Oil-binding Capacity of Prime Starch from Chlorinated Wheat Flour

M. SEGUCHI, Seibo Women's Junior College, Tayamachi-1, Fukakusa, Fushimi-ku, Kyoto, Japan 612

Cereal Chem. 61(3):241-244

ABSTRACT

Prime starch recovered by wet-fractionating serially chlorinated flour demonstrated that oil binding progressed with the rate of chlorination. This capacity was decreased when chlorinated starch was treated with proteolytic or amylolytic enzymes or with a solution of dilute acid. Chlorinated starch did not respond well to the iodine test, until it was treated with a proteolytic enzyme. The oil-binding capacity of chlorinated starch was attributed to the starch's having a surface coating of proteins. Treatment of such starch with certain lipid solvents had little effect on oil-binding capacity.

Chlorination of wheat starch has been shown to improve springiness and mouthfeel in pancakes (Seguchi and Matsuki 1977a, Gaines 1982, Gaines and Donelson 1982). Cake volume is also improved by chlorination (Sollars 1968a, 1968b; Kulp 1972a Seguchi and Matsuki 1977a; Johnson and Hoseney 1979). The improving effect on cake expansion is thought to be brought about by the modification of lipid fraction from the baking test of reconstituted flour (Kissell et al 1979; Clements and Donelson 1982a, 1982b). Chlorinated starch swells, pastes, and increases in viscosity at a faster rate and imbibes more free water than does unchlorinated starch (Kulp et al 1972b, Huang et al 1982). Thus, less free water is available in chlorinated cake crumb, and this deficiency results in a drier mouthfeel. However, the exact mechanism is unclear. One effect of chlorination is an increase in the hydrophobicity (lipophilization) of starch, as evidenced by the ability of granules to form clusters in water or to bind oil (Seguchi and Matsuki 1977a). Those clusters were broken by the addition of sucrose fatty acid ester, and the improving effects of chlorination on pancake springiness and mouthfeel also disappeared. It may be considered plausible that the increased hydrophobicity of chlorinated starch enhances the interaction of starch granules or with other batter components such as proteins, resulting in greater transfer of water to starch when swelling. The resulting decrease in free water in cake crumb would affect cake crumb dryness and springiness. When sucrose fatty acid ester was added to cake batter, chlorinated starch granules may have been coated with the substance and thus may have separated from these other components. The result may have been a decrease in the amount of water transferred to starch granules, delaying or retarding their swelling. The effect of sucrose fatty acid esters may have been to cause no change in cake crumb due to flour chlorination. One may thus conclude that the improvements in cake crumb such as springiness and mouthfeel are possibly due to the hydrophobic (lipophilic) character of chlorinated starch (Seguchi and Matsuki 1977a). This paper reports experiments conducted in an effort to examine this mechanism.

MATERIALS AND METHODS

Flour, Enzymes, and Oils

A patent-grade commercially milled U.S. Western white flour with protein and ash contents of 7.6 and 0.35%, respectively, was used. Trypsin type III, α-amylase type II-A, β-amylase type II-B, α-chymotrypsin type II, pepsin, rapeseed oil, and corn oil were obtained from commercial sources. All chemicals used were reagent grade.

Flour Chlorination and Fractionation

Sublots of flour were serially treated with chlorine gas ranging from 0 to 7.26 g per kilogram of flour and were wet-fractionated, using acetic acid as described by Seguchi and Matsuki (1977b). The prime starch fractions thus obtained were frozen as 10% suspensions in water until used.

Determination of Starch Oil-binding Capacity

Five milliliters of the 10% solution (or 0.50 g) of prime starch and 1 ml of rapeseed or corn oil in a graduated centrifuge tube (16.5 × 105 mm) were mixed vigorously on a vibrio mixer (amplitude 0.1 cm) 20 times for 10-sec periods. The mixture was then centrifuged at 600 × g for 20 min at room temperature. A minimum volume of 0.1 ml could be read. The volume of oil remaining above the aqueous phase was directly read from the graduated table to the nearest 0.1 ml. The oil-binding capacity of the starch was expressed as milliliters of oil bound per gram of prime starch. Data in this paper were derived from triplicate determinations. Standard deviation of oil binding was 0.06 ml with this method.

Treatment of Chlorinated Prime Starch Adhering to Oil Droplets with Sodium Dodecyl Sulfate (SDS) or Isoamyl Alcohol

Prime starch (5 ml of the 10% solution) was mixed with 1 ml of rapeseed oil, causing a binding, and this was followed by the addition of 100 μl of 1% SDS or the same volume of isoamyl alcohol.

