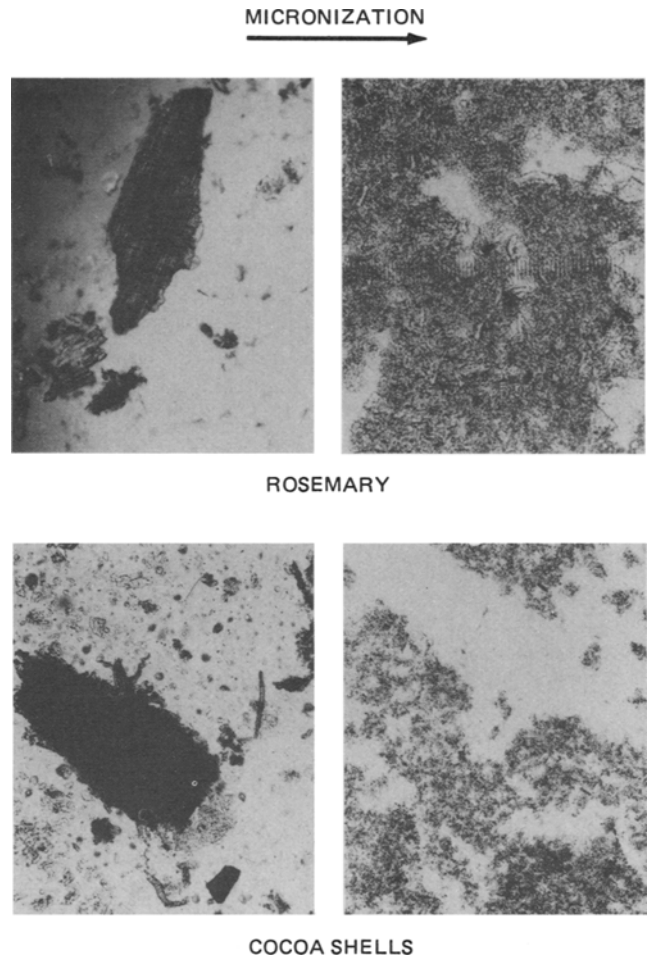



FIG. 2. Rosemary and cocoa shells before and after micronization.

by a primarily physical method. The activity of the antioxidants was evaluated by standard analytical methods as well as by practical trials with standard foods.

## EXPERIMENTAL

### Recovery of Antioxidant Material

A natural odor- and colorless antioxidant was prepared from rosemary according to the process shown in Figure 1.

The micronization of spices in edible oils is the main step of the process which: (a) ruptures the cell wall to free antioxidant from the protoplasma (Fig. 2); (b) permits intimate contact between the lipid phase and the micronized protoplasm; and (c) yields a lipid suspension for the molecular distillation step.

The micronization process used in our studies consists of 4 basic operations: (a) premilling of the antioxidant-bearing material to a particle size of ca. 2 mm on an Alpine of Forplex FLO mill (100-150 kg/hr); (b) whirl-sieving on an AZO model E-240 to collect particle size $<600\ \mu$; (c) moisturizing and mixing with an edible oil (i.e., peanut); (d) micronizing the lipid suspension on a ball-mill WAB model KD-20 (200-350 ℓ/hr).

The finely dispersed antioxidant components in the lipid phase have a molecular weight (MW) range lower than the natural triglycerides of the oil used and can therefore be physically separated by molecular distillation either on fall-film (Leybold) or on a centrifugal system (CVC).

In the fall-film system, a two-stage process was used: the lipid suspension at 80-90 C was passed through the first distillation unit (temp 140 C, pressure $0.3 \cdot 10^{-2}$ mm, condenser at 80-100 C) to eliminate volatile components, cold trapped at -196 C. The second distillation unit was operated at 205-215 C with a condenser temperature of 80-110 C, pressure lower than $1 \times 10^{-3}$ mm to obtain a distillate showing antioxidant activity and a residue consisting mainly of high MW triglycerides, which was recycled to the micronization step and reused as an antioxidant carrier.

In the centrifugal molecular distillation the micronized suspension of rosemary antioxidants in peanut oil is spread by centrifugal force in a thin, uniform film across the face of the rotor; the lighter, lower MW fraction of the feed material, showing antioxidant activity, evaporates and condenses on the internal water-cooled leaf condenser. The heavier unevaporated residue slides off the rotor into a collecting gutter and can be recycled. Material to be submitted to the centrifugal step had a temperature of ca. 60 C, was degassed at 70 C and distilled at 250 C under $8 \times 10^{-3}$ mm. Condenser temperature was fixed at 80-100 C.

