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EVIDENCES FOR A THIRD CLASS OF STARCH GRANULES IN BARLEY[1]

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1. INTRODUCTION

Starch, the main constituent of barley grains, contributes 60-64% of kernel dry weight, exists in the form of semi-crystalline granules deposited in kernels, stems, and leaves as reserve food supply for periods of dormancy, germination, and growth (*1*). In kernels, starch is deposited primarily in the endosperm, where it is synthesized and stored in amyloplasts (*2, 3*).

Several sizing techniques such as microsieving, Coulter Counter analysis, and digital image analysis (*4, 5, 6*) have been used to measure barley starch granule size, and typically these have shown a bimodal distribution for most barley genotypes. The two widely acknowledged classes of granules which comprise the bimodal distribution are the large, type A, and small, type B (*7*). The former reportedly ranges from 10 to 48 µm in diameter, and the latter from 1 to 10 µm (*4*).

MACLEOD and DUFFUS (*8*), in studying the effect of temperature on starch granules in developing barley kernels, speculated that there may be more than two classes of starch granules. More recently, BECHTEL et al. (*9*) suggested that there may be a third class composed of very small

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granules in wheat. Barley and wheat starches share many biochemical and morphological similarities.

Digital image analysis and statistical description using partial least squares curve fitting were applied in this investigation to study starch granule size distribution and learn whether the A and B granule classes fully describe the population of starch granules in barley. We have studied barley starch granule populations in mature kernels of three common barley varieties.

2. MATERIALS AND METHODS

Starch granule diameters were examined in mature kernels of three cultivars grown in Crookston, MN 1992: Alexis, Condor, and Excel. Diameters were measured by a digital image analyzer (Kontron Elektronic Gmbh, Germany), using a Sony CCD, model XC-77 video camera mounted on a Fisher microscope equipped with 40X (n.a. 0.65) objective lens.

A sample of 10 g of seed was ground on a Brinkmann sample mill (Retsch), using a 0.5 mm screen. One-half gram of the resulting flour was diluted in 50 mL sodium dodecyl sulfate (2% w/v) to solubilize crude protein, and was brought into suspension by rapid vortexing for 60 seconds. The suspension was then sonicated (Bransonic 2200) for two minutes. To stain the starch granules, a few drops of iodine solution were added to the suspension and the sample revortexed for 60 seconds. After the samples were stained with iodine, and immediately before each sample was placed on a slide for analysis, they were vortexed vigorously for 15 seconds to obtain a uniform homogenate representing the suspension. This step was important because small granules remain in suspension for a longer period than large granules. Next, 50 μL of suspension were removed by pipette and placed on a standard glass microscope slide and covered with an 18-mm square coverslip. For each cultivar seven to ten microscopic fields in each slide were measured.

Classes of granules have commonly been defined on a diameter basis (*6, 7, 9*), so "equivalent diameter" was chosen for this study. Equivalent diameter of a granule is defined as the diameter of a perfect circle with an area equal to that of the measured granule.

Collected data were transformed to a logarithmic scale and granule equivalent diameter distributions were studied using the software "PeakFit, V 3.0" (Jandel Scientific, USA). PeakFit uses a non-linear least-square curve fitting technique based on the Marquardt-Levenburg algorithm. This algorithm switches between two methods in attempting to step toward a global minimum sum of squares that represents optimum curve fitting (*10*). This procedure reduces noise and separates unresolved curve peaks, detecting the presence of subclasses in the population. The classes eventually detected were characterized for peak center, peak width, and peak amplitude and area under the curve.

