Volatile Components of Vacuum-Packed Dehydrated Pork

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Vacuum-packed stored dehydrated pork develops an off-flavor and/or -odor during storage at elevated temperatures and/or for long time intervals. Chemical methods were needed in order to determine the nature and the relative amounts of the volatile components that develop. Aeration techniques in conjunction with chromatographic methods showed the volatile components to be basic in nature and to exhibit reducing properties. Aerations of 8-ounce cans of meat by use of a specially designed can-puncturing device showed that the total basicity and reducing power of the volatile fractions increased with a rise in storage temperature. Monocarbonyl compounds were present in benzene extracts of all the meat samples. Acetaldehyde was present in dehydrated pork samples stored at both —20° and 94° F. Ammonia was detected in meat samples stored at 100° and 160° F. Schemes for the possible formation of acetaldehyde and ammonia are given. The methods presented are applicable to similar materials packed in evacuated containers.

VACUUM-PACKED DEHYDRATED PORK has desirable characteristics for use as a meat ration by the military forces, but it develops off-flavor (including off-odors) during storage at elevated temperatures. This off-flavor has been characterized as a tankage or cooked flavor, and in many cases, it renders the product highly unpalatable. The volatile chemical compounds causing the off-flavors and/or odors are unknown. Chemical methods are needed for the determination of the nature of the off-flavor and/or odor components, and also for an objective evaluation of the meat.

Most types of volatile organic compounds formed during food spoilage are oxidizable (*11*). The unpleasantness associated with used air is due to organic matter with reducing properties (*16*). Previous investigators have used total volatile basicity determinations for evaluating the condition of meat (*14*) and eggs (*18*), and aeration techniques for estimating the total volatile reducing substances present in fish samples (*2, 11*) and in a wide variety of highly fragrant products (*1*). It is therefore probable that the determination of the volatile basic and reducing substances present in a food sample would give some estimate of the amount of deterioration that has taken place.

The major objectives of these investigations were: the determination of the nature of the volatile components of vacuum-packed stored dehydrated pork, the development of a chemical method for evaluating the relative degrees of deterioration that occur in dehydrated pork stored for various time intervals at different temperatures, and the identification of the specific compound(s) present in the volatile fractions of dehydrated pork samples.

Some of the dehydrated pork samples were stored at 160° F. for one week in order to induce rapidly changes similar to those that occur in cans of meat stored at lower temperatures over longer periods.

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Determination of Total Volatile Basic and Reducing Substances by Aeration Techniques

Materials and Reagents

Vacuum-Packed Stored Dehydrated Pork

The pilot plant preparation, dehydration, and vacuum canning of the dehydrated pork were done at the Wilson and Co. Research Laboratories in Chicago in July 1952. Lean trimmings from fresh pork shoulders were ground through a 1-inch plate, and then cooked, without the addition of water, in a steam-jacketed kettle at a pressure of approximately 20 pounds per square inch 30 minutes with an internal temperature of the batch not exceeding 170° F. The meat was then ground through a $^1/_8$-inch plate, spread on trays to a thickness of $^3/_4$ inch, and placed in a three-stage vacuum dehydrator with a coil temperature of 160° F. After dehydration for 4 hours at a pressure of less than 50 microns, the meat was packed into cans of $3^3/_4$- and 8-ounce capacity and vacuum-sealed. The cans of meat were delivered to the University of Missouri Laboratories and stored at —20°, 100°, and 160° F. for various time periods.

Proximate Analysis of Dehydrated Pork. The average values for the proximate analysis of the dehydrated pork prepared in 1952 were:

Moisture, %	5.38
Fat, %	37.78
Protein, %	54.67
Reducing sugar, mg./100 g.	67

Preparation of Reagents

Potassium Permanganate, 0.11*N*

Three and one-half grams of crystals were dissolved in 1 liter of double-distilled water, and the resulting solution was heated to near boiling for 1 hour in a 2-liter beaker covered with a watch glass. The solution was allowed to stand overnight at room temperature in the covered beaker. This final solution was decanted into a dark-colored reagent bottle and standardized with sodium oxalate.

Ferrous Ammonium Sulfate, 0.11*N*. Forty-four grams of ferrous ammonium sulfate hexahydrate were dissolved in 1 liter of 1*N* sulfuric acid prepared with double-distilled water (30 ml. of concentrated sulfuric acid per liter). The resulting solution was standardized with 0.1*N* potassium permanganate.

Ceric Sulfate, 0.01*N*. Approximately 0.1*N* ceric sulfate was prepared and diluted 1 to 10 to give the 0.01*N* reagent. The 0.1*N* ceric sulfate was prepared as follows: Approximately 105.6 grams of ceric sulfate [G. Frederick Smith Chemical Co. $Ce(HSO_4)_4$] were dissolved in 1 liter of double-distilled water containing 60 ml. of concentrated sulfuric acid (solution was effected by stirring vigor-

ously with an electric stirrer). The resulting solution was diluted to 2 liters with double-distilled water, and filtered by gravity into a large reagent bottle. This solution was standardized with 0.11N ferrous ammonium sulfate using phenanthroline indicator (G. Frederick Smith Chemical Co.).

Sodium Hydroxide, 0.02N. Approximately 0.02N sodium hydroxide was prepared by dissolving 1.6 grams of pellets in freshly boiled distilled water and diluting to 2 liters. The resulting solution was standardized with potassium acid phthalate.

Sulfuric Acid, 0.02N. Approximately 0.02N sulfuric acid was prepared by dissolving 1.11 ml. of concentrated acid in water and diluting to 2 liters. The normality was checked with 0.02N sodium hydroxide.

Apparatus and Methods

Aeration Apparatus

An aeration apparatus similar to that used by Lang and others (*11*) was assembled for this investigation (Figure 1).

The aeration flask consisted of a 1-liter round-bottomed flask containing a 24/40 standard-taper glass joint and modified as follows: A 20-ml. test tube was sealed to the lower end. A 500-ml. round-bottomed flask containing a safety tube was sealed at the upper end near the glass joint. The inlet tube was made by sealing a length of capillary tubing onto a male 24/40 joint.

