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Aggregation in cooked maize starch

A. Herrera-Gómez[a,*], M. Canónico-Franco[b], G. Ramos[c], R.C. Pless[c]

[a]*CINVESTAV-Querétaro. Libramiento Norponiente 2000, Querétaro, 76230 Mexico*
[b]*Universidad Autónoma de Querétaro. Cerro de las Campanas, Querétaro, 76010 Mexico*
[c]*CICATA-IPN. José Siurob 10, Col. Alameda, Querétaro, 76040 Mexico*

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Abstract

This paper focuses on the aggregation of starch granules. The starch was cooked in a limiting amount of water and dispersed in different solvents. The size of the resulting aggregates was measured using light scattering. The solvents employed in this study (water, glycerol, ethanol, and 2-propanol) dispersed the aggregates to different degrees and resulted in different size distributions for the starch aggregates. It was observed that the size distribution of the cooked starch dispersed in the less polar solvents showed a higher representation of larger aggregates. Higher cooking temperatures resulted in larger aggregates. The methodology here presented proved to be a reproducible and practical way to study aggregation of starch. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Starch; Aggregation; Size distribution; Cluster; Swelling

1. Introduction

Many starch-based foods have characteristic rheological properties (resilience, elasticity, mouthfeel, etc.) that are perceived by the consumer as important determinants of the food quality. Some, at least, of those properties are closely correlated with the sizes of the particles constituting the finished food product or the material (dough) from which it derives, and the type and degree of interaction among them. The particles considered in this study were, on one hand, the biologically formed starch granules and, on the other, large aggregates formed from several starch granules during the processing of the food.

Khan, Des Rosiers, Rooney, Morgan, and Sweat (1982) developed a methodology to measure Particle Size Index of masa for preparation of tortilla that correlated well with the quality of the masas prepared under different conditions. It appears that the macroscopic cohesiveness of the material, perceived as texture or mouthfeel by the consumer, also dictates, at the microscopic level, the degree of aggregation of the fundamental starch granules. Therefore, the development of rapid and simple methodologies for the determination of particle size distribution in dough-like materials holds out considerable promise for the food industry, as such methodologies might permit to perform in-line testing that could predict the quality of the final product.

Studies related to the size distribution of the solid particles in different types of flour or masa have been carried out for some time (see e.g. Gomez, Waniska, & Rooney, 1991; Pflugfelder, Rooney, & Waniska, 1988; Sahai, Buendia, & Jackson, 2001). Recently they have become more convenient and, above all, they provide more detailed information, with the advent of laser-light dispersion granulometers. This type of equipment is capable of fast evaluation of the size distribution in a wide range (from under 1 μm to 2 mm) of solid particles dispersed in liquids or in air, (see e.g. Devaux, Le Deschault de Monredon, Guibert, Novales, & Abelcassis, 1998). These granulometers conveniently output the size of the particles in terms of volume proportions, i.e. the graphs presented by the instrument directly reflect the volume, and hence, by approximating to constant density, the mass of the material that has a given size (expressed as particle diameter).

We are carrying out a research program on the degree of aggregation between starch granules in masa, with a view of correlating such data with the organoleptic qualities of the final product (e.g. tortilla, bread, etc.) originating from masa prepared under different conditions. The determination of the particle size distribution is performed by laser-light dispersion granulometry. This requires disaggregating the solid materials of interest (e.g. bread dough or nixtamalized corn flour) in a suitable dispersing liquid. The disaggregation protocol should be such that the short-range

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* Corresponding author. Tel.: +52-442-211-9904; fax: +52-442-211-9937.

E-mail address: alberto@ciateq.mx (A. Herrera-Gómez).

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aggregations between the particles are retained, i.e. that the size distribution determined be reflective of the short-range aggregations existing in the masa or the flour. To develop such a protocol we have elected to first examine, as a model for a dough-like system, commercial maize starch cooked in the presence of a limited amount of water to a degree of gelatinization similar to that observed in corn masa (Gomez, McDonough, Rooney, & Waniska, 1989). The present report describes the size distribution observed for this system for different cooking conditions and using different solvents for dispersion of the samples prior to their analysis by light-dispersion granulometry.

