

X-RAY SPECTROGRAPHIC ANALYSIS OF CHLORINE IN BLEACHED FLOUR AND ITS FRACTIONS¹

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ABSTRACT

Hard red spring and soft red winter wheat flours were bleached with chlorine dioxide and chlorine, respectively. The flours were divided into portions, some of which were not treated further; some were extracted with petroleum ether. Certain flours were fractionated to provide starch, gluten, sludge, lipid, and water-soluble constituents. The flours and their fractions were analyzed for chlorine content by means of X-ray fluorescence spectroscopy. This extremely useful technique is not known to have been applied previously for the analysis of flours. Assuming an even distribution of chlorine in the fat of bleached flour, it was calculated that the lipid and the water-soluble fractions, which normally comprise about 5% of the total flour, contained in excess of 90% of the chlorine which was introduced during the bleaching process. Apart from the lipid and water-soluble portions, gluten was the third most important constituent as a repository of chlorine. However, the amount of chlorine found in gluten was extremely low. When a correction was made for the occluded lipid, no appreciable quantity of chlorine was found to be retained by the starch.

The art of applying bleaching and improving agents to flours has been practiced for many years in the flour milling industry. However, science has not elucidated the precise role played by chlorine in the bleaching process. Indeed, because of the relatively small amounts of chlorine employed, it has been difficult to learn precisely where the chlorine reacts with the various biochemical constituents of flour. In the original work on the bleaching process, J. A. Wesner (1) suggested that chlorine acted on gluten and flour oil. This suggestion apparently established the trend of subsequent thinking. Recently, it was reported (2) that all of the improving effect by chlorine had occurred in the gluten and prime starch fractions. Furthermore, a discussion of flour quality and the influence of improvers should not be undertaken with-

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out giving due consideration to the effect of the flour lipids. However, when the flour lipids are considered, the problem becomes extremely complicated.

It has been shown that it is difficult to remove all of the lipids from flour without affecting the associated components of starch and protein. Recent reviews by Bloksma (3), Cookson and Coppock (4), Glass (5), Gilles (6), Gilles and Shuey (7), and Mecham (8) demonstrate the extraordinary confusion in the literature concerning the role of flour lipids and proteins, and the many contradictions to be encountered even if theories are abandoned and only practical phenomena are considered.

Recently, the powerful tool of X-ray fluorescence spectroscopy has become relatively widely used in the area of chemical analysis (9). However, there is a paucity of information concerning the use of this technique for the analysis of cereal products. This paper describes the application of X-ray fluorescence spectroscopy for the analysis of the chlorine content of unbleached and bleached flours and their major biochemical components.

Materials and Methods

Flour. Hard wheat and soft wheat flours commercially milled were used in this work. The hard red spring wheat flour had 15.0% protein and 0.43% ash, and the soft red winter wheat flour had 9.3% protein and 0.31% ash. All values were corrected to a moisture-free basis to facilitate calculation of material balances.

Bleaching. The flour samples were bleached (10) in a MacLellan blender. Gaseous chlorine and chlorine dioxide were used as indicated in the text for soft and hard wheat flours, respectively.

X-Ray Spectroscopy. Figure 1 is a schematic diagram of this technique. X-rays are focused on a specimen of material to be analyzed. This primary white radiation causes a specimen to fluoresce. The secondary rays are collimated by parallel plates of a thin sheet. The collimated beam strikes the large crystal of known interplanar spacing; when the Bragg equation is obeyed for a certain wave length, a proportion of the waves with this wave length will be reflected into the detector.

The detector, as indicated by the counting tubes in the diagram (Fig. 1), is geared to the crystal so that it is always in a position to receive the reflected rays. By slowly rotating the crystal and the detector, one can investigate the entire spectrum of this specimen. This is the manner in which a qualitative analysis may be effected. Of particular advantage is the fact that usually no damage is done to the specimen.

