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AN INVESTIGATION OF THE PHYSICOCHEMICAL PROPERTIES OF CHLORINATED FLOUR

By

Sumei Wei

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ABSTRACT

AN INVESTIGATION OF THE PHYSICOCHEMICAL PROPERTIES OF CHLORINATED FLOUR

By

Sumei Wei

This study was conducted to examine the effects of chlorination on the physicochemical properties and cake baking performance of flour. Two levels of chlorinated flour (pH 4.8 and pH 4.2) and one non-chlorinated control flour were used, along with starch isolated from each of the treatments. Flour chlorinated at a high level (pH 4.2) showed a significant increase in flour water-binding capacity, starch hydrophobicity, and UV absorption of flour water extracts. Falling number tests indicated that chlorination significantly affected the relative viscosity of both flour and starch pastes. Rapid Visco-Analyzer measurements showed significant changes in flour or starch pasting properties. At the chlorination levels used in this study, no changes in swelling power or solubility of starch were observed. Surface tension of flour water extracts did not show an appreciable difference between treatments. Among the three types of flour, the one that was chlorinated to pH 4.8 produced cakes of the largest volume and best overall quality. This study has confirmed that optimum levels of chlorination influences the interactions between the starch fraction and other flour components significantly, thus facilitating the formation of a firm flour gel structure and a stable cake batter.

DEDICATION

To my parents, brothers and sisters in China who are so dear to me.

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1. INTRODUCTION

1.1 General

Chlorination is a process commonly used in the food industry to improve cake-baking performance, and is achieved by infusing chlorine gas into flour. The process results in changes in the physical and chemical properties of flour and flour components, which enhance processing and end product performance. However, the mechanisms by which the improvement is accomplished are not fully understood.

The quantity of chlorine gas added can be critical in enhancing cake quality. Properly chlorinated flour produces high quality cakes, while non-chlorinated or over-chlorinated flour produces cakes of inferior quality. When flour is chlorinated, its pH is lowered. Thus, in general, the pH of the flour can be used as a good measure of the chlorination level. An adequate treatment for most flours is at pH levels between 4.5 and 5.2 (Gough et al. 1978). A pH of 4.7-4.9 is commonly desired for most high-ratio cakes (Hoseney 1994). It should be noticed that the pH drop caused by chlorination is not a reason for the modification of flour components but a side effect of the chlorination process. A low pH does not contribute to the specific modifications by chlorination (Worthington 1994).

The majority of chlorine added to flour is found in the water-soluble fraction in the form of chloride ion. A large fraction of the chlorine is in flour lipids, especially in polar lipids as found by Gilles et al. (1964), Huang et al. (1982a), and Wei et al. (1984). Their studies indicated that chlorine preferentially interacts with the protein and lipid fractions of flour. Although the amount of chlorine associated with starch appears to be limited in comparison to other flour components, fractionation and reconstitution studies have indicated that the starch fraction is the major component responsible for the improvement of cake quality (Lamb and Bode 1963; and Sollars 1958; Johnson and Hoseney 1979).

Chlorine gas reacts with almost all flour constituents including starch, pentosans, proteins and lipids. The chlorination process has profound effects on some of these components. It has been reported that chlorine treatment depolymerizes starch (Huang et al. 1982a; Russell et al. 1987; Baldwin et al. 1997) and increases its hydrophobicity and hydration capacity (Seguchi 1993a; Alexander 1933; Kulp et al. 1972). This has been attributed to changes in starch surface proteins (Seguchi 1993b). Whistler and Pyler (1968) observed depolymerization of pentosans due to chlorination. Chlorination destroys normal gluten properties (Alexander 1933), increases hydrophobicity of gliadins (Sinha et al. 1997), and oxidizes cysteine, methionine and other amino acids (Ewart 1968; Tsen et al. 1971). Chlorine also combines chemically with lipids (Gough et al. 1978) and bleaches flour pigments by addition across conjugated double bonds (Bath 1995).

In addition, chlorine treatment increases the water-soluble fraction of flour as well as solubility of flour proteins (Kissell 1971; Tsen et al. 1971; Duviau et al. 1996), changes the rheological properties of flour (Ngo et al. 1985; Conforti and Johnson 1992; Worthington 1994), and alters the functional properties of flour lipids (Johnson et al. 1979; Donelson et al. 1984). The complexity of the flour system and the large number of variables which can be manipulated in a given sample have led to conflicting observations on these properties. Thus, to better understand the mechanisms involved, further research is needed on the physicochemical changes in the flour and flour components which are clearly attributable to the process of chlorination.

1.2 The Present Study

As a first approximation, a cake batter may be considered a colloidal dispersion. Among the factors that contribute to the overall physicochemical nature of a colloidal dispersion are the properties of the continuous phase, the dispersed phase and the interfacial structure. This study is part of a larger research project on flour chlorination designed to develop a molecular level of understanding of the mechanism of flour chlorination from a colloidal perspective. The goal of this part of the project was to assess the sensitivity of several techniques for detecting and characterizing the differences between chlorinated and non-chlorinated flour samples. It is expected that this study will make a significant contribution towards helping achieve the ultimate goal of promoting better understanding of the mechanisms of flour chlorination.

The objectives of this study were to: (1) examine and evaluate the effects of chlorination on the chemical and physical characteristics of cake flour and selected flour components; and (2) elucidate the factors that may control flour performance during cake baking.

This study is designed to give insight into the improvement of cake quality observed for chlorinated flour in comparison to native flour. It should also provide useful information to enable identification and exploration of alternatives to chlorination, since there are some safety concerns about using chlorine gas in foods.

2. LITERATURE REVIEW

2.1 Physicochemical Properties of Chlorinated Flour and Starch Fraction

2.1.1 Physicochemical Properties of Chlorinated Flour

The physical and chemical properties of flour and flour components greatly influence their performance in the final product. As an agent for improving cake baking, chlorine gas has profound effects on the characteristics of soft wheat flour. It has been reported that chlorine treatment increases flour water-binding capacity and solubility in water, and solubilizes and modifies flour proteins (Kissell 1971; Tsen et al. 1971; Duviau et al. 1996). In addition, the level of chlorination affects the degree of change in the physicochemical properties of the flour.

Chlorine treatment destroys normal gluten properties. Alexander (1933) reported that, after chlorination, the gluten was in a colloidal state and prevented dough formation. A weakening of gluten proteins following chlorination was also reported in a sedimentation test by Worthington (1994). Donelson and Wilson (1960b), by using fraction interchange techniques, found that gluten had the greatest effect on cake volume and structure. They noticed that gluten acts as a binder rather than as a structure element in the cake batter, and that good-quality gluten is readily solubilized.

Sinha et al. (1997) analyzed wheat gliadins extracted from chlorinated and non-chlorinated flour, using reversed-phase high-performance liquid chromatography, differential scanning calorimetry and fluorescence spectroscopy. They concluded that chlorination increased the hydrophobicity of gliadins due to conformational changes.

Tsen et al. (1971) studied protein extractability in chlorinated flour, and found that flour proteins were solubilized and modified by chlorine treatment. The chlorinated flours showed an increase in water-soluble proteins up to a chlorination level of 4 oz. per cwt. (i.e. hundredweight) flour (pH 3.63). However, the higher water extractability of flour proteins was not found with highly treated flour (16 oz. per cwt., pH 2.06). It was suggested that the chlorine cleaved the high-molecular-weight proteins into smaller, water-soluble fragments at moderate chlorine treatment, while heavy chlorine treatment altered the proteins to such an extent that they became insoluble.

Tsen et al. (1971) also detected changes in the UV spectra of the water and acetic acid extracts induced by chlorine treatment after adjusting the extracts to the same nitrogen content. They found that the absorption of the extracts at 280 nm increased with chlorine treatment up to a level of 1 oz. per cwt. (pH 4.89). Further treatment led to a decrease in absorption. They suggested that aromatic groups of amino acids such as tyrosine, tryptophan, and phenylalanine had been modified by chlorine. An increase in absorption was shown with chlorinated tyrosine but not with chlorinated tryptophan or phenylalanine (Tsen et al. 1971). Similarly, Seguchi (1985) found an increase in UV absorption with chlorinated bovine serum albumin (BSA).

Other reactions brought about by chlorination include saturation of lipids that are unsaturated in their native state (Daniels et al. 1963), and oxidation of cysteine, methionine and other amino acids in flour (Duviau et al. 1996; Ewart 1968; Tsen et al. 1971). Conforti et al. (1993) reported a decrease in unsaturated fatty acids (i.e. linoleic and linolenic acids) after chlorination, which was correlated significantly with the improvement of crumb structure and increase in cake cell evenness.

There are discrepancies in reports on the effects of chlorination on the functional role of flour lipids. Donelson et al. (1984) reported that lipids extracted from chlorinated flour by hexane were the major factor controlling cake volume and crumb grain when they were reconstituted into non-chlorinated flours. However, Johnson et al. (1979) showed that when lipids in chlorinated flour were replaced by lipids fractionated from untreated flour, there was no effect on cake baking quality.

Rheological studies on dough texture could be used to help predict the behavior of cake flour. Studies (Conforti and Johnson 1992; Worthington 1994) have shown that chlorination of flours increased water absorption in dough and decreased dough stability (tolerance to mixing) as measured by a farinograph. However, Tsen et al. (1971) reported an increase in stability at lower levels of chlorine treatments followed by a rapid decrease at higher chlorine levels. Alveograph results from the study of Worthington (1994) indicated a loss of dough extensibility and elasticity.

Viscoamylograph measurements showed that chlorine treatment increased the consistency of flour slurry (Seguchi 1977; Worthington 1994). In another study by Kulp et al. (1972), however, they reported that a high level of chlorination caused reduction in the paste consistency of flour. They attributed this to the depolymerization of starch by chlorine treatment.

2.1.2 Physicochemical Properties of Starch Isolated from Chlorinated Flour

It has been reported that chlorination can oxidize and depolymerize starch granules, and change the starch surface characteristics from hydrophilic to hydrophobic, as indicated by an increase in oil-binding capacity (Seguchi 1984a). Kulp et al. (1972) found that starch hydration capacity increased with increasing chlorine dosage. It was postulated that hydrophobicity of chlorinated starch might enhance the interaction of starch granules with other flour components such as proteins, resulting in greater transfer of water to starch during swelling (Seguchi and Matsuki 1977; and Seguchi 1984a).

Huang et al. (1982a) reported that starches from chlorinated flours showed an increase in β-amylolysis limits and a decrease in intrinsic viscosity as chlorination levels increased. A higher β-amylolysis value indicated the occurrence of cleavage of α-1,4 bonds, since cleavage of α-1,4 bonds would provide a new nonreducing end in the starch and thus a new site for β-amylase attack. A decrease of intrinsic viscosity also indicated that the starch was depolymerized by chlorination. They also reported the presence of a carbonyl absorption band on infrared scans of starch from chlorinated flours, which suggested that cleavages of C-2 and C-3 bonds occurred as a result of chlorination. Because no difference in iodine affinities was observed between the non-chlorinated and chlorinated starch, they suggested that the major effect of chlorine might be on the amylopectin fraction.

X-ray photoelectron spectroscopy (XPS) showed that there is a higher proportion of the hydrocarbon component on the surface of chlorinated starch than on the surface of non-chlorinated starch (Varriano-Marston 1985). However, by using electron spectroscopy for chemical applications (ESCA), or XPS, Russell et al. (1987) found that the portion of the carbon signal ascribed to hydrocarbons was essentially unchanged in the chlorinated starch, compared to non-chlorinated starch. They postulated that the enhanced hydrocarbon signal observed by Varriano-Marston (1985) was probably due to a greater quantity of flour lipids remaining bound on chlorinated starch. Instead they observed signals corresponding to chlorine covalently bonded to carbon within lipoprotein complexes on the starch surface, and sulphone signals indicating oxidized sulphur within the proteins as a result of chlorine treatment.

