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# Microstructure analysis of high pressure induced gelatinization of maize starch in the presence of hydrocolloids

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## ABSTRACT

The effect of high hydrostatic pressure (HHP) treatment on maize starch granules was analyzed taking into account different operating conditions (time, temperature, high pressures and starch to water ratio) and the effect of hydrocolloids ( $\lambda$ -carrageenan, guar and xanthan gum). Morphological analysis using different microscopic techniques, mainly SEM and Cryo-SEM, was carried out. This study was complemented with information acquired through Differential Scanning Calorimetry (DSC), X-ray diffractometry (XRD) and spectrophotometric determination of the amylose and amylopectin concentrations released from the granules. The gelatinization degree obtained by DSC increased accordingly with the intensity (pressure level and exposure time) of the HHP treatment, while the gelatinization temperature was scarcely affected. The presence of hydrocolloids in the suspension during HHP processing would ensure water availability. Almost complete gelatinization was observed for the sample treated at 700 MPa, both by DSC and XRD. At 400 MPa the gelatinization degree was significantly lower in starch with added xanthan gum (XG) in comparison to the other hydrocolloids, denoting a possible stabilization effect of XG on the starch granular structure during gelatinization. Maize starch granules (A-type XRD pattern), when submitted to HHP treatment, showed characteristic peaks of A and B-type patterns. Signals of V-diffraction pattern, ascribed to amylose–lipid complexes, were detected by XRD in samples submitted to either very high pressure (700 MPa), long treatment times at 400 MPa (120 min) or in presence of hydrocolloids (at lower pressures and times). The formation of V-crystalline complex would be favored by hydrocolloids presence under pressure. These results correlated well with the lower concentrations of amylose released from granules after these treatments.

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## 1. Introduction

After cellulose, starch is the second most abundant biopolymer in nature. Besides being the most important carbohydrate source for human nutrition, it has an important role in food manufacturing

processes, such as modifying the texture properties of food products. It is used worldwide in thousands of food preparations (bakery products, sauces, soups, confectionery, sugar syrups, ice cream, snack foods, meat products, baby foods, fat replacers, soft drinks, among others) (Burrell, 2003).

For most of these applications, starch gelatinization is a requirement. When granules are heated in the presence of excess of water, hydrogen bonds of amorphous regions, which are weaker, break and granules absorb water and swell up. Whereas, when starch is

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submitted to milder treatments, its structure is preserved by strong bonds of crystalline region, while it breaks and completely disrupts under harsher conditions. During this process, amylose is released and consequently the viscosity of the suspension increases (Nasehi and Javaheri, 2012). This result can be achieved either by heating or resorting to high hydrostatic pressure (HHP).

The advantage of high hydrostatic pressure process over the conventional thermal starch treatment is that a more homogeneous and better defined degree of gelatinization can be achieved by HHP treatment through more accurately controlled process parameters (Knorr et al., 2006).

It is accepted that in starch gelatinization under HHP conditions, although starch granules are still swollen, they remain undamaged or are just partly disintegrated and the solubilization of amylose is rather poor (Douzals et al., 1998; Knorr et al., 2006; Stolt et al., 2000).

Different starches show different heat-induced gelatinization temperatures and, the extent of gelatinization, can change depending on the time and temperature of heating and on the starch-to-water ratio (Aparicio et al., 2009; Hoover, 2001). In a similar way, different types of starch gelatinize over different ranges of pressure and the extent of gelatinization is dependent on the applied pressure, the treatment time and the temperature of pressurization (Bauer and Knorr, 2005).

Although there are several studies dealing with the application of HHP to a broad variety of starches and conditions (Bauer and Knorr, 2005; Katopo et al., 2002; Liu et al., 2008; Stute et al., 1996), a comprehensive study of the combination of pressure, time and temperature treatment and starch concentration for maize starch has not been reported to date. In contrast, for potato starch, all the mentioned effects related to HHP applications have been deeply studied.

Bauer and Knorr (2005) observed that the degree of gelatinization increased with increasing treatment time during the first hour of treatment for potato starch. Meanwhile at treatment times longer than 1 h, there was only a minor change in the degree of gelatinization. Also, the degree of gelatinization increased with increasing pressure. Kawai et al. (2007a, 2007b) found that gelatinization enthalpy ( $\Delta H_{\text{gel}}$ ) was significantly affected by treatment pressure and starch content and that the gelatinization peak of potato starch suspension disappeared completely when treated at 700 MPa. The decrease in gelatinization temperature at higher pressures has been attributed to some kind of disruption of the granule structure and crystallinity (Błaszczak et al., 2005). Muhr and Blanshard (1982) and Muhr et al. (1982) studied the effect of starch concentration and pressure effect and reported that the gelatinization temperature reduction was a non-linear function of pressure, being this effect more pronounced at higher pressure and higher water contents (lower starch concentrations).

Some authors have expressed their concern about the availability of water for complete gelatinization of starch, especially under pressure treatment, when starch granules are settled onto the base of a container under unstirred conditions. Stirring is not currently possible during high pressure treatments, so this situation is common to all high hydrostatic pressure studies on starch (Bauer and Knorr, 2005; Yang et al., 2017). Some workers have resorted to the addition of viscosity increasing agents, such as thermally gelatinized starch (Stolt et al., 1999, 2000; Kawai et al., 2007b) to ensure that the granules remained in suspension (and presumably more accessible to water) during pressure treatments. Thermal starch gelatinization at ratios over 30% (starch/water) is known to be incomplete and gives DSC parameters strongly dependent on this ratio (Wang et al., 2014, 2016). Contrarily, higher water contents produce complete gelatinization and the DSC parameters obtained are practically independent of the actual starch/water ratio, which makes them easier to interpret and to compare.

Literature regarding the effect of HHP induced maize starch gelatinization in the presence of different hydrocolloids is also scarce (Ahmed and Ramaswamy, 2004; Harte and Venegas, 2010). The processing of starch-hydrocolloid mixtures would allow the development of new ingredients with distinctive functional characteristics, since both ingredients co-exist in many food systems and their interaction has a strong impact on the textural properties of food products (Lai et al., 2003). Thus, it is necessary to study the effect of different

hydrocolloids addition on starch HHP gelatinization behavior and its resulting microstructure.

Promising results are expected from this investigation, considering that starch and the studied hydrocolloids are common ingredients in food formulations that could be processed by HHP and their potential interest for the design of new ingredients for food formulations with nutritional and functional added value.

The objectives of this work were to investigate the effect of HHP treatment on maize starch granules by analyzing different operating conditions (time, temperature, pressure and starch-to-water ratio). The effect of hydrocolloid addition ( $\lambda$ -carrageenan, guar and xanthan gum) and HHP treatment on starch gelatinization was also investigated. Likewise, a morphologic analysis through microscopic techniques, SEM and Cryo-SEM, was carried out, complemented with data obtained by DSC, XRD analysis and spectrophotometric determinations of the amylose and amylopectin released from the granules.

## 2. Materials and methods

### 2.1. Preparation of samples and HHP treatments

Initially, aqueous maize starch suspensions (Roquette, Laisa España, S.A.) were submitted to heating treatments, either at 60 or 75 °C, as controls of partially ( $S_{h60}$ ) and fully gelatinized starch ( $S_{h75}$ ), respectively.

