Effect of cellulose-based hydrocolloids and starch chemical modification on the rheology of gluten-free dough

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# ABSTRACT

This study was carried out to evaluate the effect of cellulose-based hydrocolloids on the rheology of flour doughs based on corn starch and potato starch. Adding cellulose-based hydrocolloids or a chemically modified waxy starch to this gluten-free dough imparted a shear-thinning behavior similar to that of wheat flour dough.

## **INTRODUCTION**

Coeliac disease is now regarded as one of the most common genetic diseases, and the only treatment at this time is a gluten-free diet<sup>1</sup>. A major drawback is that gluten is not easy to substitute as a baking ingredient since determines the viscoelastic characteristics of wheat dough, contributing to the appearance and crumb structure. To match this standard, bakery products addressed to coeliac patients would require sufficient viscoelastic properties for holding carbon dioxide released the during fermentation and enable to keep the structure during the expansion along baking. For this purpose, hydrocolloids have been widely used often in combination with proteins that can be tolerated by coeliac persons<sup>2</sup>. Recently, it was found that the addition of hydroxy-propyl-methyl-cellulose (HPMC) to the zein-based gluten-free bread improved its specific volume and slowed its staling<sup>3-4</sup>. HPMC has been successfully used also to prepare gluten-free bread with other formulations such as with rice flour or carob germ flour<sup>3</sup>. While baking is a complex process and hydrocolloids effect on bread would require an extensive study, in this work the focus is the effect of hydrocolloids chemistry on the rheology of dough containing zein as protein that substitutes gluten, as a the first step of bread making. The key to the successful role of HPMC as bread volume improver is investigated by substituting it with similar modified celluloses having systematic tailored chemistry. The physical effect of HPMC is also compared to that of octenyl succinic anhydride substituted (OSA) waxy starch, for which short octenyl succinate side chains add surface activity to the amylopectin molecule. Unlike typical surfactants, OSA starch forms strong films at the oil-water interface, providing the emulsions with resistance to agglomeration<sup>5</sup>. The effect on gluten-free dough rheology of hydrocolloids and waxy starch chemical modifications is discussed.

### MATERIALS AND METHODS

Salt, sugar, yeast, wheat flour and olive oil were purchased from local groceries. The wheat flour used was "Special Vetemjöl" produced by Kungsörnen AB (Järna, Sweden), that contained 12g/100 g of protein. The native corn starch used in this work is produced by Unilever Food Solutions (Kastrup, Denmark) under the trade name Maizena and was purchased in local food stores. Potato starch and OSA

waxy starch, sold as Trecomex-CO2, were kindly donated by Lyckeby Starch AB (Kristianstad, Sweden). The degree of substitution for OSA waxy starch used in this study was 0.017. Fiber-grade zein (F4000) with a molecular weight of 35000 and purity of 93% protein was obtained from Freeman Industries LLC (Tuckahoe, NY, USA). Hydroxy Propyl Methyl Cellulose (HPMC) Methocel<sup>™</sup> K4M with  $M_W \approx 100000$  and Degrees of substitution for methoxyl DS<sub>MO</sub>=1.9-2,4 and hydroxypropoxyl  $DS_{HPO}=0,7-1,2$ was purchased from Dow Chemicals (Midland, MI, USA). Ethyl (hydroxyethyl) cellulose (EHEC), methyl ethyl hydroxyethyl cellulose (MEHEC) and hydrophobic modified ethyl (hydroxyethyl) cellulose (HM-EHEC) were supplied by Akzo Nobel AB (Stenungsund, Sweden). The EHEC and HM-EHEC had same molecular weight (M<sub>w</sub>  $\approx$  100000) and the substitution degrees of ethyleneoxide ( $MS_{EO}$ ), ethyl ( $DS_{ethyl}$ ) were 1.8-2.5 and 0.8-1.0 for both polymers. The degree of substitution for hydrophobic tails in HM-EHEC was  $MS_{hydrophobe} = 0.008^{7-8}$ . The MEHEC used has substitution degrees of ethyleneoxide  $MS_{EO} = 0.3-1.2$ , ethyl  $DS_{ethyl} = 0.2-0.3$  and of methyl  $DS_{methyl} =$  $0.7-1.3^8$ . The chemical structures of the modified cellulose products used in this study are illustrated in Fig. 1. Dough samples were mixed for 10 minutes at room temperature using а 10-g ReoMixer (Reomix Instruments, Lund, Sweden) conforming to the AACC Mixograph standard (Method 54-40.02). All dough formulations contained 40g/100g of water and 0.2% dry yeast. The flour for hydrocolloids-containing flour had a 23% zein, 67% corn starch, 10% potato starch, 2% modified cellulose content. When HPMC was exchanged with OSA waxy starch, the starch ratio was 83% corn / 5% potato / 12% modified waxy.





