

STARCH HYDROLYSIS PRODUCTS

The present invention relates to starch hydrolysis products which have use as fat and/or oil substitutes in food compositions. It also relates to
5 a method of making the starch hydrolysis products and to the use of such products in food compositions to replace the fats and/or oils conventionally used in such compositions. The invention particularly relates to the use of the starch hydrolysis products in low-
10 fat and zero-fat salad dressings.

Starch hydrolysis products are obtained by the acid or enzymic conversion of starch. The components of a composition, and therefore the properties of a composition, obtained by acid or enzymic conversion of
15 starch depend on the operating conditions employed in the conversion process, particularly the concentration of the reactants, the temperature at which the reaction is conducted and the time for which the reaction is carried out.

20 Starch hydrolysis products (SHPs), as fat- and oil-substitutes in food compositions, have already been proposed. For instance, GB 1,423,780 discloses a process for the production of starch hydrolysis products having a dextrose equivalent (DE) below 25%,
25 wherein a starch suspension is treated with an α -amylase which is preferably bacterial α -amylase, at a temperature at which the starch granules swell, for from 5 to 30 minutes. The SHPs produced in this way form thermoreversible gels that are reportedly
30 freeze-thaw stable. Recommended food applications using these SHPs include cuttable, low-fat mayonnaise. US 4,510,166 also discloses converted starches for use as fat- or oil-replacement in foodstuffs which are characterised in that the starch has a DE of less than
35 5 and in that aqueous dispersions thereof at 10 - 50% by weight of starch solids have a hot flow viscosity

of at least about 10 seconds at 55°C and are capable of forming gels having a strength of at least 25 g within 24 hours at 4°C. The converted starches may be
5 obtained by heating an aqueous starch slurry and α -amylase until the desired conversion (indicated by DE) is reached.

We have found that salad dressings prepared using the prior art SHPs and no added oil have an
10 unacceptable pasty or gum-like consistency or have other organoleptic properties that are undesirable in such products.

α -Amylases, which catalyse hydrolytic attack of α -D-(1 $\rightarrow$ 4) linkages in starch molecules, occur
15 widely in nature; in plants, bacteria and animals. The properties of an SHP produced using α -amylase catalysed hydrolysis of starch have been found to depend on the type of α -amylase used.

We have surprisingly found that SHPs not
20 suffering from the disadvantages of the prior art SHPs may be produced using, as the conversion enzyme, porcine pancreatic α -amylase.

According to the present invention there is provided a process for the production of a starch
25 hydrolysis product which contains partly gelatinized starch granules comprising the steps of

- (i) preparing an aqueous starch suspension containing from 10 to 30% by weight (dry substance) of starch;
- 30 (ii) heating the aqueous starch suspension to a temperature in the range of from 58°C to 80°C being not significantly higher than the gelatinisation temperature of the starch;
- 35 (iii) treating the aqueous starch suspension with a porcine pancreatic α -amylase at a temperature

in the range of from 58° to 63°C for a suitable period of time to achieve the required DE and/or molecular weight for the water soluble fraction of the product; and

5 (iv) deactivating the enzyme.

The SHPs obtainable according to the process of the invention preferably have a DE in the range of from 3 to 12, more preferably 4 to 10, and are partly soluble in cold water, i.e., water of a temperature of
10 20°C and below. In addition, the SHPs are generally not pourable at 90°C and contain partly gelatinized starch granules observable by microscopic examination and typically exhibit some birefringence under crossed-polarised light. These SHPs can be used to
15 replace, partially or completely, edible fats or oils conventionally employed in food compositions. In particular, they can be used to prepare low- or zero-fat salad dressings, such as mayonnaise, which do not suffer from the textural disadvantages associated with
20 dressings prepared from prior art SHPs.

The starch hydrolysis products of the invention are partly soluble in water at 20°C at a level of 1% by weight and typically comprise from 20% to 60% by weight of a fraction which remains insoluble
25 in water even after boiling. The water soluble fraction of the products preferably shows a peak in its molecular weight profile in the range of from 6 to 30 kDa, preferably in the range of from 8 to 20 kDa. Accordingly, therefore, the present invention also
30 provides a starch hydrolysis product suitable for use as a fat and/or oil substitute in food compositions which is characterised in that it is partly soluble in cold water and its water soluble fraction has a peak in its molecular weight profile in the range of from 6
35 to 30 kDa.

For the SHPs to be produced according to the

present invention, the source of starch subjected to enzymic conversion may be a cereal or root starch, typically selected from barley starch, wheat starch, corn starch, potato starch, tapioca starch, rice starch or sago starch. The starches may be chemically modified (e.g., phosphorus cross-bonded) and may be genetic variations such as waxy corn starch and high amylose corn starch. We have found that SHPs produced from wheat starch according to the present invention are preferred since these have better fat mimetic properties than SHPs produced using the other starch sources.