HHP treated starch samples were prepared by suspending 10, 20 or 40 g of maize starch in the corresponding amount of deionized water (Milli-Q, Millipore Inc., Bedford, MA, USA) in order to obtain 10, 20 and 40% w/w suspensions. HHP treatment was performed employing a pilot-scale unit (ACB GEC ALSTHON, Nantes, France), operated at 400 MPa (Fernández et al., 2006), at different combinations of time and temperature. Starch suspensions were enclosed in polypropylene cylindrical containers (75 mm diameter, 35 mm high, approx. volume 125 mL) tightly covered with a polypropylene flexible lid that allowed compression without compromising hermeticity. These containers were manually shaken before pressurization but, in the few minutes required for introducing the containers into the HHP equipment (keeping their cylindrical axis vertical), closing it and rising the setting pressure, starch granules sedimented to the bottom of the container.

The effect of hydrocolloids incorporation on HHP-treated starch,  $Sp4$ , (400 MPa, 35 min at 40 °C) was explored.  $\lambda$ -carrageenan (Sigma-Aldrich, Saint Louis, USA), guar (Sigma-Aldrich, Saint Louis, USA) and xanthan gum (Sigma-Aldrich, Saint Louis, USA) were added at 0.3% (w/w) to the starch suspension before the HHP treatment.

To achieve a fully HHP gelatinized starch, a UNIPRESS U-111 device (High Pressure Research Center, Polish Academy of Sciences, Unipress Equipment Division, Warsaw, Poland) was used to submit maize starch to higher pressure levels. Details can be obtained in Fernández et al. (2007). The applied conditions were 10% (w/w) starch suspensions, treated to 700 MPa for 25 min, at 35 °C ( $Sp700$ ).

Notation codes for all samples are shown in Table 1.

### 2.2. Microscopy observations

#### 2.2.1. Scanning electronic microscopy (SEM)

Starch granule microstructure was observed by SEM. A ZEISS DSN-960 microscope (Oxford, UK) was used. Dry granules were attached to copper stubs using double sided adhesive tape, then coated with a layer of gold (40–50 nm) in a vacuum evaporator and examined using an acceleration voltage of 20 kV.

**Table 1 – Nomenclature of HHP treated samples: maize starch concentration (SC) and HHP conditions (time and temperature).**

Sample id	Pressure (MPa)	SC (%w/w)	Time (min)	Temperature (°C)	Type of gums			
Sp1	400	10	15	30	-			
Sp2			15	40				
Sp3			35	30				
Sp4			35	40				
Sp4C			35	40		40	Carrageenan	
Sp4G							Guar	
Sp4X							Xanthan	
Sp5			700	20		60	30	-
Sp6				40				
Sp7				10				
Sp8	25	35						
Sp700	700		25	35				

### 2.2.2. Low-temperature scanning electron microscopy (Cryo-SEM)

Observation was carried out with a Zeiss DSN-960 scanning microscope equipped with a Cryotrans CT-1500 cold plate (Oxford, UK). Cryo-SEM allows sample observation without chemical fixing or drying. The procedure consists of sample freezing by immersion in liquid nitrogen, breaking and etching. Sample holders were fitted into a special bracket on the microscope and placed in the pre-chamber cold plate (−180 °C), where samples were broken to obtain a suitable observable surface. Samples were then inserted in the microscope and etching was performed for 3 min at −90 °C. After etching, samples were gold coated and observed at −150/−160 °C under secondary and backscattering electron modes. A large number of granules corresponding to different regions of the sample on the stage were observed before representative electron micrographs were taken.

### 2.2.3. Light microscopy

Observations of starch suspensions were carried out with an Olympus BX41 microscope (Olympus, Tokyo, Japan), using transmitted light, equipped with an Olympus DP70 microscope camera (Olympus, Tokyo, Japan). Crossed Polaroid filters were employed, to investigate the gelatinization of starch granules. Granules stained after addition of a few droplets of a 0.04% I<sub>2</sub> solution were also observed.

### 2.3. Determination of amylose and amylopectin released from granules

Amylose (AM) and amylopectin (AP) concentrations were quantified in the supernatant of HHP treated samples by the spectrophotometric iodine technique (Landers et al., 1991). This method allows the simultaneous determination of amylose and amylopectin concentrations, assuming that both amylose-I<sub>2</sub> and amylopectin-I<sub>2</sub> complexes contribute additively to the total absorbance of the mixture. The absorbances of the complexes were measured according to García et al. (1995) with slight modifications. Briefly, 2–4 mL of supernatants were placed in 50 mL flasks with 0.3 mL of I<sub>2</sub>/I<sup>-</sup> solution, made up to volume and allowed to complex. Absorbances were measured at 535 and 615 nm in a LAMBDA 650 Series spectrophotometer (PerkinElmer, USA). A blank

solution without sample was also measured. For simultaneous determination of amylose and amylopectin, the following equations were applied:

$$A_{615} = \varepsilon_{615}^{AM} \times b \times CAM + \varepsilon_{615}^{AP} \times b \times CAP \quad (1)$$

$$A_{535} = \varepsilon_{535}^{AM} \times b \times CAM + \varepsilon_{535}^{AP} \times b \times CAP \quad (2)$$

where A<sub>615</sub> and A<sub>535</sub> are sample absorbances at 615 and 535 nm respectively,  $\varepsilon_{615}^{AM}$  and  $\varepsilon_{615}^{AP}$  are extinction coefficients of amylose and amylopectin iodine complexes at 615 nm (33,200 and 5900 mL g cm<sup>-1</sup>, respectively),  $\varepsilon_{535}^{AM}$  and  $\varepsilon_{535}^{AP}$  are extinction coefficients of amylose and amylopectin iodine complexes at 535 nm (17,900 and 7100 mL g cm<sup>-1</sup>, respectively), *b* is the path length (cm) and CAM and CAP are the concentrations of amylose and amylopectin in the solution (g/mL), respectively.

