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Carbohydrate Polymers 61 (2005) 162–167
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Identification of complexed native lipids in crystalline aggregates formed from jet cooked cornstarch

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Received 5 October 2004; accepted 22 April 2005
Available online 3 June 2005

Abstract

Crystalline aggregates, resulting from crystallization of helical inclusion complexes of amylose with the native lipids in jet cooked cornstarch, exhibit two distinct morphologies: smaller, torus-shaped and larger, spherical/lobed particles. Gas chromatographic analyses of extracted lipids showed that these two species contained mixtures of the same native lipids found in granular cornstarch, although in different relative amounts; especially the 16:0 (palmitic) and 18:2 (linoleic) components. The torus-shaped particles contained about twice as much palmitic as linoleic acid; whereas these ratios were reversed in the lipids extracted from the spherical/lobed particles. These findings are consistent with X-ray diffraction data. Microscopic examination of crystalline aggregates formed at different temperatures showed that the spherical/lobed particles are the first to form, and that the torus-shaped particles form at a lower temperature. The fact that the composition of the lipid mixture extracted from the spherical/lobed particles closely resembles that of the lipid mixture extracted from cornstarch itself is consistent with the fact that these particles are the first to form.
Published by Elsevier Ltd.

Keywords: Crystalline aggregates; Spherocrystals; Inclusion complex; Starch; Lipid; Amylose

1. Introduction

Steam jet cooking is a rapid and continuous process that has been used for decades to prepare aqueous starch dispersions for industrial applications (Klem & Brogley, 1981). At our Research Center, the steam jet cooking process has also been studied as a method for preparing new starch–lipid composites. In the course of these investigations, we have observed the formation of crystalline aggregates in dilute dispersions of jet-cooked cornstarch, when these dispersions were allowed to cool slowly. Particle formation in aqueous starch dispersions was also observed by earlier workers (Davies, Miller, & Procter, 1980; Jane, Kasemsuwan, Chen, & Juliano, 1996; Kitamura, Yoneda, & Kuge, 1984; Zobel, 1988).

We have shown that the particles formed in 4% dispersions of jet cooked corn starch are composed of two distinct species that differ in size, morphology, and crystal structure (Fanta, Felker & Shogren, 2002). The smaller-sized particles were disc- or torus-shaped, whereas the larger particles were more spherical and often exhibited a two-lobed or four-lobed morphology. X-ray powder diffraction patterns of the torus-shaped particles matched patterns previously reported for the 6_1 amylose V-helical complex in the hydrated form. In contrast, diffraction patterns for the spherical and lobed particles suggested a 7_1 V-helical conformation for amylose. These results are consistent with the conclusion of Davies et al. (1980) that these particles result from crystallization of helical inclusion complexes

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0144-8617/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2005.04.012

formed from amylose and the small amount of native lipid normally present in cereal starch granules.

The native lipid component of cornstarch contains mainly free fatty acids (FFA) along with smaller amounts of lysophospholipids (Morrison, 1988; Morrison, Milligan, & Azudin, 1984). Although amylose complexes are readily formed from monoglycerides (MG), neither diglycerides nor triglycerides form complexes with amylose due to their sterically hindered structure (Pomeranz, 1988). FFA and MG (i.e. lysophospholipids) are thus the dominant two lipid forms in the crystalline aggregates investigated in this study. Linoleic, palmitic and oleic (9cis-18:1) acids are the FFA present in largest amounts. Since two types of particles with distinctly different structures and morphologies were observed, it seemed likely that these differences might be caused by differences in the structures of the lipids complexed within the amylose helix. The objective of this study was to determine the FFA/MG composition of the lipids that were complexed with amylose in each of the two particle types. To accomplish this objective, we separated the small, torus-shaped particles and the larger spherical/lobed particles, extracted each to remove complexed lipids and, after conversion to fatty acid methyl esters (FAME), analyzed the extracted lipids by gas chromatography to determine their composition.

