

Formation of Amylose-Lipid Complexes by Twin-Screw Extrusion Cooking of Manioc Starch¹

C. MERCIER,² R. CHARBONNIERE,³ J. GREBAUT,⁴ and J. F. de la GUERIVIERE,⁴ Institut National de la Recherche Agronomique

ABSTRACT

Cereal Chem. 57(1):4-9

Native manioc starch with and without the addition of 2-4% of various lipids was treated by extrusion-cooking at 200°C in a twin-screw French extruder. X-ray diffraction patterns, solubility in cold water, iodine spectrum of the soluble fraction, and stability at -20°C were determined. Whereas native extruded starch gives a product devoid of crystalline organization and almost water-soluble, the products of its blends with linear fatty acids (C₂ to C_{18:2}) or with monoglycerides have a structurally organized fraction that makes them water-insoluble. The water solubility of

these products decreases with an increase in the length of the carbon chain. The iodine spectra of their solutions evolve towards the spectrum of the amylopectin type, thus confirming that amylose forms a complex with fatty acids. The products obtained by extrusion of starch with triglycerides or phosphatides have no organized fraction. The technological properties of products complexed with fatty acids or monoglycerides (reduced stickiness and freeze-thaw stability) are competitive with chemically modified starch.

Our previous work with the twin-screw French extruder showed that extrusion cooking destroys, by gelatinization, the physical structure of the various cereal and tuber starches of wheat, corn with various percentages of amylose, rice, and potato (Mercier and Feillet 1975, Action Concertée DGRST 1975, Mercier 1977). X-ray diffractometry has shown simultaneous reorganization of the gelatinized macromolecules, including the appearance of a crystalline fraction; this change was detected only in starch containing amylose and lipids, such as cornstarch (Charbonniere et al 1973, Mercier et al 1979). Extruded potato starch free of lipids (0.04%) and waxy-maize starch free of amylose (<1%) did not form any organized structure (Mercier et al 1979), although they were almost completely gelatinized. During extrusion, however, addition of oleic acid to potato starch containing amylose led to formation of the same crystalline structure, but no organized structure was detected after addition of oleic acid to waxy maize starch.

The crystalline fraction is characterized by the appearance of peaks on the x-ray patterns. The main peak is located at 9°05, 9°09 or sometimes both according to the water content of starch before extrusion. The peak at 9°05 and the two others of smaller intensity characterize the x-ray pattern of an unstable structure, termed "E_h structure" (Mercier et al 1979). The peak at 9°09 and the two peaks of smaller intensity were identified as those of a pattern derived from the butanol-amylose complex and are known as the V_{hydrate}-amylose complex structure. Examination of the extruded products reconditioned to 30% moisture content on dry basis showed transformation of the unstable E_h structure (peak at 9°05) into the stable V_h-amylose complex structure (peak at 9°09) (Mercier et al 1979).

The ability of amylose to form crystalline complexes with a large number of polar and nonpolar organic compounds is currently used to fractionate amylose from amylopectin. The amylose complex is generally prepared by addition of a complexing agent to a hot aqueous solution of starch. Based on this method of preparation, Takeo et al (1973) demonstrated, by the x-ray diffraction powder method, that complexation with various fatty acids produces different conformations, depending on the linear chain length of the acid molecules.

To better understand the complex formation during extrusion, we extruded lipid-free manioc (tapioca) starch both dry and with different fatty acids, monoglycerides, and fats and compared the

physicochemical and biochemical characteristics of the extruded starches.

MATERIALS AND METHODS

Starch Samples

A commercial sample of manioc starch (with 13.8% moisture content and 0.1% lipid content) and a chemically modified manioc starch (glycol epichlorohydrin cross-linked, named Purity D), were purchased from Roquette National Chimie (Lestrem, France).

Lipids

The fatty acids (acetic, lauric, myristic, palmitic, stearic, oleic, and linoleic), the monoglycerides (glyceryl monostearate [GMS], mixtures of glyceryl monostearate and glyceryl monopalmitate, and an emulsifier [calcium stearyl lactylate]), and the fats (lard, copra, butter, soya lecithin, and palm, peanut, and sunflower oils) were commercially available and of the purest grade.

Extrusion Cooking

Extrusion was performed in a small twin-screw extruder (type BC 45) developed by Creusot Loire (France) as described by Mercier and Feillet (1975).

Manioc starch and chemically modified starch were extruded without lipids at different barrel temperatures between 70 and 225°C with 22.0% water content on dry basis before extrusion, and with lipids (2 and 4%) at 200°C and with 21.0% water content on a dry basis before extrusion.

After treatment, all samples were ground in a laboratory Buhler mill cooled with dry ice.

