FOOD RANCIDITY

Studies on Deterioration of Walnut Meats

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Rapid deterioration in flavor often shortens the shelf life of walnuts in shelled form. A study of factors affecting such deterioration and the value of antioxidants in retarding it showed that deterioration in flavor and odor was accompanied by increase in peroxide and Kreis values, although correlation was not always close. A satisfactory method of determining the peroxide value without expressing the oil was devised. Blanching at 100° C. greatly increased the rate of peroxide formation. Lipase appeared to be present and active. Peroxide and Kreis values increased many times faster in oil stored as such than in the meats. Butylated hydroxyanisole proved more effective in retarding rancidification than did nordihydroguaiaretic acid, propyl gallate, and antioxidant salt. Light greatly hastened deterioration. Most of the deterioration is oxidative, although hydrolytic changes may also be involved. Lightproof packaging is desirable. If air is in contact with the meats, an effective antioxidant should be used for long shelf life. Packing under vacuum in cans or jars is the alternative.

CALIFORNIA HAS PRODUCED annually in recent years from about 60,000 to 80,000 tons of walnuts on the in-shell basis. An increasingly large proportion of the nuts are shelled and packaged in vacuum-sealed cans or in plastic bags. Shelf life is relatively short in the bags, but is very satisfactory in the vacuum-sealed cans. There is considerable interest in the nature and extent of the changes occurring in the oil in the nut meats in contact with air and in preventing or retarding of such deterioration.

Much research has been done in recent years on the chemistry of rancidification of fats and oils and on its quantitative measurement as exemplified in the papers of Mukerjee (*12*), Paschke and Wheeler (*13, 20*), Pool and Klose (*16*), Watts and Major (*19*), Armstrong and McFarlane (*1*), Bailey (*3, 4*), Beadle (*5*), and others. The literature on rancid deterioration of nut meats, particularly of walnuts, is much less extensive.

Wright (*21*) found that development of rancid odor and flavor in shelled pecan meats did not parallel development of free fatty acid, as free fatty acid changed very little in storage.

Bedford and Joslyn (*6*) found no close correlation between the development of rancidity in walnut meats judged organoleptically and increase in peroxide value. However, correlating peroxide values with organoleptic rancidity in chunky materials such as nut meats is very difficult, because the peroxides are developed primarily on the surface, thus affecting the taste buds at the instant of biting. The peroxide value is usually determined on the expressed or extracted oil from the entire sample.

Table I. Effect of Moisture and Heat Treatment on Increase in Peroxide Value with and Without Propylene Oxide

	Peroxide Value			
	6 weeks,	23 Weeks		40 weeks,
Treatment	no P.O.	No P.O.	P.O.	P.O.
2.2% H_2O	0.4	0.4	0.4	0.6
3.7% H_2O	0.3	0.9	1.3	0.8
6.6% H_2O	0.0	0.3	0.2	0.3
Heated	0.7	2.4	..	3.4[a]

[a] No propylene oxide used in this sample.

Cruess and Armstrong (*8*) found that development of rancidity in shelled walnut meats stored at 95° and 110° F. was retarded markedly by treatment with dilute nordihydroguaiaretic acid, or by coating with confectioners' white coating or with thin fondant.

Wright (*22*) found little correlation between the degree of rancidity of pecan nut meats judged organoleptically and the Kreis test. On the other hand McGlamery and Hood (*11*) observed that the peroxide and Kreis test values increased as the palatability of stored pecan nut meats decreased but that palatability decreased significantly before peroxide and Kreis values significantly increased. Heat-treated meats were more stable than untreated.

Cecil and Woodroof (*7*), Godkin, Beattie, and Cathcart (*9*), Pickett and Holley (*14*), and Pool and Klose (*16*) have also studied rancidification and other changes in stored nut meats.

Bailey (*4*) has defined flavor reversion in fats as the appearance of objectionable flavors from less oxidation than is required to produce true rancidity. Powick (*17*) has given a good review of the mechanism of formation of the compounds developed during rancidification of fats.

Measurement of Peroxide Value Of Walnut Meats

As no method could be found in the literature on determination of the peroxide value of walnut meats, several suggested methods were compared.

1. Direct determination on the ground meats.
2. Determination by the Wheeler procedure on the oil expressed by a Carver press.
3. Determination on oil expressed by Carver press, using the Paschke and Wheeler procedure (*13*).
4. Determination on a chloroform extract of ground walnuts.

