Aggregate Formation and Segregation of Maize Starch Granules Cooked at Reduced Moisture Conditions

The mechanism of formation of aggregates of maize starch granules was studied through the kinetics of their destruction. The cooking of the starch, which was done with limited amount of water (1:1) to imitate the conditions of starch foods, induced the formation of aggregates of different sizes. These were dispersed in ethanol and agitated for different periods, provoking their desegregation. The size distribution and its dependence on time was measured by means of laser light scattering. It was found that the aggregates are built in a fractal-type geometry: a group of starch granules form a small aggregate, then groups of the latter build into larger ones, and so on, observing up to five scales, depending on the cooking degree. The aggregates are held together by gelatinized granules that show no Maltese cross. The formation of the aggregate classes can be explained by granules swelling randomly at different points of the sample, with a lower probability of dual gelatinization of neighbor granules.

Keywords: Starch granule; Aggregation; Segregation; Size distribution; Fractal

1 Introduction

Despite the high degree of sophistication of the food industry, the one based on starch still contains an important handcraft component. An area that requires attention is the prediction of the properties of starch product from those of the material along the production process [1]. Some problems of line productions on the dough preparation are not recognized until the final cooking of the food product. This class of prediction could be possible only after understanding the interactions and the structural changes undergone by the raw material. In the initial stages of the process of production, the starch-starch interactions are responsible for the formation of aggregates, and they determine the consistency of the dough. The influence of the granule surface on the rheological behavior of dough has been observed for wheat starch [2]. The aggregation of starch granules determines the rheological properties of starch dough, which are in turn determinative to the properties of the final product. A fast characterization of the degree and class of aggregation could be employed as a tool to predict, in line, the quality of the starch product. To understand the nature of the aggregates, we measured the kinetics of their desegregation. This study gave some insights into the way granules organize within the aggregate, and on the way the latter are built. This is the second of a series of studies toward the development of an in-line characterization technique based on the aggregation of starch granules.

Previous studies in this field have concentrated on the size distribution of individual granules [3, 4, 5, 6] and of grits [7, 8]. They demonstrated, by applying similar processes on starch samples differing on their granule size distribution, that the size distribution of the granules had a strong influence on the properties of the final product. The present study is focused on the kinetics of the desegregation and on the aggregation mechanism of granules caused by wet thermal treatments. We were able to obtain fast and detailed information about the aggregation by the use of laser light dispersion [9]. An important part of the study was the use of a sample preparation protocol that provided adequate reproducibility [10]. In this way, it was possible to achieve greater precision and reproducibility than through sieve and image analysis [11, 12, 13]. We also demonstrated the correlation and sensibility of the aggregation with the cooking parameters and proposed a mechanism for the formation of aggregates.

2 Materials and Methods

2.1 Sample preparation

The sample preparation protocol is reported in our previous publication on the same issue [10]. Unmodified maize starch (23% amylose, 77% amylopectin, Sigma-Aldrich, St. Louis, MO, USA) were mixed with deionized
water to obtain a dough with 50% dry matter and 50% water. Small amounts (2 g) were sealed in polyethylene bags and cooked for 5 min in a controlled bath to temperatures of 61.5, 63, 65, and 67°C, and chilled immediately (by immersing into water) to room temperature to stop the cooking process. Under these conditions, the percentage of granules gelatinized between 62 and 63°C resembled that of corn dough for the production of tortilla (20%, as quantified by polarized light microscopy [14]). Calorimetry studies showed that the samples studied swept only half the range of gelatinization, and were still far from melting processes.

2.2 Quantification of aggregates

The determination of the particle size distribution was done with a commercial granulometer (Cilas 1640, Marcoussis, France) based on light scattering. A laser beam illuminates a region where the particles are suspended, and an arrangement of photocells provides the angular dependence of the scattered light. By assuming a spherical shape of the particles (granules and granule aggregates), and using the appropriate theory (Mie Theory and some of its approximations [15]), the dispersion patterns were transformed into particle size distributions. When the particles are not spherical, the results should be interpreted through effective diameters, that depend on the details of the geometry of the particles [16].

