Chlorine Treatment of Cake Flours. V. Oxidation of Starch[1]

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ABSTRACT

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Starches isolated from untreated flour and from Cl_2-treated flour performed equally well in cakes baked from reconstituted flours when the untreated starch was air-dried. Freeze-dried starch isolated from untreated flour gave results equal to results with untreated flours. Vacuum-drying or solvent-drying of the starch gave results similar to freeze-drying. Thus wetting and air-drying starch appeared to be equivalent to Cl_2-treatment as far as cake-baking performance was concerned. Surprisingly, commercial wheat starch responded to the wetting and drying treatment similarly to laboratory-isolated starch. Treatment of starch with base, to remove protein, reduced the effectiveness of air-drying. However, the baking quality of starch that had previously been air-dried was not affected by treatment with base. Treatment of good baking quality starches (air-dried and Br_2-treated) with β-amylase showed that they were depolymerized compared with poor-baking-quality starch (freeze-dried). Starches were debranched with pullulanase and separated into amylose and debranched amylopectin fractions by gel filtration. Both fractions from good baking quality starches (air-dried and Br_2-treated) gave lower β-amylolysis values than did the poor baking quality starch (freeze-dried). This indicates that the improving action of oxidative agents (Cl_2, Br_2, and air-drying) consists of oxidative depolymerization and probably oxidation of glucose residues in the starch chains.

The improving effect of Cl_2-treatment on cake flour is unquestioned. Chlorine-treated flours give cakes that do not collapse during baking and thus have greater volume and finer grain. Fractionation and reconstitution studies (Johnson and Hoseney 1979, Lamb and Bode 1963, Sollars 1958) have showed that the action of the chlorine on the prime starch fraction is principally responsible for Cl_2-treated flours' improved baking properties. Action of chlorine on starch was the subject of a series of studies by Whistler and co-workers (Ingle and Whistler 1964, Uchino and Whistler 1962, Whistler et al 1966, Whistler and Schweiger 1957). Although the level of chlorine used was extremely high compared with the levels used for flour improvement, these studies are useful in identifying possible modes of attack. Whistler and co-workers reported that the major effect of chlorine on semidry wheat starch was an oxidative depolymerization. They found little oxidation of the glucosidic carbons C-2 and C-3; a significant amount of oxalic acid was produced, however, indicating that some glucose residue had undergone extensive oxidation. In contrast, aqueous starch slurries treated with chlorine gave extensive oxidation of carbons C-2 and C-3 and extensive depolymerization.

The purpose of this study was to investigate various treatments of starch in an effort to understand the changes in starch caused by the Cl_2-treatment of cake flour.

MATERIALS AND METHODS

The flour was a commercially milled, soft wheat cake flour. Two flour samples were used. Both were from the same grist but one received Cl_2-treatment at the mill; the other was untreated. The flours contained 8.7% protein and 0.34% ash. When slurried with water, the Cl_2-treated flour had a pH of 4.8 and the untreated flour a pH of 5.6.

The cake baking procedure was a modification (Johnson and Hoseney 1979) of Kissell's lean cake procedure (1959). The modification included use of granular (6×) sugar, one-step mixing of 4 min (medium speed), and use of liquid shortening containing propylene glycol monostearate and stearic acid (Procter & Gamble's Nutex). The standard deviation of cake volume was 16.3 cc. A second cake-baking procedure, in which starch was used in place of flour, was that described by Cauwain and Gough (1975). The standard deviation of cake volume was 17.2 cc. All cake volumes are the average of at least duplicate bakes and generally the average of several cakes. Flour was fractionated into starch, water solubles, and gluten as described previously (Johnson and Hoseney 1979).

β-Amylolysis

Starch was dissolved in 0.1*N* potassium hydroxide 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 0.02–0.04*M* acetate buffer, pH 4.8, at 35°C for 24 hr. The maltose liberated was determined using Nelson's colorimetric copper method (1944), and the degree of β-amylolysis was calculated as follows:

$$\beta\text{-amylolysis} = \frac{\text{maltose} \times 0.95}{\text{starch wt}} \times 100$$

The procedure is essentially Whelan's (1964).

Debranching with Pullulanase

Starch was debranched at 37°C in a digest containing 2–5 mg/ml substrate and 3–5 units/ml of pullulanase (*Enterobacter aerogenes*, Sigma Chemical Co., St. Louis, MO) in 0.02*M* sodium acetate buffer, pH 5.5. Ghiasi et al (1979) had shown the pullulanase to be free of amylase activity. A drop of toluene was added to the digest to inhibit bacterial growth. After 24-hr incubation, the digest was heated to boiling to inactivate the enzymes, and insoluble material was removed by filtration (Lii and Lineback 1977).

