Comparison of Amylose Enrichment Procedures for Food Applications

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ABSTRACT

An amylose-rich product was developed using commercial corn and potato starch sources. The product is targeted to food industry applications such as biodegradable packaging, edible films, and coatings. Starch fractionation techniques such as leaching, complexing with low molecular weight alcohols, and fractional precipitation with salts were explored and compared. Optimum conditions of centrifugation speed, temperature, and leaching time were determined. The critical concentration of 1-butanol for the maximum amylose enrichment was found. The amylose-rich product was obtained by fractional precipitation with 10% $MgSO_4$. The combination of alkaline dispersion and fractional precipitation with $MgSO_4$ was the most effective technique. Optimum NaOH concentrations and temperatures for corn and potato starches were determined. Amylose-to-amylopectin ratio in the dehydrated suspension (final product) was 51:49 for corn and 50:50 for potato starch. Both ratios were similar to the ratio of starches derived from high-amylose genetic varieties. One advantage of these amylose-enriched products is the food grade quality ($MgSO_4$ residues are nontoxic). Another advantage is the nondrastic dispersion conditions for food industry applications. These amylose-rich products provide an alternative where starches derived from high-amylose genetic varieties are not available at competitive prices.

Amylose applications are based on its chemical, physical, and functional properties, particularly in its gel- and film-forming capabilities. Amylose is used in the textile industry as a sizing and finishing agent; in the food industry as a thickening, stabilizing, gelling, and encapsulating agent; and in the paper industry for adhesives, binding agents, and surface-sizing applications (Young 1984). Amylose is also a major raw material in some edible films and biodegradable packagings (Kester and Fennema 1986).

Common amylose sources are inappropriate to food industry purposes because of the chemical residues left by the fractionation methods. To overcome this restraint, high-amylose genetic varieties of corn and potato have been developed. Starch derived from high-amylose genetic varieties contains up to 70% amylose. However, these starches are not readily available in every country.

Starch fractionation has been the focus of several studies since the 1940s, but information is scarce because most of the research was disclosed only in patents (Schoch 1942, Muetgeert 1961, Banks and Greenwood 1975). Isolation of amylose and amylopectin, the main starch components, is based on differences in size and chemical nature. Both are D-glucose polymers, amylose being a linear polymer and amylopectin being branched. The more common techniques for separation include selective leaching or lixiviation, complex formation, and fractional precipitation by salts (Young 1984).

The selective leaching method employs the preferential solubilization of one of the components. When a starch suspension in excess water is heated at or above its gelatinization temperature, starch granules become disordered and swell to many times their original size. At this point, amylose molecules diffuse more easily than amylopectin molecules, which remain by hydrogen unions or crystallized inside the granule (Noel et al 1992). Solubilized amylose is separated by centrifugation. Successive leaching processes give amylose with increasing molecular size (Banks and Greenwood 1975). Different approaches or modifications to the leaching method have been published (Baum and Gilbert 1956, Muetgeert 1961, Miles et al 1985b). Selective leaching offers a preliminary separation process, but it requires additional purification techniques.

The complexing method is based on the capacity of amylose to form stable complexes with alcohols of low molecular weight (up to eight carbon atoms), which in turn give microcrystalline precipitates. The amylose in the complex assumes a V-type structure with a left-handed helix conformation; the complexing agent is located within the central cavity of the helix. Van der Waals forces stabilize this structure. The most studied complexes of this type are those of amylose-I_2. When using alcohols (1-butanol or 1-octanol), hydrogen bonds also participate in the stabilization of this type of structure. The crystals of complexed amylose might contain substantial amounts of amylopectin; thus recrystallization becomes necessary, as well as multiple washings to eliminate the complexing agent. The most commonly used complexing agent for the separation technique is 1-butanol. Its complexes are soluble in hot water but insoluble in cold water. This difference enables easy and quick recrystallization (Schoch 1942, Killion and Foster 1960, Banks and Greenwood 1975).

Gradual addition of a precipitating agent to a solution containing at least two polymers causes the polymers to precipitate separately; the polymer with the more isotactic structure will precipitate first (Young 1984). This is known as the fractional precipitation method. Sodium citrate and sulfates of sodium, ammonium, and magnesium are precipitating agents commonly used (Muetgeert 1961, Bus et al 1958), with magnesium sulfate showing the best results. Amylose can be fractionally precipitated from an aqueous starch dispersion containing 9–10% $MgSO_4$. Temperatures of 140–160°C are generally used for fractional precipitation. At these temperatures, thermal degradation reactions are unavoidable.

When salt concentration reaches 13%, amylopectin flocculates and can be separated by filtration. Fractional precipitation with salts yields an amorphous amylose precipitate. This technique can be combined with complex formation by adding 1-butanol; although no crystalline precipitates are obtained, a higher efficiency is reached (Muetgeert 1961).

The objectives of the present work were to: 1) compare different amylose fractionation techniques to obtain an amylose-rich product for the food industry with characteristics similar to those of starches derived from high-amylose commodities; 2) incorpo-

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rate suitable modifications to optimize the selected technique; and 3) analyze the influence of starch source on the method efficiency.

