

Determination of Starch Gelatinization by X-Ray Diffractometry¹

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ABSTRACT

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The potential of X-ray diffractometry for quantitative determination of gelatinization of starch was evaluated. The method, which used a "built-in" internal standard approach, had a total coefficient of variation of 99.87%. The diffractometry method also correlated well with a standard chemical method. Both procedures showed that critical moisture contents of 45-47%

for maize and 55% for pea were necessary for complete gelatinization of these starches. Results from scanning electron microscopy ran parallel to the chemical and X-ray data, indicating that both methods measure physical changes taking place during gelatinization.

Starch gelatinization is an important phenomenon in food processing, and methods for measuring it have appeared in various articles (Anderson et al 1969, Birch and Priestley 1973, Chiang and Johnson 1977, Lawton et al 1972, Shetty et al 1974, Wooton et al 1971). Most of these methods are time-consuming and, except for those of Wooton et al (1971) and Birch and Priestley (1973), they do not specifically measure gelatinization but rather both gelatinization and hydrolysis of starches.

X-ray diffractometry has been used to study starch crystallinity (Nara et al 1978); however, its use in quantitative estimation of the extent of gelatinization is limited. The crystallinity of starch is known to decrease with gelatinization; a completely gelatinized starch has an amorphous structure. The decrease in crystallinity, which can be monitored by X-ray diffractometry, could be quantitated and thus serve as a measure of the extent of gelatinization.

In this article, the potential of such X-ray quantitative analysis for starch gelatinization is evaluated and compared to the differential alkali solubility method of Birch and Priestley (1973).

Based on the theory of Alexander and Klug (1948), a relationship can be established between the X-ray diffraction intensity (I_i) from the i^{th} component in a multicomponent mixture and the weight fraction of the component (χ_i) as follows:

$$I_i = K_i \left(\frac{\chi_i / \phi_i}{\sum_1^n U_i^* \chi_i} \right) \quad (1)$$

where ϕ_i and U_i^* are the density and the mass absorption coefficient of the component i , respectively, and K_i depends on the nature of component i and the geometry of the instrument used. Considering a mixture of n components and assuming them to consist of just two components, (ie, component 1 to be analyzed and the sum of the other component designated as matrix), equation 1 could be reformulated as

$$I_i = \frac{K_1 \chi_1}{\phi_1 [\chi_1 (U_1^* - U_m^*) + U_m^*]} \quad (2)$$

where U_m^* is the mass absorption coefficient of the matrix. Equation 2 is the basic relationship underlying the quantitative analysis of multicomponent mixtures with X-ray diffractometry (Alexander and Klug 1948, Klug and Alexander 1974). The diffraction intensity of the component under study is thus related to

its concentration in the mixture and to the relative mass absorption coefficients of the component and matrix. Therefore, except for cases where $U_i^* = U_m^*$, the intensity concentration relationship is not linear. A practical way to obtain linearity for this relationship is to compare two components and relate their intensities to their weight proportions. By introducing the average mass absorption coefficient U^* for the mixture, equation 1 can be simplified to

$$I_i = K_i \left(\frac{\chi_i}{\phi_i U^*} \right) \quad (3)$$

A comparison between intensities of the i^{th} and j^{th} components produces a relation independent of U^* as follows:

$$\frac{I_i}{I_j} = \frac{K_i^1 \chi_i \phi_j}{K_j^1 \chi_j \phi_i} = K \left(\frac{\chi_i}{\chi_j} \right) \quad (4)$$

where K includes all constants, K_i^1 , K_j^1 , ϕ_i , and ϕ_j , for the two components i and j . Equation 4, which can be obtained by introducing a known weight of an internal standard to the mixture and relating the component under investigation to the standard, will show a linear relationship between the intensity ratios and the ratio of the weight fractions for the component and the standard. In the case of starch, a simpler approach could be adopted. A peak component in the starch diffractogram can be assigned to be the internal standard. The intensity or the reduction in intensity of this peak (component) with gelatinization may then be related to the initial peak height in the native starch. The use of such a "built-in" internal standard has the potential advantage of reducing the apparent error of heterogeneity associated with the conventional internal standard method.