2. Materials and methods

2.1. Materials

Samples of normal unmodified cornstarch were obtained from two different manufacturers. Pure Food Grade Cornstarch (Sample A) was a product of A.E. Staley Mfg. Co., Decatur, IL; and Globe 3005 (Sample B) was a product of Corn Products International (formerly CPC International), Westchester, IL. Percent moisture values were determined by vacuum drying weighed starch samples at 100 °C, and all weights of starch are given on a dry weight basis. Heptadecanoic acid and lipid reference standard GLC-68A were supplied by Nu-Chek Prep, Inc., Elysian, MN. A standard solution of heptadecanoic acid was made by diluting a weighed amount of the solid with toluene.

2.2. Preparation and fractionation of crystalline aggregates

Starch was jet cooked, and the starch dispersions were collected in a Dewar flask and allowed to cool for 22 h without stirring as described earlier (Fanta et al., 2002). Percent starch solids were about 4%, as determined by freeze-drying known weights of dispersion. Small variations in starch solids were observed in different experiments due to dilution of the cooked dispersions with variable amounts of condensed steam. The cooled dispersions were diluted 10-fold with water, and left to stand for about 14 days to allow the crystalline aggregates to settle. The supernatant liquid was decanted, and the particles were washed by dispersing them in excess water followed by centrifugation. Yields of crystalline aggregates were calculated from the weight of freeze-dried particles isolated from a weighed portion of jet cooked starch dispersion. Yields, based on starch, varied from 8.6 to 9.3%. Crystalline aggregates were fractionated according to particle size by dispersing the particles in excess water and then allowing the larger particles to settle, leaving the smaller particles in suspension. Light microscopy was used to monitor the progress and efficiency of this sedimentation procedure. Particles were isolated from water dispersions by freeze-drying.

2.3. Analytical methods

Lipids were extracted from the particle fractions by heating 0.2 g of freeze-dried sample for 2 h under reflux in 100 mL of 75% *n*-propyl alcohol/25% water (v/v) (Morrison, 1988). The extracted solid was separated by filtration and washed with 25 mL of 75% *n*-propanol/water. Extraction of lipids from the native cornstarch samples used in these experiments were carried out with 2 g of starch instead of 0.2 g, because of the lower lipid content of starch relative to the crystalline aggregates.

The *n*-propyl alcohol/water was removed from the lipid extracts by rotary evaporation. Heptadecanoic acid (100 μL of 0.01 mg/μL) was added as an internal standard. Toluene (about 4 mL) was added, the mixture was swirled for about one minute, and then divided into two aliquots: one to undergo hydrochloric acid/methanol esterification, and the other to undergo diazomethane esterification. The dual-esterification procedure was used to determine the percentage of each lipid (i.e. palmitic, stearic, oleic, linoleic and linolenic) that was present in the sample as FFA, as opposed to MG. HCl/methanol is an all-encompassing esterification method that forms methyl esters from MG as well as from FFA, while diazomethane is more specific and only esterifies FFA (Christie, 1982). Therefore, by adding an internal standard to the sample before division into aliquots and esterification, the percentage of FFA vs. MG in each could be quantitatively determined.

2.4. Hydrochloric acid/methanol esterification

To the first aliquot, an excess (~4 mL) of 10% HCl in methanol solution was added. This solution was stirred under argon and placed in a 65 °C oven for 2 h. The mixture was then cooled to room temperature, transferred to a 30 mL separatory funnel, and 5 mL of hexane and 5 mL of water were added. The aqueous layer was transferred to another 30 mL separatory funnel and extracted with an additional 5 mL of hexane. The hexane layers were combined and washed three times with water to neutrality. The aqueous extracts were discarded, the organic layer was transferred to a 50-mL round-bottom flask, and hexane removed by rotary evaporation. The lipid residue was dried by adding

approximately 1 mL of 19:1 (v/v) chloroform/methanol along with 1 mL of acetonitrile under rotary evaporation. The esterified lipid sample was transferred to a vial using 2,2,4-trimethylpentane as solvent. The solution was degassed with argon and was then ready for analysis by gas chromatography.

