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Macromolecular Modifications of Manioc Starch Components by Extrusion-Cooking with and without Lipids*

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SUMMARY

Macromolecular structure of manioc starch, extruded without and with lipids (oleic acid, dimodan, soya lecithin and copra) was studied, using chemical, enzymic, viscometric and chromatographic methods. Twin screw extrusion-cooking led to a macromolecular degradation of both amylose and amylopectin. The formation of lower molecular weight material was observed by a decrease of intrinsic viscosities of both components and also by their behaviour on Sepharose CL-2B, whereas no modification of β-amylolysis and iodine-binding capacity could be detected. The macromolecular degradation was increased by higher temperature and screw speed of the extruder, and was decreased by adding lipids during extrusion. Lipids such as fatty acids, mono- and triglycerides have been shown to act as lubricants (each type in its distinctive way). Lipid extraction, by different solvents, appears to have a low efficiency. Although the addition of triglycerides during extrusion reduces the macromolecular degradation, leading to a high solubility, the amylose-lipid complexes reduce the water-soluble fraction. This fraction was shown to be mainly composed of aggregated amylopectin-like material and to be highly stable after successive freeze–thaw cycles.

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INTRODUCTION

The effects of extrusion-cooking on starch have been extensively studied in the last decade and have recently been reviewed by Harper (1981) and Linko *et al.* (1982). The principal effect of this thermo-mechanical treatment is to disorganise the granular structure. Scanning electron microscopic observations of starch-based products extruded at temperatures above 150°C show that the starch granules are partially gelatinised and may form composite walls with cellulose and cellular proteins (Mercier & Feillet, 1975; Mercier, 1977; Mercier *et al.*, 1979). The native crystalline-like structure is partially or completely destroyed (Charbonniere *et al.*, 1973; Mercier *et al.*, 1979), as demonstrated by a typical amorphous X-ray pattern. However, in the presence of fatty acids or monoglycerides (at least 0·3%), extrusion-cooking leads to an organised structure of helical lipid–amylose complexes, similar to the n-butanol amylose V-complex (Mercier *et al.*, 1979, 1980). Starch gelatinisation, occurring in a dry medium, leads to the development of certain functional properties such as water solubility and water absorption capacity; these aspects have been particularly studied with sorghum (Conway *et al.*, 1968, 1971*a*,*b*; Anderson *et al.*, 1969*a*,*b*, 1970), potato (Mercier, 1977), rice (Mottern *et al.*, 1969; Spadaro *et al.*, 1971; Hennesey *et al.*, 1971) and wheat and maize (Mercier & Feillet, 1975; Olkku, 1979, 1981).

These characteristics have been observed to depend upon the amylose/amylopectin ratio and on the extrusion parameters such as moisture of the raw material, temperature, screw speed and screw geometry of the extruder.

Little attention has been paid to the importance of the macromolecular degradation of starch by extrusion-cooking. Several authors (Lorenz & Johnson, 1972; Moore, 1973; Chiang & Johnson, 1977; de la Gueriviere & Grebaut, 1975; Linko *et al.*, 1979, 1980) have suggested that starch molecules (amylose and amylopectin) could be broken down to smaller macromolecules during this process. The formation of linear oligosaccharides has only been observed on the extrusion-cooking of potato starch (Mercier, 1977). The extent of this molecular transformation in extruded starch based products would explain the variation of the functional properties with extrusion parameters (Mercier & Feillet, 1975).

The purpose of this paper is to study the changes in macromolecular structure of manioc starch components during extrusion with and without lipids.

MATERIALS AND METHODS

Extrusion-cooking

Commercial manioc starch (Roquette National Chemie, Lestrem, France) was extruded in a semi-industrial twin-screw extruder (Creusot-Loire, BC 45) at various temperatures (125, 150, 175 and 200°C) and screw speeds (40 and 80 rpm) with a moisture content of 22·0% and a feed rate of 24 kg h^{-1}.

Extrusion was also performed at 200°C with 21·0% water content (on a dry basis before extrusion) and fatty acid (oleic acid, 2%), a mixture of glyceryl monostearate and glyceryl monopalmitate (dimodan, 2%), a triglyceride (copra) and phosphatidyl choline (soya lecithin).

Materials

Sepharoses CL-2B and CL-4B were products of Pharmacia Fine Chemicals, Uppsala, Sweden. Isoamylase (EC 3.2.1.68) (Hayashibara Biochemical Laboratories Inc., Okayama, Japan) and β-amylase (EC 3.2.1.2) (Koch-Light Laboratories Ltd, England) were employed. Industrial amylose (Avebe, Veendam, The Netherlands) was used as standard amylose. All other chemicals were of analytical grade.

Gel permeation chromatography

Chromatography was performed at room temperature in a column 90 × 3·3 cm, with gel beds around 88 cm high. Elution with 0·1 N KOH was performed in an ascending mode with a peristaltic pump, at a flow rate of approximately 4·5 ml h^{-1} cm^{-2}. No variation in the bed volume took place while being continuously used over a period of several months.

Solutions of α-glucans were prepared for chromatography as follows: a sample (approx. 10 mg) was dispersed in 1 ml of 1N KOH at + 4°C

for 30 min followed by the addition of 9 ml of water. Complete solubilisation of the starch was obtained in this way. Aliquots (3–7 ml) were injected into the column. Fractions of constant volume (8·2 ml) were collected automatically. Each fraction was identified by its partition coefficient K_{av}, where $K_{av} = (V_e - V_0)/(V_t - V_0)$; V_e, V_0 and V_t are the fraction elution volume, exclusion volume (as native amylopectin elution volume) and total volume (as glucose elution volume) respectively. The concentrations of polysaccharides in the column fractions were determined automatically by the orcinol method as used by Tollier & Robin (1979). Recoveries from the columns varied from 95 to 105%. The elution profile represents the concentration ($\mu g\ ml^{-1}$) in each fraction, for 1 mg recovered, plotted against K_{av}.