2. Materials and methods

2.1. Sample preparation

The maize starch used in the experiments was a commercial product from *Productos de Maíz*, Mexico, with an initial moisture content of 10%. All of the starch used was from the same package, thoroughly mixed by shaking prior to the first use and stored at room temperature in a dry environment. Each experimental sample was prepared by adding 1.00 g of starch to 0.80 ml of water at room temperature and allowing the starch to be thoroughly wetted. The moisture content was then 50%. To minimize temperature gradients in the sample during cooking, the mixture was placed in a small plastic bag and formatted into a thin film. Temperature uniformity is of special importance because, as preliminary experiments showed, cooked starch absorbs water from its surroundings, depriving the still uncooked starch of water and exacerbating the differences in the degree of cooking. The bag was sealed, pressed, and placed in a constant temperature bath at the desired temperature for 5 min. After this treatment the sample temperature was quenched by transferring the entire bag rapidly to a large volume of water at room temperature. A paste-like product containing 50% moisture was obtained. Temperatures between 60 and 70 °C were employed because they cover the whole range of starch gelatinization at the water concentration employed (see, for example, Noel & Ring, 1992). Examination of these starch pastes under the polarized-light microscope showed a partial loss of birefringence, similar to the situation observed by Gomez et al. (1989) in nixtamalized corn masa.

2.2. Microscopy

The optical images were obtained using a Zeiss microscope fitted with a commercial 35-mm reflex camera. The samples were first dispersed by mixing typically one-tenth of the cooked starch sample in 100 ml of dispersing liquid (water, ethanol, glycerol, or 2-propanol) and vigorously stirring the mixture for 6 min to form a dilute suspension. Droplets of such suspensions were observed under the microscope using a magnification of either 45 × or 100 ×.

2.3. Laser particle size analysis

The determination of the particle size distribution was performed using a commercial Laser Particle Analyzer (CILAS 1064, France). In this case, one half of a cooked starch sample (corresponding to 0.45 g of starch and 0.45 ml of water) was suspended in 30 ml of the selected dispersant, with vigorous stirring at room temperature for 6 min. The resulting dispersion was passed through a No. 80 sieve, to retain particles larger than 500 μm. The percolate was introduced into the Laser Particle Size Analyzer which had previously been charged with 400 ml of the selected dispersant. The amount of sample used was selected according to the optical characteristics of the final dispersion, since it has to be concentrated enough to be detectable, but dilute enough not to block the laser light. For each dispersant the amount of sample was chosen to provide optical characteristics close to the optimum indicated by the manufacturer.

The quantitative analysis of the particle size distributions was obtained using the program AAnalyzer[1] (Herrera-Gómez, 1999). This program is able to perform spectral deconvolution using gaussian curves, allowing variation of the peak position and of its full width at half maximum (FWHM).

The instrument was calibrated periodically using certified reference materials (CILAS Reference Powder P_1 P_2 P_3, CILAS, France), giving the following uncertainties in the size scale: 0.2 μm at 10 μm, and 1 μm at 93 μm.

3. Results and discussion

3.1. Effect of the dispersing solvent

When starch samples cooked in water for 5 min at 65 °C were dispersed in different solvents, simple microscopic inspection of the resulting suspensions clearly revealed different size distributions. Fig. 1 shows typical bright-field photographs of suspensions in water, glycerol, ethanol, and 2-propanol. Dispersions obtained with water or glycerol overwhelmingly contained single starch granules. In no instance were aggregates of more than three starch granules observed. In contrast, dispersions obtained with ethanol or 2-propanol showed, in addition to single starch granules, a

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[1] To perform the analysis of size distribution, a fitting program called the 'AAnalyzer' was employed to apply a special algorithm for the proper treatment of the data. With this program, shared parameters among various curves, such as gaussian width and position of peaks, can be optimized simultaneously for these curves. The advantage of multiple fitting is that those curves where a particular peak is important participate to a great extent in the definition of the parameters corresponding to that peak. Other characteristics of AAnalyzer are the display of the evolution of the fitting parameters among various curves (such as that those in Figs. 7 and 8), and an easy visualization and management of the character (fix, free, or correlated) of the fitting parameter. A free copy of AAnalyzer can be requested at alberto@ciateq.mx.

