Autoxidation of Potato Granules

PART I. CHANGES IN FATTY ACIDS

RON G. BUTTERY, CARL E. HENDEL, and MILDRED M. BOGGS

Western Regional Research Laboratory, Albany 10, Calif.

PART II. FORMATION OF CARBONYLS AND HYDROCARBONS

RON G. BUTTERY

Analysis of the fatty acids in dehydrated Russet Burbank potatoes by gas-liquid chromatography of their methyl esters is reported. Linoleic, linolenic, palmitic, and stearic acids were found to be the main acids, their identity being confirmed by infrared spectra. Eight other fatty acids were also detected in small concentration and identified by their retention times. Oxidative degradation of linoleic and linolenic acids is fairly closely correlated with the actual volume of oxygen absorbed and with the degree of off-flavor of the reconstituted product. Volatile compounds present in the autoxidized dehydrated potato were found to include: methane, ethane, propane, butane, pentane, acetaldehyde, propanal, 2-methylpropanal, butanal, pentanal, 2- and 3-methylbutanal, and hexanal. These compounds have been identified by gas-liquid chromatography on at least two different columns and by chemical classification. Tentative identification is given for six other compounds.

PART I. CHANGES IN FATTY ACIDS

During 1959 in the United States, an estimated 8 million hundredweights of potatoes were processed into potato granules—i.e., instant mashed potatoes in powder form. One of the problems is the gradual development of an "oxidative off-flavor" when the product is stored in air. Several workers (*4, 10, 12*) have indicated that the small fat fraction (about 0.3% of the dry weight) is probably responsible. The present study was undertaken to determine the extent to which fat is involved.

Previous methods of studying the development of rancidity in fats, by means of peroxide values, the Kreis test, and total carbonyls, have been described by Gaddis, Ellis, and Currie (*8*) as not being very specific. This is also the opinion of the present authors. Recently, methods have been developed for the accurate analysis of fatty acids by gas-liquid chromatography, GLC, of their methyl esters (*5, 11, 23*) and it was decided to use this procedure to follow quantitatively the autoxidative loss of unsaturated fatty acids in dehydrated potatoes exposed to air. This method, although not previously used for studying oxidative deterioration in fats, would seem to be one of the most direct methods. It provides a quantitative measure of the amount of unsaturated fatty acid oxidized, in contrast to more generally used methods which usually measure one group of the complex autoxidation products. The gas-chromatography method is also a far more specific method of measuring the concentration of unsaturated fatty acids than the older methods of iodine values and absorption spectra.

Myristic (*18*), palmitic (*18*), oleic (*12*), linoleic (*12, 18*), and linolenic acids (*12, 18*) have been reported in potatoes. The most thorough quantitative study was carried out by Highlands, Licciardello, and Herb (*12*). The relative proportions of unsaturated acids in dehydrated Russet Burbank potatoes used in the present study are similar to those they found in Katahdins.

Experimental

Materials. Idaho Russet Burbank potato granules, prepared by conventional add-back process, were obtained from two firms, referred to in this paper as Company A and Company B. Sodium bisulfite at a level of 300 p.p.m. of SO_2 was the only additive. The granules were shipped to the laboratory either at a moisture level of 5.6% and sealed under nitrogen, or at 9.3% moisture and packed in air. Oxidative deterioration is very slow at the latter moisture level.

Three main lots were used for the study:

Lot 1. Company A granules dried to 5.7% of moisture in a vacuum shelf drier at 1 mm. pressure and room temperature; sealed in number 2 cans (453 grams per can) in atmospheres of air, oxygen, or nitrogen (<0.2% O_2). Storage was at room temperature, 75° F.

Lot 2. Same as Lot 1 except 4.7% of moisture, 300 grams per can, atmospheres of air or nitrogen; samples in nitrogen stored at −30° F.

Lot 3. Company B granules, treated same as Lot 1, except sealed in 6-ounce cans, 150 grams per can, 5.6% of moisture.

In some of the studies, the autoxidized granule sample was sealed under nitrogen (<0.2% O_2) and stored at −30° F. until the time of GLC analysis or appraisal. Fresh Idaho Russet Burbank potatoes were received from Idaho and stored at 50° F. until used. Authentic samples of fatty acid methyl esters were obtained from the California Corp. for Biochemical Research.

Extraction of Fatty Acids. The sample of dehydrated potato (100 grams, ground finer than 40 mesh) was mixed with a solution of KOH (20 grams) in 90% ethyl alcohol (200 ml.), and the mixture was left at room temperature in a stoppered flask with occasional shaking for 4 days. The mixture was then filtered and the residue was washed once with 100 ml. of 95% ethyl alcohol and twice with 100-ml. portions of 50% ethyl alcohol. The filtrate and washings were concentrated to about 300 ml. by distillation, then diluted to about 800 ml. with distilled water, and extracted twice with 100 ml. of petroleum ether

Table I. GLC Analysis of Fatty Acids in Samples of Vacuum Dried Idaho Russet Burbank Potatoes, and of Commercial Potato Granules

Fatty Acid	Vacuum Dried Potatoes, %	Commercial Granules, %
Decanoic[a]	Trace	Trace
Myristic	0.3	0.1
Pentadecanoic	0.4	0.1
C_{15} mono-unsaturated[a]	Trace	Trace
Palmitic	19.3	17.4
Palmitoleic[a]	Trace	Trace
Heptadecanoic	0.4	0.1
Stearic	5.4	5.4
Oleic	0.6	0.5
Linoleic	53.0	53.3
Arachidic	1.2[b]	0.5[b]
Linolenic	19.7	22.6

[a] Identity tentative.
[b] Approximate.

