Table III. Fate of Extractable Potato Nitrogen Due to Frying

Sample	N (Kjeldahl) μmoles/g. dry wt.	N (Kjeldahl) % loss on frying	Free Amino Acid Nitrogen[a] Recovery, μmoles/g. dry wt.	Free Amino Acid Nitrogen[a] % loss on frying	% N (Kjeldahl) as Free Amino Acids[a] Recov.	% N (Kjeldahl) as Free Amino Acids[a] Loss
Fresh potato extract	812.1		742.50		91.4	8.6
Fresh chip extract	741.0	8.8	355.74	52.1	48.0	52.0
Stored potato extract	858.7		643.55		74.8	25.2
Stored chip extract[b]	761.1	11.4	98.68	84.7	12.9	87.1

[a] Also includes amides and unknown peaks on analyzer. [b] Extract of chips made from stored potatoes.

Table IV. Fate of Potato Sugars Due to Frying

Sample	Sucrose Mg./g. dry wt.	Sucrose % loss on frying	Reducing Sugars[a] Mg./g. dry wt.	Reducing Sugars[a] μmoles/g. dry wt.	Reducing Sugars[a] % loss on frying
Fresh potato extract	13.43		9.73	54.01	
Fresh chip extract	13.15	2.0	3.25	18.04	66.6
Stored potato extract	70.58		91.13	505.83	
Stored chip extract[b]	64.37	8.8	13.42	74.49	85.3

[a] As fructose. [b] Extract of chips made from stored potatoes.

and amide nitrogen, accompanied by a loss of 85.3% of the reducing sugars, with less than a 9.0% decrease in sucrose. The actual ratio of the decrease in nitrogen to reducing sugar (as fructose) was in the order of 5 to 4 μmoles, respectively, per gram of dry weight of potato. The quantity of this nitrogen which combined directly with reducing sugars is not known. However, if we assume uniform losses of nitrogen from hydrolysis, cyclization, oxidation, etc., in the analysis of the potatoes and chips both before and after storage, the increase in reducing sugars on storage appears to be responsible for the additional 33% decrease in nitrogenous constituents when chips from these potatoes are fried.

Weast and Mackinney (*13*), working with a dark pigment from dried apricots stored for several years, indicated the combination of 1 mole of amino acid with 3 moles of sugar. It is commonly acknowledged, however, that in browning reactions, at least in those occurring in fruit, additional forces come into play. Haas and Stadtman (*4*) show that organic acids are involved, in addition to sugars and amino acids. The picture becomes more complex when a product such as the potato is subjected to frying at high temperatures which probably increase the speed and amplitude of the formation of the browning products.

Finally, the amino acid analysis of these potatoes confirms the presence of the amino acids found in Florida potatoes by Furuholmen *et al.* (*3*). Furthermore, the more sensitive analytical procedures employed revealed that these potatoes, qualitatively, contain the same full spectrum of amino acids found in potatoes grown in other areas of the country (*9, 10, 12*); however, some quantitative differences are present.

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FOOD COLOR STABILITY

Factors Affecting Oxidative Stability of Carotenoid Pigments of Durum Milled Products

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The combined effect of lipoxidase, tocopherols, and free fatty acids on the oxidative stability of the carotenoid pigments of durum milled products was investigated. Free polyunsaturated fatty acids, which increase on the aging of wheat and flour, play a significant role. Tocopherols, if present in sufficient quantity, can influence oxidative pigment stability, and lipoxidase alone is an inadequate criterion by which to predict yellow pigment loss during macaroni and spaghetti processing.

THE carotenoid pigments of durum milled products (semolina and flours) are mainly responsible for the yellow color of macaroni and spaghetti. A bright yellow color is a mark of quality for the consumer and thus is an object of concern to the macaroni processor. The carotenoid pigments consist primarily of xanthophylls, with small amounts of xanthophyll esters and carotenes. The xanthophylls have been found to be mainly lutein and taraxanthin (*7*). These pigments are subject to oxidation by a coupled reaction involving the oxidation of polyunsaturated fatty acid lipids. The preponderant fatty acid present in flour lipids is linoleic, representing about 60% of the total fatty acids (*3*).

When a carotene-linoleate mixture is allowed to autoxidize, the carotene

color fades as oxidation proceeds. Holman (*6*) allowed a mixture of carotene and ethyl linoleate to autoxidize and followed the stages of oxidation with a spectrophotometer. The carotene was oxidized quickly in the early stages of linoleate oxidation.