The molecular distillation flow on the condenser unit can be improved by using a lipid-like, low MW component such as mid-chain triglycerides or glycerol monoester. They can be solubilized into the peanut oil and codistilled with the active antioxidant components, playing a double role during the distillation process (codistillation) and at the condenser level (flow improvers).

Table I shows the processing parameters used for processing rosemary and cocoa shells.

TABLE I

**Processing Parameters for Antioxidant Recovery**

| | Rosemary (%) | Cocoa shells (%) |
|---|---|---|
| Micronization | | |
| Peanut oil | 78 | 82 |
| Rosemary | 18.48 | – |
| Cocoa shell | – | 17.20 |
| Water | 3.52 | 2.80 |
| Characteristics | | |
| Moisture before moisturizing | 6.0 | 14.0 |
| Moisture after moisturizing | 16.0 | 20.0 |
| Rosemary (% in peanut oil) | 22.0 | – |
| Cocoa shell (% in peanut oil) | – | 17.20 |
| Particle size (μ) | 5-20 | 5-15 |
| Viscosity (cp at 20 C) | 325 | 140 |
| Specific density | 0.98 | 1.05 |

## Analytical Investigations

The antioxidant material isolated from rosemary was separated by column chromatography (length: 30 cm; 2.5 cm id) on Kieselgel (0.05-0.2 mm), using solvents with increasing polarity. The fractions obtained were further investigated by mass spectrometry, coupled thin layer-mass spectrometry and UV-absorption. Moreover, antioxidant activity was checked in edible oils and fats and fat-containing, high oxygen-sensitive products, such as potato flakes.

Oxygen absorption according to Astell (15) and Hadorn (16) gave a fast and reliable indication of the antioxidant activity of the natural distillates in fats and oils. Specific methods were developed for evaluation in food products such as potato flakes to follow their oxidative degradation during storage. In potato flakes the following analyses were performed.

*Residual oxygen in the head space.* The more oxygen is left in the head space, the better are the keeping qualities of the sample.

*Carotenoids.* Several methods are used for the determination of carotenoids, but the simplest one is a visual assessment of the color of the samples. This method proved to be worthless for dehydrated vegetables, i.e., potatoes. It was therefore necessary to apply a method based on solvent extraction and spectrophotometric evaluation of the pigments (17,18).

*Pentane.* This hydrocarbon is a secondary degradation product of linoleic acid oxidation; the less pentane found in the head space, the better are the keeping qualities.

We combined the pentane determination with an accelerated storage test for foods, in which ultraviolet light is used as catalyst (J. Loeliger, Swiss patent pending). The functional description of the different parts of this UV-oxidation equipment is given in Figure 3.

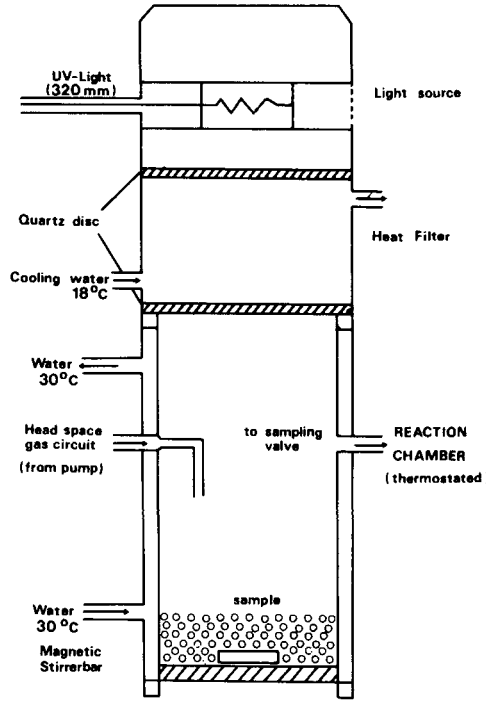



FIG. 3. Scheme of the UV-Irradiation unit.

This all stainless steel (with the exception of the heat filter, which is quartz) UV-irradiation unit is equipped with a high pressure mercury arc light bulb, Hanau TQ-150, with the corresponding electric starter unit. The quartz discs (Ø = 60 mm, 3 mm thick, Société Electrothermique, La Tour-de-Treme, Fribourg) are transparent for the high pressure mercury arc light. The heat filter is installed to give the flexibility of temperature control to the reaction chamber, which in turn can be set at any temperature between 10 C and 80 C. The substance to be oxidized is placed in the reaction chamber, where it is subjected to UV-light and the temperature applied to the reactor. This system is hooked up to a gas liquid chromatograph for headspace gas analysis by means of 1/8 in. nylon tubing. The gas is constantly circulated within the reaction vessel-pump and sampling valve. The headspace gas analysis is performed according to Arnaud (19) on a 2 m x 1/8 in. stainless steel column filled with alumina.