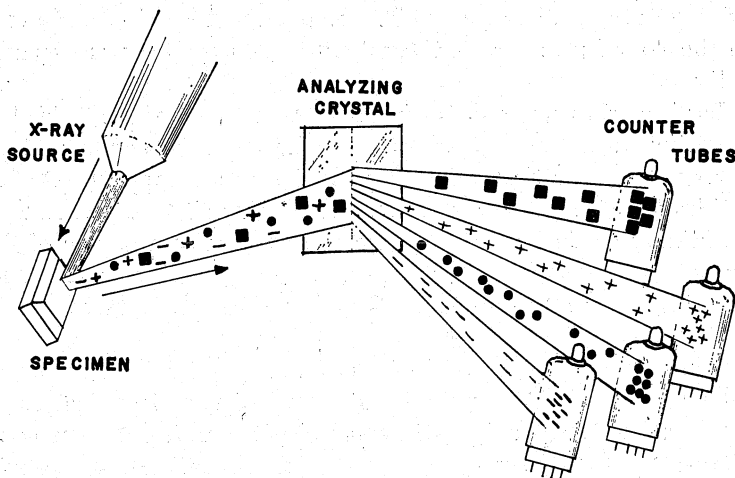


Fig. 1. X-ray emission diagram schematically illustrating the operating principles.

When a specimen is to be quantitatively analyzed, the analyzing crystal and the detector are adjusted to the specific position for this specific element. It was by this means that the analytical results described in this work were accomplished. The analyzing crystal and detector were set in the specific position for maximum detection of rays emitted by chlorine.

In using X-ray spectroscopy, quanta arriving in a random fashion are counted; to increase precision, it is merely necessary to increase the total number of quanta recorded or, in other words, to increase the length of time of counting. Thus, to get a 1% accuracy, as little as 30 sec. may be devoted to counting a single major constituent, whereas 5 to 10 min. per element may be required for counting purposes to estimate minor constituents. These times are remarkably fast when compared with the hours required for many conventional methods of chemical analysis.

There is no doubt that as a nonroutine laboratory tool, X-ray fluorescence spectroscopy presents a number of advantages. It is much faster than ordinary volumetric methods, and is nondestructive of the sample. The sensitivity in low concentration is usually great. For example, as little as 10^{-8} g. of many elements in solution when applied to a filter paper will give an adequate response for purposes of identification; in many cases, less than 1 p.p.m. can be detected. The X-ray fluorescence spectrometer with associated equipment (Fig. 2) was used; Fig. 3 is a close-up, showing the sample holder filled with the sample ready for analysis. Equipment and operating conditions were: standard

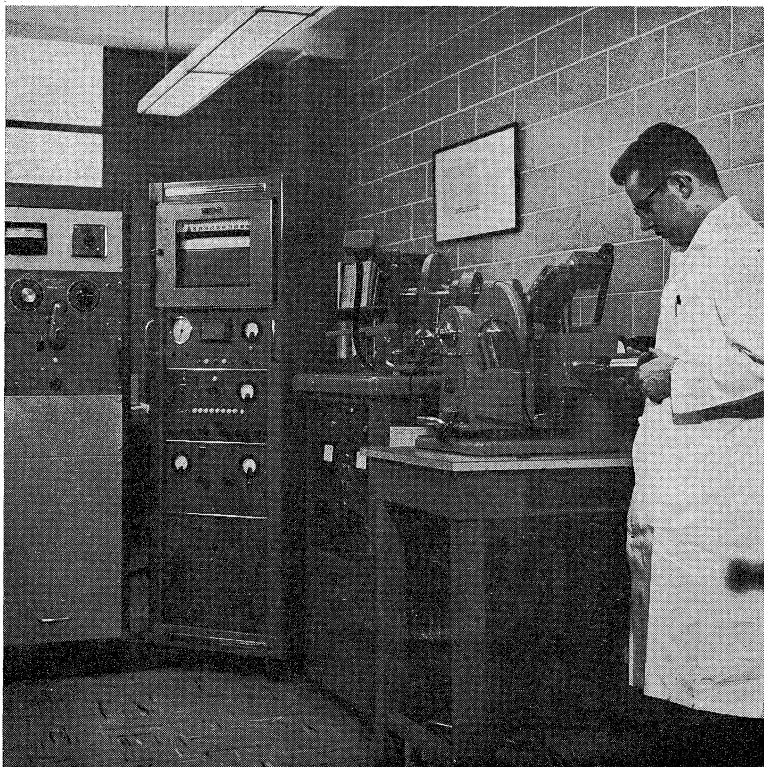


Fig. 2. X-ray laboratory equipment.

