Carbonyl Compounds Developed in Freeze-Dried Meat as a Measure of Lipid Deterioration During Storage

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ABSTRACT

Saturated, $\alpha - \beta$ unsaturated carbonyl compounds and three classes of monocarbonyls were separated in freeze-dried beef stored at room temperature. This study was an attempt to correlate values obtained for the above compounds with the progressive autoxidation of beef lipids.

In the determination of carbonyl compounds both methods of Henick *et al.* (8), and Keith and Day (10), which involve colourimetric estimation of 2,4-dinitrophenylhydrazone derivatives in basic solution, were used with some modifications. Under conditions of this investigation, carbonyl compounds were present at different concentrations at the intervals studied. Apparently, these modified methods of analysis could not be relied upon entirely to follow the progressive deterioration of lipids in freeze-dried meat.

INTRODUCTION

Freeze-drying is considered one of the most promising methods for preservation of food (2,7). This process protects food against microbial spoilage because of the very low moisture content in the final product. However, it does not prevent completely changes in lipids leading to oxidative rancidity. Such oxidation reactions in lipids or fatty portions of food degrade its quality during storage. Generally, the oxidized food develops off odours and flavours as well as changes in colour.

Historically, objective tests for rancidity began many years ago with the development of the Kreis test (11). Although many methods are available to make quantitative evaluation of rancidity, very few have proved to be satisfactory. Carbonyl compounds, especially volatile monocarbonyls, have been directly related to flavour deterioration of oxidized lipids (1,5,10). Determination of carbonyl compounds appears to give good indication of deteriorative changes of foods rich in fats and oils.

In this study the carbonyl content was determined in freeze-dried meat during storage at room temperature. Results were used to estimate rate of degradation and type of changes occuring under conditions of this investigation.

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MATERIAL AND METHODS

Meat Samples

Commercial grade, top round cuts of beef were obtained from the local market. The samples were closely trimmed of separable fat and connective tissue. The trimmed meat was cut into cubes of approximately 1″ × 1″ × 1″ and divided into two lots. One lot was freeze-dried raw, and the other was cooked prior to freeze-drying. Cooking was carried out in a Radarange Microwave oven, Model 1161, in a single layer for five minutes. Both meat lots were freeze-dried in a Stokes Freeze-Drier at approximately 40°C and a vacuum of 0.1 mm of Hg. The freeze-dried samples were stored in loose-covered glass jars at room temperature (24 ± 2°C), for 12-weeks. Samples were withdrawn for chemical analysis at different intervals.

Carbonyl-free Benzene (CFB)

The two litres of benzene were refluxed for 1-hour with 10 grams of DNP- hydrazine and 2 grams of TCA. The CFB was obtained by distillation through a Vigreau column.

Carbonyl-free Absolute Ethanol (CFAE).

Fourteen grams of aluminium granules and 18 grams of KOH were added to 2 litres of ethanol and the mixture was refluxed for one hour. During distillation the first and last 100 ml portions of the distillate were discarded.

DNP-hydrazine Solution

One half gram of DNP-hydrazine, twice recrystallized from carbonyl-free methanol, was dissolved in CFB to obtain a 0.05 percent solution (w/v).

Trichloroacetic Acid (TCA).

A 4.3% solution of TCA in CFB was prepared (w/v).

Ethanolic KOH

A 4% solution (w/v) of KOH in CFAE was freshly prepared and centrifuged for 10 minutes at 2,000 r.p.m. immediately before use.

Hydrated Alumina

Activated alumina, F-20 grade (Aluminum Company of America, East St. Louis, Ill., U.S.A.) was modified by mixing with 15% hydrated alumina and equilibrated for 1 hour before use.

Extraction of carbonyl compounds

A sample of 5–7 grams of freeze-dried meat was soaked in 20 ml of CFAE for 1 minute. Then 40 ml of CFB were added and the mixture was blended in a Virtis' 45 homogenizer while cooling the container to about 5°C. The slurry was filtered through a Buchner funnel and washed with 40 ml of benzene-methanol mixture (2 : 1, v/v). About 5 ml of the filtrate were used for analysis.

Determination of Carbonyl Compounds

The method described by Henick (8), was used in this investigation with a few modifications and after standardization with known compounds. A reaction mixture made up of 3 ml of TCA solution and 5 ml of DNP-hydrazine was placed in a 50 ml volumetric flask. A 5 ml of DNP-hydrazine extract was added to the above mixture and the flask was loosely stoppered and heated in a water-bath at 60°C for 30 minutes then cooled. For colour development, 10 ml of ethanolic KOH were added and the volume was brought to 50 ml by adding CFAE. The contents were mixed thoroughly. After 10 minutes the wine-red developed colour was measured at 430 and 460 mu., using a Beckman DU Spectrophotometre. A mixture of CFB-CFAE (2:1, v/v) was used as a blank. The following equations were used to calculate saturated as well as $\alpha - \beta$ unsaturated carbonyls as micromoles per 50 ml solutions:

$$\text{Saturated carbonyls} = 5.803\ A_{430} - 4.412\ A_{460}$$
$$\text{Unsaturated carbonyls} = -3.366\ A_{430} + 4.338\ A_{460}$$

where A_{430} and A_{460} indicate absorbancy at the wave lengths 430 and 460 mu., respectively.