Chlorinated Prime Starch Reactions with Solvents, Sodium Dodecyl Sulfate (SDS), or Dilute Acid

Thirty milliliters of the 10% solution (or 3.00 g) of chlorinated prime starch were subjected to the following actions:

1. 30 ml of chloroform-methanol (2:1, v/v), boiled 5 min under reflux, two times successively
2. 20 ml of water-saturated butanol (WSB), extracted 4 min at 1,000 rpm with a National MK-210 mixer, three times successively (Seguchi and Matsuki 1977c)
3. 30 ml of SDS (1%) solution, extracted 3.7 hr at 37°C
4. 30 ml of HCl (7.0%) solution, extracted three days at room temperature (Kainuma and French 1971)

After each treatment, the suspension was centrifuged 10 min at 7,500 × *g*, then washed five times with 10 ml of water each time. After being washed, the suspension was centrifuged under the same conditions. The washed starch was suspended in 2 ml of water as a 10% suspension, and was tested for oil-binding capacity.

TABLE I
Chlorinated Prime Starch Treated with 20 ml of Enzyme Preparations

Starch Quantity (g)	Enzyme	Enzyme Quantity (g)	Medium
0.5	Pepsin	0.5, 5.0, 50	HCl solution, pH 2.0
0.5	Trypsin	50	0.1 *M* Tris/HCl, pH 6.9
0.5	α-Chymotrypsin	50	0.1 *M* Tris/HCl, pH 6.9
0.5	α-Amylase	5	0.02 *M* Na-phosphate buffer, pH 6.9, containing 0.0067 *M* NaCl
0.5	β-Amylase	8.25	0.0016 *M* Na-acetate buffer, pH 4.8

TABLE II
Recovery of Prime Starch and Tailings Fractions from Fractionation of 100 Grams of Serially Chlorinated Flours

Chlorination Rate[a] (g/kg)	Flour pH	Prime[b] Starch (g)	Tailings[b] (g)
0.00	5.70	54	30
0.40	5.40	53	33
1.20	5.00	58	30
2.26	4.62	49	42
3.26	3.32	27	65
4.26	2.96	0	89
7.26	2.15	0	92

[a] Grams of chlorine gas per kilogram of flour.
[b] Based on 10% moisture content.

TABLE III
Oil-binding Capacity of Prime Starch Fraction from Serially Chlorinated Flours

Chlorination Rate[a] (g/kg)	Oil-binding Capacity (ml/g of starch)
0.00	0.2
0.40	0.4
1.20	0.8
2.26	1.4

[a] Grams of chlorine gas per kilogram of flour.

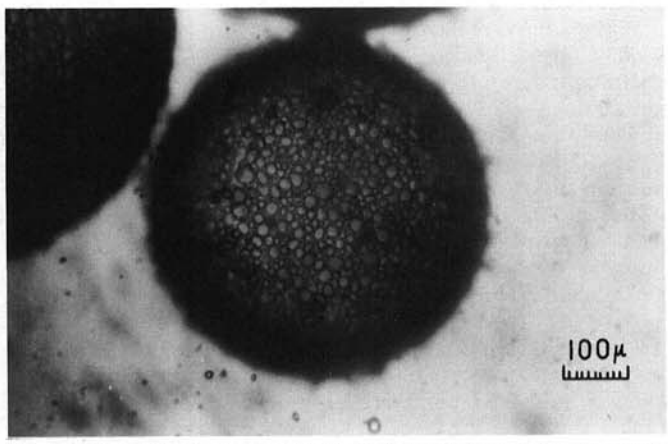

Fig. 1. Photomicrograph of chlorinated prime starch granules adhering to an oil droplet.

Treatment of Chlorinated Prime Starch with Enzymes

Sublots of 10% solution of chlorinated prime starch were treated with 20 ml of enzyme preparations under the conditions given in Table I.

For each reaction, the starch-enzyme suspension was incubated for 60 min at 37° C, washed, dialyzed against water, resuspended in water and tested for oil-binding capacity as described for starch subjected to solvent action.

Staining of Chlorinated Prime Starch

Sublots of 10% solution of chlorinated prime starch were tested for reaction with iodine as described by Schoch (1964). Fifty microliters of a 10% suspension of starch was reacted with an equal volume of iodine solution on a glass slide, and the field was observed under a microscope after a few minutes.