Norelco X-ray spectrograph with helium attachment; Philips FA-60 tungsten target tube operated at 50 kv., 45 ma.; NaCl analyzing crystal (2θ for Cl K-alpha = 113.91°); flow proportional detector gas P-10 (90% argon, 10% methane) and 0.3 cu. ft./hr.; parallel plate collimator with 0.02-in. spacings; and Atomic Instruments Company pulse height analyzer.

The samples were contained in open Teflon dishes. Powders were tamped and smoothed with a small glass plate and liquids were added by pipet. Depending on the chlorine concentration of the sample, counting times ranged from 0.5 to 6 min., and from 8,000 to 256,000 counts were taken. Each sample was flushed for 1 min. with helium prior to counting; this caused the total analysis time to be 1.5 to 7 min. Sample preparation time is negligible, since a sample can be prepared while the previous one is running. In comparison, the Parr-Volhard bomb technique (11) requires at least 25 min. per analysis.

To compensate for day-to-day fluctuations in the X-ray instrument,

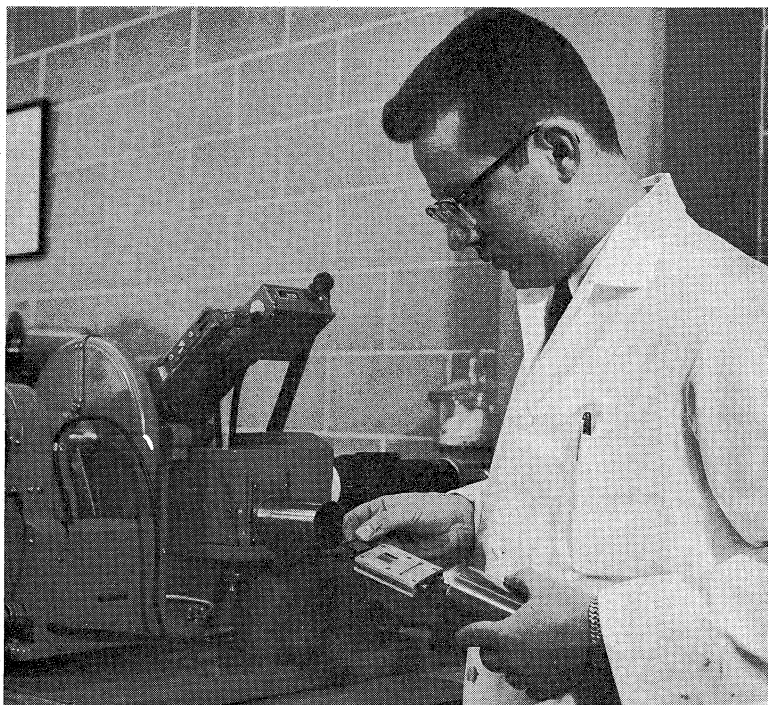


Fig. 3. Close-up of sample holder.

one of the flour samples was designated as the "day-to-day" standard and was run each day. The instrument was then adjusted to give a uniform response.

Intensity of the chlorine K-alpha peak was used to determine chlorine concentration. Results were read off standard curves relating peak intensity to percent chlorine. Since the lipid, glyceride, and phospholipid fractions were viscous liquids and the remainder of the samples were finely divided powders, it was necessary to prepare two series of standards, one for powders and one for liquids. In preparing synthetic standards for X-ray spectroscopy, it was necessary to simulate the elemental composition of the unknown samples as closely as possible.

Reference Standards. For the preparation of synthetic standards, sucrose was chosen as the matrix material because of its elemental similarity to flour and because of its ready availability in Cl-free form. Mallinckrodt reagent-grade sucrose was certified to contain less than 0.0005% Cl; the X-ray spectrograph was found to be insensitive to this amount. The crystalline sucrose was reduced to a fine powder by passing it twice through a hammer mill.

For the Cl-containing material to be blended with the sucrose, a substance was sought which had the following properties: organic, low Cl content, available in pure form, and high-melting solid. Quinine hydrochloride met all of these qualifications. Mallinckrodt USP-grade compound was reduced to 325-mesh and weighed into the sucrose. Thirty minutes in a vibratory Spex Mixer-Mill plus mixing with mortar and pestle was found necessary to produce homogeneous blends.