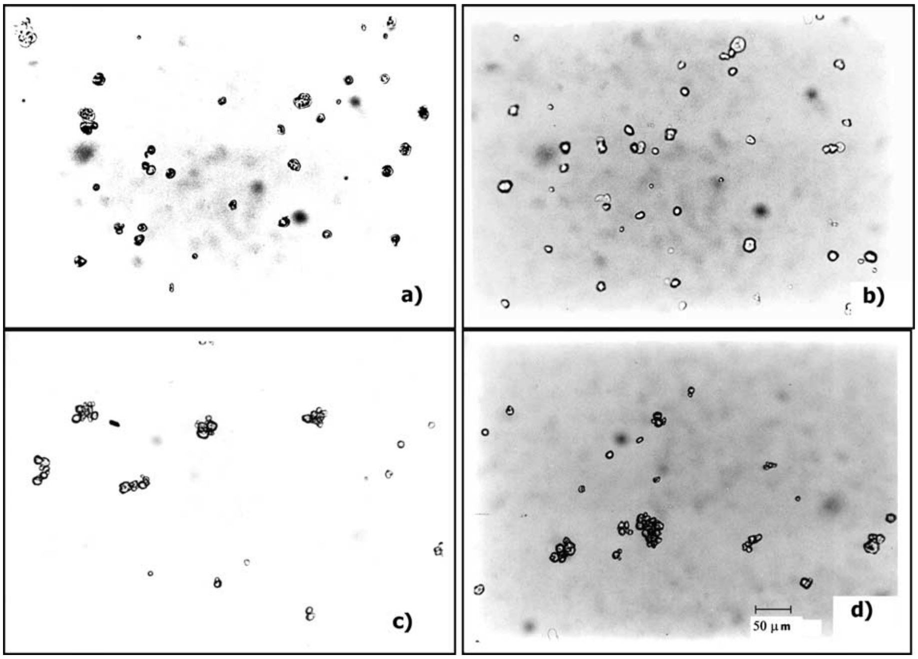

Fig. 1. Starch cooked at 65 °C and dispersed with different solvents: (a) water, (b) glycerol, (c) ethanol, and (d) 2-propanol. All microphotographs are on the same scale.

significant presence of larger aggregates, containing dozens of granules.

Fig. 2 shows the distribution of particle size of the starch samples with 50% of moisture content, cooked at 65 °C and then dispersed in the different solvents, as determined by light scattering. In the particle size distributions the *y*-axis represents the population of particles of a determined size weighted for volume, while the *x*-axis represents the effective diameter of the particles. In the cases of water and glycerol, the predominant peak was centered on a particle size of around 15–17 μm, which corresponds to the size of single maize starch granules. Some smaller particles were also observed in water (a shoulder around 1.6 μm) and with glycerol (a shoulder centered around 5 μm). These could correspond to oil and protein bodies present in maize grains.

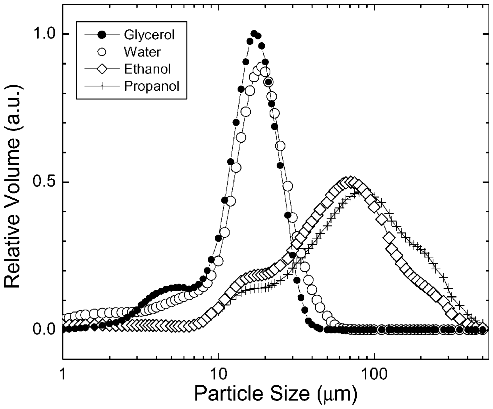

Fig. 2. Size distribution of starch cooked at 65 °C and dispersed in different solvents, as determined through light scattering. The higher-polarity solvents were more efficient in destroying the aggregates and in dispersing the cooked starch down to the starch granule constituents. The aggregates present in the samples dispersed in alcohol were constituted from cluster classes with defined sizes.

Very different distributions were observed for ethanol and 2-propanol. While particles with a size around 18 μm were still clearly evident as a shoulder, the distribution maxima occurred at 75 and 90 μm for ethanol and 2-propanol, respectively. For these two solvents, a significant amount of material up to sizes of 250 μm was present, while particles smaller than 7 μm could not be observed; it is possible that the alcohols dissolved oil bodies and protein bodies. These distributions were consistent with the microscopic observations: essential absence of larger particles for glycerol and water, and aggregates of significant size in 2-propanol and ethanol.

Cooking of maize starch at 65 °C in the presence of a limiting amount of water resulted in a significant degree of aggregation between the starch granules. While glycerol and water were able to disaggregate the agglomerates practically down to single granules, when 2-propanol or ethanol were employed these aggregates were retained, at least partially.