The enzyme lipoxidase powerfully catalyzes the oxidation of methylene-interrupted polyunsaturated fatty acids and the coupled oxidation of carotenoid pigments and these acids. Oleic acid is not acted upon by this enzyme. Irvine and Winkler (*9*) found that lipoxidase is a major factor in the oxidative destruction of carotenoid pigments during macaroni and spaghetti processing and that the most potent inhibitors of this reaction are the phenolic lipid antioxidants.

The work described explores the effect of the tocopherols and free fatty acids o durum semolina and flour upon the oxidative stability of the carotenoid pigments. It has been found that the free fatty acid content is an important variable factor. Free fatty acids increase naturally as wheat and its products age during storage (*2, 4, 10*). Thus, during any given crop year, the lipoxidase content may remain essentially constant, but the oxidative stability of the wheat and its products decreases because of increasing free fatty acids. Many practical observations have shown that knowledge of pigment content and lipoxidase activity is not sufficient for an adequate prediction of the color of pasta products.

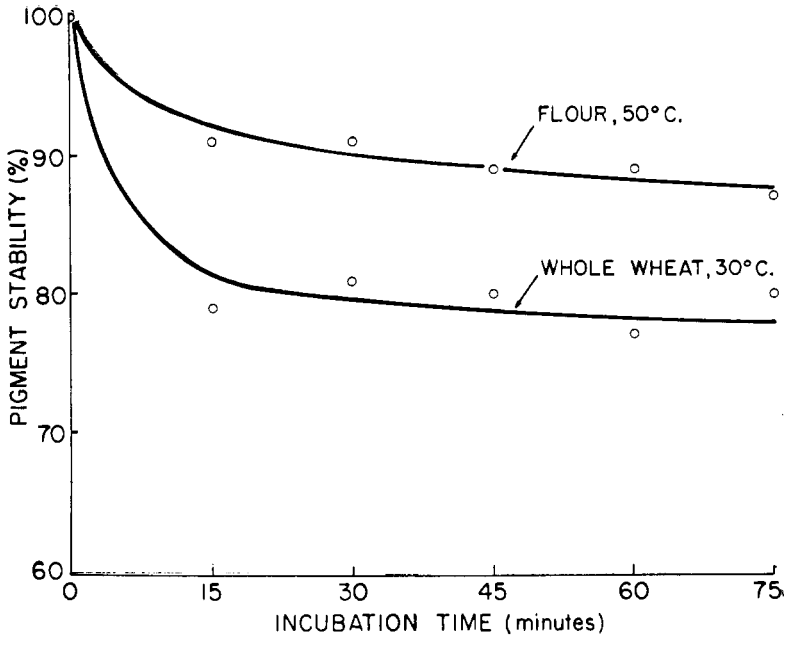

Figure 1. Pigment stability of aqueous slurries of ground whole durum wheat and durum flour on incubation

Procedure

Lipoxidase Activity. Holman (*5*) found that, over long periods of incubation at 0° C., linoleic acid is oxidized by lipoxidase in linear relation to lipoxidase content. At higher temperatures (20° to 30° C.), reaction inactivation occurs after relatively short periods of incubation.

For the experiments reported here, a colorimetric procedure was devised for assaying lipoxidase, based on measurement of the lipid peroxides formed from the oxidation of linoleic acid at 0° C. by lipoxidase extracts. Lipoxidase was extracted from ground wheat and/or milled product by steeping 10 grams in 100 ml. of water at 50° to 55° C. in a stoppered flask for 75 minutes, followed by centrifugation and recovery of the supernatant liquid (enzyme extract). Separate experiments indicated that virtually all the lipoxidase was extracted in less than an hour of steeping. The amount obtained compared well with the quantity extracted by the procedure of Irvine (*8*), in which the material was ground with sand and water in a mortar. Lipoxidase is thermally stable (*5*) and is not inactivated over fairly long periods at 50° to 55° C.

The substrate was prepared just prior to use by dissolving 1.0 gram of linoleic acid in 10 ml. of acetone and then dispersing 5.0 ml. of this solution into 45 ml. of phosphate buffer (pH 8.2).

One milliliter of enzyme extract was added to 10 ml. of buffer in an ice-water bath attached to an Eberhard shaker. After this mixture attained the temperature of the bath, 2.0 ml. of chilled substrate was added and gentle shaking started. After $^1/_2$ hour, the reaction was stopped by adding 1.0 ml. of chilled concentrated hydrochloric acid. Ten milliliters of chilled butanol were added; the mixture was shaken vigorously to ensure complete mixing and extraction of the peroxidized linoleic acid and then centrifuged. The butanol volume increased to 11 ml. because of water uptake.