For the liquid standards to simulate the lipid fractions, Wesson oil (cottonseed) was chosen as the matrix material. It contained no detectable chlorine. For the Cl-containing material pentachlorophenol, recrystallized, was used; carbon tetrachloride was too volatile.

Lipid Extraction. The flours were placed in heavy muslin bags, securely tied, and extracted with petroleum ether (b.p. 37°–39°C.) for 48 hr. The particular Soxhlet-type device extracted the flour with petroleum ether which had been refluxed and condensed prior to the time the solvents contacted the flour. The lipid extracts were concentrated under vacuum in a glass rotary concentrator at approximately 40°C. on a water bath.

Lipid Analysis. The conventional analysis of the lipids, namely iodine value, acid hydrolysis, and refractive index, were performed by AOAC methods (12).

Flour Fractionation. The flour fractionation technique used to prepare the samples for our study is outlined schematically in Fig. 4. Two series of samples were prepared from each control and bleached flour.

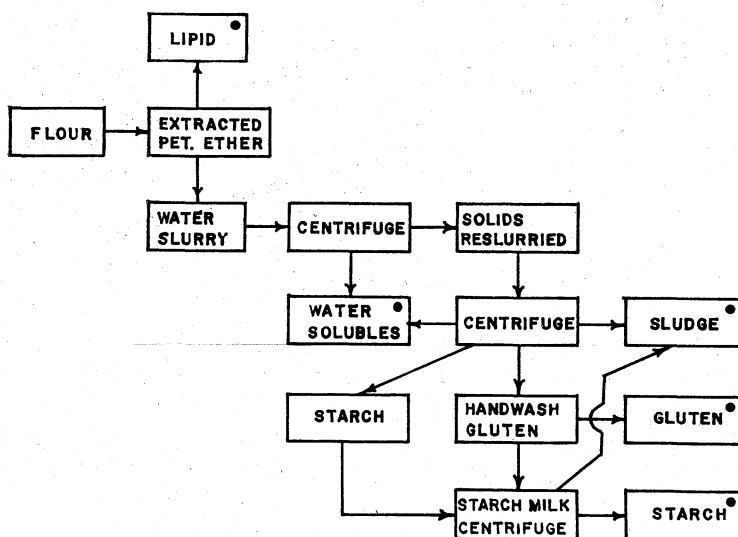


Fig. 4. Flow sheet of flour fractionation.

In one series, the lipids were not extracted with petroleum ether prior to dispersion of the flour in water. In the other series, the flour was extracted with petroleum ether, which upon concentration gave the lipid fraction. The extracted residue was air-dried to remove the solvent, then slurried in two and one-half volumes of water under an atmosphere of nitrogen, and centrifuged. The supernatant liquid was saved and the solids were resuspended in water and again centrifuged. This supernatant liquid, when combined with that liquid from the previous centrifugation, comprised the water-soluble fraction. This fraction was shell-frozen immediately in preparation for freeze-drying.

In this process, three distinct layers formed at the base of the centrifuge cup. The most dense layer, the starch, was at the bottom; the gluten was directly above the starch, and a layer primarily of protein-carbohydrate material, which, for lack of a better term, we called sludge, was the uppermost layer in the tube. The sludge fraction may be considered similar to the "amylodextrin" or "tailings" fraction described by MacMasters and Hilbert (13), who have commented on the complexity of these ill-defined fractions. With the use of a flat-tipped spatula, the three layers were separated. The gluten was hand-washed and all of the wash water was retained. The starch was added to the gluten wash water and resuspended. This enabled the operator to retrieve gluten which had been lost in the wash water; it also assisted in purifying the starch.

After centrifugation, the nongluten material was combined with the sludge fraction, and the starch was resuspended in water, recentrifuged, and dried in air. The sludge fraction was dispersed in a minimum amount of water and dried from the frozen state. The gluten was placed in a large flask and evacuated directly on the freeze-dryer. This puffed gluten mass, when dried, was readily friable and removable from the flask. The five major fractions analyzed in this work are indicated in Fig. 4 by the black dots: lipids, water-solubles, sludge, gluten, and starch.