RESULTS AND DISCUSSION

Recovery data for fractions from serially chlorinated flours showed that prime starch could not be readily recovered from flour

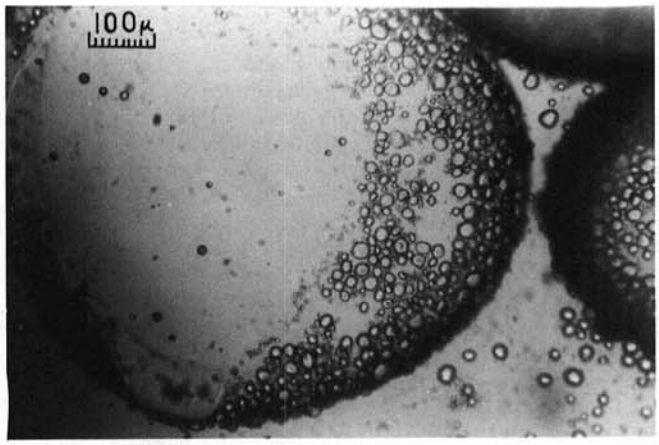

Fig. 2. Photomicrograph showing separation of chlorinated prime starch from an oil droplet surface when sodium dodecyl sulfate was added to the suspension.

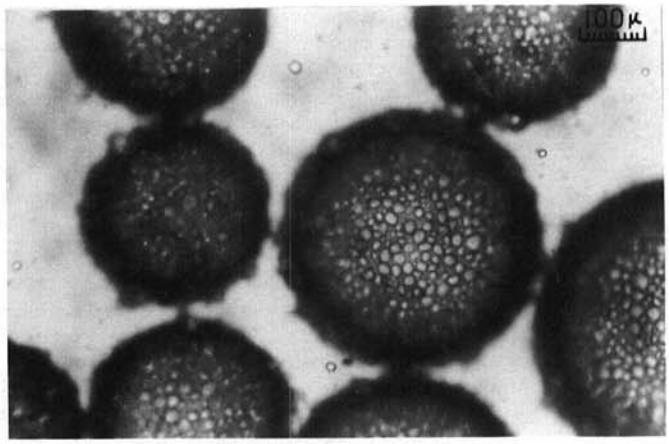

Fig. 3. Photomicrograph showing renewed adherence of chlorinated prime starch separated from an oil droplet through the addition of sodium dodecyl sulfate to the suspension. The recovered starch is then washed and dialyzed.

chlorinated at the rate of 4.26 g/kg of flour or greater (Table II). This difficulty may be attributed to an effect of polymerizing grafts, as suggested by Whistler.[1] As a result, prime starch recovered from flour chlorinated at 2.26 g/kg flour was selected as the starting material for subsequent experiments with solvents and enzymes.

Effect of Chlorination on Oil-binding Capacity of Starch

The oil-binding capacity of starch increased with increments of chlorine (Table III), reaching 1.4 ml of oil/g of starch at the chlorination rate of 2.26 g/kg. Figure 1 is a photomicrograph of starch granules on an oil droplet.

Effects of Addition of SDS or Isoamyl Alcohol on Oil-binding Capacity of Starch Adhering to Oil Droplets

The addition of SDS or isoamyl alcohol to a suspension in which chlorinated prime starch was bound to oil droplets decreased the ability of starch to bind oil (Fig. 2). However, when starch thus separated was recovered, washed thoroughly with water, and dialyzed, it regained its oil-binding ability (Fig. 3). These results suggest that SDS and isoamyl alcohol exert preferential bonding, which is reversible, to chlorinated prime starch.

Effects of Proteolytic and Amylolytic Enzymes on Oil-binding Capacity of Chlorinated Prime Starch

Pepsin in relatively high concentration destroyed the oil-binding capacity of chlorinated starch (Table IV), and at lower concentrations it had proportional effects. However, when the enzyme preparation was heat-denatured by being boiled for 5 min, it had little effect in lowering oil-binding capacity. Similar effects were observed with trypsin (Table IV). At the same concentration as pepsin or trypsin, α-chymotrypsin exhibited less, though still positive, effects in reducing oil-binding capacity of starch. This suggests that protein on the starch granule is involved in the binding of oil.

Chlorinated prime starch, as examined under the microscope, was only partially stained by iodine (Fig. 4A). But when starch was incubated with proteolytic enzyme and then reacted with iodine, the granules were fully stained (Fig. 4B). It is probable that the chlorination of starch resulted in a reaction which blocked access of iodine reaction sites but that much of this blockage was removed through proteolysis. It thus appears probable that protein comprises the iodine-blocking entity. Since increased dispersion of flour proteins is one effect of the chlorine-flour reaction, one may surmise that a reaction between starch and solubulized protein occurs on the starch surface immediately upon flour wetting. The nature of that bonding is of such strength that the complex resists separation even after repeated washing with water.