MATERIALS AND METHODS

Commercial corn starch (Refinerías de Maíz, Argentina) with an amylose-to-amylopectin ratio of 25:75, containing 11.3% water, 0.3% proteins, 0.6% lipids, and 1.3% ash (wb) was used. Commercial potato starch (PROINT, Argentina) with an amylose-to-amylopectin ratio of 23:77 was used only in the fractional precipitation assay. Amylose and amylopectin of reagent grade (Sigma) were used to prepare standard solutions. The other chemicals used were also reagent grade.

Determination of Amylose and Amylopectin Content

To compare the effectivity of the fractionation methods, appropriate analytical techniques to determine amylose and amylopectin concentration are needed (Williams et al 1970, Banks et al 1971). The spectrophotometric iodine technique was selected because of its simplicity and precision. This method allows the simultaneous determination of amylose and amylopectin concentrations, assuming that both amylose-I_2 and amylopectin-I_2 complexes contribute additively to the total absorbance of the mixture. Absorbances of the complex were measured at the wavelengths of maximum absorption (Landers et al 1991).

The stock iodine solution contained 2 mg/ml of I_2 and 20 mg/ml of KI (Lyne 1976). Amylose and amylopectin standards were solubilized in 50 ml of boiling water. Samples were centrifuged in a Sorval RC-5B refrigerated, superspeed centrifuge (Du Pont Instruments) at 2,310 × *g* for 10 min to separate the insolubles. In all cases, aliquots of supernatant were diluted 25 times. Iodine solution (0.3 ml) was added to the supernatants, and the absorbances were measured in a Shimadzu Double Bean Spectrophotometer (UV-150-02, Seisakusho Ltd., Kyoto, Japan). Solutions of both complexes were scanned between 400 and 700 nm. Calibration curves were used to determine the absorption coefficients of the complexes.

Starch Fractionation by Leaching

Corn starch dispersions of 10% were used. To detect the optimum centrifugation speed, the dispersions were heated in a water bath at 72°C for 30 min. Samples were centrifuged for 10 min at 577, 2,310, 5,190, and 9,220 × *g*. To determine the optimum incubation temperature, the dispersions were heated in a water bath at 75, 80, 85, 90, 95, and 98°C. Optimum incubation time was tested at 15, 20, 30, 45, 60, and 90 min. The optimum conditions were determined by measuring the absorbance of the amylose-I_2 complex at the wavelengths of maximum absorption.

Fractionation by 1-Butanol Complexing Agents

Corn starch dispersions of 10% were incubated at 85°C for 20 min to allow amylose to leach. Samples were cooled and then centrifuged at 2,310 × *g* for 10 min. Supernatants were collected and mixed with 1-butanol at 50°C in a decanter and stirred thoroughly for 10 min. The volumetric fraction of 1-butanol varied from 0.0125 to 0.1. Aqueous phase was decanted and allowed to cool to favor complex precipitation. Then, samples were centrifuged for 15 min at 2,310 × *g* to determine noncomplexed amylose in the supernatants. The complexing agent effectivity was calculated as the difference between the absorbances of amylose-I_2 complex after leaching and after complexing.

Fractionation by 1-Octanol Complexing Agents

A procedure similar to that using 1-butanol was followed. The aqueous-to-organic phase ratio was 50:1. The mixture was agitated at ≈60°C for 30 min and then centrifuged for 15 min at 2,310 × *g*.

Fractional Precipitation with $MgSO_4$

Samples of either 5% corn or potato starch were treated with 10% $MgSO_4$ and small amounts of Na_2SO_3 to prevent degradative reactions of the amylose. Starch dispersion is the greatest disadvantage of this technique (Bus et al 1958, Muetgeert 1961). Three dispersion methods were assayed: alkaline, acid, and autoclaving. The dispersion treatment with dimethyl sulfoxide (DMSO) was not considered in the present study because of the high toxicity of the DMSO.

Samples were dispersed in 0.2*N* NaOH at room temperature and then heated at 85°C for 30 min. Neutralization was performed with 50% H_3PO_4 with phenolphthalein as an indicator. Samples were centrifuged at 2,310 × *g* for 10 min, and supernatants were spectrophotometrically analyzed.

The acid dispersion was made with 0.2*N* HCl, then neutralized with 1*N* NaOH. The other steps were performed as described for alkaline dispersion.

A third group of samples was autoclaved for 15 min at 1.5 atm and processed as described previously.

To find the best conditions for alkaline procedure, 0.25, 0.50 and 0.75*N* NaOH were tested. Dispersions temperatures assayed were 25, 55, 60, 65, 75, 80, and 85°C. Heating time was 30 min for all experiments.

The efficiency (η) of the fractional precipitation technique represents the amylose recovery evaluated as:

$$\eta = (p/a_i)100 \quad (1)$$

where p is the amylose content of the amylose-rich product determined spectrophotometrically (dwb), and a_i is the amylose content of the initial starch samples (dwb).

Fractionation of the Amylopectin-Rich Product

Samples of either 5% corn or potato starch were treated with 13% $MgSO_4$. The procedure followed was the same as that described for amylose fractionation and efficiency determination.