Methodology

The physicochemical characteristics of the extruded samples, such as expansion, water absorption index, water solubility index, and viscosity, were determined as described by Mercier and Feillet (1975).

The modification of the physical structure was studied by an x-ray diffraction powder method (Mercier et al 1979).

X-ray diffraction patterns were obtained by transmission with a diffractometer equipped with a Guinier monochromator selecting the K_α radiation of a Cu target and with a scintillator counter. Each diagram was directly recorded. The extruded starches were measured either with their initial water content after extrusion or with a 30% water content after reconditioning by air moisture sorption. The water content of starch or starchy products is known to modify their x-ray diffraction patterns.

Characteristics of the water-soluble carbohydrate were determined by the iodine-carbohydrate complex spectra and by measurement of the wavelength of peak absorption (λ_{max}), as performed by Bourne et al (1948).

The percentage of retrogradation of the water-soluble

¹Presented to the Sixth International Cereal and Bread Congress, Winnipeg, Canada, September 1978.

²Centre de Recherches Agro-Alimentaires, Laboratoire de Biochimie des Aliments, Chemin de la Géraudière, 44072, Nantes Cedex, France.

³Laboratoire de Technologie Alimentaire, au Cerdia, 91305, Massy, France.

⁴CTU - 45 bis, Avenue de la Belle Gabrielle, 94130, Nogent-Sur-Marne, France.

carbohydrate at room temperature, +4°C, and -20°C after five successive freeze-thaw cycles was measured on a part of the water-soluble fraction prepared from a 3% extruded sample dispersion and held overnight at the cited temperatures. The solutions were reequilibrated at 20°C and centrifuged at 4,000 rpm at room temperature. The carbohydrate in the supernatant was determined on dry matter basis. The difference between the total dry matter and the soluble dry matter after centrifugation corresponds to the retrograded fraction expressed in percent.

RESULTS

Extrusion Cooking of Native Manioc Starch

Extrusion cooking of manioc starch with 22.0% water content completely destroyed the organized structure, as shown by the x-ray patterns (Fig. 1a). Extrusion temperature as low as 70°C disorganized the granule, and an amorphous pattern was observed for all the temperatures studied. Reconditioning the samples to 30% moisture content (Fig. 1b) did not improve any of the spectra and confirmed the absence of complex formation in manioc starch, which contains only 0.1% lipid.

Simultaneously, the starch was partly gelatinized. Maximum expansion (Fig. 2) was observed around 200°C. As the water-solubility index increased with the extrusion temperature, the water-absorption index and the viscosity at 50°C decreased. The λ_{\max} of the iodine-soluble carbohydrate fractions was 590 nm, corresponding to the λ_{\max} of a swollen starch.

Therefore, the reaction of manioc starch to extrusion was similar to that of potato starch and different from that of cereal starches (Mercier et al 1979). No V-amylose structure was formed because the level of lipids was very low (0.1%) and extrusion produced a highly gelatinized starch.

Extrusion Cooking of Manioc Starch with Fatty Acids and Monoglycerides

Addition of 2% fatty acids, except acetic acid, during extrusion led to formation of an organized structure (Fig. 3) with the two

structures E_h and V_h superimposed for a moisture content lower than 20%. Reconditioning to 30% moisture content transformed the organized E_h structure to the amylose-stable V_h -amylose structure.

Complex formation did not occur with acetic acid because at the 200°C extrusion temperature, acetic acid was volatile. An attempt to extrude manioc starch at a temperature (90°C) lower than the boiling temperature of acetic acid (118°C) did not show any complexation, probably because the granules of starch were partly solubilized to 20% and not disorganized enough to liberate the amylose fraction.

The phenomenon with fatty acids was observed in extrusion of manioc starch with 2% monoglycerides (Fig. 4). In all the cases, a complex formed between amylose and lipid as shown by the x-ray patterns of the extruded samples. Extrusion of the chemically modified starch (Purity D) with 2% oleic acid also led to the structure E_h (Fig. 4), which became the V_h -amylose complex after rehydration of the product. The x-ray pattern indicated that the very low level of substitution of the amylose fraction did not interfere.

In addition to forming the amylose complex, starch was partly gelatinized. However, the water-soluble fraction formed by extrusion with fatty acids and monoglycerides decreased to as low as 25% in contrast to the amount (90%) produced by extrusion of manioc starch without lipids (Fig. 5). As the number of unsaturated bonds in the fatty acids increased (stearic C_{18} , oleic $C_{18:1}$, linoleic $C_{18:2}$ acids), solubility seemed also to be reduced. GMS showed the same decrease of solubilization of the extruded starch ($\approx 40\%$) as did stearic acid.