MATERIALS AND METHODS

Materials

Raw commercial cornstarch was obtained from St. Lawrence Starch Co., Mississauga, Ont., Canada. Crude pea starch was provided by the National Research Council of Canada, Saskatoon, Sask. The crude starch was further purified, following the procedure of Biliaderis and Grant (1979) except that four successive washings were made and a speed of $2,500 \times g$ was used in the centrifugation step. The purified pea starch was vacuum dried at 58°C and 3.33 kPa. For identification purposes, both starch samples were analyzed for protein, fat, and moisture.

Determination of Extent of Gelatinization of Standards

Standards representing 0-100% gelatinized starch were prepared. Predried raw commercial cornstarch was used to represent the 0% gelatinized sample, and the 100% gelatinized sample was prepared following a modified procedure of Owusu-Ansah et al (1980). A 5:2 water:maize slurry of commercial

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cornstarch was heated in a steam-jacketed kettle for 25 min. The paste was homogenized in a blender with 2 L of water, then drum dried on a laboratory twin drum drier at 4 rpm and a pressure of 207 kPa. The scraped flakes were further dried in a vacuum oven and milled in a laboratory hammer mill to pass a 200-mesh screen. Twenty-gram mixtures of the two standards, ranging from 0 to 100% gelatinization, were prepared and analyzed by an X-ray diffraction and alkali solubility method of Birch and Priestley (1973).

Both conventional and built-in internal standard approaches were used to analyze the samples. Duplicate 5-g samples of each of the mixtures were weighed into aluminum dishes, with one set equilibrated overnight at 20°C and a relative humidity of 91%. To

each sample in the other sets, 0.263 g of calcium carbonate was added as an internal standard (Priestley 1975) forming approximately 5% of the total weight. These samples were thoroughly mixed, first manually using a mortar and pestle and then with a magnetic stirrer for 30 min, and then equilibrated at the same temperature and relative humidity as the first set. For X-ray diffraction, samples from each set were filled into the instrument sample compartment and were tamped and packed into place with the edge and face of a microscope slide. The finished surface was smoothed and made flush with the face of the sample holder. The packed sample was then mounted vertically into the X-ray diffractometer (General Electric Co., modified XRD-6) equipped with a monochromator that selected $K\alpha$ radiation from a cobalt target. The scan was made from a diffraction angle (2θ) of 12–30°, using a slit width of 0.2 mm, a scale factor of 2,000, and a time constant of 10. The goniometer and the recording chart speeds were 2° and 1 cm/min, respectively. Duplicate scans were made in each sample and the diffraction patterns recorded directly. The peak heights of the internal standard and the components at a diffraction angle of 26.3° were recorded. The ratio of the peak height of the component at 26.3° to that of the internal standard was calculated and expressed as a percentage. For the built-in (native) internal standard method, the scans were made directly on the milled samples without any premixing. The ratio of the decrease in peak height with gelatinization (peak at 26.3°) relative to the corresponding peak height in the native starch was also calculated from the results and plotted against the degree of gelatinization of the test samples to obtain a standard curve that served as the measure of extent of gelatinization.

The extent of gelatinization of the standard mixtures was determined chemically by the differential alkali solubility method of Birch and Priestley (1973) except that the samples were dispersed in 50 ml of 0.25 M and 0.7 M KOH. The absorbance of the amylose-iodine complex in 0.25 M KOH relative to that at 0.7 M KOH was calculated as a percentage and plotted against the degree of gelatinization of the reference mixtures to produce a standard curve.

Determination of Moisture Requirements for Gelatinization of Corn and Pea Starches

To evaluate the practicability of the X-ray method in determining the extent of gelatinization of processed starch, the X-ray and chemical methods were used to measure the extent of gelatinization of pea and corn starches heated at different moisture contents.