Native Starch Lipids Extraction

The presence of native starch lipids could reduce the amount of free amylose by complex formation. Native starch lipids consist of free fatty acids (monoglycerides) and lysophospholipids (Tester and Morrison 1992). The monoglycerides can be combined with amylose to form known amylose-lipid complexes that are more stable than those of amylose-I_2 (Juliano et al 1981). To increase fractionation efficiency, the need of a previous extraction of native lipids was evaluated. The method of Sowbhagya (Sowbhagya and Bhattacharya 1979) was modified. Lipids were extracted from both starches with a Soxhlet extractor at 45–55°C. The first extraction was performed with petroleum ether and the second one was performed with methanol; extraction times were 4 and 8 hr, respectively, for corn starch, and 3 and 6 hr, respectively, for potato starch. Samples were then processed according to the predetermined optimum parameters.

Drying and Solubilization Procedures

To analyze the effect of drying on solubilization properties of fractionated amylose and amylopectin precipitated with $MgSO_4$, several drying procedures were tested: a) precipitates were washed with distilled water and dried at 60, 70, 80, and 100°C in an oven; b) precipitates were washed with ethanol and dried at 70°C in an oven; and c) precipitates were freeze-dried in Thermovac equipment. Drying times and precipitate characteristics were recorded.

Solubility was determined with 30 mg of the dried precipitates to which 40 ml of boiling distilled water was added. Suspensions were cooled at room temperature and then centrifuged for 10 min at 2,310 × *g*. The supernatants were transferred to 100-ml volumetric flasks and brought to volume. Then samples were analyzed spectrophotometrically to determine solubilized amylose and

amylopectin. The differences between the weight of the tubes with the dried samples and those with the insoluble residue after drying gave the amounts of solubilized sample. The purity of the solubilized fraction was calculated by the spectrophotometric method.

Determination of Residual Amount of Sulfates

A stock solution of K_2SO_4 containing 100 mg/L was prepared to obtain a series of dilutions to determine the calibration curve. Different amounts of the stock solution were transferred to 100-ml volumetric flasks; 4 ml of conditioning solution (50 ml of glycerol, 30 ml of HCl, 300 ml of water, 100 ml of 96 ethanol, and 75 g of NaCl), and 0.3 g of $BaCl_2$ were added. The solution was brought to volume. Solutions were carefully mixed and measured immediately in the spectrophotometer at 400 nm. The blank was a 0 mg/L of K_2SO_4 solution.

To measure the residual sulfates of the precipitates, 30 mg of sample was weighed and solubilized as described for the solubility determination. Aliquots of 10 ml were transferred to a 100-ml volumetric flask, as for the calibration curve; equal amounts of $BaCl_2$ and conditioning solution were added and solution was brought to volume. Three replicates were analyzed with the spectrophotometer.

Microscopy Observation

Treatment effect on starch granule swelling was analyzed by phase-contrast microscopy. A drop of each sample was placed on a slide and micrographed (Ortholux 2, Vario Orthomat, Leitz, Germany).

Statistical Analysis

Software (Systat 1990) was used for all statistical analyses, including analysis of variance (ANOVA), Fisher least significant difference (LSD), mean comparison test, and regression analysis.

RESULTS AND DISCUSSION

Determination of Amylose and Amylopectin Concentrations

The maximum absorbance of the amylose-I_2 complex corresponded to 615 nm for corn starch and to 620 nm for potato starch. The maximum absorbance of the amylopectin-I_2 complex corresponded to 535 nm for corn starch and to 540 nm for potato starch. The differences between the maximum absorbances of both starches should be attributed to the different distribution of molecular chain lengths, which depends on the vegetable origin. The uptake of I_2 varies because the complex stoichiometry is determined by the number of bound I_2 molecules per amylose helix (Murdoch 1992).

Figure 1 shows the absorbance spectra of amylose-I_2 and amylopectin-I_2 complexes (solid and dotted lines respectively).