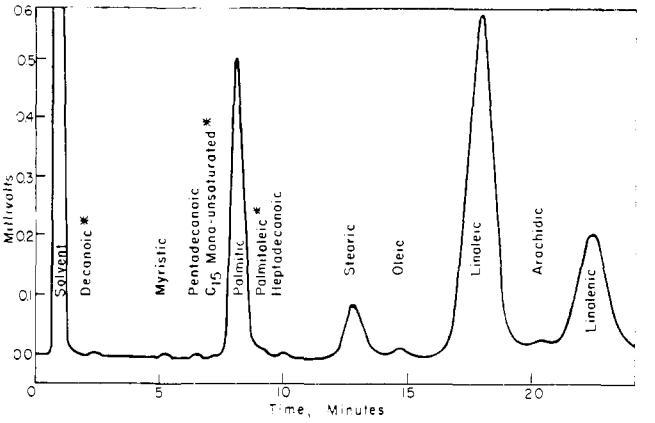

Figure 1. GLC curve of fatty acid methyl esters from a fresh sample of potato granules

Tentative identification indicated by asterisk

(b.p. 40° to 60° C.) to remove unsaponifiable material. The extract was discarded.

The solution was then acidified with excess hydrochloric acid and the fatty acids were removed by extraction three times with 100-ml. portions of petroleum ether. The extract was dried over Na_2SO_4 and the acids were converted to their methyl esters with excess diazomethane. The petroleum ether was removed mostly on the water bath and finally under vacuum to constant weight.

Gas-Liquid Chromatography. The GLC apparatus was laboratory-built of stainless steel construction and used katharometer detection. The column was 5 feet by 1/4 inch stainless steel, packed with 80- to 100-mesh diatomaceous earth firebrick coated with 20% diethylene glycol succinate polyester. Conditions for analysis were 215° C., helium flow rate, 80 ml. per minute; sample size 10 μl. of a 25% solution of the fatty acid methyl esters in benzene. The area of a peak was measured by multiplying the peak height by the peak width at 0.5 peak height. For appraisal studies, fatty acid values are the average of analyses carried out on at least two samples.

Oxygen Analysis. Gas analysis was carried out with a modified Orsat gas-analyzer of the gas absorption type. Each gas analysis of the can atmosphere was made on the same sample as used for GLC analysis.

Organoleptic Appraisal. Experiments were first carried out to choose judges who could detect the oxidative off-flavor of potato granules. The product was reconstituted in the normal way with hot water and salt and served to the judges in a darkened room. Each judge sat in a separate booth lighted by a green 7.5 watt bulb to mask possible appearance differences. The specific appraisal procedures are described later.

Results and Discussion

Extraction of Fatty Acids. The method used involved direct saponification of the dehydrated potato with 10% potassium hydroxide in 90% ethyl alcohol for 4 days at room temperature as described under Experimental. Such a method was desirable for extraction of fatty acids from the stale (oxidized), dehydrated potato. Unsaturated fatty acids are well known to form sparingly soluble polymers on autoxidation, and it is possible that an unoxidized fatty acid molecule could be held in such a polymer through the glycerol linkage and thus would not be removed by solvent extraction. Direct saponification would split these linkages and thus allow quantitative removal of the unoxidized fatty acids. This method gave a slightly higher weight of fatty acid methyl esters (0.26% dry weight) than the weight of petroleum ether-soluble lipide extracted with ethyl alcohol (0.24%) from a freshly prepared sample of potato granules, and also a much larger weight of methyl esters (0.17%) than the ethyl alcohol-extraction method (0.03%) in a sample of granules that had been stored 2 years in air.

For the main group of potato granules (Lot 1), the amount of fatty acid methyl esters extracted by direct saponification was 0.26% (average of three determinations). For a variety of freshly dehydrated samples studied, including vacuum-dried potatoes, the concentration of fatty acids (as their methyl esters) varied between 0.2 and 0.3% dry weight.

Identification of Fatty Acids. The fatty acids found in Lot 1 granules are given in Table I. Figure 1 illustrates the GLC curve obtained. The fatty acids in a sample of vacuum-dried Idaho Russet Burbank potatoes are also listed in the table for comparison. It can be seen that there is no major difference in the identity and the relative amounts of fatty acids. The retention time relative to that of methyl palmitate as an internal standard was used as the main means of identification. Authentic samples of methyl esters were used to obtain the standard relative retention times except in the cases of palmitoleic, pentadecanoic, heptadecanoic, and a possible C_{15} monounsaturated acid. These were identified by plotting the logarithms of retention times against the number of carbon atoms in the chain. It has been shown that for homologous series, such plots form smooth curves which are closely linear for higher homolgs (*5, 14*) such as fatty acids in potatoes. The retention time ratio of an unknown can be predicted from the curve if enough other points are known to plot an accurate one. The identity of the main fatty acids—palmitic, stearic, linoleic, and linolenic acids—was also corroborated by their isolation by gas chromatography, measurement of their infrared spectra, and comparison with those of authentic samples. These spectra showed also that linoleic and linolenic acids were present in their *cis*-forms (as is usual for plant lipides) by the absence of any maxima at 10.3 mμ.

The relative percentages of fatty acids given in Table I were determined by measuring areas and using calibration figures obtained by injecting standard mixtures of authentic samples into the GLC apparatus. This was done for the main fatty acids: palmitic, stearic, oleic, linoleic, and linolenic. The actual relative percentages are very close to the relative areas (*11*) and, for the rest of the analyses in this study, the relative areas were used without correction.

It is somewhat surprising to find C_{15} and C_{17} saturated acids in potatoes. Acids with an odd number of carbon atoms are known to be rare in plant lipides. The use of gas-liquid chromatography, however, may show that such acids are more widely distributed than formerly thought. It may be noted

that heptadecanoic acid was recently identified in spinach by gas-liquid chromatography (*20*).