A set of 20-g samples of the purified pea starch and raw commercial cornstarch of known moisture content were weighed, and distilled water was added to each sample to produce a series of samples with moisture contents ranging from 13 to 70%. The water was added slowly to the samples while mixing them in a Hobart

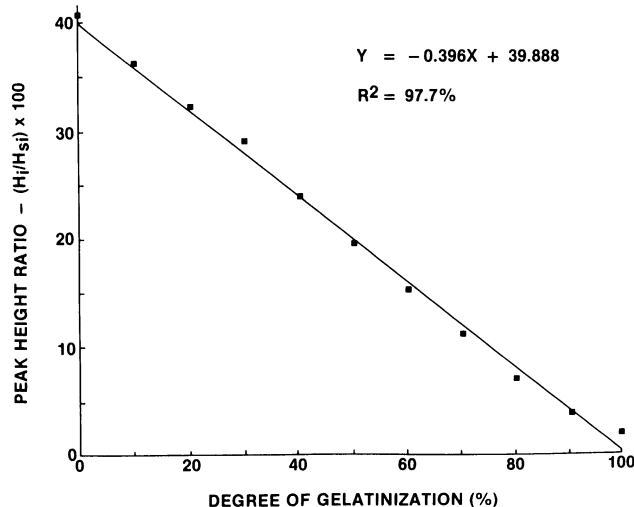


Fig. 1. Percentage ratio of peak height for mixtures of 0–100% gelatinized standard samples, using calcium carbonate as internal standard. H_i = peak height of starch undergoing gelatinization, H_{si} = peak height of CaCO_3 internal standard.

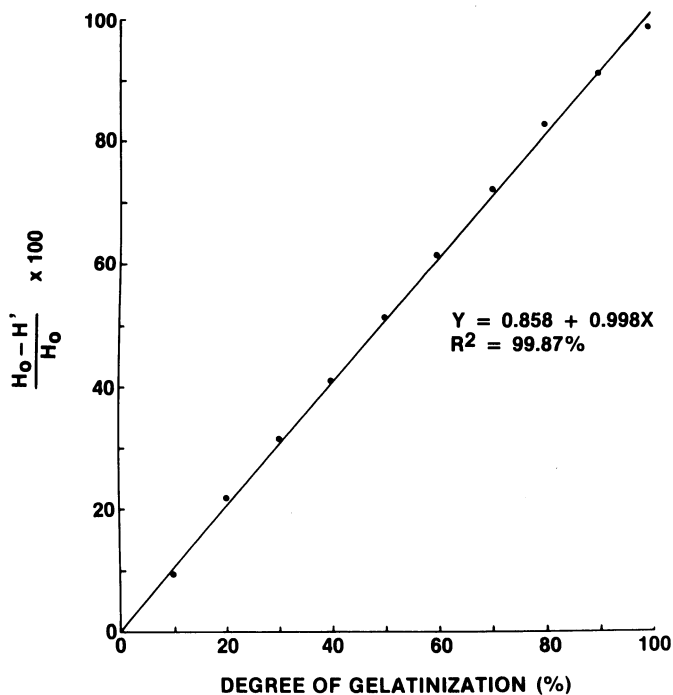


Fig. 2. Percentage ratio of peak decrease for mixtures of 0–100% gelatinized standard samples, using a component in the native starch as the internal standard. H_0 = peak height of raw starch used as internal standard, H' = peak height of starch undergoing gelatinization.

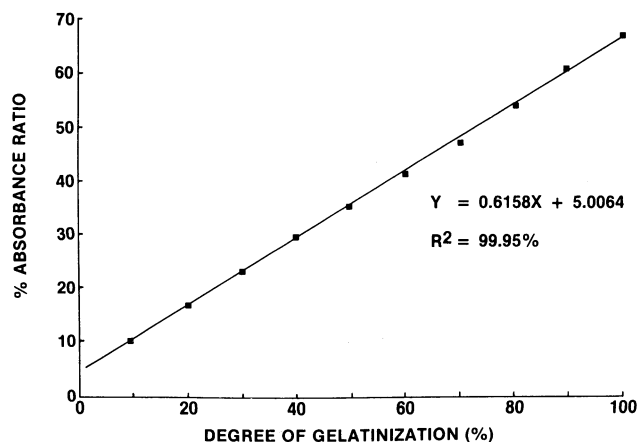


Fig. 3. Percentage absorbance ratio for mixtures of 0–100% gelatinized standard samples, using the alkali solubility method.