2.5. Diazomethane esterification

The second aliquot was transferred to a 4 mL vial and the toluene removed by evaporation with a stream of argon. Diazomethane solution was prepared according to the method of Lombardi (1990). Approximately 3 mL of diazomethane solution was added, the mixture was stirred using a vortex mixer, and the reaction was allowed to proceed at room temperature under argon. The solvents and excess diazomethane were removed using a stream of argon, and the sample was stored in 1 mL of 2,2,4-trimethylpentane under argon.

2.6. Gas chromatography

The gas chromatograph used was a Varian 3900 (He carrier gas) with FID detector using a Supelco SP2380 column (30 m × 0.32 mm × 0.2 μm) and driven by Varian Star Chromatography Workstation software version 5.52. For each run, column temperature started at 100 °C and rose 3 °C/min to a final temperature of 205 °C. Retention times for the esterified lipid peaks were identified using lipid reference standard GLC-68A (Nu-Chek Prep, Inc.).

2.7. Microscopy

Particle samples in aqueous dispersions were examined with an Axioskop light microscope (Carl Zeiss, Inc., Thornwood, NY) using phase contrast optics. Representative fields were photographed using a Nikon D100 digital camera (Nikon Corp., Tokyo, Japan). For scanning electron microscopy (SEM), an aqueous dispersion of particles (20 μL) was added to 20 mL of absolute ethanol and allowed to settle. Particles were washed with ethanol and then critical point dried onto aluminium stubs. Dried specimens were sputter coated with Au–Pd and examined with a JEOL 6400 V (JEOL USA, Inc., Peabody, MA) scanning electron microscope.

3. Results

Fig. 1 shows SEM and phase contrast images of representative samples of the torus-shaped and the spherical/lobed particles. Relative percentages of the different lipids extracted from these particles, as well as from the cornstarch samples used as starting materials, are shown in Table 1. The percentage of FFA in each of the lipid components is shown in parentheses. To obtain data from a diverse series of cornstarch samples, cornstarches obtained