There were not enough authentic samples available to plot accurate curves of the logarithm of retention times against the number of carbon atoms in the molecule for unsaturated acids. The identification of palmitoleic acid and the monounsaturated C_{15} acid is therefore tentative. The identification of decanoic acid is also tentative because it was overshadowed by the benzene peak used as solvent in most cases.

The identification of C_{15}, C_{17}, and of other saturated acids except decanoic, as well as of monounsaturated acids was corroborated by GLC analysis using a 5-foot by $^1/_4$-inch column of Apiezon L on 40- to 60-mesh firebrick at 248° C. Further corroboration for all acids mentioned, except decanoic, was obtained by GLC analysis using a 75-foot capillary column coated with diethylene glycol succinate polyester, which gave a chromatogram showing an efficiency of about 20,000 theoretical plates. The method of plotting logarithms of retention times against number of carbon atoms was used for both columns. The capillary column also indicated the possible presence of traces of acids with longer retention times than linolenic acid.

Autoxidation of Linoleic and Linolenic Acids. The granules were stored as already described and removed at intervals to carry out GLC analysis of the fatty acids and gas analysis of the can atmosphere. Table II shows a comparison of the relative percentages of the main fatty acids at different storage times in air and for one sample stored in oxygen.

For such a study it would seem convenient to have a single numerical index to express the degree of degradation of the fats. Linoleic and linolenic acids are the main unsaturated acids present; oleic acid makes up less than 1% of the total. The results of this work show that in potato lipide, the two acids, linoleic and linolenic, are oxidized at about the same rate. This would be expected from the known relative rates of oxidation of the pure acids (*13*), and the effect of concentration on rate (*3*)—i.e., the relative rate of autoxidation of pure linolenate:linoleate is 2.4:1 and their relative concentration in potato lipide is 1:2.4. The saturated fatty acids are oxidized at a much slower rate than the unsaturated ones and for practical purposes can be considered not autoxidized at room temperature.

Oleic acid is also oxidized at a much slower rate than linoleic and linolenic acids (*13*), especially at the low concentration present in potato lipide. A convenient index would seem to be the ratio of linoleic plus linolenic acids to the saturated acids, palmitic plus stearic.

Table II. Relative Percentages of the Main Fatty Acids and Unsaturation Ratio for Potato Granules Stored at 75° F. in Air and Oxygen

Storage Time and Conditions	Acids, %				Unsaturation Ratio[a]
	Palmitic	Stearic	Linoleic	Linolenic	
Fresh	20.3	5.4	53.6	20.5	2.9
29 days, air, 75° F.	21.7	5.9	53.4	19.1	2.6
42 days, air, 75° F.	29.8	7.2	46.9	16.1	1.7
140 days, air, 75° F.	35.5	9.2	42.7	12.6	1.2
115 days, O_2, 75° F.	45.6	12.4	31.1	10.8	0.7

[a] (Linoleic + Linolenic)/(Palmitic + Stearic).

Table III. Relative Percentages of Main Fatty Acids and Unsaturation Ratio for Samples of Fresh Dehydrated Potato

Sample	Acids, %				Unsaturation Ratio[a]
	Palmitic	Stearic	Linoleic	Linolenic	
Lot 1, produced Dec. 1958	19.4	5.4	52.9	22.4	3.04
Lot 3, produced Jan. 1959	20.2	5.5	55.1	19.2	2.9
Company A, produced Jan. 1956, held at −30° F. in N_2	18.5	5.3	52.3	23.0	3.0
Vacuum dehydrated sample 1	17.9	6.5	53.8	21.9	3.1
Vacuum dehydrated sample 2	21.7	5.4	53.1	20.1	2.7
Vacuum dehydrated sample 3 (extensive sprouts)	20.6	6.0	54.5	19.0	2.8
Fresh potato (no dehydration)	16.5	4.2	56.1	23.4	3.8

[a] (Linoleic + Linolenic)/(Palmitic + Stearic).

For convenience, this ratio will be referred to as the unsaturation ratio. As the amounts of palmitic and stearic acids should remain constant during autoxidation, this ratio gives a direct measure of the amount of linoleic and linolenic acids present.

A study of several samples of freshly dehydrated potato granules and of fresh vacuum-dried potato showed that the unsaturation ratio of most was close to 3.0, with a range of 2.7 to 3.8. Table III gives the unsaturation ratios for several samples of freshly vacuum-dried potatoes and freshly dehydrated potato granules. The Lot 1 granules in the present study had an unsaturation ratio of 3.04 (average of three determinations). The average deviation from the mean for three determinations on the fresh sample was 0.05. On partly oxidized granules with an unsaturation ratio of 2.6, the average deviation was 0.07 for determinations on samples from five different cans all stored for the same period in air. For Katahdin potatoes, Highlands and coworkers (*12*) found 41.3% of linoleic, 28.4% of linolenic, and 23.3% of saturated acids. This corresponds to an unsaturation ratio of 3.0.

The change in unsaturation ratio with storage in air and oxygen is shown in Table II. The first four samples in the table are from Lot 2; the last is from Lot 1. This ratio decreases markedly during storage.

The solid line in Figure 2 illustrates the relation found between the weight of linoleic and linolenic acids oxidized and the actual volume of oxygen absorbed.