This similarity indicates that the stearyl group, not the glycerol moiety, is involved in the complex. This was confirmed by the absence of V-amylose structure when the starch was extruded with glycerol (Fig. 6); this effect was attributed to the stearic hindrance of the glycerol molecule inside the internal helical section of amylose.

The cross-linkages (glycol epichlorohydrin) in manioc starch do not affect its behavior in extrusion. Under the same extrusion

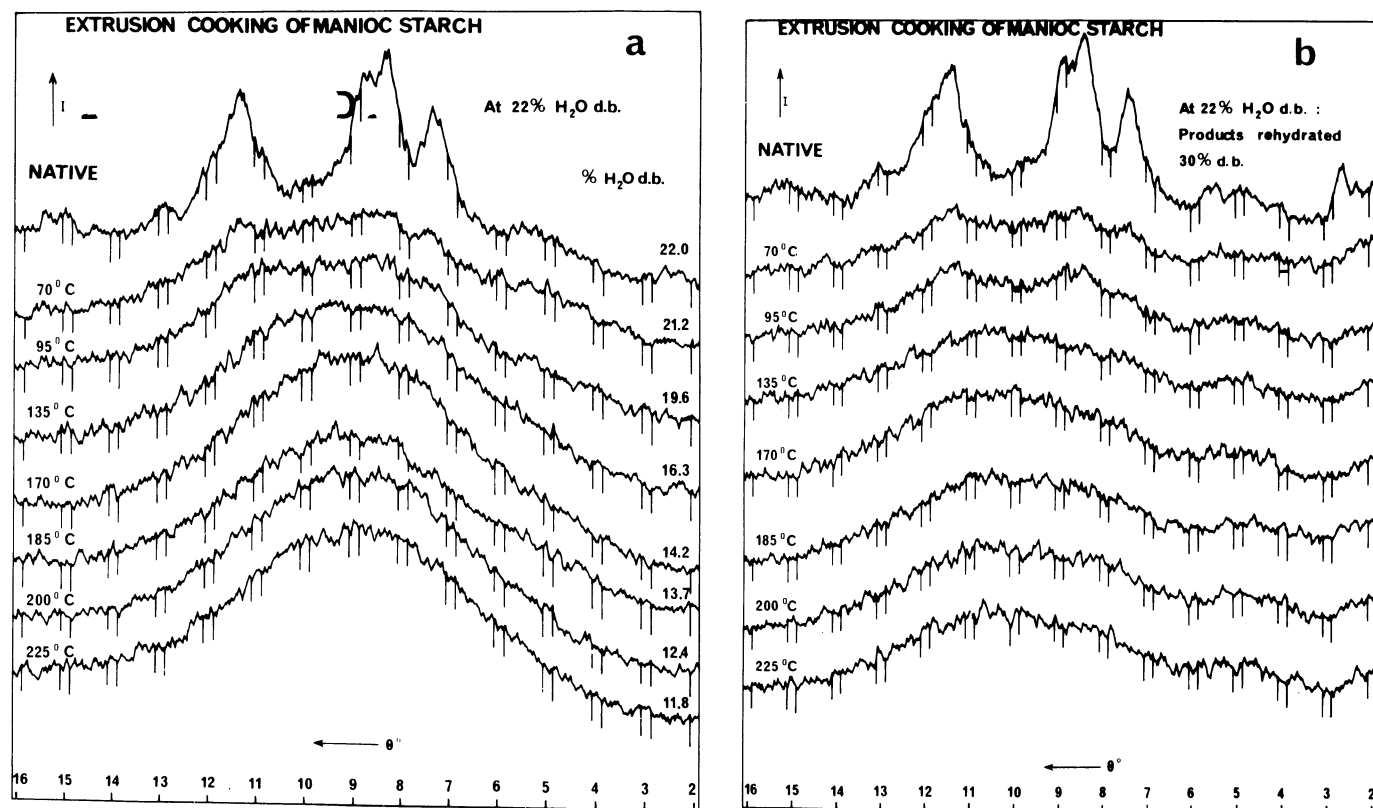


Fig. 1. X-ray patterns of native and extruded (70–225°C) manioc starch with an initial moisture content before extrusion of 22% db: a, final products; b, products rehydrated to 30% db.

conditions (water content and temperature), both native and Purity D manioc starch become soluble to 90%, which decreases to 25% in both cases by extrusion in the presence of 2% oleic acid (Fig. 5).

