Flour Chlorination. I. Chlorine Location and Quantitation in Air-Classified Fractions and Physicochemical Effects on Starch[1]

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ABSTRACT

Cake flour was treated with 0, 1, or 4 oz of chlorine per cwt and dry-fractionated by an air classifier. Fractions containing high levels of protein showed the highest chlorine content as determined by biamperometric titration. X-ray microanalysis of the chloride on the protein and starch indicated that protein absorbs significantly more chloride than does starch at all levels of chlorine treatment, and that the chloride uptake by the protein increases with increasing chlorine dose. Conversely, chloride uptake by starch granules reached a plateau at a dose of 2 oz/cwt (no significant increase at 4 oz/cwt). Starches washed from untreated and chlorinated flours showed an increase in β-amylolysis and a decrease in intrinsic viscosity when treated with increased levels of chlorine, indicating that the starch was depolymerized during chlorination. In addition, the presence of a carbonyl absorption band on the infrared scans of the treated starches suggested that an oxidation occurred at carbons C-2 and C-3 during chlorination.

Many studies have dealt with the action of chlorine on flour components (Gough et al 1978). Unfortunately, the amount of chlorine used in those studies was higher than the 1,100–2,300 ppm used in practice. Furthermore, methods employed in fractionating the flour components before testing the various fractions may be the major factor contributing to the varying reported results. The first objective of this study was to localize and quantitate the distribution of chlorine in air-classified flour by using biamperometric titration and X-ray energy dispersive analysis.

Some authors have suggested that the reaction of chlorine with the prime starch fraction is responsible for the beneficial effect of chlorine on flour (Johnson and Hoseney 1979; Lamb and Bode 1963; Sollars 1958a, 1958b). However, experimental evidence for chemical attack on starch at the relatively low concentrations used in the chlorination of cake flour is limited. Sollars (1961) wet-fractionated flours and found that the ionic chlorine content in the treated starch fraction increased and suggested that this was possibly due to the oxidation of starch. Using air-classification to separate the flours, Wilson et al (1964) also found that the high-starch fraction had taken up chlorine mainly as chlorine ion and suggested that the reaction was straight oxidation of functional groups, ring scission, or chain scission. However, further studies by Sollars (1964) on the effect of chlorine bleaching on cake-baking quality showed that a small amount of chlorine (relative to that required for flour) effected the full improvement of the cake-baking quality of starch and that improvement was not related to the increase in chloride and carbonyl contents.

More recently, Johnson et al (1980) found that starches from untreated flour, when air-dried or treated with bromine, showed the same improvement in cakemaking as did chlorine treatment. The increase in the β-amylolysis of the air-dried and bromine-treated starches indicated oxidative cleavage of α-1,4 bonds. In addition, the amylose and amylopectin fractions of those starches, when treated with β-amylases, produced low β-amylolysis values, indicating that the glucose residues had also undergone oxidation between carbons C-2 and C-3. Based on those data, Johnson et al (1980) suggested that chlorine oxidation of starch produced the improving effect in chlorinated flour. They did not, however, present β-amylolysis values for starches from chlorine-treated flours, so the effect of chlorine on starch could not be compared with the effects of drying on unchlorinated starch. The second objective of our study was to determine the physicochemical effects of chlorination (1, 2, and 4 oz/cwt) on starch isolated from air-classified fractions.

MATERIALS AND METHODS

Materials

Untreated, pin-milled cake flour was obtained from Mennel Milling Company (Fostoria, OH). Its protein (N × 5.7) and ash contents were 8.3 and 0.3%, respectively (14% mb).

Methods

Flour Chlorination. Chlorine gas was added to cake flour at 0, 1, 2, and 4 oz/cwt in a laboratory bleaching mixer. Treated and untreated flours were dry-fractionated with an air classifier (566-lb laboratory classifier) in a four-stage operation as described by Daftary et al (1966). Five fractions of each flour were obtained and their protein, ash, and moisture contents determined by standard methods (AACC 1976). Table I shows the pH of the flours after chlorination as well as before air classification (two months of storage at 5°C) as determined by the AACC method (1976).

