RAPID ASSESSMENT OF STARCH PASTING USING A RAPID FORCE ANALYZER

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Running tittle: Rapid force analysis of starch gels

Abstract

1) Background and objectives: The falling number is a rapid method developed for assessing amylase level on flours, based on viscosity changes. This measurement records the time required for a stirring rod to travel through a flour paste. The Chopin Amylab FN, developed for assessing the FN, allows working as a Rapid Force Analyzer (RFA), recording the force changes of a starch/flour slurry under controlled mixing/heating conditions. The objective of this research was to underline the starch changes occurring along 90 s under continuous mixing and heating.
2) Findings: Four different starches (corn, rice, wheat and potato) were analyzed using the RFA, evaluating step-by-step the structural and textural modifications. Scanning electron micrographs revealed the progressive gelatinization process, that was specific for each type of starch. Nevertheless, the 90 s procedure was sufficient to ensure complete gelatinization of all starches. Parameters recorded from the RFA showed strong significant correlations with onset and peak gelatinization temperature, besides gelatinization enthalpy.
3) Conclusion: RFA could be used as a rapid method for starch pasting assessment, being valid for discriminating among different types of starches.
4) Significance and novelty: Study shows the potential of Amylab as RFA that records starches pasting performance in 90 s.

A test of 90 seconds allows determining starch paste performance using a rapid force analyzer.

Keywords: starch; gel; rapid force analyzer; microstructure; texture; Amylab

Introduction

Starches are common ingredients for numerous industrial food applications, which explains why their quality control have been the objective of much researches. Gelatinization is a primary physicochemical property of the starches, because of that viscosity measurements are widely applied for assessing the starch quality, particularly consisting of heating-cooling cycles to follow gelatinization and gelling phenomena (reviewed by Ai and Jane 2015). Different systems (Brabender amylograph and viscograph, Rapid Viscoanalyzer) have been developed for assessing the starch viscosity. The majority of them are empirical methods based on recording the relative or apparent viscosity, derived from a torque, of a starch paste subjected to thermal and mechanical constraints (Suh and Jane 2003). Those systems record the starch gelatinization in an excess of water, going from granular state to pastes, because of that it is widely referred as pasting performance. Differences on those systems rely on the amount of sample (2-80 g), the rotational speed, temperature gradient and so on. As consequence, differences in the pasting parameters obtained from each instrument have been reported, which have been attributed to differences in the spindle structure (Suh and Jane 2003). One of the most successful devices for assessing apparent viscosity of starches has been the rapid viscoanalyzer (RVA) designed back in 1987 (Walker et al. 1988), owing to the lower amount of sample required and the short-time assay (within minutes), besides the versatility of the operational conditions (Balet et al. 2019; Batey and Curtin 2000). Nowadays, similar versatility and benefits can be obtained with the micro-visco-amylograph (Wang and Shi 2020). In addition, the change of operational conditions is continuously extending those instruments' applications, for instance to assess ingredients impact (Abdel-Aal et al. 2019), batter characteristics (Rios et al. 2018), breeders selection (Gil-Humanes et al. 2012), or even more fundamental

information like proteins impact on starch pasting properties (Li et al. 2020), among others. All those applications reveal the endless interest for assessing pasting performance of starches using rapid methods.

Apart from starch/flour quality assessment, viscosity has been the basis for predicting α– amylase activity and cereal sprouting in a rapid test (measurement in seconds), widely known as falling number (FN) parameter (Best and Muller 1991). The Hagberg falling number method approved by AACC International (AACCI) consists in a rapid heating of a starch suspension, with simultaneous stirring during the first 60 s, till reaching a point where the stirrer falls following the gravity force and it is recorded the time required for the rod to cross the viscous suspension (Chang et al. 1999). This methodology is extensively used and the FN is one of the most extended parameters in wheat quality surveys, although its repeatability and precision have been lately investigated (Delwiche et al. 2015). Therefore, there is extensive use of instruments for assessing the pasting properties of starches. Likewise, the falling number methodology is also based on apparent viscosity, recording the travelling time of the stirring rod through a starchy paste.

Recently, Chopin Technologies launched the Amylab FN to measure the Hagberg FN. Although this device was originally design to determine the falling number index of the flours, which is related to the α–amylase activity, such a rapid test might be very helpful for assessing starch performances if provided enough comprehensive information for understanding the process. This research aims to study the starch changes underlining during a short (90 seconds) heating cycle with simultaneous stirring. The rheological properties of different starches during their Rapid Force

Analysis (RFA) carried out in the Chopin Amylab have been related with the changes undergone on their microstructure and textural features.

Materials and methods

Materials

Commercial native starches of food grade for wheat starch (ADM, Chicago, US), corn starch (Tate & Lyle, London, UK) and potato starch (Tereos, Zaragoza, Spain) were directly used in the study. Rice starch was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Other reagents were of analytical grade.

Starches characterization

Water (WBC) and oil (OBC) binding capacities were determined to evaluate the hydration properties of the different starches. Those properties were assessed as previously described (Cornejo and Rosell 2015). Briefly, starch (100 mg) was suspended into one milliliter of water, in the case of WBC, or vegetable oil for the OBC. Suspensions were vortexed for 5 min and then centrifuge at 5000 x *g* for 5 min. The sediment was weighed after draining the tubes. Results were expressed as grams of water or oil adsorbed by gram of starch. Five replicates were made for each experimental result.