Hydrophobically modified EHEC



M-EHEC



Figure 1. Chemical structures of the hydrocolloids used for this study: Hydroxy Propyl Methyl Cellulose (HPMC), ethyl (hydroxyethyl) cellulose (EHEC), hydrophobically modified ethyl (hydroxyethyl) cellulose (HM-EHEC), methyl ethyl hydroxyethyl cellulose (MEHEC).

The rheological properties of the dough were measured at room temperature using an Ares-G2 (TA instruments, Waters LLC, USA) strain controlled rotational rheometer equipped with a plate-plate geometry having a 20 mm diameter. Steady viscometry measurements shear were carried out directly after mixing in the range 0.01-20 s<sup>-1</sup>. Dough samples were coated with silicon oil to prevent evaporation. Oscillatory time sweeps were carried out for 30 minutes at a constant frequency of 1Hz and in a strain range of 0.5-1% within the viscoelastic linear region. The microstructures of the dough were examined with confocal laser microscopy (CLSM) using a Leica TCS SP2 confocal laser scanning microscope (Leica Microsystems, Heidelberg, Germany). Akriflavin and Texas Red<sup>®</sup> sulfonyl chloride were used as fluorescent dyes that bind preferentially to starch and protein, respectively. Akriflavin was dissolved in ethanol and Texas Red® chloride in water prior sulfonyl to application to the sample. The light sources were a HeNe laser with  $\lambda_{ex}$ =594 nm (Texas Red) and an Ar/ArKr laser with  $\lambda_{ex}$ =488 nm (Akriflavin). Signals from the samples were captured at wavelengths of 608-673 nm and 502-549 nm for Texas Red and Akriflavin, respectively. In the resulting images, proteins are stained in green and starch in red. Images were recorded with Leica Confocal Software.

#### **RESULTS AND DISCUSSION**

All gluten-free doughs displayed a similar torque response to mixing, as shown in Fig. 2. The standard curve for hydrocolloids containing gluten-free doughs showed an increase to  $\sim 8$  mixograph units in less than a minute, as a result of water absorption, followed by a decay ending within 5 minutes of mixing. The dough containing chemically substituted waxy starch, displays produced a curve with lower peak and faster decay.



Figure 2. Mixograph curves for gluten containing and gluten-free dough formulations. Wheat based dough – black dashes, M-EHEC – grey dashes, EHEC – grey dots, HPMC – continuous black, HM-EHEC – dashes/dots, and substituted starch – black dots.

The wheat flour based dough reaches 6.5 torque units initially, as hydration occurs followed by a slight decay within 3 minutes and a steady increase afterwards.



Figure 3. Flow curves for gluten containing and gluten-free dough formulations. Wheat based dough – crosses, M-EHEC – empty squares, EHEC – full diamonds, HPMC – empty triangles, HM-EHEC – full circles, substitued starch – empty squares.

In Fig. 3 it can be observed that at low shear rates the two gluten-free formulations containing EHEC and HM-EHEC display a lower viscosity than wheat flour dough and

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a shear thickening behaviour in the range of 5-20 s<sup>-1</sup>. The other gluten-free formulations instead display a flow behaviour very similar to that of wheat. These differences in rheology can be explained by analysis of the dough microstructures shown in the CLSM micrographs in Fig.4. The wheat flour dough microstructure in Fig. 4a presents gluten fibers, in orange, along with the starch granules, in green. The gluten network forms upon kneading and that produced the torque increase after hydration observed in Fig. 2.

