A Simplified Method for the Evaluation of Antioxidants

ABSTRACT

A procedure is described for rapid evaluation of antioxidants. Dilute aqueous emulsions of an antioxidant, carotene and lipid were prepared in spectrometer tubes. The oxidative destruction of carotene in the emulsion was observed directly with a colorimeter. The antioxidants were then evaluated according to their effect on the rate of carotene decolorization.

Numerous techniques have been developed for the evaluation of antioxidants, some of which have been reviewed recently by Marco (1). The method developed by Marco provides a rapid, reliable system for analyses that alleviates many of the shortcomings encountered with other techniques. A simplification of Marco's method is described in this paper.

A dilute, oxygenated emulsion was prepared in a similar manner to that described by Marco. A 2.0 gm sample of crystalline β-carotene was dissolved in 10 ml of chloroform. One milliliter of this solution was then pipetted into a round-bottomed flask which contained 20 mg of purified linoleic acid and 200 mg of Tween 40 emulsifier. After removal of chloroform on a rotary evaporator, 50 ml of oxygenated, distilled water was added to the flask with vigorous stirring. A 5 ml aliquot of the aqueous emulsion which formed was then pipetted into each of a series of spectrometer tubes which contained 0.2 ml portions of ethanolic antioxidant solution. A zero reading was taken at 470 nm on the reaction mixture in each tube immediately after addition of the emulsion to the antioxidant solution. (A Bausch and Lomb Spectronic 20 colorimeter and Bausch and Lomb spectrometer tubes were used.) The tubes were then stoppered and placed in a water bath at 50 C. Subsequent readings were taken at regular intervals until the carotene had been decolorized (about 1-3 hr). About 5 sec were required to take a spectrometer reading. This is not enough time for a temperature change to occur in the reaction media which would cause a significant change in the reaction rate. The results of a typical experiment are shown in Figure 1. This procedure is simple and gives quick results. The experimental setup is uncomplicated, and 10 or more samples can be evaluated simultaneously without difficulty.

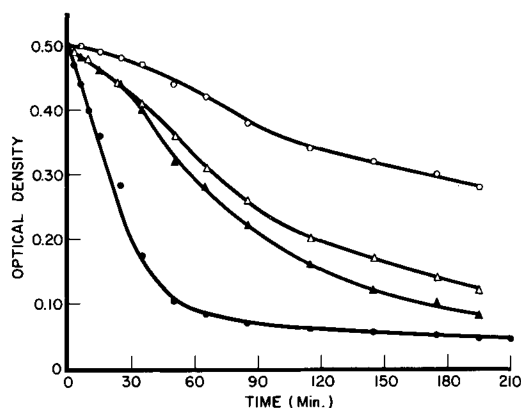

FIG. 1. Carotene destruction in the presence of BHT and cocoa hull extractives. The control sample with no antioxidant added is represented by •; BHT (8 mg/liter) by ○; cocoa hull extract A (30 mg/liter) by △; and cocoa hull extract B (80 mg/liter) by ▲.

H.E. MILLER
General Mills, Inc.
James Ford Bell Technical Center
9000 Plymouth Avenue
Minneapolis, Minnesota 55427

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[Received June 17, 1970]

High Levels of Acetone Found in Male Turkey Skin[1]

ABSTRACT

Investigation of the carbonyl compounds in the hexane extractable lipid of raw poultry skin revealed that the only monocarbonyl present was acetone. Furthermore, in male turkeys the level of acetone was significantly greater than the level observed in either chickens or female turkeys. During maturation, increased acetone concentrations were accompanied by low lipid deposition in the tissue.

Investigations in our laboratory on poultry flavor have involved the characterization and quantification of carbonyl compounds associated with the lipids isolated from the skin of chickens and turkeys (1,2). This report illustrates the unique variation in the concentration of acetone in the raw skin of both groups of birds due to sex and age.

Ten chickens and five turkeys of each sex were raised on standard commercial rations and slaughtered at each of three ages. The main body skin was removed immediately after slaughter and the hexane extractable lipids were isolated from homogenized samples with carbonyl-free hexane. The carbonyl analyses were conducted in triplicate on the hexane extracts passed through Celite impregnated

[1]Authorized for publication as paper No. 3831 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

TABLE I

Concentration[a] of Total Carbonyl and Acetone Derivatives Present in Male and Female Poultry Skin at Three Ages

Age at slaughter, weeks	Total carbonyl		Acetone	
	Male	Female	Male	Female
Chickens				
8	46.5	65.3	2.8	1.2
10	49.2	55.2	1.1	0.9
12	38.8	33.1	0.6	1.1
Turkeys				
20	263.4	30.4	110.1	2.0
24	397.5	48.6	182.2	4.3
30	50.0	69.3	2.9	2.7

[a]Mean of triplicate samples in μmoles/10 g lipid.

with 2,4dinitrophenyl (DNP) hydrazine, phosphoric acid and water to convert the carbonyls to their 2,4-DNP hydrazone derivatives (3). The monocarbonyl derivatives were eluted from the total carbonyls on a Celite 545-Sea Sorb 43 (Fisher) column and freed of ketoglycerides on a neutral alumina column (4). The monocarbonyl derivatives were further fractionated into classes by column chromatography (5). Quantificiation of the 2,4-DNP hydrazones were determined spectrophotometrically using a Beckman spectrophotometer, model DB-G.