### 2.4. Differential Scanning Calorimetry (DSC) conditions

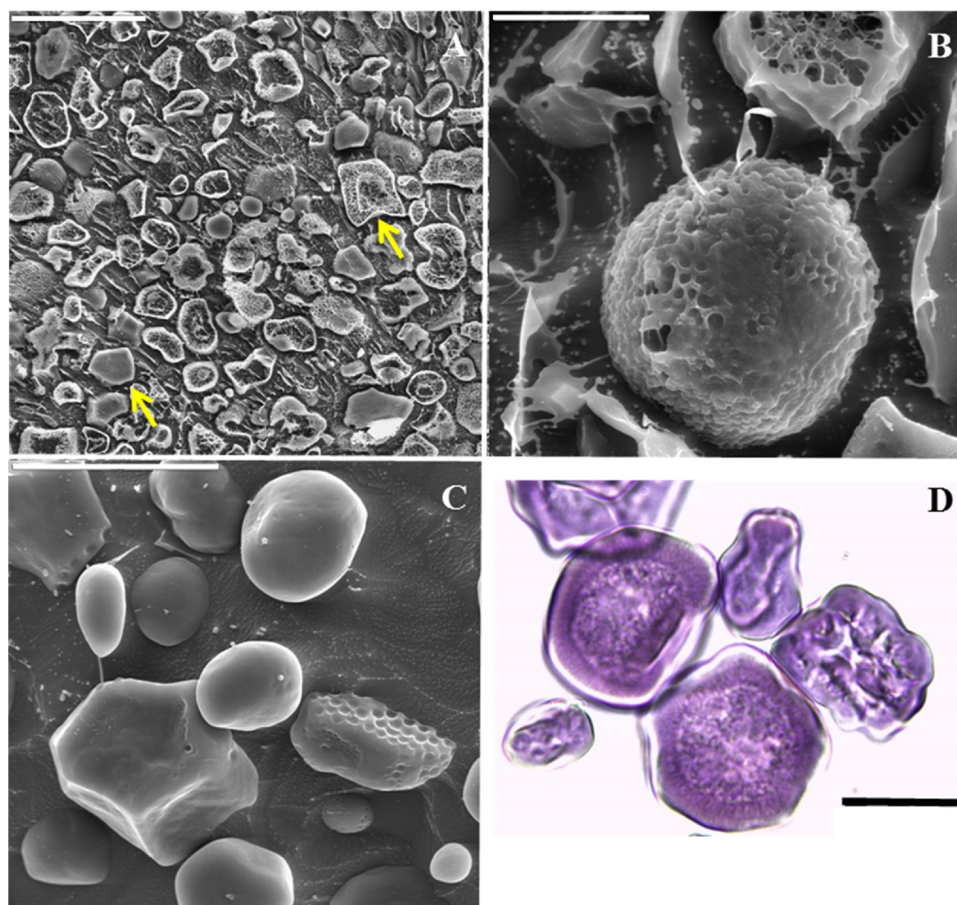
The degree of gelatinization of the samples was determined using a DSC Q1000 (TA Instruments, New Castle, DE, USA). Hermetic aluminum pans (TA) (to avoid water evaporation) were used in all DSC experiments and an empty pan was employed as reference. A scan rate of 10 °C/min (between 10 and 100 °C) was employed. The samples weighted around 10 mg. Data were analyzed using the specific analysis program TA Universal Analysis 2000 (TA, Twin Lakes, WI, United States) that yields the desired process thermal parameters (T<sub>o</sub>: onset temperature; T<sub>p</sub>: peak temperature (starch gelatinization temperature); ΔH<sub>G</sub>: enthalpy on dry basis).

The Range of Gelatinization (RG) was estimated through the difference between T<sub>p</sub>-T<sub>o</sub>, and the gelatinization degree (GD) was calculated according to Błaszczak et al. (2007):

$$GD(\%) = \left\{ (\Delta H_{ns} - \Delta H_{ts}) * \Delta H_{ns}^{-1} \right\} \times 100 \quad (3)$$

where ΔH<sub>ns</sub> and ΔH<sub>ts</sub> are the melting enthalpies of native and pressure-treated starches, respectively.

After DSC runs, the pans were punctured and dried until constant weight at 105 °C to obtain the dry weight of the sample. Calorimetric data were collected from at least two replicates per treatment.



**Fig. 1** – Cryo-SEM micrographs of (A) and (B) maize starch partially gelatinized at 75 °C ( $S_h75$ ), (C) maize starch partially gelatinized at 60 °C ( $S_h60$ ) and (D) Optical micrograph of maize starch treated at 400 MPa, 15 min at 40 °C ( $S_p2$ ). Nomenclature is referred in [Table 1](#). Bar = (A) 50  $\mu\text{m}$ , (B) 10  $\mu\text{m}$ , (C) and (D) 20  $\mu\text{m}$ .

## 2.5. X-ray diffraction

X-ray diffraction (XRD) patterns of the starch samples were analyzed between  $2\theta=4^\circ$  to  $2\theta=60^\circ$ , with a step size of  $2\theta=0.02^\circ$ , in the X-ray diffractometer X'Pert Pro PANalytical Model PW3040/60 (Almelo, The Netherlands) using a  $\text{CuK}\alpha$  radiation ( $\lambda=1.543 \text{ \AA}$ ), 40 kV and 40 mA. Starch diffractograms are characterized by sharp peaks associated with the crystalline diffraction peaks and an amorphous zone (background). The amorphous fraction of the sample can be estimated by the area between the smooth curve drawn following the scattering hump, and the baseline joining the background within the low and high-angle points. The crystalline fraction (CF) was estimated by the upper region above the smooth curve ([Mali et al., 2006](#); [Snyder, 1992](#); [Teixeira et al., 2015](#)) as follows:

$$\text{CF} (\%) = \frac{\text{crystalline area} \times 100}{\text{total area}} \quad (4)$$

## 2.6. Statistical analysis

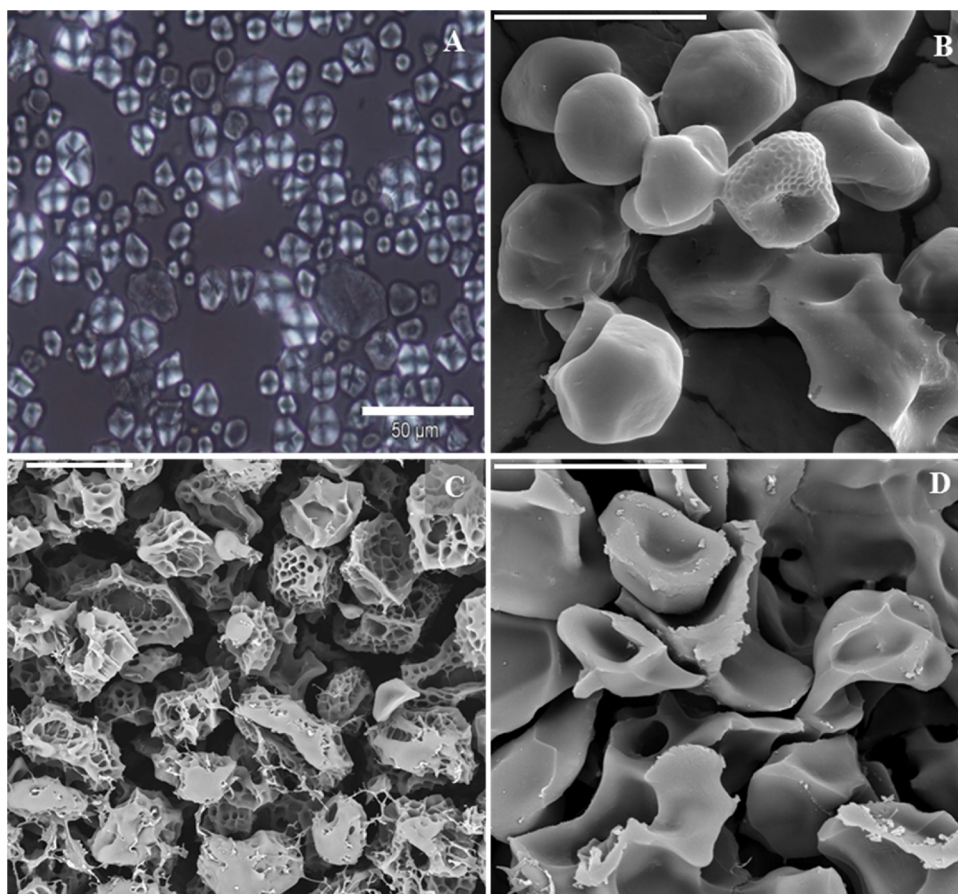
Analysis of variance (ANOVA) and mean comparisons were performed with the software SYSTAT INC. (Evanston, USA). Unless indicated, a level of 95% confidence ( $\alpha=0.05$ ) was used.