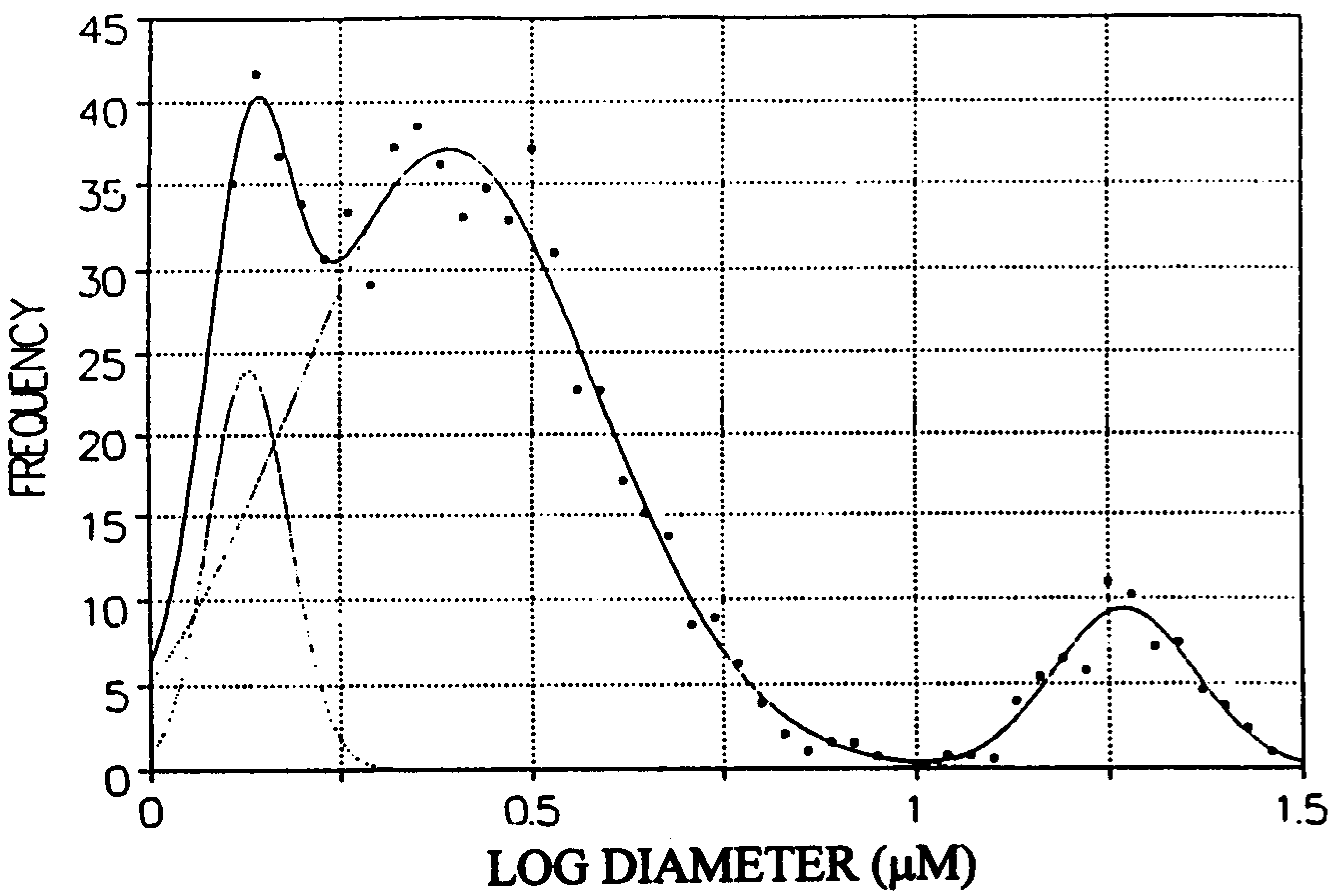
3. RESULTS AND DISCUSSION

Granule frequency was plotted as a function of equivalent granule diameter, expressed in logarithmic scale. Peak amplitude (PkAmpl), peak center (PkCtr), width at half of the maximum frequency (Wid@HM), area under the curve, area under the curve expressed in percentage of the total, and coefficient of determination are presented for granule distributions in Figures 1 to 3. The area under the curve represents the total number of starch granules for a specific diameter range.

Barley starch granule distribution has been examined by different researchers. Typically these studies have indicated only two subpopulations of granules, i.e., starch granule diameter falls into two classes (*6, 7*). In contrast, our analyses of granule distribution indicated the presence of three Gaussian subpopulations (Figure 1 to 3). The three subpopulations (or three granule classes), are visible in each figure. Significantly, the two peaks with the smallest diameter centers are difficult to identify in the original spectrum. However, these analyses indicate the existence of a second class of starch granules, within what is generally called the B granule class. To facilitate the discussion, the three identified subpopulations of granules are labeled A, B, and C.

The best curve fit for granule diameter distribution for variety Alexis (Figure 1) shows three Gaussian curves. The C granule Gaussian curve stands by itself (dotted line), while the B granule is shown in dotted and continuous lines. The A granule Gaussian curve corresponds to diameters between 1.0 and 1.5 in logarithmic scale (Figure 1). There was a substantial overlap between B and C granule distributions. This kind of overlap is probably the main reason why less-discriminating analytical tools failed to detect the third class of starch granules. A granule diameters peaked at 1.269, while B granules had a peak center at 0.389. The smallest granules, C type, had a peak center at 0.131 (Figure 1). A, B, and C Alexis-starch granules were 10.0%, 80.2% , and 9.8% of the total number of granules, respectively.