The water-soluble fraction formed by extrusion is characterized by its iodine-absorption capacity. The iodine spectra (Fig. 7) of the water-soluble carbohydrate material obtained after extrusion without and with fatty acids show a displacement of the λ_{max} from

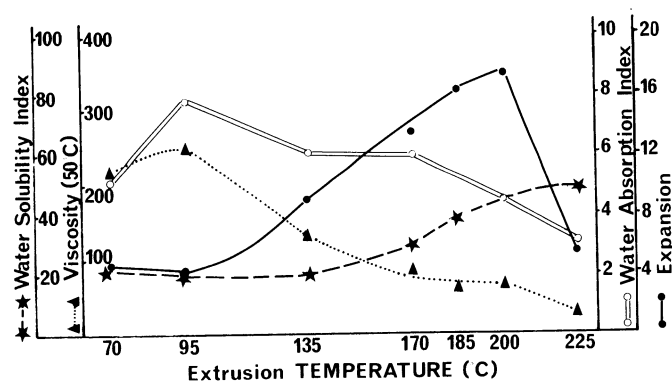


Fig. 2. Effects of extrusion temperature on expansion (●—●), water absorption index (□—□), water solubility index (★—★), and viscosity at 50°C (▲—▲) of manioc starch. Initial moisture content before extrusion was 22.0% db by weight.

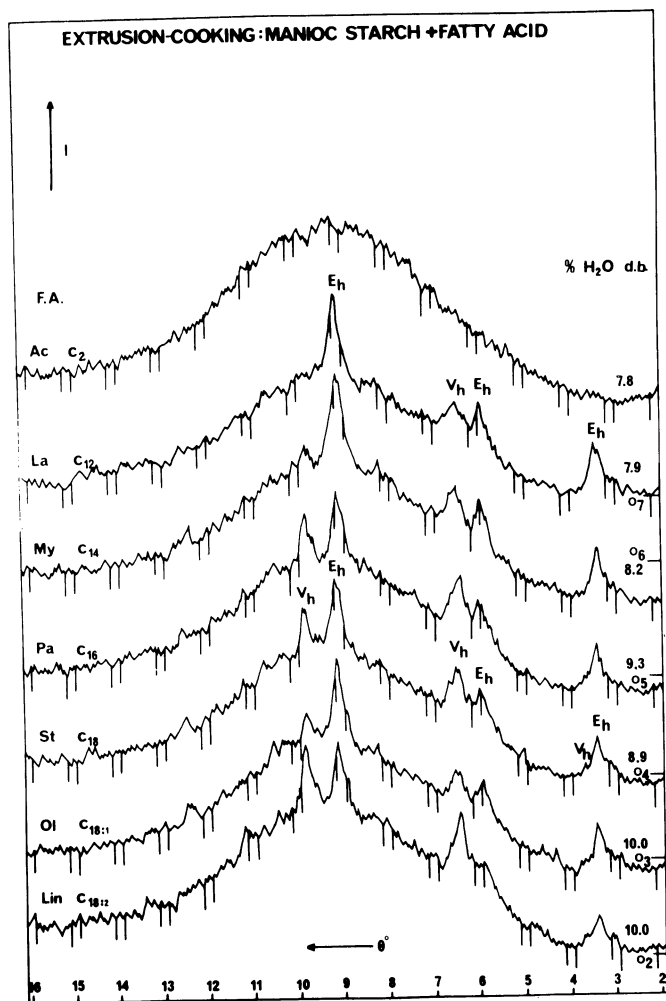


Fig. 3. X-ray patterns of manioc starch extruded with 2% of acetic (Ac C₂), lauric (La C₁₂), myristic (My C₁₄), palmitic (Pa C₁₆), stearic (St C₁₈), oleic (OI C_{18:1}), linoleic (Lin C_{18:2}) at 200°C and 21.0% db initial moisture content before extrusion.

590 nm to 538 nm with the increase of the chain length of the fatty acid. The same phenomenon occurs with monoglycerides (Fig. 8).