Peroxides were measured by a slight modification of the thiocyanate procedure described by Wagner, Smith, and Peters (*11*). One gram of ammonium thiocyanate was dissolved in 200 ml. of methanol. One-tenth gram of ferrous ammonium sulfate was dissolved in 1.0 ml. of 25% sulfuric acid. These solutions were combined to form the color reagent just prior to use. One milliliter of butanol extract was combined with 10 ml. of the color reagent and, after 10 minutes, the solution was read at 478 mμ in a Coleman Junior spectrophotometer. As a standard, 0.2542 μeq. of ferric ion per ml. of water-saturated butanol was employed. The lipoxidase activity is expressed as micromoles of peroxide per gram of wheat or milled product per hour.

Tocopherol Assay. In the standard method for assaying tocopherols, the Emmerie-Engel procedure, color is developed from the ferrous ion resulting from the interaction of the ferric ion and the tocopherols. This color development is influenced by other lipid material such as the triglycerides. Thus, lipid extracts of semolina and durum first clear flour were used as a solvent medium for tocopherol increments from which a standard curve was constructed. Since the conditions of the standard curve duplicate those of assay, tocopherols in a sample were measured without separation from the other lipid material. It is assumed that essentially all endogenous ferric ion–reducing material in a lipid extract of milled products derives from the tocopherols.

Tocopherols were extracted from 10 grams of ground wheat and/or milled products with 20 ml. of Skellysolve B. Two milliliters of extract were combined with 10 ml. of the color reagent and measured at 520 mμ after 10 minutes. The tocopherol concentration, in parts per million, was read from the appropriate standard curve. The color reagent consisted of 100 mg. of bipyridine and 50 ml. of ferric chloride in 200 ml. of glacial acetic acid.

Incubation Procedure. Amber durum wheat was coarsely ground in a LabConCo mill; then 3.0-gram samples were weighed into flasks, 15 ml. of water were slurried into each flask, and the flasks were incubated at 30° C. with shaking. The flasks were removed at 15-minute intervals and the samples were assayed for yellow pigment by extracting with 15 ml. of water-saturated butanol and measuring the absorbance at 440 mμ. The ratio of incubated to unincubated pigment is expressed as per cent pigment stability. A comparable procedure was followed with durum flour, except that a higher incubation temperature (50° C.) was employed to obtain measurable pigment losses. The results are shown in Figure 1.

The curves indicate a rapid loss in the first 15 minutes of incubation, followed by a gradual loss. Irvine (*9*) also observed a rapid initial loss of pigment in his kinetic studies of pigment destruction during mixing of macaroni pastas.

Fat Acidity. Free fatty acids were assayed by the method of the American Association of Cereal Chemists (*1*).

Results and Discussion

Effect of Free Fatty Acids on Pigment Stability. The fatty acid content of wheat and its products increases with storage time. Cuendet *et al.* (*4*) reported that, over a year, the free fatty acids of patent flour may increase from about 4 to 16 μmoles per gram. Accordingly, increments of linoleic acid from 0 to 10 μmoles per gram were added to durum flour containing 1.21 μmoles of free fatty acid per gram. The appropriate amounts of linoleic acid were added to the flour as a petroleum ether solution, the mixture was slurried, and then the solvent was removed in a vacuum desiccator. The pigment stability was determined by incubation at 50° C. for 1 hour. Decreasing pigment stability accompanies increasing linoleic acid content (Figure 2).

Relative Effect of Lipoxidase, Tocopherols, and Free Fatty Acids on Pigment Stability of Feed Fractions. Bran, shorts, and red dog were assayed for lipoxidase, tocopherols, and free fatty acids and then tested for pigment stability at 30° C. for 1 hour. The relatively low lipoxidase content of the feed fractions was atypical, but was confirmed on reassay using Irvine's technique of extraction. The pigment-stability procedure was modified by acidification of the water-saturated butanol to restrict pigment measurement as much as possible to nonindicator carotenoid pigments (Table I).

Although the precise bearing of these three factors upon the whole is not known, the ranking of the three mill fractions in order of pigment stability might be rationalized as follows.

The bran and shorts had the highest tocopherol content and the highest pigment stability. Although the shorts contained the greatest amount of tocopherols, its lower pigment stability can be attributed to the higher free fatty acid content. Although the red dog had the least lipoxidase, its tocopherol content apparently was not sufficient to offset the free fatty acids.

Effect of Aging on Pigment Stability of Semolina and Flour as Reflected by Free Fatty Acid Content. Freshly milled and aged (12 months at 50° F. in cotton bags) semolina and flour were assayed for lipoxidase, tocopherols, and free fatty acids. They were tested for pigment stability by incubation at 50° C. for 1 hour (Figure 3).