mixer. Each sample was then quantitatively transferred into a 300×407 can and sealed. The samples were coded and allowed to equilibrate overnight and then retorted at 120°C for 1 hr. After cooling, they were removed and the starch was manually disintegrated into small pieces that were vacuum dried at 58°C and 3.33 kPa for 12 hr and then milled to pass a 200-mesh screen. The extent of gelatinization of the retorted samples was then determined by the X-ray and chemical methods described previously, except that the scan for the pea starch was made from a diffraction angle of 10 – 28° and the intensity at 24.8° was used for quantitation. For the chemical method, the pea starch samples were dispersed in 50 ml of 0.2M and 0.7M KOH. Calculated absorbance ratios were plotted against the moisture content.

An examination of the extent of gelatinization was also made by scanning electron microscopy (SEM). Dried samples were tapped onto a copper adhesive tape and the samples were flushed with short blasts of Freon gas after mounting to remove any loose particles. They were then coated in vacuo with gold/platinum for 5 min and examined in an ETEC Autoscan electron microscope operating at 10 kV.

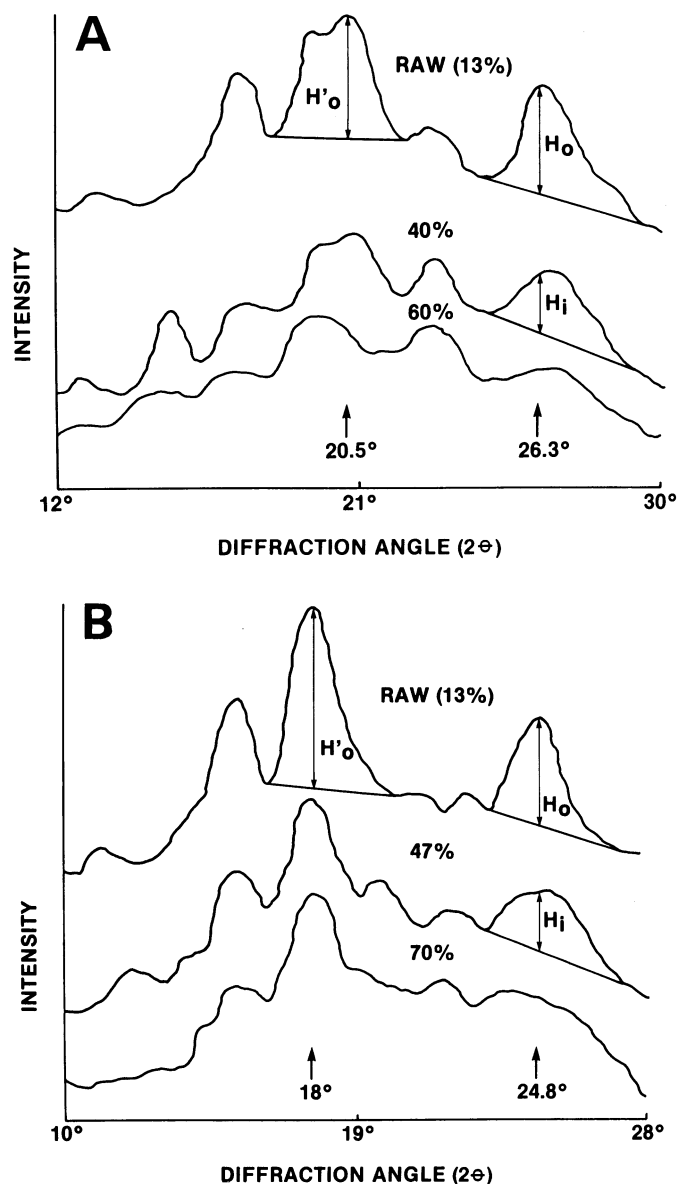


Fig. 4. X-ray diffractograms of cornstarch (A) and pea starch (B) retorted at various moisture contents (slightly smoothed). H'_o = peak height of raw starch initially used as internal standard, H_o = peak height of raw starch used as internal standard, H_i = peak height of raw starch undergoing gelatinization.