Starches from different sources may have different gelatinisation temperatures. For instance, not only is it the case that the gelatinisation temperatures of wheat starch and tapioca starch are different but it is also the case that a European wheat starch gelatinizes at a temperature different from the temperature at which an American wheat starch gelatinizes. This difference is, at least partly, due to the difference between the climatic conditions prevailing at the different locations. To take into account these differences, the process of the invention requires the starch, in an aqueous suspension, to be heated to a temperature in the range of from 58° to 80°C but which temperature is not significantly higher than the gelatinisation temperature of the starch being used. This gelatinisation temperature can be determined experimentally. As a result, the starch granules are caused to swell and the starch may become partially gelatinised before or during the onset of enzyme-catalyzed hydrolysis. Preferably, the aqueous starch suspension is heated to a temperature which is slightly below, e.g., 1 - 2°C below, the temperature

at which swelling of the starch begins.

5 The aqueous starch suspension having been heated as described above is then subjected to treatment with a porcine pancreatic α -amylase at a temperature within the range of from 58° to 63°C, preferably 58 - 60°C for a suitable period of time. A typical porcine pancreatic α -amylase that is suitable for use in the present invention is E.C. 3.2.1.1 Type
10 VI-B (Sigma) No. A-3176 which has a reported activity of 25 units/mg solid at pH 6.9 and 13% moisture. One unit of this enzyme is defined as liberating 1 mg of maltose from starch in 3 minutes at 20°C and pH 6.9. Reported β -amylase activity is 7.4 units/mg solid at
15 pH 4.8. The reported pH optimum for this enzyme is 6 - 7 and the enzyme is thermolabile above 50°C. The concentration of the enzyme used in the invention is typically in the range of from 700 to 2100 units/litre.

20 The period of time suitable for the enzymatic conversion of the starch to give a product having the preferred DE in the range of from 3 - 12 and/or the preferred molecular weight profile having a peak in the range of from 6 to 30 kDa for its water soluble
25 fraction is generally from 5 to 30 minutes and typically from 11 - 15 minutes for a product having a DE in the most preferred range of from 4 to 10.

When the desired DE and/or molecular weight for the water soluble fraction of the product is
30 achieved it is necessary that further hydrolysis of the substrate is prevented by inactivating the enzyme. Inactivation is typically achieved by heating the composition containing the enzyme to a temperature of at least 80°C, preferably 90°C - 95°C.

35 Following enzyme inactivation, the SHP is cooled prior to use. The SHP may, optionally, be

dried by techniques known per se in the art, such as by freeze-drying or spray drying.

5 The SHPs obtained according to the present invention preferably have a DE in the range of from 3 to 12, more preferably from 4 to 10 and are partly soluble in cold water (i.e., water at a temperature of about 20°C). Typically, the SHPs are not pourable at 90°C. Also the SHPs obtained according to this
10 invention contain partly gelatinized starch granules and generally exhibit birefringence under cross-polarised light indicating the presence of some starch crystals. We have also found that the SHPs of the invention will typically have a break strength in the
15 range of from 5 to 160 g, preferably 5 to 55 g, and a gel strength not greater than 25 g/mm, preferably 2 to 13 g/mm, as measured on a 20% paste of the modified starch (matured for 24 hours), and a peak viscosity of 80 to 1200 BU (Brabender units) measured at a
20 temperature of 51 - 59°C in a Brabender amylograph.

The SHPs may be used as oil- and/or fat-substitutes in food compositions, particularly in 0% oil or 0% fat compositions. They are especially useful as fat mimetics (body-imparting and/or
25 consistency-imparting carriers) in 0% oil salad dressings, such as in low calorie mayonnaises.

EXPERIMENTAL

30 SHPs according to the present invention were prepared as described in the following Examples. The enzyme used in the Examples was porcine pancreatic α -amylase (Product A-3176, Type VI-B obtained from Sigma Chemical Company with a reported activity of 25
35 units/mg solid at pH 6.9). One unit was defined as liberating 1 mg of maltose from starch in 3 min at 20°C

and pH 6.9. Reported β -amylase activity was 7.4 units/mg solid at pH 4.8. The reported pH optimum was 6 - 7 and the enzyme was thermolabile above 50°C.