Baldwin et al. (1997) used time-of-flight-secondary ion mass spectrometry (TOF-SIMS) to study the surface chemistry of starch granules. They detected neither direct addition of chlorine to starch polymers, nor oxidation of the C2 and C3 hydroxyls to carbonyl groups. But they reported that chlorination modified the phosphocholine group of starch surface phospholipids. They also postulated that chlorination depolymerized starch (cleavage of α-1,4 and/or α-1,6 bonds of the glucan chains), based on the evidence that some strong mass carbohydrate ions resulting from glucan chains (found with non-chlorinated starches) were diminished in the spectra of chlorinated starch.

The starch surface is covered with proteins and lipids. Chlorination of wheat prime starch granules changes the surface characteristics from hydrophilic to hydrophobic. However, it is unknown whether the hydrophobic nature of starch granules from chlorinated wheat flour is caused by a direct chlorination of prime starch or by interaction with other flour components (Seguchi 1984b). Seguchi (1986) showed the presence of proteins on wheat starch granule surfaces by using protein-specific dyes. Treatment with 7% HCl or digestion by α-amylase or pepsin caused the chlorinated wheat prime starch to lose its oil-binding ability. This would appear to indicate that the oil-binding may be reduced when proteins are released or dissolved from starch granule surfaces. Based on this result, Seguchi (1986) attributed the oil-binding capacity of chlorinated prime starch to the presence of proteins complexed with starch. Treatment of chlorinated starch with water-saturated butanol (WSB) or chloroform-methanol, generally used as solvents for lipid extraction, had little effect on decreasing oil-binding ability (Seguchi 1984a and 1984b). This indicates that lipids did not affect the oil-binding ability of chlorinated starch.

In a model experiment, Seguchi (1985) found that a water-soluble protein, bovine serum albumin (BSA), formed water-insoluble films after chlorination. Results from sodium dodecyl sulfate (SDS) disc gel electrophoresis analysis indicated that the water insolubility of BSA was not due to polymerization or decomposition. Since an increase in absorption in the wavelength range of 260-400 nm was observed for chlorinated BSA, Seguchi (1985) suggested that these spectroscopical changes were indicators of chemical modifications, which may be responsible for the insolubility of BSA after chlorination. In general, protein's insolubility is evidence of an increase in hydrophobicity. He further chlorinated amino acids, and found that chlorination increased the hydrophobicity of tyrosine, lysine, and cystine, as determined by paper chromatography. It was suggested that the hydrophobicity of chlorinated starch granules could be attributed to chlorinated amino acids in the proteins on the starch granule surface.

Seguchi (1990) also studied the surface proteins on starch isolated from chlorinated flour, using sodium dodecyl sulfate (SDS) slab gel electrophoresis and Sephadex G-150 column chromatography. He observed that some large molecular weight protein bands (near M_r = 45,000, and M_r = 24,000-45,000) increased gradually with chlorination, which indicated the occurrence of polymerization of proteins during chlorination. It was also reported that the amount of starch surface protein increased gradually with chlorination levels (Seguchi 1990 and 1993b). In an earlier study, however, Seguchi (1987) observed no increase or decrease of protein content by chlorination in the starch samples. It should be noted that in these two studies, the same wet-fractionation method with acetic acid was used to separate the starch from flour.

It was suggested that chlorine treatment increased the swelling capacity and enhanced the gel-forming properties of starch (Alexander 1933; Ngo et al. 1985). Since chlorine treatment depolymerizes starch granules, the starch granules may have a less rigid molecular network and show a greater swelling after chlorination. However, the evidence for an increase in starch swelling following chlorine treatment is conflicting (Gough et al. 1978).

Gelatinization involves a progressive loss of order in starch granules when heated in water, which leads to granule swelling and hydration, and solubilization of starch molecules. The enthalpy of gelatinization can be determined quantitatively using differential scanning calorimeter (DSC), which records heat flow as thermal events occur during heating of starch. It was shown that the heat of gelatinization for starch-water slurry produces an endothermic peak on the DSC thermogram (Stevens and Elton 1971).

Allen et al. (1982) studied the effect of chlorination on the gelatinization endotherm of wheat flour as well as starch isolated from the flour using DSC. It was found that chlorination did not significantly affect the transition temperatures, or enthalpies of either the flour or the starch. But the enthalpy values observed for the chlorinated samples tended to be a little lower, which indicated that less energy is needed for starch gelatinization after chlorination. The transition temperatures indicate the onset and conclusion temperatures of gelatinization while the enthalpy change measures the energy required for disrupting the order structure of starch granules during gelatinization.

Kulp et al. (1972) examined the pasting behavior of starch isolated from flours of different chlorination levels using Brabender Viscograph. They observed little changes in paste viscosity, unless very high chlorine doses were used (above 4.0 oz. chlorine per cwt. of flour). A decrease in paste viscosity was found with the starch isolated from highly chlorinated flour. It was attributed to the degradation of starch by chlorination.

2.2 Cake Baking Performance of Chlorinated Flour

2.2.1 Cake Baking Mechanism

There are two types of cakes: those made with fat, called shortened or butter cakes, and those made without fat, called sponge or foam cakes. Cakes made with fat include plain yellow, white, chocolate, spice, and pound cakes. Sponge or foam cakes include angel cakes, yellow sponge, and mock sponge cakes (Freeland-Graves and Peckham 1987).

Cake batter is a multi-component and heterogeneous dispersed system. The continuous phase is a liquid mixture of water, and soluble components of egg, sugar and flour. The dispersed phase consists of gas bubbles, flour particles, oil and fat. During the early stages of cake baking, the viscosity of the batter decreases as temperature increases, and batter stability is reduced. This causes foam drainage and bubble coalescence. In the middle stages (from about 65°C), the starch becomes gelatinized, causing the viscosity to increase slightly. At the same time, the batter expands, due to the pressure within the air cells. The final stages begin at about 85°C, when starch gelatinization is nearly complete and protein coagulation is accelerating. At this point, batter expansion ceases, and gas is released. Egg and flour proteins coagulate and continue to strengthen the structure until the end of baking (Mizukoshi 1985).

To produce a good cake, the cake batter must have sufficient viscosity to prevent the incorporated air bubbles from rising to the surface and being lost during the initial heating. Cake setting must be timed to allow proper batter expansion by carbon dioxide gas and water vapor. Sufficient gel strength is also needed for the cake to resist the stresses exerted upon it as it cools, so complete baking can be achieved without collapse (Kim and Walker 1992).

2.2.2 Cake Batter Characteristics of Chlorinated Flour

Guy and Pithawala (1981) conducted compression tests on cake batters using a sponge cake formulation. They found that the batters of both chlorinated and heat treated flour were significantly firmer between 80 and 90°C than that of untreated flour. Adding egg albumen greatly increased the overall gel strength of the batters made from the treated flours. However, untreated flour batters did not show any significant increase in firmness following addition of egg albumen. Similar results were observed for the batters made from starches isolated from treated and untreated flours. This indicated substantial starch-protein interactions in the treated systems that were absent in the untreated flours.

Dynamic rheological studies by Ngo et al (1985) showed that the batters prepared with chlorinated flour had a higher storage modulus G', a measure of elasticity, than the batters prepared from untreated flours. However, pure flour-water systems prepared with chlorinated and untreated flours did not show any difference in G'. This observation appears to indicate that chlorination has a large influence on the interactions of flour components with ingredients in cake mixes.

2.2.3 Cake Baking Performance of Chlorinated Flour

Cake shrinkage or collapse is one of the most undesirable results in cake making (Mizukoshi 1985). It was reported that high baking powder ratio or high sugar to water ratio causes dips in cakes (Kissell 1967; Kissell and Marshall 1962). Chlorination enables the flour to carry higher levels of sugar and shortening so that high-ratio cakes can be made without fear of collapse (Tsen et al. 1971; Kissell and Yamazaki 1979; Gaines 1982). Cakes made from flour optimally chlorinated have higher volume, drier mouthfeel, finer grain and whiter crumb color than those made from non-chlorinated flour.

Johnson and Hoseney (1979) studied the effect of certain ingredients in the cake formula. At shortening levels above 24% of flour weight, the cakes made from non-chlorinated flours showed a decrease in volume and had a poor grain. Over a range of shortening contents (0-36%) used in their study, however, the volume and grain of cakes made from chlorinated flours did not show any change. When wheat or corn starch was used to replace part of the wheat flour, cakes baked from non-chlorinated flour did not collapse. Although the starch improved the volume, it did not improve the grain of the cakes. The quality of these cakes was still inferior to those made from chlorinated flour. The replaced starch did not affect the baking quality of chlorinated flour. This may indicate that chlorination brings about different interactions between starch and other flour components.

By using a sponge cake formulation, Guy and Pithawala (1981) observed batter behavior during baking. They found that cakes of both chlorinated and non-chlorinated flours reached approximately the same height before the cakes made from non-chlorinated flour collapsed in the last 5 minutes of the baking period. The larger volumes were obtained for the cakes made from chlorinated flour. Conforti and Johnson (1992) found that angel food cakes prepared with non-chlorinated flour had a larger volume but an inferior structural quality compared to those prepared with chlorinated flour. On the contrary, the angel cakes made from chlorinated flour were low in volume but showed less shrinkage, more even contour, and better crumb. Similar baking results were found with Japanese-type sponge cakes, with lower volumes but better symmetry for those baked with chlorinated flours (Worthington 1994).

Kissell and Yamazaki (1982) studied the effects of chlorination level on batter expansion during baking and on final cake volume. They found that batter expansion was always better for cakes made from chlorinated flours than those from untreated flours. The white layer cakes had the highest expansion and volume at low levels of chlorination.

3. MATERIALS AND EXPERIMENTAL METHODS

3.1 Materials

3.1.1 Wheat Flours

Chlorinated and non-chlorinated commercial soft wheat flours were all obtained from one source (King Milling Company, Lowell, MI). The flour was chlorinated by the milling company to pH levels of 4.8 and 4.2. Non-chlorinated flour from the same source was used as a control in this study.

3.1.2 Chemicals

Glacial acetic acid and sodium hydroxide were purchased from Fisher Scientific (Pittsburgh, PA). Blue dextran was purchased from the Sigma Chemical Company (St. Louis, MO). All chemicals used in this study were of reagent grade. Vegetable oil and cake ingredients were obtained from commercial sources.

3.2 Experimental Methods

3.2.1 Flour Fractionation

In order to examine chlorination effects on the starch fraction, starch was separated from flour, by using the acetic acid fractionation method according to Sollars (1958), with some modifications as described in this section. The amount of flour fractionated was reduced from 600 g to 500 g, and the quantities of reagents were also reduced accordingly. The overall fractionation scheme is illustrated in Figure 1. All the fractions obtained were freeze-dried for a week, instead of being air-dried as was done by Sollars (1958).

1). Water extraction: Five hundred grams (500 g) of flour was weighed and mixed into 1500 ml of distilled de-ionized water, and stirred for 10 minutes with a Hobart mixer (Hobart Corporation, Troy, OH) at the lowest speed, instead of the propeller-type laboratory stirrer that was used in Sollars' method. The suspension was centrifuged for 10 minutes at 2000 g. The supernatant was decanted and filtered through Whatman No.1 paper, instead of through glass wool. The supernatant containing water-solubles was freeze-dried instead of being concentrated and stored in the liquid state.

2). Acid extraction: The dough was transferred from the centrifuge bottles to a Waring Blender cup, with half of the dough transferred at a time. Five hundred milliliters (500 ml) of an acetic acid solution of sufficient strength (4 ml glacial acetic acid and 496 ml distilled de-ionized water) was added to obtain a pH of 3.5. The suspension was agitated for 2 minutes, then centrifuged for 10 minutes at 2000 g. The acid extract was decanted into a beaker. The same procedure as above was applied to the other half of the dough. The residues were combined and extracted for 1 minute with an acetic acid solution of sufficient strength (1 ml glacial acetic acid and 500 ml distilled de-ionized water) to maintain the pH at 3.5. Then the suspension was centrifuged and the supernatant was decanted.