The reaction flasks were constructed from 250-ml. Erlenmeyer flasks containing 24/40 glass joints. The side arms consisted of a length of tubing containing the male part of a ball and socket joint. The inlet tubes were made by sealing a length of capillary onto the lower end of a 24/40 male joint, and attaching a length of tubing containing the female part of a ball and socket joint to the upper end of the 24/40 joint. Smaller values for blank runs were noted when

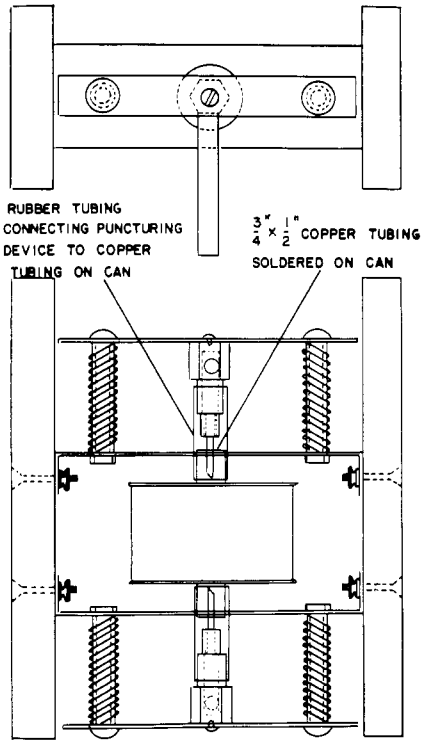


Figure 2. Can-puncturing device

rubber tubing connections were made as short as possible or replaced by glass joints.

Approximately 10-gram samples of dehydrated meat either in their normal granular consistency or as slurries were placed in the aeration flask. Each of the reaction flasks contained 10 ml. of standard permanganate (0.1N) and 10 ml. of 2N sodium hydroxide or 15 ml. of 0.02N sulfuric acid in addition to 25 to 50 ml. of water. The vacuum pump was turned on with stopcock B at the large trap in its open position, and the three-way stopcock, A, was set to allow the flow of air from the washing bottles to pass through the system. After 5 to 10 minutes, the pressure in the system was adjusted to 640 mm. by means of stopcock B. The pressure remained fairly constant throughout an aeration period of 3 hours. In order to prevent the drawing back of solution from the reaction flasks, the following precautions were carried out before turning off the vacuum pump: Stopcocks A and B were turned to their open positions, and the inlet tube to the first reaction flask was disconnected.

After aeration, the alkaline permanganate was made acid with 15 ml. of 6N sulfuric acid, and 15 ml. of 0.11N ferrous ammonium sulfate were added, followed by 1 drop of phenanthroline indicator. The excess reducing agent was titrated with 0.01N ceric sulfate. The results were expressed in terms of the number of microequivalents of the standard permanganate reduced. The 0.02N sulfuric acid was titrated to a methyl red end point with 0.02N sodium hydroxide. These results were expressed as milliequivalents of standard acid neutralized.

As noted in Figure 1, the incoming air was passed through a train of bubblers: concentrated sulfuric acid, distilled water, three bubblers containing 10% alkaline permanganate, and finally distilled water.

Calibration of Flowmeters. Two flowmeters were calibrated to give a flow rate of air approximating 56 to 58 liters per hour. They were calibrated with an air flow obtained by displacing air with water, and maintaining a constant flowmeter mercury level by variation of the manometer reading.

Can-Puncturing Device. A can-puncturing device designed at the Missouri Experiment Station Laboratories for aeration purposes has been used with success. Its operation is indicated in Figure 2. The insertion of this device into the aeration apparatus permitted aerations on cans of dehydrated pork without exposure of the contents to the atmosphere.

The can-puncturing device replaces the aeration flask, while other parts of the original aeration apparatus remain unchanged. Because the cans of meat had been closed at a reduced pressure, it was necessary to puncture them first at the end nearest the washing bottles. This permitted the adjustment of the pressure in the can to that of the atmosphere by a flow of air through the washing bottles into the can.

Prior to puncturing the cans, the vacuum pump was turned on and the manometer adjusted to read approximately the same pressure as during the normal aeration. Immediately after one end of the can had been punctured as previously mentioned, the other end was punctured and aerations were carried out at a normal rate for various periods of time.

Figure 1. Aeration apparatus

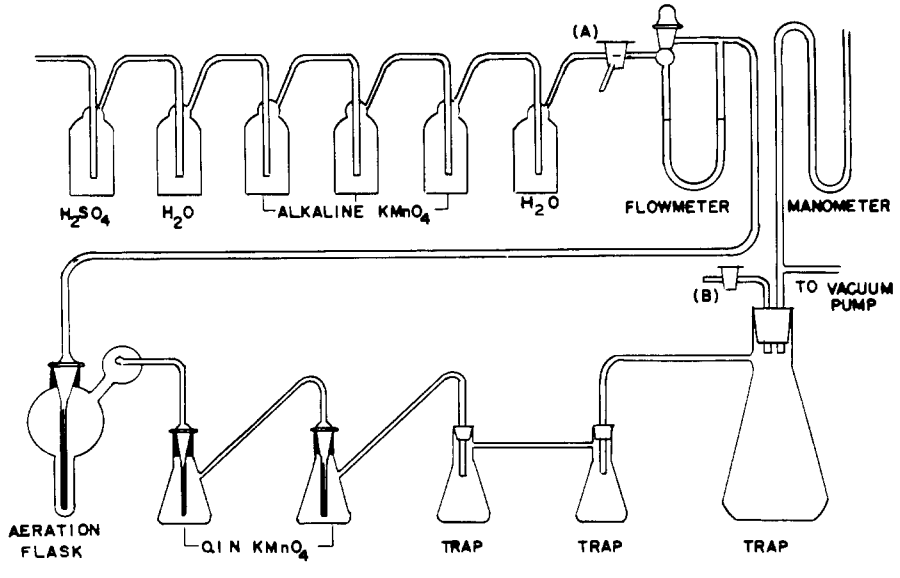


Table I. Microequivalents of Reduction for Various Samples of Dehydrated Pork[a]

Lab. No.	Description	Storage Conditions	Odor and Color	Microequiv. of Reduction
A	Dehydrated	100° F., 36 weeks	Disagreeable odor, some browning	18.0, 17.5
B	Dehydrated	−20° F., 36 weeks	Slight odor, light color	23.5, 23.4
17	Not dehydrated	−20° F., 1 week	Little color or odor	19.0, 19.7
17	Dehydrated	160° F., 1 week	Reddish brown color, disagreeable odor	18.0, 20.5

[a] 10-gram samples.