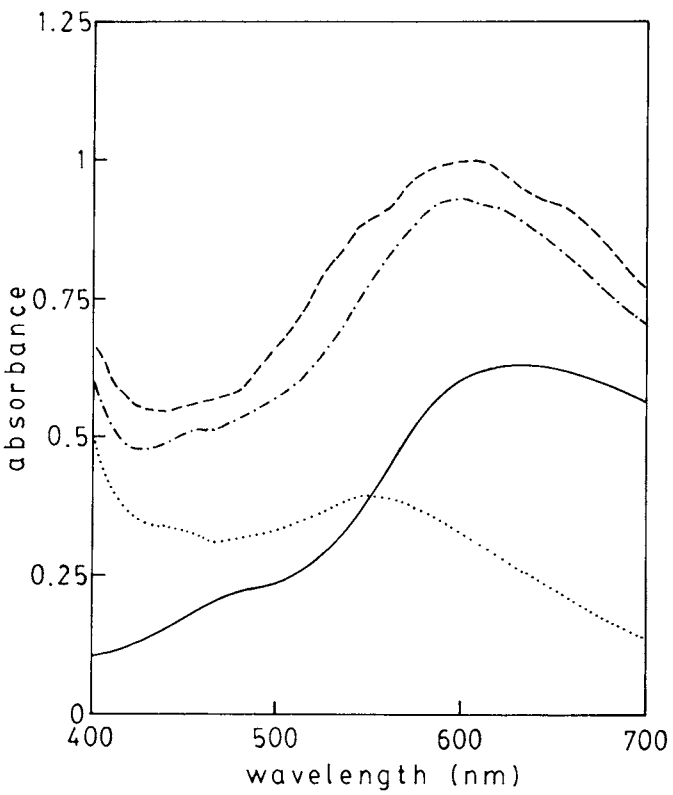

Fig. 1. Absorbance spectra of amylose-I_2 and amylopectin-I_2 complexes. —— = Amylose standard solution (2.4×10^{-5}g/ml). · · · · · = Amylopectin standard solution (1×10^{-4}g/ml). – · – · – = Amylose-amylopectin mixture (2.4×10^{-5}g/ml in amylose and 1×10^{-4}g/ml in amylopectin). - - - - - - = Curve obtained by adding amylose and amylopectin absorbance values.

TABLE I
Amylose and Amylopectin Absorption Coefficients[a]

	Absorption Coefficients, ml/g cm	
Wavelength, nm	Amylose	Amylopectin
535	17,900 (600)	7,100 (700)
540	19,800 (800)	7,300 (700)
615	33,200 (800)	5,900 (400)
620	33,500 (800)	5,800 (400)

[a] Standard deviation in parentheses

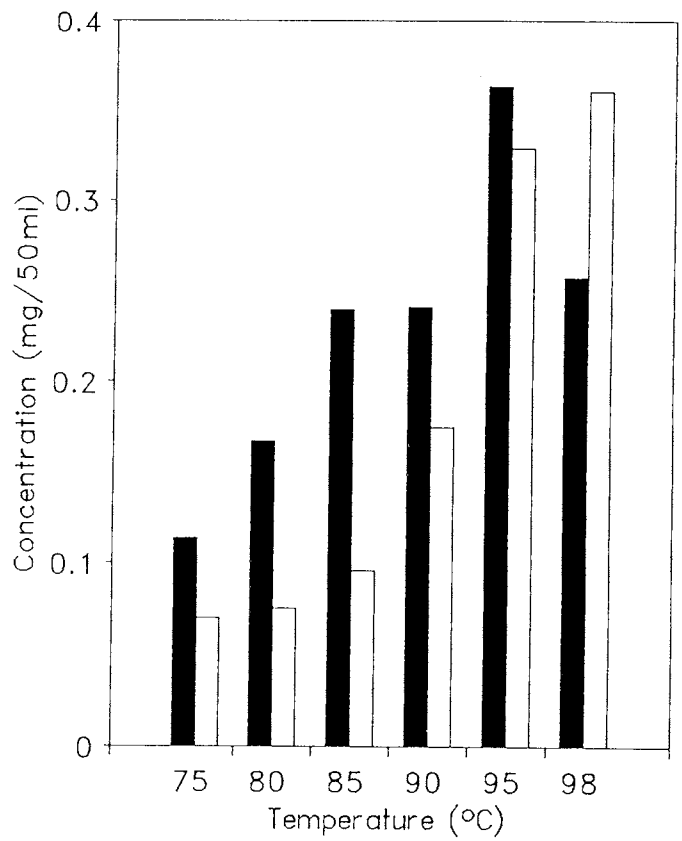

Fig. 2. Effect of temperature on amylose and amylopectin leached. ■ = Supernatant amylose concentration. ❑ = Supernatant amylopectin concentration.

Maximum absorbances of amylose and amylopectin complexes are not clearly separated. Thus, amylopectin absorbance at the maximum wavelength of amylose complex could not be ignored. The absorbances additivity was confirmed when the curves of the mixture (dashed line) and the addition of the individual curves (center line) were compared.

The absorption coefficients of amylose and amylopectin complexes were obtained from the calibration curves (Table I). Due to the flat shape of the spectrum curve in that region, no significant differences ($P < 0.05$) were found between the absorption coefficients at 615 and 620 nm for either amylose or for amylopectin. Correlation coefficient of the calibration curve was >0.969 for amylose and >0.843 amylopectin. Landers et al (1991) reported similar correlation coefficients of 0.97 and 0.72 for amylose and amylopectin, respectively. The lower correlation coefficient of amylopectin, compared to that of amylose, could be attributed to the limited amylopectin solubility. As the obtained amylopectin concentrations were low, the absorbances values were low too, and thus subjected to a higher error. Solubility was a critical factor in both, quantification and later uses of amylose and amylopectin. Similar problems occurred when standard solutions were prepared.

