CHANGES IN GELATINISATION AND PASTING PROPERTIES OF VARIOUS STARCHES (WHEAT, MAIZE AND WAXY MAIZE) BY THE ADDITION OF BACTERIAL CELLULOSE FIBRILS.

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ABSTRACT

The aim of this work was to analyse the effect of bacterial cellulose fibrils (BCF) on the gelatinization profile and pasting properties of starches from different sources (wheat, maize and waxy maize) and amylose contents. Blends of 8% starch with different BCF levels (0, 0.5, 2, 6 and 10% based on the dry weight of starch) were prepared and tested by Rapid Visco-Analysis (RVA), Differential Scanning Calorimetry (DSC) and both Optical and Polarized Light Microscopy. Results showed that BCF produce a significant modification of pasting properties. The pasting temperature was reduced but viscosities (peak, final, trough, breakdown and final) increased. The reduction in pasting temperature at the highest BCF addition was 20^0C higher for maize and wheat starches but only 2^0C higher for waxy maize starch. In contrast to the pasting temperature, the gelatinisation temperature by DSC for all three starches slightly varied upon BCF addition, but the gelatinisation enthalpy was reduced to a greater extent than values reported for the addition of other hydrocolloids to starch blends. Optical and polarized light microscopy showed the presence of domains rich in starch and highly aggregated BCF in all three starches evaluated. The increase in viscosity and decrease in pasting temperature are discussed in terms of changes in starch concentrations in the starch rich domain. These results open interesting perspectives in the use of bacterial cellulose and plant cell walls to design novel bio-composites to structure foods.

Keywords: bacterial cellulose, starch, gelatinisation, pasting properties, advanced materials.

1. INTRODUCTION

Cellulose is the most abundant biopolymer in nature. It is mainly produced by plants, trees and bacteria by condensation of glucose units during the photosynthesis process in plants and trees. Long chains of anhydro-glucose units, joined via β-1,4-glycosidic linkages (C–O–C), are formed during this process (Eichhorn, 2011). Cellulosic materials consist of both crystalline and amorphous domains, in different proportions depending of cellulose source (Ciolacu et al., 2011). The presence of para-crystalline or amorphous cellulose is often significant, although it varies from species to species (Eichhorn, 2011). The physical properties of cellulose, as well as their chemical behaviour and reactivity, are strongly influenced by the arrangement of the cellulose molecules with respect to each other and to the fibre axis (Ciolacu et al., 2011). One type of cellulosic material that has received much recent attention as potential new functional material for industrial applications is bacterial cellulose.

Bacterial cellulose is a biopolymer formed by nanofibrils, which is synthetized mainly by *Acetobacter xylinus* and *Acetobacter hansenii* (Shah etal., 2013). These microorganisms are able to create in their extracellular matrix a complex network of cellulose fibres by a highly regular intra- and inter-molecular hydrogen bonds network resulting in a weak gel structure. This is the basis of Nata de Coco a traditional sweet candy desert originating in the Philippines (Tabuchi, 2007). Bacterial cellulose has a unique structure, composed by nanofibrils forming a nanostructured network characterized by high purity (free of components such as lignin and hemicellulose) Because of its nanostructure bacterial cellulose shows a high mechanical stability, high water absorption capacity in the wet state and full biocompatibility making this material feasible to be used in wide variety of applications (Picheth et al., 2017; Lee, Buldum, Mantalaris, & Bismarck, 2014; Shah et al., 2013). More recently the production, structure and applications of bacterial cellulose has been reviewed in the context of food use (Grishkewich, Mohammed, Tang, & Tam, 2017; Shi, Zhang, Phillips, & Yang, 2014; Ullah, Santos, & Khan, 2016). Incorporation of hydrocolloids, particularly pectin and mannans during the synthesis of bacterial cellulose has frequently been used to prepare models of the plant cell wall structure (Lopez-sanchez et al., 2017; Whitney, Brigham, Darke, Reid, & Gidley, 1998). Remnants of plant cell walls can be used to replace soluble hydrocolloids in structuring foods and may give health benefits (Foster, 2011; Padayachee, Day, Howell, & Gidley, 2017).