Table II. Reducing Power and Basicity of Vacuum-Packed Stored Dehydrated Pork[a]

Storage Temp., ° F.	Length of Aeration, Hours	Reduction, μeq.	Basicity, Meq.
−20	1	39.1	...
160 (1 week)	1	125.2	...
−20	3	...	0.074
160 (1 week)	3	...	0.18

[a] Per 8-ounce can.

Results and Discussion

The results of the 60-minute aerations (10 grams) are shown in Table I. Samples A and B were placed in the aeration flask in their normal granular consistency. Samples 17 were blended with 50 ml. of double-distilled water in a Waring Blendor for 10 minutes. During the preparation, the temperature of the slurries varied between 45° and 59° C., depending on the room temperature, which ranged from 19° to 29° C. The slurries were transferred to the aeration flask and aerated as such. All samples were held unopened at room temperature for approximately 2 hours prior to weighing.

The results shown in Table I do not give a true evaluation of the condition of the meat when the cans were opened. A disagreeable odor was noted in dehydrated pork stored at 100° and 160° F., but only a slight odor in meat stored at −20° F. This indicated that some of the odor-producing components were very volatile, and were lost on opening the cans. Thus, to give a true objective rating to the meat samples, a method was required for determining the headspace gases present in the cans of dehydrated pork. A can-puncturing device was needed in order to prevent exposure of the meat prior to aeration.

Aerations with the can-puncturing device were made on meat stored at −20° and 160° F. for 1 week (Table II). In each case a blank aeration was carried out for the same length of time as the sample aeration, on cans aerated in a vertical position. Significant differences were obtained. Storage of dehydrated pork at an elevated temperature resulted in the evolution of greater amounts of volatile basic and reducing materials than when held at lower temperatures.

Solutions of ammonia, propionaldehyde, and the methylamines were aerated to obtain data for comparison purposes. Ammonia could be formed in samples of dehydrated pork from deamination of amino acids by aerobic and anaerobic microorganisms. Carbonyl-type compounds may result from fat oxidation, the oxidative deamination and decarboxylation of amino acids, and the decarboxylation of keto acids. Amines may be present due to anaerobic decarboxylation of amino acids (Table III).

Table III. Reducing Power and Basicity of Individual Compounds

Compound	Reduction, μeq.	Basicity, Meq.
Ammonia (0.346 meq.)	None	0.329
Methylamine (0.0018 g.)	34.4	0.172
Dimethylamine·HCl (0.006 g.)	244[a]	0.089
Trimethylamine·HCl (0.0025 g.)	130[a]	0.050
Propionaldehyde (0.003 g.)	220	None

[a] Solutions made basic prior to aeration.

Detection of Carbonyls and Determination of Total Monocarbonyl

This phase of the dehydrated pork studies reports the investigations undertaken to prove the existence of carbonyl compounds in the volatile fractions, and gives in detail the method adopted for determination of total monocarbonyl in benzene extracts of dehydrated pork samples.

Reagents

Carbonyl-Free Methanol and Ethanol. Carbonyl-free methanol and ethanol were prepared as follows: Five hundred milliliters of C.P. alcohol, 5 grams of 2,4-dinitrophenylhydrazine (2, 4-DNP), and 5 drops of concentrated hydrochloric acid were placed in a 1-liter glass-jointed round-bottomed flask. A condenser containing a calcium chloride drying tube at its upper end was attached to the flask, and reflux was carried out for 2 hours on the steam bath. A fractionating column was then connected to the flask, and the condenser was set up for distillation. The receiving flask consisted of a 1-liter distillation flask connected to the condenser by means of a cork stopper. A calcium chloride drying tube was attached to the side arm of the distillation flask. Distillation was carried out on the steam bath.

Aqueous-Alcoholic Potassium Hydroxide, 10%. Ten grams of analytical reagent potassium hydroxide pellets were dissolved in 20 ml. of double-distilled water, and the resulting solution was diluted to 100 ml. with carbonyl-free methanol.

Purification of 2,4-Dinitrophenylhydrazine. To purify the 2,4-dinitrophenylhydrazine used for the Lappin and Clark test, approximately 5 grams of reagent grade 2,4-dinitrophenylhydrazine were placed in a 500-ml. round-bottomed flask with 150 ml. of carbonyl-free methanol, and the resulting mixture was refluxed for 30 minutes on the steam bath. The hot 2,4-dinitrophenylhydrazine solution was then filtered through a preheated Büchner funnel and allowed to cool to room temperature and the crystals were collected. Recrystallization was carried out a second time in the same manner. The resulting crystals were collected on a Büchner funnel, air-dried, and placed in a desiccator for future use.

The 2,4-dinitrophenylhydrazine used for determination of total monocarbonyl according to the method of Pool and Klose (*15*) was purified as follows. One gram of 2,4-dinitrophenylhydrazine was dissolved in 500 ml. of benzene, and the resulting mixture was refluxed and filtered. Recrystallization was carried out a second time in the same manner, and the crystals were collected and dried as directed above.

Absolute Alcohol. The absolute alcohol used in the total monocarbonyl determinations was prepared using calcium oxide according to the directions of Fieser (*3*).

Methods and Materials

Detection of Carbonyls Using Aeration Techniques. Four milliliters of an aqueous solution of propionaldehyde (0.0003 gram of aldehyde per ml. of solution) were aerated for 60 minutes using the aeration apparatus as described above. The resulting gases were collected in 20 ml. of a saturated, carbonyl-free, methanolic solution of 2,4-dinitrophenylhydrazine containing 5 drops of concentrated hydrochloric acid. After the aeration, the contents of the reaction flask were transferred to a large

test tube, and the volume was made to 25 ml. with methanol. The tube was lightly stoppered and heated on the steam bath for 5 minutes. After cooling, 10 ml. of the resulting solution were made alkaline by the addition of 5 ml. of a 10% aqueous-alcoholic solution of potassium hydroxide. A black color formed immediately, and within 1 minute a wine-red color was obtained. A blank aeration resulted in the formation of a light yellow-green colored solution.