Results and Discussion

The standard curves for X-ray analysis (Fig. 5) were compared with the comparable data derived by the conventional Parr-Volhard bomb technique for determination of Cl. Accuracy of the X-ray results, estimated from the spread of points on the standard curves, was at least 1% relative for a sample containing 3% Cl. At the 0.05% Cl level, accuracy of at least 10% relative was obtained. The standard deviation of the determination, based on 21 replicates, was 0.0015% Cl absolute.

In this work, the samples ranged from 0.011 to 5.99% Cl. The cor-

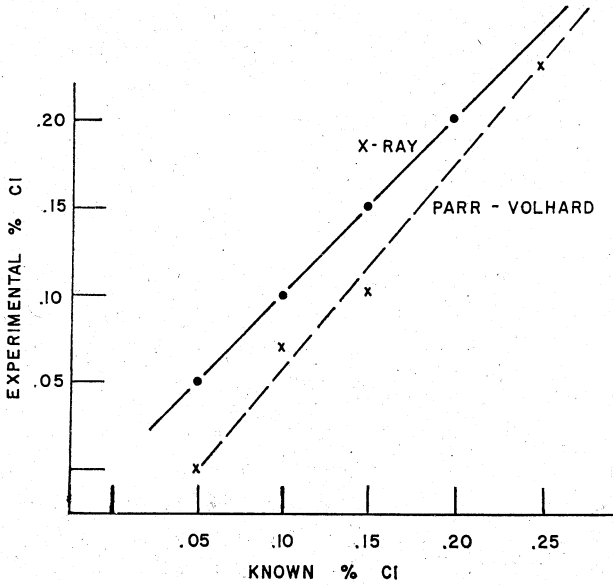


Fig. 5. Standard curves for chlorine analysis.

responding range of peak intensities (background subtracted) was 4.68 to 9,603 counts per sec. Use of a pulse height analyzer, which reduced background from 74.21 to 16.67 counts per sec., was, therefore, obligatory with the low-Cl samples.

Hard Wheat Flour. A spring wheat flour was subdivided into five portions and treated with various amounts of chlorine dioxide (Dyox), as indicated in the left column of Table I. The petroleum ether re-

TABLE I
ANALYSIS OF THE LIPID AND PROTEIN CONTENT OF HARD WHEAT FLOURS
BEFORE AND AFTER EXTRACTION WITH PETROLEUM ETHER

| TREATMENT | LIPID | | PROTEIN | |
|---------------------|-----------------|-----------------|---------|-------|
| | Acid Hydrolysis | Petroleum Ether | | |
| <i>g. Dyox/cwt.</i> | % | % | % | |
| Control flours | 0.0 | 2.01 | 0.93 | 14.94 |
| | 0.05 | 1.97 | 0.89 | 15.05 |
| | 0.1 | 1.76 | 0.85 | 15.10 |
| | 0.2 | 1.90 | 0.85 | 15.05 |
| | 1.0 | 1.72 | 0.92 | 15.05 |
| Extracted flours | 0.0 | 1.05 | - | 14.68 |
| | 0.05 | 1.17 | - | 14.71 |
| | 0.1 | 1.20 | - | 14.84 |
| | 0.2 | 1.05 | - | 14.63 |
| | 1.0 | 0.96 | - | 14.61 |

moved approximately 50% of the lipid constituents as evaluated by the acid hydrolysis method. However, the acid hydrolysis was a destructive method, and consequently lipids assessed by this technique could not be used in the current studies. For analytical calculations it was assumed that the lipids not extracted by petroleum ether were of the same composition as the materials which were extracted.

The flours treated in the extractor were not extracted further with petroleum ether but were analyzed for lipid content only by means of acid hydrolysis. The protein content of these flours was approximately 0.4% lower than that of the original untreated flour, which indicated that some nitrogenous material, such as the phospholipids, was removed by the petroleum ether extraction.

Analysis of the five major flour fractions of the hard wheat flour series is shown in Table II. For illustration and simplicity, only the unbleached and the normally bleached (0.1 g. Dyox/cwt.) flours are shown. In the unbleached series, the water-solubles contained the major amount of chlorine, 0.6%. A small amount was found in each of the sludge and lipid fractions. In the bleached bread flours, the chlorine dioxide which was added appeared to concentrate in the water-soluble fraction; this was the only fraction that appeared to increase in chlorine content. This effect is readily perceived in Fig. 6, which shows the chlorine content of each of the fractions derived from the hard wheat flours.