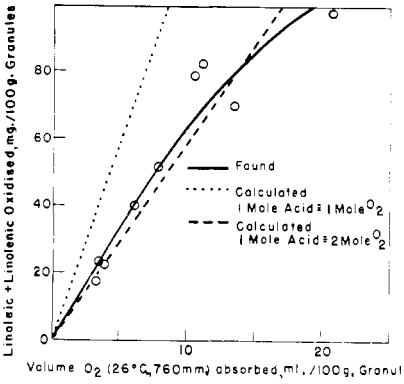

Figure 2. Relation between weight of oxidized linoleic and linolenic acids and volume of oxygen absorbed during storage

Points are taken from both Lot 1 and Lot 2 samples stored in air at 75° F. For comparison, the dashed line in Figure 2 shows the calculated relation based on the assumption that two molecules of oxygen are absorbed per molecule of oxidized linoleic or linolenic acid. The dotted line shows the calculated relation for one molecule of oxygen absorbed per molecule of oxidized acid. The curve found for potato lipide lies fairly close to that for two molecules o oxygen per molecule of acid. It has not been rigorously established how much oxygen is absorbed per molecule of linoleic and linolenic acids oxidized in a system like potato granules where the fat is finely dispersed throughout an inert medium. With finely dispersed linoleate in the form of a colloidal

solution of its sodium salt, Bergström, Blomstrand, and Laurell (*7*) have found that two molecules of oxygen are absorbed per molecule of linoleate. Linolenate would probably behave in approximately the same way in the earlier stages of oxidation, absorbing a greater ratio of oxygen in the later stages.

If this relation holds true for a system such as potato granules, the main bulk of oxygen absorption could be accounted for by the autoxidation of lipides, and oxygen absorption by other chemical groups is likely to be relatively minor at least in the earlier stages of autoxidation.

Off-Flavor and Degree of Autoxidation. Two types of organoleptic studies were used to relate degree of off-flavor development in potato granules to degree of autoxidation of the unsaturated fatty acids, expressed as th unsaturation ratio. In the first type of study, seven judges scored the flavor of six samples per taste period, with three replicate taste periods. The six samples were Lot 3 granules stored as air or oxygen packs for varying times at 75° F. to provide a graded series of unsaturation ratios.

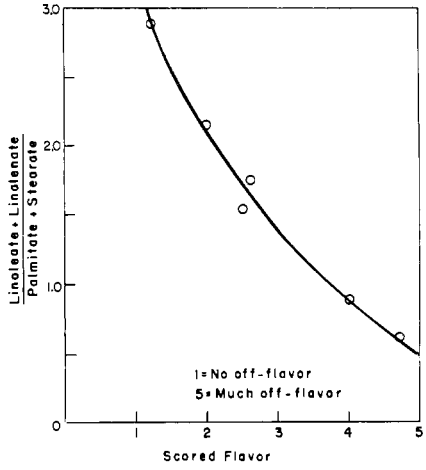

Figure 3. Relation between unsaturation ratio and off-flavor scores

Figure 3 shows that off-flavor increased regularly with decreases in unsaturation ratio. Analysis of variance and the Duncan test (*6*) showed that the scores of different replicates and judges agreed closely. Score difference of 0.2 between any two treatments was significant at the 5% level. Treatments were the only significant source of variability of scores.

The second judging procedure included features not previously reported. The method was developed for use in a study with frozen peas (*2*). The purpose of the method was to follow off-flavor development rather precisely throughout storage up to an advanced stage of deterioration in order to determine whether off-flavor developed at a uniform rate. The method involves measurement o a series of independent detectable differences. The samples were prepared from Lot 2 granules.

First, a nitrogen-packed, control sample stored at −30° F. (essentially equivalent to unstored granules) was compared by the duo-trio test with each of a series of samples held for varying times in air at 75° F., until a storage time was found for which 85% of the judgments showed a difference between the stored and unstored samples. The days to 85% correct matchings of duplicates were determined from the straight line portion of a plot of per cent correct matchings and days of storage (11, 13, 15, 19, 21, 22, 25, 26, 27, 28, and 29 days).

Thirteen judges appraised 4 duo-trios, giving 52 judgments per storage time. The storage time which gave 85% correct judgments was 29 days. This time was called the first detectable difference. Then 15 cans of the 29-day sample were packed in nitrogen (<0.2% O_2) and transferred to −30° F. storage, and used as a control for determining the second detectable difference.

The new control was compared with another series of air, 75° F. samples stored longer than 29 days, until the storage time was found at which 85% of the judges distinguished the sample from the 29-day control. The third detectable difference was determined in a similar way. The study was terminated at this stage because judges seriously objected to the off-flavor present at this time.

Figure 4 shows a plot of volume of oxygen absorbed against storage time, indicating where the three detectable differences occurred and the unsaturation ratios at these points. It can be seen that the curve is typical of fat autoxidation—i.e., it exhibits an induction period, a period of rapid oxidation, and an eventual tailing off. Judges' results showed the same trend. They found that equal amounts of flavor deterioration occurred between no-storage and 29 days, between 29 days and 42 days, and between 42 and 140 days at 75° F. It is clear that oxygen absorption and the degree of linoleic and linolenic acids autoxidation are important factors related to off-flavor development in air-packed potato granules held at 75° F.

PART II. FORMATION OF CARBONYLS AND HYDROCARBONS

THE OXIDATIVE off-flavor of dehydrated potatoes is not characteristic of normal fat rancidity, and it is possible that other chemical groups in the potato may also be involved. It seemed desirable, therefore, to establish the identity of the compounds causing off-flavor in order to understand better the nature of its development and to utilize this knowledge in methods to control off-flavor.