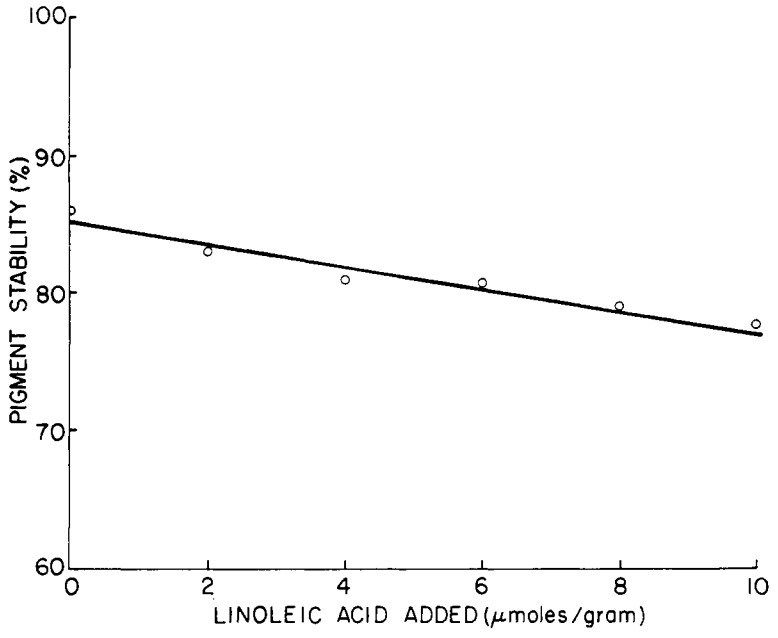

Figure 2. Effect of added linoleic acid on pigment stability of durum flour on incubation as aqueous slurry at 50° C. for 1 hour

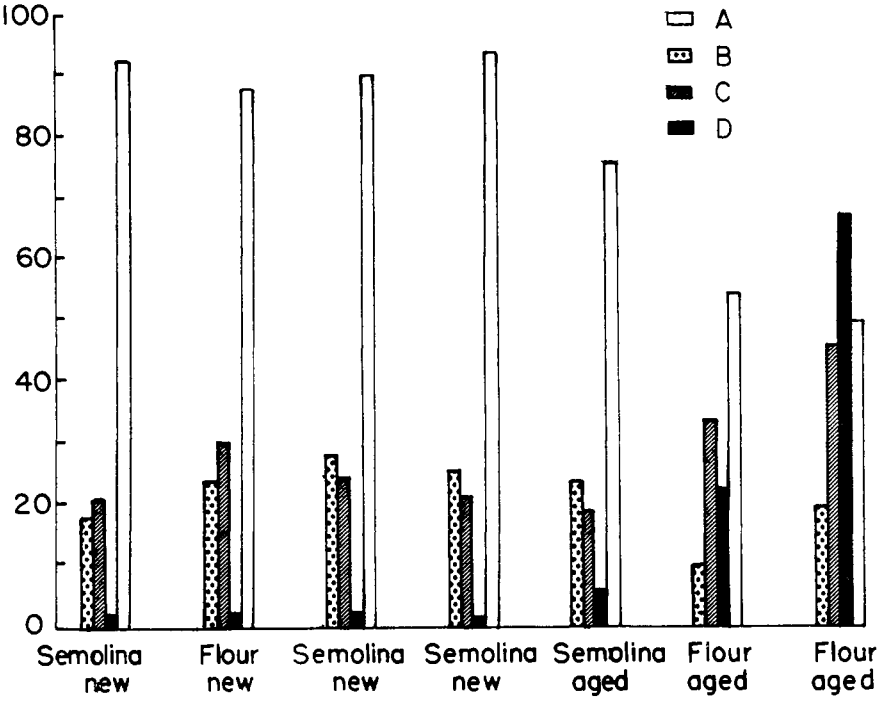

Figure 3. Effect of aging on pigment stability of semolina and durum flour as reflected by lipoxidase activity, tocopherol content, and free fatty acid content

A. Pigment stability, %
B. Lipoxidase activity, μmoles peroxide per gram flour per hour 0° C.
C. Tocopherol content, p.p.m.
D. Free fatty acid content, micromoles per gram

Table I. Relative Effect of Lipoxidase, Tocopherols, and Free Fatty Acids on Pigment Stability of Feed Fractions

Sample	Tocopherol, P.P.M.	Lipoxidase Activity, μmoles Peroxide/ Gram/Hr., 0° C.	Free Fatty Acid, μmoles/Gram	Pigment Stability
Bran	120.0	33.2	25.5	100.0
Shorts	138.0	35.0	72.6	84.8
Red dog	71.0	28.7	28.3	39.4

The free fatty acid content was the determining variable in this series. The aged samples had generally less lipoxidase than the fresh samples. The two aged flours showed the highest tocopherol content, but the aged semolina and flour contained severalfold more free fatty acids and correspondingly lower pigment stability.