[1]R. L. Whistler. 1967. Recent Developments in Starch Chemistry and Technology. Special lecture at the 1967 Special Meeting of the Technological Society of Starch, Tokyo, on August 18.

TABLE IV
Oil-binding Capacity of Chlorinated Prime Starch Following Treatment with Various Enzymes and Certain Solvents

Treatment	Oil-binding Capacity (%)	Oil-binding Capacity (ml/g)[a]
None	100	1.4
Pepsin		
50 mg	0	0.0
5 mg	7	0.1
0.5 mg	20	0.3
Heat-denatured pepsin,[b] 50 mg	79	1.1
Trypsin, 50 mg	0	0.0
α-Chymotrypsin, 50 mg	29	0.4
α-Amylase, 5 mg	20	0.3
β-Amylase, 8.25 mg	20	0.3
Chloroform-methanol	150	2.1
Water-saturated 1-butanol	100	1.4
Sodium dodecyl sulfate	130	1.8
7% HCl at room temperature	0	0.0

[a]Milliliters of oil per gram of starch.
[b]Boiled 5 min.

Digestion of chlorinated starch granules with amylase preparations also resulted in reduced oil-binding capacity (Table IV). Without having further evidence, I may speculate that amylolytic action weakens the starch-protein bonding such that the protein is released from the starch surface through starch hydrolysis.

Effects of Solvents and Dilute Acids on Oil-binding Capacity

Treatment of chlorinated starch with SDS, chloroform-methanol, or water-saturated butanol (WSB) following with wash and dialysis against water did not discernibly reduce the oil-binding capacity of chlorinated starch (Table IV). The result obtained by protein dispersing effects of SDS indicated that protein on the starch granule was probably not dispersed by SDS. Sodium dodecyl sulfate is also known to disperse inner lipids that are complexed with amylose (Fujii and Oba 1962). The result of the SDS treatment indicates that lipid-amylose complexes probably also were not related to the oil-binding capacity of starch. Chloroform-methanol is known as a solvent for extraction of whole lipid, and WSB is known for the extraction of polar and bound lipids (Seguchi and Matsuki 1977c). The absence of change in oil-binding capacity by WSB indicates that polar flour lipids are probably not directly involved in the increased capacity of chlorinated starch to bind oil. The data for chloroform-methanol and SDS were increased to 150 and 130%, respectively, suggesting that lipids near hydrophobic sites may be released by these solvents; this may increase oil-binding capacity.

The solvent action of 7% hydrochloric acid in negating oil-binding capacity (Table IV) may be due to its releasing of proteins from the starch surface or, alternatively, to the formation of Nägeli amylodextrin from starch by weak acid at room temperature (Kainuma and French 1971). Because noncrystalline starch is more readily degraded by acid than its crystalline counterpart, the starch-protein complex is probably a combination of dispersed protein and noncrystalline starch.