Separation of free mono-carbonyl compounds

The method used by Keith and Day (10), was modified to separate three classes of mono-carbonyl compounds extracted from the freeze-dried meat. A chromatographic column of 15 mm (i.d.) and 30 cm long was packed with 15% hydrated alumina, using CFB, to a depth of 3 cm. This was followed by the addition of 10 gm of DNP-hydrazine and sufficient amount of hydrated alumina to increase the depth of the column by 1 cm. More alumina, suspended in CFB, was added till the column height reached 8 cm. The column was then washed with CFB before adding the extract containing the carbonyl compounds, to the top of the column. The column was eluated by 100 ml of CFB at a flow rate of 5 ml/min. The eluate was collected in standard, round-bottomed flasks. The

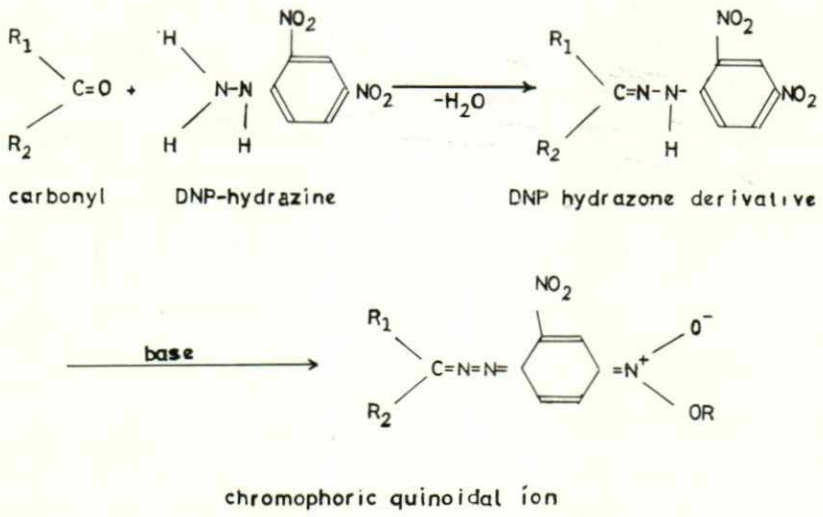

Fig. 1. Reaction of DNP-hydrazine with a carbonyl compound and the chromophoric quinoidal ion developed in the presence of base.

solvent was evaporated under reduced pressure at room temperature. The following reagents were added in the following order: 5 ml of C.F.B., 10 ml of 4% ethanolic KOH, and 35 ml of CFAE. The contents of the flask were thoroughly mixed and after 10 minutes from the addition of ethanolic KOH the absorbancy values for the contents of the flask were determined at 430, 460 and 480 mu against a blank. The blank was prepared by passing a portion of the sample extract over an alumina column free from DNP-hydrazine

The quantity of each of the three mono-carbonyl classes was calculated in micromoles per 50 ml solution by applying the following simultaneous equations, derived by matrix simultaneous equations, derived by matrix inversion from average molar absorptivities of DNP-hydrazone derivatives of mono-carbonyl compounds:

$$\text{Alkanals} = 7.158\ A_{430} - 11.142\ A_{460} + 6.496\ A_{480}$$

$$\text{Alk}-2-\text{enals} = -5.477\ A_{430} + 15.374\ A_{460} - 11.090\ A_{480}$$

$$\text{Alk-2, 4-dienals} = 1.514\ A_{430} - 6.634\ A_{460} + 6.423\ A_{480}$$

RESULTS AND DISCUSSION

Carbonyl compounds especially the mono-carbonyls have been associated with food lipid rancidity (1,5,10,12). This study was carried out to correlate free carbonyl content of freeze-dried raw and cooked meat, held at room temperature, with the extent of oxidative rancidity taking place in the samples used in this investigation.

In order to obtain reproducible results the following observations should be considered when applying the modified methods described in this study. The absorbancy values of quinoidal ion should be read exactly 10 minutes after the addition of ethanolic KOH to the carbonyl extract reaction mixture. This was found necessary due to the fact that the colour of quinoidal ion reached its maximum at the time specified following which it faded (9,13).

The use of rubber tubing was eliminated from all equipment used in this study because it was found to contaminate the sample extract with rubber carbonyl compounds. Teflon stopcocks were used with the chromatographic columns to avoid solvent leakage.