## 3. Results and discussion

### 3.1. Effect of temperature or HHP on gelatinization of starch granules

#### 3.1.1. Comparison between thermal gelatinization and HHP treatment

[Fig. 1](#) shows HHP treated starch granule images, obtained by different microscopic techniques. The comparison of the images obtained at the initial stages of the gelatinization process, either thermally induced or by HHP treatments, suggests different gelatinization mechanisms. In this way, [Fig. 1A](#), shows (by cryo-SEM) a granule ensemble where some of the individual granules can be appreciated as completely gelatinized (see upper arrow), meanwhile, in others, the process is less advanced, appearing nearly intact (see lower arrow). [Fig. 1B](#) focuses on a single granule, showing a complete gelatinization, characterized by amylose released by the granule, which has created a network that gives rise to porous and broken granules. Both images were cryo-SEM pictures of starch thermally gelatinized at 75 °C ( $S_h75$ ). The effect of thermal treatment at lower temperatures (60 °C) was also observed by cryo-SEM ([Fig. 1C](#),  $S_h60$ ). This temperature is too low for complete maize starch gelatinization, and it can be appreciated that, while those granules of smaller size appear gelatinized, larger ones do not. In this sense, [Ratnayake and Jackson \(2006\)](#) studied the gelatinization and solubility of corn starch during heating in excess water and stated that complete granular



**Fig. 2 – (A) Polarized light micrograph of maize starch treated at 400 MPa, 15 min at 30 °C (Sp1), (B) SEM micrographs of maize starch treated at 400 MPa, 35 min at 30 °C (Sp3), (C) and (D) SEM micrographs of maize starch treated at 700 MPa, 25 min at 35 °C (Sp700). Nomenclature is referred in Table 1. Bar = (A), (C) and (D) 20 μm and (B) 50 μm.**

disruption and the formation of a gelatinized solution did not occur below 70 °C.

In Fig. 1D (a light microscopy picture of I<sub>2</sub> solution-dyed starch treated at 400 MPa, during 15 min at 40 °C, Sp2), the advance of the gelatinization process can be followed as it starts from the interior of the granule, where a higher disorder degree is shown, while an external “crown” appears to keep the native non-gelatinized granular ordering. This observation is in agreement with the fact that HHP gelatinization products exhibit differences from the thermally treated ones, such as a smaller degree of amylose released from the granules, a reduced granule swelling and starch granules appearing intact or just partially disintegrated (Stute et al., 1996; Knorr et al., 2006).

### 3.1.2. Pressure and temperature effect on starch gelatinization

The gelatinization degrees achieved by maize starch suspensions treated at 400 and 700 MPa were compared. Process duration and temperature were taken into consideration, which is shown in Fig. 2A, corresponding to a 15 min treatment at 30 °C and 400 MPa (Sp1), using polarized light microscopy, where most granules can be seen as nongelatinized, while a few have actually completed the gelatinization process and others are still in different intermediate stages. Besides, Fig. 2B corresponding to a 35 min treatment at 30 °C and 400 MPa (Sp3), shows partial gelatinization while granular identity is preserved. Oh et al. (2008) found similar results for normal

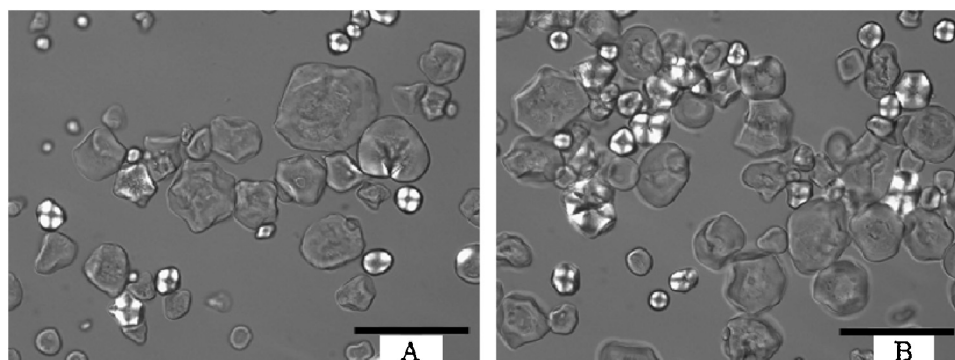
corn starch, showing that some granules lost birefringence after treatment at 400 MPa, for 30 min at 20 °C.

Fig. 2C and D, corresponding to a sample treated for 25 min at 35 °C and 700 MPa (Sp700), clearly shows in its right part how this drastic treatment causes damage in the granules, how their surfaces are eroded and how the granule integrity is lost. The built up of a gel network was also appreciated and it was associated to the gelatinization process.

### 3.1.3. Effect of hydrocolloid addition on HHP gelatinization of maize starch

Maize starch was also pressure treated (400 MPa, 35 min and 40 °C) after addition of hydrocolloids. Optical microscopic observations were performed, using transmitted and polarized light, on samples obtained from the surface immediately after treatment (Fig. 3A) and the bottom (Fig. 3B) of the container. At the top of the vessel, more gelatinized starch granules can be observed, some of them, partially gelatinized, shown by the Maltese cross fading away from the center to the edge of the granules. At the bottom of the container, the described effects were less intense, indicating the presence of a higher proportion of gelatinized granules at the surface.

A possible function of the presence of hydrocolloids in the suspension during HHP processing could be ensuring water availability. Ferrero et al. (1996) showed that even at a gum concentration of 1%, the availability of water was not sufficiently reduced by the presence of hydrocolloids to induce a second starch transition peak in the DSC curves (characteristic of starch gelatinization at low water content). In the



**Fig. 3** – Optical micrographs using transmitted and polarized light in the sample Sp4G. (A) At the surface and (B) at the bottom, respectively. Nomenclature is referred in Table 1. Bars = 50 µm.

present work, where 0.3% hydrocolloids were used, the availability of water for starch gelatinization was guaranteed and consequently, a single DSC transition peak was observed. Fully gelatinized starch granules (Fig. 3A) were more frequent in samples taken from the surface of the container, than in those taken from the base, apparently responding to water availability in these two zones. However, as an alternative explanation, this could be due to a possibly lower density of gelatinized starch over native granules. There is no explicit data available about the density of these partially gelatinized maize starch granules, but observations on wheat starch indicate that the density of the granule decreases from approx.  $1.5 \text{ g cm}^{-3}$ – $1.3 \text{ g cm}^{-3}$  upon hydration and incipient swelling (Dengate et al., 1978). Although the buoyancy of partially gelatinized granules, or rather their ability to keep in suspension for a longer time, will not only depend on their density, the counter-sedimenting effect of a lower density would be helped by a more extensive interaction with the network of gelatinized granules, with their mane of protruding amylose chains. Thus, the observed difference in the ratio of gelatinized/native granules could be a result of their sedimentation behavior, rather than reflecting a persisting gelatinization degree distribution resulting from a preferential gelatinization at the top of the 35 mm high container.