Table II. Formation of Free Fatty Acid in Stored Samples of Nut Meats with and Without Propylene Oxide as Preservative

Treatment	Free Fatty Acid, % as Oleic			
	Initial	7 days	30 days	90 days
Control, not ground	0.17	0.18	0.23	0.33
Control, P.O. added	0.17	0.18	0.23	0.33
Not ground, 5% water	0.18	0.19	0.35	0.45
Not ground, 5% water + P.O.	0.18	0.19	0.35	0.36
Ground, 2.5% water	0.17	0.25	0.39	0.58
Ground, 2.5% water + P.O.	0.17	0.24	0.39	0.56
Ground, control	0.17	0.28	0.61	0.81
Ground, control + P.O.	0.17	0.28	0.57	0.78
Ground, 4.5% water + P.O.	0.17	0.33	0.62	1.09
Ground, 5.0% water + P.O.	0.18	0.34	0.74	1.32
Ground, 5.5% water + P.O.	0.21	0.37	0.80	1.40
Ground, 6.5% water + P.O.	0.24	0.40	2.30	4.40
Ground, 6.5% water. No P.O.	0.24	0.40	19.60	19.10
Blanched, 4% water	0.17	0.17	0.19	0.54
Blanched, 4% water + P.O.	0.17	0.17	0.19	0.19
Blanched, 6.5% water	0.19	0.17	9.5	22.00
Blanched, 6.5% water + P.O.	0.19	0.17	0.24	0.28

Direct determination using a ground sample of walnut meats gave negative results.

In Method 2, the Wheeler method, the ground sample was pressed in a Carver press at 15,000 pounds per square inch for 25 minutes. A 6-gram sample of the oil was dissolved in 50 ml. of an acetic acid–chloroform mixture made up of 3 parts of C.P. glacial acetic acid and 2 parts by volume of C.P. chloroform. One milliliter of saturated potassium iodide was added and stirred by rotating the flask. After exactly 1 minute's standing in a darkened place, 100 ml. of water was added. The liberated iodine was titrated with 0.002*N* sodium thiosulfate, using starch indicator. This very weak thiosulfate solution was used because the peroxide value of the oil expressed from the walnut meats was very low. If high values were encountered, 0.01 or 0.10*N* thiosulfate was used. Use of a 3-gram sample of the expressed oil gave a somewhat higher peroxide value than did a 5-gram sample of oil.

In Method 3, the Paschke and Wheeler procedure, 3 grams of oil were used. The mixture in the flask was washed with a stream of carbon dioxide gas, the flask was stoppered and was allowed to stand 1 hour, and the liberated iodine was titrated as in Method 2. This gave a somewhat higher peroxide value than did Method 2.

In Method 4, 50 grams of the ground sample was mixed with 10 grams of anhydrous sodium sulfate in a beaker. The mixture was transferred to a Waring Blendor and disintegrated 5 minutes with 100 ml. of chloroform. The mixture was transferred to a beaker, the blender was rinsed with 50 ml. of chloroform, and the rinsings were added to the sample in the beaker.

On a 12.5-cm. filter paper in a Büchner funnel was laid down a layer of Filter-cel by filtering 100 ml. of a 5% suspension in chloroform. After standing 1 hour, the disintegrated mixture of chloroform and walnut meats was filtered by suction in the prepared Büchner funnel; 50 ml. of chloroform was used to wash the filter and was added to the filtrate.

The filtered solution was diluted to 200 ml. in a volumetric flask. To 20 ml. of the solution was added 30 ml. of glacial acetic acid and the peroxide value was determined as in Method 2.

For ready comparison the peroxide values obtained by the several methods are given in the following tabulation:

Method	Reaction Period	Size of Sample, Grams	Peroxide Value, Meq./Kg. Oil
1. Not extracted	1 min. in air	5	0
2. Carver press, Wheeler method on oil	1 min. in air	5	1.5
2. Carver press, Wheeler method on oil	1 min. in air	3	2.0
3. Carver press, Paschke-Wheeler method on oil	1 hour, CO_2	3	3.2
4. Extracted with chloroform, 20-ml. sample used	1 min. in air	20 ml. of solution (about 3.25 g. oil)	1.4

As a Carver press was not available for most of the experiments, Method 4 was used in subsequent analyses. In most cases 65% of the weight of the walnut meats was dissolved by the chloroform. This value was taken as standard, and assumed to be oil. In each determination the actual per cent of oil dissolved was determined and the observed peroxide values were corrected to the 65% oil basis.