The only class of carbonyl present in the monocarbonyl fraction was methyl ketone derivatives with an absorbance maximum of 365 mμ in chloroform. Further characterization of this fraction utilizing thin layer chromatography to separate the methyl ketone 2,4-DNP-hydrazones into their carbon lengths (6) revealed only the three carbon methyl ketone (acetone) to be present. To confirm that the only derivative present was the DNP hydrazone of acetone, melting points were determined on recrystallized samples of the methyl ketone fraction. The melting point of 126 C confirmed the exclusive presence of acetone. Continuous monitoring showed no significant acetone contamination in the solvents.

Mean concentrations of the total carbonyls and acetone are shown in Table I. Age produced significant variations in the acetone concentration in turkey skin. An increased acetone concentration from 20 to 24 weeks was seen in both sexes; by 30 weeks of age both males and females had dropped to low levels, being similar to each other. The sex differences were particularly striking in the turkeys. While female turkey skin showed an increase in acetone content at 24 weeks, the level at any of the three ages was not high. The greater acetone level in the male turkeys was statistically significant. The interesting finding is the decrease which occurred in the quantity of lipid in the skin of the 24-week-old turkeys. Normally birds are thought to be

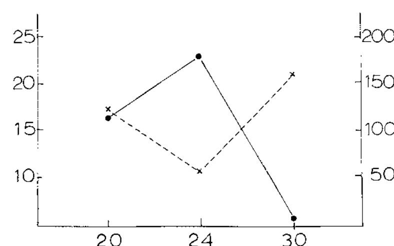

FIG. 1. Relationship between lipid and acetone concentrations in raw male turkey skin. The solid line is acetone, the broken line is lipid. left ordinate, gram lipid/50 g skin; abscissa, age in weeks; right ordinate, μ moles of acetone/10 g lipid.

continually laying down skin lipids during this stage of maturation. This period where a decrease in lipid deposition occurs was marked by increased acetone concentrations in the tissue (Fig. 1). Whether this unusually high level of acetone in the male turkey's skin is also reflected in other tissue is unknown at present. The source of the acetone, however bears consideration. Lipogenesis in avian species occurs primarily in the liver (7), unlike other animals in which extrahepatic tissue also may be an active site of lipid synthesis. In the liver acetyl CoA is formed directly from glucose or from pyruvate via the tricarboxylic acid cycle. This acetyl CoA, independent of its source, is then available for hepatic synthesis of fatty acids for glyceride formation. The triglycerides are transported via low density or β-lipoproteins in the plasma to the adipose tissue. The decreased lipid accumulation in the skin of the 24-week-old male turkeys therefore may be a reflection of depressed hepatic lipogenesis and result in the diversion of acetyl CoA to acetoacetate and thus acetone. However, one cannot discount the possibility of depletion of the lipid stores in the skin by oxidation yielding C-2 units which, because of limited lipogenic capacity at this site, may be diverted into ketone bodies.

C.P. THOMAS
P.S. DIMICK
J.H. MACNEIL
Division of Food Science
The Pennsylvania State University
University Park, Pennsylvania 16802

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[Received September 24, 1970]

The Contribution of Saturated Acyl Groups to the Volatiles of Oxidized Soybean Oil[1]

ABSTRACT

U-^{14}C-Stearic acid was interesterified into soybean oil and the resulting oil was oxidized to high peroxide values at 25 C and at 125 C. Volatile oxidation products were isolated and found to have a count that was not significantly above background.

[1]Journal paper no. 6715 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project 1856.

Pure saturated fatty acids and esters are quite resistant to oxidation at temperatures below 100 C, but the presence of unsaturated fatty acids decreases their stability. In reviewing the oxidation of saturated fatty acids, Brodnitz (1) made the interesting suggestion that, in the oxidation of natural fats and oils, oxidation of saturated acyl groups may be enhanced by concurrent oxidation of unsaturated acyl groups. He suggested that the oxidation products of saturated acyl groups might make a significant contribution to volatile products that are produced and account for