The samples were fed into the granulometer by suspending 0.90 g of the cooked sample in 30 mL of ethanol and agitated for different periods, from 1.5 min to 10 h. As discussed elsewhere [10], ethanol was chosen as the solvent because it retains the aggregation structure for enough time to be observed. These dispersions were filtered through a sieve #80 that retained particles larger than 500 μm, and then placed in the granulometer that contained 400 mL of ethanol. The particle concentration was optimized accordingly to the indications of the granulometer manufacturer (Cilas 1640).

The distributions were analyzed simultaneously with an algorithm that allowed multidimensional parameter optimization [17]. By a simultaneous analysis of the data it was possible to discriminate features hidden in one set of data but apparent in others [10, 18].

2.3 Light and electron microscopy

Two microscopic techniques, environmental scanning electronic microscopy (ESEM, Phillips, Eindhoven, Netherlands) and polarized light microscopy (Zeiss, Oberko-chen, Germany), were employed. The optical images were obtained from two drops of a solution of 0.1 g of sample suspended in 100 mL of ethanol and agitated for 6 min. The determination of the birefringence was carried out in three images containing around 80 granules each. For ESEM, the sample was agitated for only 1.5 min to retain larger aggregates. Two drops were placed in a conducting slide and, after evaporating the solvent, placed in the ESEM chamber. Low values for vacuum and electron beam were elected to minimize the modifications in the samples.

3 Results and Discussion

3.1 Gluing together granules into aggregates

As previously reported, the particle size distribution curves of maize starch cooked in limited amount of water show well defined peaks and shoulders that suggest the existence of a set of aggregate sizes classes [10]. Evidence of the formation of starch aggregates of characteristic sizes have been reported previously in other starch systems [19]. Microscopic observations of these samples have been performed using ESEM and light microscopy. Fig. 1a shows ESEM images at low magnification of starch aggregates formed at a cooking temperature of 65°C and dispersed in ethanol. This particular image shows three types of particles: individual granules, small aggregates and larger aggregates. This observation was also made in many other microscopic images at different magnifications (not shown). The high magnification image (Fig. 1 b) shows an aggregate formed by a few starch granules. The individual granules exhibit two distinct shapes: either spherical or with an irregular shape. The spherical shape suggests that the granule is not gelatinized, while the irregular shape suggests that the granule has been gelatinized, at least partially.

In order to clarify the role of gelatinized granules in the formation of aggregates a series of light microscopic observations were made. Fig. 2 shows a starch aggregate in bright field and in polarized light. The size and boundaries of each granule can be identified. Granules in the boundary seem to be not deformed. The granule denoted with the number “2” appears to paste the granules glued around the granule marked as “1”, to the granules on the other side of granule “2”. Fig. 2b reveals the birefringence of the different granules. Granules “1” and “2” show no birefringence and have to be considered gelatinized, while other granules show different degrees of birefringence, indicating that they are not or only partially gelatinized [20]. This image suggests that aggregates form around gelatinized granules. Since prior to cooking the
moisture is limited, the probability of fully gelatinizing two adjacent starch granules is low. A fully gelatinized granule can be surrounded only by a limited number of partially or non-gelatinized granules, giving rise to aggregates of a characteristic dimension. This type of aggregates, consisting of two to five granules glued to a gelatinized granule, will be called Class 1. It is important to emphasize that the gelatinized granule can be more easily deformed, which allows a larger contact area to the other granules, thus forming more stable aggregates. This mechanism can explain the formation of a class of aggregate containing few granules and could correspond to the small aggregates shown in Fig. 1a. Aggregates of larger sizes could be explained in terms of Class 1 aggregates glued together, and could correspond to the larger aggregates shown in Fig. 1a. These will be called Class 2 aggregates.