Thermal properties were determined using a Differential Scanning Calorimeter (DSC-TAQ2000, TA Instruments Ltd., New Castle, DE, US). Starch samples (8 mg) were weight in stainless steel pans and distilled water was added in the proportion 1:4 (starch: water, w/w). Pans were heated at 10 ºC/min from 30 to 120 ºC, an empty pan was used as reference. Onset temperature (To), heat of transition (ΔH, in joules per gram of starch) and peak temperature (Tp) were determined. All measurements were done at least in triplicate.

Rapid force analyzer of different starches

The Chopin Amylab® (Chopin Technologies, Villeneuve-la-Garenne, Cedex, France) in its testogram mode was used as a rapid force analyzer (RFA) to record the gelatinization of the different starches. This device operates with a continuous up and down motion of the stirrer rod during 90 s at a constant temperature of 100 °C. A slurry containing 7 g (14% mb) of starch and 25 ml of distilled water were placed into the precision test tubes of the device and manually shaken vigorously for 30 s. After immersing the stirring rod into the slurry, the tube was capped with a plunger and placed into the holder of the device. An insulated thermocouple (type K) was inserted and the wire leads attached to the bottom of the rod to record the temperature changes during measurements with a Comark N2014 multi sensor temperature data logger (Comark Instruments, Norwich, Norfolk, UK). Temperature readings were recorded every second. Plot recorded shows the force, expressed in Newtons, of the slurry/gel under continuous heating/shearing (Figure 1). Parameters defined include: onset force (F0) before force increase due to gelatinization, onset time (s) at which F0 is detected, 50% (t1) and 75% (t2) of time to onset force, maximum force (F1) and final force at 90 s (F2). Other parameters calculated from the previously identified forces included: α (slope between F0 and F1), gel stability as the elapsed time in which force was kept ±10% of the maximum force (F1) and the force difference between F1 and F2 was associated to starch breakdown. To understand starch changes along the gelatinization carried out with this device, the equipment was stopped to allow sampling at different times (t1, t2, onset, time to reach F1 and F2) as indicated in Figure 1. Three replicates were carried out for each sampling point and type of starch and results showed the average of experimental data.

At each point, samples obtained from the RFA were immediately poured into cylindrical containers of 25 mm internal diameter, covered with lids and kept at room temperature to cool down up to 25 ºC in the center of the gel. Simultaneously, the slurry/gel samples obtained from each stage were immediately frozen in liquid nitrogen and then freeze-dried to avoid microstructure alteration during freezing.

Gel texture and degree of gelatinization

In each stage, gel firmness analysis was conducted in a TA-XT2 Texturometer fitted with a 5 kg load cell (Stable Microsystems, Surrey, UK). Then, gels were measured through a single compression test using a 10 mm diameter aluminum round probe. Measurements were carried out at 1 mm/s crosshead speed and 50% of strain. Firmness was considered as the maximum penetration force and adhesiveness defined as the area required to remove the probe from the gel. All textural analysis in each stage were done at least four times.

The degree of gelatinization (DG %) for samples from the different stages was assessed by running DSC test as previously described for native starches, but using the freeze-dried powder of the samples taken from rapid force analyzer. DG was calculated using the equation suggested by Ozge Sakiyan et al. (2011):

$$\text{DG}\ (\%) = (1 - (\Delta\text{H sample})/(\Delta\text{H native})) \cdot 100$$

Scanning electron microscopy (SEM)

Native starches and freeze-dried samples, from slurries/gels taken at different times from the rapid force analyzer, were observed using SEM (Hitachi S-4800, Tokyo, Japan). All samples (native and gels) were coated with gold using a vacuum evaporator (JEE 400; JEOL, Tokyo, Japan) for 5 min. The images taken at 10 kV acceleration voltage were captured using 900x magnification. Four micrographs captured at each

stage were analyzed by digital image analysis using ImageJ software (ImajeJ 1.52p, National Institutes of Health, Bethesda, Maryland, US) to characterize microstructure. All micrographs were modified as 8-bit color and improved in contrast and brightness as reported by Espinosa-Ramirez et al. (2018). Threshold was assessed by adapting the software algorithm to each micrograph. Finally, sample analysis was carried out and mean cell area (μm^2) and gel porosity (%) were calculated.

Statistical analysis

Each quality parameter was subjected to a one-way analysis of variance (ANOVA) using Statgraphics Centurion XVII V 17.2 (Statgraphiscs Technologies, Inc., The Plains, Virginia, US). Fisher least significant difference test was used to assess significant differences ($P< 0.05$) among samples that might allow discrimination among them. Additionally, Pearson correlation analysis was applied to establish possible relationships among experimental variables extracted from the different analysis.

Results and Discussion

Starches characterization

To understand the starch changes when subjected to a rapid pasting procedure (90 s), different starches were selected, three from cereals (corn, rice and wheat) and one tuber starch from potato. Hydration properties and thermal parameters of those starches were determined because they might be potentially related to the pasting performance in this rapid procedure. Specifically, water and oil binding capacities reflect the hydrophilic and hydrophobic surface of the starch granules, which might affect the water uptake during granule swelling. Likewise, thermal properties have been related to changes in granular structure and endothermic gelatinization. Cereal starches showed much higher values for OBC than for WBC (Table 1), indicating greater superficial hydrophobicity,

likely their A-type polymorphs with the double helixes closely packed are responsible of that behavior (Waterschoot et al. 2015). Wheat starch exhibited the lowest WBC compared to the other starches. Conversely, potato starch showed similar values for WBC and OBC, likely its B-type polymorphs that allow more water within the loosely packed helices could be responsible of that result.