Each fraction was characterised by determining the λ_{max} of its iodine complex produced by mixing not more than 8 ml of eluent (corresponding to 200 μg of material), 0·8 ml of 1N HCl and 0·208 ml of aqueous iodine solution (0·2% iodine + 2·0% potassium iodide).

Enzymic methods

The percentage of α-1,6 linkages in native and extruded starches was determined by measuring the percentage of glucose reducing power liberated after hydrolysis by isoamylase, and calculated as:

$$\%\ \alpha\text{-1,6 linkage} = \frac{\text{reducing power (glucose equivalents)}}{\text{polysaccharide in digest (glucose equivalents)}}$$

Debranching by isoamylase in DMSO (20% v/v), followed by a β-amylolysis to verify the complete hydrolysis of α-1,6 linkages, was performed as by Mercier & Kainuma (1975) as well as the preparation of β-limit dextrins which have been further dialysed to remove maltose before any chromatography. The total concentration of starch-type polysaccharide was measured in water–DMSO (v/v : 4/1) by the amyloglucosidase method as described by Colonna *et al.* (1981). All other determinations were performed as described previously (Colonna *et al.*, 1982).

Chemical methods

In all determinations, lipids were removed previously by boiling ground samples in methanol–water (v/v : 85/15).

Starches were fractionated into amylose and amylopectin by selective precipitations with thymol and then n-butanol (Banks & Greenwood, 1967).

Iodine-binding capacities (IBC) were measured by an amperometric method (Larson *et al.*, 1953) at 25°C.

The intrinsic viscosity $\{\eta\}$ was determined in 0·2 M KOH at 25°C with an Ostwald viscometer (solvent flow time, 88 s). For amylose, the viscosity average molecular weights ($\bar{M}_v$) were calculated according to Banks & Greenwood (1969) ($K_a = 6{\cdot}92 \times 10^{-3}$; $a = 0{\cdot}78$).

Number average molecular weights ($\bar{M}_n$) of amylose were determined by measuring their reducing end groups (Nussenbaum & Hassid, 1952).

Water soluble fraction (WSF)

Water soluble fractions were prepared according to Mercier & Feillet (1975). Stable water soluble fractions (SWSF) at low temperature were obtained after five freeze–thaw cycles (−20°C), followed each time by a centrifugation at 3400 g, for 15 min at 20°C.

Quantitative determination of lipids

In all cases, ground, extruded products, with a particle size less than 0·5 mm were vacuum dried over phosphorus pentoxide at room temperature before analysis.

Four methods of determination were used:

Procedure a

After hydrolysis by hydrochloric acid in the presence of formic acid and ethanol, lipids were recovered in n-hexane by liquid–liquid extraction and determined gravimetrically (Drapron, 1975).

Procedure b

Samples (weight, 5 g) were washed with n-hexane (100 ml) five successive times at room temperature during 4 h. Lipids were recovered in the hexane fraction and weighed after solvent evaporation.

Procedure c

Lipids were extracted in a Soxhlet apparatus with n-hexane–ethanol (v/v : 75/25) (sample weight, 10 g).

TABLE 1
Characteristics of Native and Extruded Manioc Starches without and with Lipids

	IBC (g of I_2/ 100 g of starch)	*β-amylolysis, %*	*α-1,6 linkages, %*	*Intrinsic viscosity ($ml\,g^{-1}$)*	*Elution profile on Sepharose CL-2B*		
					% material with $K_{av} \leqslant 0.1$	*K_{av} of last fraction with λ_{max} = 540–550 nm*	*K_{av} of first fraction with $\lambda_{max} \geqslant$ 610 nm*
Native starch	3·5	61·8	4·1–4·2 (103)[a]	197·3	61·7	0·08	0·45
Extrusion without lipids							
$T = 125°C$ $v = 40$ rpm	3·5	62·7	ND	104·1	31·4	0·23	0·57
$T = 150°C$ $v = 40$ rpm	3·6	61·7	4·0–4·3 (103)[a]	103·5	31·8	0·25	0·57
$T = 150°C$ $v = 80$ rpm	3·6	60·3	4·1–4·2 (102)[a]	71·0	23·0	0·31	0·63
$T = 175°C$ $v = 40$ rpm	3·5	61·9	ND	75·0	24·1	0·35	0·58
$T = 200°C$ $v = 40$ rpm	3·6	62·8	4·3 (102)[a]	69·5	15·9	0·34	0·77
Extrusion with lipids (200°C, 40 rpm)							
2% copra	3·4[b]	ND	ND	101·5	22·5	0·24	0·55
4% copra	3·5[b]	ND	ND	137·5	31·6	0·13	0·47
2% soya lecithin	3·5[b]	ND	ND	139·2	34·3	0·10	0·48
2% dimodan	3·4[b]	ND	ND	123·0	28·2	0·16	0·52
2% oleic acid	3·3[b]	ND	ND	78·4	18·8	0·26	0·65

ND, not determined. [a] Degree of β-amylolysis obtained after debranching. [b] Measured after defatting.

Procedure d
Lipids were extracted by boiling methanol–water (v/v: 85/15) (sample weight, 5 g).