RESULTS AND DISCUSSION

The results for the extent of gelatinization of the standard samples as determined using the conventional internal standard method (CaCO_3 as internal standard) are shown in Fig. 1. The extent of gelatinization bears an inverse linear relationship to the ratio of the peak heights. However, by choosing the peak height of a component of the native starch as the internal standard (built-in internal standard) and relating its decrease in crystallinity to the original peak of this component, a direct linear relationship was obtained between the decrease in crystallinity and the extent of gelatinization (Fig. 2). The results from the latter approach had a slightly higher coefficient of variation (99.87) than did the internal standard approach (97.70). The improved precision might be due to the heterogeneity of test components usually inherent in the conventional internal standard approach (Klug and Alexander 1974). The alkali solubility method yielded similar results; the standard mixtures were linearly related to the extent of gelatinization of the samples (Fig. 3). The coefficient of variation was high (99.95) and similar to that obtained using the assumed internal (built-in) standard approach. Thus, although the two methods are based on different principles, they seem to measure the same change with almost the same precision.

To evaluate the practicability of the X-ray method, the built-in X-ray diffraction approach and the chemical method were used to study the moisture requirements for the gelatinization of corn and pea starches. The X-ray diffractograms of the corn and pea starches retorted at various moisture contents are shown in Fig. 4. In all cases, the crystallinity of the starches decreased as gelatinization (ie, moisture content) increased. The peaks at the diffraction angles of 26.3 and 24.8° were chosen as references for quantitation of the extent of gelatinization in the corn and pea starch samples, respectively, due to lower background interference in these regions than at the initial diffraction angles of 26.3 and 24.8° .

The results from the X-ray diffraction and the alkali solubility methods are shown in Figs. 5 and 6, respectively. The percent decrease in peak height increased with moisture content (Fig. 5), with these decreases more pronounced in maize starch than in pea

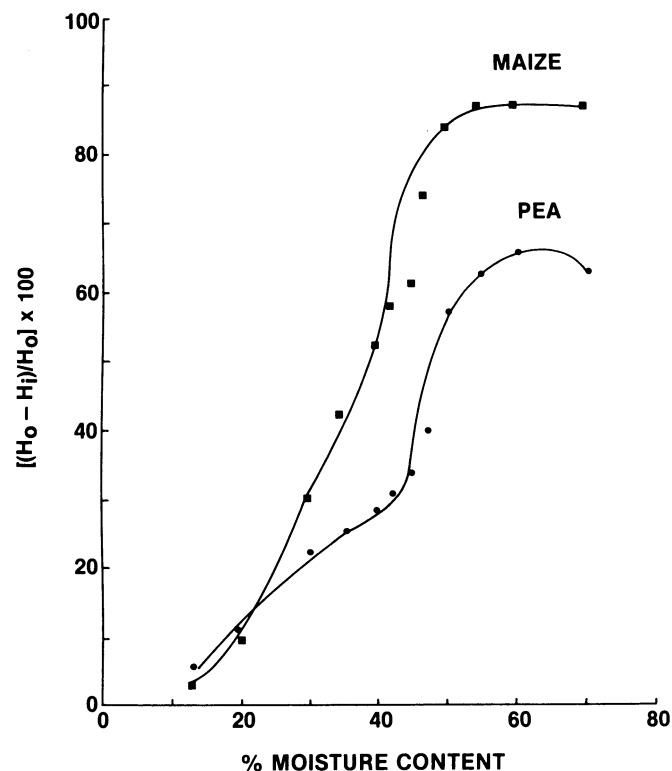


Fig. 5. Percentage decrease in X-ray peak height as a function of moisture content. H'_o = peak height of raw starch used as internal standard, H_i = peak height of starch undergoing gelatinization.