3). Recovery of gluten: The pH of the combined acid extracts was adjusted with 5N sodium hydroxide to 6.4. The extracts were centrifuged at 2000 g for 30 minutes. The supernatant was decanted, and the gluten was removed, and cut into thin strips, and freeze-dried.

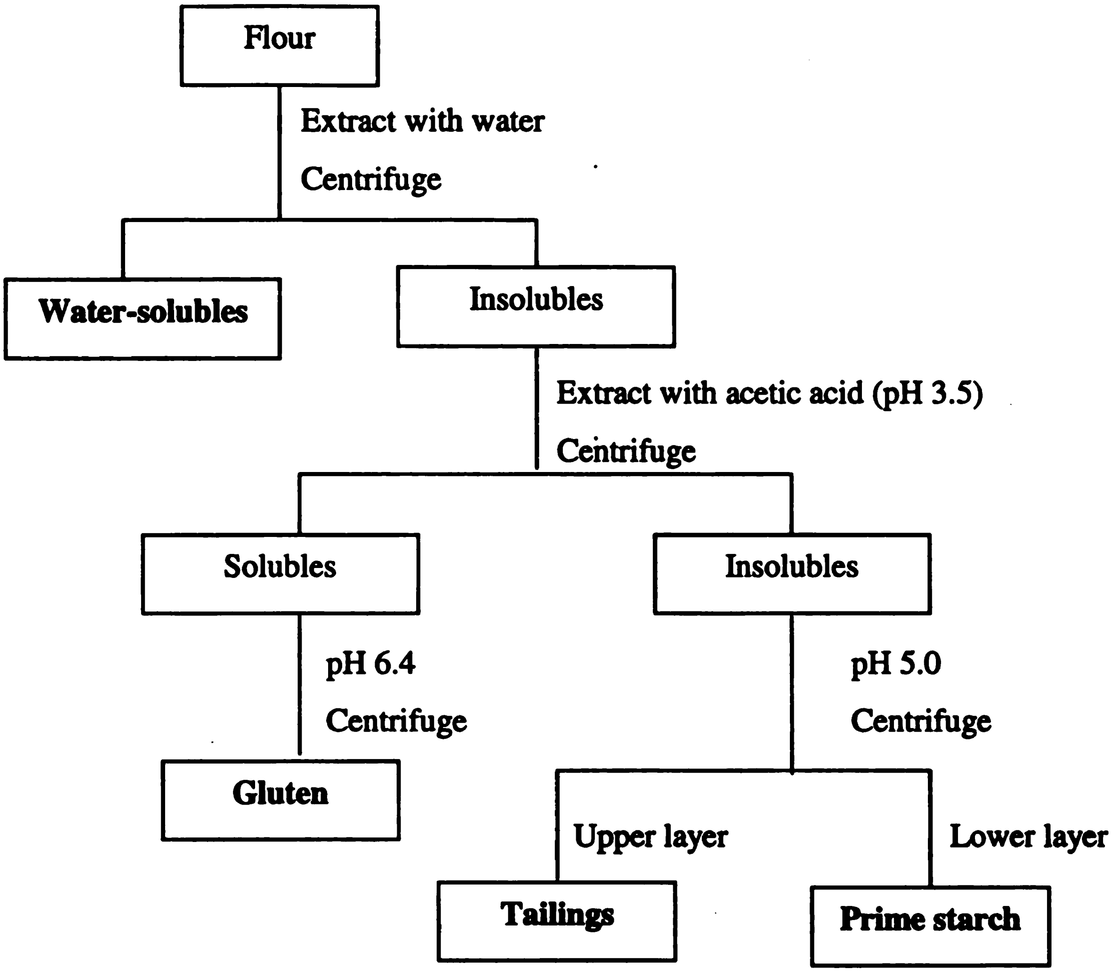


Figure 1. Fractionation scheme for non-chlorinated and chlorinated flours.

4). Recovery of tailings and prime starch: Five hundred milliliters (500 ml) distilled de-ionized water was added into the residue from step 2, and the pH was adjusted from 3.6 to 5.0 by adding 0.5N NaOH while stirring. The suspension was centrifuged for 10 minutes at 2000g. The supernatant was decanted. The upper layer of the residue (tailings) in the centrifuge bottles was removed with a spatula, then suspended in distilled de-ionized water and freeze-dried. The lower and white prime starch layer was suspended in water, and the liquid was removed by filtration in a Buchner funnel. The moist starch cake was re-suspended in distilled de-ionized water, and freeze-dried.

3.2.2 Analytical Tests

3.2.2.1 Moisture Content

The moisture contents of the flours and starches were determined in duplicate, using the air-oven method (AACC 1990, method 44-15A). Two grams (2 g) of flour samples or 1 g of starch samples was used, since starches were limited in quantity. The samples were weighed in a pre-weighed aluminum weighing dish, and dried at 130 °C for an hour in an Isotemp Oven (Fisher Scientific, Pittsburgh, PA), cooled in a desiccator and then re-weighed. The percent moisture was calculated as the weight loss per 100 g of wet sample.

3.2.2.2 Protein Content

The protein contents of the whole flours and starches were determined in duplicate, using a standard micro-Kjeldahl procedure (AACC 1990, method 46-13).

Sample size varied with sample type. A 0.25 g sample size was used for whole flour. For starches, a 0.4 g sample size was used due to their low protein contents. Each sample was digested with sulfuric acid in the presence of catalysts, and the total organic nitrogen was converted to ammonium sulfate. The digest was neutralized with 50ml alkali and distilled into 10 ml of boric acid solution, then titrated with a standard acid. Methylene blue was used as an indicator in the sample titrations. The percentage of nitrogen was calculated according to the titration result. A conversion factor of 5.7 was used to convert the percent nitrogen (N) to percent protein (AACC 1990, method 46-13).

3.2.2.3 Damaged Starch

Damaged starch contents of flours and starches were measured in duplicate, according to AACC standard method 76-30A (AACC 1990). The determination is based on the fact that damaged starch is susceptible to fungal α-amylase, while undamaged starch is not. One gram (1g) of sample (14% moisture content) was digested by 0.05 g fungal α-amylase in 45 ml acetate buffer (pH 4.6-4.8) for 15 minutes (incubated in a 30 °C water bath). Then 3 ml sulfuric acid (H_2SO_4) and 20 ml sodium tungstate solution were added to terminate the reaction. The mixture was filtered, and 5.0 ml of filtrate transferred into test tube, then 10 ml alkaline ferricyanide solution was added. The α-amylase converted the damaged starch to maltose and reduced ferricyanide to ferrocyanide. Extra ferricynide reacted with starch-potassium iodide (KI) and produced iodine, which was titrated with sodium thiosulfate. The titration result was converted to maltose content according to Table 22-18 in AACC standard method 22-18 (AACC 1990). The percent damaged starch was evaluated according to the content of maltose.

3.2.3 Water Binding Capacity

The water binding capacity of flour and starch was determined according to Shelke et al (1992). The flour (2.5 g, as is) was weighed in duplicate into centrifuge tubes, dispersed with 25 ml of distilled water, held for 30 minutes at room temperature, then centrifuged at 1500 g for 10 minutes. The centrifuge tubes were then drained for 10 minutes at an angle of 30° to remove supernatants, and the residues in the centrifuge tubes were re-weighed. For starches, a 2.0 g sample size was used, and accordingly dispersed with 20 ml of distilled water. The water binding capacity was calculated as the amount of distilled water retained by the flour or starch as a percentage of the original sample weight, on a 14% moisture basis.

3.2.4 Concentration of Water Solubles

Flours (100 g on a 14% moisture basis) were weighed into the mixing bowl of a Hobart mixer. Half of the total water to be added was poured into the bowl, and mixed for 1 minute at a slow speed. Flour sticking to the wall of the mixing bowl was scraped down, and the other half of the water was added. The mixture was stirred for 1.5 minutes at a medium speed. The flour on the wall of the mixing bowl was scraped down again, and mixed for 1.5 minutes at a slow speed, then 6 minutes at a medium speed. The batters were transferred to centrifuge bottles, and centrifuged at 1100 g for 15 minutes.

One milliliter (1 ml) of solution was taken in duplicate to weighing dishes, and heated at 120°C for an hour in the oven to determine the soluble content.

The total water added was 135 ml, consistent with the water level used during baking. The above procedure is the standard one used in batter mixing for cake baking (AACC 1990, method 10-90).

3.2.5 Ultraviolet Absorption of Water-solubles

Aqueous flour extracts (1 to 5% weight/volume) were obtained by stirring 1.00 to 5.00g flour on 14% moisture basis into 100 ml distilled de-ionized water for 4 minutes and centrifuging at 3000 rpm for 10 minutes. The absorption of the supernatant was determined over the UV range of 200-350 nm using a Diode Array Spectrophotometer Hewlett Packard 8452A. The absorbance at UV 280 nm was recorded as an indication of the concentration of proteins in the extracts.

3.2.6 Surface Tension of Aqueous Flour Extracts

Aqueous flour extracts (0.05 to 20% flour on 14% moisture basis) were prepared according to Evers et al. (1990). Flour of 0.05 to 20 g was stirred gently into 100 ml distilled de-ionized water. The suspension was left for 20 minutes in an ice-bath to minimize enzymatic activity, then centrifuged at 1100 g for 15 minutes. The supernatant was decanted to the sample vessel of a Kruss K12 Interfacial Tensiometer (Kruss USA, Charlotte, NC). Bubbles and dust particles were removed with a glass pipette (Evers et al. 1990). After equilibrating at 30°C for 30 minutes, the surface tension was measured, by using the Wilhelmy plate method according to the procedures described in the operational manual of the tensiometer. The mean of 10 values at 30-second intervals was recorded as the surface tension at that concentration.

3.2.7 Falling Number Test on Flour and Starch

The falling number was determined for flours and starches in duplicate, according to AACC method 56-81B (AACC 1990). Seven grams (7 g) of sample (14% moisture basis) were added to 25 ml of distilled de-ionized water in a test tube, which was then vigorously shaken and immersed in a boiling water bath. The suspension was stirred by moving the stirrer up and down for 60 seconds. The stirrer was then allowed to drop by its own weight from its uppermost position. The total time, in seconds, for the stirrer to reach the bottom was recorded as the "falling number." The rate of the stirrer's fall is related to the viscosity of the slurry.

3.2.8 Pasting Properties of Flour and Starch

The pasting properties of flour and starch were determined in triplicate and duplicate, respectively, according to a 23-minute test procedure described in the Rapid Visco-Analyzer (RVA) operational manual (Newport Scientific Pty. Ltd. 1995, Australia). A weighed sample (3.5 g of flour or 3.0 g of starch at 14% moisture basis) was added to 25.0 ml of distilled water in a disposable aluminum sample cup. A disposable plastic paddle was placed in the sample cup to mix the contents during heating. After seven seconds of vigorous stirring (900 rpm) to disperse the sample and help induce rapid equilibration of temperature, a stirring speed of 160 rpm was used for the remainder of the test. While stirring, the mixture was equilibrated at 50°C for 1 minute, then put on a heating cycle of 95°C in 7.5 minutes, held at 95°C for 5 minutes, cooled down to 50°C in 7.5 minutes, and then held at 50°C for 2 minutes. A pasting curve was obtained and evaluated for the following parameters: pasting temperature, peak viscosity, time to peak, breakdown, minimum viscosity (trough), setback, and final viscosity (see Appendix I for description).

3.2.9 Swelling Power of Starch

The influence of chlorination on starch swelling power was examined by both the traditional water uptake method (Leach et al.1959) and the blue dextran dye exclusion method (Tester and Morrison 1990).

1). Water uptake method: 0.2 g starch was suspended in 20.0 g distilled water in a centrifuge tube, incubated and shaken in a water bath for 30 minutes at a constant temperature between 40° and 95°C, then centrifuged at 4000 rpm for 20 minutes. The supernatant was carefully drawn off. An aliquot of the supernatant was dried in an oven at 120°C for 3 hours. The percentage of solubles extracted from starch was calculated on a dry basis. The tube and the remaining starch paste were re-weighed. The swelling power was calculated as the weight of sedimented paste per gram of dry starch. This value was corrected for solubles to measure the swell of the undissolved portion of the starch.