5

EXAMPLES 1 TO 9

10 Wheat starch was dispersed in 0.02 M sodium phosphate buffer (pH 7), with or without 0.02 M sodium chloride added, to give a final starch concentration of 30%. Thus, 9 kg starch was mixed with 21 litres of buffer; 12 kg starch with 28 litres of buffer; and 20 kg starch with 46.7 litres of buffer. The enzyme was dissolved in the same buffer and added to the starch
15 slurry to give the appropriate activity (units/litre of starch slurry) at time zero. The hydrolysis was carried out in a 100-litre surface-scraped, steam-jacketed mixer (Agemore) with a central paddle counter-revolving at 45 rev/min. The temperature of
20 the vessel was controlled by the application of intermittent steam pressure at 5 psig during the reaction and at 15 psig during the enzymic inactivation stage (90 - 95°C) to the steam jacket.

25 The enzymic hydrolysis of the starch was allowed to take place at 58 - 60°C and the concentration of enzyme added was 700 - 2100 units/litre. The time of hydrolysis (holding time) was varied from 11 - 15 minutes to produce DEs from
30 3.8 to 9.0. The reaction was terminated by rapid heating of the starch mix to above 90°C to inactivate the enzyme. The hot SHP was poured into metal trays, allowed to cool and blast frozen at -30°C prior to freeze-drying. Once dry, the material was ground in a
35 hammer mill to obtain a powder.

The course of hydrolysis was monitored by periodic removal of samples of the starch mixture

which were boiled to inactivate the enzyme, followed by freeze-drying and measurement of reducing sugars by the DNSA method.

5 Examples of reaction parameters are shown in Table I.

Table I

10 Reaction parameters used in the preparation of SHPs

	<u>Example</u>	<u>NaCl</u>	<u>DE</u>	<u>Amount of enzyme</u> (units/l)	<u>Holding</u>
					<u>time at 59-63°C</u> (min)
15	1	Yes	4.4	1400	30
	2	Yes	4.5	1400	5
	3	No	4.9	1400	17
	4	Yes	6.7	1400	12
	5	Yes	8.4	1400	19
20	6	Yes	9.0	1400	22
	7	No	9.2	2100	22
	8	No	9.6	2800	27
	9	Yes	11.0	1400	30

25 Slurries were prepared by dispersing 80 g of the SHPs in distilled water to make a total volume of 400 ml. The slurries were heated in a Brabender Viscograph (Type 801201) fitted with an eight-prong sample bowl, a seven-prong sensor and a 700-cmg measuring head from 25° to 97°C at 1.5°C per minute. The slurries were held at 97°C for 15 minutes, followed by cooling to 30°C. Viscosities were monitored on a chart recorder throughout the heating and cooling cycle, the recorder being set to zero viscosity between each sample with 400 ml of distilled water in the bowl. Peak viscosity was recorded in

Brabender Units.

The viscosifying properties of a range of SHPs prepared are shown in Table II.

Table II

**Viscosities of starch pastes measured in a
Brabender Viscograph**

(All pastes contained 20% wt/vol sample unless
otherwise indicated)

	<u>Example</u>	<u>DE</u>	<u>Starting</u>	<u>Pasting</u>	<u>Peak viscosity</u>	
	<u>No</u>		<u>viscosity</u>	<u>temp.</u>	<u>(BU)</u>	<u>Temp.</u>
			(BU)	(°C)		(°C)
15	1	4.4	100	28	1163	53
	2	4.5	38	27	1200	57
	3	4.9	365	34	615	53
	4	6.7	50	25	410	54
20	5	8.4	60	27	220	55
	6	9.0	460	37	460	51
	7	9.2	140	35	180	59
	8	9.6	65	40	83	58
25	9	11.0	40	27	350	52

The Gelling Properties of SHPs

The SHPs were dispersed in distilled water at a concentration of 20% (wt/vol), heated in a Brabender Viscograph as described above, held at 97°C for 15 minutes and poured, whilst hot, into 120-ml capacity glass jars, the necks of which had been extended with tape. The jars were filled to above the rim, cooled and allowed to mature at 20°C or at 4°C for 24 hours.

The matured starch gels formed a tough skin, which was removed at the rim with a wire cheese-

5 cutter. The gels were analysed with an LFRA/Stevens
Texture Analyser fitted with a hemispherically tipped
Perspect probe (diameter 13 mm x 3.5 cm). The probe
was programmed to penetrate the centre of the gels to
a maximum depth of 20 mm at a speed of 0.5 mm/s. The
load on the probe was continuously recorded on a chart
recorder as a function of penetration distance. The
texture analyser was calibrated with a standard 200 g
weight such that a response range of 0.200 mV
10 (corresponding to a load of 200 g) was obtained as a
full-scale deflection on the chart recorder paper.

The gelling properties of a range of SHPs are
shown in Table III.