Chlorine Content of Flour Fractions. One gram of each air-classified flour fraction was added to 5 ml of deionized water, shaken for 2 hr, and centrifuged at 5,000 rpm for 15 min. Chlorine content in the supernatant was measured with a Buchler Digital Chloridometer (Searle G. D. and Co., Fort Lee, NJ), a biamperometric titrator.

SEM. To study the surface structure of the flour fractions, samples were sprinkled onto double-faced tape, coated with gold-palladium (60:40), and viewed in an ETEC U-1 scanning electron microscope (SEM) at 5 kV.

X-Ray Microanalysis. Carbon planchets were coated with a thin layer of colloidal graphite and cemented to aluminum stubs with colloidal graphite. The E fractions (high-starch fractions) were then sprinkled onto the carbon planchets. Excess sample was removed by passing a stream of air over the stub, and then the samples were coated with carbon. X-ray microanalysis (energy dispersive) was done using an Ortec detector (E. G. & G. Ortec, Oak Ridge, TN) having a revolution of 155 eV (from manganese K_α) and an IT-5300

TABLE I
pH of Water-Flour Suspensions

Chlorine Level (oz/cwt)	Immediately After Chlorination	After Two Months of Storage at 5°C
0	5.70	5.45
1	4.95	4.78
2	4.35	4.54
4	3.40	3.60

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Norland multichannel analyzer (Norland Inst., Fort Atkinson, WI). All analyses were performed with the electron beam perpendicularly incident upon the specimen at a working distance of 11 mm. Chloride analyses were done at an operating voltage of 10 kV; counting time was 400 sec. Two replicates of each sample were analyzed. For each replicate, we made three chloride determinations of starch-granule surfaces (free from protein), of protein, and of the stub surface. The latter determinations were used for background corrections. The corrected percentage of chloride associated with the starch and protein was calculated as follows:

$$\text{Counts for C peak} = \text{Counts of Cl window} \times \frac{\text{Area of Cl peak}}{\text{Total area of Cl window}} \quad (1)$$

$$\text{Uncorrected chloride (\%)} = \frac{\text{Counts for Cl peak}}{\text{Counts in all channels}} \times 100 \quad (2)$$

$$\text{Corrected chloride (\%)} = \text{Percent Cl on starch (or protein)} - \text{Percent Cl on stub surface} \quad (3)$$

β-Amylolysis. Starch was dissolved in 1*N* KOH and neutralized with 1*N* HCl. An aliquot representing 20 mg of starch was incubated with 750 units of β-amylase (Crystalline, type I-B, Sigma Chemical Co., St. Louis, MO) in a total of 5 ml of 0.02*M* acetate buffer, pH 4.8, at 35°C for 24 hr. Maltose was determined by Nelson's (1944) colorimetric copper method, and the degree of β-amylolysis was calculated according to Whelan (1964).

Intrinsic Viscosity. Starch washed from the E fraction was freeze-dried. Intrinsic viscosity was determined at 35°C according to Leach (1963); a Cannon-Fenske viscometer, capillary size 100, was used.

Infrared Spectroscopy. Starch was formed into a KBr pellet, and infrared analysis was done on a Perkin-Elmer model 180 infrared dispersive spectrophotometer. Spectra were recorded between 4,000 and 500 cm^{-1}.

Iodine Affinity. Iodine affinity of starches was determined according to Schoch (1964). Samples were defatted with methanol before the determination.

Lipid Extraction. Free lipids were extracted with petroleum ether (38°–50°C boiling point) in a Goldfish apparatus for 14 hr. The lipid content was expressed as percent of the total flour on a 14% mb.

RESULTS AND DISCUSSION

Chlorine Content and Location

Protein, ash, and pH of flour fractions obtained from the air classifier are presented in Table II. The B fractions had the highest protein content, whereas E fractions showed the lowest protein content for all levels of chlorine treatment. In addition, regardless of the treatment, E fractions contained essentially the same amount of protein (~4%).