The DSC parameters confirmed the significantly lower onset temperature of wheat starch, whereas the highest one was exhibited by corn starch. The peak temperature of the endothermic peak showed the same tendency described for the onset temperature. Nevertheless, potato starch required greater enthalpy for gelatinization, and the opposite behavior was observed in the rice starch. Results are within the range of gelatinization properties previously reported for those starches (Ai and Jane 2015).

Starch gelatinization recorded with a Rapid Force Analyzer

The Chopin Amylab has been designed for inducing starch gelatinization in a rapid test (90 s) applying heating to a starch slurry subjected to continuous stirring (Figure 1). The device records the force changes during the starch gelatinization that occurs within the 90 s, acting as a Rapid Force Analyzer (RFA), although there is no previous information about changes occurring during the assay. A brief explanation of the plot recorded is following. In the present study 7 g of starch (adapted to 14% moisture content) suspended on 25 ml water was used, which corresponds to the best precision for measuring the FN (Chang et al. 1999). Firstly, the starch or flour slurry is in a liquid form that does not require any force for stirring. As the heating progresses, swelling of starch granules increases viscosity and in consequence the force require for keeping homogenous shearing increases significantly. The initial force (F0) of the slurry, before granules swelling, indicates the slurry consistency and might be related to the rapid

starch water uptake on the surface besides the potential impact of granules size. The time at which F0 is detected, is referred as the gelatinization onset and it could be related to the gelatinization temperature. The slope (α -slope) was also quantified to evaluate if the rate of starch swelling could be related to granules morphology. It is well known that granules swelling continues till their disintegration or breaking down that leads to a force decrease, which lasted until an even gel is formed (Figure 1). The maximum force detected was referred to F1, and the time of holding force was defined as stability. To understand morphological and textural changes during the gelatinization process, the assay was stopped at the points (t1, t2, onset, time at F1 and at 90 s) where major changes were expected (Figure 1). Microstructure on those points was compared with the native granular structure of each starch (Figure 2).

Micrographs of corn and rice starches at t1 were rather similar to the native starches, showing intact granules that kept their polyhedral shape forming agglomerates (Figure 2). Therefore, t1 was not enough to alter those starches structure and initiate the gelatinization, which agrees with their higher onset temperature (Table 1). Conversely, potato and wheat starches even at t1 exhibited signals of either distortion (wheat) or leaching (potato). In wheat starch, the two granules populations were detected but the bigger A-type displayed flat morphologies and some small B-type granules showed a deep groove in the center. In the case of potato starch, swollen granules together with some amylose leaching were observed, although it seems that those changes were not sufficient to modify the stirring force (Figure 3). Despite potato has a B-type starch its early gelatinization has been attributed to the negative charges of the phosphate-monoester derivatives that destabilize double helical structure (Ai and Jane 2015). Considering the different thermal properties of the tested starches (Table 1), significant correlations were detected between t1 recorded in the RFA with DSC parameters, To

(*r*= 0.78) and Tp (*r*= 0.89). In t2, which corresponded to 75% of the total time required for the onset, potato starch was completely gelatinized and a network was already observed, although it was not completely homogenous, showing irregular cavities sizes. At that point wheat starch was even more distorted, with flat structures that were held together by the leached molecules. Wheat granules perimeter was still defined in some granules and granules were interacting one to another. Similarly, corn and rice starches started their gelatinization with the deformation of the structures; thus, deformed granules were surrounded by leaching material and granules fragments resulting in an irregular mass. At the onset force (F0) the potato gel was completely formed, exhibiting an uniform network structure, whereas granules deformation progressed in cereal starches. At this point, some gel network was envisaged in corn and rice starches, but wheat granules were even thinner adopting flakes-like structures, with still defined perimeters.

Micrographs captured at the maximum force (F1) confirmed the gels formation in all the starches although some irregularities were observed in wheat gel. Considering that the maximum force is the inflection force point corresponding to the balance between swollen granules and fragmented ones, all starches lost their integrity and the granules interaction led to the gel mass. Further heating and gel shearing (till 90 s) did not provoke additional changes in the gel mass that could be visually detected, with the exception of the rice starch that exhibited more packed network at the end of the assay (90 s), likely better organization was achieved with the continuous stirring motion that allowed air bubbles removal. Similar structural changes were described for corn starch when gelatinization was sufficiently extended with the RVA and sampling was carried out at different times (Nelles et al. 2003). Therefore, considering the micrographs

information, the force plot recorded with the RFA reproduced the rheological changes that has been reported to describe starch gelatinization (Singh et al. 2003).