It is not clear if a starch granule has to be fully gelatinized in order to paste other granules together, as partially gelatinized granules having their surface gelatinized should also be able to glue to non-gelatinized granules. As a first approach we can consider only fully gelatinized
granules responsible for the aggregation mechanism [21]. This mechanism is also consistent with the observation of different aggregate sizes for different cooking temperatures: at low cooking temperatures the fraction of gelatinized granules is small and only Class 1 aggregates can be formed. With increasing cooking temperature the gelatinized fraction increases and more complex aggregates can be formed.

### 3.2 Disintegration of the aggregates

The aggregates are formed during the cooking process in a packed configuration with limited moisture, and therefore cannot be directly observed. To be observed they had to be dispersed; ethanol was chosen as the dispersant for reasons explained elsewhere [10]. To characterize the stability of the aggregates dispersed in ethanol, we studied the kinetic of their disintegration caused by agitation. Fig. 3 shows the size distribution of a sample cooked at 65°C and dispersed in ethanol using two different dispersing times. For 1.5 min, a larger relative volume can be observed for sizes larger than about 150 µm, compared to a dispersion time of 6 min. The difference between these two curves is labeled “volume lost”. For sizes between 50 and 80 µm an increment in volume can be observed with the increase in dispersion time (labeled “volume gained” in Fig. 3). The two volumes are approximately equal. The loss of large aggregates (volume lost) was not reflected into the increase of the population of single starch granules. Instead, most of that material became part of another class of aggregates. This strongly suggested that the destruction of the large aggregates does not occur by disintegrating into single granules, but through large fractures to form smaller aggregates.

The kinetics of disintegration contributed valuable information to explain the aggregation mechanism. The agitation caused gradual disappearance of the aggregates in favor of single granules. Fig. 4 shows the influence of the dispersion time in the population of starch aggregates cooked at different temperatures and dispersed in ethanol. The times used range from 1.5 up to 390 min. After dispersing the 61.5°C sample for 4 min, two clear peaks could be observed (Fig. 4a). With increasing dispersion time the height of the peak with the larger sizes decreased continuously, while the other peak increased. At around 150 min of dispersion only one peak could be distinguished. At the longest time used (390 min of agitation) only a peak centered around 18 µm remained.

For a cooking temperature of 63°C (Fig. 4b) the initial size distribution (at 1.5 min dispersion) was centered at sizes around 50 µm. Up to dispersion times of 15 min, the changes on the distribution were not very appreciable.
Fig. 4. Influence of agitation time on the size distribution of starch aggregates for samples cooked at various temperatures (61.5°C, 63°C, 65°C and 67°C) and dispersed in ethanol at room temperature.

This apparent lack of evolution is very interesting, and is qualitative and quantitatively explained later in Section 3.5. At 60 min of dispersion the distribution started to change: the shoulder around 15 μm increased its relative volume, while the population with sizes between 30 and 80 μm decreased. This process was almost completed at 150 min of dispersion. Longer dispersion times practically did not influence the distribution. The final distribution, again, consisted of a single peak around 18 μm.

For the cooking temperature of 65°C (Fig. 4c) the changes on the distribution were apparent only after 60 min of agitation. This phenomenon is also explained in Section 3.5. The most pronounced changes happened between 150 min and 210 min of dispersion, reaching a final distribution similar to the other previous cases. For these first three cooking temperatures the final distribution reached at 390 min is practically the same.

For the cooking temperature of 67°C (Fig. 4d) the initial distribution was more complex: an important population with sizes about 200 μm appeared. In this case, a transition to a distribution centered around 60–80 μm was observed (between 6 and 15 min). This distribution remained stable for a certain period of time and for times longer than 210 min moved to another distribution. For larger periods (390 min), the distribution differed from the final distribution for the other temperatures. It is possible that in this case, even longer times were needed to reach a distribution similar to the previous cases.