SEM pictures of air-classified flour fractions (Fig. 1) indicated that the high-protein fractions (fractions B and C) contained aggregates of small starch granules and protein (Fig. 1a and b), whereas the high-starch fractions (fractions D and E) contained a preponderance of large-sized starch granules having little or no protein adhering to them (Fig 1c and d), along with some free protein aggregates.

Chamberlain (1962) and Wilson et al (1964) reported that during flour chlorination, the major portion of the chlorine reacts with the protein fraction, whereas little or no chlorine is found in the high-starch fraction. Our chlorine determinations on air-classified fractions (Table III) indicated that fractions containing high levels of protein also showed the highest chlorine content ($P<0.05$).

Gilles et al (1964) found that the major portions of the chlorine were associated with the petroleum ether-extractable (free) lipids in the flour. On the other hand, Sollars (1961) found that most of the chlorine in the butanol-extractable lipids (free and bound) was associated with the gluten proteins. Our data showed no significant differences in the chlorine content of defatted (petroleum ether-extracted) and undefatted E fractions.

Although biamperometric determinations of chlorine provide information on the total chlorine present in the fraction, they do not indicate the location of chlorine in single fractions; ie, they do not answer the question: Is it concentrated mainly on the starch component or the protein component of a particular fraction? Therefore, we used X-ray microanalysis to determine the location of chlorine in the E fraction.

Starch granule and protein sites chosen for analyses were selected to minimize cross contamination of chloride among components. Typical X-ray spectra showing chloride analysis for starch and protein is shown in Fig. 2. Spectra of the protein component also revealed a large peak for sulfur as well as for chloride.

TABLE II
Analytical Results for Cake Flours: Four-Stage Air Classification

Chlorine Level (oz/cwt)	Fraction	Yield (%)	Protein (%)[a]	Ash (%)[a]	pH[b]
0	B	1.0	28.6	0.39	5.36
	C	13.0	16.6	0.41	5.52
	D	18.7	5.6	0.30	5.58
	E	19.3	3.9	0.29	5.68
	EE	38.8	8.5	0.34	5.70
1	B	4.6	24.3	0.38	4.76
	C	12.9	16.0	0.37	4.82
	D	18.1	5.6	0.29	4.96
	E	18.2	4.0	0.27	5.05
	EE	37.7	8.2	0.32	5.10
2	B	2.9	25.7	0.38	4.20
	C	13.3	15.8	0.37	4.40
	D	18.7	5.4	0.29	4.48
	E	17.8	4.0	0.28	4.50
	EE	38.1	8.5	0.34	4.68
4	B	3.0	25.8	0.37	3.34
	C	12.6	15.8	0.36	3.42
	D	18.7	5.7	0.31	3.56
	E	17.6	4.0	0.31	3.60
	EE	38.2	8.7	0.35	3.60

[a] Based on 14% mb.
[b] AACC method 02-52 (1976).

TABLE III
Chlorine Content (meq/g × 10^{-2}) of Air-Classified Flour Fractions

Fraction	Chlorine Level (oz/cwt)[a] 0	1	2	4
B	2.13	3.07	5.91	10.00
C	1.83	3.02	4.86	7.59
D	1.29	1.88	2.66	4.17
E	1.23	1.65	2.29	3.25
E[b]	1.16	1.55	2.22	3.27
EE	1.35	1.92	2.85	4.96

[a] Least significant difference = 0.14 for treatment at $P<0.05$. Least significant difference = 0.16 for fraction at $P<0.05$.
[b] Petroleum ether-extracted flour.

TABLE IV
Chloride (%) on Starch Granules and Protein Components of Flour as Determined by X-Ray Microanalysis

Fraction	Chlorine Level (oz/cwt)[a] 0	1	2	4
Starch	0.48	0.93	1.43	1.47
Protein	0.49	1.13	2.56	3.20

[a] Least significant difference = 0.45 for treatment at $P<0.05$. Least significant difference = 0.32 for fraction at $P<0.05$.