TABLE II

**Column Chromatography and Mass Spectrometry Identification of Rosemary Antioxidant Components**

| Fraction | Solvent | ml | Identified components and molecular mass of unidentified components |
|---|---|---|---|
| 1 | Hexane | 75 | Amyron |
| 2 | Hexane/dichloromethane (1:1) | 230 | Amyron + α-tocopherol + 419 + 286 |
| 3 | Dichloromethane | 75 | Amyron + α-tocopherol + amyrin + 330 + 284 + 286 + 298 |
| 4 | Dichloromethane/diethylether (1:1) | 135 | Triterpene alcohols |
| 5a | Diethylether | 200 | 419 + methylcarnosol + 310 + 300 + 298 |
| 5b | Diethylether | 200 | 286 + triterpene acids |
| 5c | Diethylether | 200 | Carnosic acid, Me-carnosic acid (carnosol + Me carnosol) |
| 6 | Acetone | 750 | Carnosol + Me Carnosol |
| 7 | Ethanol | 470 | Flavons |

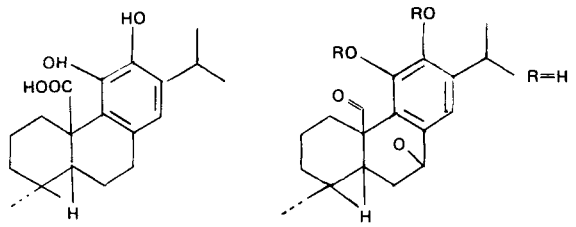
*Taste testing.* The 1 to 8 scale allows one to assess the organoleptic quality of the tested samples; code 1 corresponds to a fresh, unoxidized sample, code 8 to a deteriorated product.

## RESULTS AND DISCUSSION

### Chemical Structures of Rosemary Antioxidants

Sixteen compounds belonging to classes of flavones, diterpenes, steroids and triterpenes were found and collected for further antioxidant activity investigation; they are reported in Table II, in which identified components and molecular masses of unidentified components also are reported.

Our preliminary studies suggested that the antioxidant activity of rosemary extracts must be mainly related to its carnosic acid and carnosol content (Scheme I).




[I]

Carnosic acid Carnosol

Carnosol has already been described as the most active antioxidant constituent found in rosemary (20). This phenol belongs to the diterpene class and it has been shown to be an oxidative artifact of carnosic acid. Other constituents related to carnosic acid (e.g., methylcarnosol, methyl-carnosic acid, methoxy-carnosol), although still possessing antioxidant activity, are probably artifacts

TABLE III

**Induction Period (hr) on Chicken Fat (wt of substrate = 4 g, temp = 90 C)**

| Code | | Hr |
|---|---|---|
| 1 | Chicken fat control | 4 |
| 2 | Chicken fat + 1,000 ppm rosemary antioxidant | 25 |
| 3 | Chicken fat + 1,000 ppm rosemary antioxidant + monoglyceride as codistillant | 20 |
| 4 | Chicken fat + 1,000 ppm sage antioxidant | 30 |
| 5 | Chicken fat + 1,000 ppm cocoa shell antioxidant | 20 |
| 6 | Chicken fat + 1,000 ppm BHA + BHT (1:1) | 20-25 |

From these data, it appears that rosemary and sage prolong the induction period in chicken fat and shown antioxidant activity comparable with BHA and BHT. Moreover, they are more effective than cocoa shell distillate as antioxidants. The antioxidant properties of rosemary distillate were also evaluated in potato flakes according to the methods already described. Figure 4 gives the antioxidant activity measured as residual oxygen (%) and pentane (IU $10^4$) in the headspace of the potato flakes packaging, the amount of residual carotenoids (mg/kg) in the product and the organoleptic scores.