For many years there has been extensive interest in hydrocolloid:starch blends because of their inclusion in a wide range of food products. A review by Bemiller (2011) identified a large number of starch hydrocolloid blends, however we are not aware of any studies where bacterial cellulose has been added to starch to modify pasting behaviour. This paper describes a preliminary study to determine how bacterial cellulose fibrils modify the gelatinisation profile and pasting properties of starch. Starch gelatinisation is a physical transition that takes place in a starch granule and modifies the functional properties (e.g. solubility, viscosity, water holding capacity) as a response to high temperature and water. Although there is not a formal definition, gelatinisation has been described as "the collapse of molecular order inside the starch granule which produce irreversible structural changes related with an increase in granule volume, melting of crystalline form, loss of birefringence and increasing in starch solubility due to effect of temperature in an environment of high moisture" (Belitz, Grosch, & Schieberle, 2009; J. N. BeMiller & Huber, 2008). Normally the gelatinisation is measured by microscopy, differential calorimetry, X-Ray diffraction among other techniques. The modification of the gelatinization profile of starches by other biostructures is important for a number of reasons, including its potential effect on the extent of retrogradation on cooling and presumably on generation of low-digestive and resistant starch (Mishra, Hardacre, & Monro, 2012). Appelqvist et al (1995) also described an application of freeze-thaw resistance in starch sauces when mixed with hydrocolloids. In the case of starch pasting, it is regarded as a consequence of gelatinisation and is generally followed by viscosity changes. Indeed, as a result of starch gelatinisation, a viscoelastic mass is obtained (called paste), which consists of a continuous phase that is a molecular dispersion of suspended starch polymer molecules forming a network and a discontinuous phase of swollen granules, granules ghosts and granule fragments (Bemiller, 2011b). A common technique used to follow starch pasting is the Rapid-Visco Analysis (RVA) which was developed from the well-known bravender curves of starch viscosity used in the industry.

Studies looking at starch hydrocolloid interactions have generally involved only one starch source and several hydrocolloids. In this study maize, wheat and waxy maize starches were selected because of their industrial importance, but also due to some structural differences between them. For instance, the waxy maize starch contains only traces of amylose whereas the amylose content of maize and wheat starches is ~25-29% but this could vary with source and extraction method (Bertoft, 2017). Swelling of granules on heating will be influenced by the presence of amylose-lipid complexes, which could be more present in high amylose cereal starches than do normal and waxy starches (Debet & Gidley, 2006; Pérez, Baldwin, & Gallant, 2009). In terms of starch granule size, they have been well characterised. 5-20 μm (diameter) in maize and waxy maize and 2-36 μm (diameter) in wheat. However, wheat starch shows a bimodal distribution in size. Considering the typical X Ray diffraction pattern all these starches correspond to type-A starch (Buléon, 1998; Jane, 2009).

The objective of this work was to determine how the addition of bacterial cellulose fibrils modify the gelatinisation profile and pasting properties of starch from different sources (wheat, maize and waxy maize).

2. MATERIALS AND METHODS

2.1. Materials

Native wheat, maize and waxy maize starches were purchased from Sigma Aldrich (Germany) in powder form. Dried sheets of bacterial cellulose fibrils (BCF) were kindly provided by Membracel (Brazil). The starches and bacterial cellulose were used as received without further purification and stored at room temperature until further use.

2.2. Preparation of starch-BCF suspensions

BCF was added to each starch in a concentration of 0, 0.5, 2, 6 and 10% w/weight dry starch (Equation 1), using distilled water as solvent. BCF dried sheets were processed prior mixing following the protocol proposed by Quero et al. (2015). In the first step a well defined amount of BCF was held overnight in excess of distilled water in order to promote full hydration. In the next step, the BCF suspension was homogenized using a high power kitchen blender (Thomas “Premium”, Germany) for 20 minutes, then followed by vacuum filtration using 8 μm diameter filter papers (Whatman 541, USA). At the same time, starch water suspensions at 8% (w/v) were prepared for each starch type. In the final step, the filtered BCF was added to each starch suspension and stirred for 15 min at room temperature in order to get homogeneous suspensions.

$$BCF\ concentration\ (\%, w/w) = \left(\frac{BCF\ weight}{BCF\ weight + Starch\ weight}\right) x100$$

(Equation 1)

A control sample, in this case BCF in the absence of starch, was prepared following the same protocol.