In recent years, sensitive techniques have been developed by which analysis of minute concentrations of volatile compounds responsible for flavor has been made possible. The most useful of these is gas-liquid chromatography coupled with ionization detection (*19*,

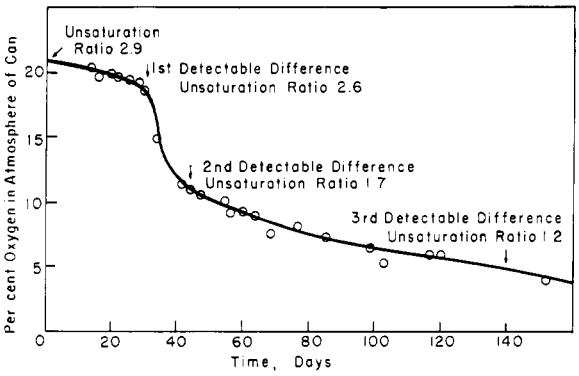

Figure 4. Plot of headspace oxygen against time, showing points of detectable difference of flavor and unsaturation ratio at these points

Effective gas volume of can containing sample, was 370 ml.

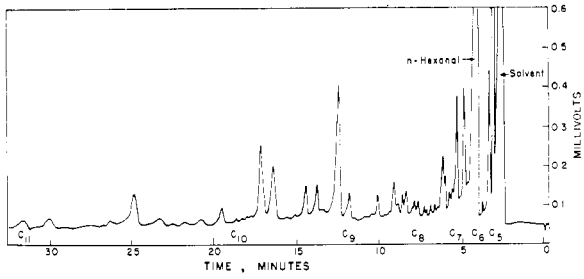

Figure 5. GLC analysis of pentane concentrate of the steam distillate from lot 2 autoxidized potato granules

Conditions:
Column. 75-Foot, 0.01 inch in I.D. stainless steel capillary coated with Tween-20 at 100° C.
Flow rate. 1 Ml./minute
Sample size. Using a split stream, 1/1000 of 8 μl.

27). Another sensitive technique is analysis of carbonyl flavoring compounds by paper chromatography of their 2,4-dinitrophenylhydrazones (9, 22). Both of these methods were used in the present study.

Experimental

Materials. Two main lots of potato granules were used.

Lot 3. Same as for Part I. Samples were analyzed after storage for 12 to 18 months.

Lot 4. A 100-pound quantity, dried to 6.4% moisture, was placed in a galvanized steel cylinder 4 feet high and 10 inches in diameter. The cylinder was airtight except for a tube inlet at the bottom and a tube outlet at the top. Oxygen at a flow rate of 50 ml. per minute was passed through the apparatus for 5 months at room temperature. A dry ice trap was placed at the outlet to trap compounds carried along by the oxygen. After passage of oxygen, the granules were stored in air for 10 months before further isolation of flavoring compounds by steam distillation.

Reagents. Authentic samples of aldehydes and ketones were obtained from reliable commercial sources or were synthesized by known methods. Purification, if necessary, was carried out by using bisulfite derivatives for aldehydes and/or fractional distillation, and in some cases by GLC.

Pentane, 99% purity, obtained from a commercial source was further purified by fractionation through a 20-plate Nichrome helix-packed column. This was used for extraction and as an authentic sample for GLC. Butane, propane, and ethane were synthesized from the corresponding alkyl bromide by reduction with zinc dust and dilute acid. Methane was synthesized by heating sodium acetate with solid potassium hydroxide.

Isolation of Flavoring Compounds

Steam Distillation. On a moderate scale, this step was carried out by placing 1000 grams of granulated sample in a 12-liter flask containing 6 liters of distilled water. The mixture was then steam-distilled and 2 liters of distillate were collected. For solvent extraction, the distillate was extracted with purified pentane (3 × 100 ml.). The pentane extract was dried over sodium sulfate and concentrated to 1 ml. by using a 250-mm. in over-all length, 9 mm. in O.D. column, packed with Nichrome helices for 120 mm. of its length. If the carbonyl compounds were isolated as their 2,4-dinitrophenylhydrazones, 50 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* HCl was added to the receiver prior to steam distillation. After standing overnight, the 2,4-dinitrophenylhydrazones were filtered, washed with a large volume of water, and dried.

Steam distillation on a large scale was carried out with 50-pound quantities of lot 4 autoxidized granules in a stainless steel steam still with a stainless steel condenser. The pentane extracts from two such distillations were combined and concentrated to 2 ml. by passage through helix-packed columns.

Can Headspace or Vapor above Reconstituted Sample. This step was carried out by drawing up 1 to 10 ml. of the can headspace or of the vapor above the hot reconstituted product in a syringe and injecting it directly into the GLC apparatus.

Dry Ice Trap. In the case of lot 4 granules, the aqueous dry ice-trapped material, after 5 months' exposure to passage of oxygen, was extracted with purified pentane (20 ml.). The extract dried over sodium sulfate was concentrated to 5 ml. by using the 250-mm. helix-packed column.

Gas-Liquid Chromatography

The apparatus was laboratory constructed. The column was heated by an oven consisting of a 12-inch in diameter, 7-inch long aluminum cylinder heated by Nichrome wire wound evenly around the cylinder, electrically insulated from the aluminum by a sheet of asbestos paper. The oven was heat-insulated with a 2-inch layer of glass fiber which completely enclosed the oven. Two types of flame ionization detectors were used. A single flame ionization detector was constructed according to the design of Thompson (26). The second detector, a dual flame unit, was constructed according to the design of McWilliam and Dewar (21); its sensitivity was slightly greater than that of the first one.