This color test for traces of carbonyl compounds was developed by Lappin and Clark (*12*). The method has been used in conjunction with aeration methods for detecting carbonyl compounds in the volatile fraction of vacuum packed stored dehydrated pork.

Total Monocarbonyl Determinations. A method using column chromatography in conjunction with colorimetry has been utilized for the quantitative determination of total monocarbonyls in benzene extracts of dehydrated pork (*15*).

The chromatographic tubes shown in Figure 3 were packed with deactivated alumina, which had been prepared by thoroughly mixing reagent alumina with alumina that had been hydrated in a desiccator over water for several hours. The final mixture contained 15 to 25 grams of hydrated alumina per 100 grams of total mixture.

The deactivated alumina was added to the column to a depth of 3 cm.; then 6 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in benzene were added. More deactivated alumina was added to the column to a depth of about 10 cm. By this procedure the alumina was packed by the flow of benzene through the column. The 2,4-dinitrophenylhydrazine was adsorbed by the alumina and cannot be eluted with benzene. When all of the 2,4-dinitrophenylhydrazine solution had entered the column, 4 ml. of benzene were added for washing. A second 4-ml. portion of benzene was added after all of the first portion had entered the column. Two milliliters of the standard solution of a carbonyl or of the meat extract were added and collection of the eluate in a 25-ml. graduated cylinder was begun. Elution with benzene was continued until 19 ml. of eluate were collected. The eluate contained the mono- 2,4-dinitrophenylhydrazone (2,4-DNPH) derivatives which were formed in the column. According to Pool and Klose, dicarbonyl derivatives cannot be eluted with benzene.

The eluate was transferred to a 50-ml. colorimetric test tube. To the tube were then added 6 ml. of a solution of potassium hydroxide in ethanol prepared by dissolving 6 grams of potassium hydroxide pellets in 100 ml. of carbonyl-free absolute alcohol and filtering through a sintered-glass funnel. Solution was effected by heating the alkali-alcohol mixture contained in a 250-ml. glass-stoppered flask on a hot plate. Attached to the flask was an air condenser. Atmospheric moisture was excluded by connecting a calcium chloride drying tube to the upper end of the condenser.

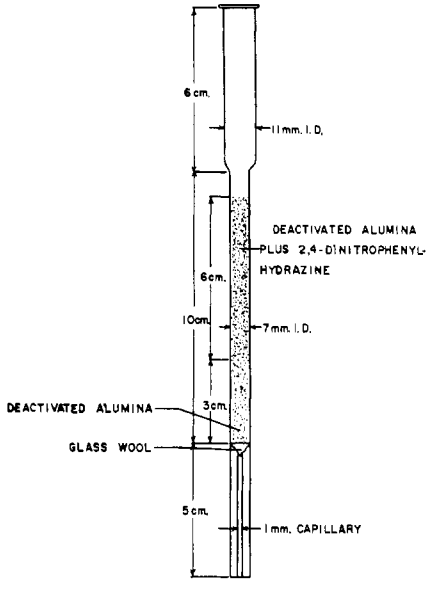


Figure 3. Column for quantitative carbonyl determination

After the addition of the potassium hydroxide solution (final volume, 25 ml.), thorough mixing resulted in the formation of a color ranging from a sherry-wine to a red-wine color, depending on the concentration of the monocarbonyl derivatives present. The per cent transmittance of each solution was read on the Evelyn colorimeter using the 420 filter. In each case, the instrument was set at 100% transmittance with a blank obtained by passing benzene through a column containing 2,4-dinitrophenylhydrazine and treating the resulting eluate in the same way as a standard or unknown solution.

The following carbonyl compounds were subjected to the preceding method of analysis for the preparation of a standard curve: propionaldehyde, heptaldehyde, isovaleraldehyde, and 4-methyl-2-pentanone. In each case, the shelf reagent was fractionally distilled. A 1° to 2° fraction was collected for preparing the standard solutions.

For each carbonyl, a benzene solution (solution A) was prepared containing the calculated amount of the carbonyl to give solutions $5.6 \times 10^{-3} M$. Solution A was diluted 1 to 100 with benzene to give a solution $5.6 \times 10^{-5} M$. Two milliliters of the latter solution were passed through the column, the final volume being 25 ml.—a dilution of 1 to 12.5. This gave solution B with a final concentration of $4.5 \times 10^{-6} M$. In addition, the following solutions were prepared and passed through the column:

C. Final concentration $9.0 \times 10^{-6} M$; 2 ml. of A were diluted to 100 ml. and 2 ml. of this chromatographed.

D. Final concentration $18 \times 10^{-6} M$; 4 ml. of A were diluted to 100 ml. and 2 ml. of this chromatographed.

E. Final concentration $27 \times 10^{-6} M$; 6 ml. of A were diluted to 100 ml. and 2 ml. of this chromatographed.

F. Final concentration $13.5 \times 10^{-6} M$; 3 ml. of A were diluted to 100 ml. and 2 ml. of this chromatographed.

The results may be observed on the curve of absorbance *vs.* concentration ($M \times 10^{-6}$) (Figure 4). For each carbonyl, three separate runs were carried out on different days.

The total monocarbonyl determinations were carried out on 8-ounce cans of vacuum-packed dehydrated pork stored at −20°, 100°, and 160° F. for various time intervals.

Sampling Procedure. The contents of a can (8 ounces) were transferred to a wide-mouthed bottle and thoroughly mixed. A 10-gram sample was weighed into a 250-ml. glass-stoppered Erlenmeyer flask, 10 ml. of benzene were added to the sample in the flask, and the flask was stoppered and shaken continuously by hand for 3 minutes. The resulting mixture was then filtered, using suction, through a muslin filter pad on a Büchner funnel. The residue on the funnel was washed with 25 ml. of benzene and the resulting filtrate transferred to a 100-ml. volumetric flask and made to volume.