Leaching Method

Significant differences ($P < 0.05$) in absorbance measurements were obtained by centrifugation at 2,310 × g, thus this speed was selected for maximum amylose enrichment. Figure 2 shows the results used to determine the incubation temperature of 85°C, although the supernatant concentrations obtained at 95°C were higher. The LSD test did not show significant differences ($P > 0.05$) between the amount of leached amylose at 85 and at 90°C. However, the amylose purity varied, since the amount of leached amylopectin was not significantly different ($P < 0.05$). At temperatures higher than 90°C, the granule became more fragile, and the probability of amylopectin leaching also increased. Although a higher amount of amylose was obtained, it was contaminated with amylopectin. These results are in accord with other findings regarding amylose leaching (Tester and Morrison 1992, Miles et al 1985a). Thus, the best compromise condition for both quantity and purity was 85°C.

The highest amylose contents were obtained at 20 and 30 min of incubation; no significant differences ($P > 0.05$) were found between these times. A longer incubation time led to a decrease in the amount of amylose in the solution, which could be due to a gel formation involving amylose. This gel-like structure increased its viscosity with increasing incubation time. When samples were agitated, a significant effect ($P < 0.05$) on amylose leaching was observed. However, the uniform gel structure developed made the amylose recovery difficult.

Fractionation by Complexing Agents

Amylose-I_2 complex used to quantify the amount of amylose has a lower affinity than that of amylose and 1-butanol or amylose and 1-octanol complexes. So in the spectrophotometric assays, the measured amylose is the amylose that remains soluble, not the amylose complexed with the alcohol.

Complexing agent concentrations in aqueous systems are of critical concern. Each complexing agent has its own specific concentration region in which it exhibits optimum fractionating properties (Muetgeert 1961). The optimum concentration of a complexing agent is also known as critical concentration. Muetgeert et al (1956) found that complexing agent (critical concentration) in quantities lower than the solubility yielded better amylose separations. Otherwise, complexes may form with the outer branches of the amylopectin and reduce the effectiveness of the separation. The optimum concentration of 1-

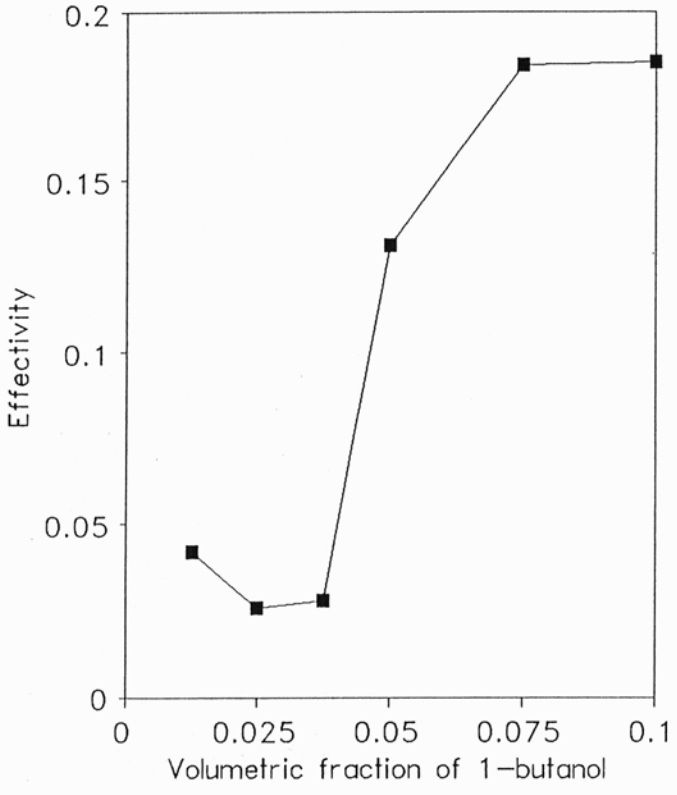

Fig. 3. Influence of 1-butanol volumetric fraction on the effectivity of the complexing agent. Effectivity was the difference between the absorbance of amylose-I_2 complex of the leaching supernatants (before addition of the complexing agent) and the absorbance of amylose-I_2 complex after 1-butanol addition.