Nitrogen was the carrier gas and hydrogen was led into the end of the column just below the detector. The capillary column was 75 feet long, 0.01 inch in I.D. stainless steel coated with Tween-20. The other columns were 1/4 inch in O.D., 5 mm. in I.D. stainless steel tubing. Column packings were 30% Apiezon *M* on 60- to 80-mesh firebrick (5- and 10-foot columns), 20% Carbowax 1540 on 40- to 60-mesh firebrick (10-foot column), 20% diethylene glycol succinate polyester on 60- to 80-mesh firebrick (5-foot column), and 30- to 60-mesh molecular sieve-type 5-*A* (no stationary liquid; 10-foot column). In the case of the 5-foot Apiezon *M* firebrick column, "wet" nitrogen was used as carrier gas to minimize tailing in the analysis of carbonyls. Nitrogen was passed over distilled water before entering the GLC apparatus.

All retention times or ratios listed are an average of three separate runs carried out on the same day or on consecutive days. GLC runs of authentic sample and unknown were carried out alternately. In the case of the vapor above the hot reconstituted samples, authentic samples were injected with 5 ml. of the vapor above hot distilled water.

Results and Discussion

Isolation of Oxidative Flavoring Compounds. Three methods were used: (1) steam distillation, coupled with pentane extraction of the distillate or precipitation of 2,4-dinitrophenylhydrazones, (2) direct use of headspace vapor of the can or of the vapor above the hot reconstituted material; (3) use of the dry ice-trapped material from passage of oxygen through the 100-pound quantity of granules. The method of using headspaces directly, coupled with ionization detection, was developed for other products (25).

Desirable methods of extraction are, of course, those which do not change the chemical nature of flavoring compounds. To this extent, the second method has considerable advantages. The other methods, however, gave concentrates which when diluted had the typical odor of autoxidized dehydrated potato, indicating that there was no serious change in the chemical nature of extracted compounds.

The second method is also a rapid and sensitive method of detecting oxidative deterioration in dehydrated potatoes and

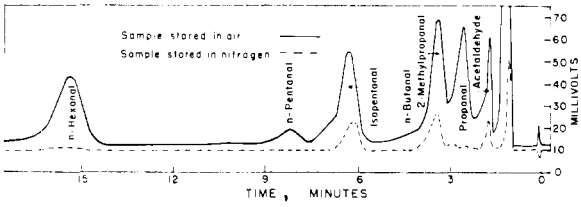

Figure 6. GLC analysis of vapor above hot reconstituted samples of lot 2 potato granules both autoxidized and "fresh"

Conditions:
Column. 5-Foot, 0.20 inch in I.D. Apiezon M at 114° C.
Flow rate. Nitrogen gas (wet) carrier at 25 ml./minute
Sample size. 5 Ml.

Table IV. GLC Analysis of Volatile Compounds in Vapor above Hot Reconstituted Autoxidized Granules (Lot 1), Using a 5-Foot Apiezon *M* Column at 114° C. with a Nitrogen (Wet) Carrier Gas Flow Rate of 25 Ml./Minute

Peak	Retention Time, Cm. Chart Speed, 101.6 Cm./Hour	Apparent Identity	Retention Time of Authentic Compound
B	3.02	Acetaldehyde	3.02
C	4.42	Propanal	4.48
D	5.85	2-Methyl-propanal	5.77
E	7.43	Butanal	7.48
F	10.6	2- and 3-methylbutanal	10.7[a]
G	13.8	Pentanal	13.8
H	25.7	Hexanal	25.7

Authentic Compound[b]	Retention Time, Cm.
Acetone	7.0
2-Butanone	10.8
Acrolein	4.67
Crotonaldehyde	19.5
2,3-Butanedione	8.48

[a] This figure is for 3-methylbutanal; the 2-methyl isomer occurred at very close to the same position.
[b] Under same conditions.

Table V. Flash Hydrazone Keto Acid Exchange GLC Analysis of Volatile Aldehydes in Autoxidized Potato Granules (Lot 1), Using a 5-Foot Diethylene Glycol Succinate Polyester Column at 100° C. and a Dry Nitrogen Carrier Gas Flow Rate of 20 Ml./Minute

Peak	Relative Retention Time Compared to Propanal	Apparent Identity	Relative Retention Time of Authentic Sample
A	0.73	Acetaldehyde	0.737
B	1.00	Propanal	1.00
		2-Methyl-propanal	1.05
C	1.15	?	...
D	1.36	Butanal	1.39
E	1.56	2- and 3-Methylbutanal	1.59[a]
F	2.10	Pentanal	2.10
G	3.26	Hexanal	3.26

Authentic Compound[b]	Relative Retention Time
Acetone	1.22
2-Butanone	1.66
Acrolein	1.28
Crotonaldehyde	3.49
2,3-Butanedione	2.44

[a] This figure is for 3-methylbutanal; the 2-methyl isomer occurred at very close to the same position.
[b] Under same conditions.

Table VI. Identification of Hydrocarbons in Headspace of Autoxidized Granules (Lot 1)

Retention Time, Cm.[a] Apiezon M Column			Retention Time, 5-A Molecular Sieve Column, Cm.[a]		Retention Time Carbowax 1540 Column, Cm.[a]	
Apparent identity	Headspace	Authentic compound	Headspace	Authentic compound	Headspace	Authentic compound
Methane	1.94[b]	1.91	1.65[e]	1.67		
Ethane	2.53[b]	2.53	7.49[e]	7.50		
Propane	4.07[b]	4.04	10.7[f]	10.8		
Butane	3.34[c]	3.34	38.5[f]	38.3		
Pentane	8.52[d]	8.54			6.27[g]	6.27

[a] Chart speed 60.95 cm./hour.
[b] N_2 exit flow rate 23 ml./minute, column temperature 32° C.
[c] 46 ml./minute, 32° C.
[d] 46 ml./minute, 40° C.
[e] 150 ml./minute 100° C.
[f] 120 ml./minute 170° C.
[g] 24 ml./minute 50° C.

could easily be used as a control method in industry.