By comparing the effect of the different dispersing solvents, we observed a qualitative correlation between the presence of large aggregates and a low polarity of the solvent molecules. The dielectric constants of the liquids used in this study are: water, $D = 78.54$; glycerol, $D = 42.5$; ethanol, $D = 24.3$; 2-propanol, $D = 18.3$. The use of the low-polarity solvents (ethanol and 2-propanol) as dispersants resulted in a reduced population of single starch granules in favor of larger aggregates. This trend was more pronounced for the alcohol with the lower polarity, viz.

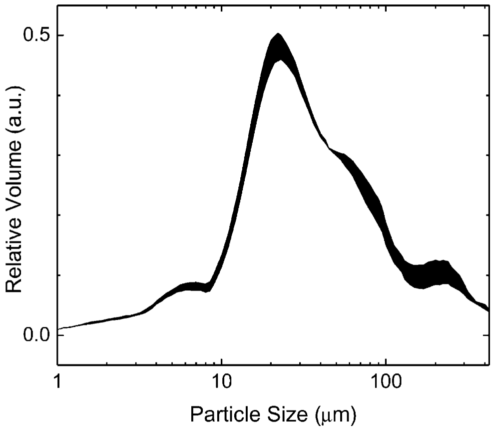

Fig. 3. The particle size distribution measurements were highly reproducible. The shaded area shows the range of relative volume values found in four starch samples cooked independently (different days) at 69 °C and dispersed in water. The maximum variation of the relative volume was smaller than that caused by changing the cooking temperature by 1 °C.

2-propanol. This suggests that the interaction that keeps the granules together is of a polar type, and that solvent molecules with larger polarity are more efficient in masking those interactions, allowing more disaggregation.

3.2. Reproducibility

The sample preparation protocol proposed in this study, together with the use of light scattering, solved a number of important issues related to reproducibility in the measurement of particle size distributions. Light scattering has strong advantages over other techniques also employed for this type of measurements. Its advantage over Image Analysis is that it can generate large statistics in a small period, and over Sieve Segregation is that it provides much more detailed information in a much shorter period. Proper sampling of powders requires an elaborate protocol because gravity causes the powder to segregate by size. By a proper mixture of the starch powder, and by a homogenous cooking, it was possible to obtain size distribution measurements that were reproducible in great detail. This reproducibility allowed the observation of clear tendencies of the characteristics of the size distributions. The shaded region of Fig. 3 shows the ranges of values for the volume fraction for samples prepared on different days under the same conditions. Similar measurements for other temperature–solvent combinations (not shown here) also demonstrated good reproducibility.

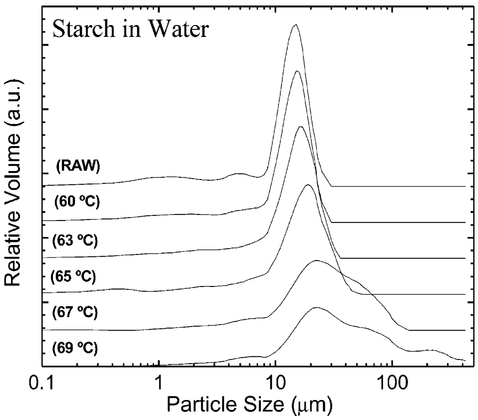

Fig. 4. Particle size distribution of starch cooked at different temperatures and dispersed in water, as determined by light scattering. Although it shows a clear dependence on the cooking temperature, the sensibility was smaller than in the ethanol case (see Fig. 5).

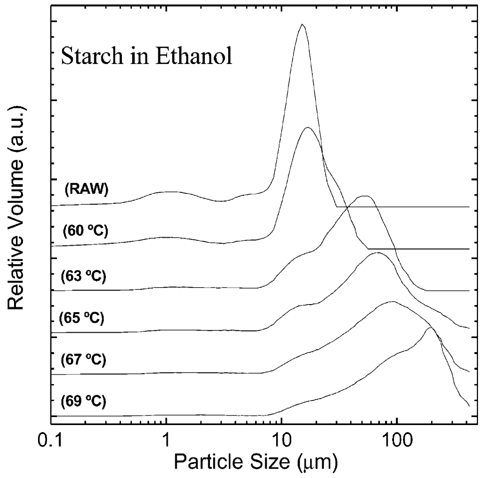

Fig. 5. Particle size distribution of starch cooked at different temperatures and dispersed in ethanol. These distributions showed a high dependence on the treatment temperature.