Two-milliliter aliquots of the diluted extracts were used for analysis. As with the standards, 19 ml. of eluate were collected, and to this were added 6 ml. of alkaline ethanol. In each case, the samples were run in triplicate.

Results and Discussion Eight-ounce cans of vacuum-packed dehydrated pork stored at −20° and 160° F. for 1 week were aerated for the detection of volatile carbonyls. Carbonyls were not detectable in the −20° F. sample. The volatile fraction of the 160° F. sample yielded an amber color in the test for carbonyls; this was indicative of a positive test. The rapid fading of the color rendered the procedure unsatisfactory for quantitative determinations.

The results of the determinations of the total monocarbonyl content in benzene extracts of dehydrated pork are given in Table IV. There is good agreement of results among the individual samples. No two samples came from the same can, and no two samples were run on the same day.

The average per cent transmittance readings were converted to absorbance, and the corresponding molar concentrations were taken from an average of the two lines shown on Figure 4. An average molecular weight of 100 was assumed for the carbonyls present, and the total

Table IV. Comparison of Total Monocarbonyls per 8-Ounce Can of Meat

Sample	Storage Conditions	% T	Average % T	Total Monocarbonyls per 8-Ounce Can, Mg.
I	−20° F.	77.3 73.3 72.5	74.4	18.6
II	100° F., 12 months	64.5 64.0 64.5	64.7	28.7
III	160° F., 1 week	51.5 46.4 52.6	50.2	43.7

monocarbonyls per 8-ounce can calculated. The data (milligrams of carbonyls per 8-ounce can) in Table IV are presented for comparative purposes only. The results clearly show that the total monocarbonyl content increased with higher storage temperatures.

Identification of Acetaldehyde and Ammonia in Volatile Fraction

Materials and Reagents

Laboratory-Prepared Vacuum - Packed Stored Dehydrated Pork. In order positively to identify the volatile carbonyl compounds detected in samples of dehydrated pork as described above, it was necessary to prepare approximately 10-pound quantities of dehydrated pork and to store the meat in specially designed flasks. Approximately 80 pounds of defatted hams were ground through a 1-inch plate, frozen, and then allowed to thaw for 24 hours at 38° F. prior to cooking. The meat was cooked in a steam-jacketed kettle for 30 minutes with continuous stirring. The steam pressure was gradually increased from 5 to 25 pounds per square inch during the cooking period. A small amount of water was added to the meat during cooking to prevent "case-hardening." After cooking, the meat was ground through an $^1/_8$-inch plate, spread on trays, and cooled at 38° F.

In the initial dehydration phase, the meat was spread in a thin layer on 2 × 43 × 63 cm. trays (about 1.0 to 1.3 pounds per tray), and placed in the convection oven set at 65° C. Dehydration was then carried out for 8 hours. The meat was then transferred to two 22-liter round-bottomed flasks and one small vacuum desiccator. Each flask was fitted with a 45/50 standard-taper female glass joint, to which a 45/50 male joint was fitted, containing both inlet and outlet tubing typical of the common laboratory gas-washing bottle. Both inlet and outlet tubing contained high-vacuum stopcocks. The inlet tubing extended about two-thirds of the depth of the flask. A sample was placed in the desiccator for determination of fat, protein, moisture, and trace elements, in order to avoid opening the round-bottomed flasks during the storage period.

Approximately 10 pounds of meat were placed in each flask and 1 pound in the desiccator. The flasks and desiccator were then immersed in a water bath (55° C.) and attached to a vacuum pump. Evacuation was carried out for 10 to 12 hours with a manometer reading of 1 cm. One 10-pound sample of dehydrated pork was stored at −20° F. and the second at 94° F.

Proximate Analysis of Dehydrated Pork. The values for the proximate analysis of the laboratory-prepared and vacuum-packed stored dehydrated pork were:

Moisture, %	2.95
Fat, %	23.47
Protein, %	68.31
Ash, %	3.08
Reducing sugar, mg./100 g.	70

Element analysis, p.p.m. of ash

Cu	50	Mn	100
Zn	300	Mo	—
Co	—	B	—
Fe	1000		

Saturated Solution of 2,4-Dinitrophenylhydrazine in 2*N* Hydrochloric Acid. Twice recrystallized 2,4-dinitrophenylhydrazine was added in excess to 2*N* hydrochloric acid, and stirred at room temperature with an electric stirrer. The saturated solution was then filtered by gravity into a standard reagent bottle and stored for future use.

Ferrous Ammonium Sulfate Solution, approximately 0.2*N*, was prepared by dissolving the salt in 1*N* sulfuric acid.

Fieser's Solution. This is a solution of sodium anthraquinone β-sulfonate (2 grams) and sodium hydrosulfite (15 grams) in aqueous potassium hydroxide (20 grams of potassium hydroxide per 100 ml.). It was prepared according to the method of Fieser (*4*).

Saturated Lead Acetate. This was prepared by saturating water at room temperature with basic lead acetate.

Apparatus and Methods

Collection of Volatile Carbonyls. The apparatus used for preliminary experimentation in the collection of volatile fractions is outlined in Figure 5.

The 10-pound flasks of dehydrated meat were removed from storage after 8 weeks at −20° and 94° F., and placed in the train shown in Figure 5 for aerations. The resulting gases were collected by passing them through two washing tubes, each of which contained 50 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid. The washing tubes were 45 mm. in diameter and 5.5 inches in depth. They contained a standard-taper 45/50 female joint, and were fitted with an inlet-outlet connection from a standard gas-washing bottle by means of a 45/50-29/42 standard-taper reducing joint. The aerations were carried out with the collection tubes and meat samples at room temperature.

After the flasks had been placed in the aeration train, the pressure was equalized by opening the stopcock on the inlet tube of the flask of meat with the washing bottles exposed to the atmosphere. Nitrogen from a cylinder was then passed into the washing bottles at a pressure of about 2 pounds per square inch. The stopcock attached to the outlet tube of the flask of meat was opened immediately after the nitrogen had been introduced into the aeration train. Aeration at a pressure of 2 pounds per square inch was carried out for 4 hours.