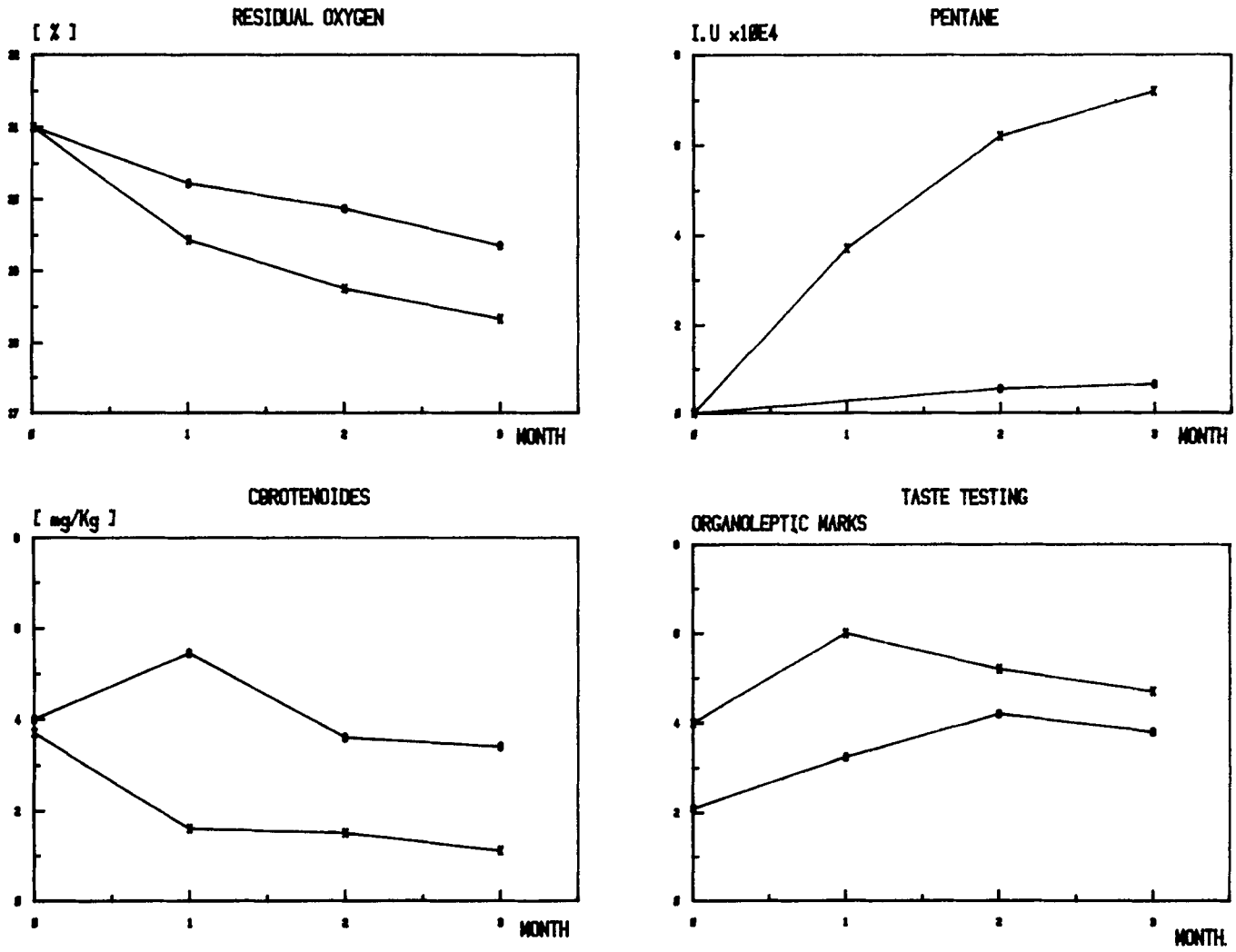



FIG. 4. Potato flake stabilization. ~~ooo~~: 700 ppm rosemary; ~~xxx~~: control.

formed during the isolation of rosemary antioxidant. Triterpene alcohols and acids (erythrodiol, betulin, ursolic and oleanolic acids) could be associated as synergists with the antioxidant activity of the rosemary complex.

### Antioxidant Activity

Table III shows reports the induction period (hr) to record a decrease in the oxygen pressure on the surface of the sample (chicken fat) showing an oxygen absorption on the substrate, according to the Astell method (11).

From these data, it appears that rosemary and sage prolong the induction period in chicken fat and show antioxidant activity comparable with BHA and BHT. Moreover, they are more effective than cocoa shell distillate as antioxidants. The antioxidant properties of rosemary distillate were also evaluated in potato flakes according to the methods already described. Figure 4 gives the antioxidant activity measured as residual oxygen (%) and pentane (IU $10^4$) in the headspace of the potato flakes packaging, the amount of residual carotenoids (mg/kg) in the product and the organoleptic scores.

From these data, it is clear that rosemary antioxidant retards linoleic degradation (pentane), carotenoid loss and protects lipids and lipid-like materials from oxygen attack. This protection has been confirmed by taste testings which show satisfactory correlation with the analytical figures.

We conclude that natural antioxidants, obtained as described here and added to oxygen-sensitive foods, improve their shelf-life and avoid deterioration of the organoleptic properties.