RESULTS

Macromolecular structure of native manioc starch

The native manioc starch had an iodine binding capacity (IBC) of 3·5 g I_2 per 100 g of dry starch, corresponding to an apparent amylose content of 18·9% and a β-amylolysis limit of 61·8% (Table 1). The isoamylase-debranching led to a percentage of α-1,6 linkages of 4·1–4·2 corresponding to a CL of 24·1. The intrinsic viscosity of native starch, solubilised in 0·2 N KOH was 197·3 ml g^{-1}. The elution profile on Sepharose CL-2B (Fig. 1) showed a sharp peak located at the void volume, representing 61·7% of the total material, followed by a tail up to the total volume. The wavelength (540 nm) of the maximum absorption of the iodine–polysaccharide complex (λ_{max}) identified amylopectin in the first peak. As the K_{av} of a given fraction increased, so also did λ_{max}, from 540 to 620 nm, which indicated an increasing contamination of amylopectin by amylose, pure amylose being observed at $K_{av} = 0{\cdot}45$. When subjected to chromatography on Sepharose CL-4B, the profile of the β-limit-dextrin (Fig. 2) from native manioc starch was characterised by a main peak (74·4% of the total material), eluted at the void volume, followed by a long tail until $K_{av} = 0{\cdot}82$.

After fractionation of native starch into amylose (19%) and amylopectin (78%), the elution profile on Sepharose CL-2B of the isolated amylopectin ($[\eta] = 167{\cdot}9$ ml g^{-1}) (Fig. 3) showed mainly a sharp peak at the void volume with a λ_{max} of 540 nm and a very low tail up to $K_{av} = 0{\cdot}75$. In contrast, the elution profile (Fig. 4) of fractionated amylose ($[\eta] = 243{\cdot}1$ ml g^{-1}; $\bar{M}_n = 275\,400$; IBC = 18 g I_2 per 100 g α-glucan) was composed essentially of a wide peak from $K_{av} = 0$ to $K_{av} = 0{\cdot}9$, with a λ_{max} of 620 nm at $K_{av} = 0{\cdot}45$.

Effects of extrusion temperature and screw speed

Changes in some of the characteristics of the extruded products compared to the native starch are seen in Table 1.

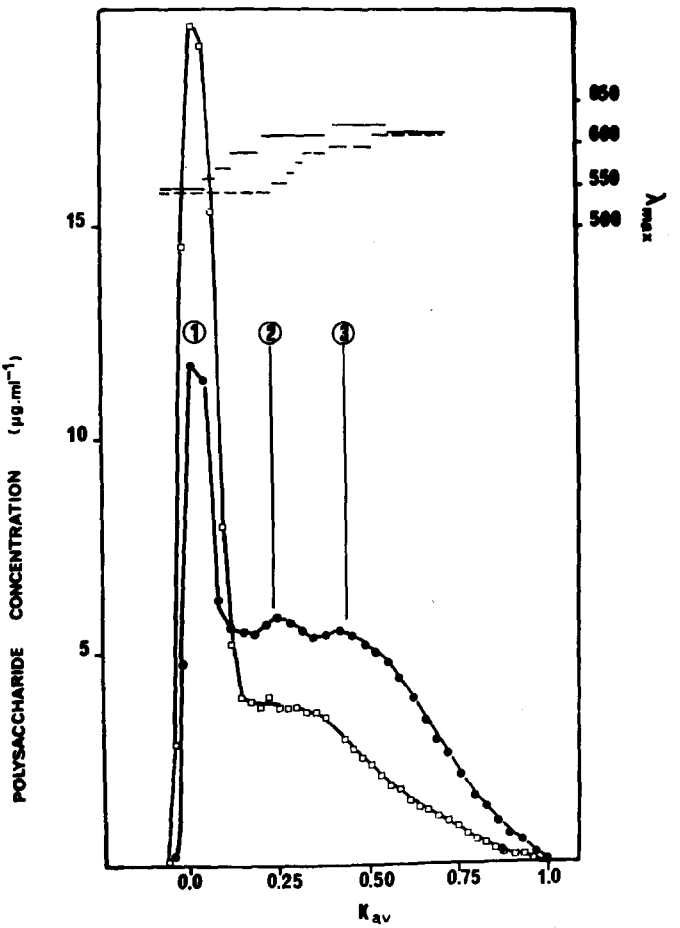

Fig. 1. Elution profiles from Sepharose CL-2B of native (□) and extruded (150°C, 40 rpm) (●) manioc starches.

No difference can be observed for the IBC (3·5–3·6 g I_2/100 g of dry starch), β-amylolysis limit (60·3–62·8%) and percentage of α-1,6 linkages (4·0–4·3%) between native and extruded starches after solubilisation in either 0·1N KOH or water–DMSO (v/v : 4/1). These results indicate that there is no preferential splitting of α-1,6 linkages.

In contrast, the intrinsic viscosity is highly reduced by extrusion to the range 104·1–69·5 ml g^{-1}: this decrease is observed both on increasing the extrusion temperature from 125°C (104·1 ml g^{-1}) to 200°C