These results indicate a concentration of the amylopectin content

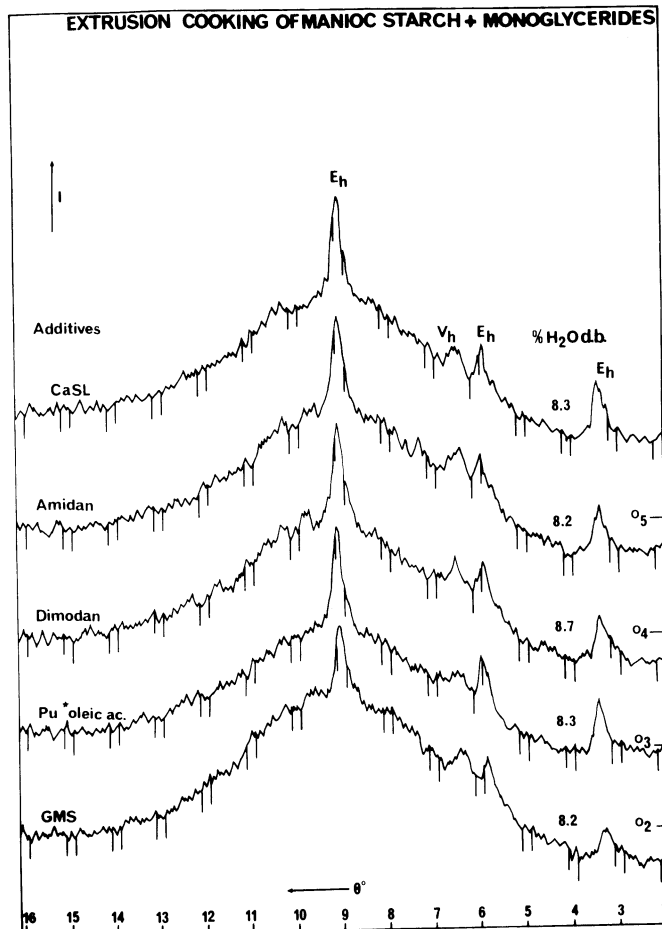


Fig. 4. X-ray patterns of manioc starch extruded with 2% of calcium stearyl lactylate (CaSL), glyceryl monostearate (GMS), Amidan and Dimodan (mixtures of GMS and glyceryl monopalmitate), and Purity D (Pu) with 2% oleic acid at 200°C and 21.0% db initial moisture content before extrusion.

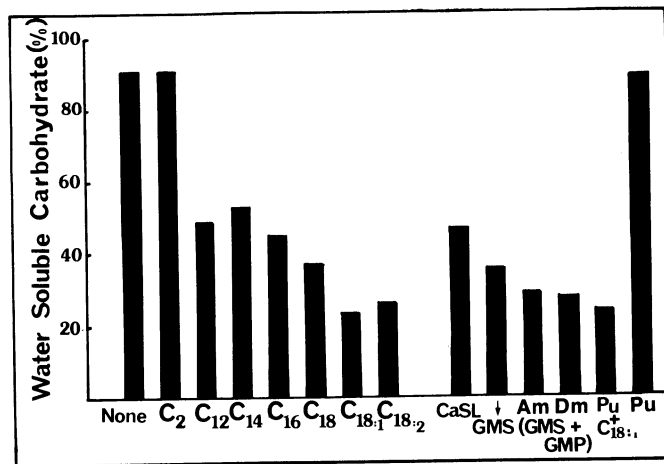


Fig. 5. Water solubility of manioc starch and chemically modified starch (Purity D) extruded without and with 2% fatty acids and monoglycerides at 200°C and 21.0% db initial moisture content before extrusion. C₂ = acetic, C₁₂ = lauric, C₁₄ = myristic, C₁₆ = palmitic, C₁₈ = stearic, C_{18:1} = oleic, C_{18:2} = linoleic, CaSL = calcium stearyl lactylate, GMS = glyceryl monostearate, Am = Amidan and Dm = Dimodan (mixtures of GMS and GMP [glyceryl monopalmitate]), Pu = Purity D.

of starch in the soluble fraction. This concentration was confirmed by the insolubilization of the amylose, which was complexed by the fatty acids and monoglycerides.

The minimum amount of fatty acid required to form a complex was studied with oleic acid. The x-ray pattern of manioc starch extruded with 0.12% oleic acid was amorphous. Formation of the complex became obvious only after 0.29% oleic acid was added. Increases of the complexed material were seen with additions of 0.59, 1.5, 3.0, and 4.5% oleic acid. The complex cannot be quantified from the x-ray pattern, although the amount of the water-soluble material seems to be related to the complex, as shown in