3.3. Effect of the cooking temperature

In order to test the capability of the particle size analysis to distinguish starch with different degrees of cooking, various samples were prepared at different cooking temperatures ranging from 60–69 °C. The corresponding distributions of the dispersions in water are shown in Fig. 4. The raw sample showed a single predominant peak for single starch granules (around 15 μm). The sample cooked at 60 °C showed a similar distribution as the raw sample, but with a small shift of the position of the maximum to larger sizes. As the cooking temperature increased, the centroid of the single-granule peak shifted slightly to larger sizes and its height decreased. Material in the larger-size classes began to appear for cooking temperatures of 67 °C or higher.

The distributions corresponding to the dispersions in ethanol are shown in Fig. 5. The trend of the single-granule peak was roughly similar to that observed in water, but its height decreased more rapidly as the cooking temperature increased. The presence of material with a size larger than 40 μm was observed already at 63 °C. At the highest cooking temperature employed (69 °C) the dominant peak centered on 200 μm. For cooking temperatures of 63 °C and higher, the single-granule peak was smaller than the

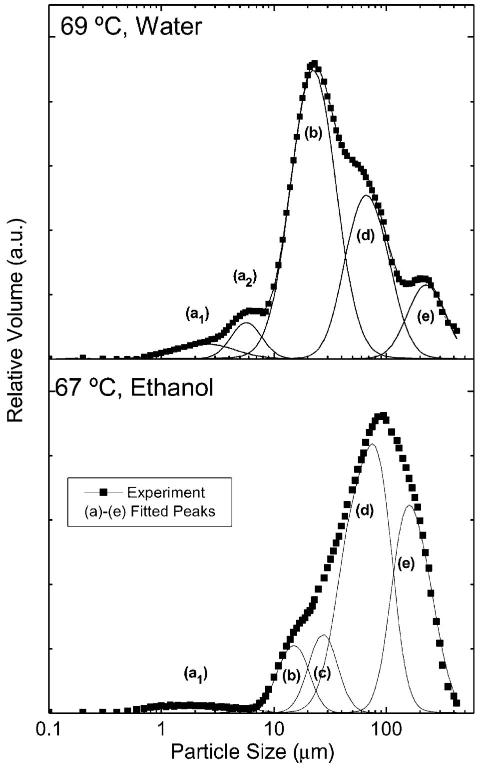

Fig. 6. Typical decomposition of the size distribution in different particle classes. Particles of type (a) may be oil and protein bodies that were dissolved by alcohol. The single granules are represented by the particles of type (b). Particles of types (c)–(e) are granule clusters. Water was able to destroy the small clusters represented by (c).

peaks corresponding to the aggregates, but it was still present up to the highest temperature used.

3.4. Detailed analysis

From the raw data it was possible to distinguish clear peaks, which probably corresponded to different cluster classes. Detailed analysis reinforced this interpretation. The distributions were decomposed in a set of gaussian peaks. Fig. 6 shows the typical size distribution of samples dispersed in water and ethanol together with the peaks employed in the decomposition of these curves. An attempt was made to fit the experimental distributions with the least number of peaks that could reproduce the experimental data. The peaks are labeled (a) through (e); with (b) representing the single starch granules. Peaks (a_1) and (a_2) probably correspond to oil and protein bodies and will be referred to as corpuscles. Peaks (c), (d), and (e) correspond to aggregates of granules of different size. They will be referred to as first, second, and third cluster class, respectively. In both figures, the continuous line passing through the experimental data corresponds to the calculated curve (sum of the peaks). Similar cluster classes were found in samples dispersed in both water and ethanol. It is interesting to observe that peak (c) was not necessary to fit the data of the dispersion in water, which suggests that water is able to destroy the first clusters.

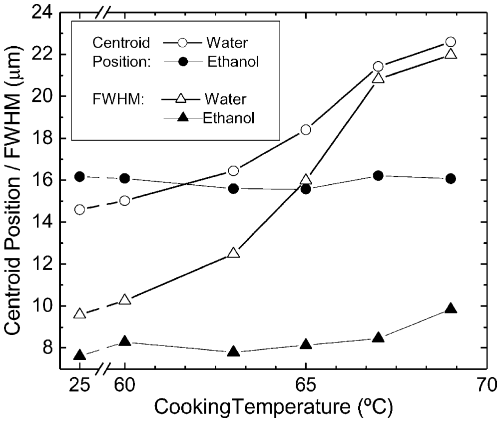

Fig. 7. Centroid and FWHM for the peak corresponding to single granules as a function of cooking temperature. The size increase of the granules dispersed in water was due to the extra swelling undergone by cooked starch granules when extra water was available. This water absorption occurred even at room temperature. In contrast, the cooked granules dispersed in alcohol did not show any size increase. The difference in size for the water and alcohol cases at the lower temperatures is not real. It is originated from imprecise values for the optical constant of cooked starch that were fed into the light scattering analyzer.