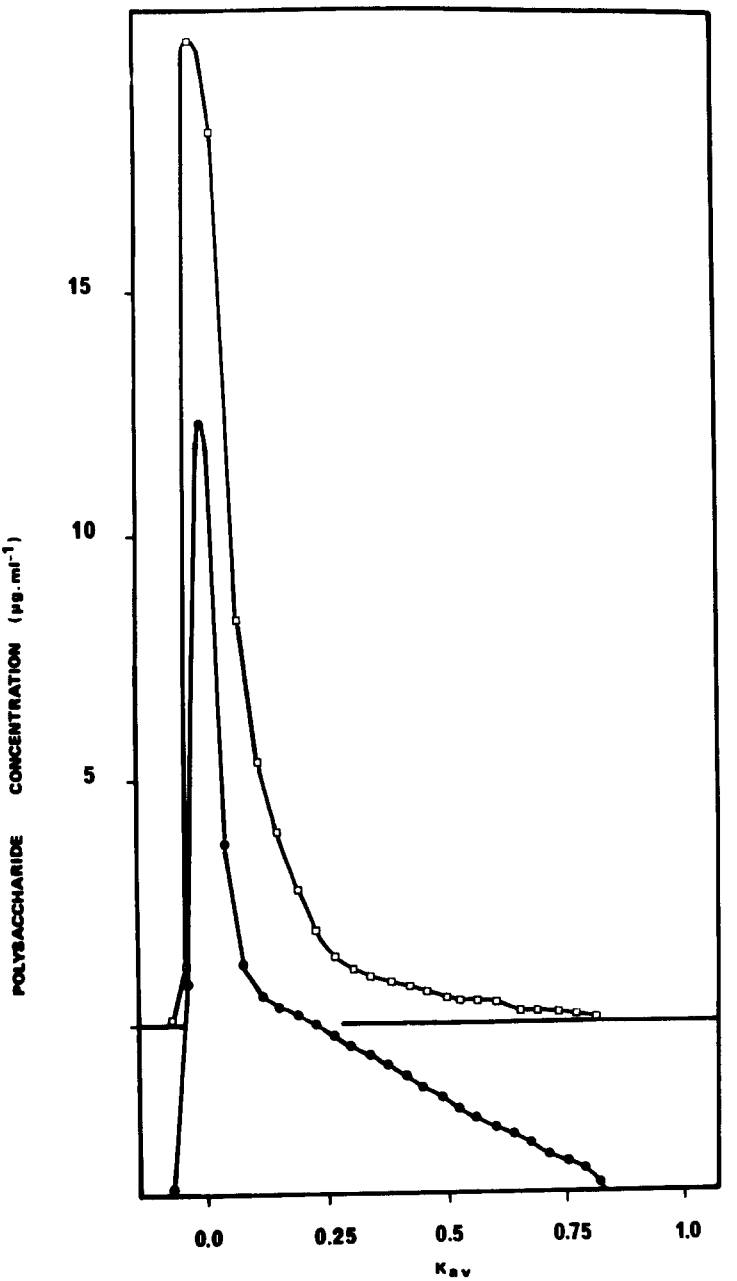

Fig. 2. Elution profiles from Sepharose CL-4B of β-limit-dextrin from native (□) and extruded (150°C, 40 rpm) (●) manioc starches.

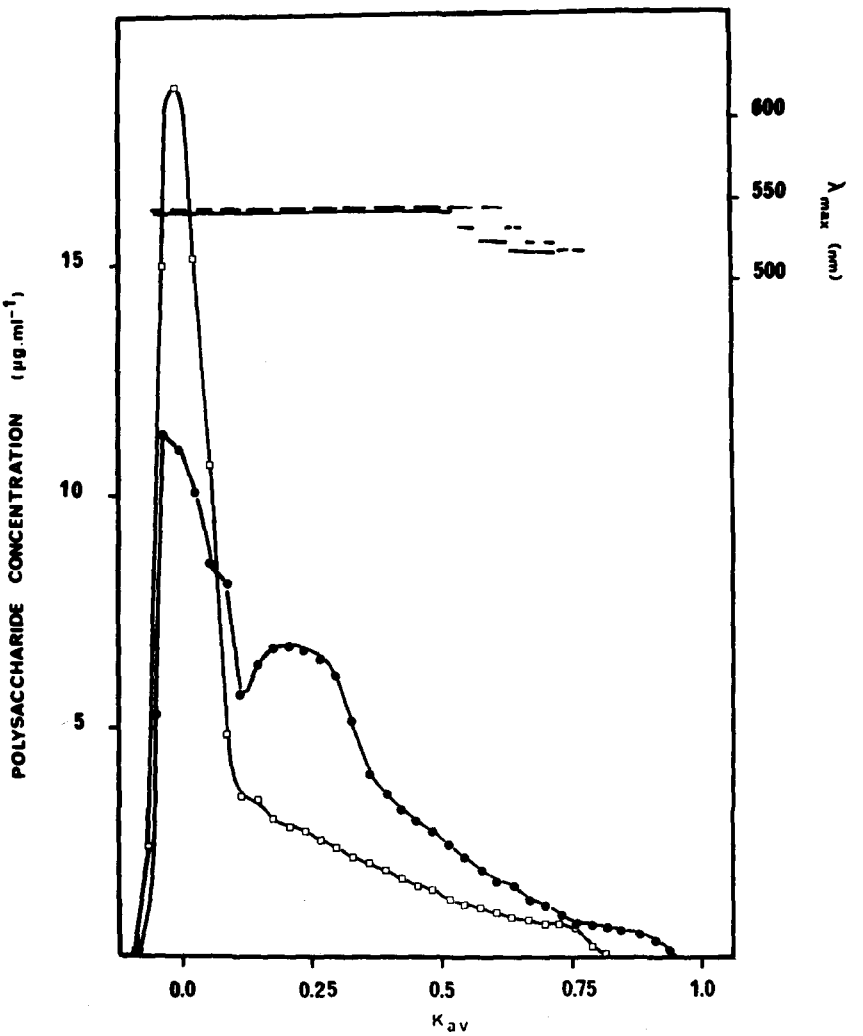

Fig. 3. Elution profiles from Sepharose CL-2B of amylopectins extracted from native (□) and extruded (150°C, 40 rpm) (●) manioc starches.