Kreis Test

The Kreis test of rancidity has been used extensively in judging oxidative rancidity of oils and has undergone several modifications since its introduction. In the present investigation the Pool and Prater modification (*15*) was used, although the amounts of reagents were doubled and the reaction was conducted at room temperature instead of 45° C. and the chloroform extract of the ground nut meats (Method 4) was used instead of a chloroform solution of the expressed oil.

To 10 ml. of the chloroform extract previously described were added 10 ml. of a solution of 150 grams of trichloroacetic acid and 2 ml. of a solution of 1 gram of phloroglucinol in 100 ml. of glacial acetic acid. The solution was mixed 2 to 3 seconds by bubbling a stream of air through it. It was allowed to stand 1 hour at room temperature, after which 8 ml. of 95% ethyl alcohol was added. Readings of the developed color were made with a Klett colorimeter fitted with a green filter (500 to 570 mμ). As a blank, a reading was made on the solution prepared as above, except that the phloroglucinol was omitted. Readings were reported in per cent transmittance with the green filter.

Effect of Moisture Content and Heat Treatment of Walnuts on Oxidative Rancidity

Shelled walnuts of sound quality and free of rancidity as judged organoleptically were ground with a 16-bladed knife in a small kitchen-type food grinder. They were divided into four lots treated as follows:

1. Control. Natural moisture content of 3.7%.
2. Dried at 100° F. to 2.2% moisture approximately 3 hours.
3. Brought to 6.6% moisture by addition of water and mixing and standing overnight at room temperature.
4. Heated in steam at 100° C. for 30 minutes; then dried to 3.7% moisture at 100° F. Time of drying about 2 hours.

The samples were stored in heat-sealed polyethylene bags semipermeable to gases but very resistant to passage of moisture vapor. To one set of samples of each lot except the heated samples propylene oxide was added to prevent microbial growth (Table I). Differences in peroxide value among the unheated samples were not very marked, although the sample of 3.7% moisture appeared to increase in peroxide value somewhat more rapidly than did those of 2.2 and 6.6% moisture. The heated sample definitely increased more rapidly in peroxide values than did the unheated, perhaps because heating inactivated natural

Table III. Formation of Free Fatty Acid in Fresh Walnut Meats Stored in Sealed Jars at Room Temperature

Sample	Free Fatty Acid, % as Oleic				
	Initial	1 day	9 days	21 days	30 days
In shell, 27% water	0.02	..	..	..	0.22
Shelled, 27% water	0.02	0.03	0.13	5.1	5.3
Shelled, 7.8% water	0.02	..	0.04	..	0.11

antioxidants in the oil or brought oil to the surface of the nut pieces, thus exposing it to more rapid oxidation.

In another experiment walnut meats in halves were brought to 5% moisture content by storage in a desiccator which contained a beaker of water, and then stored. At 3 months meats of 5% moisture content were distinctly more rancid in taste and odor than untreated meats of 4% moisture content stored in closed containers in the dark at room temperature.

Hydrolytic Deterioration

In this series of experiments data were obtained on hydrolysis of the oils of walnut meats as determined by development of free fatty acid. The samples were stored in 0.0015-inch polyethylene bags that were then heat-sealed using paper headers and stored in sealed jars. Propylene oxide was added to some samples to prevent microbial growth.

The following lots were prepared:

1. Quartered pieces, not ground.
2. Ground, dried at 100° F. to 2.5% water.
3. Ground, brought to 4.5% water, by adding water at room temperature.
4. Ground, brought to 5.0% water, by adding water at room temperature.
5. Ground, brought to 5.5% water, by adding water at room temperature.
6. Ground, brought to 6.5% water, by adding water at room temperature.
7. Not ground, brought to 5.0% water, by adding water at room temperature.
8. Blanched in steam, then ground and dried to 4.0% water, at 100° F.
9. Like 8 but dried to 6.5% water at 100° F.

The samples were stored at room temperature and free fatty acid was determined at 7, 30, and 90 days on the oil expressed from the nut meats by a Carver press. The customary AOAC method of titration of a weighed sample in ethyl alcohol was used (*2*). Table II presents the data.

Blanching apparently inactivated the agent responsible for increase in free fatty acid. The great increase in the blanched sample of 6.5% water content without propylene oxide was due to microbial growth, which also occurred in the unheated sample of 6.5% moisture content where no propylene oxide was used.