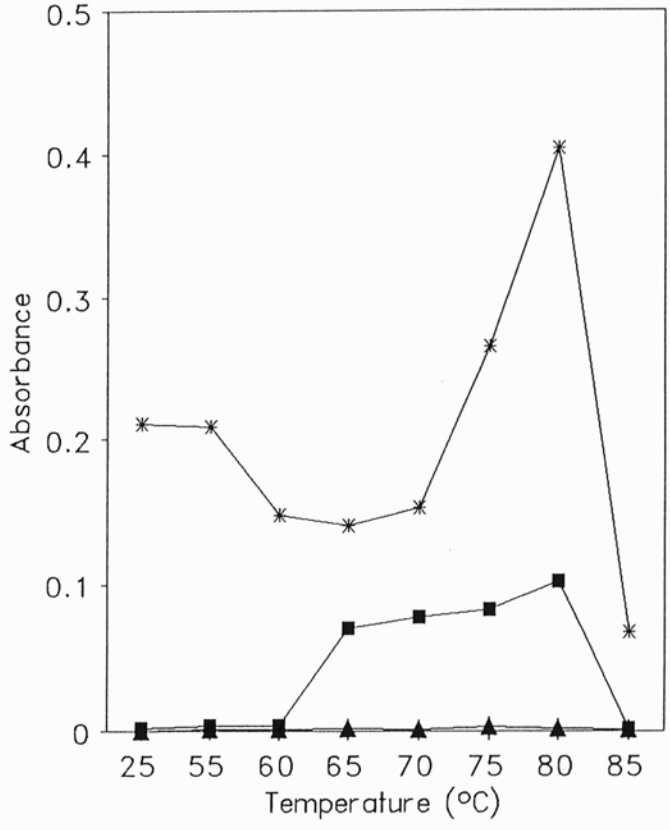

Fig. 4. Typical absorbance curves for alkaline dispersion (0.5N NaOH) of 5% corn starch as a function of dispersion temperature. Absorbances of amylose-I_2 complex: ■ = supernatants without washings; ▲ = after one washing; ∗ = solubilized precipitate.

butanol was determined; working conditions were within the range of maximum solubility of 1-butanol in water. Muetgeert et al (1956) reported a critical concentration for 1-butanol of 4.2 g/100 ml at 20°C and a solubility of 7.9 g/100 ml; for 1-octanol a critical concentration was 0.04 g/100 ml and solubility of 0.13 g/100 ml.

Figure 3 shows the effectivity of 1-butanol as a function of the volumetric fraction of 1-butanol. In the present work, increasing 1-butanol fraction increases the complex amount up to the solubility limit. The amylose-to-amylopectin ratio obtained with 1-butanol was 67.4:32.6.

Mechanisms of amylose complex formation with I_2, 1-butanol, and 1-octanol are similar (Whittman et al 1989). The 1-octanol was more effective for complexing amylose, because the complexing agent effectivity (previously defined) was 0.080 for 1-butanol and 0.154 for 1-octanol.

Unless the amylose-alcohol complex was centrifuged immediately after the precipitate was formed, the efficiency was lowered. Once the complex is isolated, the amylose should be recovered. Although several techniques were proposed (Schoch 1942, Miles et al 1985b), the isolated amylose maintained a residual amount of 1-butanol of 5–10%. Taking into account that 1-octanol is hard to eliminate and is also more toxic than 1-butanol, the use for food purposes of 1-octanol as complexing agent is not recommended.

Starch Fractionation by Fractional Precipitation

Acid treatment resulted in insufficient dispersion. Autoclaving was too drastic; it produced a stable gel structure. Amylose molecules that take part in the gel network were not available for $MgSO_4$ precipitation. Moderate conditions like those used in the alkaline dispersion were the most effective. In a first step, the alkali favored amylose leaching, then solubilized amylose was induced to precipitate by the salt without gel development.

Although Na_2SO_3 is added, some degradative reactions may occur: mainly the inclusion of anomalous groups and the alteration of the molecular size, because starch is very sensitive to oxidation and hydrolysis (Baum and Gilbert 1956). Baum and Gilbert (1956) fractionated amylose from 0.79% starch slurries with cold dilute alkali. Dispersion was performed without $MgSO_4$ and after 2 hr of centrifugation at 40,000 × *g*, they obtained an amylose enrichment of 58%. When this technique was adapted to our conditions (10% starch slurry and centrifugation at 20,800 × *g* for 30 min), no amylose separation was obtained because of gel formation. The technique proposed in the present work is important because it incorporates the combined use of the alkaline medium as a dispersing agent with fractional precipitation by $MgSO_4$. It should be pointed out that the alkali should be added to the starch solution containing the salt to avoid gel formation. Granule breakdown should be avoided since amylopectin might diffuse outside the granule and contaminate the precipitate, which is rich in amylose, although some amylopectin will be eliminated during the washings.

The optimum conditions for corn and potato starch were found. Figure 4 shows typical absorbance curves for alkaline dispersion of corn starch as a function of dispersion and temperature. The amylose lost with the supernatants and during the washings was negligible. When two and three extra washings were assayed, the amylopectin content did not differ significantly ($P > 0.05$), thus only one washing was enough for amylopectin elimination.

The variables starch source, dispersion temperature, and dispersion medium concentration, and their interactions were statistically analyzed. ANOVA showed a significant ($P < 0.05$) effect of the three variables and a very significant ($P < 0.01$) interaction between medium concentration and dispersion temperature. The highest temperature assayed was 90°C; as described in leaching,