Since the calculation of swelling power with this method is based on the weight of the sedimentation paste, it includes the water within the swollen granules and the water in the interstices between swollen granules.

2). <u>Blue dextran dye exclusion method</u>: This method is based on the observation that blue dextran ($M_r = 2x10^6$) does not penetrate swollen granules, hence it remains in the free water outside the swollen granules. Since only intragranular water is counted in calculation of the swelling factor, this method measures true granule swelling. Fifty milligrams (50 mg) starch (corrected to 0.1 mg) was suspended into 5 ml distilled de-ionized water in a centrifuge tube. The tube was incubated horizontally with constant shaking in a water bath for 30 minutes at a constant temperature between 40° and 80°C. The tube was cooled down rapidly, and 0.5 ml of blue dextran (5 mg/ml) was added and mixed. After the mixture was centrifuged at 1500 g for 6 minutes, the absorption of the supernatant (A_S) was measured. The absorption of a blank reference (A_R) without starch was also measured. Samples were measured in duplicate.

The calculation of the swelling factor (SF) was based on starch weight (corrected to 12% moisture) and a density of 1.4 g/ml. The swelling factor was calculated as the volume of the swollen starch granules divided by the initial volume of the starch of weight W (in milligrams). The equations used were as follows:

The volume of free water (ml) in the system is given by

$$FW = 5.5\left(\frac{A_R}{A_S}\right) - 0.5$$

The volume of water absorbed in the intra-granular space can then be calculated as

$$V_1 = 5.0 - FW$$

The initial volume of starch of weight W in mg is

$$V_o = \frac{W}{1400}$$

Now, the volume of the swollen granules can be determined from the sum of the two volumes above:

$$V_2 = V_1 + V_2$$

Finally, the swelling factor can be calculated:

$$SF = \frac{V_2}{V_0}$$

3.2.10 Determination of Starch Hydrophobicity

This experiment was designed to observe the behavior of starches in water-oil mixtures and the effect of chlorination on starch hydrophobicity. Non-chlorinated, chlorinated pH 4.8, or pH 4.2 starch (0.2 g) was each weighed into seven small glass tubes. Then water and vegetable oil were added to the seven tubes at a ratio of 1:11, 1:9; 1:6; 1:5; 1:2; 1:1; and 2:1. The total volume of water and vegetable oil was 6 ml. The tubes were stopped by a rubber stopper, shaken for one minute with a Fisher Vortex Genie2 (Bohemia, NY), and then set on stand. Each tube was then observed over a period of time to determine if the starches had a preference for the water or the oil phase, or whether a stable mixture would result.

3.2.11 The Environmental Scanning Electron Microscopy Study

The starch samples were dispersed in distilled de-ionized water (starch: water = 1: 2). Two drops of the suspension were loaded into the sample holder on the hot stage of a microscope. The sample was covered with a ceramic plate and heated up to 100°C at a rate of 5°C/min. When the temperature reached 100°C, the ceramic cover was removed. Holding at 100°C, the sample was imaged with a 20KV accelerating voltage at a sample chamber pressure of ~6 torr.

The samples were then cooled down from 100°C to 30°C at a rate of 5°C/min. Images were taken at 100°C, 90°C, 80°C, 70°C, 60°C, 50°C, 40°C, and 30°C on the same focused spots for comparison.

Two points at each side of the particle were selected, and the particle diameter was calculated by a computer. Seventy-two (72) readings were recorded for each sample from the images taken at 100°C. Thirty-six (36) readings were recorded from the images taken as samples cooled down from 100°C to 30°C.

3.2.12 Baking of High-Ratio Cake

3.2.12.1 Baking Procedure

The high-ratio white layer cakes were baked in duplicate according to the AACC method (AACC 1990, method 10-90). The formula used was: 100 g flour, 140 g sugar, 50 g shortening, 12 g non-fat dry milk, 9 g dried egg whites, and 3 g salt. The quantity of baking powder was determined from the table given in this procedure, based on the laboratory elevation and the barometric pressure on the day of baking. A water-to-flour ratio of 135:100 was used for chlorinated and non-chlorinated flours. The batter was mixed in a Hobart mixer (model N-50, Hobart Canada, North York, Ontario, Canada). The cakes were baked at 190°C for 25 minutes in a Rotary Hearth Test Baking Oven (National Mfg. Co., Lincoln, NE).

3.2.12.2 Batter Specific Gravity

Batter specific gravity was determined in duplicate using a container of known volume. The ratio of batter weight to the weight of an equivalent volume of water was reported as the batter specific gravity.

3.2.12.3 Cake Evaluation

Cake volumes were measured by the rapeseed displacement method. The internal texture was subjectively graded according to AACC method 10-90 (AACC 1990). Indices for cake volume, uniformity and symmetry were determined, based on objective measurements from a cake template provided for AACC method 10-91 (AACC 1990).

3.3 Statistical Analysis of Data

Using the data analysis tools of Microsoft Excel 7.0, one-way analysis of variance (ANOVA) was performed to determine if any significant differences existed among the treatments (non-chlorinated, chlorinated pH 4.8 and pH 4.2 flours or starches isolated from the flours). A significance level of 0.05 was used. When a significant difference was found among the treatments, protected least significant difference (LSD) was used to compare and rank them. When there was no significant difference among the treatments, Turkey's test was used to compare the means with the critical value -- honestly significant difference (HSD) (see Appendix II).

4. RESULTS AND DISCUSSION

4.1 Flour Fractionation

The flours were separated into four fractions: water-solubles, gluten, tailings, and prime starch, using the acetic acid method described above. The total recovery and the relative amounts of each fraction obtained are shown in Table 1. The total recoveries of the three types of flour were in a narrow range of 94.9-96.0%. Chlorinated pH 4.2 flour yielded slightly more water-solubles than the other two flours.

Table 1. Flour fractionation results showing percentage by weight of flour fractions on a dry basis.

Flour	Water-solubles (%)	Gluten (%)	Prime Starch (%)	Tailings (%)	Total Recovery (%)
Unchl.	6.3	6.4	53.1	29.1	94.9
Chl.-pH 4.8	6.2	5.8	60.0	24.0	96.0
Chl.-pH 4.2	6.6	5.7	54.6	28.5	95.4

Compared to the non-chlorinated flour, the chlorinated flours produced slightly less gluten when extracted by acetic acid. This result indicates that gluten was probably destroyed during the chlorination process. This is in agreement with the study of Tsen et al. (1971), in which it was reported that the amount of acetic acid extractable protein decreased with increasing levels of chlorination.

The pH 4.8 flour produced more prime starch and less tailings in comparison to both the pH4.2 and the non-chlorinated flours. This suggests a strong interaction between the starch and tailings in pH 4.8 flour which affects the separation of tailings from the starch. Seguchi (1984) found similar results for the recovery of starch and tailings from flours at different levels of chlorination. He reported that prime starch could not readily be recovered from flour chlorinated at a level of 4.26 g/kg of flour (pH 2.96) or greater.

The flour fractionation results with chlorinated pH 4.8 flour are in agreement with the findings of Sollars (1958), who reported that flours chlorinated to a pH of 4.82 yielded smaller amounts of tailings and gluten and a larger amounts of prime starch compared to non-chlorinated flour.

4.2 Physico-chemical Properties of Flour and Starch

4.2.1 Moisture Content

The moisture contents of the flours were 9.38, 10.82, and 10.73% for non-chlorinated, pH 4.8, and pH 4.2, respectively. The higher moisture contents of the chlorinated flours were probably due to moisture pick-up by the flours during handling and/or storage. The moisture contents of starches isolated from the flours were in the range of 0.29-0.43% after freeze-drying.

4.2.2 Protein Content

The protein contents of flours and starches (14% moisture basis) are shown in Table 3. The protein contents of the flours were in the range of 7.5-8.5%, which are

Table 2. Protein content, damaged starch, and water binding capacity of flour and starch samples

Treatment		Protein content (%) (n = 2)		Damaged starch (%) (n =2)		Water binding capacity (%) (n = 3)	
		Flour	Starch	Flour	Starch	Flour	Starch
Non-chl.	Mean	8.43 a	0.29 a	6.97 a	2.17 a	62 b	74 b
	S.D.	0.03	0.02	0.00	0.17	1	1
Chl.-pH 4.8	Mean	7.96 b	0.24 b	6.48 a	2.30 a	64 b	74 b
	S.D.	0.01	0.01	0.11	0.00	1	1
Chl.-pH 4.2	Mean	7.92 b	0.25 b	6.77 a	1.95 a	69 a	82 a
	S.D.	0.01	0.00	0.29	0.14	3	1

Note: There is no significant difference ($P<0.05$) between means in a column followed by the same letter.
S.D. denotes the standard deviation.

typical protein levels in cake flour for high-ratio cakes. The non-chlorinated flour had a higher protein content than the chlorinated flours. This result was unexpected, given that the chlorinated and non-chlorinated flours were supposed to have come from the same source. A difference in flour sources may cause a difference in protein contents. In addition, it is possible that chlorination may offer another explanation for this difference, since chlorine treatment alters flour proteins and may affect the recovery of nitrogen in the flour.

The starches isolated from non-chlorinated flour had a higher protein content than the starch from chlorinated flours. This would indicate that chlorination did not increase the surface protein of the starch granules. The results agree with findings of Seguchi (1987), who reported no increase in the surface proteins on the starches isolated from chlorinated flours by the acetic acid procedure.

4.2.3 Damaged Starch

The damaged starch contents for non-chlorinated, pH 4.8 and pH 4.2 flour were 6.97, 6.48, and 6.77%, respectively, showing no significant difference among the treatments. It should also be noted that the levels of damaged starch in these flours are acceptable for cake baking purposes. For the starches isolated from the control, pH 4.8 and pH 4.2 flour, the damaged starch contents were 2.17, 2.30, and 1.95%, respectively (Table 3), again with no significant differences among treatments ($p>0.05$).

4.2.4 Water Binding Capacity

The water binding capacity of each flour or starch was determined as the amount of water retained by the hydrated flour or starch. As shown in Table 3, water-binding capacity of pH 4.2 flour and starch were significantly higher ($p<0.05$) than for the other treatments. There was no significant difference between the non-chlorinated and pH 4.8 samples. Having a higher water-binding capacity implies that chlorinated pH 4.2 flour may have a higher batter viscosity, and thus a higher ability of its batter to hold gas bubbles in.

Shelke et al (1992) studied the water binding capacity for chlorinated and aged flours. They found that chlorinated flours had higher water binding capacity than the untreated control flour. Aged flour showed higher water binding capacity than freshly milled flour, and chlorination increased the rate of age-related change in water binding capacity. They also found that flour water binding capacity was highly correlated with batter viscosity, cake volume, and cake symmetry index.

The above measurements gave the water-binding capacity of flour or starch in excess water. In this study, it was also observed that the chlorination affected the absorption of moisture by dry starch. After dry starches with similar initial moisture contents (0.29 - 0.43%) were held in an atmosphere of 50% relative humidity at room temperature for 2 hours, the moisture contents of the starches isolated from non-chlorinated, pH 4.8 and pH 4.2 flours were measured as 4.02, 4.08 and 4.91%, respectively. The starch from chlorinated pH 4.2 flour absorbed significantly more water than that from non-chlorinated and chlorinated pH 4.8 flours during the 2 hours. When the starches were held overnight under the same conditions, the moisture contents increased to 10.25, 9.92, and 9.65% for non-chlorinated, and chlorinated pH 4.8 and pH 4.2, respectively. There was no significant difference in the final moisture content among the treatments. The final moisture contents tended to decrease with chlorination levels (Figure 2). These observations may indicate a difference in the mechanism for water absorption between the starch isolated from chlorinated flour and that from non-chlorinated flour.