The plots recorded by the rapid force analyzer (Figure 3) confirmed differences among the different starches. The temperature profile shows that 40±3 s was required to reach 95 ºC, which is much faster than the over 150 s reported for the Perten FN (Chang et al. 1999). Initially, all slurries required low force for stirring, but after variable heating time (30-40 s) a fast increase of the recorded force was detected due to starch gelatinization. At that moment, the rapid heating rate underwent a decrease due to the energy required for the endothermic gelatinization, which agrees with reported results for the Perten FN (Chang et al. 1999). Cereal starches showed some minor decay after reaching the maximum force. In opposition, potato starch showed a well-defined peak of gel force with a large force decrease when heating-stirring progressed.

Parameters defined from the plots are included in Table 1. Potato and rice starches required significantly ($P< 0.05$) higher force (F0) for keeping homogenous slurries, which might be related to their WBC. In fact, a significant correlation ($r= 0.89$) was identified between F0 and WBC, in accordance to the relationship reported among the capacity to hydrate and swell and the starch viscosity (Cornejo-Ramirez et al. 2018). The lower onset observed for potato and wheat starches indicated that their gelatinization started at lower temperature, which agrees with data from DSC. In fact, a very strong positive correlation was encountered between the RFA onset time and the DSC To ($r= 0.90$) and Tp ($r= 0.97$). Potato starch displayed the faster gelatinization (α-slope) and rice starch had the lowest rate of gelatinization, with higher time (Time F1) to reach the maximum gel force (F1). Interestingly, a significant positive correlation ($r= 0.87$) was detected between the α-slope and the gelatinization enthalpy (ΔH).

As expected potato starch exhibited the highest force (F1) followed by wheat starch, whereas corn and rice starches did not differ significantly ($P< 0.05$) on their maximum force. A negative correlation ($r= 0.88$) was observed between the OBC and the F1. To assess behavior of gels after complete granule disintegration, gels stability was defined as the time holding the maximum force, which confirmed the short stability of the potato gel, and the longer stability of rice and wheat gels. Potato and wheat gels required higher forces indicating their higher viscosity. The force decay indicated by the breakdown reflected the granules resistance to physical rupture, which has significantly higher for potato starch, supporting that starches exhibiting higher swelling are less resistant to breakdown on cooking (Singh et al. 2003). In fact, a highly significant correlation ($r= 0.90$) was observed between F1 and breakdown.

Starch gels properties along gelatinization in RFA

The texture, RFA parameters, gelatinization degree (GD) and microstructure of the samples taken along the gelatinization carried out in the RFA were evaluated (Table 2). The statistical analysis of the variance showed that the starch factor significantly ($P< 0.05$) affected all the parameters tested, with exception of the degree of gelatinization. Likewise, samples taken at the different stages of the gelatinization (different times along RFA analysis) showed significantly ($P< 0.05$) different properties.

The RFA provokes a rapid gelatinization of the different starches, which was confirmed with the 100% gelatinization degree determined with the DSC. As it was previously described for the microstructure changes, the GD was reached at different times depending on the type the starch. Corn and wheat starches required 28 s and 23 s (Force time column in Table 2), respectively, to reach 100% GD. Conversely, potato and rice starches reached 100% GD at 30 s and 36 s, respectively. Surprisingly, according to the

311 GD, corn and wheat starches were rapidly gelatinized, when the SEM micrographs still
312 revealed granular structures at those times. Considering that the gelatinization degree
313 was calculated based on the transition enthalpy, it might be that the changes required to
314 obtain a complete lattice structure do not require additional energy, and in consequence
315 no enthalpy was detected when evaluated those samples.
316 Potato gel had the highest firmness (5.5 N), which agree with the highest force value
317 recorded with the RFA. But those maxima values were reached at different times, that is
318 the firmest potato gel was obtained after 30 s, whereas its maximum force recorded with
319 the RFA was observed at 38 s. This result agrees with the SEM observation that
320 indicated smaller air voids in the potato gel at F0 than at F1 (Figure 3), leading to firmer
321 gels. On the contrary, for cereal based gels the highest firmness was in accordance to
322 the maximum force. Rice gel was the softer one with the lowest firmness. Nonetheless,
323 in cereal based gels, there was not a direct trend between the firmness of the gels and
324 the maximum force (F1) detected with the RFA. Previous studies carried out with
325 potato and wheat starches stated the linear relationship between macroscopic and
326 microscopic viscosity determined with creep and rotational measurements, respectively,
327 within the temperature range 30-50 ºC (Yamano et al. 1996). Nevertheless, with this
328 rapid analysis carried out at higher temperatures, no linearity was observed between F1
329 and firmness. The firmness of the gels obtained after the 90 s (at F2) tended to decrease,
330 although no significant differences were observed. Presumably, the molecular order of
331 the gels was kept till a point where the thermal and mechanical constraints caused their
332 weakening, and that effect was more noticeable in firmer gels like potato. Similarly, a
333 significant ($P< 0.05$) decrease in the force values was observed in all starches as
334 gelatinization progresses, which might be related to the thixotropic (shear thinning)
335 behavior of the starch pastes with respect to time (Sikora et al. 2015).