Gel Filtration of Debranched Starch

Debranched samples (5–10 mg) were fractionated on a Bio-Gel P-10 column (2.6 × 70 cm) at room temperature (Lii and Lineback 1977). Elution was performed with 0.01*M* phosphate buffer, pH 7.0, containing 0.02% sodium azide. Fractions (~6 ml) were collected every 30 min. Total carbohydrate content in each fraction was measured by the phenolsulfuric acid method (Dubois et al 1956).

Alkali Treatment of Starch

Some starch samples were steeped in 0.2% NaOH (1:3 w/v) at 5°C for 12 hr (Goering 1967). The suspension was adjusted to pH 6.0 with 1*N* HCl and then filtered. The wet starch was washed three times by suspending it in distilled water, stirring with a magnetic stirrer for 10 min, and then filtering. The starch was dried in a stream of air and stored at 4°C.

Treatment of Starch with Bromine

Starch was treated with Br_2 by adding 1 ml of a solution containing 10 mg of Br_2 per 1 ml of chloroform to 100 g of starch in

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a 1-lb tin can and shaking the can vigorously for 3 min. The solvent was removed by placing the starch in an airstream for 30 min. The starch was stored at 4°C until used.

RESULTS AND DISCUSSION

The prime starch fraction has been shown to be the component of flour responsible for the beneficial effect of chlorine on flour. However, wheat starch used to replace the flour in commercial cake formulas performed satisfactorily even though not treated with chlorine (Cauwain and Gough 1975, Cauwain et al 1977, and Howard et al 1968). An explanation for the apparent difference in baking response of starch was discovered accidentally. Starch isolated from untreated flour was dried in air rather than freeze-dried. When this air-dried starch was reconstituted with the other untreated flour fractions and baked into cakes, the cakes were of quality comparable to that of cakes made with Cl_2-treated flour (Table I). Thus, from the cake-baking standpoint, air-drying of the starch apparently performs the same function as Cl_2-treatment of the flour.

The quality of cakes baked from reconstituted untreated flours depended on the method used to dry the starch (Table I). When untreated flour was fractionated by the scheme given previously (Johnson and Hoseney 1979) and the wet starch freeze-dried, the reconstituted flours gave cakes similar to those obtained using the unfractionated untreated flour.If the starch was air-dried rather than freeze-dried, however, the reconstituted flours gave cakes similar to those from unfractionated Cl_2-treated flours. When starch was isolated from untreated flour, freeze-dried, and then slurried in water and air-dried, it gave reconstituted flours with baking properties essentially equal to those of unfractionated Cl_2-treated flour. If the starch was first air-dried, then slurried in water and freeze-dried its baking properties were equal to those of air-dried starch. Starch isolated from Cl_2-treated flour gave very similar baking responses regardless of whether it was air-dried or freeze-dried. The data show that the method used to dry starch isolated from untreated flour determines the baking properties of the reconstituted flour.

Effect of Different Starch Treatments on Cake Quality

Cauwain and Gough (1975) developed a yellow cake-baking procedure that permitted more direct comparison of the baking properties of chlorinated and nonchlorinated starches. In their procedure, starch was substituted for flour in cake baking.

Cauwain's yellow cake-baking procedures were used to study the baking properties of air-dried, freeze-dried, vacuum-dried, and solvent (methanol)-dried starch isolated from Cl_2-treated and untreated flours.

Starch isolated from Cl_2-treated flour performed satisfactorily

TABLE I
Comparison of the Baking Properties (in White Layer Cakes) of Air-Dried and Freeze-Dried Starch Isolated from Cl_2-Treated and Untreated Flours

Source of Starch	Volume (cc)	Grain
Cl_2-treated flour	575	Fine
Untreated flour	400	Coarse
Reconstituted Cl_2-treated flour		
Air-dried	600	Fine
Freeze-dried	580	Fine
Reconstituted untreated flour		
Air-dried	540	Fine
Freeze-dried	385	Collapsed
Freeze-dried/air-dried	520	Fine
Air-dried/freeze-dried	530	Fine

TABLE II
Effect of Air-Drying and Freeze-Drying on the Baking Quality of Starches (in Yellow Cakes[a]) Isolated from Cl_2-Treated and Untreated Flours

Source of Starch	Volume (cc)	Collapse	Grain[b]
Cl_2-treated flour			
Freeze-dried	630	No	S
Air-dried	600	No	S
Untreated flour			
Freeze-dried	450	Yes	U
Air-dried	590	No	S
Freeze-dried/air-dried[c]	600	No	S
Air-dried/freeze-dried[c]	640	No	S

[a] Starch substituted for flour.
[b] S = satisfactory, U = unsatis
[c] Dried, wetted, and then redrie

TABLE III
Effect of Freeze-Drying, Vacuum-Drying, Methanol-Drying, Air-Drying, and Combinations on the Baking Quality of Starch from Untreated Flour (in Yellow Cakes[a])

Starch Drying Methods	Volume (cc)	Collapse	Grain[b]
Freeze-dried	450	Yes	U
Vacuum-dried	455	Yes	U
Methanol-dried	440	Yes	U
Methanol-dried/air-dried	600	No	S
Freeze-dried/air-dried	470	Yes	U

[a] Starch substituted for flour.
[b] S = satisfactory, U = unsatisfactory.