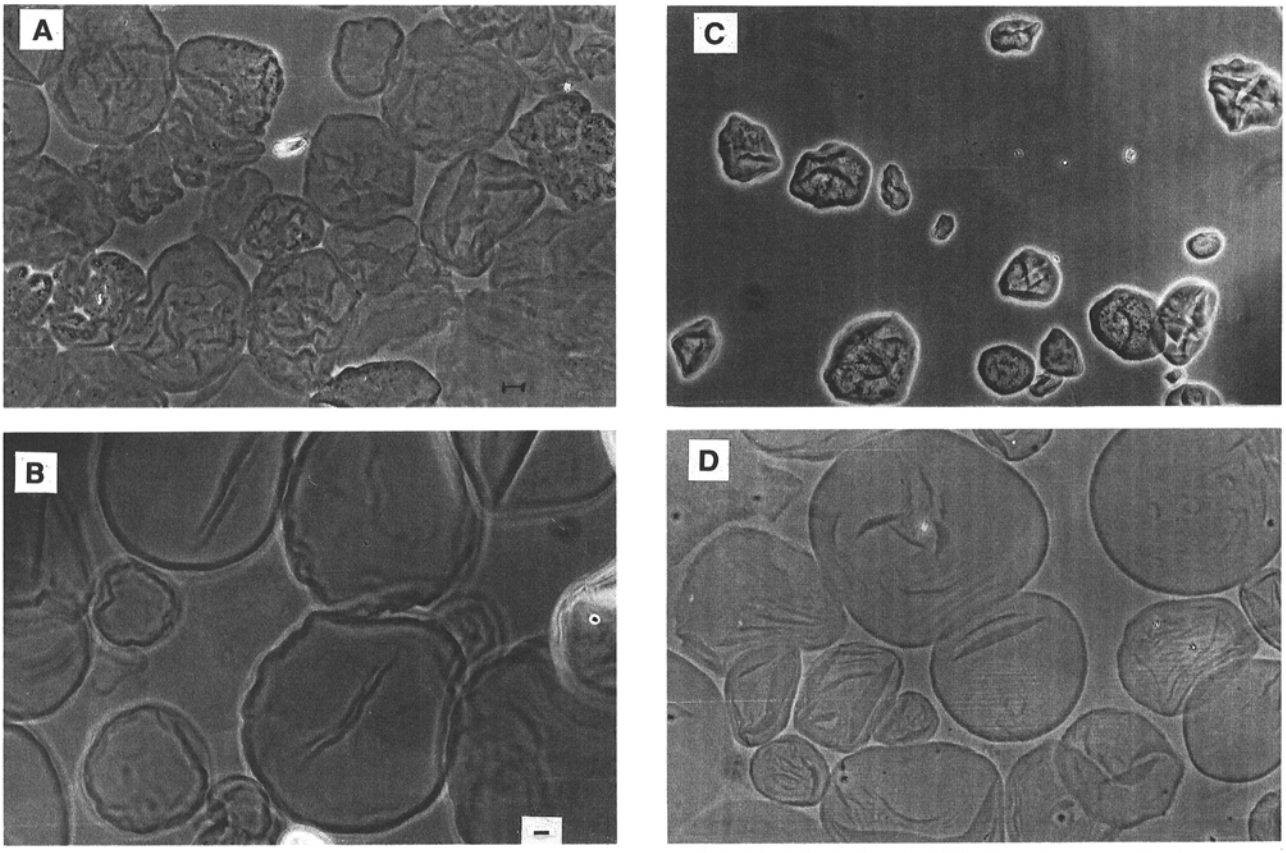

Fig. 5. Micrographs of 5% starch dispersions: **A,** corn starch in 0.5 NaOH; **B,** potato starch in 0.25 NaOH; **C,** corn starch in distilled water; **D,** potato starch in water. Bar = 10 μm.

increasing the temperature led to a higher amylopectin contamination.

Increasing the $MgSO_4$ concentration to 13% produced an alternative product enriched in amylopectin. The efficiencies of this procedure were 89.9 and 72.9% for corn and potato starch, respectively. The amylose-to-amylopectin ratios were 14.0 ± 3.58:86 and 15.0 ± 6.1:85 for corn and potato starches, respectively.

Effect of Native Lipids in Amylose Fractionation

The effect of lipid extraction was analyzed comparing the amount of amylose obtained. Statistical analysis showed no significant differences ($P > 0.05$) between treated and nontreated samples for both corn and potato samples. Thus, this treatment is not necessary to increase the method efficiency. Also, because the extraction procedure involves toxic solvents, the use of this treatment for food purposes is discouraged.

Microscopy Observation

The micrographs show 5% dispersions of corn starch (Fig. 5a) and potato starch (Fig. 5b) treated with NaOH; the starch granules are swollen, eroded, and broken. Control dispersions (Fig. 5c and d) without NaOH help to distinguish the effect of the alkaline treatment. Typically, the potato granules (Fig. 5b and d) are spherical shaped and larger than corn starch granules. When the $MgSO_4$ was added, the leached amylose was visualized as a diffuse zone around the granules. Samples were stained with iodine solution to confirm the enrichment in amylose of the precipitates.

Drying Procedure and Solubility of Amylose Precipitates

The optimum oven-drying temperature was 70°C for 8–9 hr for samples washed with water. Although freeze-drying operation required 16 hr, the precipitates were fine powders with better appearance as compared to oven-dried precipitates. Corn and potato precipitates exhibited similar characteristics for ethanol and oven-drying, or freeze-drying treatments. Drying times were independent of starch source.

Figure 6 shows the solubilities in hot water of amylose-rich product (powder) from corn and potato starch from each drying technique. The starch source significantly ($P < 0.05$) influenced the solubilities of oven-dried and freeze-dried samples; it showed no differences for samples washed with ethanol.