From the image analysis of the gels structure it was calculated the porosity of the network and the median cell area, the former to avoid the misrepresentation that using the average value could create. Porosity observed in the micrographs corresponded to the regions initially occupied by water that was removed by freeze-drying. Those parameters were identified as soon as a gel was detected, which was reached in the early stages in the case of potato starch. From the porosity results it was evident in all the gels, the progressive increase in the porosity as the RFA progresses, indicating the gradual formation of an even gel structure, as was observed in the micrographs. The continuous stirring might help to obtain a more uniform structure since the shear force favors the alignment of the molecules within the lattice structure of the gels (Nelles et al. 2003). The higher median area of the lattice voids of potato gel indicated a more open structure of this gel with bigger cavities than those obtained for cereal based gels. Moreover, in the last stages it could envisage a decrease in the median area of the holes, with exception of wheat gel. Although the network structure visualized in the micrographs resulted from the removal of water leading to voids, changes in the last stages after complete gelatinization might be associated to the removal of air bubbles entrapped within the gel forced by the stirring motion.

A correlation matrix within the measured parameters confirmed the significant relationship among parameter recorded from the RFA and resulting gel features (Table 3), allowing better understanding of the changes undergone in the RFA along gelatinization. Only a very strong positive correlation ($r \geq 0.7$) was observed between the force measured in the RFA and the porosity of the gels, indicating that higher force gels would lead to more porous gels. Positive moderate ($0.4< r <0.7$) correlations were

observed between force and the gelatinization degree and median cell area, confirming the total gelatinization of the starches and higher force resulted from gels with thicker walls and big holes. There was a highly significant ($P< 0.0000$) correlation ($r = 0.66$) between force and time to reach those forces, which suggested longer time in the RFA was required for the stronger gels, like it was observed in the case of potato. The correlation between force and gel firmness, although statistically significant, was rather weak ($r < 0.3$). In the same sense, no correlation was found by Gaines et al. (2000) between pasting properties and gel hardness. Force time was also positively correlated with the GD and gel porosity. Other important correlations were detected among the gel firmness with adhesiveness ($r = 0.66$; $P= 0.0000$), GD ($r = 0.52$; $P= 0.0005$) and porosity ($r = 0.62$; $P= 0.0000$). The moderate negative correlation observed between the adhesiveness and median cell area suggested that gels with smaller voids were more adhesive.

Conclusions

The underlying mechanism occurring during a rapid starch gelatinization carried out with a Rapid Force Analyzer was investigated by stopping the measurement at different stages. Different parameters have been defined from the RFA plots to characterize starch performance during pasting. The microstructural changes observed with four different starches (corn, potato, rice and wheat) confirmed the complete starch gelatinization within the 90 s test, although time to reach gelatinization was dependent on the starch source. The force plots obtained from the RFA allowed the discrimination among the different starches. Significant correlations were detected between the maximum force (F2) recorded by RFA with the gelatinization degree and gel microstructure (porosity and median cell area).

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Conflict of Interest The authors declare that they have no conflict of interest.

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Figure captions

Figure 1. Typical plot recording force vs time along starch gelatinization using a Rapid Force Analyzer (Amylab). The main parameters used to evaluate slurries/gels are detailed in the drawing. Parameters defined include: onset force (F0) or starting force before gelatinization, onset time (s) at which F0 is detected, 50% (t1) and 75% (t2) of time to onset force, maximum force (F1), final force at 90 s (F2), α (slope between F0 and F1), gel stability as the elapsed time in which force was kept ±10% of the maximum force (F1) and breakdown (force difference between F1 and F2).

Figure 2. SEM micrographs captured along the Rapid Force Analyzer (RFA) cycle showing the starch changes along heating and stirring. Micrographs were taken at 900x magnification. Micrographs were taken at the different RFA stages described in Figure 1.

Figure 3. Plots from the Rapid Force Analyzer for corn, potato, rice and wheat starches assessed with the Amylab. Temperature was simultaneously recorded with a multi sensor temperature data logger (plot with secondary y-axis).

485 **Table 1.** Characterization of raw starches regarding hydration properties, calorimetric
486 parameters and performance during rapid force analysis assessed with the Chopin
487 Amylab.

	Corn	Potato	Rice	Wheat
Hydration properties				
WBC	2.09 ± 0.23 [a]	2.05 ± 0.17 [ab]	2.16 ± 0.18 [a]	1.85 ± 0.05 [b]
OBC	2.56 ± 0.09 [a]	2.17 ± 0.06 [c]	2.60 ± 0.06 [a]	2.44 ± 0.08 [b]
Calorimetric properties				
To (ºC)	69.90 ± 0.11 [a]	62.90 ± 0.54 [c]	65.62 ± 0.23 [b]	60.66 ± 0.23 [d]
ΔH (J/g)	12.76 ± 0 [b]	14.46 ± 0.25 [a]	6.56 ± 0.04 [d]	11.42 ± 0.11 [c]
Tp (ºC)	74.37 ± 0.21 [a]	67.77 ± 0.60 [c]	72.13 ± 0 [b]	65.29 ± 0.04 [d]
RFA-Amylab				
t1 (s)	19 ± 0 [a]	15 ± 0 [b]	20 ± 0 [a]	15 ± 0 [a]
t2 (s)	28 ± 1 [a]	23 ± 1 [b]	30 ± 0 [a]	23 ± 0 [a]
F0 (N)	2.45 ± 0.07 [b]	3.76 ± 0.16 [a]	3.76 ± 0.01 [a]	2.92 ± 0.36 [b]
Onset (s)	36 ± 0 [c]	30 ± 0 [b]	36 ± 0 [c]	29 ± 0 [a]
α-slope	1.83 ± 0.01 [b]	2.07 ± 0.04 [a]	0.38 ± 0.02 [d]	0.65 ± 0.01 [c]
F1 (N)	11.51 ± 0.07 [c]	21.04 ± 0.30 [a]	11.34 ± 0.29 [c]	15.33 ± 0.20 [b]
Time F1 (s)	47 ± 0 [c]	38 ± 0 [d]	67 ± 0 [a]	57 ± 3 [b]
Stability (s)	12 ± 2 [b]	4 ± 1 [c]	22 ± 1 [a]	22 ± 1 [a]
F2 (N)	7.70 ± 0.04 [c]	12.78 ± 0.34 [a]	9.56 ± 0.05 [b]	12.21 ± 0.47 [a]
Breakdown (N)	3.81 ± 0.10 [b]	8.26 ± 0.64 [a]	1.78 ± 0.24 [c]	3.12 ± 0.28 [b]