### 3.1.4. Differential Scanning Calorimetry (DSC) studies of the gelatinization process

The gelatinization extent on HHP treated samples was measured by DSC (Table 2). Melting enthalpies ( $\Delta H$ ) of native and pressure-treated starches were calculated to analyze the effect of the HHP treatment conditions on the gelatinization degree (GD). The  $\Delta H$  parameter represents the starch fraction that gelatinizes during DSC experiments; lower enthalpy values would be obtained if the starch granules were previously gelatinized during the HHP treatment, which means that higher GD values would be obtained accordingly.

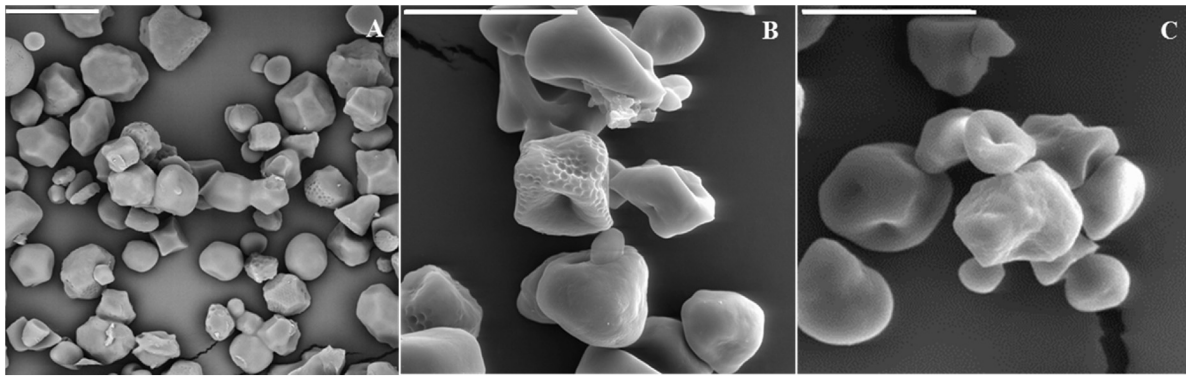
It must be remembered that the situation of a flat cylindrical container where starch granules sediment under a layer of clearer water is common to the usual experimental DSC procedures. DSC pans are smaller but have a similar dimensional ratio and, no matter how samples were previously stirred, they would settle down in the DSC oven well before gelatinization onset, which will take place, again, with most starch granules close to the container bottom.

Other reported data (Błaszczak et al., 2005; Oh et al., 2008) and the results presented in the present work when studying the effect of concentration, are consistent with the water amount available at the container top being enough for granting complete gelatinization. The only difference with the

**Table 2** – Thermal parameters obtained from DSC thermograms for HHP treated starches with and without gums addition. Nomenclature is referred to that in Table 1.

Sample	To	Tp	$\Delta HG$	GD (%)	RG
	(°C)	(°C)	(J/g)		
Maize starch	64.37 <sup>bc</sup> ± 0.41	69.88 <sup>d</sup> ± 0.45	11.06 <sup>e</sup> ± 0.48	0 <sup>a</sup>	4.59 <sup>b</sup> ± 0.79
Sp1	64.52 <sup>b</sup> ± 0.75	68.75 <sup>bc</sup> ± 0.88	9.14 <sup>d</sup> ± 1.38	17.35 <sup>b</sup> ± 3.71	4.22 <sup>a</sup> ± 0.43
Sp3	64.84 <sup>bc</sup> ± 1.06	68.82 <sup>cd</sup> ± 0.82	8.30 <sup>cd</sup> ± 0.36	24.95 <sup>bc</sup> ± 3.28	4.02 <sup>ab</sup> ± 0.12
Sp7	64.82 <sup>bc</sup> ± 0.20	68.10 <sup>b</sup> ± 0.19	7.67 <sup>bc</sup> ± 0.47	33.90 <sup>d</sup> ± 4.29	3.27 <sup>a</sup> ± 0.27
Sp8	63.98 <sup>b</sup> ± 0.36	67.67 <sup>b</sup> ± 0.17	6.20 <sup>ab</sup> ± 0.89	43.94 <sup>e</sup> ± 8.05	3.85 <sup>ab</sup> ± 0.19
Sp700	54.18 <sup>a</sup> ± 1.42	60.35 <sup>a</sup> ± 0.36	4.23 <sup>a</sup> ± 0.09	61.75 <sup>f</sup> ± 0.89	6.16 <sup>c</sup> ± 2.08
Sp6	65.37 <sup>bc</sup> ± 0.36	69.27 <sup>cd</sup> ± 0.03	7.85 <sup>bc</sup> ± 0.43	28.99 <sup>cd</sup> ± 3.90	3.89 <sup>ab</sup> ± 0.33
Sp4	65.43 <sup>c</sup> ± 0.64	68.98 <sup>cd</sup> ± 0.54	8.47 <sup>cd</sup> ± 0.37	23.41 <sup>cd</sup> ± 2.19	3.55 <sup>a</sup> ± 0.35
Sp4C	64.80 <sup>bc</sup> ± 0.24	68.47 <sup>bc</sup> ± 0.37	8.52 <sup>cd</sup> ± 0.46	22.98 <sup>bc</sup> ± 4.20	3.58 <sup>a</sup> ± 0.25
Sp4G	64.59 <sup>bc</sup> ± 0.04	67.77 <sup>b</sup> ± 0.83	8.77 <sup>cd</sup> ± 0.39	20.71 <sup>bc</sup> ± 3.58	3.18 <sup>a</sup> ± 0.88
Sp4X	64.48 <sup>bc</sup> ± 1.31	67.93 <sup>b</sup> ± 0.93	9.23 <sup>d</sup> ± 0.51	16.53 <sup>b</sup> ± 2.94	3.30 <sup>a</sup> ± 0.27

To: onset temperature; Tp: peak temperature (starch gelatinization temperature);  $\Delta HG$ : Enthalpy in dry basis; GD: gelatinization degree; RG: Range of Gelatinization: Tp – To. Different letters within the same column indicate statistical differences ( $p < 0.05$ ) between samples.



**Fig. 4 – SEM micrographs of maize starch treated at 400 MPa and 30 °C, (A) 15 min (Sp1), (B) 35 min (Sp3) and (C) 120 min (Sp8), respectively. Nomenclature is referred in Table 1. Bars = 20 μm.**

scenario of pressure gelatinization would be a higher temperature (approximately 30 °C higher) in the DSC, which would increase granular mobility and water diffusion rate, though not drastically. However, this might have a promoting effect on the complex starch gelatinization phenomenon.

Water content is an important factor when studying starch gelatinization (Stute et al., 1996; Bauer and Knorr, 2005; Kawai et al., 2007a). Le Bail et al. (1999) explained that the term “gelatinization” is more often used for starch processing in excess water (60–70% wet basis), when the process parameters become independent of the water content, whereas “melting” designates intermediate and low water contents. In our study, the effect of starch concentration on the obtained gelatinization degree, in samples treated at 400 MPa at 40 °C, was not significant ( $p > 0.05$ ) as it can be observed in Table 2 for samples with 10% of starch (Sp4) vs. samples with 40% starch (Sp6). Stute et al. (1996) found similar results for pressurized wheat starch (600 MPa, 15 min, 20 °C), in relation to their water content. However, in the case of potato starch, Kawai et al. (2007a) found that the gelatinization degree decreased with starch content (10–70%) in the range of 700–1200 MPa at 40 °C.