TABLE IV
Effect of the Drying Method on the Baking Properties of Commercial Starch (in Yellow Cakes[a])

Drying Method	Volume (cc)	Grain[b]
None	430	U
Freeze-dried	450	U
Air-dried	605	S
Methanol-dried	440	U
Vacuum-dried	455	U

[a] Starch substituted for flour.
[b] S = satisfactory, U = unsatisfactory.

TABLE V
Effect of Purification with 0.2% NaOH Solution on the Baking Properties of Air-Dried Starch (in Yellow Cakes[a])

Treatment	Volume (cc)	Grain[b]
None	450	U
Air-dried	640	S
Purified/air-dried	506	U
Air-dried/purified/air-dried	600	S
Purified/Br_2-treated	640	S

[a] Starch substituted for flour.
[b] S = satisfactory, U = unsatisfactory.

TABLE VI
Values from β-Amylolysis of Starches with Different Baking Properties

Starch	β-Amylolysis (%)
Freeze-dried	62.4
Air-dried	74.4
Br_2-treated	65.4
Amylose fraction from P10[a]	
Freeze-dried	86.5
Air-dried	75.2
Br_2-treated	64.4
Debranched amylopectin from P10[a]	
Freeze-dried	91.7
Air-dried	71.5
Br_2-treated	75.9

[a] Bio-Gel P10 column.

regardless of whether the starch had been dried in air or freeze-dried (Table II). The cakes did not collapse when cooled and had good volume and a fine grain. Starch isolated from untreated flour performed satisfactorily if the starch had been air-dried. Cakes from this flour did not collapse when cooled; they had good volume and a fine grain. However, if the starch isolated from untreated flour had been freeze-dried only, it failed to give good cakes. These cakes collapsed when cooled and thus had low volume. Starch isolated from untreated flour, freeze-dried, then slurried with water, and air-dried gave cakes with good volume and a fine grain; they did not collapse when cooled. The baking quality of air-dried starch was unchanged when the starch was slurried with water and freeze-dried.

The baking performance of starch isolated from untreated flour was therefore benefitted by air-drying and unchanged by freeze-drying. Further, when starch from untreated flour was either freeze-dried, wetted, and air-dried or air-dried, wetted, and freeze-dried, it performed identically to starch isolated from Cl_2-treated flour. Thus, starch from untreated flour when air-dried undergoes some change beneficial to cake-baking properties. Starch isolated from Cl_2-treated flour and freeze-dried performed identically to the same starch air-dried. Apparently starch from Cl_2-treated flour underwent a change during chlorine-treatment that made it insensitive to drying conditions.

The nature of the change that starch isolated from untreated flour undergoes during air-drying was unclear. To determine whether exposing wet starch to air was essential for its improved properties, starch was dried under a vacuum and in methanol.

Starch isolated from untreated flour was dried in a vacuum desiccator under reduced pressure and used to bake cakes (Table III). The cakes collapsed when cooled, had low volume, and collapsed structure.

Another sample of starch was isolated from untreated flour and dried in methanol; the methanol was removed by exposing the starch to a stream of air. That starch also gave cakes that collapsed when cooled and had low volume and a collapsed structure. However, if the starch dried with methanol was wetted with water and air-dried, cakes produced with it equaled those from air-dried starch. Thus, methanol did not affect the baking quality of the starch. The data indicate that exposing the wet starch to air was necessary for the starch's baking performance to be improved by drying. The data further suggested that, for the desired change to take place, the starch must have reasonably high moisture content when exposed to air. This suggestion was further supported by the fact that freeze-dried starch was not improved when exposed to a stream of air for three days, the time used to air-dry wet starch.

The treatment with methyl alcohol would also have removed bound lipid from the starch. Thus, changes in the lipid associated with the starch did not appear to be important to the starch's improved baking properties.

In commercial isolation of wheat starch, the wet starch is dried. Therefore, we assumed that the starch would have good baking properties. Surprisingly, this was not true (Table IV). The air-drying procedure improved the baking properties of the commercial starch just as it had our laboratory-isolated starch. Freeze-drying, solvent-drying (methyl alcohol), and vacuum-drying were also ineffective in improving the baking properties of the commercial starch.