(69·5 ml g^{-1}) at 40 rpm or screw speed from 40 rpm (103·5 ml g^{-1}) to 80 rpm (71·0 ml g^{-1}) at 150°C. By gel permeation chromatography on Sepharose CL-2B, elution profiles (Fig. 1) of extruded starches show three peaks. The percentage of material eluted at the void volume (peak 1) decreases from 61·7% for native starch to the range of 31·4%

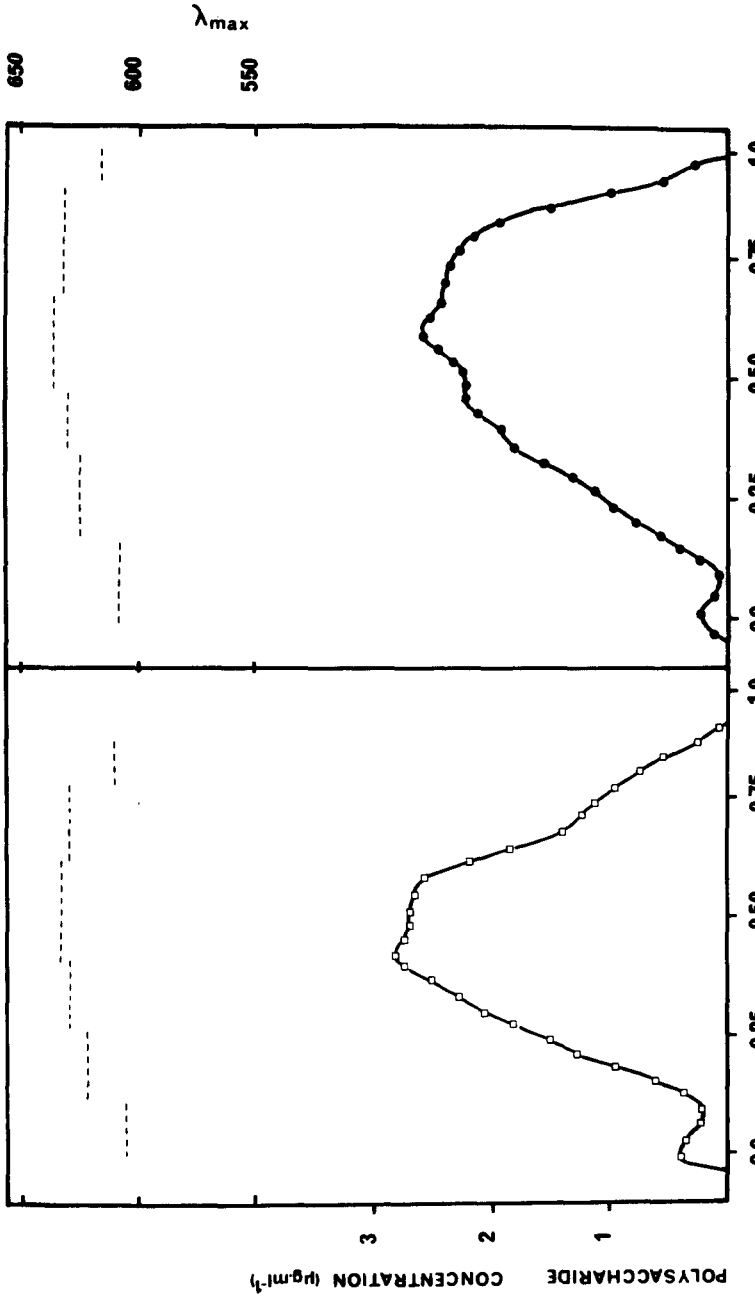

Fig. 4. Elution profiles from Sepharose CL-2B of amyloses extracted from native (□) and extruded (150°C, 40 rpm) (●) manioc starches.

to 15·9%, when the extrusion temperature and/or screw speed increase. The λ_{max} of 540–545 nm identifies this peak 1 as still pure amylopectin. The importance of material eluted at $K_{av} = 0{\cdot}25$ (peak 2) increases from native to extruded starches. The λ_{max} value (540–550 nm) still indicates an amylopectin-like molecule, although the K_{av} of the peak is increased from 0·23 to 0·35 with increasing macromolecular degradation.

The third peak (peak 3), which was not well resolved from peak 2, was observed only after extrusion, at K_{av} varying from 0·57 to 0·77, with an increase of temperature and screw speed. The λ_{max} of peak 3 never reached higher values than 610 nm and both K_{av} and λ_{max} indicated a macromolecular degradation of amylose, since in native starch amylose was observed at $K_{av} = 0{\cdot}45$ with a λ_{max} of 620 nm. A further increase in screw speed (150°C, 80 rpm) degraded material 3 into smaller chains (2·9%), eluted at the total volume.

The profiles of the β-limit-dextrins of all these extruded starches (Fig. 2), when analysed by chromatography on Sepharose CL-4B, showed only quantitative differences with the native starch one. The percentage of material excluded from the Sepharose CL-4B gel decreased to the range 43·5% (125°C, 40 rpm)–33·5% (200°C, 40 rpm), whereas the second part of the chromatography profile was increasing and finished around $K_{av} = 0{\cdot}9$. The elution profile on Sepharose CL-2B of the amylopectin fraction ($[\eta] = 98{\cdot}3$ ml g^{-1}) isolated (recovery: 77%) from the extruded starch (150°C, 40 rpm), showed two peaks (Fig. 3), both with λ_{max} of 540 nm, corresponding to material 1 and 2 of the total extruded starch. Similarly, the fractionated amylose (recovery 17%) ($[\eta] = 110{\cdot}0$ ml g^{-1}; $\bar{M}_n = 90\,200$; IBC = 18 g of I_2 per 100 g of α-glucan) from extruded starch (150°C, 40 rpm) (Fig. 4), showed one peak, located at $K_{av} = 0{\cdot}60$. These results indicate that both amylose and amylopectin have been degraded by extrusion into lower molecular weight material.