488 Means within the same row followed by different letters indicate significant differences by LSD multiple
489 range test *P<0.05.*

490

Table 2. Characterization of the starchy gels obtained along the gelatinization process including texture, RFA parameters, gelatinization degree (GD) and microstructure.

Starch	RFA-Stage	Force time (s)[a]	Force (N)	Firmness (N)	Adhesiveness (N·s)	GD (%)	Porosity (%)	Median cell area (μm²)
Corn	t1	19 ± 0^{e}	1.1 ± 0^{d}	n.d.*	n.d.	40 ± 4	n.d.	n.d.
	t2	28 ± 1^{d}	1.1 ± 0^{d}	2.3 ± 0.9^{b}	1.4 ± 0.9^{b}	100 ± 0	n.d.	n.d.
	F0	36 ± 0^{c}	2.5 ± 0.1^{c}	4.7 ± 0.5^{a}	3.0 ± 0.7^{a}	100 ± 0	37.8 ± 0.3^{b}	24 ± 8^{a}
	F1	47 ± 0^{b}	11.5 ± 0.1^{a}	4.8 ± 0.6^{a}	2.6 ± 0.7^{ab}	100 ± 0	45.5 ± 2.8^{ab}	18 ± 4^{ab}
	F2	90 ± 0^{a}	7.7 ± 0.0^{b}	4.4 ± 0.4^{a}	3.4 ± 0.3^{a}	100 ± 0	52.2 ± 3.7^{a}	6 ± 1^{b}
Potato	t1	15 ± 0^{e}	1.3 ± 0.1^{d}	n.d.	n.d.	49 ± 4	n.d.	n.d.
	t2	23 ± 1^{d}	1.4 ± 0.2^{d}	4.8 ± 0.8^{b}	1.4 ± 0.3^{a}	84 ± 3	49.3 ± 3.5^{b}	27 ± 9^{a}
	F0	30 ± 0^{c}	3.8 ± 0.2^{c}	5.5 ± 0.5^{a}	1.3 ± 0.3^{a}	100 ± 0	52.4 ± 3.0^{b}	28 ± 2^{b}
	F1	38 ± 0^{b}	21.0 ± 0.3^{a}	2.5 ± 0.3^{c}	0.9 ± 0.2^{b}	100 ± 0	67.8 ± 2.0^{a}	62 ± 5^{a}
	F2	90 ± 0^{a}	12.8 ± 0.3^{b}	2.1 ± 0.1^{c}	0.7 ± 0.1^{b}	100 ± 0	64.6 ± 3.8^{a}	53 ± 3^{a}
Rice	t1	20 ± 0^{e}	1.5 ± 0.1^{d}	n.d.	n.d.	22 ± 2	n.d.	n.d.
	t2	30 ± 0^{d}	1.5 ± 0.1^{d}	0.5 ± 0.1^{b}	0.5 ± 0.2^{b}	96 ± 1	n.d.	n.d.
	F0	36 ± 0^{c}	3.8 ± 0.0^{c}	0.9 ± 0.1^{a}	1.0 ± 0.1^{a}	100 ± 0	15.0 ± 2.5^{c}	8 ± 1^{b}
	F1	67 ± 0^{b}	11.1 ± 0.3^{a}	1.0 ± 0.1^{a}	1.1 ± 0.1^{a}	100 ± 0	35.0 ± 2.5^{b}	29 ± 5^{a}
	F2	90 ± 0^{a}	9.5 ± 0.1^{b}	1.0 ± 0.1^{a}	0.7 ± 0.2^{b}	100 ± 0	51.0 ± 2.8^{a}	18 ± 2^{b}
Wheat	t1	15 ± 0^{e}	1.1 ± 0.1^{d}	n.d.	n.d.	73 ± 1	n.d.	n.d.
	t2	23 ± 0^{d}	1.1 ± 0.2^{d}	1.4 ± 0.1^{c}	1.5 ± 0.2^{b}	100 ± 0	n.d.	n.d.
	F0	28 ± 0^{c}	2.9 ± 0.4^{c}	2.7 ± 0.6^{b}	1.9 ± 0.9^{b}	100 ± 0	n.d.	n.d.
	F1	57 ± 3^{b}	15.3 ± 0.2^{a}	4.4 ± 0.4^{a}	3.5 ± 0.8^{a}	100 ± 0	16.7 ± 2.1^{b}	12 ± 2^{a}
	F2	90 ± 0^{a}	12.2 ± 0.5^{b}	4.2 ± 0.2^{a}	3.2 ± 0.5^{a}	100 ± 0	64.9 ± 0.5^{a}	16 ± 1^{a}
***P*-value**								
Starch		**0.0006**	**0.0016**	**0.0000**	**0.0000**	0.0506	**0.0000**	**0.0000**
RFA-stage		**0.0000**	**0.0000**	**0.0000**	**0.0006**	**0.0000**	**0.0000**	**0.0157**

[a] Force time: time to reach sampling point as displayed in Figure 1.