Huang et al (1982b) measured the water activity of flours held over water in a desiccator (a relative humidity of 100%) for 15 hours at room temperature. They found that non-chlorinated flour and starch fractions adsorbed more water. The final moisture contents were 17.74, 15.55, and 15.43% for non-chlorinated, pH 4.95 (1-oz chlorine per cwt. flour), and pH 4.35 (2-oz chlorine per cwt. flour) chlorinated flours, respectively.

Gur-Arieh et al. (1967) reported that the high-starch fraction of hard wheat flour bound the first 7% of water (mass/mass on a dry basis) according to a Brunauer, Emmet, and Teller (BET) monolayer. Further absorption of water up to 26% built up hydration layers on the surface. Starch from chlorinated flour picked up more moisture first, compared to non-chlorinated starch. Measurements of isotherms during water vapor sorption on starch surfaces would help to elucidate these differences.

4.2.5 Concentration of Water Solubles

In the liquid phase of the flour-water mixture, the water-soluble contents were 5.54, 5.68, and 6.53% for non-chlorinated, pH 4.8 and pH 4.2 flour, respectively. The

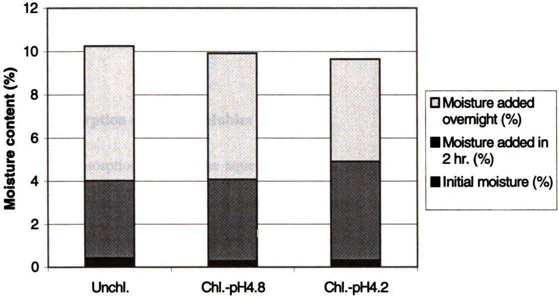


Figure 2. Changes in moisture contents for three starches (non-chlorinated, chlorinated pH 4.8 and pH 4.2) held at 50% relative humidity and 25 °C.

water-soluble content was significantly higher ($p<0.05$) in the mixture of pH 4.2 flour. There was no significant difference in water-soluble contents between pH 4.8 flour and non-chlorinated flour. Flour water-solubles was reported to have an influence on cake

volume. An increase in water-solubles beyond the normal level tended to decrease cake volume (Donelson and Wilson 1960a).

4.3 UV Absorption of Water-solubles

The absorption peak of the aqueous flour extracts was in a wavelength range of 250-310 nm. Since proteins show strong absorption at 280 nm primarily due to tryptophon and tyrosine residues in the proteins. The absorbance at UV 280 nm was read as an indication of protein content. As shown in Figure 3, the water extract of pH 4.2 flour gave a higher absorption at 280 nm. However, there was little difference between the absorption of non-chlorinated and chlorinated pH 4.8 flour.

The differences in the absorbance at UV 280 nm are possibly due to differences in protein extractability. Kissell (1971) analyzed the water-soluble fractions of cake flour for soluble protein using the Kjeldahl method, and reported that flour chlorination increased the amount of water-extractable proteins.

Modification of aromatic amino acids in proteins by chlorination may be another reason for the increase in the absorption values. Tsen et al. (1971) found an increase in absorbance at UV 280 nm by chlorinated tyrosine. By adjusting all the extracts to the same nitrogen content, Tsen et al (1971) detected changes in the UV spectra of water and acetic acid extracts after chlorine treatment. The absorption at 280 nm increased with chlorine treatment up to a level of 1 oz. per cwt. (pH 4.89). Further treatment (pH 4.51

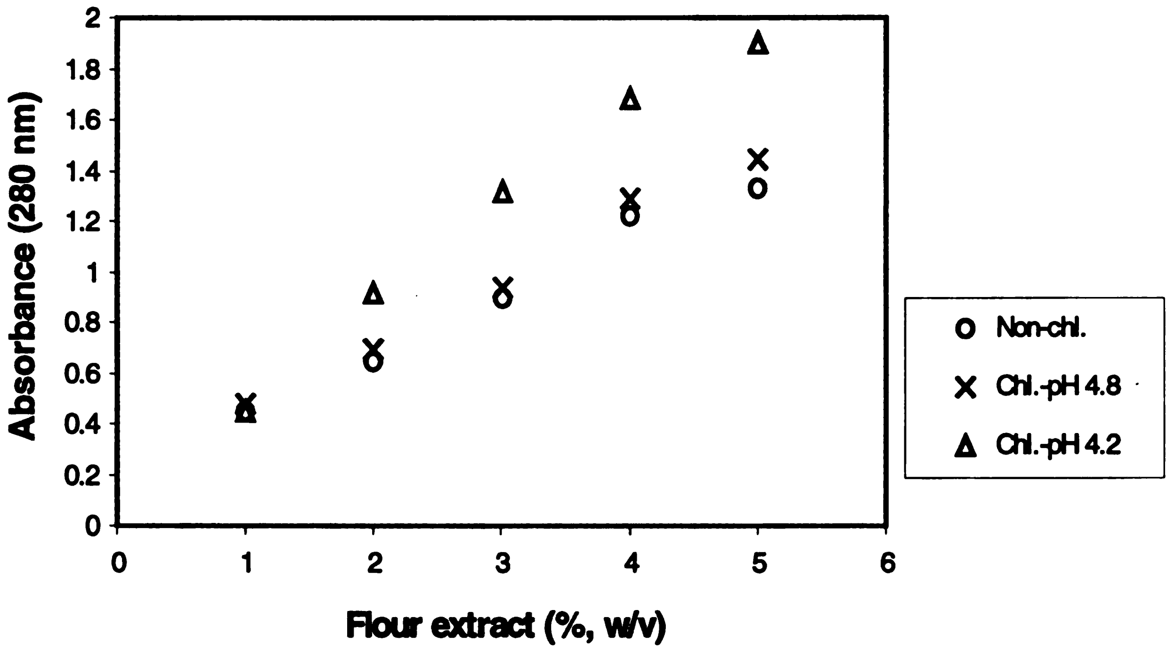


Figure 3. UV absorption of water extracts from non-chlorinated control and chlorinated flours.

and below) led to a decrease in UV absorption, which means that high level of chlorination did not cause an increase in UV absorption. If this finding is true, then the large increase in absorbance at 280 nm for pH 4.2 chlorinated flour observed in this study could be due mainly to solubilization of flour proteins by chlorination.

4.4 Surface Tension of Aqueous Flour Extract

During batter processing, more liquid-air interfaces are produced. The surface-active components at the interface determine the gas retention capacity and baking quality. Surface-active components in flour such as proteins, lipids and pentosans are all involved in reducing the surface tension of water and stabilizing the liquid-air interface.

Figure 4 shows that there was no significant difference in the surface tension values measured on the water extracts of chlorinated and non-chlorinated flours. In addition, there was no significant change in surface tension over the concentrations of the flour extracts used in this study. It is possible that the surfactants may have saturated the air-water interface at the lower concentration, thus further increases in soluble concentration did not reduce the surface tension any further.

4.5 Falling Number Test on Flour and Starch

The falling number test is commonly used to determine α-amylase activity in cereal grain. It relies on the ability of α-amylase to liquefy gelatinized starch. The starch polymer is cleaved by α-amylase into smaller polysaccharide units. Thus the viscosity of the flour-water slurry is reduced. A high α-amylase activity is related to a low sample

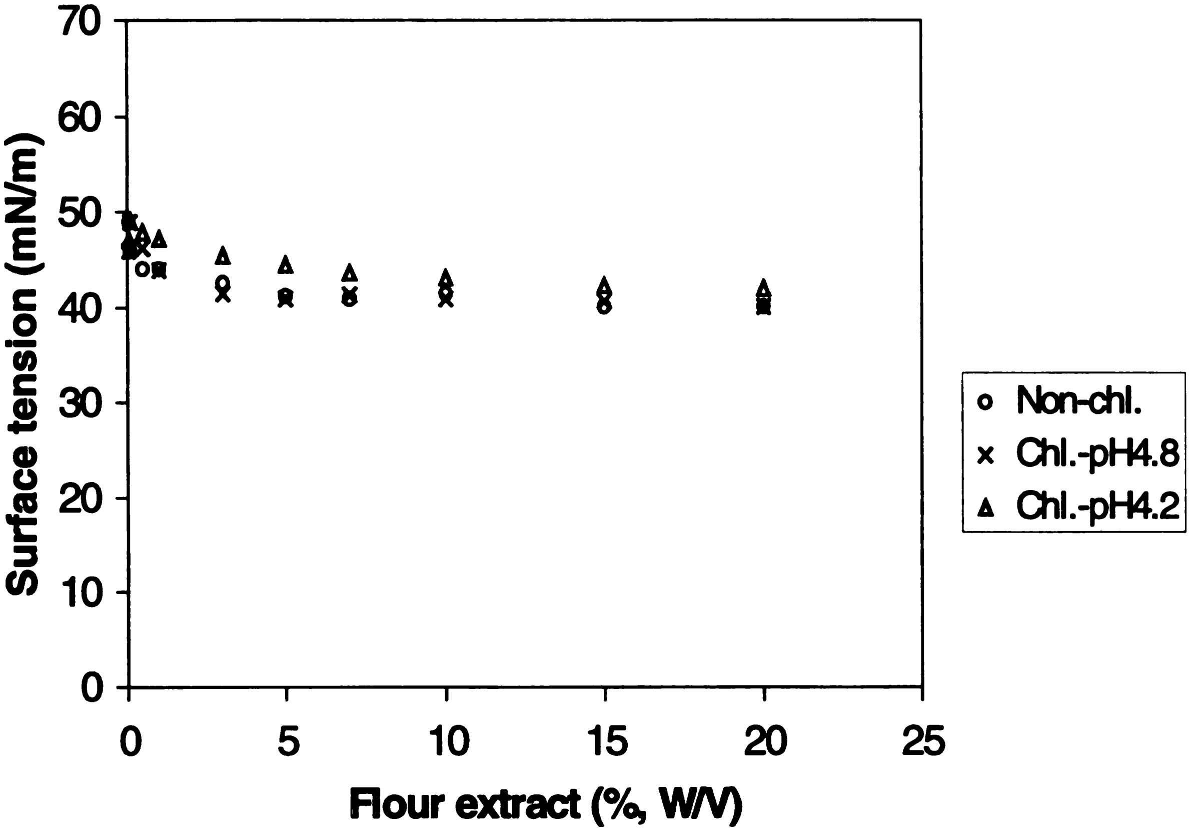


Figure 4. Surface tension of aqueous flour extracts as a function of concentration (0.05-20 g flour on 14% moisture basis extracted per 100 ml water).

viscosity and a low falling number value. Differences in both amylase activity and starch susceptibility to enzyme attack contribute to the falling number values. Any factor affecting the relative viscosity of the flour or starch paste may affect the falling number values.

The values of the falling numbers of chlorinated flour and starch were significantly higher ($p<0.05$) than for non-chlorinated flour and starch. However, there was no significant difference in the falling numbers for pH 4.8 and pH 4.2 flours or starches (Table 3). For the flours, chlorination may inactivate α-amylase in the flour, thus giving the chlorinated flour a higher falling number value due to its lower α-amylase activity. The low pH of the chlorinated pH 4.2 flour is not favorable for α-amylase activity, and may provide an explanation for the higher falling number value for chlorinated flour at pH 4.2.

Table 3. Falling number (FN) values measured for non-chlorinated and chlorinated flours (pH 4.8 and pH 4.2), and for the starches isolated from the flours. A higher FN value indicates a higher relative viscosity.

Chlorination level	FN of flour	FN of starch
Non-chl.	283 b	592 b
Chl.-pH 4.8	384 a	718 a
Chl.-pH 4.2	447 a	722 a

Note: Each value is the mean of replicates. Means followed by the same letter in the same column are not significantly different ($P<0.05$).

It was reported that lower levels of chlorination increased α-amylase activity, while higher levels of chlorination decreased α-amylase activity (Huang et al. 1982b). Flours with a high α-amylase activity should give a low falling number. In this study, the pH 4.8 flour did not show a lower falling number value than non-chlorinated flour. It suggests that α-amylase activity may not be the only factor affecting the falling number of the flours.