The results of the experiments reported here indicate the importance of the amount of free fatty acids (chiefly linoleic) of wheat and its milled products upon the oxidative stability of the carotenoid pigments. This is not surprising, since ordinary oxidative rancidity of many fat-containing substances is accelerated by accompanying hydrolytic rancidity. Free fatty acids are more rapidly autoxidized than the esterified form of triglycerides. It would appear that free fatty acids constitute a prime substrate for wheat lipoxidase action.

Acknowledgment

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FISH OIL ODORS

Volatile Acids from Menhaden Oil

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The acidic constituents of a highly volatile fraction collected during molecular distillation of menhaden oil have been examined by paper chromatography. Tentatively identified were formic (or acetic), acrylic, propionic, crotonic, butyric, and valeric acids, and an unknown compound with a polarity greater than formic acid but less than pyruvic or lactic acids.

Oils carefully rendered from fresh fish tissues are relatively bland or possess, at most, a mild, not unpleasant odor. The strong, undesirable odors usually associated with marine oils are caused mainly by the use of tissues in various stages of decomposition and by deterioration of the oil after it has been prepared. The first factor gives dark oils containing relatively large quantities of nitrogen and with odors of a putrid character. The second effect is associated with autoxidation of the highly unsaturated fatty acids of the oil.

The great complexity of the carbonyl compounds that can be isolated from fish oils by various techniques has been reported (*14, 19, 20*). Furthermore, fishy odors and flavors have been detected in fats of nonmarine origin, such as soybean oil, beef tallow, butter, and other dairy products. Under certain conditions, fishy flavors can be reproducibly developed in butter (*13*). According to Forss *et al.* and Stark and Forss, the fishy flavor of butterfat results from a combination of compounds responsible for an oily flavor (*n*-hexanal, *n*-heptanal, and hex-2-enal) (*7*) and a metallic flavor (oct-1-en-3-one) (*16*). However, other workers (*4, 6, 10*) have isolated carbonyl compounds identical or very closely related to those of Forss *et al.* from butterfat which was oxidized but not considered fishy.

On the other hand, Davies and Gill (*3*) concluded that fishiness developed when highly unsaturated fatty acids and tertiary nitrogen compounds were present together under autoxidizing conditions. The concept that tertiary nitrogen is necessary for fishiness to occur is supported also by Stansby (*15*) and by observations that trimethylamine, triethylamine, and even ammonia, at low concentrations, have odors akin to those encountered in products with a mild degree of fishiness. Chang *et al.* (*2*) also consider that the fishy odor of hydrogenated soybean oil is due to noncarbonyl compounds.

These observations made it desirable to examine the volatile material obtainable from fish oils for compounds other than the carbonyls which are usually associated with the odors of oxidized fats. This report describes the acidic volatile compounds isolated from menhaden oil.

Experimental

Starting Materials. The highly volatile fraction used in this study was accumulated in a cold trap maintained at dry ice–acetone temperature during fractional molecular distillation of 5 gallons of cold-pressed menhaden oil. After a degassing period at 500 microns and 60° C., six distillate fractions were collected at a pressure of 17 to 18 microns and temperatures between 190° and 212° C.

A total of 25 ml. of volatile compounds was collected during the distillation of 5 gallons of menhaden oil. When the fraction was melted, it separated into a nearly colorless lower aqueous layer with a volume of approximately 8 ml. and 17 ml. of a dark upper organic phase.

Examination of Total Volatiles. Both aqueous and organic phases had similar sharp unpleasant odors which were described by four observers as a combination of putrid, slightly fishy, and very strongly rancid. Qualitative tests indicated that both layers contained acids and carbonyls, but a Kjeldahl determination showed that the lower aqueous layer contained more than 15 times the concentration of nitrogen found in the upper layer. Spot tests for amines were positive for the lower phase, but negative for the upper organic layer. Tests for sulfhydryl groups (*5*) were negative for both layers. As a result of these observations, it was decided to examine the lower aqueous layer more thoroughly.

Separation of Acids from Aqueous Phase. A 1-ml. portion of the aqueous layer was neutralized to a pH of 10 (pH paper) with 0.1*N* NaOH (6.9 ml.).