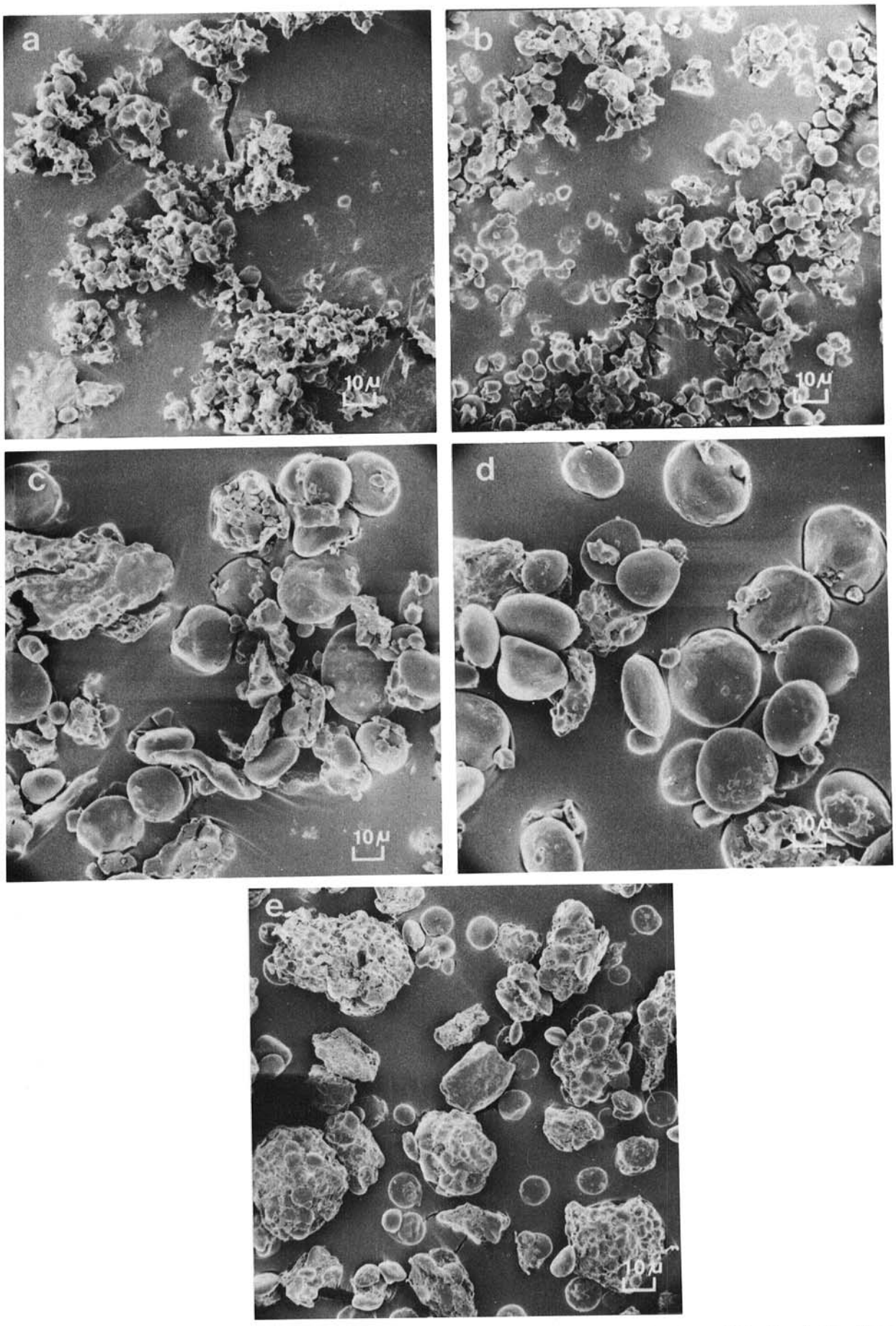

Fig. 1. Scanning electron micrographs of flour fractions obtained from the air classifier: **a,** B fraction; **b,** C fraction; **c,** D fraction; **d,** E fraction; and **e,** EE fraction.