TABLE II
COMPARISON OF UNBLEACHED AND BLEACHED HARD WHEAT FLOUR FRACTIONS
AND THEIR RESPECTIVE CHLORINE CONTENTS

| FLOUR FRACTION | FRACTION | | CHLORINE IN FRACTION | |
|-------------------|------------|-----------------------|----------------------|-----------------------|
| | Unbleached | Bleached ^a | Unbleached | Bleached ^a |
| | % | % | % | % |
| Water-solubles | 5.81 | 5.27 | 0.60 | 0.67 |
| Gluten | 12.33 | 11.61 | 0.00 | 0.00 |
| Sludge | 25.44 | 25.38 | 0.02 | 0.01 |
| Starch | 52.30 | 49.89 | 0.00 | 0.00 |
| Lipid | 0.93 | 0.85 | 0.03 | 0.03 |

^a 0.1 g. Dyox/cwt.

Soft Wheat Flour. A patent cake flour was divided into four portions. One was untreated; the other three were treated with gaseous chlorine at the rate of 1, 2, and 4 oz./cwt., respectively. The protein content of this series was approximately 9.4%. Note in Table III that again approximately one-half of the lipids was removed by petroleum ether. However, the lipid content of the soft wheat flours was approximately 0.5% lower than that of the hard wheat flour series. Moreover,

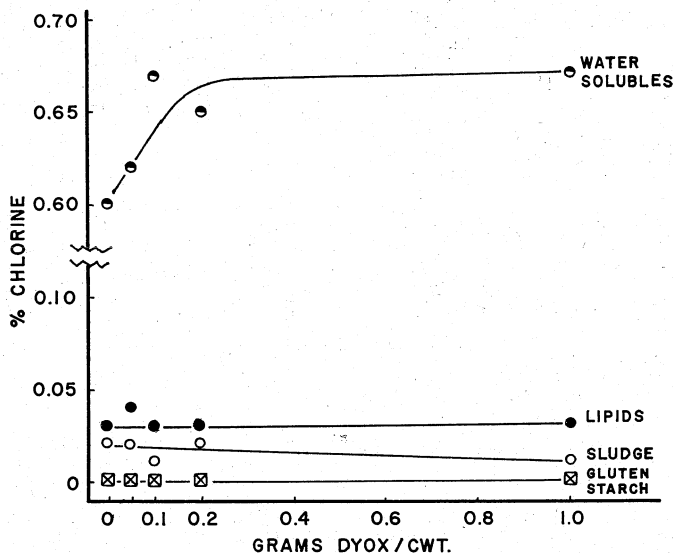


Fig. 6. Chlorine percent present in fractions of hard wheat flour bleached with chlorine dioxide.

the protein content of the extracted flours appeared more comparable to the protein content of the untreated flour in the soft wheat flour series than in the hard wheat flour series.

TABLE III
ANALYSIS OF THE LIPID AND PROTEIN CONTENT OF SOFT WHEAT FLOURS
BEFORE AND AFTER EXTRACTION WITH PETROLEUM ETHER

| TREATMENT | LIPID | | PROTEIN |
|----------------------|-----------------|-----------------|---------|
| | Acid Hydrolysis | Petroleum Ether | |
| <i>oz. Dyox/cwt.</i> | % | % | % |
| Control flours | | | |
| 0.0 | 1.48 | 0.69 | 9.32 |
| 1.0 | 1.48 | 0.76 | 9.50 |
| 2.0 | 1.42 | 0.77 | 9.40 |
| 4.0 | 1.63 | 0.68 | 9.17 |
| Extracted flours | | | |
| 0.0 | 0.93 | | 9.37 |
| 1.0 | 0.85 | | 9.22 |
| 2.0 | 0.91 | | 9.13 |
| 4.0 | 1.01 | | 9.31 |

Table IV shows the major flour fractions derived from the soft wheat flours. Again, the unbleached and normally bleached (1 oz. Cl/cwt.) flours are used for illustration. One observes that in the unbleached cake flour, the chlorine appears to be concentrated in the

water-solubles and in the lipids. Moreover, there appears to be about 50% more chlorine in the water-soluble portion of the unbleached soft wheat flour than in the unbleached hard wheat flour. This undoubtedly reflects the nature of the minerals inherent in the region where the wheat was grown.