2.3 Measurement Viscoelasticity of BC Suspensions

A preliminary characterization of the viscoelasticity of BCF suspensions in water in the absence of starch was carried out using a rheometer (Physica MCR 301, Anton Paar, Germany) equipped with parallel plate geometry. BCF suspensions were prepared at concentration of 0.05, 0.1 and 0.2% (w/v). Measurements were made in the linear viscoelastic region at a frequency of 1Hz and strain of 0.5%. The temperature was scanned from 10ºC to 40ºC at a rate of 5ºC/min.

2.4 Measurements of Pasting Properties

Pasting properties of starch-BCF blend were analysed by Rapid-Visco-Analysis (RVA super 4, Newport Scientific, Australia) in accordance with the methodology proposed by Sullo & Foster (2010) with minor modifications. 25-28 g of each suspension was weighed in aluminium canisters and inserted into the instrument. Pasting profiles were obtained as a function of temperature as follow: holding at 25ºC during 5 min, heating between 25-95ºC at 5ºC/min, holding at 95ºC during 5 min, cooling to 25ºC at 5ºC/min and holding at 25ºC during 5 min. The analysis was performed under constant stirring (160 RPM).

The pasting properties measured were: 1) pasting temperature (temperature at which starch granules begin to swell and gelatinise due to water uptake, which is recorded from the onset of the viscosity peak); 2) peak viscosity (maximum paste viscosity achieved in the heating stage of the profile); 3) through viscosity (minimum paste viscosity achieved after holding at the maximum temperature); 4) breakdown viscosity (difference between peak and trough viscosities); 5) final viscosity (viscosity at the end of the run); 6) setback viscosity (difference between final viscosity and trough viscosity). All the measurements were carried out at least in quadruplicate.

2.5 Differential Scanning Calorimetry (DSC)

Gelatinization temperature (ºC) and enthalpy (ΔH, J/g_{starch}) were measured by differential scanning calorimetry (DSC 1, Mettler-Toledo, Switzerland) following the protocol reported by Karlsson & Eliasson (2003) with minor modifications. In order to improve the resolution signal a higher of the starch weight suspensions were use, keeping the BCF weight fractions indicated previously (0, 0.5, 2, 6 and 10% w/w). The starch concentration was 20% w/v. ~20 uL of starch-BCF blend suspensions were loaded into 40 μL aluminium pans and then hermetically sealed. The DSC was calibrated using indium (melting temperature and enthalpy of 156.5 ± 1.56ºC, $\Delta H = 28.6 \pm 1$ J/g), and an empty pan was used as a reference. Thermal properties of the suspensions were measured as follow: holding temperature at 5ºC during 3 min, heating from 5ºC to 85ºC at 10ºC/min, and holding at 85ºC during 3 min. Gelatinization temperature (ºC) was recorded from the onset of endothermic peak associated to starch granule swelling and structural changes (Biliaderis, 2009), while gelatinisation enthalpy was considered as the area under the endothermic peak. Gelatinisation enthalpy was normalised in terms of starch dry mass and was expressed in J/g_{starch}. All measurements were performed in triplicate.

2.6 Polarized Light Microscopy

Structural features of the starch-BCF blends after starch complete gelatinisation were analysed by light and polarized light microscopy (DIAPLAN, Leitz, Germany). One aliquot was transferred from each fully gelatinised starch-BC from RVA canister immediately after analysis and deposited directly on a clean dry glass surface and covered by a clean coverslip. Light and polarized light images were obtained using a magnification 10X. Pictures were taken by a digital camera connected to the microscope (PixeLINK PL-A662, Canada). A set of pictures were captured and those most representative of each blend were selected for analysis.

2.7 Statistical Analysis

Where appropriate, the statistical significance was assessed by a paired t-test (same variances) and ANOVA using the Solver tool in Excel (Office 2016, Microsoft Corp.).

3 RESULTS

3.1 Viscoelasticity of BCF suspensions

The viscoelasticity of BCF suspensions in the absence of starch was only weakly dependent on temperature. Values of G′ of 0.74, 5.10 and 34.6 Pa were obtained at BCF concentrations of 0.05, 0.1 and 0.2 w/v respectively (Supplementary data, Figure S-1). The corresponding values of tanδ (G′′/G′) were 0.17, 0.16 and 0.14 respectively. These low values of tanδ support the description of a weak gel structure at low concentrations in the temperature range studied (Ikeda & Nishinari, 2001).