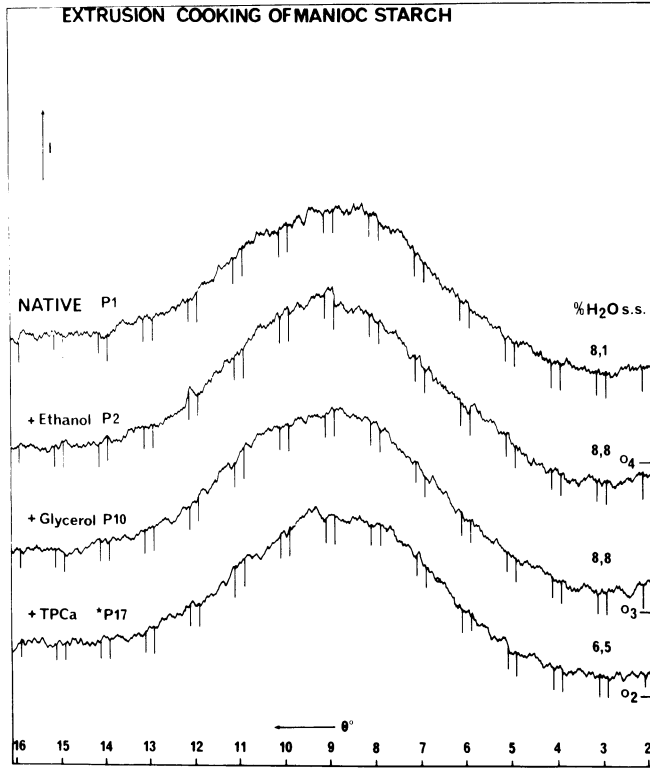


Fig. 6. X-ray patterns of native and extruded manioc starch with ethanol, glycerol, and Ca tripolyphosphate (TPCa) at 200°C and 21.0% db initial moisture content before extrusion.

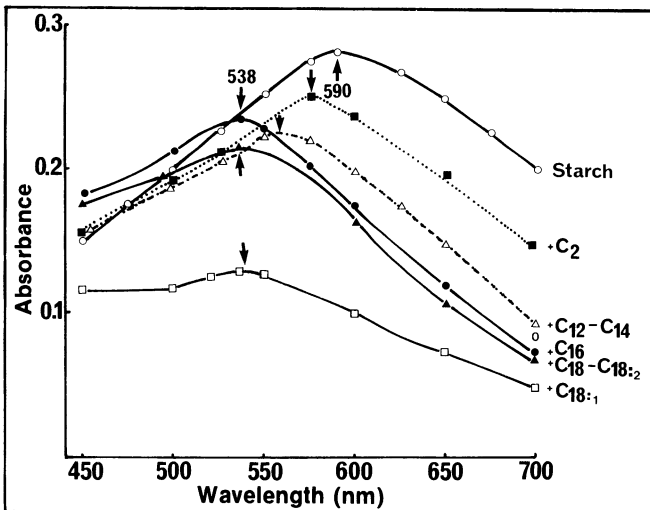


Fig. 7. Iodine spectra of water-soluble manioc starch extruded with and without 2% fatty acids. C₂ = acetic, C₁₂ = lauric, C₁₄ = myristic, C₁₆ = palmitic, C₁₈ = stearic, C_{18:1} = oleic, C_{18:2} = linoleic.

Fig. 9. Furthermore, the temperature of extrusion also affects the amount of complexation. The complexing between amylose and oleic acid was higher at 200°C, which is the temperature of maximum expansion of manioc starch, than at 250°C. A maximum of complexation can probably be obtained by using an extrusion temperature that produces maximum expansion.

Extrusion Cooking of Manioc Starch with Various Fats

Addition of 2 and 4% of various fats did not affect manioc starch. The x-ray patterns of starch extruded with lard, copra, soya lecithin, butter, and palm, peanut, and sunflower oils showed an amorphous structure. No complex can be formed because the fats are bigger than the internal helix section of amylose.

In all the cases, starch was solubilized up to 90% (Fig. 10) and the iodine spectra of the soluble extruded fractions were all similar, with an λ_{max} at 590 nm.

Technological Interest of Extrusion with Fatty Acids

By extrusion of native manioc starch with lipids, an amylose-lipid complex can be formed using only free fatty acids and monoglycerides. As more amylose is complexed, the water-soluble fraction decreases and therefore becomes more concentrated into amylopectin. This phenomenon is of technological interest.

The amount of water-soluble material seems to be directly

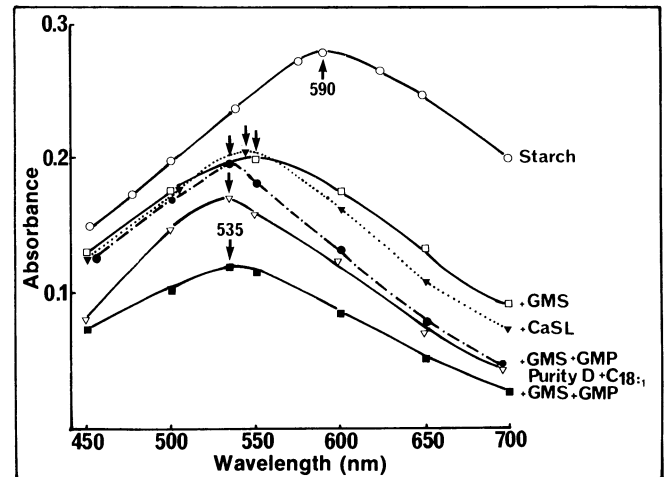


Fig. 8. Iodine spectra of water-soluble manioc starch and chemically modified starch (Purity D) extruded with and without 2% monoglycerides. GMS = glyceryl monostearate, CaSL = calcium stearyl lactylate, GMP = glyceryl monopalmitate, C_{18:1} = oleic acid. (GMS + GMP mixtures: ● = Dimodan, ■ = Amidan.)