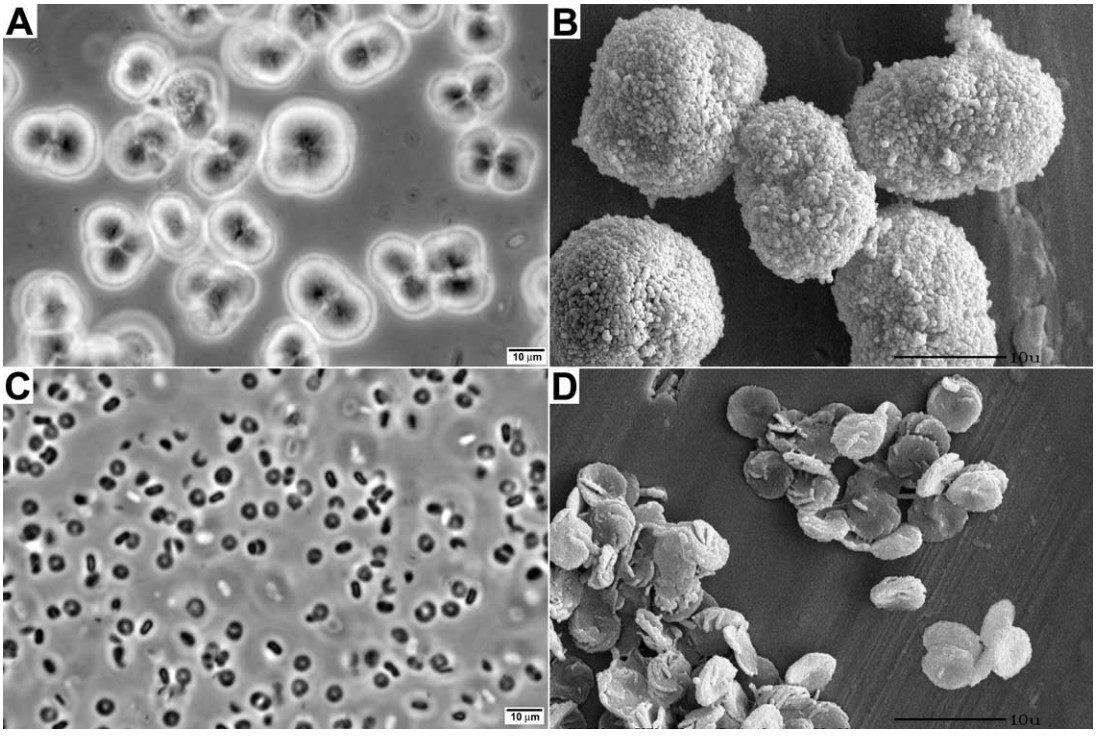

Fig. 1. Micrographs of crystalline aggregates formed in slowly-cooled dispersions of jet cooked cornstarch. (A) Phase contrast image of spherical/lobed particles. (B) SEM image of spherical/lobed particles. (C) Phase contrast image of torus-shaped particles. (D) SEM image of torus-shaped particles.

Table 1
Composition of lipids extracted from crystalline aggregates and from native cornstarch

Sample	No. of jet cooking experiments	Total no. of extracts analyzed	Relative percentages of lipid components[a]				
			Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
Cornstarch Sample A, Lot 1							
Native cornstarch	n/a	3	27.2±3.0 (79±9)	3.1±0.1 (79±10)	13.5±0.8 (79±11)	52.7±2.1 (82±9)	3.4±0.2 (85±10)
Torus-shaped particles	4	8	55.7±3.3 (73±4)	6.8±0.9 (88±7)	13.3±0.7 (88±4)	22.8±3.4 (92±5)	1.5±0.4 (90±7)
Spherical/lobed particles	4	8	28.9±2.8 (65±7)	3.4±0.8 (81±15)	13.8±0.3 (84±2)	50.8±2.2 (86±6)	3.1±0.2 (92±7)
Cornstarch Sample A, Lot 2							
Native cornstarch[b]	n/a	3	27.4±0.5 (72.9±0.7)	3.0±0.4 (74±10)	11.4±0.2 (87±4)	44.9±0.4 (85±2)	2.9±0.1 (89±1)
Torus-shaped particles	1	1	49.9 (73)	7.3 (77)	13.9 (81)	27.0 (85)	1.9 (85)
Spherical/lobed particles	1	2	29.4±5.5 (59±4)	2.8±0.1 (76±4)	13.2±0.8 (80±2)	51.5±4.5 (83±2)	3.1±0.2 (89±4)
Cornstarch Sample B							
Native cornstarch	n/a	2	33.1±0.7 (71±4)	1.5±0.1 (92±3)	11.0±0.2 (94±9)	51.2±0.5 (94±8)	3.3±0.1 (98±3)
Torus-shaped particles	1	2	53.2±0.1 (72±13)	4.3±0.1 (86±7)	12.6±0.1 (86±4)	28.3±0.1 (92±4)	1.6±0.1 (96±6)
Spherical/lobed particles	1	2	29.7±0.2 (59±4)	2.0±0.1 (85±3)	12.5±0.3 (86±3)	52.6±0.5 (91±6)	3.2±0.1 (96±6)

[a] Values in parentheses are the percentages of FFA (as opposed to MG).
[b] Contained roughly 10% myristic acid (14:0), which did not appear in the crystalline aggregates or any other sample.

from two different companies were used as starting materials (Samples A and B). Two different sample lots from the same company (Lots 1 and 2) were also used. Except for two samples of torus-shaped particles (where sample size was not sufficient for more than one analysis), each sample in Table 1 was subjected to 2–3 repetitive extractions and analyses. To confirm the reproducibility of these experiments, four replicate jet-cooking experiments were carried out under the same conditions with starch sample A, lot 1. The results of Table 1 are summarized in pie-chart form in Fig. 2.