The percentage of chloride in each component as determined by X-ray analysis is reported in Table IV. The protein component absorbed significantly more chloride than did the starch component at all levels of chlorine treatment, and the chloride uptake increased with increased doses of chlorine. On the other hand, the uptake of chloride by starch granules showed a high level at 2 oz/cwt with no further significant increase at 4 oz/cwt. That suggested that the starch component can only absorb a limited amount of chlorine, whereas the reaction of chlorine with protein will increase as the dosage is increased. The amount of chlorine taken up by the starch component coincided with the level normally used in commercial practice (about 1–2 oz/cwt), suggesting that the beneficial effect of chlorination on cake flour is due, at least in part, to the starch component.

Interaction of Chlorine with Starch

The degree of β-amylolysis of starches washed from chlorinated and unchlorinated flours provides evidence for the chemical effects of chlorine on starch (Table V). Starches were freeze-dried using the same procedure as Johnson et al (1980). Higher β-amylolysis limits were observed for 2- and 4-oz chlorinated starches than for the control or the 1-oz treated samples. Because β-amylolysis limits increased rather than decreased, the major effect of chlorination appears to be depolymerization of the starch rather than oxidation of carbons C-2 and C-3.

Intrinsic viscosities of the starches also indicated that chlorination effected starch depolymerization (Table V). As the level of chlorine treatment increased, the intrinsic viscosities decreased. The fact that the iodine affinities of the control and chlorinated starches were not different suggested that the major effect of chlorine might be on the amylopectin fraction.

Our infrared scans of potassium bromate starch pellets (Fig. 3) showed the presence of a carbonyl absorption band (~1,720 cm^{-1}) in starch washed from chlorinated flours, which was not present in the unchlorinated sample, suggesting that a cleavage of carbons C-2 and C-3 bonds occurred because of chlorination. Apparently, low doses of chlorine have the same qualitative effects on starch as do the high doses used by Whistler and coworkers (Ingle and Whistler 1964, Uchino and Whistler 1962, Whistler and Schweiger 1957, Whistler et al 1966). The main difference appears to be at the quantitative level.

TABLE V
Intrinsic Viscosity and Iodine Affinity of Starches Washed from E Fractions

Chlorine Level (oz/cwt)	β-Amylolysis[a] (%)	Intrinsic Viscosity[b] (ml/g)	Iodine Affinity (%)
0	66.5	2.20	4.13
1	68.1	2.05	4.24
2	72.3	1.98	4.16
4	71.2	1.53	4.20

[a] Least significant difference = 1.2 at $P < 0.05$.
[b] Least significant difference = 0.08 at $P < 0.05$ level, $[\eta] = C \rightarrow 0 \frac{\ln rel}{C}$.