3.2 Pasting Properties of BCF starch blends

Changes in viscosity and pasting parameters of starch-BCF blends produced by RVA as a function of temperature and BCF weight fraction are presented in Figure 1 and Figure 2 respectively. Figure 1 shows the viscosity profile for the different starches (wheat, maize and waxy maize) at different concentrations of BCF. It is evident that BCF has a significant effect ($p<0.05$) on the viscosity of all three starches during gelatinisation and re-association (retrogradation) stages. The presence of BCF generates a significant increase in viscosity, which is observed even with the lowest BCF concentration (0.5% w/w), indicating that very low concentrations of BCF can produce an increment in starch viscosity. This behaviour was observed in all three starches considered in this study (wheat, maize and waxy maize), although the magnitude of change in viscosity observed in waxy maize starch was lower than the one observed in wheat and maize starches (Figure 1). Figure 2 shows effect of BCF concentrations on the pasting temperature and the viscosity-pasting parameters (Peak, Trough, Breakdown, Final and Setback). These parameters were derived from the results shown in Figure 1. Most interesting is the pasting temperature which can be defined as the temperature at which the increase in viscosity (onset) is observed and can be related with the starting point of starch gelatinisation (Phimolsiripol, Siripatrawan, & Henry, 2011). A marked shift to a lower pasting temperature was observed for wheat and maize starch when the concentration of BCF was increased (Figure 1a and 1b, Figure 2a). However, in the case of waxy maize starch the presence of BCF produced a much lower effect on the pasting temperature. These results are in agreement with those shown in Sullo & Foster (2010), and Sullo (2012), who identify that when starch is embedded in a hydrocolloid solution the composite viscosity would be affected by changes in the two phases, and the way the two phases interact with each other. They also highlight that an increase in effective concentration of starch in the starch phase would promote interactions between starch granules, and that the higher viscosity of the continuous phase might enable the detection of the early stages of granule swelling which would be undetected when starch is dispersed in water. Sullo (2012) also showed that waxy maize behaved differently from native maize starch, upon gelatinisation in the presence of guar gum, methylcellulose or hydroxypropylmethylcellulose. Abdulmola, Hember, Richardson, & Morris (1996) explained an unexpected increase in moduli in a xanthan:starch system in terms of xanthan promoting an interaction between gelatinised starch granules due to a depletion flocculation mechanism, by which low concentration of xanthan promotes the attraction of gelatinised starch granules and therefore the bringing of them togheter.

3.3 Thermal Properties

Both gelatinisation temperature (ºC) and gelatinisation enthalpy (ΔH, J/g_{starch}) of starch-BCF blends are shown in Figure 3. The presence of BCF significantly reduces ($p<0.05$) the gelatinisation enthalpy in all three starches studied, however BCF addition had little effect on the gelatinization temperature recorded from the onset of endothermic peak associated to starch granule swelling (Section 2.5).

3.4 Morphological Analysis

Morphological analysis of starch (wheat, maize and waxy maize) blended with 10% BCF after complete gelatinisation by RVA are presented in Figure 4. Results using polarised and non-polarised light are shown. These images show the presence of two material domains in the blend. The BCF can be seen as birefringent bundles with dimensions up to 500 microns. No birefringence ascribed to the starch domain is observed.

DISCUSSION

4.1 Viscoelasticity of Bacterial Cellulose Suspensions

Early work on the rheology of a series of celluloses ranging from microcrystalline cellulose to bacterial cellulose was interpreted in terms of the length to diameter ratio (L:D) of the rod shaped particle (Tatsumi, Shioka, & Oto, 2002). The dynamic viscosity of the bacterial cellulose used in this previous work was higher than found here. This could reflect differences in the L:D ratio of cellulose but could also be due to differences in the degree of dispersibility. Although interpretation in terms of the length to diameter L:D was successful in predicting rheology particularly the yield stress, microscopic examination by SEM and AFM of bacterial cellulose blends has repeatedly shown the presence of a continuous network of ultrafine fibres (Evans, Clarck, & Morrison, 1998; Shi et al., 2014; Tatsumi et al., 2002). The weak gel structure for BCF has some analogy to xanthan gum though values of G' in this current work are higher than reported for xanthan gum. For example the relatively recent study of Choi, Mitchell, Gaddipati, Hill & Wolf (2014) gave a value of G' of ~4Pa for a 0.4% w/w solution xanthan. Biopolymers which have a stiff rod shaped structure have a strong tendency to associate partly because the entropy decrease (which prevents association) is lower than for more flexible polymers. This will not only promote weak gel structures but also make dispersibility more difficult. In these respects, there are some similarities between xanthan and bacterial cellulose though the analogy should not be taken too far. Xanthan is a water soluble polyelectrolyte whereas bacterial cellulose is not water soluble. However, Foster (2010) and Lad, Samanci, Mitchell & Foster (2010) hypothesise that xanthan is driven into a nematic liquid crystalline state when competing for water with swollen starch granules, affecting the viscosity of the composite in different ways to that expected, and seen for more random coil polysaccharides. Similar differences in xanthan and guar gum have also been reported more recently by Heyman, Vos, Depypere & Meeren (2014). Therefore, xanthan as a more rigid hydrocolloid could be seen to be more similar to cellulose, given those observations and the ones reported here, and rigid rods would be more effective at promoting depletion flocculation as described by Abdulmola et al. (1996).