The falling number tests on flour indicated that chlorinated flour pastes were firmer (higher falling number) than those of non-chlorinated flour. The firmer structure of chlorinated flour pastes may prevent cakes from collapsing during baking. This is in agreement with Guy and Pithawala's (1981) compression tests on cake batters. They found that the firmness of the chlorinated flour batter was much higher than that of untreated flour batter.

The falling numbers of chlorinated starch were significantly higher ($p<0.05$) than non-chlorinated starch. It is unlikely that α-amylase activity contributed to this since α-amylase activity is generally negligible in starches isolated from flours by the acetic acid procedure. In the three treatments (see section 4.7, Swelling Power of Starch), there was no difference in the water solubilities of starch during heating. Thus, the water solubilities did not contribute to the higher falling numbers for starches isolated from chlorinated flours.

4.6 Pasting Properties of Flour and Starch

The pasting behavior of flour or starch during heating and cooling in water was measured with a Rapid Visco-Analyzer. The viscosity curves for flours and starches are shown in Figure 5 and Figure 6, respectively. The viscosities are reported in rapid visco units (RVUs, 1 RVU = 12 cP). The pasting parameters for flours and starches are reported in Table 4 and Table 5, respectively. There were significant differences in the pasting characteristics of the flours, including peak, trough (hot paste), breakdown, setback and final viscosity (see Appendix I for definition of the parameters).

The peak viscosity for the pH 4.2 flour is significantly higher than non-chlorinated and pH 4.8 flours, while non-chlorinated and pH 4.8 chlorinated flours had about the same peak viscosity. Inhibition of α-amylase activity by chlorination or low flour pH may cause an increase in peak viscosity observed for pH 4.2 chlorinated flour. Trough viscosity, final viscosity, and setback increased with chlorination level (i.e. pH 4.2 > pH 4.8 > non-chlorinated control). The chlorinated pH 4.8 flour showed less breakdown value, which is defined as the peak viscosity minus trough viscosity. The higher final viscosity values for chlorinated flours indicate that chlorination increased flour gel strength, which could help prevent post-baking collapse of the cakes. The pH 4.2 flour had the highest breakdown value followed by non-chlorinated control flour, with pH 4.8 flour showing the least breakdown. The breakdown value indicates the resistance of the flour to mechanical disintegration.

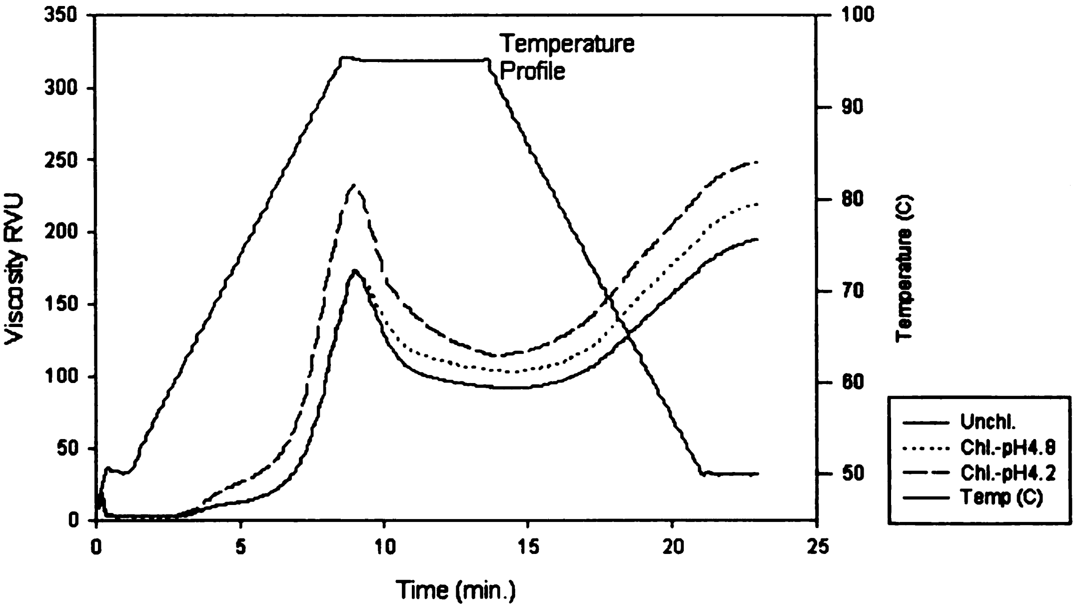


Figure 5. Pasting curves of flours measured by the Rapid Visco-Analyzer using a 23-min test profile. The viscosities shown are the average of three trials for non-chlorinated, pH 4.8 chlorinated, and pH 4.2 chlorinated flours, along with the temperature profile.

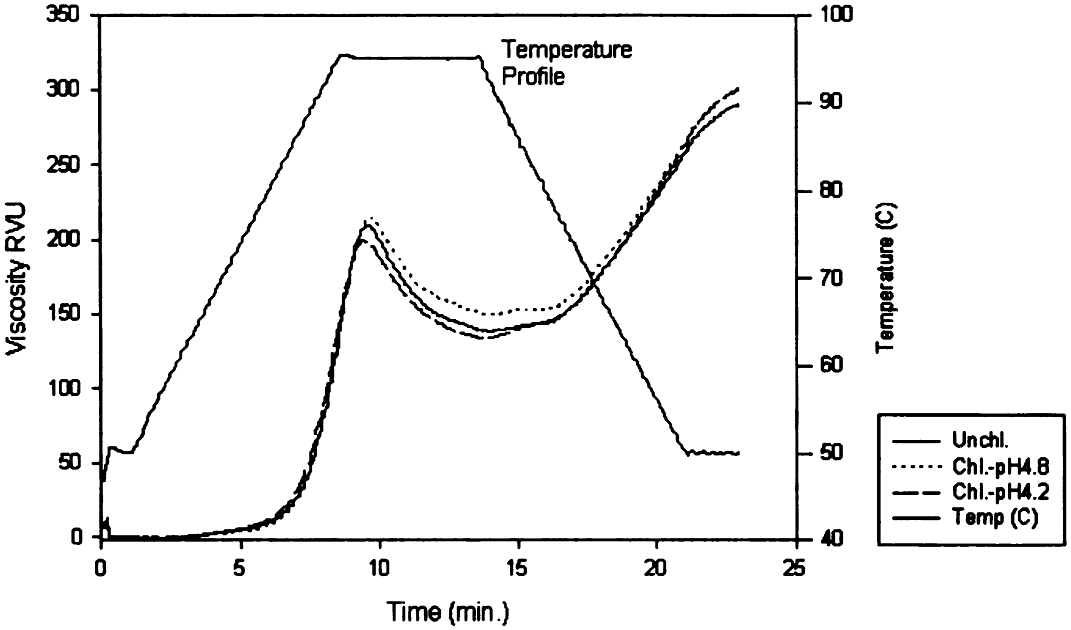


Figure 6. Pasting curves of starches measured by the Rapid Visco-Analyzer using a 23-min test profile. The viscosities shown are the average of two trials for the starches isolated from non-chlorinated, pH 4.8 chlorinated, and pH 4.2 chlorinated flours, along with the temperature profile.

Table 4. Pasting parameters of flour (non-chlorinated, pH 4.8 chlorinated, and pH 4.2 chlorinated flours) measured by Rapid Visco-Analyzer 23-min test.

Flour	Peak 1	Trough 1	Breakdown	Final Visc	Setback	Peak Time	Pasting Temp
Unchl.	173.69 b	91.53 c	82.17 b	195.25 c	103.72 c	9.00 ab	82.93 a
Chl.-pH 4.8	172.56 b	103.44 b	69.11 c	219.47 b	116.03 b	9.04 a	82.50 a
Chl.-pH 4.2	232.92 a	114.44 a	118.47 a	248.36 a	133.92 a	8.96 b	64.62 a

Note: Each value is the mean of three replicates. Means followed by the same letter in the same column are not significantly different ($P<0.05$).

Table 5. Pasting parameters of starch (isolated from non-chlorinated, pH 4.8 chlorinated, and pH 4.2 chlorinated flours) measured by Rapid Visco-Analyzer 23-min test.

Starch	Peak 1	Trough 1	Breakdown	Final Visc	Setback	Peak Time	Pasting Temp
Unchl.	209.33 b	138.75 b	70.58 a	290.96 b	152.21 b	9.57 a	83.15 a
Chl.-pH 4.8	215.54 a	152.38 a	63.17 b	302.33 a	149.96 c	9.67 a	83.35 a
Chl.-pH 4.2	200.04 c	133.92 b	66.13 ab	301.08 a	167.17 a	9.43 b	82.75 a

Note: Each value is the mean of two replicates. Means followed by the same letter in the same column are not significantly different ($P<0.05$).

The difference in the pasting properties between starches was not as large as that between flours. However, what appear to be minor differences should not be neglected. The highest peak viscosity was obtained from starch fractionated from pH 4.8 flour, followed by starch from non-chlorinated control flour, with the lowest peak viscosity and an earlier peak time for starch from pH 4.2 flour. Starch from pH 4.8 flour was more resistant to breakdown than that from non-chlorinated flour, and showed the least setback.

The earlier peak time for starch from pH 4.2 flour indicated an earlier swelling. This is in agreement with the DSC study of Allen et al. (1982), who found that the enthalpy values observed for chlorinated samples tended to be a little lower. Since starch swelling, thermal and mechanical disintegration, and solubles leached from the granules are all involved in pasting, it is difficult to give one simple explanation for the complicated pasting behavior.

The significant differences in the pasting characteristics of the flours are indications of the effect of chlorination. The pasting behavior of the starches was different from the flours from which they originated. This suggests that other flour components have a significant effect on the pasting characteristics of the starch, following chlorination.

4.7 Swelling Power of Starch

The starch fraction of chlorinated flour is considered the major factor in improving cake-baking quality. Starch swells during the heating of cake batter. The influence of chlorination on starch swelling power was examined by both the traditional water uptake method (Leach et al, 1959) and the blue dextran dye exclusion method (Tester and Morrison, 1990). The water uptake method does not distinguish water within the swollen starch granules (intragranular water) from water in the interstices between the swollen granules.

1) Water uptake method: The changes in the swelling power and water solubility of the starches during heating are shown in Figure 7. There was no significant difference in swelling power between chlorinated and non-chlorinated starches. Moreover, chlorination did not increase the solubility of starches in this experiment.

A two-stage swelling power was observed. The starches gradually swelled from 50° to 70°C. There was a period of slow swelling between 70° to 85°C. The swelling increased again rapidly above 85°C. The two-stage swelling pattern indicates different bonding forces involved in the different stages of starch swelling process. The pattern of starch solubility is different from that of swelling power. In this study, the solubility of starch showed an exponential increase over the temperature range of 40° - 95 °C.

2) Blue dextran dye exclusion method: This method is very sensitive to many factors, and has not been used widely. The standard deviation of a two-replicate measurement was up to 2.11 swelling factor (SF) units. The difference in swelling factor shown in Figure 8 can not be considered as the difference in swelling factor between the three starches since the variations were greater than the differences between treatments.