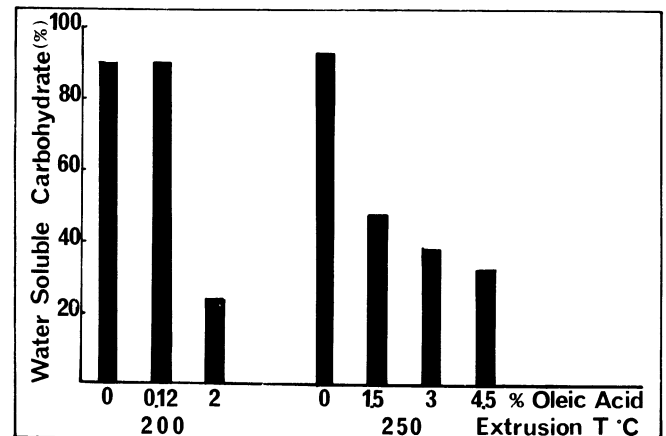


Fig. 9. Water solubility of manioc starch extruded with and without 1.5, 3.0, and 4.5% oleic acid at an extrusion temperature of 250°C and with and without 0.12 and 2.0% oleic acid at an extrusion temperature of 200°C.

related to the stickiness of the extruded product. As extruded starch becomes soluble, it becomes sticky in the mouth. Because the presence of fatty acids during extrusion reduces the solubility, the

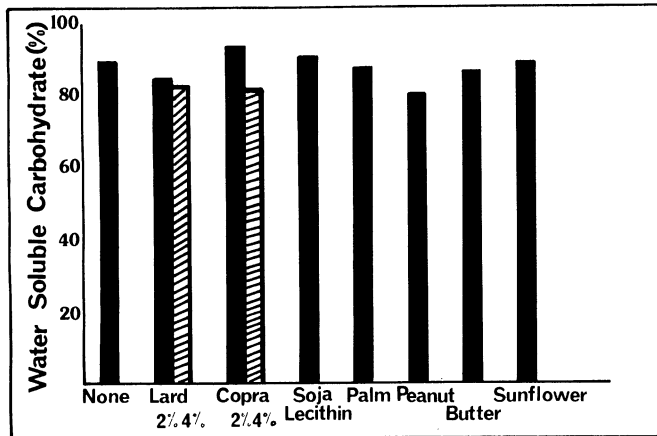


Fig. 10. Water solubility of manioc starch extruded with and without 2 or 4% fats, at 200°C and 17.6% initial moisture content before extrusion.

stickiness is also reduced, which improves the palatability of the end product.

After extrusion with fatty acids, the water-soluble fraction, which is mainly amylopectin, was more stable at low temperature than were the total solubles from starch extruded alone.

At room temperature and +4°C, overnight, the extruded soluble fraction was stable, and almost no retrogradation appeared for the sample extruded without or with lipids.

At -20°C after five successive freeze-thaw cycles, the amount of retrograded material decreased (Fig. 11), a change that is related to the chain length of the fatty acid and, for chains of the same length, to the number of unsaturated bonds.

The same results were obtained with monoglycerides. Stearic acid and GMS gave similar temperature stability to the starch solutions, which is understandable because both precooked starches released mainly amylopectin into the solutions (Fig. 7 and 8).

The behavior of the cross-linked manioc starch (Purity D), reputed commercially to have high and low temperature stability, was similar after extrusion to that of native manioc starch (Fig. 11). The soluble fraction retrograded at -20°C to the same level of 35%. Extrusion of manioc and Purity D with 2% oleic acid reduced the retrogradation for both starches, which behaved similarly.

Because no complexation of amylose was observed by extrusion of starch with fats and because the soluble fraction was as high as the one obtained from extruded starch without lipids, the amount of retrograded material is important and similar to the native starch (Fig. 12).