4.2 Starch Bacterial Cellulose Blends

When compared with other hydrocolloids, addition of bacterial cellulose has a greater effect on the gelatinisation and pasting properties of starch. This can be most clearly seen by the large reduction in enthalpy (Figure 3).

It is appropriate to consider the system as a dispersion of BCF in the starch phase in a similar way to the structure of BCF gelatine blends (Quero et al., 2015). The degree of dispersion will be governed by the volume fraction occupied by the BCF network. In our case, the volume occupied by the BCF network will increase as it is observed. If starch is partly excluded from this network then even low amounts of BCF will increase significantly the concentration of starch in its own domain. The gelatinisation temperature of starch measured by viscosity changes will correspond to the temperature when the starch concentration of swollen starch granules approaches a critical concentration given approximately by the equation $cS = 1$, where S is the extent of swelling and c the concentration of starch (Steeneken, 1989). The rheology of phase separated mixtures of biopolymers has been extensively studied in terms of the behaviour of mixed gels. Increasing the concentration in both domains will increase the overall modulus and the viscosity though the exact model will depend on nature of the deformation and interactions between the two domains. Although this hypothesis has attractive features, a large number of questions remain, some of which could be resolved by determining the phase diagram for the blend. The assumption we have is that there is less starch in the cellulose region of the BCF fibre-network. The microscopy images support this but it has not been quantified.

Therefore, the questions of interest for discussion are: Why should waxy maize starch behave differently from the other two starches? and what is the origin of the large decrease in gelatinisation enthalpy?.

These two questions will be briefly discussed in turn. On gelatinisation of wheat and maize starches, amylose will be released from the swollen starch granule. This released amylose would be expected to interact with the bacterial cellulose, as reported by Lin, Lopez-sanchez & Gidley (2015) where it was shown that there was extensive binding of dextrins extracted from potato during fermentation of bacterial cellulose. This interaction was non-specific and reversible compared with the well-known interactions between mannan based polysaccharides and bacterial cellulose (Whitney et al., 1998). However waxy maize starch does not contain the linear amylose fraction, and therefore the absence of interaction with amylose occuring outside the swollen starch granule may explain the very small change in the pasting temperature with BCF concentration observed in waxy maize starch compared with the large change observed for the two amylose containing starches. Indeed, the viscosity differences between maize and wheat starch are small reflecting the similar amylose contents. Interestingly, when comparing these observations with Sullo & Foster (2010) and Sullo (2012), who saw similar effects, the explanation of ‘binding’ of amylose to other hydrocolloids would need to be assumed also. This is not a phenomenon that has been studied extensively, and may open up a new area of research. An alternative explanation is that the previously described ‘depletion flocculation effect’ (Abdulmola et al., 1996) would indicate that the waxy maize granules are inherently softer than the wheat or maize starches, upon gelatinisation, and that the viscous continuous phase does not promote the detection of the early onset of gelatinisation of these ‘softer’ granules, or that the effect would only be noticeable at much higher granule packing fractions for waxy maize starch.

The lower peak viscosity for the blend containing waxy maize may also reflect the lower rigidity and integrity of the gelatinized waxy maize starch granule. A consequence of this will be a weaker concentration dependence of the viscosity in the concentrated starch phase (Steeneken, 1989). This could explain the lower viscosity observed in the blends containing waxy starch. This lack of integrity because of the lack amylose network may allow some of the waxy maize starch to penetrate the cellulose network, in other words it is suggested that the phase diagram for the system containing waxy maize starch would be different to the other two starches.