Fig. 7 shows the centroid and the full width at half maximum (FWHM) of the single-granule peak as a function of the cooking temperature for dispersions in both water and ethanol. FWHM is given here simply as a measure of the spread in the particle sizes subsumed under a given peak in the deconvolution: the larger the FWHM of a peak, the broader is the distribution of the particles represented in that peak. In the case of the samples dispersed in water, the centroid position shifted from 14 μm at 25 °C to 23 μm at 69 °C, and the FWHM increased from 9.5 μm at 25 °C to 22 μm at 69 °C. This behavior suggests that starch granules originally cooked with limited access to water continue to swell when more water becomes available, even at room temperature.

The behavior of the population of the different aggregate classes for samples dispersed in ethanol showed a clear tendency: as the cooking temperature increased, the fractional population of the smaller clusters decreased in favor of larger-size cluster classes. Fig. 8 shows the population of the size classes of the aggregates as a function of the cooking temperature. At 25 °C about 80% of the population corresponded to single granules. This population decreased steadily with increasing cooking temperature and represented less than 10% of the whole population for samples cooked at 69 °C. The corpuscles showed the same trend, starting at about 10% at 25 °C, and then practically vanishing above 65 °C, though this is most likely due to dissolution. All of the three cluster types were initially absent and started to appear at 60, 63, and 65 °C, respectively. After their first appearance and an initial increase in their population at the lower cooking temperatures, the small clusters

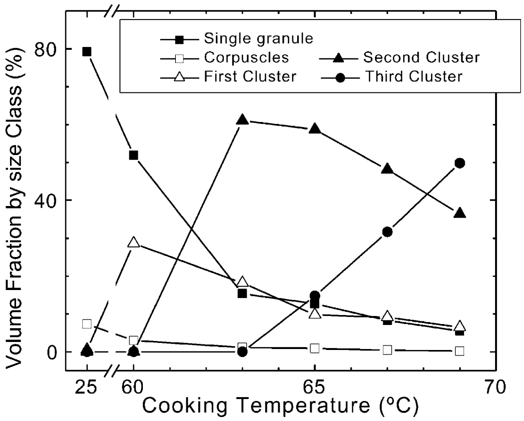

Fig. 8. Population of the size classes of the aggregates, as a function of the cooking temperature, for dispersion in ethanol. The larger clusters appeared at the higher cooking temperatures, at the expense of the smaller clusters and the single-granule population.

decreased in number, in favor of the larger clusters, at the higher temperatures.

The mechanism underlying the formation of the agglomerates observed in these studies is still unknown, but it appears clear that the experimental methodology explored in this study allows to perform reproducible studies that could show the dependence of aggregation phenomena on treatment parameters, allowing the development of physical models about the clustering mechanism.

4. Conclusions

The aggregation of starch was investigated for various cooking temperatures and under the dispersion in different solvents. Light scattering proved to be a practical technique for the measurement of aggregate population for these systems. All four solvents considered in this study dispersed the cooked samples, but different solvents gave different distributions for the various size classes of starch aggregates. It was found that molecules with larger polarity are more capable of disaggregating the starch granules, which suggests that the granule–granule interaction is of a polar type and that highly polar solvent molecules are able to mask this interaction.

A detailed comparison of the results obtained with dispersion in water and with dispersion in ethanol showed, for the latter, a much stronger representation of the higher aggregate classes and a higher sensitivity to the cooking temperature. Our study showed a marked dependence of the particle size distribution on the starch cooking temperature employed: higher temperatures resulted in a strong bias in favor of larger aggregates. The use of water as dispersant shows that starch granules cooked in a limited water environment continued to swell when in contact with additional water, even at room temperature. Conversely, when the cooked starch was in contact with ethanol, they remained essentially unchanged.

An interesting result of this study is that the aggregates formed in cooked starch can be subsumed under three different size classes (around 30, 70, and 200 μm) recognizable in water dispersion as well as in ethanol dispersion.

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