*n.d. (not detected)

Means within the same column in each starch followed by different letters indicate significant differences by LSD multiple range test *P<0.05*

RFA: Rapid force analyzer (Chopin Amylab working in its testogram mode).

506 **Table 3**. Correlation matrix among texture properties, RFA parameters, gelatinization

507 degree (GD) and microstructure obtained from the different starches.

	Firmness (N)	Adhesiveness (g·s)	Force (N)	Force time (s)	GD (%)	Porosity (%)
Adhesiveness (g·s)	**0.66** (0.0000)					
Force (N)	**0.33** (0.0371)	0.13 (0.4885)				
Force time (s)	0.27 (0.0869)	0.20 (0.3041)	**0.66** (0.0000)			
GD (%)	**0.52** (0.0005)	0.16 (0.3947)	**0.47** (0.0022)	**0.50** (0.0010)		
Porosity (%)	**0.62** (0.0000)	0.08 (0.6656)	**0.70** (0.0000)	**0.68** (0.0000)	**0.48** (0.0016)	
Median cell area (µm2)	-0.14 (0.5089)	**-0.49** (0.0248)	**0.44** (0.0298)	-0.15 (0.5587)	-0.05 (0.8312)	**0.55** (0.0052)

Upper row= *Pearson* correlations, bold values indicate significant correlations.
In parenthesis *P*-values for statistical significance of estimated correlation.

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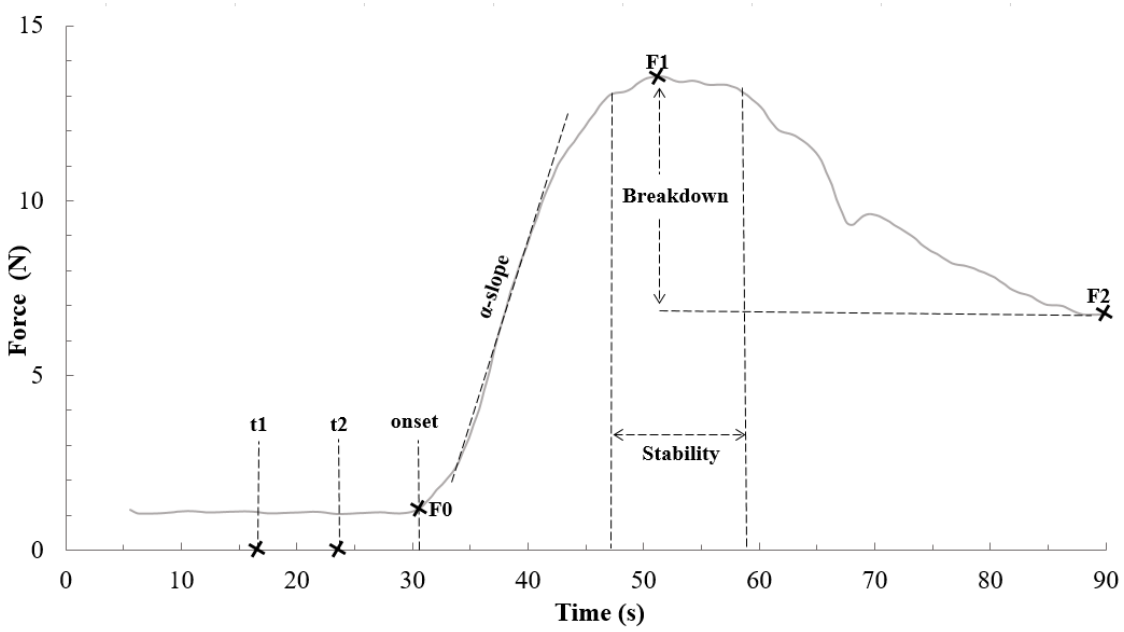
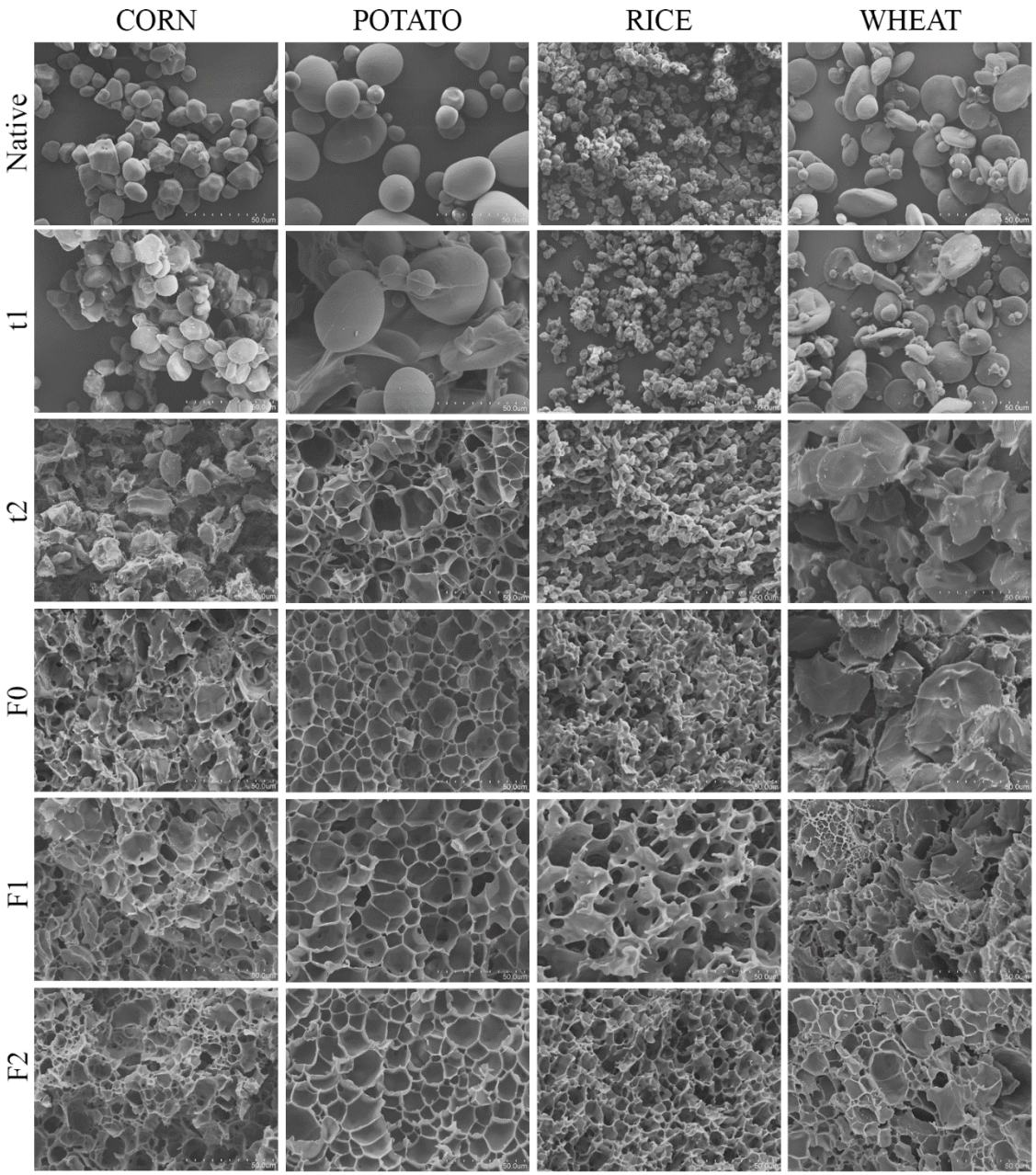
Figure 1.