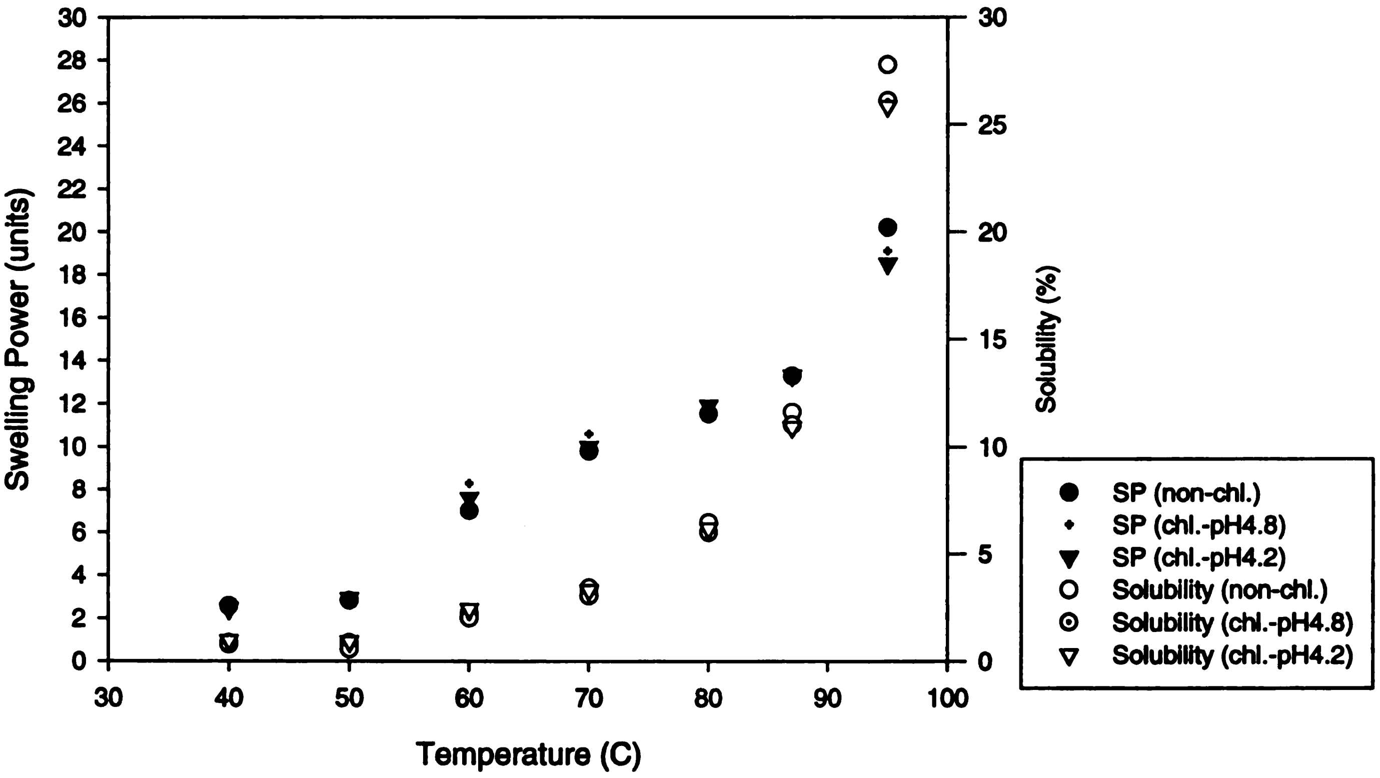


Figure 7. Swelling power (SP) and water solubility of starches, measured by the water uptake method according to Leach et al. (1959). Each datum is the average of two replicates, with a standard deviation of less than 0.7 units.

4.8 Study of Starch Hydrophobicity

At lower oil-to-water ratios (1:11 and 1:9), the oil-bound starch flocs settled at the bottom of the tubes for each of the three starches. The flocs of starch isolated from chlorinated flour looked fluffy and constituted a larger volume, compared to the starch isolated from non-chlorinated flour.

At higher oil-to-water ratios (above 1:6), the mixture containing starch isolated from non-chlorinated flour was separated rapidly into a bottom water layer with starch sediment and an upper starch floc bound with oil. However, the mixture containing chlorinated starch separated very slowly into a clear bottom water layer and an upper starch floc bound with oil (Figure 9). After one hour, there was a thin layer of oil on top of the mixture in the tube containing non-chlorinated starch. The separation time of the mixture increased with chlorination level. When the oil-to-water ratio was increased to 2:1, the mixtures separated into three layers, i.e. an upper oil layer, a middle starch floc, and a bottom water layer.

This study provides evidence that the starch isolated from chlorinated flour is more hydrophobic and is more successful in binding oil. In all cases, the starch isolated from pH 4.8 flour had an intermediate behavior between non-chlorinated flour and pH 4.2 flour. The hydrophobic nature of chlorinated starch may improve incorporation of air during batter mixing, and increase cake volume. It may also help to disperse fat or oil evenly in the cake batter and stabilize it.

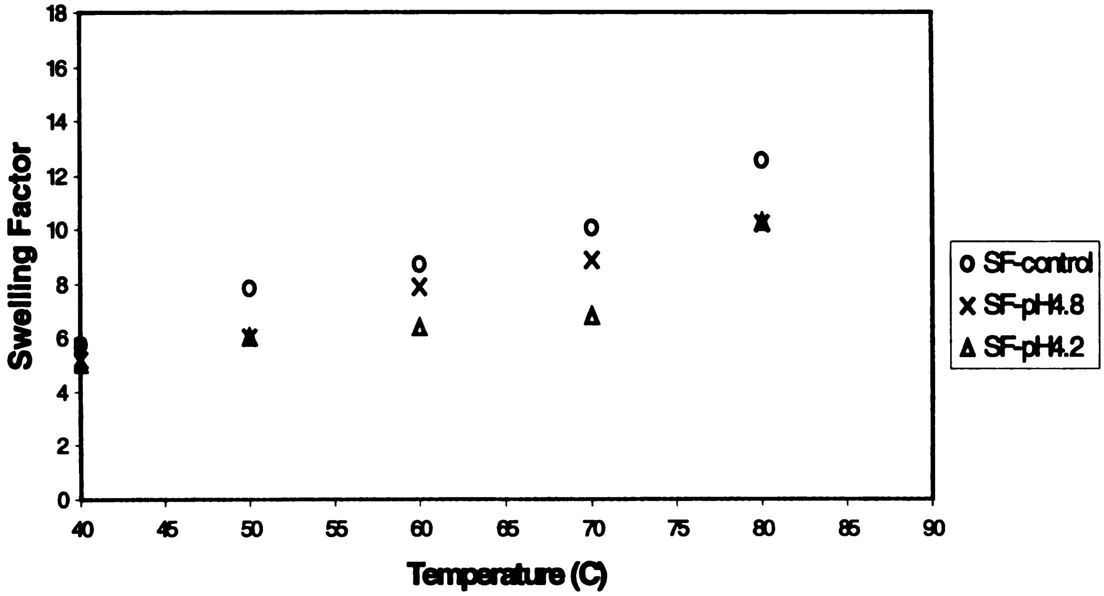
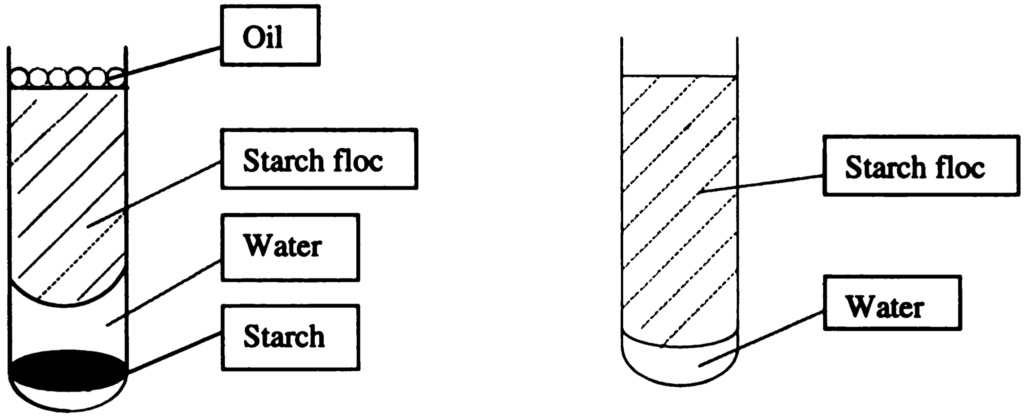


Figure 8. Swelling factor (SF) of starch measured by the blue dextran exclusion method according to Tester and Morrison (1990). Each datum is the average of two replicates, with a standard deviation of up to 2.11 units.



Non-chlorinated starch mixture

Chlorinated starch mixture (pH 4.2)

Figure 9. Separation of initially uniform starch-oil-water mixtures into various layers.

4.9 The Environmental Scanning Electron Microscopy Study

The results of ESEM experiments (Table 6) showed that the starch from chlorinated flour did not swell any more than that from non-chlorinated flour when heated up to 100°C in water with a starch to water ratio of 1:2. This observation is in agreement with the results from the swelling power measurements in this study. The initially swollen starch of chlorinated flour shrank less than that of the control flour when cooled down from 100°C to 30°C.

Table 6. Starch size determined by environmental scanning electron microscopy (ESEM).

Starch	Mean Diameter at 100°C (μm)	Shrinkage Index
Non-chl.	17.82	0.08
Chl.-pH 4.8	16.88	0.06
Chl.-pH 4.2	16.76	0.02

Note: Shrinkage index is calculated as:
(mean diameter at 100°C - mean diameter at 30°C) / mean diameter at 100°C.

An equatorial groove was observed on the starch isolated from pH 4.2 flour when heated to 100°C. However, it was not observed for starches isolated from non-chlorinated and chlorinated pH 4.8 flours. This morphological change in the starch may indicate a structural change due to flour chlorination. This study was conducted on starch heated in limited water to mimic baking procedure, in which limited water is actually used. The chlorination effect may be amplified by heating the starch in excess water.

4.10 Baking of High-Ratio Cake

4.10.1 Batter Specific Gravity

Batter specific gravity is an important factor in the evaluation of the cake-baking quality of flour. The specific gravity values measured for batters of non-chlorinated, pH 4.8 and pH 4.2 flour were 0.92, 0.87 and 0.85, respectively. Although there was no significant difference in batter specific gravity between these flours ($p > 0.05$), there was a slightly declining trend for chlorinated flour. The increase in starch hydrophobicity may increase the amount of air incorporated into the cake batter, hence giving the batter a lower specific gravity.

4.10.2 Cake Evaluation

The high-ratio white layer formulation (AACC 1990, method 10-90) was used to test the cake-baking potential of the flours. The cross-sectional views of cakes made from non-chlorinated and chlorinated flour are shown in Figure 10. The subjective scores and objective measurements are summarized in Table 7. The volumes of cakes baked from non-chlorinated, pH 4.8 and pH 4.2 flour were 863, 900 and 791 ml, respectively. There were significant differences in cake volume ($p < 0.05$) with the biggest volume obtained from pH 4.8 flour, followed by non-chlorinated, and the pH 4.2 flour, respectively. Cakes made from pH 4.8 flour had less shrinkage than those made from pH 4.2 flour.

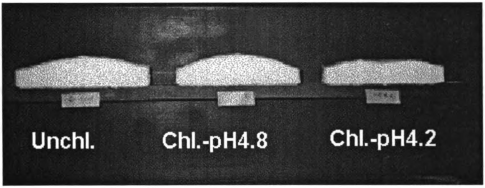


Figure 10. Cross-view of cakes made from control and chlorinated flours.

Table 7. Baking results of control and chlorinated flours.

Flour	Volume (ml)	Volume Index (cm)	Shrinkage Value (cm)	Symmetry Index (cm)	Uniformity (cm)	Internal texture
Unchl.	863 b	10.9 b	1.75 ab	1.85 a	-0.15 a	68
Chl.-pH 4.8	900 a	11.5 a	1.50 b	1.85 a	0.05 a	96
Chl.-pH 4.2	791 c	9.9 c	1.85 a	-0.10 b	0.10 a	68

Note: Each value is the mean of two replicates. Means followed by the same letter in the same column are not significantly different ($P<0.05$).

The cakes made from both non-chlorinated and chlorinated pH 4.8 flours gave positive symmetry indices, which indicated peaked cakes. A negative symmetry index value was obtained for cakes made from pH 4.2 flour. A negative symmetry index indicates a collapsed cake and is undesirable.