REFERENCES


1. Mueller, W.S., J. Dairy Sci. 37:754 (1954).
2. Mueller, W.S., Can. Inst. Food Tech. J. 1:110 (1968).
3. Miller, J.E., JAOCS 48:91 (1971).
4. Nataratan, C.P., J. Sci. Ind. Res. (India) 17c:145 (1958).
5. Inge, M., German Patent 2,426,269 (1975).
6. Korobkina, G.S., Vopr. Pitan. 15:48 (1957); Chem. Abstr. 51:6026a (1957).
7. Baker, D.L., and N.N. Hellman, Food Technol. 12:33 (1958).
8. Tsuchiya, T., Japanese Patent 7,352,804 (1973).
9. Chipault, J.R., Food Eng. 29:134 (1957).
10. Rac, M., and B. Ostric, Rev. Fr. Corps Gras 2:796 (1955).
11. Berner, D.L., and G.A. Jacobson, U.S. Patent 3,732,111 (1973).
12. Chang, S.S., B. Ostric-Matijevic, O.P.L. Hsieh and Cheng-Li Huang, J. Food Sci. 42:1102 (1977).
13. Chang, S.S., U.S. Patent 3,950,266 (1976).
14. Palitzsch, A., Fleischwirtschaft 54:63 (1974).
15. British Food Manufacture Industry Research Association (BFMIRA) Tech. Circular no. 654, May 1978.
16. Hadorn, H., and K. Zuercher, Dtsch. Lebensm. Rundsch. 70:57 (1974).
17. O'Conner, M., Ind. Eng. Chem. Anal. 18:557 (1946).
18. Simeonova, W., Nahrung 9:307 (1965).
19. Arnaud, M., IV Intern. Congress of Food Science and Technology Madrid, September 1964).
20. Breiskorn, C.H., Z. Lebensm. Unters. Forsch. 141:10 (1969).


[Received May 1, 1980]

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# Absence of the Cotton Dust/Bract Antigen in House Dust, Clean Cotton Fibers and Cottonseed Hulls and Proteins

**A.A. SEKUL** and **R.L. ORY**, Southern Regional Research Center[1], New Orleans, LA 70179


## ABSTRACT

Aqueous extracts of cotton dust and cotton bract induced the formation of antibodies in rabbits. The antisera cross-reacted with both extracts as well as with extracts of stem, leaf, baled cotton, and gin trash. No reaction was obtained with extracts of cottonseed hulls, cottonseed proteins, noncontaminated cotton fibers, or house dust. None of the anitgens reacted with normal rabbit serum.


## INTRODUCTION

Byssinosis, a respiratory disease, has been shown to affect the respiratory function of susceptible textile workers (1-3) as well as workers who handle flax, soft hemp and sisal (4,5). Neither the causative agent(s) nor the mode of action by which byssinosis is produced is known. The symptoms are generally ascribed to the prolonged inhalation of dust in textile mills that process raw fibers. Cotton dust is a heterogeneous material which varies in composition among work areas (6). Cotton bract has been identified as one of the major contaminants (32-52%) of the cotton plant trash which is associated with the fiber (7). Botanically, cotton dust at the textile mill is composed mainly of micronized plant parts such as bract, stem, leaf and cotton fiber. In the cottonseed oil mill, the botanical nature of the dust in the linter handling and processing consists largely of seed-coat fragments and linter trash microparticulates (8).

In the only study done in the U.S. oil mills, Jones et al. (9) reported a low prevalence of chronic airways diseases (2.3% byssinosis and 4% chronic bronchitis) among workers in several oil mills. This byssinosis prevalence level is about the level of false positives one would expect to find. Studies by Noweir et al. (10) showed that extremely high dust levels (73-590 mg/m$^3$) generated in cottonseed-handling operations (loading and unloading) in oil mills led to some respiratory complaints, but that dust from other operations (grinding and oil extraction) was inert.

Opinions regarding the etiology of byssinosis are conflicting. The symptoms are presumed to be caused by airway constriction brought about by induction of histamine release by cotton and hemp dust (11,12). Recently, reports have appeared (13) as well as our own studies (14), showing that cotton dust activates the complement system of proteins. Previous studies in this laboratory (15,16) showed that antibodies to water extracts of cotton card-room dust, as well as cotton bract and a purified fraction of dust, gave positive immunological responses in rabbits. In view of the low prevalence of byssinosis in oil mills, the purpose of this investigation was to test water extracts of clean cotton, cottonseed kernels (proteins plus aller-

[1] One of the facilities of the Southern Region, Science and Education Administration, U.S. Department of Agriculture.