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Impact of chlorine treatment on properties of wheat flour and its components in the presence of sucrose

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ABSTRACT

Chlorine treatment increases the surface hydrophobicity of starch granules of soft wheat flour and reduces its gluten network forming capacity and apparent content of the flour lipids, the latter presumably by forming chlorinated derivatives. It also increases its solvent holding capacity as shown by proton nuclear magnetic resonance (^{1}H NMR) experiments in presence of sucrose. The exchanging protons in the extra-granular space are present in an environment with restricted mobility, indicating strong interactions between chlorinated soft wheat flour (CSWF) components and solvent. Chlorination reduced the capability of free flour lipids to shield starch granules during rapid visco analysis (RVA). The higher viscosity reached in RVA with CSWF was attributed to not only its lipids having different properties but also its protein, resulting in improved starch swelling and amylose leaching. Finally, ^{1}H NMR indicated that starch and protein networks in CSWF-sucrose gels were better structured than in gels prepared from non-chlorinated flour.

1. Introduction

In the United States, soft wheat flour (SWF) has been chlorinated since the 1930 s. Chlorinated soft wheat flour (CSWF) is *e.g.* used in the production of high-ratio cakes of which the recipes include more sugar than flour. When made from regular SWF, such cakes tend to collapse after removal from the oven. The use of CSWF prevents such collapse and yields cakes of increased volume and white, finer and more even crumb (Gough, Whitehouse, Greenwood, & Miller, 1978). Chlorine gas (Cl_2) is commercially applied at a rate of 300–1500 mg/kg of flour (Pyler, 1988). It reacts with water in the flour [flour typically has a moisture content (MC) of about 14.0%] according to the equation:

$$Cl_2 + 3H_2O \rightarrow 2H_3O^+ + OCl^- + Cl^-$$

The formed hypochlorite ion (OCl^-) is a strong oxidizing agent. It acts on flour carotenoid pigments that are part of the lipid fraction, and decreases their levels rapidly. This bleaches the flour (Tsen, Kulp, & Daly, 1971). The formed hydrogen ions lower flour pH (Gough et al., 1978) and the decrease in pH is monitored to obtain the desired level of chlorination (Sollars, 1958).

Many researchers have tried to understand the effects of Cl_2 treatment on individual flour components, but no consensus has been reached concerning the modification which is most important for the changes in flour functionality. Whatever be the case, OCl^- reacts with starch, gluten, lipids and arabinoxylan. The exact portion reacting with each flour component is however difficult to determine.

We further elaborate on the components reacting with Cl_2 as well as on fractionation-reconstitution experiments of control and chlorinated

Abbreviations: ^{1}H, proton; ASE, accelerated solvent extractor; C*, close packing concentration; Cl_2, chlorine; CPMG, Carr-Purcell-Meiboom-Gill; CSWF, chlorinated soft wheat flour; dm, dry matter; DSC, differential scanning calorimetry; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); FID, free induction decay; FL, free lipids; HPLC-ELSD, high-performance liquid chromatography coupled to an evaporative light scattering detector; MC, moisture content; MW, molecular weight; NMR, nuclear magnetic resonance; OCl-, hypochlorite ion; RP-HPLC, reversed-phase high-performance liquid chromatography; RT, room temperature; RVA, rapid visco analyser; SDS, sodium dodecyl sulfate; SE-HPLC, size-exclusion high-performance liquid chromatography; SH, sulfhydryl; SHC, solvent holding capacity; SRC, solvent retention capacity; SS, disulfide; T_2, spin-spin; T_C, conclusion temperature of starch crystal melting; TD, time domain; T_O, onset temperature of starch crystal melting; T_P, peak temperature of starch crystal melting; WSB, water saturated butan-1-ol; ΔH, gelatinization enthalpy

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flour as a way to explore which chlorinated flour components are most responsible for the changes in flour functionality.

1.1. Components reacting with chlorine gas

Sollars (1961) used consecutive extractions with water and acetic acid solution to separate (C)SWF into water solubles, gluten, prime starch and starch tailings (*i.e.* small and disrupted larger starch granules). He found that most of the Cl_2 reacts with the water extractables and gluten, while only minor levels react with the tailings and the prime starch. However, more than half of the Cl_2 in the gluten fraction was held by lipids. Chamberlain (1962), by air classification to fractionate CSWF, found that half of the Cl_2 is retained by proteins, one third by lipids and one seventh to one fifth by starch. However, calculations were based on a lipid content of 1.0% such as determined by petroleum ether extraction. This procedure only detects free lipids (FL) and not those bound to protein or starch, making it possible that Cl_2 in the protein and starch fractions was that bound to lipids (Gough et al., 1978). Indeed, flour lipids can be divided into starch internal and non-starch lipids. The former can only be extracted following starch gelatinization, while the latter are extractable at room temperature (RT). Non-starch lipids are either free (extractable with non-polar solvents, *e.g.* hexane) or bound [extractable with more polar solvents, *e.g.* water saturated butan-1-ol (WSB)] (Pareyt, Finnie, Putseys, & Delcour, 2011). Based on air classification also, Huang, Finn, and Varriano-Marston (1982a) found proteins to absorb more Cl_2 than does starch. Still, they stated that at least part of the beneficial effects of chlorination is due to changes in the starch fraction.