Alkali-Treated Starch

Sodium hydroxide (0.2%) has been used to purify starch (Goering 1967). The alkali effectively removes protein associated with the starch. Commercial wheat starch was treated with 0.2% NaOH; the solution was neutralized with HCl (1*N*) and the starch was air-dried. Cakes baked from this starch were slightly better than those baked from untreated starch but much poorer than those baked from air-dried starch that had not been treated with NaOH (Table V). Treating previously air-dried starch with 0.2% NaOH did not alter its baking properties. Starch that was treated with 0.2% NaOH and later treated with bromine gave excellent baking results.

Effect of Treatments on Starch

The treatments that improved the baking properties of starch (Cl_2-treatment, Br_2-treatment, and air-drying) are oxidative or potentially oxidative. As shown by Whistler and co-workers (Ingle and Whistler 1964, Uchino and Whistler 1962, Whistler et al 1966, Whistler and Schweiger 1957) high levels of Cl_2-treatment can attack starch by oxidative cleavage of the α-1,4 bond between glucose residues or by oxidative cleavage between the C-2 and C-3 carbons in the glucose residues.

Cleavage of α-1,4 bonds would provide a new nonreducing end in the starch and thus a new site for β-amylase attack. Thus, if the starch were undergoing significant oxidative cleavage due to the treatment, the total starch should give higher β-amylolysis limits than untreated starch. Three samples of starch—a freeze-dried starch with poor baking properties, a Br_2-treated starch with good baking properties, and an air-dried starch with good baking properties—were subjected to hydrolysis with crystalline β-amylase (Table VI). The higher β-limits for the two treated starches with good baking properties clearly show the starch to have undergone oxidative cleavage of α-1,4 bonds.

To determine if the glucose residues were also oxidatively cleaved between the C-2 and C-3 carbons, the dissolved starch was debranched with pullulanase and placed on a Bio-Gel P10 Column. The material eluting at the void volume of the column is essentially amylose and is separated from the debranched amylopectin. Both fractions should have high β-amylase limits, but a glucose residue cleaved between C-2 and C-3 would provide a barrier to further β-amylolysis. The data (Table VI) show clearly that the two starches with good baking properties (Br_2-treated and air-dried) had lower β-amylolysis values than the fractions from the freeze-dried starch. Thus the improving action of the treatments on starch appears to involve both types of oxidative cleavage.

LITERATURE CITED

CAUWAIN, S. P., and GOUGH, B. M. 1975. High-ratio yellow cake. The starch cake as a model system for response to chlorine. J. Sci. Food Agric. 26:1861.

CAUWAIN, S. P., GOUGH, B. M., and WHITEHOUSE, M. E. 1977. The role of starch in baked goods, part 2. The influence of purification procedure on the surface properties of the granule. Staerke 29:91.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

GHIASI, K., HOSENEY, R. C., and LINEBACK, D. R. 1979. Characterization of soluble starch from bread crumb. Cereal Chem. 56:485.

GOERING, K. J. 1967. New starches. II. The properties of the starch chunks from Amaranthus retroflexus. Cereal Chem. 44:245.

HOWARD, N. B., HUGHES, D. H., and STROBEL, R. G. K. 1968. Function of the starch granule in the formation of layer cake structure. Cereal Chem. 45:329.

INGLE, T. R., and WHISTLER, R. L. 1964. Action of chlorine on semidry starch. Cereal Chem. 41:474.

JOHNSON, A. C., and HOSENEY, R. C. 1979. Chlorine treatment of cake flours. III. Fractionation and reconstitution techniques for Cl_2-treated and untreated flours. Cereal Chem. 56:443.

KISSELL, L. T. 1959. A lean-formula cake method for varietal evaluation and research. Cereal Chem. 36:168.

LAMB, C. A., and BODE, C. E. 1963. Quality Evaluation of Soft Winter Wheat. Ohio Agric. Exp. Stn. Res. Bull. 926. Wooster, OH.

LII, C. Y., and LINEBACK, D. R. 1977. Characterization and comparison of cereal starches. Cereal Chem. 54:138.

NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375.

SOLLARS, W. F. 1958. Fractionation and reconstitution procedures for cake flour. Cereal Chem. 35:85.

UCHINO, N., and WHISTLER, R. L. 1962. Oxidation of wheat starch with chlorine. Cereal Chem. 39:477.

WHELAN, W. J. 1964. Hydrolysis with β-amylose limit dextrin of amylopectin. Methods Carbohydr. Chem. IV:261.

WHISTLER, R. L., MITTAG, T. W., and INGLE, T. R. 1966. Mechanism of starch depolymerization with chlorine. Cereal Chem. 43:362.

WHISTLER, R. L., and SCHWEIGER, R. 1957. Oxidation of amylopectin with hypochlorite at different hydrogen ion concentrations. J. Am. Chem. Soc. 79:6460.

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