Results from DSC thermograms shown in Table 2 did match the observations extracted from micrographs shown in Fig. 4A (Sp1), where the degree of starch gelatinization was low after a treatment of 15 min at 400 MPa at 30 °C. Increasing time to 35 min (Fig. 4B, Sp3) did not show differences with the 15 min treatment, as presented in the gelatinization degree column of Table 2. However, increasing pressurization time to 120 min, at the same temperature, caused a noticeable increase of gelatinization degree, as appreciated in Fig. 4C (Sp8) and Table 2. Buckow et al. (2007) found that the degree of gelatinization continuously increased with exposure time at different pressures and temperature combinations, for suspensions of maize starch in distilled water (5% w/w).

With respect to the effect of temperature (Sp3 at 30 °C vs. Sp4 at 40 °C), a difference of 10 °C in pressurization treatment temperature did not significantly increase ( $p > 0.05$ ) the gelatinization degree (Table 2), which means that temperature was not an important factor under the assayed conditions.

Based on the intermediate gelatinization degree obtained, the condition of 10% starch concentration, 35 min and 40 °C for HHP treatment (Sp4), was selected to study the effect of gum addition. DSC results for samples with the addition of different hydrocolloids are also shown in Table 2. Samples with  $\lambda$ -carrageenan and guar gum showed similar gelatinization degrees (20–23%) to Sp4, which were samples in the same conditions but without gum addition. However, the observed

gelatinization degree (GD) was significantly lower in the case of Sp4X (with added xanthan gum) ( $p < 0.05$ ), denoting a possible stabilization of the granular structure by this hydrocolloid during starch gelatinization (Biliaderis et al., 1997). In this sense, Díaz-Calderón et al. (2018) working on wheat, maize and waxy maize starch stressed that gelatinization enthalpy was reduced by the addition of bacterial cellulose fibrils, which is in agreement with the results obtained in the present work.

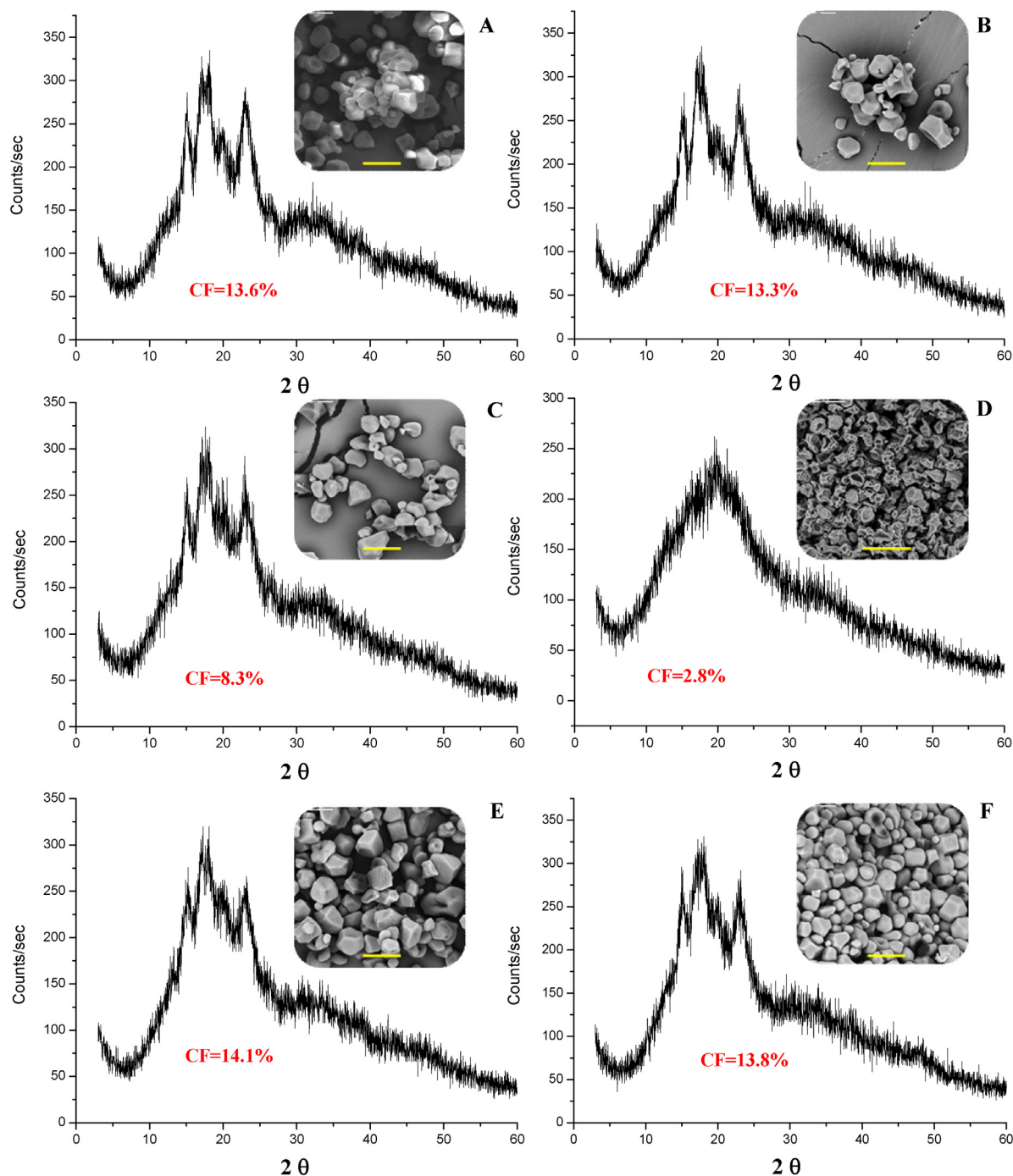
An almost complete gelatinization was found for the sample treated at higher pressure of 700 MPa (Sp700) showing a 62% of GD; gelatinization temperature was nearly 10 °C lower than that of native maize starch (Table 2). Stute et al. (1996) also found similar results when applying a 500 MPa treatment, of 25 min at 20 °C.

HHP application (400 MPa) provoked a slight decrease in gelatinization temperature ( $T_p$ ), with respect to native starch; hydrocolloid addition did not introduce significant differences ( $p > 0.05$ ). Similar results were obtained by Tester and Sommerville (2003) when working with native maize starch with water ratios of 1:5 and 0.5% of guar, carrageenan or xanthan. Only the sample treated at 700 MPa (Sp700) exhibited a gelatinization temperature 10 °C lower, which was significantly different from all samples ( $p < 0.05$ ).

### 3.1.5. X-ray diffraction studies

Much of the information available in literature about starch granule crystalline properties has been acquired from X-ray powder diffraction studies. According to several studies, starch can be classified in A, B and C forms (Cheetham and Tao, 1998; Hibi et al., 1993; Le Bail et al., 1999; Zobel, 1988). In the native granular forms, the A pattern is associated mainly with cereal starches, while the B form is usually obtained from tuber starches. The C pattern is a mixture of both A and B types, but also occurs naturally, e.g. in smooth-seeded pea starch and various bean starches (Cheetham and Tao, 1998) as well as in those derived from unripe fruits.