Relative percentages (by weight) of the torus-shaped and spherical/lobed particles isolated in each of these experiments are shown in Table 2. Although these percentages varied, the spherical/lobed particles were formed in larger amounts in all of these experiments.

In a separate experiment, we observed the formation and growth of the two types of particles by removing samples for microscopic examination from a hot, jet cooked starch dispersion at intervals of 2 °C, as the dispersion was allowed to slowly cool. Examination of samples removed at 86, 84 and 82 °C showed that the spherical/lobed particles were the first to form. Phase contrast images of these samples are shown in Fig. 3. Small, two-lobed and four-lobed particles were first seen at 86 °C. At 84 °C the lobed particles increased in size and number, and further increases were observed at 82 °C. Although not easily seen in the phase

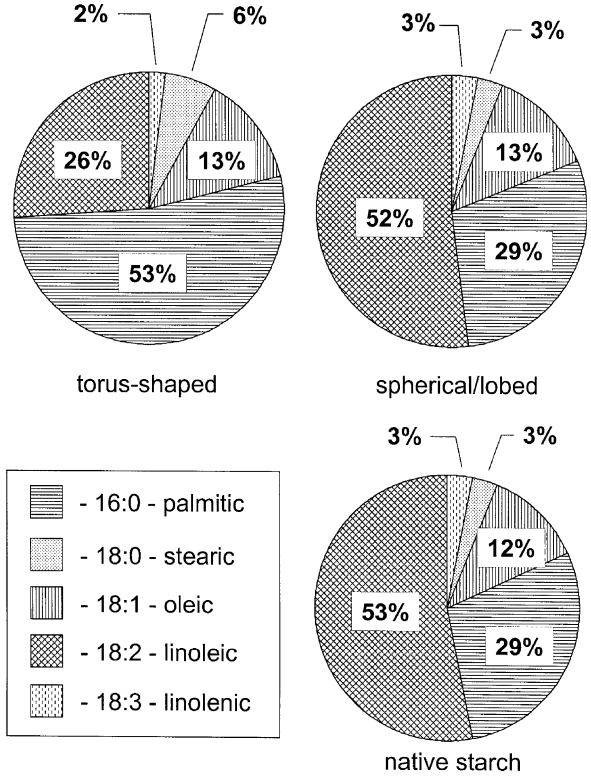

Fig. 2. Summary of Table 1 in pie-chart form. Composition of the lipid mixtures extracted from torus-shaped particles, spherical/lobed particles and native cornstarch.

Table 2
Relative percentages (by weight) of crystalline aggregates isolated from jet cooked dispersions

Native cornstarch sample	Torus-shaped particles (%)	Spherical/lobed particles (%)
Sample A, Lot 1, Exp. 1	14	86
Sample A, Lot 1, Exp. 2	2	98
Sample A, Lot 1, Exp. 3	5	95
Sample A, Lot 1, Exp. 4	5	95
Sample A, Lot 2	9	91
Sample B	3	97

contrast image, a few small, torus-shaped particles were first observed at 84 °C. These particles increased in size and number, as the dispersion was cooled to 82 °C. Cooling the starch dispersion below 82 °C did not significantly change the appearance of the particle mixture.

The FAME in the extracts obtained from the two types of particles (Table 1), as well as from the cornstarch samples used in their preparation, consisted primarily of 16:0, 18:2 and 18:1. The 18:0 and 18:3 FAME were present in smaller amounts. Cornstarch sample A, lot 2, also contained about 10% of 14:0 (myristic acid), a component not listed in Table 1. The sum of the five lipid percentages for this sample is therefore less than 100%. The 14:0 lipid component was not observed in significant amounts in any of the other lipid mixtures. The lipids extracted from the various particle fractions, as well as from their cornstarch precursors, were composed largely of FFA, with MG (lysophospholipids) present in lesser amounts. The lowest percentages of FFA (approximately 60%) were observed in the 16:0 lipid component extracted from the spherical/lobed particles. Standard deviations for a few of the values in Table 1 are large enough to show FFA amounts in excess of 100% (e.g. the 18:3 entries for the Cornstarch Sample B series). The reason for this anomaly is that in quantifying lipid concentrations in the diazomethane vs. HCl/methanol esterifications, random errors occurred with very small (<0.1 mg detectable) lipid amounts.