Figure 2 shows the granule diameter distribution for variety Condor. A granules averaged at 1.263 and B granules peaked at 0.447. Condor's smallest granules, type C, peaked at 0.164. A and B granules were 9.6% and 72.1% of the total number of granules, while C granules accounted for 18.2% of the total starch granule number, up 8.4% in relation to Alexis C granules (Figure 1).

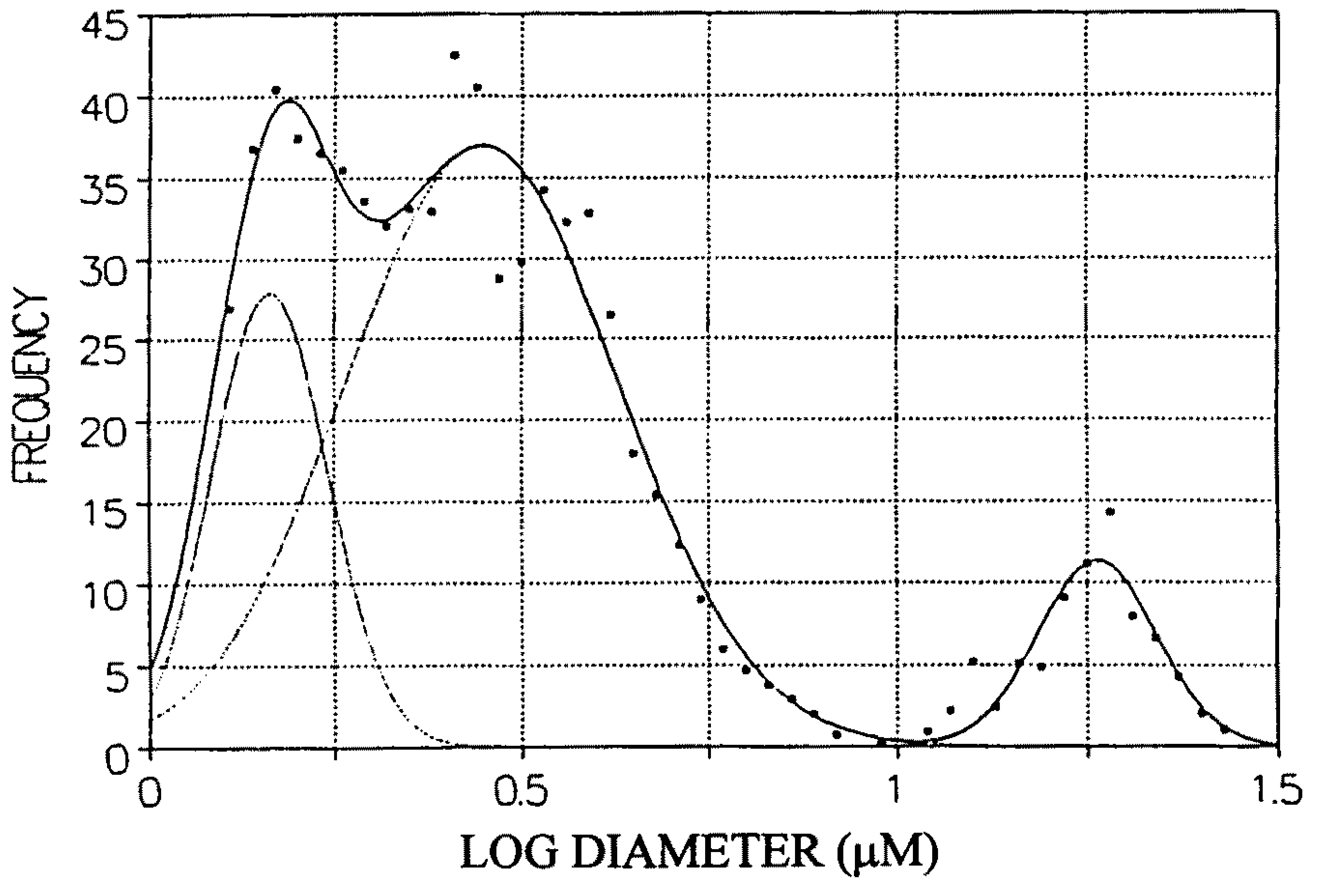


Curve-Fit Parameters:
Curve-Fit Std Error= 1.8563 r^2= **0.9865**

Measured values:

Peak#	Granule class	Type	PkAmpl	PkCtr	Wid@HM	Area	%Area
1	C	Gaussian	23.9581	0.13143	0.12224	2.0581	9.7969
2	B	Gaussian	37.0089	0.38926	0.46397	16.8490	80.2035
3	A	Gaussian	9.3452	1.26939	0.21515	2.1007	9.9999
		Total				21.0079	100.0000

FIGURE 1 - Starch granule diameter distribution and statistical summary of fit for Alexis.