Effect of lipids on extruded manioc starch

Native manioc starch has a very low lipid content (0·1%). When lipids are added during extrusion at 200°C with a screw speed of 40 rpm, the total lipid contents (Table 2), estimated by the hydrolysate lipid value (Procedure *a*) agree with the theoretical amount (2%). In contrast, the amounts of lipid recovered by hexane (Procedure *b*) and hexane–ethanol (Procedure *c*) represent only 6–30% and 14–65% respectively

TABLE 2
Yields (%, Dry Basis) of Lipids from Manioc Starch Extruded with Lipids According to the Four Described Procedures (*a*, *b*, *c* and *d*)

Sample	*Procedure*			
	a *hydrolysate lipids*	*b* *hexane*	*c* *hexane-ethanol*	*d* *boiling methanol-water*
Native starch	0·1	ND	ND	ND
Extruded starch (200°C, 40 rpm)				
without lipids	0·1	ND	ND	ND
with 2% copra	2·4	0·8	1·9	2·2
with 2% soya lecithin	1·6	0·1	0·6	1·4
with 2% dimodan	1·8	0·2	0·5	1·6
with 2% oleic acid	2·2	0·2	0·3	1·8

ND, Not determined.

of the total lipids, showing that lipid extraction is incomplete. It is only with boiling water–methanol (v/v: 15/85) that results are near those obtained by the *a* procedure – 82–92% of the total lipid values. Therefore, the Procedure *d* has been applied to all samples before any characterisation.

When the fat content (copra) added to starch before extrusion increased from 0 to 4%, the IBC did not change whereas the intrinsic viscosity of the extruded starch increased from 69·5 to 137·5 ml g^{-1} (Table 1). The chromatography profiles on Sepharose CL-2B were characterised by an increase of the material excluded from the gel (from 15·9 to 31·6%). The amylopectin-like and amylose materials were shifted towards lower K_{av} (from $K_{av} = 0{\cdot}34$ to $K_{av} = 0{\cdot}13$ for peak 2 and from $K_{av} = 0{\cdot}77$ to $K_{av} = 0{\cdot}47$ for peak 3), which are almost similar to those of native starch. Therefore these results demonstrate that extrusion in the presence of copra modified the macromolecular weight distribution of manioc starch components.

Manioc starches, extruded with 2% of monoglyceride (dimodan) or soya lecithin, exhibited intrinsic viscosities and modified chromatographic profiles on Sepharose CL-2B, in the same order as those with copra. However, 2% of soya lecithin had the same effect on macromolecular structure as 4% of copra, whereas 2% of dimodan led to intermediate results. In contrast, manioc starch extruded with 2% oleic acid showed the same low intrinsic viscosity (78·4 ml g^{-1}) and the same profile on Sepharose CL-2B as those for starch extruded (200°C, 40 rpm) alone.

Water solubility of extruded starches

Whereas native starch is insoluble in water at room temperature, extruded starches become partly soluble, leading to a turbid solution. A quantitative study of the water soluble fractions (WSF) (Table 3) confirmed our previous work (Mercier *et al.*, 1980); there is an increase of solubility with the addition of triglyceride and soya lecithin, but a notable decrease of solubility in the presence of fatty acid or monoglyceride.

The WSF of extruded starch (150°C, 40 rpm) has a high turbidity (OD = 0·385), estimated by the optical density of the freshly prepared WSF (concentration, 16·5 mg ml^{-1}) at 500 nm. This turbidity may be greatly decreased by adding to 9 ml of the WSF, 1 ml of 1 N KOH (OD = 0·085), or urea up to 8 M (OD = 0·153), or by heating in a boiling water-bath for 3 min (OD = 0·050).

The WSFs of starches extruded without lipids have lower IBC values (0·7–0·8 g of I_2 per 100 g of α-glucan) and lower β-amylolysis limits (58·4–58·8%) than the total extruded starches. The intrinsic viscosities are reduced to 64·7, 68·6 and 50·3 ml g^{-1} for WSF from samples extruded at 150°C 40 rpm, 150°C 80 rpm and 200°C 40 rpm respectively. In comparison with their corresponding extruded starches, the WSFs have chromatographic profiles on Sepharose CL-2B, characterised by lower amounts of material eluted at the void volume (amylopectin-like: 17·7–23·8%), a shift towards 1 of the fraction's K_{av}, containing mainly amylose and therefore a wide peak located between $K_{av} = 0$ and 1. Their β-limit-dextrins also show low amounts of material at the void volume (37·5 and 30·3% respectively) on Sepharose CL-4B.

Chromatographic profiles on Sepharose CL-2B of the WSF from starches extruded with fat (Table 3), such as soya lecithin and copra,

TABLE 3
Importance and Characteristics of Water Soluble Fractions (WSF) and Stable Water Soluble Fractions (SWSF) from Extruded Manioc Starches without and with Lipids

	Total starch, %	*Elution profile on Sepharose CL-2B*		
		% material located at $K_{av} \leqslant 0{\cdot}1$	K_{av} *of last fraction with* $\lambda_{max} = 540$–*550 nm*	K_{av} *of first fraction with* $\lambda_{max} \geqslant 610$ *nm*
Extrusion without lipids				
150°C, 40 rpm				
WSF	86·4	23·8	0·39	0·70
SWSF	46·2	18·9	0·38	nd
150°C, 80 rpm				
WSF	86·3	17·3	0·28	0·67
SWSF	24·4	10·6	0·51	nd
200°C, 40 rpm				
WSF	82·0	17·7	0·41	0·60
SWSF	38·1	10·6	0·55	nd
Extrusion with lipids (200°C, 40 rpm)				
2% oleic acid				
WSF	27·5	1·1	0·90	nd
SWSF	26·7	0·4	0·90	nd
2% dimodan				
WSF	27·6	23·2	0·40	nd
SWSF	27·3	10·9	0·85	nd
2% soya lecithin				
WSF	90·4	30·2	0·20	0·58
SWSF	40·5	26·2	0·45	nd
2% copra				
WSF	90·0	19·2	0·44	0·75
SWSF	46·4	13·3	0·37	nd

nd, Non-detectable.