Free fatty acid formed more rapidly in the ground than in unground samples, perhaps because of liberation and mixing of the oil and enzymes. However, a measurable increase occurred in the unground samples. Increase was more extensive at 6.5% moisture content than at 5.0%, indicating that the critical water content may lie between these two values.

An unsuccessful attempt was made to substantiate the presence of lipase by the methods of Longenecker and Haley (*10*) and of Rose (*18*). However, as Rose points out, existing tests for lipase in some oil-bearing materials including walnuts are unreliable. The data of Table II strongly indicate that walnuts contain an enzyme that can hydrolyze walnut oil.

In another experiment freshly picked walnuts were hulled (outer green tissue removed) and stored whole, unshelled in sealed jars at a natural moisture content of 27%. Others were hulled and cracked and the meats of 27% moisture content were quartered and stored in sealed jars. Other meats were dried at 100° F. to 7.8% moisture before storage.

Table III gives the free fatty acid determinations at several periods of storage.

At 30 days several pieces at 27% water content showed mold growth, but only meats free of mold were used for analysis. Evidently free fatty acid formation in the unshelled nuts was very slow, but it was rapid in the shelled meats of 27% water content.

Effect of Light on Rancidification Rate

In this experiment the effect of light on the rate of rancidification of walnut oil and on walnut meats as judged organoleptically and by increase in peroxide value was observed.

Walnut Oil Commercially prepared walnut oil with a peroxide value of 4.2 was used. The following lots were made up and stored as indicated. Portions of 150 ml. each were used.

1. Glass jar, stored in the open under a bright fluorescent light during the day, but in the dark at night.
2. Like 1 but stored continuously in the dark.
3. Polyethylene bag inside a jar, in light.
4. Polyethylene bag inside a jar, in dark.
5. Saran bag inside a jar, in light.
6. Saran bag inside a jar, in dark.
7. Like 2, but a small piece of cardboard was placed upright in the sample. The jars were covered but not sealed and air could enter freely.

The peroxide value was determined at 2.5, 11, and 24 weeks with the results given in Table IV.

Light increased the rate of rancidification of the oil very markedly; thus at 24 weeks the ratio of the peroxide value in glass was 8.8 to 1. In polyethylene the rate of increase was similar to that in glass, but it was slower in saran plastic, which evidently screened out more of the actinic rays than did the glass or polyethylene. It was thought that oil might be drawn up the cardboard strip in sample 7 and its rate of oxidation might be increased because of the greater surface exposed; but surface exposure appeared to have little effect on the rate of increase in peroxide value. After an oil has become organoleptically unacceptable (through rancidification), further increase in peroxide value is perhaps of no great significance.

Table IV. Increase in Peroxide Value of Walnut Oil Stored in Light and in Dark at Room Temperature

Sample	Peroxide Value, Meq./Kg. Oil		
	2.5 weeks	11 weeks	24 weeks
1. In glass, in light	39.1	279	690
2. In glass, in dark	4.9	32.5	77
3. In polyethylene, in light	37.0	267	645
4. In polyethylene, in dark	4.9	33.7	80.3
5. In saran, in light	32.3	165	384
6. In saran, in dark	5.2	42.8	100
7. In glass, with cardboard strip, in dark	4.9	34.2	95

The oxidation of the oil in this test was far more rapid than that of the oil in the ground or whole pieces of walnut meats. When stored in a shallow layer the oil oxidized to a thick, gummy consistency in a few weeks; in fact, it is a semidrying oil used generally in artist's paints because of this property.

Walnut Meats In this experiment commercially prepared walnut meats were quartered and stored as indicated in Table V. Peroxide and Kreis values were determined at 4, 14, and 24 weeks. In this experiment, as in the experiment with oil, rancidification was more rapid in the light than in the dark.

Organoleptically the nut meats were judged detectably rancid when the peroxide value reached 2.0 or above. Thus the sample in light at 14 weeks was

slightly rancid in odor and taste at a peroxide value of 1.9 while that stored in the dark was still sweet in odor and taste at a peroxide value of 0.7. However, in other experiments there seemed to be no close correlation between organoleptic rancidity and peroxide value at low peroxide values. There is needed a better quantitative method of measuring rancidity of nut meats in the initial stages of oxidative deterioration. At very low values, 1.0 or less, the determination peroxide value is not very reliable and is subject to considerable error.