Capillary Column Analysis. Figure 5 shows the GLC curve of the concentrate from the pentane extract of the steam distillate of lot 4 autoxidized granules. The column used was a 75-foot, 0.01 inch in I.D. stainless steel capillary coated with Tween-20. This gives a general picture of the range of compounds present. Indication of the approximate length of the carbon chain based on plots of logarithms of the retention times (with corrections for the dead volume of the column) against the number of carbon atoms in the molecule for *n*-aldehyde homologs is also shown.

As can be seen, the mixture is complex, containing at least 40 individual components. Complete analysis is therefore not a simple problem. The present paper is a study concerned mostly with the major low-boiling components and with tentative identification of some of the high-boiling components. It is also limited to samples at an advanced stage of autoxidation. This seemed valid because, for conditions of this study, degree of off-flavor was found to increase uniformly with degree of autoxidation as measured by oxygen absorption.

The pentane concentrate from the steam distillate of lot 3 autoxidized granules showed peaks similar in number and position to those of Figure 5 but it could not be concentrated enough to show appreciable size peaks with the capillary column.

Components Boiling Lower than Hexanal. Several techniques were used to establish and corroborate the identity of the volatile compounds with boiling points less than hexanal. Figure 6 shows the chromatogram obtained on injection, into the GLC apparatus, of 5 ml. of the vapor above the hot reconstituted lot 3 autoxidized granules. A 5-foot nonpolar, stationary liquid phase Apiezon *M* column was used at 114° C. Also shown is the curve for a sample of granules stored in nitrogen at −30° F. (essentially fresh). Table IV lists peaks found in Figure 6 with their retention times. Listed also are their apparent identity and the retention time of the authentic substance run under the same conditions. Each retention time for both unknown and authentic sample is the average of three different runs.

The most desirable method of corroborating identification by GLC is to separate the compound and measure its infrared absorption spectra or another suitable physical property. This was done in the present work for hexanal which formed by far the largest proportion of the steam-volatile compounds. Its identity was confirmed both by infrared spectra and nondepression of a mixed melting point of its 2,4-dinitrophenylhydrazone with that obtained from an authentic sample. Isolation of sufficient quantities of other volatile compounds for infrared spectra was, however, difficult. The level of hexanal in lot 3 autoxidized granules was 2.5 p.p.m. as calculated by GLC analysis of the pentane extract of the steam distillate. Hexanal amounted to four times the concentration of any other component and to ten times that of the majority of compounds.

Methods of separating compounds into chemical classes are useful because the possibility that two compounds of the same chemical class will have the same GLC retention time is much smaller. In this work, the method of flash hydrazone–keto acid exchange (*24*) which is specific for only aldehydes and ketones was used to establish the chemical class of components shown in Table IV. The method described by Ralls (*24*) was used except that carbonyl compounds were first flash-exchanged into a 5-ml. syringe opened to the 1-ml. mark and vapors were then injected into the GLC apparatus. Thus, material for the flame ionization detector was readily and more conveniently obtained than with

the method of direct introduction. Using this method, identifications in Table IV were corroborated by separation of the 2,4-dinitrophenylhydrazones from the steam distillate of lot 3 autoxidized granules and GLC analysis by flash-exchanging as above. GLC conditions were the same as those in Table IV.

Further confirmation was obtained by carrying out the GLC analysis of the flash-exchanged carbonyls using the 5-foot polar, stationary liquid phase diethylene glycol succinate polyester column at 100° C. Results of this analysis, shown in Table V, further corroborate the identifications of Table IV. It is unlikely that two different compounds of the same class would show identical retention times on such widely differing stationary liquid phases. Analysis of smaller fragments up to 2- and 3-methylbutanal at a lower temperature on the 5-foot Apiezon *M* column (85° C.) and the 10-foot Carbowax 1540 column (70° C.) confirmed the identity of compounds listed in Table IV, particularly 2-methylpropanal, but could not distinguish between 2- and 3-methylbutanal.

To corroborate further the identifications shown in Tables IV and V, the method of Nonaka, Pippen, and Bailey (*22*) of paper chromatography of 2,4-dinitrophenylhydrazones was used. By this method, spots corresponding to R_h values of acetaldehyde (R_h 0.23), propanal (R_h 0.40), butanal (R_h 0.51), pentanal (R_h 0.63), 3-methylbutanal (R_h 0.74), and hexanal (R_h 0.83) were found. Ultraviolet absorption spectra of all of these spots (on paper) showed absorption maxima close to 370 mμ, confirming their saturated identity. There were indications of other minor spots also. The authentic sample of 2-methylpropanal 2,4-dinitrophenylhydrazone (DNP) had an R_h of 0.66, which is about the same as pentanal 2,4-DNP and therefore could not be distinguished by this method.

Analysis of dry vapors in the headspace of the cans of lot 3 autoxidized granules gave a qualitative analysis similar to that for aldehydes in Figure 6, except that the butanal peak was larger, the 2- and 3-methylbutanal peak much smaller, and several minor peaks were more evident. There were also several strong peaks in the early part of the chromatogram which did not correspond to any authentic aldehyde or ketone. These were found to be sharp even on a nonaged Apiezon *M* firebrick column with dry nitrogen at room temperature. Aldehydes and ketones and other oxygen-containing compounds gave very wide peaks with much tailing on this column. The only compounds which did not show appreciable tailing were hydrocarbons. Table VI lists identifications of these. Methane, ethane, propane, and butane were identified by their retention times on both the 10-foot Apiezon *M* column and the type *5-A* Molecular Sieve column. The molecular sieve column, however, was not suitable for pentane, which was identified by its retention times on the 10-foot Apiezon *M* column and a 10-foot Carbowax 1540 column.