3.3 Protocol of analysis of the size distribution of aggregates

These phenomena suggested a methodology to obtain the information that is manifested in the structure of the distribution curves of aggregates size. To this end the
curves were described by representative peaks of the different classes of aggregates (Fig. 5a). It is important to mention that, although these distribution could be fitted better with an ad-hoc set of peaks, the demand that the same group of peaks adjusted all the data in Fig. 4, for all temperatures, imposed much stricter conditions on the acceptable values of the parameters. The minimum set required is shown in Fig. 5a. The fitting to all the curves using these peaks corresponds to the continuous lines of Fig. 4. The analysis of the kinetics of disintegration for samples cooked at different temperatures demanded practically identical groups of peaks, indicating that the same class of aggregates existed in samples cooked to the different temperatures in the range considered.

3.4 Structure of the aggregates

The representative diameters (T) of the peaks shown in Fig. 5a are plotted as a function of the class number (Fig. 5b). It was interesting to observe a regular geometrical behavior \( T = 18.1 \times 1.6^n \), where \( n \) is the aggregate class number. This behavior indicated that the representative diameter of Class \( n \) aggregates was 1.6 times larger than the diameter of Class \( n - 1 \) aggregates. As this applies to all classes, the overall structure resembled a fractal system. It should be noticed that \( 1.6^3 \) is approximately equal to 4. This means that the representative volume of Class \( n \) aggregate was 4 times larger than the volume of Class \( n - 1 \) aggregates, which could be explained by constructing each Class \( n \) aggregate by four Class \( n - 1 \) aggregates.

Although this suggested tetrahedron-shaped aggregates, it did not imply that linear aggregates were not formed in the first place, but rather that those in a more compact structure have a much larger lifetime. The fractal theory has been used to describe the interaction among granules of starch [22] as well as the structure of the aggregation of other powders; the existence of different classes of aggregates and the destruction of some to form others accumulated evidence in this direction.

3.5 Quantitative study of the kinetics of the destruction of the aggregates

Fig. 6 shows the progression of the population of each aggregate class and the single starch granule for samples cooked at different temperatures (61.5°C, 63°C, 65°C and 67°C) as a function of the dispersion time in ethanol. In Fig. 6a (61.5°C) only three classes (0, 1 and 2) are present. The population of Class 2 aggregates decreases continuously with time; the population of Class 1 aggregates first increases then decrease for times longer than 60 min. In contrast, the single starch granule population continuously increased. The final distribution consisted of 100% single starch granules. It was interesting to observe that the population of Class 1 aggregates first increased. Again, this suggests that Class 2 aggregates does not break down to single starch granules in one step, but break first in Class 1 aggregates. This observation was useful for proposing a model that could describe the evolution of each population at the expenses of others, in a set of differential equations:
Aggregate Formation and Segregation of Maize Starch Granules

\[
\frac{d}{dt} V_0 = \frac{V_1}{\tau_1}
\]

\[
\frac{d}{dt} V_1 = -\frac{V_1}{\tau_1} + \frac{V_2}{\tau_2}
\]

\[
\frac{d}{dt} V_i = -\frac{V_{i-1}}{\tau_{i-1}} + \frac{V_{i+1}}{\tau_{i+1}}
\]

\[
\frac{d}{dt} V_4 = -\frac{V_4}{\tau_4} + \frac{V_5}{\tau_5}
\]

\[
\frac{d}{dt} V_5 = -\frac{V_5}{\tau_5}
\]

(1)

Where \( V_i \) is the volume fraction of the \( i \)-th population (0 = single starch granule, 1 = aggregate Class 1, and so on), and \( \tau_i \) its mean lifetime. This model was established under rather simple assumptions:

- The destruction of the aggregates is of a random character. The fracture speed is proportional to the number of aggregates without containing aging effects.
- The desegregation take place through fractures, so that the population of the class aggregates \( i \) is only fed by the disintegration of the aggregates of Class \( i + 1 \).
- Once the aggregates are fractured they are not formed again. A finite probability of reformation of the aggregates would have been reflected in a distribution where several classes of aggregates coexist, and not in the complete desegregation at long times of agitation.