For amylose-rich corn starch (ACS), no significant differences in solubilities ($P > 0.05$) were found between the drying techniques. However, for amylose-rich potato starch (APS), ethanol washings and oven drying significantly improved powder solubility. APS had higher water content than did ACS, and APS showed a vitreous appearance. Precipitates washed with ethanol retained less water than those washed with water and did not exhibit vitreous characteristics.

Determination of Residual $MgSO_4$

The SO_4^{-2} anion concentration of the amylose-rich product was determined from the calibration curve. After the first washing, ACS and APS had $MgSO_4$ contents of 23.75±0.58 and 25.8±1.83%, respectively. After the second washing, the $MgSO_4$ content was reduced to 13.78±0.16 and 14.12±2.17%, respectively. Increasing the number of washings also reduced the amount of amylose. Since $MgSO_4$ is nontoxic, two washings was selected as optimum.

Efficiency of Fractional Precipitation with $MgSO_4$

Table II shows the calculated efficiencies as defined in Eq. 1. Dispersion values may be attributed to the variability in the chemical composition of the commercial starches used. It is also affected by cumulative errors caused by the precipitation technique. As shown in Table II, amylose-to-amylopectin ratio is similar for ACS and APS products. However, corn starch provides higher efficiency. The amylose enrichment obtained matches the amylose content of starches derived from high-amylose genetic varieties (e.g., Amaizo 5 and Amylomaize VII), which are not available in many countries.

CONCLUSIONS

The complexing method with 1-butanol leads to a high amylose-to-amylopectin ratio, but the toxic residues are hard to eliminate. Given the toxicity of the 1-butanol, the use of this complexing technique is not recommended in foods. Amylose purification is also costly and difficult. Generally, none of the products available in the market meet food standards because amylose is usually isolated by complexing.

On the other hand, fractional precipitation with $MgSO_4$ has several advantages: simplicity, nontoxicity, and relative low cost. Using corn starch as raw material is highly recommended because it produces efficiencies similar to that of potato starch. In ACS, minimal amounts of amylose are lost in supernatants and wash-

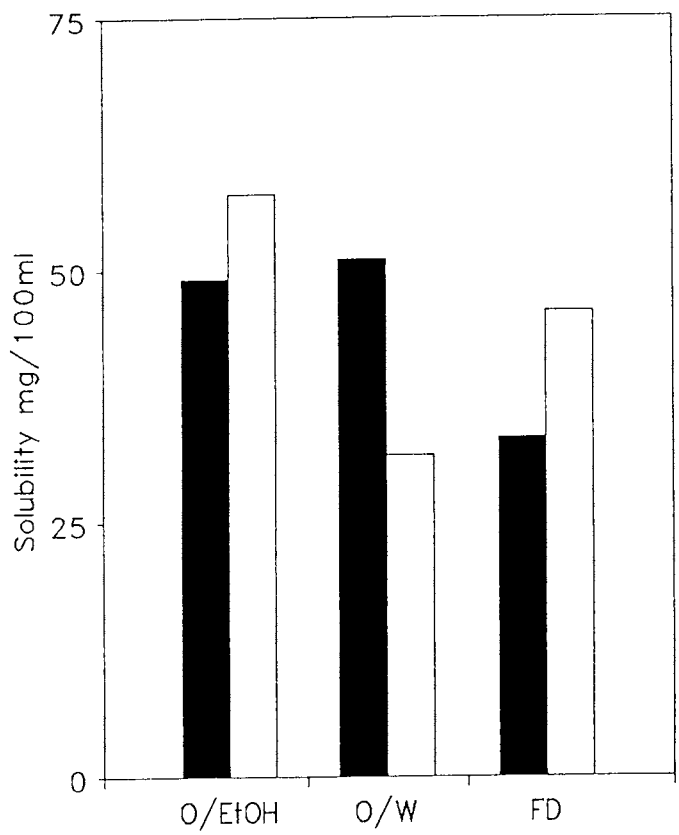

Fig. 6. Effect of drying method on the solubility of amylose-rich corn and potato starch powders: washed with ethanol and oven-dried (O/EtOH); washed with water and oven-dried (O/W); freeze-dried (FD). ■ = ACS (amylose-rich corn starch); ❑ = APS (amylose-rich potato starch).

TABLE II
Efficiency of the Fractional Precipitation

Amylose Source	Efficiency[a]	Amylose/Amylopectin Ratio: Initial Starch	Amylose/Amylopectin Ratio: Amylose-Rich Product	Amylose Standard Deviation
Corn	90.60	25/75	51/49	1.47
Potato	45.33	23/77	50/50	3.32

[a] Defined in Eq. 1.

ings, it retains less water after drying, and ACS is more soluble than APS.

Although the ACS and APS products have amylose-to-amylopectin ratios similar to those of starches derived from high-amylose genetic varieties, the amylose of the latter is within the granule, while the amylose in ACS and APS is partially outside. High-amylose starches need high temperatures (>160°C) to leach the amylose; these drastic conditions are highly favorable to degradative reactions. However, ACS and APS solubilize at 100°C, minimizing degradative reactions. Thus, it can be concluded that fractional precipitation with $MgSO_4$, proposed in the present work, is a highly recommended method for food applications.

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