Decreases in enthalpy on hydrocolloid addition to starches has been reported before, and it is important to appreciate the role of water. Interesting results have been reported by Khanna & Tester (2006) and Torres, Moreira, Chenlo & Morel (2013) on starch konjac mannan and guar gum blends, respectively, but these studies have been carried out at lower water contents than used in our study. At these lower water contents of below 1.5 g_{H2O}/g_{starch} two endotherms are generally observed (Donovan, 1979). The high temperature endotherm, which is dependent on water content, is often interpreted in terms of the Flory relationship for the melting of polymer crystallites. The level of water in the current system is much higher than this limiting value and single endotherms are observed in all cases (data not showed). When excess water is present (>2g_{H20}/g_{starch}) the value of enthalpy will be independent of the water content (Donovan, 1979), therefore a dilution effect is not to be expected under our conditions.

In the work of Cooke & Gidley (1992), where NMR was used to determine the starch double helical content, it was showed that the loss in enthalpy can be well explained by a change in the double helical content. On the other hand, the decrease in enthalpy has been also interpreted as due to water binding by the cellulose. We are a bit uncomfortable in terms of such a non-specific interpretation, however if there is starch in the cellulose domain we may suggest that if starch is protected for losing the double helical structure of amylose during heating because of water binding in the BCF network, or by changes in mobility and possibly interactions with the cellulose surface, this could help to explain the decrease in enthalpy observed in this study. Hence, in the bacterial cellulose system it would be of interest to measure the starch double helical content (e.g. by NMR), as a function of temperature, to confirm this hypothesis. Other possible explanation could be related with as starch is dispersed and phase concentrated in a cellulose continuous phase, the water available to the starch in the starch phase is insufficient for helix melting and solvation which may therefore occur at a much higher temperature.

CONCLUSIONS

The presence of low levels of BCF modifies the pasting properties of starch granules (wheat, maize and waxy maize) during gelatinisation in RVA testing. All three starches show a large increase in peak viscosity on BCF incorporation but the reduction of pasting temperature is much lower for waxy maize than the two native cereal starches. In contrast, the gelatinisation temperature measured by DSC did not show significant differences on incorporation of BCF, but the gelatinisation enthalpy was reduced, which can be explained by the failure of starch within the cellulose domain to lose double helical structure on heating because of water binding in the BCF network. Following pasting in the RVA, morphological analysis by optical and polarised light microscopy showed the presence of two clearly defined domains, one of them containing fully gelatinised starch granules and the other rich in a complex structure of highly aggregated bacterial cellulose fibrils. The viscosity increase on heating may be due to increase in the starch concentration in the starch domain upon the addition of BCF, and possibly as a result of previously reported interactions between amylose and cellulose. The lack of this second mechanism for waxy maize starch could be used to explain the lower decrease in pasting temperature when compared with maize and wheat starches. As reported previously when dispersed in water, BCF forms weak, elastic gel structure at low concentrations. These results open interesting perspectives in the use of bacterial cellulose in the presence of starch for designing bio-composites with advanced properties and tailored structures to be used in food structure design and for enhanced or personalised nutrition, as well as in other related biopolymer applications. Specifically, the results could also be relevant for the structuring of foods by plant cell wall fractions rather than soluble hydrocolloids, which is an area of current interest.

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Figure Captions

Figure 1. RVA profiles in starch from different sources: a) wheat, b) maize, c) waxy maize, as a function of BCF concentration. From bottom to top: 0, 0.5, 2, 6 and 10% db. Green line correspond to bacterial cellulose RVA profile. Red line correspond to temperature scan used during analysis.

Figure 2. Pasting properties of wheat, maize and waxy maize starch modified by BCF: a) pasting temperature, b) peak viscosity, c) trough viscosity, d) breakdown viscosity, e) final viscosity, and f) setback viscosity. Continuous lines correspond only to guide to eye.

Figure 3. Gelatinisation enthalpy and temperature of starch from different sources as a function of BCF concentration: a) wheat, b) maize, and c) waxy maize. Black circles correspond to temperature while empty circles to enthalpy. Continuous lines correspond only to guide to eye.

Figure 4. Images taken by Optical Microscopy (top) and Polarized Light Microscopy (bottom) in starch blended with 10% BCF after complete gelatinisation by RVA: wheat (A1 and A2 respectively), maize (B1 and B2 respect.), and waxy maize (C1 and C2 respect). White bar represents 100 microns.