Comparison of Several Antioxidants

Commercially prepared walnut meats of good quality and free of rancidity as judged by odor and flavor were treated as indicated in the following paragraphs. Lots of 150 grams from each treatment and one untreated sample were packed in 0.0015-inch polyethylene bags and the bags were heat sealed. Polyethylene film allows rapid transmission of oxygen.

1. Untreated.
2. Nordihydroguaiaretic Acid. Nordihydroguaiaretic acid (0.5 gram) and citric acid (0.5 gram) were dissolved in 95% ethyl alcohol and diluted to 100 ml. with alcohol. This solution was diluted with water to 0.005% nordihydroguaiaretic acid before use. The nut meats were dipped in the dilute solution, drained, and air-dried on a screen overnight at room temperature before packaging.
3. Soluble G-4. A 10% solution was made up in water and sprayed on the nuts to give 0.25% of G-4 calculated to a dry basis on the nuts. They were air-dried overnight at room temperature before packaging. This is a proprietary product (made by The Griffith Laboratories, Inc.), whose composition is not known to the authors.
4. Propyl Gallate. The meats were dipped in a 0.1% solution of the gallate, drained, and allowed to dry overnight at room temperature.
5. Tenox II. A solution of Eastman Tenox II containing propyl gallate and butylated hydroxyanisole was prepared by first soaking gum tragacanth overnight in water to swell, then adding Tenox II equal to the weight of the gum tragacanth preparation, and diluting the mixture to 5% Tenox II. The nuts were sprayed with this solution, drained, and air-dried overnight at room temperature. The dried nuts carried about 0.15% of Tenox II by weight on the dry basis.
6. Antioxidant Salt, Diamond Crystal, said to contain butylated hydroxyanisole, propyl gallate, citric acid, and propylene glycol, which together total 0.25%. When 3% of the salt was added in dry form to the nuts and packaged with them, but little adhered to the nuts. A better procedure would have been to prepare a brine, wet the nuts with it, and dry in air.

The various lots were stored 9 months at room temperature at about 70° F. They were then examined organoleptically only; peroxide and Kreis values were not determined.

Treatment	Odor and Flavor after 9 Months' Storage at Room Temperature
1. Untreated	Very rancid
2. NDGA	Rancid and slightly bitter
3. Soluble G-4	Very rancid
4. Propyl gallate	Rancid
5. Tenox II	Not rancid, slight stale odor, edible
6. Antioxidant salt	Very rancid

In a more recent experiment with nut meats stored at 80° to 85° F., the untreated nuts soon became rancid, whereas those treated with Tenox II were still sound (not rancid) after 6 months' storage.

In another experiment nuts dipped in 0.005% nordihydroguaiaretic acid became rancid in less than 80 days at 110° F.; those dipped in 0.05% nordihydroguaiaretic acid did not become rancid in this period but were disagreeably bitter in taste. Quercitrin, digallic acid, and tannin did not prevent rancidification in 80 days' storage at 110° F. Additional treatment and storage tests are under way. Of the antioxidants reported upon in the present paper, Tenox II gave the best results.

Summary

Comparison of several methods of determining the peroxide value of the oil in walnut meats showed that blanching of walnut meats at 100° C. greatly increased the rate of peroxide formation during subsequent storage.

There was considerable evidence of an undetermined enzyme that exhibited lipolytic activity in walnut meats. It was more active in the ground walnuts than in halves and quarters.

Free fatty acid increase was very slow in unshelled walnut meats of high moisture content (27%) but was fairly rapid in the shelled nuts of similar moisture content.

Peroxide value of walnut oil increased many times more rapidly when the oil was stored as such than it did in stored meats. Light markedly increased the rate of rancidification.

The deterioration in flavor of walnut meats on storage as judged organoleptically was accompanied by an increase in peroxide values and in Kreis values. Consequently, at least part of the deterioration is due to oxidation.

Table V. Effect of Light on Rate of Rancidification of Walnut Meats Stored at Room Temperature

Sample	4 Weeks		14 Weeks		24 Weeks	
	P.V.[a]	Kreis[b]	P.V.	Kreis	P.V.	Kreis
1. Glass, in light	0.2	8	1.9	63	4.3	116
2. Glass, in dark	0.0	7	0.7	36	2.3	74
3. Polyethylene, in light	..	..	2.8	87	..	..
4. Polyethylene, in dark	..	..	3.5	102	5.6	..

[a] Peroxide value as meq./kg. oil.
[b] Kreis value as % transmittance with green filter (500 to 570 mμ).

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