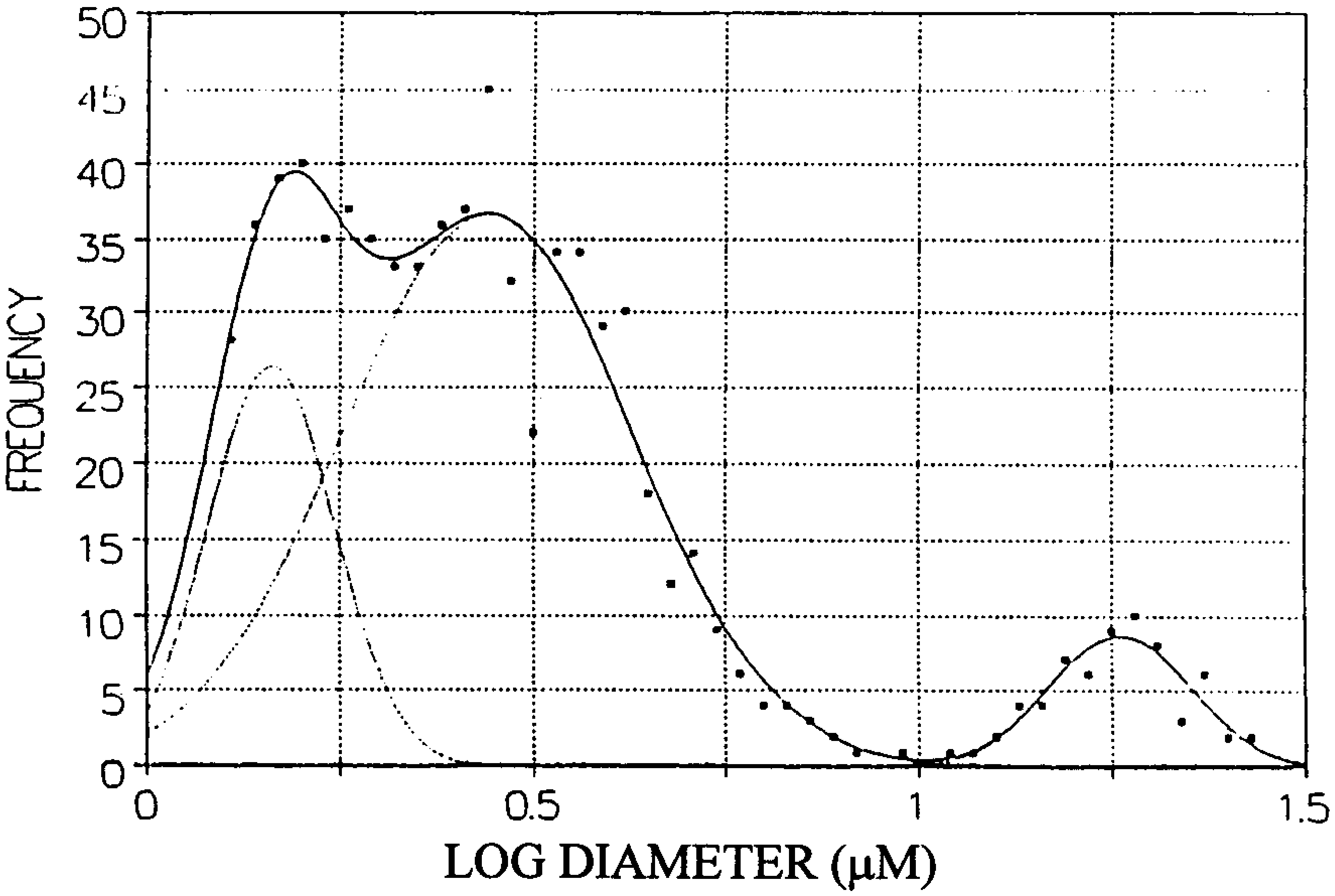
<u>Curve-Fit Parameters</u>:
Curve-Fit Std Error= 2.7261 r^2= 0.9722

<u>Measured values</u>:

Peak#	Granule class	Type	PkAmpl	PkCtr	Wid@HM	Area	%Area
1	C	Gaussian	27.9375	0.16377	0.18206	4.0966	18.2292
2	B	Gaussian	36.9655	0.44761	0.42504	16.2116	72.1407
3	A	Gaussian	11.2608	1.26334	0.18350	2.1638	9.6295
	Total					22.4712	100.0000

FIGURE 2. Starch granule diameter distribution and statistical summary of fit for Condor.