Further confirmation of the saturated nature of these compounds was obtained by shaking the headspace vapors with concentrated sulfuric acid which would absorb any oxygen-containing compound or olefin. GLC analysis of vapors after shaking with sulfuric acid showed no appreciable loss of the above compounds.

Identification of Compounds Boiling Higher than Hexanal. For a preliminary study of compounds with boiling points higher than hexanal, the dry ice-trapped concentrate from passage of oxygen through 100 pounds of lot 4 granules was used. GLC analysis was carried out on three different columns, Apiezon *M*, Carbowax 1540, and diethylene glycol succinate polyester. With increasing molecular weight, there is considerable increase in the number of possible isomers for each empirical formula. This results in a greater likelihood of two or more compounds having the same retention time. Identification of higher molecular weight compounds in a complex mixture such as Figure 5 by GLC analysis alone, even if several columns are used, can therefore, only be tentative. As a basis for further work, this has, however, been done for several compounds. Work is in progress to isolate each compound and determine its infrared spectrum; however, it is difficult because of the very low concentration available.

Besides compounds already mentioned, peaks corresponding to retention times of octanal, 2-pentenal, and 2-octenal were found with the three different columns. Peaks corresponding to heptanal, nonanal, and 2-hexenal were found with two of the three columns. The compound corresponding to 2-octenal was isolated by GLC and shown to contain an α,β-unsaturated carbonyl group, by infrared absorption spectra.

Authentic samples were not available for all compounds. Only acrolein, crotonaldehyde, 2-pentenal, 2-heptenal, and 2-octenal were available as members of 2-alkenal homologs. Acetone, 2-butanone, 2-heptanone, and 2-octanone were available as members of the 2-alkanone series. The whole series from C_2 to C_9 was available for the normal aldehyde homologs. For identification of compounds not available, use was made of the property of homologous series to show closely linear plots of the logarithms of relative retention times against the number of carbon atoms in the homolog (*14*).

The dry ice-trapped material of lot 4 granules would be expected to contain compounds of the early stages of autoxidation. The 2-alkenals found in the dry ice-trapped material were much less concentrated in the pentane extracts of the steam distillates from both lots 3 and 4 autoxidized granule samples.

Origin of Volatile Oxidative Flavors. The products identified seem to arise mainly from autoxidation of the unsaturated fatty acids linoleic and linolenic acids in the dehydrated potato granules. Various workers have studied the autoxidation products of linoleate and linolenate (*7*, *15*, *16*). Acetaldehyde, propanal, butanal, 2-butenal, 2-pentenal, 2-hexenal, 2-heptenal, 2,4-heptadienal, and 2,4-nonadienal have been reported as autoxidation products of linolenate. It might be expected that compounds in autoxidized potato granules would be similar. However, the condition of fat in dehydrated potato granules is quite different from that used by the workers mentioned above, who usually oxidized the fatty acid ester in bulk form by flushing with oxygen or air for a few days. In the latter case, there is a relatively high concentration of fatty acid ester and a low concentration of air. With potato granules, however, the fat is finely dispersed throughout an inert medium and was exposed to an abundance of oxygen for a year. It might therefore be expected that the autoxidation products of the potato granules would be more degraded than those of liquid fatty acid esters. This seems to be actually the case from the identification of the hydrocarbons and the small concentration of the labile unsaturated aldehydes.

The mechanism of the formation of hydrocarbons can be readily understood from some recent work of Kerr and Trotman-Dickenson (*17*), who showed that the peroxide-catalyzed free radical degradation of pentanal gave propene, carbon monoxide, and butane as the major products with methane, ethylene, ethane, propane, and octane as minor products. It might be expected that hexanal, the compound found in highest concentration in the present study, could be degraded by a similar mechanism by the fat peroxide to give butene, pentane, and carbon monoxide as major products with methane, ethylene, ethane, propene, propane, butane, and decane as minor products. All of the saturated hydrocarbons predicted by this mechanism were found except decane. No unsaturated hydrocarbons were found, however. It is possible that the unsaturated compounds are polymerized.

The branched-chain aldehydes, 2-methylpropanal, and 2- and 3-methylbutanal present in rancid potatoes could result from breakdown of amino acids, valine and leucine (or isoleucine), although it is not certain how this occurs.

Recent work by Gaddis, Ellis, and Currie (*9*) has indicated that a large

part of the determinable carbonyls in rancid fat do not originally exist in the oxidized fat as free carbonyls. They are apparently produced through breakdown of precursors by reaction conditions used in isolation. This seems to be also characteristic of autoxidized dehydrated potatoes. Samples stored in open air do not show appreciable compounds in their headspace vapors on enclosing in a flask, but show large concentration of carbonyls on steam distillation or hot reconstitution. Such conditions are brought about in normal food preparation and, therefore, these "bound" compounds are important in considering flavor.

Acknowledgment

The author is indebted to representatives of member firms of the Instant Potato Granule Manufacturers Association for continued interest and helpful suggestions, in particular to R. M. Stephenson, American Potato Co., J. M. Fogelberg, R. T. French Co.; and R. W. Kueneman, J. R. Simplot Co. The author also thanks R. Teranishi and C. E. Hendel, Western Utilization Research and Development Division, for helpful discussion and suggestions and Delpha Venstrom for technical assistance with the organoleptic appraisal.

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Received for review September 16, 1960. Accepted March 30, 1961. Work carried out in cooperation with the Instant Potato Granule Manufacturers Assoc., who supplied potato granules and other materials used. Mention of suppliers of chemicals or equipment does not imply recommendations over comparable products of other manufacturers. Work done at a laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

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