The major difference between the lipids extracted from the torus-shaped particles and the spherical/lobed particles was the relative percentages of 16:0 and 18:2. In the spherical/lobed particles, the linoleic (18:2) percentages were about twice as high as the palmitic (16:0); and the relative percentages of the five lipid components also resembled those observed for the native cornstarches. These relative percentages were reversed in the lipid mixtures extracted from the torus-shaped particles, i.e. the palmitic percentages were about twice as high as the linoleic.

4. Discussion

Crystalline aggregates are formed from helical inclusion complexes of amylose with the native lipids normally present in cornstarch granules. Previously obtained X-ray diffraction patterns (Fanta et al., 2002) have suggested that

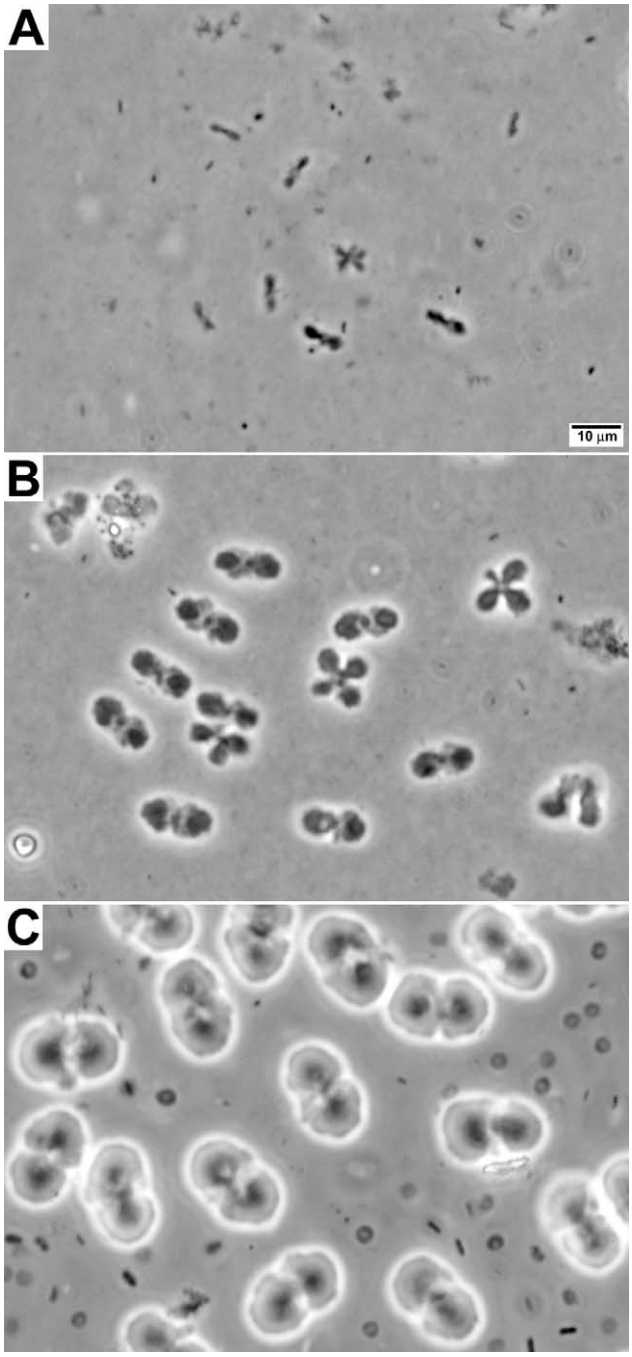

Fig. 3. Phase contrast micrographs of crystalline aggregates formed from jet cooked cornstarch at various temperatures. (A) 86 °C. (B) 84 °C. (C) 82 °C.