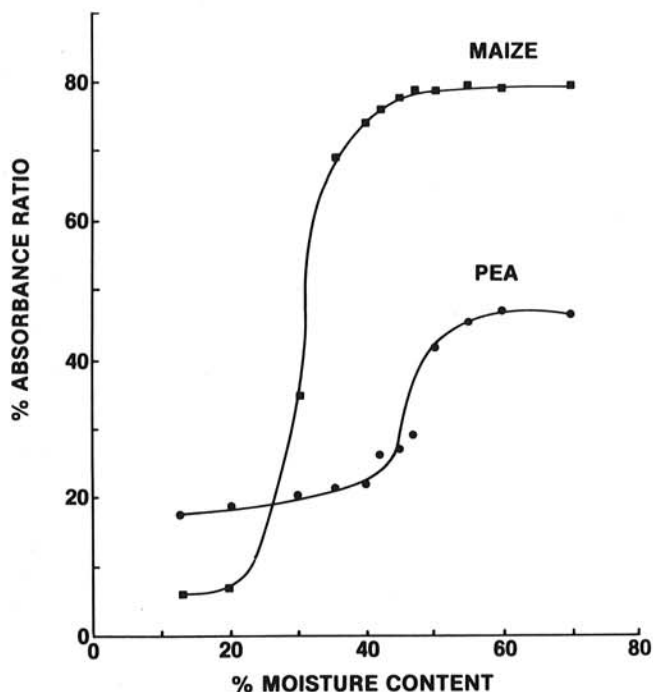


Fig. 6. Changes in the percentage absorbance ratio as a function of moisture content.

starch. In both starches, a critical moisture content was observed (about 47% for maize starch and 55% for pea starch), after which no appreciable decrease in the peak heights was found as the moisture content increased. The lack of further decrease in crystallinity might indicate complete gelatinization of the starches. Thus, below 47% (maize) and 55% (pea) moisture, the starches might have been partially gelatinized. Similar results were obtained for the alkali solubility method (Fig. 6) but, unlike the X-ray method, this method showed no change in the absorbance ratios of the samples below 20% moisture. Gelatinization responses of pea starch from both methods were less pronounced, suggesting that it released less amylose into solution when gelatinized and did not lose as much of its crystallinity. The high moisture level (55%) needed for complete gelatinization in pea starch might be caused by the high amylose-amylopectin ratio in this material (Banks et al 1974). The high amylose component could have bound much of the water so that only after higher moisture levels had been attained did any appreciable gelatinization take place. Collison and Chilton (1974) reported a similar observation in potato starch and attributed it to a high amylose-amylopectin ratio. The high hydration requirement for the gelatinization of pea starch might generally reflect the hard-to-cook phenomenon of peas (Sefaddeh et al 1979).

SEM was used to follow gelatinization of starches as a function

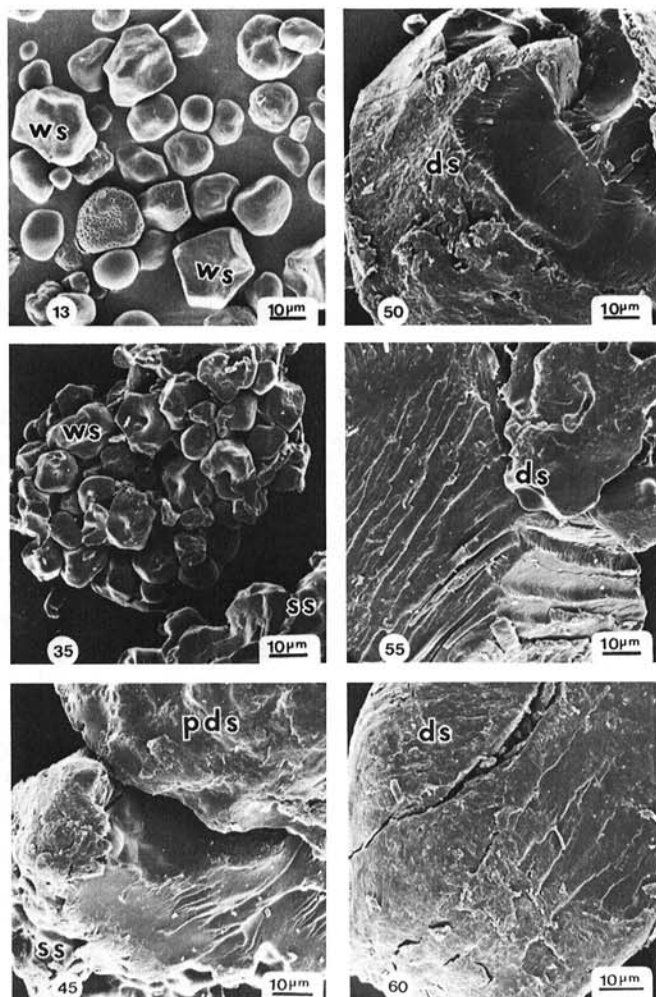


Fig. 7. Scanning electron micrographs of cornstarch retorted at various moisture contents. The numbers in circles indicate the retorting moisture contents of the samples; ws = whole intact starch, ss = swollen starch, pds = partially disintegrated starch, ds = disintegrated starch.