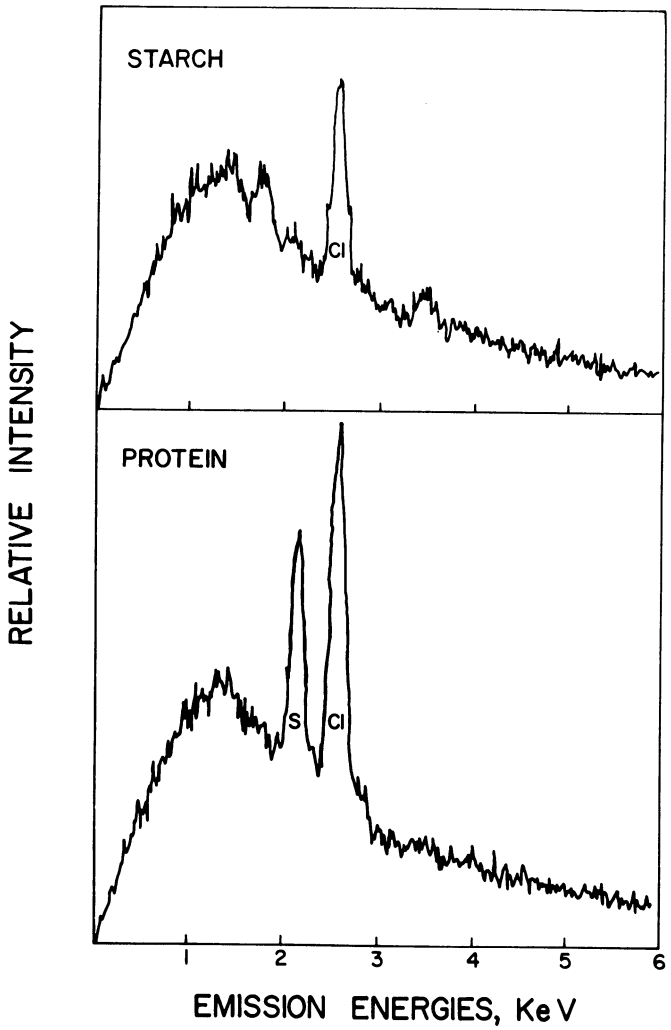

Fig. 2. Sample spectra showing chloride in starch and protein as determined by X-ray microanalysis. (The window for chloride is at 2.52–2.75 KeV.)

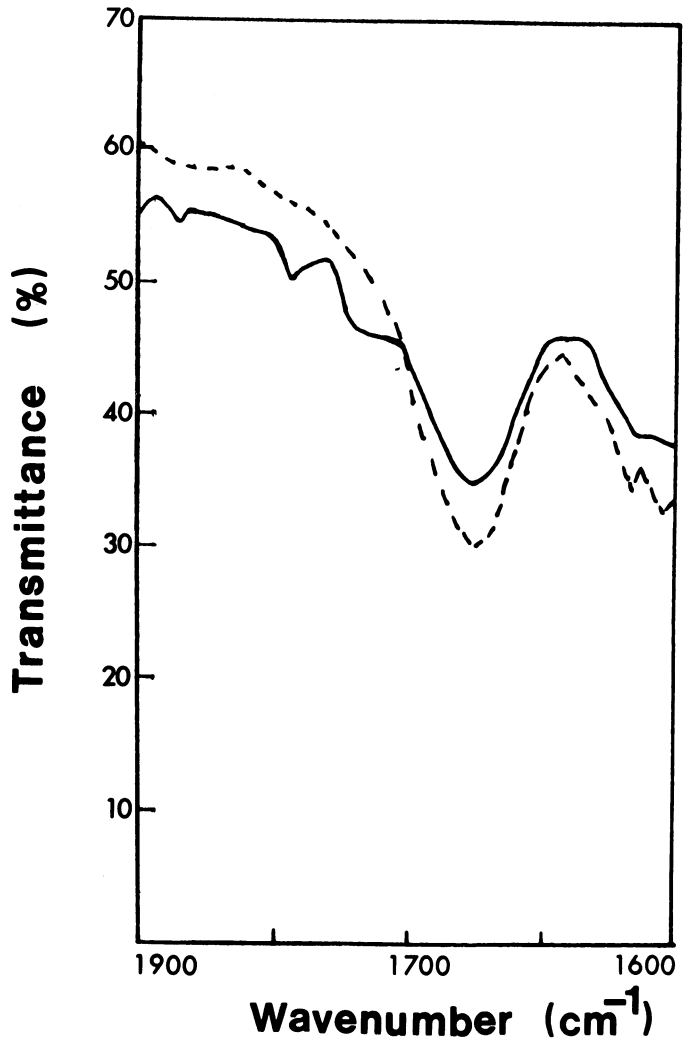

Fig. 3. Infrared scans of KBr starch pellets. — — indicates starch from untreated flour; —— indicates starch from flour chlorinated to a level of 4 oz/cwt.

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