Heikes (1992) found several compounds in CSWF which were not present in SWF. These included chlorinated derivatives of endogenous fatty acids. The most abundant chlorinated derivatives were 9,10-dichloro-12-octadecenoic and 12,13-dichloro-9-octadecenoic acid. These were presumably formed from linoleic acid by addition of Cl_2 to their unsaturated bonds. Lipids are closely associated to flour proteins and starch granules and these associations may well be affected by chlorination, which in turn can impact cake quality (Gough et al., 1978). Despite the low lipid contents in wheat flour (*ca.* 2%) (Pareyt et al., 2011), a large part of the applied Cl_2 reacts with them.

Kulp, Tsen, and Daly (1972) observed starch depolymerization, but only in excessively CL_2 treated flour (flour chlorinated to a pH below 4.0). However, later Huang et al. (1982a) stated that starch depolymerizes during Cl_2 treatment but also that oxidation at C-2 and C-3 occurs, even with Cl_2 doses corresponding to those used for chlorination of flour. Baldwin, Melia, and Davies (1997) did not observe oxidation of starch hydroxyl groups but agreed with the hypothesis of depolymerization. They did not observe any direct addition of Cl_2 to the starch polymers. In addition, chlorination changes neither the Kofler hot stage polarization microscopy starch gelatinization temperature (Kulp et al., 1972) nor that determined with differential scanning calorimetry (DSC) (Huang, Finn, & Varriano-Marston, 1982b). It also does not impact its X-ray diffraction pattern (Huang et al., 1982a). Seguchi (1984) reported that chlorination increases the hydrophobicity of starch granules and suggested this to be the effect of the granule-surface proteins becoming more hydrophobic.

Tsen et al. (1971) observed a larger water extractable protein fraction in CSWF than in SWF. They attributed this to disruption of inter- and intramolecular hydrogen bonds and certain peptide bonds. Altered properties of flour proteins may also be attributed to oxidation by Cl_2 which may result in degradation of aromatic amino acids and oxidation of free sulfhydryl (SH) groups. Duviau, Yamamoto, Ng, and Kobrehel (1996) also observed changes in protein extractability and found a slight decrease in SH groups as a result of chlorination. However, no changes in disulfide (SS) bond levels were detected.

Only little research has been performed on the action of Cl_2 on arabinoxylan, a minor constituent of wheat flour (*ca.* 2%) (Goesaert et al., 2005). Kweon, Slade, Levine, and Souza (2010) performed solvent retention capacity (SRC) tests and attributed the increased viscosity development of CSWF suspensions during heating in a rapid visco analyzer (RVA) to the oxidative gelation of solvent-accessible arabinoxylan induced by Cl_2.

1.2. Fractionation-reconstitution experiments of (chlorinated) soft wheat flour

To understand the impact of chlorination of the different flour components on the quality of high-ratio cakes, several authors performed flour fractionation-reconstitution experiments. Sollars (1958) separated (C)SWF flour into water solubles, gluten, prime starch and starch tailings (see above). Afterwards he reconstituted flour samples with each time one of the components interchanged with its chlorinated counterpart and *vice versa*. He concluded that changes induced in the gluten and prime starch fractions by chlorination play a key role in preventing collapse during cooling of freshly baked high-ratio cakes from CSWF. However, lipids were not extracted as a separate fraction but were present in the prime starch and gluten fractions. Donelson, Yamazaki, and Kissell (1984) extracted FL with hexane from SWF and CSWF and interchanged them between the two flour types. They concluded that FL in CSWF improve cake volume, but did not explain the cause of this improvement.

It follows from the above that many studies have dealt with the impact of chlorination on flour components and the quality of high-ratio cakes. Most studies agree that chlorination induces changes in the starch and gluten fractions possibly by altering the composition of FL. However, it is still not clear how the altered starch, gluten and lipid properties in CSWF result in improved high-ratio cake quality. In this study, the impact of chlorination on the properties of starch, gluten and lipids when in the presence of sucrose is investigated. Beside more conventional techniques such as DSC and RVA, time domain (TD) proton (^{1}H) nuclear magnetic resonance (NMR) is used here for the first time to study the effects of chlorination on solvent distribution and flour properties before and after heating of model systems with a sucrose concentration that resembles that in high-ratio cake recipes. It is hypothesized that chlorination induces changes in the gluten, starch and lipid fractions that result in strong interaction of CSWF with the cake batter's solvent, leading to well-structured networks in the resulting cake.

2. Materials and methods

2.1. Materials and composition analysis

SWF (13.1% MC, 8.3% protein, 70.4% starch, 2.6% damaged starch, pH 5.9) and its chlorinated counterpart (CSWF, 12.0% MC, 8.4% protein, 74.4% starch, 2.6% damaged starch, pH 4.5) were kindly provided by The Mennel Milling Company (Fostoria, OH, USA). Protein, starch and damaged starch contents of both flours were not significantly different. Sucrose was from a local supermarket.

Flour MC was determined according to AACCI Approved Method 44-15.02 (AACCI, 1999b). Protein content was analyzed using an adaptation of the AOAC Official Method (AOAC, 1995) to an automated Dumas protein analysis system (EAS vario Max C/N, Elt, Gouda, The Netherlands), with 5.7 as the nitrogen to protein conversion factor. Flour pH was determined according to AACCI Approved Method 02-52.01 (AACCI, 1999a). Starch content was calculated as 0.9 times the glucose level estimated by gas chromatography following acid hydrolysis and conversion to alditol peracetates (Courtin, Roelants, & Delcour, 1999). Damaged starch levels were analyzed according to AACCI Approved Method 76-31.01 (AACCI, 1999d) using the damaged starch assay procedure of Megazyme (Bray, Ireland). All reagents, solvents and chemicals were of analytical grade and obtained from Sigma-Aldrich (Bornem, Belgium) unless indicated otherwise.

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