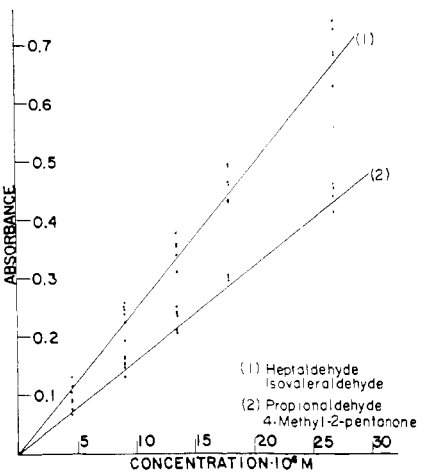


Figure 4. Analytical curve for carbonyls

At the end of 4 hours, both stopcocks were closed and the aeration was discontinued. Ten liters of petroleum ether (boiling point 65° to 110° C.) were poured into a carboy, which was placed on a stand so that it was higher than the flask of meat. The petroleum ether was siphoned onto the dehydrated pork with both stopcocks open. When all of the solvent had entered the flask, the flask was connected back into the train, and the slurry aerated for 4 more hours. The total aeration time was 8 hours. Twelve cubic feet of nitrogen were passed through the system.

After aeration, the contents of the two washing tubes were combined (a total of

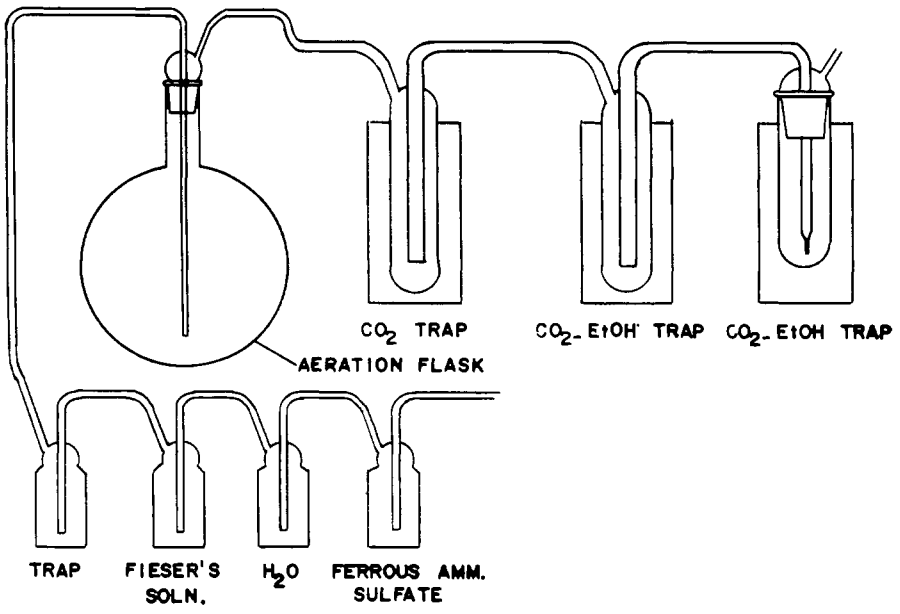


Figure 5. Apparatus for collection of volatile constituent

100 ml. of reaction medium) and transferred to a 500-ml. separatory funnel.

Purification of 2,4-Dinitrophenylhydrazone Derivatives. The 100-ml. of reaction medium were washed with three 100-ml. portions of ethyl ether, and the combined ether extracts were washed with three 100-ml. portions of water. The washed ether extract was then concentrated to 15 ml. on the steam bath. To the concentrated ether solution 15 ml. of 95% ethanol were added. The resulting ether-ethanol solution was then concentrated to approximately 12 ml. on the steam bath, and allowed to stand overnight at room temperature.

Orange crystals separated from the concentrated ethanol solution on standing overnight, and were washed onto a Büchner funnel with cold, 80% ethanol. The crude material was then dissolved in 25 ml. of benzene and applied to a 2.5 × 28 cm. column packed to a height of 7.5 cm. with alumina which contained 26% deactivated alumina. One wide band appeared on the column on elution with 170 ml. of benzene.

The resulting benzene eluate was concentrated to 8 to 10 ml. on the steam bath, 10 ml. of 95% ethanol were added to the concentrated benzene solution, and the benzene-ethanol solution was concentrated to 8 to 10 ml. on the steam bath. This final concentrated solution was set aside and allowed to stand overnight at room temperature. Yellow-orange needles separated on standing, and to ensure more complete crystallization the resulting mixture was cooled to 0° C. for several hours.

The yellow-orange needles were finally collected on a Büchner funnel and washed with suction. From the meat stored at 94° F. 23.5 mg. of a 2,4-dinitrophenylhydrazone derivative of melting point 155.5° C. were obtained; from the meat stored at −20° F. about 1 mg. of derivative of melting point 157.5° C. was obtained.

Collection of Volatile Basic Constituents. The apparatus shown in Figure 6 was used for the collection of volatile basic constituents. Approximately 1125 grams of dehydrated pork (prepared in the Wilson and Co. laboratory, Chicago), previously stored for 1 week at 160° F., were placed in the three-necked flask. A solution of 22.5 grams of potassium carbonate in 3800 ml. of water was added to the flask. The resulting slurry had a pH of 7.5. Nitrogen was passed through the system at a rate of 60 liters per hour for 7 hours, during which the slurry was stirred. The resulting solution in the reaction flask (30 ml. of ethanol plus 5 drops of concentrated hydrochloric acid) was transferred to a small Erlenmeyer flask and taken to dryness on the steam bath; 0.01 mg. of a grayish white material was obtained. An attempt was made to take a melting point of this material. It did not melt at a temperature of 285° C.

Two 8-ounce cans of dehydrated pork (Wilson and Co. laboratory, Chicago) previously stored at 100° F. for 19 hours were aerated for 60 minutes each, using the aeration apparatus and can-puncturing device described. The resulting gases were passed through 30 ml. of ethanol containing 2 drops of concentrated hydrochloric acid. After being transferred to a 50-ml. Erlenmeyer flask, the resulting solution was taken to dryness on the steam bath. A heterogeneous residue containing white granular material and a yellow material was obtained, which appeared to be partially crystalline. The residue was subjected to paper chromatographic analysis (*5*) using the method originated by Schwyzer for aliphatic amines (*17*). An R_f value of 0.20 was obtained for both the unknown hydrochloride and ammonium chloride.