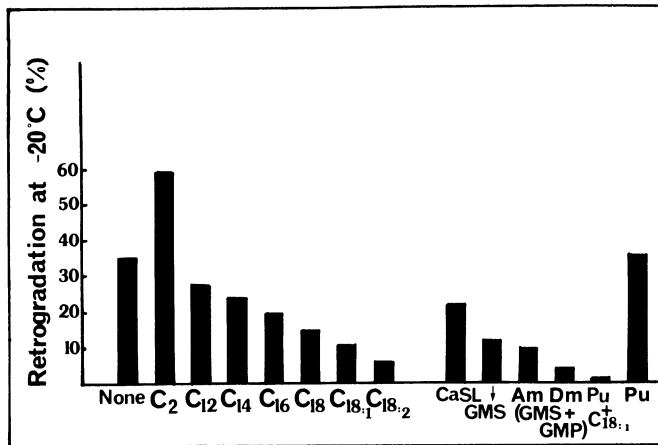


Fig. 11. Percentage of retrogradation at -20°C after five successive freeze-thaw cycles of manioc starch and chemically modified starch (Purity D) extruded with 2% fatty acids and monoglycerides. C₂ = acetic, C₁₂ = lauric, C₁₄ = myristic, C₁₆ = palmitic, C₁₈ = stearic, C_{18:1} = oleic, C_{18:2} = linoleic, CaSL = calcium stearyl lactylate, GMS = glyceryl monostearate, Am = Amidan and Dm = Dimodan (mixtures of GMS and GMP [glyceryl monopalmitate]), Pu = Purity D.

DISCUSSION AND CONCLUSIONS

The present work has shown that twin-screw extrusion cooking of manioc starch disorganizes the granule as it does in the starches already studied (Mercier and Feillet 1975, Mercier 1977). An amorphous structure, previously found for potato starch, was observed by x-ray diffractometry, and the extruded manioc starch became almost completely water-soluble.

Extrusion of manioc starch in the presence of at least 0.3% fatty acids or monoglycerides caused the amylose constituent to be complexed in the stable V-amylose structure. This behavior confirms the previous results (Mercier et al 1979) obtained from cereal and tuber starches. Cereal starches containing natural fatty acids and amylose form an amylose-lipid complex when extruded, whereas potato and manioc starches with a lipid content lower than 0.1% do not. After addition of at least 0.3% oleic acid, the V-amylose structure was observed.

Cross-linkages due to the reaction of starch with epichlorohydrin could be located mainly around the α 1-6 bonds of amylopectin, with very few on the amylose, as has been shown for the O-2-hydroxypropyl distarch phosphate of manioc starch (Hood and Mercier, 1978).

The reaction of Purity D to extrusion with and without oleic acid was similar to that of manioc starch and seemed to reinforce the finding that the chemical cross-links are located on the amylopectin fraction.

In addition to the insolubilization of amylose involved in a lipid complex, the soluble fraction is reduced when the complexing increases. The soluble fraction is richer in amylopectin, the branched component that is much more soluble at low temperature. Indeed, as more complex is formed with fatty acids, the soluble fraction is decreased and the low temperature stability is better.

The complexing of amylose with different fatty acids, alcohols, and pyridine is well known and is used in starch technology to fractionate amylose from amylopectin. By extrusion, however, the complex can be formed under low-moisture conditions. Starch is gelatinized to solubilize amylose, and the lipids enter the internal helix of the amylose molecules. However, the reagents must be stable at extrusion temperature. The boiling temperatures of acetic acid and ethanol are too low to simultaneously obtain gelatinization and a stable complex. On the other hand, extrusion of manioc starch with glycerol or fats did not produce complexation because

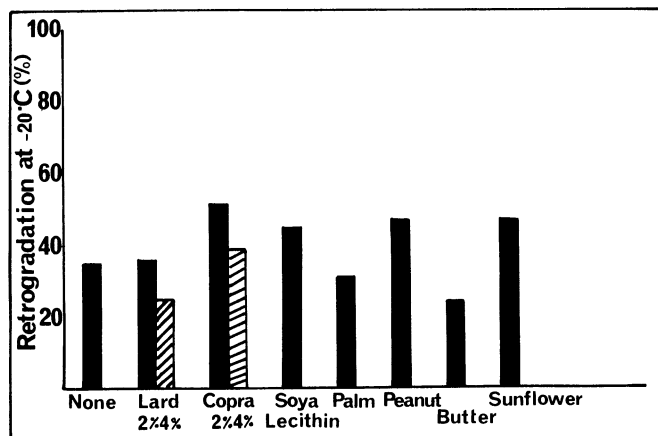


Fig. 12. Percentage of retrogradation at -20°C after five successive freeze-thaw cycles of manioc starch extruded with and without 2 or 4% fats.

of the bulky size of these lipids.

Under proper conditions, extrusion can produce a modified starch having technological properties, such as solubility, reduced stickiness, and freeze thaw stability, which are competitive with those of chemically modified starch.

ACKNOWLEDGMENTS

We thank Marie-Jeanne Crepeau for technical assistance. This work was supported by a grants from La Délégation Générale à la Recherche Scientifique et Technique, No. 75-7-0616 et 77-7-0432.

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[Received February 20, 1979. Accepted July 1, 1979]