TABLE IV
COMPARISON OF UNBLEACHED AND BLEACHED SOFT WHEAT FLOUR FRACTIONS
AND THEIR RESPECTIVE CHLORINE CONTENTS

| FLOUR FRACTION | FRACTION | | CHLORINE IN FRACTION | |
|----------------|------------|-----------------------|----------------------|-----------------------|
| | Unbleached | Bleached ^a | Unbleached | Bleached ^a |
| | % | % | % | % |
| Water-solubles | 4.09 | 4.08 | 0.91 | 1.47 |
| Gluten | 8.06 | 7.28 | 0.00 | 0.05 |
| Sludge | 26.39 | 30.78 | 0.00 | 0.01 |
| Starch | 56.07 | 52.95 | 0.00 | 0.00 |
| Lipid | 0.69 | 0.76 | 0.44 | 2.13 |

^a 1 oz. Cl/cwt.

When one observes the chlorine content in the bleached cake flour, one immediately recognizes that the gaseous chlorine appeared to concentrate in the lipid portion and that the water-soluble material, likewise, was a prime repository for chlorine. This effect is illustrated in Fig. 7. The starch fraction, when corrected for the lipids sorbed on

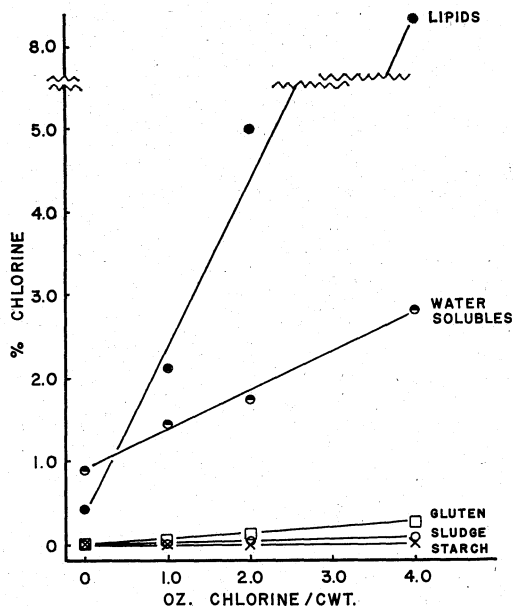


Fig. 7. Chlorine percent present in fractions of soft wheat flour bleached with chlorine.

the starch, did not increase in chlorine content. There was an insignificant increase in the sludge fraction and a slight increase in the gluten. One must conclude that the lipids and the water-solubles were the primary sites for the repository of chlorine used in bleaching the soft wheat cake flours.

An analysis of the soft wheat lipids appears in Table V; flour treatment is indicated in the left column. As the chlorine content increased with progressive increase in treatment, the iodine value of these lipids decreased. This indicated that the degree of unsaturation of the polyethanoic acids decreased because of the addition of chlorine. The refractive index, likewise, changed: a slight increase as the amount of chlorine treatment increased.

TABLE V
ANALYSIS OF SOFT WHEAT FLOUR LIPIDS

| TREATMENT | CHLORINE | IODINE VALUE | $[n]_D^{20}$ |
|-----------------|----------|-----------------|--------------|
| <i>oz./cwt.</i> | % | | |
| 0.0 | 0.44 | 105 | 1.4788 |
| 1.0 | 2.13 | 87 | 1.4810 |
| 2.0 | 5.02 | 69 | 1.4870 |
| 4.0 | 8.34 | 50 | 1.4880 |

Thin-film chromatographs of the normal and bleached flour lipids derived from soft wheat flour indicated that the constituents of the lipid mixture were changed as chlorine bleaching levels increased. Further work on lipid constituents is contemplated.

While these analyses do not indicate the precise mechanism for the reaction of bleaching agents and specific flour components, it is apparent that the lipid and water-soluble fractions of flour were primary repositories of the chlorine. Moreover, differences observed for the two flours were probably due to the fact that different reagents were used and not to inherent differences in flours. It is conceivable that the previously reported improving effect of chlorinated bleaching agents on the proteins and starch may be due to protein-lipid and starch-lipid complexes. The presence of chlorine in the lipids and water-solubles may alter the colloidal properties of the associated starch and proteins to a greater degree than was recognized heretofore.

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