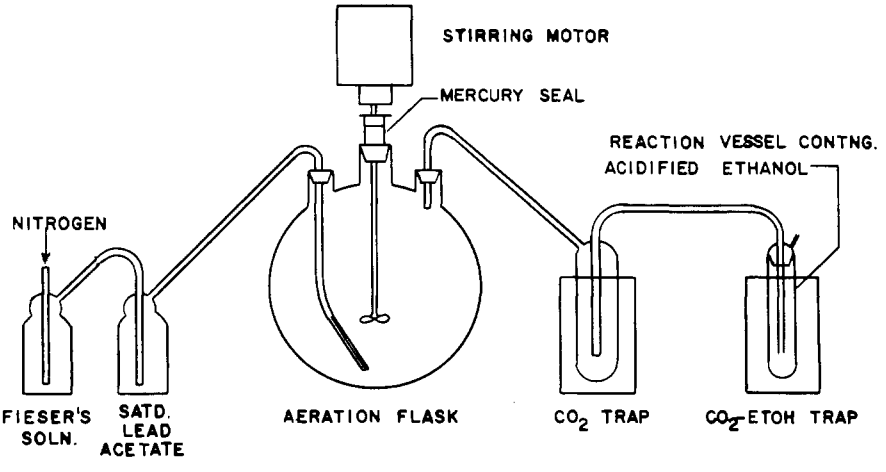
Results and Discussion The 2,4-dinitrophenylhydrazone derivative of melting point 155.5° C. isolated from the dehydrated pork stored at 94° F. and the 2,4-dinitrophenylhydrazone derivative of melting point 157.5° C. isolated from the −20° F. stored meat were mixed together and found to melt at 157.5° C. Each of the isolated 2,4-dinitrophenylhydrazone derivatives was subjected to mixed melting point determinations with laboratory-prepared acetaldehyde 2,4-dinitrophenylhydrazone of melting point 157.5° C. In both cases the resulting melting point was 157.5° C.

The 2,4-dinitrophenylhydrazone derivatives isolated from the dehydrated pork stored at −20° and 94° F. were subjected to paper chromatographic analysis (*5*) by the method originated by Meigh (*13*). An R_f value of 0.45 was obtained for both unknown 2,4-dinitrophenylhydrazone's and for the known acetaldehyde 2,4-dinitrophenylhydrazone.

As further proof that both of the isolated 2,4-dinitrophenylhydrazones were derivatives of acetaldehyde, absorption spectra were obtained for the following:

2,4-Dinitrophenylhydrazine
Acetaldehyde 2,4-dinitrophenylhydrazone

Figure 6. Apparatus for collection of basic component



2,4-dinitrophenylhydrazone from dehydrated pork stored at 94° F.
2,4-dinitrophenylhydrazone from dehydrated pork stored at −20° F.

The absorption spectra were observed in the range of 390 to 210 mμ by means of the Cary recording spectrophotometer, Model 11. The solvent used was 95% ethanol. The compounds were measured at a concentration of 0.023 to 0.025 mg. per ml.

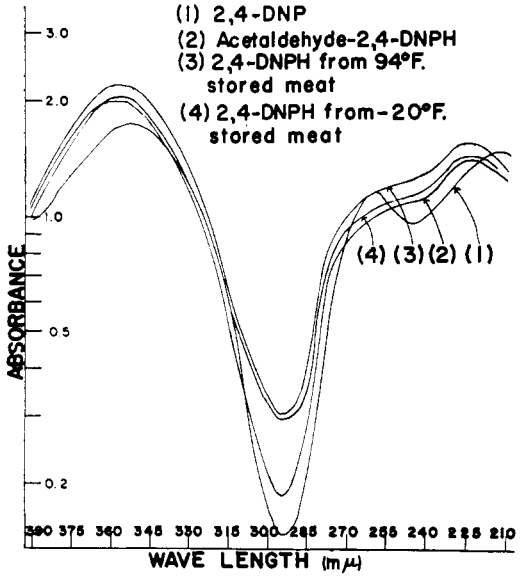


Figure 7. Absorbance curves for 2,4-dinitrophenylhydrazine and 2,4-dinitrophenylhydrazone

The absorption spectra are shown in Figure 7. The spectra of 2,4-dinitrophenylhydrazine and acetaldehyde 2,4-dinitrophenylhydrazone are decidedly different, but the spectra of acetaldehyde 2,4-dinitrophenylhydrazone and of the isolated 2,4-dinitrophenylhydrazone derivatives are similar. The criterion used in comparing the spectra was the ratio between the absorbance values observed at the two maxima on the same curve. In this case, a value of 1.42 was obtained for the ratio for all three 2,4-dinitrophenylhydrazone curves when comparing the maximum at 356 mμ to that observed at 224 mμ. Thus, acetaldehyde was shown to be present in meat stored at −20° F. as well as at 94° F.

Acetaldehyde may be formed in biological materials by the oxidation of fats, the oxidative deamination and decarboxylation of alanine, or the decarboxylation of pyruvic acid.

Storage in a vacuum is an effective method for inhibiting the oxidation of fats. Earlier investigators have stated, however, that it is not an absolute safeguard against the oxidation of some fats (*9*). Fat oxidation could occur to a small degree without the production of an intense tallowy odor. Gortner has mentioned that, whereas the oxidation of oleic acid is mainly responsible for the tallowy odors in fats, the oxidation of linoleic acid produces less off-odors, and the oxidation of linolenic acid produces a very slight amount of off-odors (*8*).

Alanine has been shown to be present in extracts of dehydrated pork (*5*). According to Karrer, amino acids may be decomposed by anaerobic microorganisms to yield aldehydes of one less carbon atom as intermediate products (*10*).