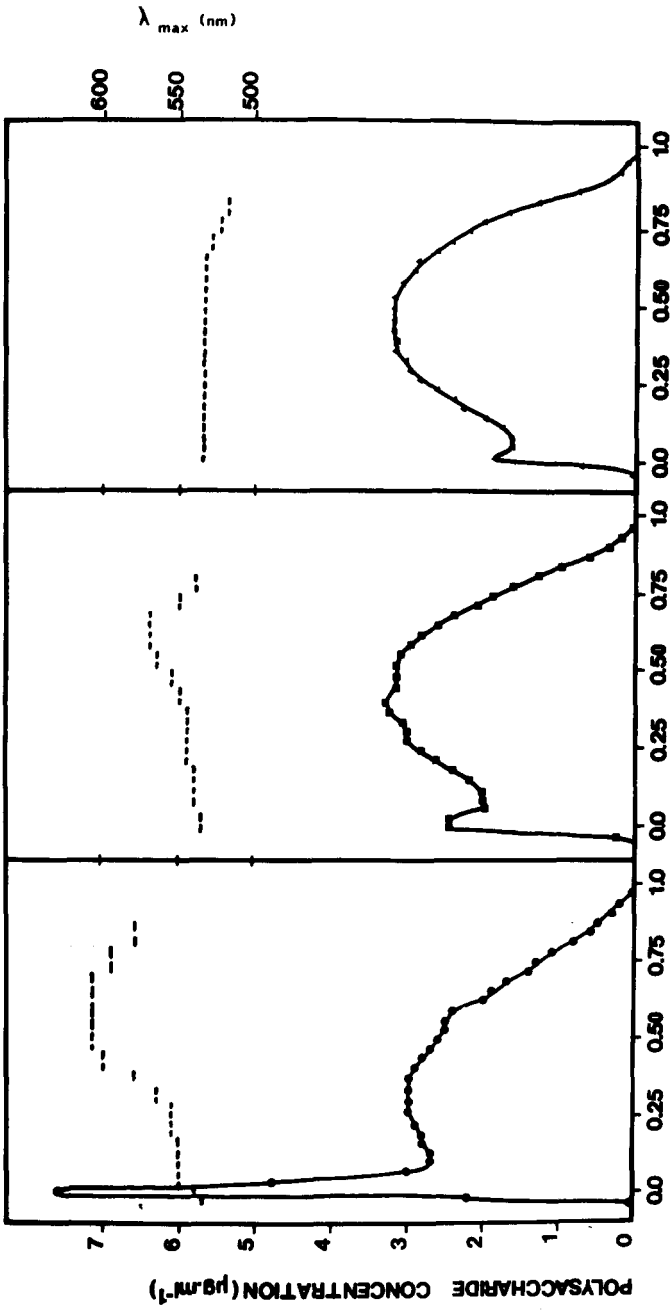

Fig. 5. Elution profiles from Sepharose CL-2B of (left) manioc starch extruded (200°C, 40 rpm) (●) with 2% dimodan; its water soluble fraction (WSF) (middle) (■) and its stable water soluble fraction (SWSF) (right) (▲).

are also characterised by a low amount of material (amylopectin) at the void volume (1·1–30·2%), and amylose-like material is observed at K_{av} values higher than 0·58 and 0·75 respectively. With dimodan (Fig. 5), there is only amylopectin-like material until $K_{av} = 0{\cdot}40$, but above $K_{av} = 0{\cdot}40$, no fraction has a λ_{max} higher than 610 nm, whereas with fatty acid (oleic acid), the second part of their profile is free of amylose, as shown by a constant λ_{max} of 540–550 nm.

Only part of the WSF is stable after five successive freeze–thaw cycles (−20°C) (SWSF) for starches extruded alone and with copra or soya lecithin. In contrast, almost all the WSFs from starches extruded with oleic acid or dimodan are highly stable.

When extrusion is carried out with and without fatty acid or monoglycerides, their profiles (Table 3) are characterised by lower amounts of material extruded from the gel (10·6–18·9%) than for their corresponding WSF, λ_{max} never reaching a value higher than 610 nm during the whole elution. The SWSF from starch extruded alone at 150°C and 40 rpm, has an IBC of 0·6 g of I_2 per 100 g of α-glucan and an intrinsic viscosity of 62·6 ml g^{-1}. The SWSF from starches extruded with lipids are still characterised by minor amounts of material (0·4–26·2%) eluted at the void volume; the main difference in their Sepharose CL-2B profile is the presence of a material, whose iodine complex is absorbing at a λ_{max} always lower than 540 nm (dimodan, Fig. 5).

DISCUSSION

Our results on the characteristics of native manioc starch are in complete agreement with previous studies (Greenwood & Thomson, 1962; Hood & Mercier, 1978; Hizukuri *et al.*, 1981). Manioc amylose has a high molecular weight ($\bar{M}_n = 275\,400$; $\bar{M}_v = 672\,300$) with a great polydispersity ($I = 2{\cdot}44$). The observed values agree also with an exponential distribution for the molecular weight of manioc amylose, as generally suggested by Banks & Greenwood (1975). The gel permeation chromatography on Sepharose CL-2B facilitates a rough separation of amylose (19%) from amylopectin (78%), but the selective fractionation by complexing with thymol and n-butanol (Banks & Greenwood, 1967) is still the best way to separate the two starch components.