Cakes with a uniformity index close to zero are good in uniformity. A positive or negative value indicates that one side of the cake is higher than the other side. The cakes baked from chlorinated pH 4.8 flour were better in uniformity than those from non-chlorinated and chlorinated pH 4.2 flours. From the statistical analysis, however, there were no significant differences in uniformity index among the three flours.

Based on the objective measurements, the pH 4.8 and non-chlorinated flours had very similar overall baking performance in terms of shrinkage, symmetry, and uniformity. The internal texture of cakes made from pH 4.8 flour scored higher than those from non-chlorinated flour. The parameters used to evaluate the internal texture are shown in Appendix III.

5. SUMMARY AND CONCLUSIONS

5.1 Summary

This study investigated the effects of chlorination on the physicochemical characteristics of cake flour and selected flour components. Two levels of chlorinated flours (pH 4.8 and pH 4.2) along with an non-chlorinated flour were used. Starch was isolated from the flours by the acetic acid wet-fractionation procedure and freeze-dried. Property changes as a result of chlorination were examined by physicochemical means on flour, flour water extract, and the starch component.

Analytical tests were conducted on all samples. Surface properties and UV absorption were measured on flour water extracts. The effects of chlorination on the flour and starch components were evaluated by falling number tests, pasting characteristics with a Rapid Visco-Analyzer, and water-binding capacity. The swelling power, solubility and hydrophobicity of the starches were also measured. The behavior of starches during a heating and cooling cycle was examined visually by an Environmental Scanning Electron Microscope. The effects of chlorination on the flour and starch properties were correlated with cake-baking properties.

In fractionating the flours, water-solubles were first obtained by extracting flour with water, then extracting gluten with a dilute acetic acid. The final two-layer residue was separated into tailings and prime starch. All these fractions were freeze-dried. The water-solubles content was higher in pH 4.2 flour, but there was no significant difference in water solubility between pH 4.8 and non-chlorinated flours. Compared to the non-chlorinated control, chlorinated flour yielded less gluten, which would indicate that gluten was destroyed during chlorination. The prime starch recovery increased when the flour was chlorinated to pH 4.8, but decreased when the flour was chlorinated further to pH 4.2. The pH 4.8 flour produced more prime starch and less tailings than the other two treatments, which indicates that the interaction of pH 4.8 starch with other flour components is different from those in the other two flours.

Physicochemical analysis was conducted on the samples to determine the moisture content, protein content, damaged starch content, water-binding capacity and water extractability. The moisture content, protein content and damaged starch for the flours were all acceptable for soft wheat flours used for cake-baking. There was no increase in protein content in the starches isolated from chlorinated flours. The chlorinated pH 4.2 flour showed a higher water-binding capacity and water solubility than the non-chlorinated flour and the chlorinated pH 4.8 flour. The starch isolated from pH 4.2 flour also showed a higher water-binding capacity than the other starches.

UV absorption of flour extracts at 280nm showed that the water extract of pH 4.2 flour gave a higher absorption, while there was little difference between the non-chlorinated control and pH 4.8 flours. This result indicated that a high chlorination level increases the amount of water-extractable proteins. In addition, the increase of ultraviolet absorption may be due to the modification of aromatic groups of amino acids by chlorine. However, the changes in water-solubles as a result of flour chlorination were not evident in the surface behavior as measured by surface tension.

In falling number tests, chlorinated flours gave a higher falling number than the non-chlorinated control, which indicated that chlorination increased the gel strength of the flour. Similar results were observed for starches isolated from those flours. The firmer structure of chlorinated flour gel may prevent cakes from collapsing during baking.

Pasting properties were measured on flours and starches by the Rapid Visco-Analyzer method. The highest peak viscosity was observed for chlorinated pH 4.2 flour. There was practically no difference between the peak viscosities of the non-chlorinated control and pH 4.8 flours. However, pH 4.2 flour had the highest breakdown value, with pH 4.8 flour showing the least breakdown. Trough viscosity, final viscosity, and setback all increased with increasing chlorination levels. A different pasting behavior was observed for the starches isolated from the flours. The highest peak viscosity was obtained for the starch fractionated from pH 4.8 flour, with the lowest peak viscosity and an earlier peak time for starch from pH 4.2 flour. Starch from pH 4.8 flour was more resistant to breakdown than that of the non-chlorinated control, and showed the least setback. The fact that the pasting properties of the starches were different from the flours from which they originated suggests that other flour components may have a significant effect on the pasting characteristics of the starch. It can also be postulated that chlorination of flour modified the starch, and hence changed the way in which the starch interacts with other components.

Swelling power and solubility studies on starches did not show appreciable differences between chlorinated and non-chlorinated flours. Therefore, the higher viscosity observed for chlorinated flour could not be attributed to starch granule swelling or solubles leaching from the swollen granules. Hydrophobicity studies on the starches indicated that chlorination increased the hydrophobicity of the starch granules in the flour. Starch hydrophobicity increased as the chlorination level increased.

The results of ESEM tests confirmed that starch isolated from chlorinated flour did not swell any more than that from non-chlorinated flour when heated up to 100°C. However, the swollen starch from chlorinated flour shrank less than that from the non-chlorinated control flour when cooled down from 100°C to 30°C. An equatorial groove was observed on the starch from pH 4.2 flour when heated in limited water.

The high-ratio white layer formula was used to evaluate cake-baking performance of the flours. The specific gravity of cake batter made from chlorinated flour tended to be lower. This result suggests that chlorinated flour enhanced the incorporation of air into the cake batter. Objective evaluation of the cakes included measurements of the cake volume, volume index, shrinkage value, symmetry index, and uniformity. Cakes made from chlorinated pH 4.8 flour gave the highest volume. Based on the objective measurements, the chlorinated pH 4.8 and non-chlorinated flours had very similar overall baking performances in terms of shrinkage, symmetry and uniformity. In the subjective evaluations which were based on the internal texture, however, the cakes made from non-chlorinated flour scored lower than those from pH 4.8 flour.

5.2 Conclusions

The flour that was chlorinated to pH 4.8 produced cakes of the best volume and overall quality. Flours chlorinated to pH 4.2 flour were over-chlorinated and produced smaller cakes of inferior quality. The chlorination of flour produced changes in the overall characteristics of both the flour and the starch components. These changes include an increase in flour solubility, water-binding capacity, flour pasting viscosity, and solubilization of flour proteins. Evidently, chlorination increased starch hydrophobicity and hydration capacity. At the chlorination levels used in this study, no changes in swelling power and solubility of starch were observed.

Chlorination had a significant effect on the flour pasting profiles. The highest peak viscosity was observed for chlorinated pH 4.2 flour. Although there was no difference in peak viscosity between the non-chlorinated control and the chlorinated pH 4.8 flour, the pH 4.8 flour showed the least breakdown. Trough viscosity, final viscosity, and setback all increased as chlorination levels increased. Since no such effects were observed with starches isolated from the chlorinated flours, this suggests that the interaction between the starch isolated from chlorinated flour and other flour components play a significant role. The interaction facilitated formation of a firm flour gel structure and a stable cake batter.

6. RECOMMENDATIONS FOR FUTURE RESEARCH

1. Chlorine treatment modifies the surface of the starch granules, thus the interactions between starch and other flour components. An approach based on surface and colloid science is needed to examine the starch granule surface modification by flour chlorination and the interaction between the starch granule and other flour components or cake ingredients.
2. Because of the large range of starch granule sizes (1-30 μm), which is in the upper range of colloidal particles (10^{-6} – 10^{-9} m), some common surface and colloidal techniques may not be readily applicable. Modeling of the surface characteristics of chlorinated starch may provide a way to study the interactions.
3. A well-designed microscopic approach can be used to physically examine the starch surface morphology and dynamic changes in starch and batter behavior during heating. This visual examination will help interpret results from macroscopic observations.
4. Development of alternatives to the chlorination process can be tried by imitating the physicochemical characteristics of chlorinated flour through formulation or physical treatment.

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APPENDICES

APPENDIX I

APPENDIX I

Explanation of the parameters measured by the Rapid Visco-Analyzer

1. Peak viscosity:
   Maximum viscosity developed during or soon after the heating portion of the test, in RVU (Rapid Visco Unit).

2. Peak time:
   Time at which the peak visocity occurred, in minutes.

3. Pasting temperature:
   Temperature where viscosity first increases by at least 2 RVU over a one minute period using profile STD2, in °C.

4. Peak temperature:
   Temperature at which the peak viscosity occurred, in °C.

5. Holding strength:
   Minimum viscosity after the peak, normally occurring around the commencement of sample cooling, in RVU.

6. Breakdown:
   Peak viscosity minus trough viscosity, in RVU.

7. Final viscosity:
   Viscosity at the end of the test, in RVU.

8. Setback (from Trough):
   Final viscosity minus trough viscosity, in RVU.

9. Setback from Peak:
   Final viscosity minus peak viscosity, in RVU.

APPENDIX II

APPENDIX II

Analysis of Variance (ANOVA)

Table II-1. ANOVA for flour and starch properties

Attribute	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Flour protein content	Treatment	0.3152	2	0.1576	525.4	0.0002	9.5521
	Error	0.0009	3	0.0003			
	Total	0.3161	5				
Starch protein content	Treatment	0.0033	2	0.0016	14.0	0.0301	9.5521
	Error	0.0003	3	0.0001			
	Total	0.0036	5				
Flour water binding capacity	Treatment	0.0086	2	0.0043	12.1	0.0078	5.1432
	Error	0.0021	6	0.0004			
	Total	0.0108	8				
Starch water binding capacity	Treatment	0.0140	2	0.0070	69.8	0.0001	5.1432
	Error	0.0006	6	0.0001			
	Total	0.0146	8				
Flour falling number	Treatment	27226.3	2	13613.2	29.4	0.0107	9.5521
	Error	1390.5	3	463.5			
	Total	28616.8	5				
Starch falling number	Treatment	44587.5	2	22293.8	14.3	0.0023	4.4590
	Error	12438.7	8	1554.8			
	Total	57026.2	10				
Flour solubility	Treatment	1.1557	2	0.5778	59.7	0.0038	9.5521
	Error	0.0290	3	0.0097			
	Total	1.1847	5				
Damaged starch in flour	Treatment	0.2422	2	0.1211	3.8	0.1526	9.5521
	Error	0.0968	3	0.0323			
	Total	0.3391	5				
Damaged starch in starch	Treatment	0.1249	2	0.0625	3.7	0.1569	9.5521
	Error	0.0513	3	0.0171			
	Total	0.1762	5				

Table II-2. ANOVA for cake evaluation

Attribute	***Source of Variation***	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Volume	Treatment	12289.3	2	6144.7	118.2	0.0014	9.5521
	Error	156.0	3	52.0			
	Total	12445.3	5				
Volume index	Treatment	2.79	2	1.395	167.4	0.0008	9.5521
	Error	0.02	3	0.008			
	Total	2.81	5				
Shrinkage value	Treatment	0.13	2	0.065	6.5	0.0812	9.5521
	Error	0.03	3	0.010			
	Total	0.16	5				
Symmetry index	Treatment	4.08	2	2.042	87.5	0.0022	9.5521
	Error	0.07	3	0.023			
	Total	4.15	5				
Uniformity index	Treatment	0.07	2	0.035	3.5	0.1643	9.5521
	Error	0.03	3	0.010			
	Total	0.1	5				

APPENDIX III

APPENDIX III

Cake Internal Texture Grading

Table III-1. Grading for internal texture of cakes based on the AACC standard method 10-90

Internal Factors		Score (Points)		
		Unchl.	Chl.-pH 4.8	Chl.-pH 4.2
Cells (30pts.)	Uniformity (10pts.)	2	10	6
	Size (10pts.)	6	8	6
	Thickness of walls (10pts.)	2	10	6
Texture (34pts.)	Moisture (10pts.)	10	10	8
	Tenderness (14pts.)	12	14	10
	Softness (10pts.)	8	10	4
Grain (16pts.)		10	16	10
Crumb color (10pts.)		8	8	8
Flavor (10pts.)		10	10	10
Total		68	96	68