Reference carbonyl compounds were used to test the accuracy of the modified method of Henick *et al.* (8). Almost 100% of carbonyls were recovered when their concentration did not exceed 250×10^{-6} moles.

The data obtained and presented in Table 1, indicated quite noticeable changes in carbonyl content during the storage of freeze-dried raw and cooked beef, at room tem-

Table 1 Changes in carbonyl content of freeze-dried, raw and cooked beef held at room temperature.

Sample	Carbonyl compounds	Storage period (week)				
		0	1	2	4	12
Raw	Saturated	4.12	5.33	5.21	5.10	7.34
	Unsaturated	0.00	0.04	0.23	0.12	0.36
	Total	4.12	5.37	5.43	5.22	7.70
Cooked	Saturated	6.86	6.41	5.38	3.08	6.12
	Unsaturated	0.06	0.06	1.86	0.16	1.16
	Total	6.92	6.47	7.24	3.24	7.28

perature. Values for free carbonyl compounds determined showed highest concentration by the end of the 12-week storage period.

The method of Henick *et al.* (8), although simple and convenient, it was found inadequate to give a clear picture of carbonyl distribution in the deteriorated freeze-dried beef.

Alkanals, alk-2-enals and alk-2, 4-dienals (Fig. 2), are the major classes of mono-carbonyl compounds produced in oxidized lipids (4,6,10). A modification of the method of Keith and Day (10) described in this paper was used to separate the above classes of mono-carbonyls in this study. The alumina reaction column impregnated with DNP-hydrazine was washed with ethanol to get rid of undesirable substances such as excess DNP-hydrazine and DNP-hydrazone derivatives of polycarbonyls present in the column.

The simultaneous equations used in this study were valid only for the three components, alkanals, alk-2-enals and alk-2, 4-dienals formed under the same conditions as those for which the equations were developed. The equations were derived by matrix inversion from average molar absorptivities of DNP-hydrazone derivatives of mono-carbonyl compounds used as references.

The eluate obtained from the alumina chromatographic column by the use of benzene-ethanol solvent system was found to contain more than three components. However, the total mono-carbonyl content of the freeze-dried meat extract showed a steady rise in concentration.

Results presented in Table 2, showed a maximum carbonyl content in freeze-dried raw

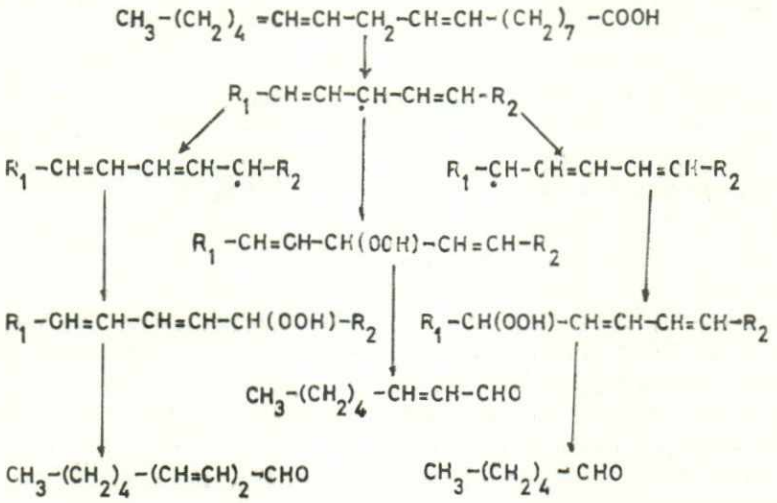

Fig. 2. Scheme for formation of hydroperoxides and dismutation to monocarbonyl compounds.

Table 2 Changes in free monocarbonyls of freeze-dried raw beef held at room temperature.

Class of Monocarbonyls	Storage period (day)					
	0	1	2	6	9	12
Alkanals	N.D.	N.D.	0.020	0.182	1.680	0.820
Alk-2-enals	N.D.	N.D.	N.D.	0.420	N.D.	N.D.
Alk-2, 4-dienals	N.D.	N.D.	N.D.	0.124	0.242	0.310
Total carbonyls	N.D.	N.D.	0.020	0.726	1.922	1.130

N.D. Non-detectable values

meat after 9-days storage period. Failure to detect some of the mono-carbonyl classes at the testing intervals (Table 2), may be due to the reaction of such carbonyls with some other components in the system. Another probability would be their further oxidation to form products differing in their properties from mono-carbonyls.

Generally, results indicated the accumulation of carbonyl compounds in the freeze-dried raw and cooked meat as shown in Table 1. Due to the complexity of the autoxidation process, it seems that at present there is no simple analytical method which can be directly applied for the evaluation of deteriorative changes taking place in freeze-dried meat.

In conclusion, it is felt that further investigation should be carried out to evaluate this procedure with other standard methods for measuring oxidative changes in food lipids.

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