The primary chemical structure of starch is preserved during extrusion, since glucose, the monomer, is completely recovered after an

amyloglucosidase action (Mercier & Feillet, 1975; Mercier, 1977; Mercier *et al.*, 1979); the β-amylolysis limits and the percentages of α-1,6 linkages were identical with those of native starch, which explains the similar iodine binding capacities of the extruded starches.

In contrast, the intrinsic viscosities of both the fractionated amylose and the total starches decrease strongly after extrusion-cooking. Banks & Greenwood (1969) have given the parameters K_a and a of the Mark-Houwinck-Sakurada equation for amylose in 0·2 N KOH. Therefore any variation in the intrinsic viscosity of amylose can be related to a variation in the viscometric average-molecular-weight $\bar{M}_v$, leading to an easy quantitative determination of macromolecular degradation by extrusion-cooking at 150°C and 40 rpm. No information on the parameters K_a and a is available for amylopectin, preventing a parallel study of its macromolecular degradation. Whereas the intrinsic viscosity of amylose decreases by 54·8%, the decrease in the intrinsic viscosity of amylopectin is reduced by 41·5%, which is due to its branched structure. As this line of argument applies also to mixtures of amylose and amylopectin, the measurement of intrinsic viscosity of a modified starch, in comparison to a native starch, can be used as a tool for detecting any degradation of the macromolecular structure of starch components. The results obtained with the viscometric method correlate well with the profiles found by gel permeation chromatography which also exhibit the molecular degradation of starch by extrusion-cooking either on the fractionated components or on the total starch. However, it is not possible at the present time to use the gel permeation chromatography for the determination of average-molecular-weights, in view of the absence of suitable standards for calibration purpose. The calibration of the column with commercial characterised dextrans (Biliaderis *et al.*, 1979) is not correct as their primary chemical structure is completely different from those of amylose and amylopectin.

Both methods demonstrate that amylose and amylopectin are degraded by extrusion-cooking into lower molecular weight materials. Nevertheless, the amylose splitting is not drastic enough to create substantial amounts of glucose oligomers with DP < 200, otherwise the IBC should decrease (Banks *et al.*, 1971). Knowing that β-amylase completely converts linear amylose and the outer chains of amylopectin, from dispersed starch, into maltose and maltotriose, leading to β-limit-dextrins, differences in elution profiles of β-limit-dextrins from native and extruded starches would indicate differences in the molecular

weight distribution of the internal structure of the branched molecules, mainly amylopectin, after extrusion. Indeed this has been observed on Sepharose CL-4B (Fig. 2), which is a gel with a higher resolution for this type of macromolecule than Sepharose CL-2B. However, our methods are unable to prove whether starch components are broken in pure random chain scission. The slight increase of the polydispersity of amylose after extrusion-cooking and the absence of substantial amounts of oligosaccharides are supporting arguments for this view (Banks & Greenwood, 1975; Jellinek, 1978). Nevertheless, it does not prove that all chain links are of equal accessibility, irrespective of their location in the chains, especially so for amylopectin.

The macromolecular degradation is confirmed to be a positive function of extrusion parameters such as temperature and screw speed. However, this modification is less extensive when lipids are present, suggesting that lipids may act in extrusion as a lubricant, each type of lipid having its distinctive qualities. Manioc starch with a very low lipid content (0·01%–0·1%) has been chosen because it has been demonstrated that no complex is formed by extrusion alone (Mercier *et al.*, 1980). It was therefore interesting to observe the behaviour of such a starch with added lipids during extrusion.

Lipids are not easily extracted from the extrudates by hexane at room temperature and if almost all the lipids are to be removed, then polar solvents at the boiling point are needed. Accurate determination is difficult without acid hydrolysis, as substantial quantities of lipids may be trapped inside impermeable cells, where they are inaccessible to solvents under normal conditions of extraction. Diffusion of solvents in the particles, with variable density and size, is a problem. The low proportion (8–18%) of lipids, which are not extracted by boiling methanol, may possibly be linked to polysaccharides by ester-linkages. It is also worth noticing that extrudates, with lipids that will complex (e.g. dimodan and oleic acid), give always lower recoveries than with copra and soya lecithin, indicating that only a part of the oleic acid and dimodan are involved in complex formation during extrusion-cooking.

Whereas native starch is completely insoluble at room temperature, extruded starches are partly soluble (Mercier *et al.*, 1980) giving a highly turbid WSF. The turbidity was observed to be due to some aggregates of starch components, linked by hydrogen bonds since they are broken down by chemical (urea, alkali) or thermal factors. From the IBC measurements and the gel permeation profiles, it appears that

amylose is less solubilised than amylopectin in the WSF, in comparison with its concentration in the native starch. Moreover, the WSFs are preferentially composed of material of lower molecular weight, arising from amylose and amylopectin. The stabilities at low temperature of these fractions (SWSF) are due to the degraded amylopectin, since the amylose fraction disappears from the SWSF. This is confirmed by the behaviour of the starches extruded with complexing lipids (oleic acid and dimodan), which give a WSF quite free of amylose. This higher water-solubility of degraded amylopectin may be due to either a higher solubility when the molecular weight decreases and/or a greater facility in leaching from the entangled starch molecules during swelling in water.

The use of complexing lipids (oleic acid and dimodan) induces the insolubilisation of amylose in the WSF, by complex formation in exactly the same way as during the selective precipitation of amylose by thymol and n-butanol.

Our results show that the effects of extrusion-cooking on starch must not be interpreted simply in terms of the gelatinisation of starch granules. The macromolecular degradation of both amylose and amylopectin, leading to lower molecular weight material, confers to the extruded starches, typical functional properties. The addition of lipids, acting as a lubricant, can modify some of their properties. Further work is in progress to determine the molecular weights of degraded starch components and to relate their hydrodynamic behaviour to structural characteristics.

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