the structures of these particles may be governed to a large extent by the chemical structures of the lipids complexed within the amylose helix. For example, diffraction patterns of the small, torus-shaped particles were consistent with a 6_1 V-helical complex (i.e. six glucose units per turn of the amylose helix), suggesting complex formation with saturated, straight chain FFA with low steric hindrance. The 7_1 V-helical pattern for the larger, spherical/lobed particles, requires seven glucose units per turn of the helix, indicating that the amylose helix is larger in diameter, due to complex

formation with unsaturated, bulkier (i.e. kinked) lipids. These X-ray diffraction results are supported by the present study, which shows that the bulkier 18:2 (linoleic) component is the major lipid component in the spherical/lobed particles exhibiting the 7_1 V-helical conformation; whereas the more linear 16:0 (palmitic) lipid is the major lipid component in the torus-shaped particles exhibiting the 6_1 V-helical pattern. It is interesting that neither the torus-shaped nor the spherical/lobed particles contain any lipid components that are not found in the other. The structure and morphology of these particles therefore does not depend upon complex formation with any one particular lipid component to the exclusion of others, but upon the relative amounts of the different native lipids in the complex, particularly palmitic vs. linoleic.

Microscopic examination of the particles formed at different temperatures showed that the spherical/lobed particles are the first to form at the highest temperature, and that the torus-shaped particles form at lower temperature (see Fig. 3). The fact that the spherical/lobed particles are formed in the largest amounts in all of our experiments is consistent with this observation. Table 1 and Fig. 2 show that the composition of the lipid mixture extracted from the spherical/lobed particles closely resembles that of the lipid mixture present in cornstarch itself (i.e. a predominance of 18:2 lipid). This finding is also consistent with the observation that the spherical/lobed particles form first at the highest temperature. Formation and growth of the torus-shaped particles is slower because of the lower concentration of lipid remaining in the jet cooked dispersion.

We still do not know why the high temperature formation of crystallites with the 7_1 V-helical conformation leads to the spherical/lobed morphology; whereas the lower temperature formation of crystallites having the 6_1 V-helical conformation produces the smaller, torus-shaped particles. We are currently examining the crystalline aggregates formed by jet cooking defatted cornstarch with a variety of different pure FFA to obtain further information on the formation of these two types of particles.

5. Conclusions

Gas chromatographic analyses of extracted lipids show that the small, torus shaped particles and the larger, spherical/lobed particles contain mixtures of the same lipids found in native cornstarch, although in different relative amounts. Lipid components were present largely as FFA, with lesser amounts of MG. The major difference between the lipid mixtures extracted from the two different types of particles was in the relative amounts of 16:0 (palmitic) and 18:2 (linoleic) FFA. The torus-shaped particles contained about twice as much palmitic as linoleic; whereas these ratios were reversed in the spherical/lobed particles. These findings are consistent with previously observed X-ray diffraction patterns, since the 6_1 V pattern exhibited by the torus-shaped particles suggests the predominance of a straight chain lipid component. Linoleic acid is a bulkier molecule than palmitic acid because of its two olefinic bonds; and the spherical/lobed particles' 7_1 V pattern, indicative of seven glucose units per turn of the amylose helix, would be expected.

Microscopic examination of crystalline aggregates formed at different temperatures shows that the spherical/lobed particles are the first to form at the highest temperature, and that the torus-shaped particles form at a lower temperature. The fact that the composition of the lipid mixture extracted from the spherical/lobed particles closely resembles that of the lipid mixture extracted from cornstarch itself is consistent with the observations that the spherical/lobed particles are formed in the largest amounts and that they are the first to form at the highest temperature. Formation and growth of the torus-shaped species takes place more slowly due to the lower concentration of lipid remaining in the jet cooked dispersion.

Acknowledgements

The authors would like to thank Lynne Copes and Erin Walter for technical assistance, and Karen Ray for preparation and donation of the diazomethane solution.

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