Pyruvic acid may be formed from the oxidative deamination of alanine (*21*) and from the anaerobic dissimilation of glucose (*19*). Pyruvic acid was not detected in extracts of dehydrated pork, but evidence of the existence of lactic acid was obtained (*5*). A number of species of anaerobic bacteria are capable of forming lactic acid from pyruvic acid (*20*); hence, pyruvic acid may have been present to a small extent in the samples of dehydrated pork. Pyruvic acid could be the precursor of acetaldehyde, because pyruvate may be anaerobically decarboxylated by yeast to form acetaldehyde (*7*).

Very good evidence has been obtained for the presence of ammonia in the volatile fraction of dehydrated pork stored at 100° F. for 19 hours, and in meat stored at 160° F. for 1 week. A hydrochloride salt was obtained from the 100° F. stored meat, which exhibited an R_f value on paper corresponding to that of ammonium chloride. The hydrochloride salt isolated from the meat stored at 160° F. had a melting point greater than 285° F., which is higher than the melting points of the hydrochloride salts of the lower, volatile amines.

Ammonia could result from both aerobic and anaerobic deamination of amino acids by microorganisms (*6*). The aerobic reactions result in the production of a saturated acid possessing one less carbon atom than the original acid or in production of a hydroxy acid having the same number of carbon atoms as the original amino acid. Anaerobic deamination may occur with and without reduction, forming saturated and unsaturated acids.

Summary

Methods were developed for the aeration of dehydrated vacuum-packed pork samples. Using aeration techniques, the volatile components of the stored pork were found to have both basic and reducing properties. At higher storage temperatures there was an increase in the volatile constituents. Theoretical considerations indicated the possibility of the existence of ammonia, amines, and carbonyl-type molecules in the volatile fraction. The total basicity and reducing values for these compounds corresponded closely to those found for the volatile fractions of meat and could therefore account for the values observed. Comparisons with the basic and reducing values of known compounds suggested that the basic compounds contributed a minor part to the total reduction and that other volatile compounds may have been present in greater amounts, as was indicated by the reducing value for propionaldehyde. There was no evidence to exclude the existence of volatile acidic components.

Using aeration techniques in conjunction with a colorimetric scheme, a positive test for carbonyls was obtained from the volatile fraction of vacuum-packed dehydrated pork stored at 160° F., but not from the meat stored at −20° F. Total monocarbonyl determinations carried out on benzene extracts of the meat samples showed that carbonyl compounds were present in all stored samples of dehydrated pork, and that the content increased with a rise in storage temperature.

A 2,4-dinitrophenylhydrazone derivative was isolated from the reaction flask after 10-pound samples of vacuum-packed stored dehydrated pork had been aerated into solutions of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid. The amount of the 2,4-dinitrophenylhydrazone derivative isolated from the dehydrated pork stored at − 20° F. was about 1 mg., whereas 23.5 mg. of derivative were isolated from the dehydrated pork stored at 94° F. The 2,4-dinitrophenylhydrazone derivatives were positively identified as acetaldehyde 2,4-dinitrophenylhydrazone by means of melting points, mixed melting points, paper chromatography, and absorption spectra.

Hydrochloride salts were formed when 1 pound of dehydrated pork (100° F. for 19 hours) was aerated into acidified ethanol. Paper chromatographic analysis of the residue gave an R_f value for both the unknown hydrochloride and ammonium hydrochloride.

Further evidence of the existence of ammonia in the volatile fraction was obtained by aerating 1125 grams of meat (160° F. for 1 week) into acidified ethanol. The resulting salt had a melting point greater than 285° F., which is higher than the melting points of the hydrochloride salts of the lower amines.

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NUTRITIVE VALUES OF CROPS

Nutrient Content and Protein Quality of Quinua and Cañihua, Edible Seed Products of the Andes Mountains

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Quinoa (*Chenopodium quinoa,* Willd) and cañihua (*Chenopodium pallidiculae*) produce edible seeds that have been included in the diets of the Andean Indians for centuries. They are cultivated by primitive methods, usually at altitudes above 8000 feet. The total estimated production of both quinoa and cañihua in Peru is approximately 50,000 tons. Rat growth studies using either young rats or depleted adult rats showed that at equal levels of protein intake the proteins of quinoa and cañihua produced weight gains equal to or superior to those obtained with dried whole milk. Mixtures of quinoa and milk did not produce greater gains than quinoa alone. The excellent over-all nutritive value of these products and particularly the high quality of their proteins are emphasized.

Quinua (*Chenopodium quinoa*, Willd) and cañihua (*Chenopodium pallidiculae*) are plants which have been growing, either wild or cultivated, in the high Andes Mountains since long before the time of the Inca Empire. While believed by many to be native to this area, these or similar plants are found in many regions of the world (*17*). They are stock plants, 4 to 6 feet high, with large clusters of small seeds produced at the end of the stock. The seeds of both are edible and have been included in the diets of Andean Indians for centuries, although probably less so now than in the time of the Incas. Primitive agricultural methods are still used by the indigenous population in the cultivation of these products. The nature of the plants and the fact that the small seeds (2 to 4 mm. in diameter) are very loosely attached to the stock would probably make mechanized production difficult. Among other than the Andean Indians these foods have low prestige value. Many varieties of each are known; certain types of quinua possess the disadvantage that the seeds are covered with a saponin, which has a very bitter taste and must be removed prior to use by vigorous washing and scrubbing.

The total yearly production of quinua in Peru is estimated as approximately 40,000 tons and that of cañihua as 10,000 tons (*4*). Yields of quinua in the order of 440 to 800 pounds per acre are obtained with the primitive methods employed for the major proportion of its production, but yields as high as 4400 pounds per acre have been obtained under experimental conditions (*11*, *13*). Although the average yield is about the same as that of wheat, quinua may be produced on land that will not support common cereals, and most of the quinua produced in Peru is grown at altitudes over 8000 feet above sea level. It may be grown at altitudes as high as 13,000 feet above sea level. It is a sturdy plant resistant to frosts and blights, and needs little water. Depending upon the variety, quinua matures in from 5 to 7 months. Although of lesser importance than quinua in terms of total production, cañihua is produced under similar conditions.

There have been several reports of the high food value of these plants, particularly that of quinua. Alcazar