The microstructures for both EHEC and HM-EHEC containing dough presented some islands in orange, indicated by the white arrows in Fig. 4b, among the strach granules. These islands comprise zein protein, hydrocolloids and water. This microstructure indicates phase separation of hydrophobic hydrocolloids from the starch matrix. The hydrocolloid-containing lumps in Fig. 4b do not hinder the starch granules aggregation at high shear. These immiscible domains would act as a filler producing a higher viscosity of the dough but not suppressing or mitigating the shear thickening behaviour typical of a starch dough without any hydrocolloid added, namely oobleck fluid.

The micrograph of Fig 4c is a structure representative of shear-thinning gluten-free doughs. No orange pockets can be observed as it was for shear thickening formulations, but a good dispersion within the starch granules. This is likely related to the presence of methyl groups in the HPMC and M-EHEC hydrocolloids, and in the OSA waxy starch molecules. These hydrophilic methyl groups prevent the phase separation EHEC observed for and M-EHEC containing doughs. The shear-thinning behaviour that we observe for the glutenfree dough cannot be related to a protein network held together by covalent bonds as for the gluten-containing dough.



Figure 4. Confocal laser micrographs representative of the microstructure of a) wheat flour dough, b) shear-thickening gluten free dough and c) shear- thinning gluten-free dough.

At this low concentration, zein does not form fibers (Figs. 4b and 4c) and by removing the zein in the HPMC containing gluten-free formulation presented, the shearthinning flow behaviour persists (data not shown). For our formulation zein acts simply as filler rather than forming a protein network that imparts elasticity and shear thinning behaviour as for gluten. The chemical structure and hydrophobicity of hydrocolloids added to the zein-starch gluten-free dough affects also its thickening over time, as shown by the oscillatory time sweeps in Fig. 5.



Figure 5. Oscillatory time sweep curves for gluten containing and gluten-free dough formulations. Wheat based dough – black dashes, M-EHEC – grey dashes, EHEC – grey dots, HPMC – continuous black, HM-EHEC – dashes/dots, and OSA waxy starch – black dots

The hydrocolloids containing methyl groups, HPMC and M-EHEC, induce a thickening within the initial 10 minutes. This is likely an effect of formation of noncovalent bonds and gelling of the hydrocolloid. The thickening induced by the more hydrophobic EHEC and HM-EHEC instead is more pronounced and continues up till 30 minutes. This suggests a different mechanism, likely phase separation with formation of domains containing immiscible hydrocolloids, as those observed in Fig. 4b.

#### CONCLUSIONS

The hydrocolloids' effect on the rheology of the zein-starch dough could be linked to their chemistry. While hydrocolloids do not help the formation of a protein network similar to that of gluten, those containing methyl groups mix with starch granules and induce a shear thinning behaviour similar to that of gluten-containing dough. Their miscibility is linked to the presence of hydrophilic groups while the change of rheology those induce in starch-water dough can be possibly explained by non-covalent interactions. A similar mechanism was proposed by Smith et al.<sup>9</sup> for gluten-free doughs containing zein. Non-covalent interactions have been invoked to explain the ability of the low molecular weight zein protein to form viscoelastic materials, rather than chemical and physical interactions of molecular very high weight protein complexes found gluten. in Similar interactions may occur also between hydrocolloids when mixed into starch-zein dough. The emulsifying properties of OSA starch may help to prevent aggregation of the granules and the consequent shear thickening behaviour of the dough. When the hydrocolloids molecule hydrophobicity induces phase separation instead (EHEC and HM-EHEC), this does not affect the flow rheology in the same way and the dough is shear thickening as would be a pure starchwater mixture. The phase separation induces starker thickening than that of HPMC. For M-EHEC hydrocolloids HPMC and thickening is likely produced by gelling.

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