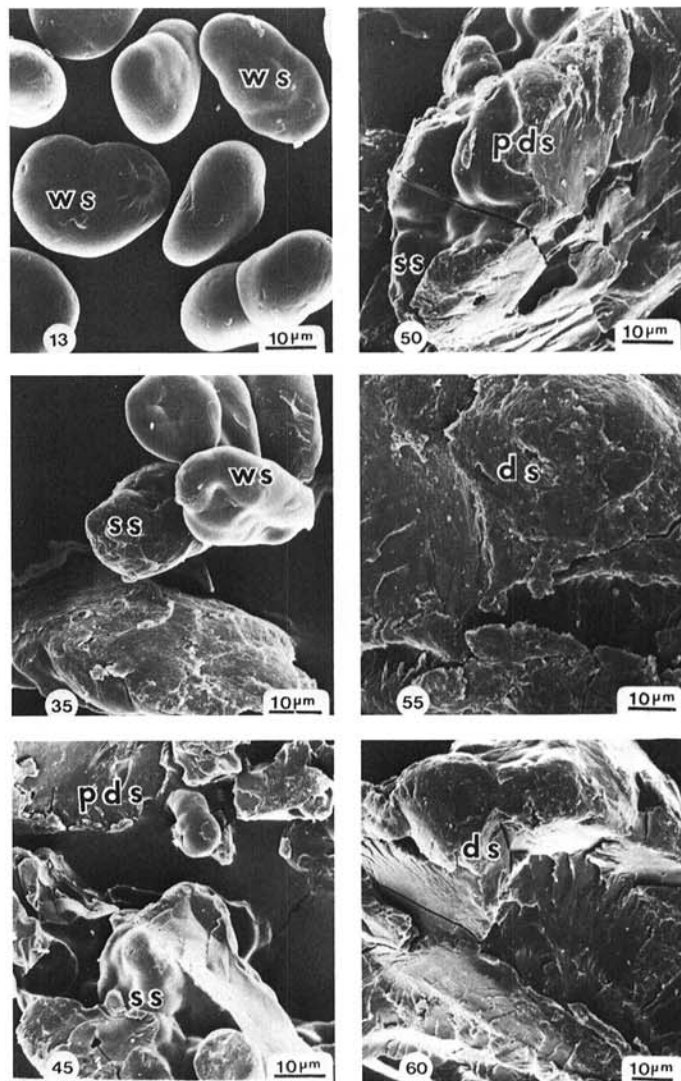


Fig. 8. Scanning electron micrographs of pea starch retorted at various moisture contents. The numbers in circles indicate the retorting moisture contents of the samples; ws = whole intact starch, ss = swollen starch, pds = partially disintegrated starch, ds = disintegrated starch.

of moisture content. Although not quantitative, it was the most appropriate approach to conclusively determine whether the instrumental and chemical methods reflected the physical changes taking place. Observations made with SEM (Figs. 7 and 8) paralleled the results obtained using both the X-ray diffraction and the alkali solubility methods. At 13% moisture content, the maize starch showed typical angular and rounded granules, whereas the pea starch exhibited the characteristic bilobed structure of legume starch. As the moisture content increased, the starches started swelling and eventually disintegrated. Complete gelatinization (total loss of characteristic structures) occurred at moisture levels of 45 and 55% and beyond for the maize and pea starches, respectively. Thus, both analytical methods seemed to measure the physical changes taking place during gelatinization.

CONCLUSION

X-ray diffractometry can be used to quantitatively estimate gelatinization of starches. The technique might be useful in estimating gelatinization of dry processed starchy products and, with the advent of new X-ray instruments with computer interfacing capabilities, for routine quality control purposes.

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