Literature shows that HHP treatment converts starches displaying A-type pattern into B-type-like starches, whereas B-type starches keep their original pattern (Hibi et al., 1993; Katopo et al., 2002; Yang et al., 2013). Katopo et al. (2002) attributed the difference between A- and B-type starches to their amylopectin structures. They suggested that, for B-type starches, water fills up the channel in the cell unit of the crystallite, stabilizing the structure. In contrast, A-type crystallite has a more scattered amylopectin branching structure, which is more flexible, and therefore allows double helices rearrangement to generate a channel in which water molecules



**Fig. 5 – X-ray diffraction pattern of (A) Sp1; (B) Sp3; (C) Sp8; (D) Sp700; (E) Sp5 and (F) Sp6. Nomenclature is referred in Table 1. Crystalline fraction (CF) values are included for each sample. SEM micrograph bars = 20  $\mu\text{m}$ .**

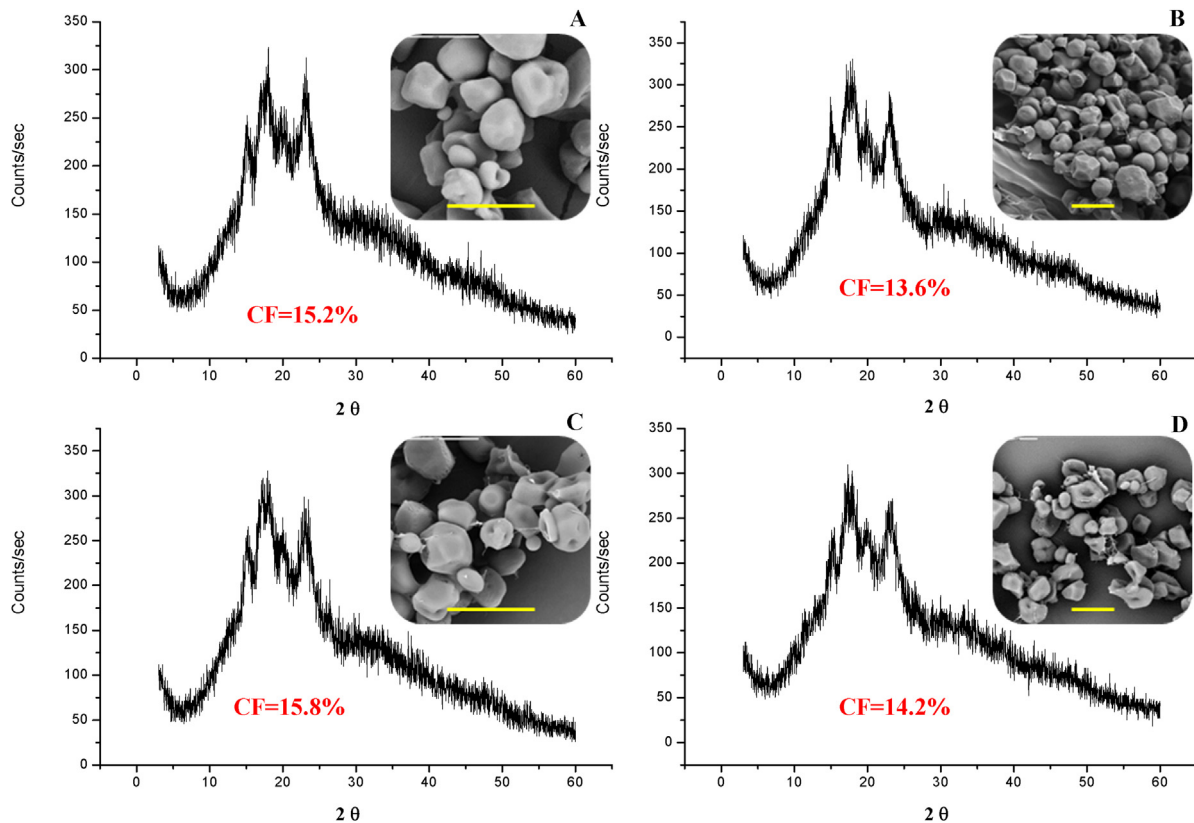
are included under pressure. So, the B-type structure is more favored by HHP because of the higher number of associated water molecules stabilizing the helix structure (Yang et al., 2017).

Native maize starch showed a typical A-type XRD pattern, with characteristic peaks at  $2\theta = 5.2, 5.8, 15.3, 17.1, 18.2$  and  $23.5$  (Teixeira et al., 2015). In HHP treated samples, results obtained in the present work evidenced that the pattern assignment was complex, since characteristic peaks from A-type and B-type patterns, with typical peaks at  $14.4, 17.2$  and  $22.2$ , coexist. It is known that whereas A-type pattern is the form thermodynamically more stable, B is the kinetically favored one. Fig. 5 shows diffractograms of starch samples at 10% w/v treated at 400 and 700 MPa and their respective crystalline fraction (CF), calculated as indicated in Section 2.5.

Taking into account that native maize starch exhibited a CF close to 24% (Teixeira et al., 2015), the treatment at 400 MPa, at a temperature of  $30^\circ\text{C}$  for 15 and 35 min (Sp1 and Sp3, respectively) preserved the crystallinity of native starch in a 54.2%, since Sp1 and Sp3 exhibited CF values of 13.6% and 13.3%, respectively. In contrast, the sample treated at 700 MPa (Sp700) with a CF of 2.8%, only retained 11.7% of the native granule crystallinity.

The diffractogram of maize starch treated at 700 MPa shows a considerably reduced crystalline area, indicating an advanced degree of starch granule gelatinization during HHP treatment (Fig. 5D). This statement was verified by DSC measurements, where GD was close to 62% (Table 2). Similar results were obtained by Katopo et al. (2002) when working with starches from different sources at 690 MPa for 5 min, indicating





**Fig. 6 – X-ray diffraction pattern of (A) Sp4; (B) Sp4C; (C) Sp4X and (D) Sp4G. Nomenclature is referred in Table 1. Crystalline fraction (CF) values are included for each sample. SEM micrograph bars = 20  $\mu\text{m}$ .**

a total loss of the native crystalline structure and molecular order.

Therefore, HHP treatments at 700 MPa would lead to granular structure irreversible alterations, with loss of its integrity, while 400 MPa treatments would cause a partial gelatinization, maintaining granular integrity.

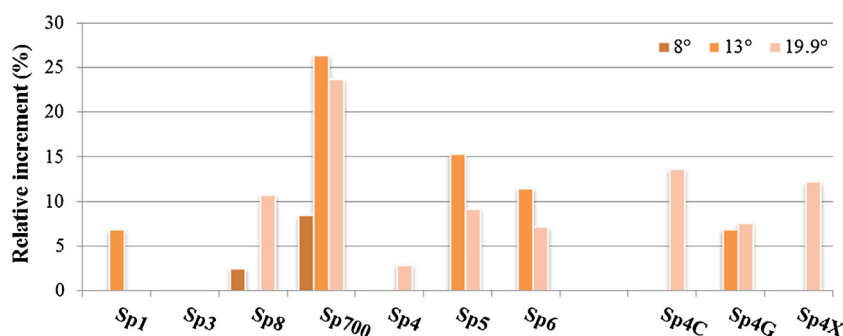
The crystallization degree decreased with increasing treatment time (15, 35 and 120 min) (Fig. 5A–C). Accordingly, the remaining crystalline fraction, showed an increasing contribution of B-type crystallite for longer times. A similar trend was found when pressure was increased from 400 MPa to 700 MPa, being the B-type crystallite increase more drastic in the latter case. These results agree with the GD obtained from DSC (Table 2). Similar results, corresponding to a mixture of A and B-type patterns after HHP treatment, were reported by Choi et al. (2009), who suggested that B-type crystallites may be produced by recrystallization immediately after pressure induced-gelatinization.