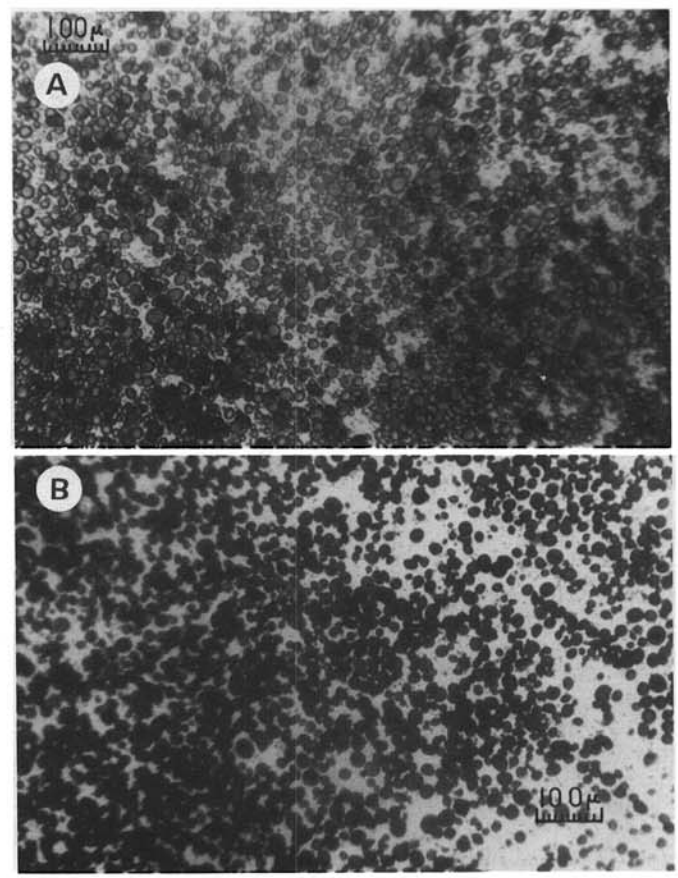

Fig. 4A, Chlorinated prime starch granules incompletely stained with iodine solution. **B,** Chlorinated prime starch granules treated with proteolytic enzyme, washed, and stained with iodine solution.

Based on the above data, I may attribute the oil-binding capacity of chlorinated prime starch to the presence of protein on the surface of the starch. The protein, complexed with the starch, provides sites for oil binding. Treatments that modified the protein (by a possible solvent action by hydrochloric acid) lowered the oil-binding capacity of the starch. Similarly, enzymic amylolysis may have removed the protein from the surface of the granule. One study of proteins on the surface of yeast cells (Eddy and Rudin 1958) indicated that such proteins are important in the flocculation of brewer's yeast. The subject of proteins on the surface of chlorinated prime starch warrants further study.

ACKNOWLEDGMENTS

The author is very grateful to H. Matsumoto and W. T. Yamazaki for help in the preparation of this manuscript. Thanks are also due to Y. Tani for his advice.

LITERATURE CITED

CLEMENTS, R. L., and DONELSON, J. R. 1982a. Role of free flour lipids in batter expansion in layer cakes. I. Effects of "aging." Cereal Chem. 59:121.

CLEMENTS, R. L., and DONELSON, J. R. 1982b. Role of free flour lipids in batter expansion in layer cakes. II. Effects of heating. Cereal Chem. 59:125.

EDDY, A. A., and RUDIN, A. D. 1958. Part of the yeast surface apparently involved in flocculation. J. Inst. Brew. 64:19.

FUJII, T., and OBA, K. 1962. Purification of starch by surface active agents. XII. Fatty substance in sweet potato starch and removal of "fat-by-hydrolysis" by surface active agents. J. Ferment. Technol. 40:390.

GAINES, C. S. 1982. Technique for objectively measuring a relationship between flour chlorination and cake crumb stickiness. Cereal Chem. 59:149.

GAINES, C. S., and DONELSON, J. R. 1982. Contribution of chlorinated flour fractions to cake crumb stickiness. Cereal Chem. 59:378.

HUANG, G., FINN, J. W., and VARRIANO-MARSTON, E. 1982. Flour chlorination. II. Effects on water binding. Cereal Chem. 59:500.

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.

KAINUMA, K., and FRENCH, D. 1971. Nägeli amylodextrin and its relationship to starch granule structure. I. Preparation and properties of amylodextrins from various starch types. Biopolymers 10:1673.

KISSELL, L. T., DONELSON, J. R., and CLEMENTS, R. L. 1979. Functionality in white layer cake of lipids from untreated chlorinated patent flours. I. Effects of free lipids. Cereal Chem. 56:11.

KULP, K. 1972a. Some effects of chlorine treatment of soft wheat flour. Bakers Dig. 46(3):26.

KULP, K., TSEN, C. C., and DALY, C. J. 1972b. Effect of chlorine on the starch component of soft wheat flour. Cereal Chem. 44:194.

SCHOCH, T. J. 1964. Iodimetric determination of amylose. Potentiometric titration. Methods Carbohydr. Chem. 4:157.

SEGUCHI, M., and MATSUKI, J. 1977a. Studies on pan-cake baking. I. Effect of chlorination of flour on pan-cake qualities. Cereal Chem. 54:287.

SEGUCHI, M., and MATSUKI, J. 1977b. Studies on pan-cake baking. III. Effects of pH, mixing time, and moisture level on quality of pan-cake from reconstituted flour. Cereal Chem. 54:1056.

SEGUCHI, M., and MATSUKI, J. 1977c. Studies on pan-cake baking. II. Effect of lipids on pan-cake qualities. Cereal Chem. 54:918.

SOLLARS, W. F. 1968a. Fractionation and reconstitution procedures for cake flours. Cereal Chem. 35:85.

SOLLARS, W. F. 1968b. Cake and cookie flour fractions affected by chlorine bleaching. Cereal Chem. 35:100.

[Received March 3, 1982. Accepted January 12, 1984]