The system of differential equations (1) was analytically solved and the values of \( \tau_i \) that best reproduced the behavior were obtained (Fig. 7). The model also applied to the more complex situations where more classes of aggregates were present. For the samples cooked at 63°C, five classes were present; the single granules (Class 0) did not represent the largest population at the beginning of the segregation process. Class 4 aggregates were destroyed after 20 min of agitation. The population of Class 3 aggregates slightly increased around 20–30 min then decrease for larger agitation times. The apparent
lack of changes within the first 15 min can now be understood. The destruction of Class 4 aggregates feed the population of aggregates Class 3, and maintained its population roughly constant. Similar phenomena occurred with aggregates Class 2 and 1. The lifetime of Class 1 aggregates was large enough to prevent an appreciable increase in Class 0 aggregates (single granules). The final distribution consisted, as in the case of 61.5°C, only of single starch granules.

A similar picture can be observed for the cooking temperature of 65°C, where all six populations were present. The apparent lack of evolution within the first 60 min is explained similarly. Class 5 aggregates did disappear, but its concentration was initially small enough to be appreciated from the distribution curves of Fig. 4c. Their disappearance feed the population of Class 4 aggregates and prevented it from decreasing. Although slightly, the population of Class 4 even increased by disintegration of Class 5 aggregates. The final distribution consisted mainly of single starch granules.

The life time of the aggregates of smaller size did not change drastically with the cooking temperature (see Fig. 7). This can also be observed directly in Fig. 4, which shows that Class 1 aggregates were the most persistent for all the considered temperatures. This indicated that the increase in the temperature in the considered range did not radically change the gluing capability of gelatinized granules, but rather the main effect resided in the number of gelatinized granules.

The increment in the temperature increased the population of gelatinized granules, giving place to the conformation of larger aggregates. However, sticking together various Class 1 aggregates to form a Class 2 aggregate, required more powerful gluing. Thus, the lifetime of Class 2 aggregates was expected to exhibit an stronger dependence on the gelatinization degree, and, therefore, on the cooking temperature. This also applies to larger aggregates (see Fig. 7).

4 Conclusions

Starch aggregates are glued together by gelatinized granules. The cooking process of starch in a packed configuration with limited water, resulted in random swelling of granules and in the formation of aggregate classes in a fractal-type geometry. The destruction of the aggregates occurred by large fractures to form aggregates of smaller size, and not through the disintegration into single granules. The kinetics of desegregation could be explained through a model of random destruction with well defined life times for each aggregate class. The relative independence of the life time of the first class of aggregates indicated that cooking at higher temperatures was reflected in an increased number of gelatinized granules (those granules gluing together the aggregates), rather that in a higher degree of gelatinization of these granules.

References


The algorithms were written into a software called AAnalyzer®, which is available at www.qro.cinvestav.mx/~aanalyzer.


That neighboring granules exhibit different degrees of gelatinization, even though they were exposed to similar conditions, is caused by the limitation on the moisture. If a granules begin its gelatinization process, it will rapidly absorb the water surrounding it, and will prevent close by granules to gelatinize.

This presumption will be tested in future work by comparing the activation energy of the chemical reaction that causes a granule to become an aggregation agent, to the activation energy of the chemical reactions involved in the gelatinization process. Although the gelatinization process is not understood in detail, it has been established that it involves a migration of amylose from inside the granule. Partially released amylose could serve as a sticking point to other surrounding granules. If this is correct, then the reactions for both processes (glue and gelatinization) would be the same (Carlos Gomez Aldapa, private communication).