X-ray diffractograms of samples with different starch concentration (10, 20 or 40%, Sp4, Sp5 and Sp6, respectively), subjected to 400 MPa, did not show significant modifications in peak positions and intensities. Moreover, the crystal fraction was not affected by starch concentration, in agreement with DSC results (Table 2).

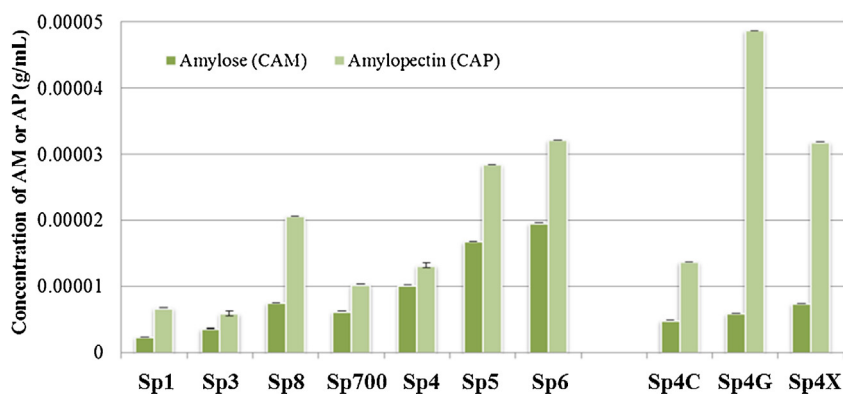
Fig. 6 shows the effect of hydrocolloid addition on X-ray diffractograms of maize starch samples treated at 400 MPa (Sp4). Typical peaks positions and intensities of A and B patterns also coexist in these samples (which is referred to as C-pattern). Samples containing guar,  $\lambda$ -carrageenan and xanthan hydrocolloids showed similar crystalline fraction than the control without hydrocolloids (Sp4) ( $p > 0.05$ ) (Fig. 6).

V-diffraction pattern is a result of amylose being complexed with substances such as aliphatic fatty acids, emulsifiers, linear alcohols of up to 8 carbon atoms or iodine (Cheetham and Tao, 1998; Shi et al., 2017) and it can appear after starch granule gelatinization (Gallant et al., 1992). Wang et al. (2008), while studying high pressure treated maize starch (9 min at 650 MPa), observed that samples with higher amylose contents still retained their granule structure, although they suffered a decrease in crystallinity after HHP treatment. They attributed this behavior to the ability of amylose to form complexes with lipids present in maize starch granules, stabilizing the granular structure and restricting its swelling. Katopo et al. (2002) also suggested that amylose–fatty acid complexes could be formed in starches under HHP, and that these complexes, in addition to a high amylose content, may restrict swelling and gelatinization.

According to Shamaï et al. (2003) and Yoon and Lee (1998), peaks located at  $2\theta = 8, 13$  and  $19.9^\circ$  are characteristic of the V-type polymorph. Fig. 7 shows the effect of HHP treatment on the relative increase of intensity of peaks located at  $2\theta = 8, 13$  and  $19.9^\circ$  with respect to native maize starch. The relative intensity of these peaks increased with treatment time (as can be observed in samples treated for 120 min, Sp8) and with the increment of starch concentration (20 and 40% of starch, Sp5 and Sp6, respectively). A special behavior was observed for the extreme condition of sample Sp700 (700 MPa, 25 min,  $30^\circ\text{C}$ ), where the V-pattern characteristic peak at  $19.9^\circ$  showed remarkable intensity (Fig. 5D). This observation was related to the lower degree of amylose released from granules, determined by the spectrophotometric iodine technique in sample supernatants (Fig. 8); in this case, it can be assumed that a higher concentration of amylose forms part of the



**Fig. 7** – Effect of HHP treatment on the relative increment of diffraction peaks located at  $2\theta = 8, 13$  and  $19.9^\circ$  with respect to native maize starch. Nomenclature is referred in [Table 1](#).



**Fig. 8** – Effect of HHP treatment on the amylose and amylopectine concentration in sample supernatants. Nomenclature is referred in [Table 1](#).

complex, decreasing free amylose concentration in the supernatant. Besides, as observed by DSC, this sample (Sp700) was almost totally gelatinized under high pressure, which may contribute to a lower amylose leaching. Le Bail *et al.* (1999) found similar results when studying the crystallization of amylose–lipid complexes in samples of maize starch with different water contents by DSC and X-ray diffraction. They found that crystallinity of the amylose–lipid complexes increased with temperature, as revealed by peak intensity and sharpness, which was attributed to the crystallization of the complexes upon heating.

All samples with hydrocolloids showed higher intensities of the peak of V-type polymorph situated at  $2\theta = 19.9$ , in comparison with the control without gum (Sp4). These results correlated with the lower amylose concentration in the supernatants shown in [Fig. 8](#), compared to that of the control samples. Thus the formation of the V-crystalline complex would be favored by hydrocolloid presence under pressure. In these cases, hydrocolloids, which are essentially linear polysaccharides, could contribute to the lixiviated amylose stabilization, leading to lower amylose contents in the supernatants of HHP treated samples ([Fig. 8](#)).

#### 4. Conclusions

Both, morphologic observations, mainly SEM and Cryo-SEM and microstructural studies performed by DSC and X-ray diffraction analysis, allowed a detailed understanding of high pressure induced gelatinization of maize starch granule. The degree of starch gelatinization measured by DSC increased accordingly with the intensity (pressure level and exposure time) of the HHP treatment.

The presence of hydrocolloids in the suspension during HHP processing would ensure water availability. None of the

hydrocolloids employed altered the starch gelatinization temperature in DSC thermograms. At 400 MPa the gelatinization degree was significantly lower in starch with added xanthan gum (XG) in comparison to the other hydrocolloids, denoting a possible stabilization effect of XG on the starch granular structure during gelatinization. An almost complete gelatinization was observed for the sample treated at a pressure of 700 MPa both by DSC and XRD. HHP treated samples, evidenced that characteristic peaks from A-type and B-type patterns coexist, which is indicative of the partial gelatinization that occurs during HHP treatment.

V-diffraction pattern ascribed to the formation of amylose complexes was detected by XRD in samples submitted to very high pressure (700 MPa) and long treatment times at 400 MPa. Samples with hydrocolloids showed higher intensities of the peak corresponding to the V-type polymorph situated at  $2\theta = 19.9$ , in comparison to the control without hydrocolloid addition. These results agreed with the lower concentrations of amylose released, and then the formation of V-crystalline complex would be favored by hydrocolloids presence under pressure.

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