15
10
5
0
Force (N)
0
10
20
30
40
50
60
70
80
90
Time (s)
F1
F2
F0
t1
t2
onset
α-slope
Breakdown
Stability

514 **Figure 2**.



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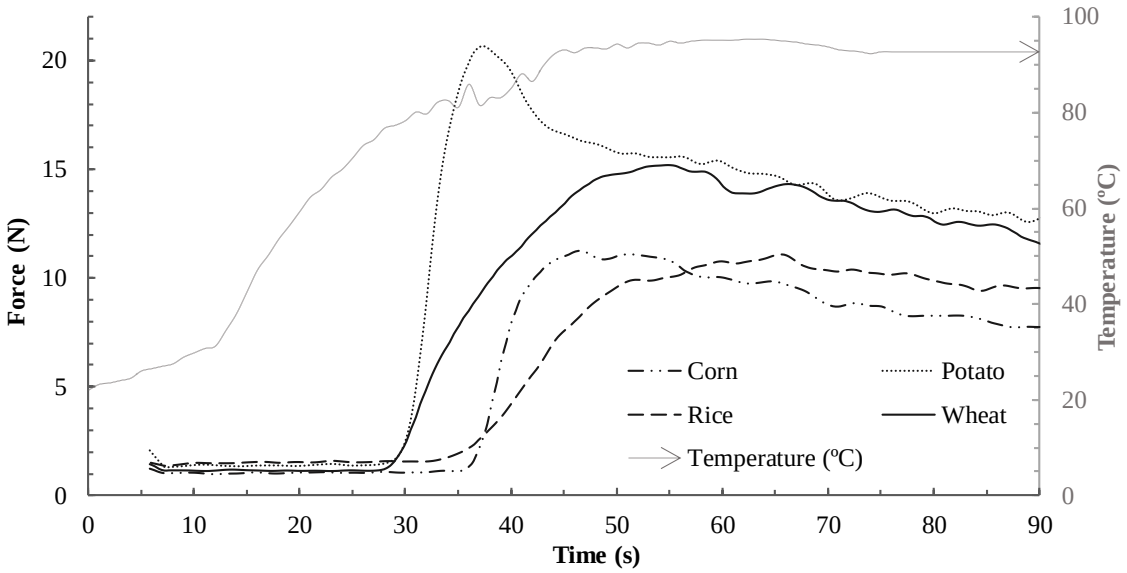
517 **Figure 3**.



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