Likewise, variety Excel granule diameter distribution, shown in Figure 3, had peak centers at 1.261, 0.441 and 0.162, for A, B, and C granules, respectively. Excel had the lowest percentage of A granules among the three cultivars, although no statistical procedure was available to test this hypothesis. The most frequent subclass of granules, B type, accounted for 73.6% of the total.



Curve-Fit Parameters:

Curve-Fit Std Error= 3.3745 r^2= 0.9591

Measured values:

Peak#	Granule class	Type	PkAmpl	PkCtr	Wid@HM	Area	%Area
1	C	Gaussian	26.3276	0.16236	0.19078	3.9616	17.7937
2	B	Gaussian	36.6609	0.44108	0.43619	16.3934	73.6304
3	A	Gaussian	8.6038	1.26052	0.21535	1.9093	8.5757
		Total				22.2645	100.0000

FIGURE 3 - Starch granule diameter distribution and statistical summary of fit for Excel.

Our results are similar to those of KARLSSON and OLERED (*11*) and PEDERSEN (*6*) in revealing a paucity of granules around diameter of 10 µm. Our results, however, indicated that granules smaller than 10 µm also fit a distribution that is bimodal, leading to the hypothesis that three subpopulations of starch granules exist.

The non-linear least-square curve fitting algorithm permitted us to identify less distinct granule classes than in the past. This newer procedure is potentially superior to those used by other workers (*6, 7 ,11*).

The existence of a third class of starch granules does not mean that pooling B and C granules together in a single class, as has been the case, invalidates inferences about them, but rather implies that a reassessment of granule properties, considering them as two subpopulations, could shed new light on their functions and ontogeny. Evaluations of potential differences in behaviour of these different sub-populations during processing are underway.

4. CONCLUSIONS

In conclusion, the results suggest the existence of three classes of starch granules rather than the widely accepted two classes. In this case, the barley starch granule size distribution would be trimodal.

5. SUMMARY

Starch constitutes 60-64% of barley grains, and occurs in the form of granules which range in size from 1 to 48 µm in diameter and traditionally are considered to fall into two major size classesThe objective of this study was to investigate starch granule size distribution in mature kernels of Alexis, Condor and Excel barley cultivars. A non-linear least square curve fitting technique was used to study granule diameter distributions. The curve fitting procedure revealed a trimodal distribution of starch granules, characterized by three Gaussian curves, indicating three classes of granules, A, B and C. The A granule distribution was distinct from B and C granule distributions, while there was substantial overlap between B and C granule distributions. On average, A granules were characterized by a curve with peak center at a diameter of 18.68 µm, while B and C granules had peak centers at 2.60 µm and 1.42 µm, respectively. A, B, and C granules were 9.4%, 75.3% and 15.3% of the total number of granules, respectively. These results suggest the existence of three classes of starch granules rather than the widely accepted two classes.

6. RESUMO

(EVIDÊNCIAS DE UMA TERCEIRA CLASSE DE GRÂNULOS DE AMIDO EM CEVADA)

O amido, constituindo aproximadamente 60 a 64% do peso dos grãos de cevada, ocorre em forma de grânulos que variam de 1 a 48 μm de diâmetro e são comumente agrupados em duas classes. O objetivo deste estudo foi investigar a distribuição dos tamanhos dos grânulos de amido em grãos maduros das variedades de cevada Alexis, Condor e Excel, por meio de uma técnica não linear de ajustamento de curvas. O processo de ajustamento das freqüências dos grânulos revelou distribuição trimodal, caracterizada por três curvas gausianas, indicando a existência de três classes de grânulos, A, B, e C. A distribuição dos grânulos A foi distinta das distribuições dos grânulos B e C, uma vez que ocorreu substancial sobreposição entre as classes B e C. Em média, os grânulos A foram caracterizados por um pico de diâmetro de 18,68 μm , enquanto os grânulos B e C tiveram 2,60 μm e 1,42 μm, respectivamente. Os grânulos A, B, e C corresponderam a 9,4%, 75,3% e 15,3% do número total de grânulos, respectivamente. Esses resultados sugerem a existência de três classes de grânulos de amido de cevada, em vez das duas classes amplamente reconhecidas.

7. REFERENCES

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