Table III

Gel preparations of starch pastes measured in a
Stevens/LFRA Texture Analyser

(All gels contained 20% starch unless otherwise
indicated)

25	<u>Example</u> <u>No</u>	<u>DE</u>	<u>Break</u> <u>strength</u> (g)	<u>Elastic</u> <u>limit</u> (mm)	<u>Gel</u> <u>strength</u> (g/mm)
	1	4.4	50.0	3.9	12.8
	2	4.5	159	6.3	25.4
30	3	4.9	42.5	3.5	12.1
	4	6.7	38.2	3.8	10.1
	5	8.4	24.8	4.3	5.8
	6	9.0	54.2	4.9	11.1
	7	9.2	8.0	3.7	2.2
35	8	9.6	5.0	2.1	2.4
	9	11.0	46.4	5.4	8.6

Microscopy

5 All SHPs prepared contained some partially
gelatinised starch grains, as determined by light
microscopy. Thus, compact, angular fragments, 30 -
100 μ m in size containing tightly packed starch grains
were observed. The fragments hydrated slowly in
10 water. Some birefringence was also observed. In
contrast, a commercial potato SHP such as Paselli SA2
from Avebe, contained no intact or partially
gelatinised granules.

EXAMPLES 10 - 18

15 In the following Examples low DE SHPs were
prepared from some Latin American starches (Brazilian
corn starch, Brazilian wheat starch and Mexican
tapioca starch). In these examples the α -amylase was
20 the same porcine pancreatic α -amylase as was used in
Examples 1-9.

The wheat starch SHPs were prepared by
dispersing the starch (1.2 kg) in 2.8 litres of 10 mM
sodium phosphate buffer (pH 7) to give a final starch
25 concentration of 30%. The enzyme was dissolved in 10
ml of the same buffer and then added to the starch
slurry at time zero. The starch mixture was stirred
using an overhead paddle mixer until the onset of
gelatinisation at around 60°C. Following this, the
30 reaction was stirred manually as it was too thick to
be stirred by the mixer arrangement. At the end of
the reaction period (the 'holding time') the
temperature of the mixture was raised to 80°C to
inactivate the enzyme.

35 The hydrolysis of the tapioca starch was
carried out essentially as above except that the

starch slurry was heated to 70°C and allowed to cool (at ambient temperature) to 60°C before addition of the enzyme (the porcine amylase would otherwise be thermally-inactivated). At the end of the reaction period, the temperature of the mixture was raised to 80°C to inactivate the enzyme.

The moisture content of all the SHPs prepared in this study was very similar. Thus, SHPs of Examples 10 and 11 prepared from Brazilian wheat contained 2.4 and 2.6% moisture, respectively; SHPs from Examples 13 and 14 from Mexican tapioca contained 2.8 and 2.1% moisture, respectively; and SHPs from Examples 17 and 18 from Brazilian corn contained 2.9 and 3.1% moisture, respectively.

Table V shows the rheological properties of the Latin American starches and their SHPs as measured in a Brabender Viscograph. The results obtained with Brazilian wheat were similar to those obtained for SHPs prepared from European wheat. For example, SHP from Example 10 with a DE of 4.9 prepared from Brazilian wheat had a peak viscosity of 1300 BU whilst SHP from Example 2 prepared from European wheat with a DE of 4.5 had a peak viscosity of 1200 BU.

Mexican tapioca starch and its products were more viscous than Brazilian wheat starch and its products, as shown in Table V. Although Brazilian corn and its SHPs were not as viscous as the Mexican tapioca and its products, they were more viscous than the Brazilian wheat starch and its products. Therefore, if the Latin American starches were listed in order of decreasing viscosity the order would be: Mexican tapioca Brazilian corn Brazilian wheat. These general trends in viscosity were paralleled in European starches from tapioca, corn and wheat.

The corn starch SHPs were prepared as above

except that the starch slurry was heated to 75°C and allowed to cool to 60°C before addition of the enzyme. Problems were experienced during production of the first corn SHP (Example 15) at a concentration of 30%, as the starch thickened to such an extent that stirring became difficult and it was not possible to prepare a homogeneous product. Therefore, subsequent corn SHPs (Examples 16 to 18) were prepared at a concentration of 10%. At the end of the reaction period, the temperature of the mixture was raised to 80°C to inactivate the enzyme.

The course of each hydrolysis reaction was monitored by periodic sampling (5 - 10 ml) of the reaction mixture and boiling for 10 min to inactivate the enzyme. These samples were frozen before freeze-drying in a Lyolab BII laboratory freeze dryer (Life Science Labs. Ltd., Luton, Beds., UK). The bulk of the SHPs at the end of reaction were poured hot into metal trays, cooled at ambient temperature, and frozen before freeze-drying in an Edwards 'Mini-Fast' laboratory/pilot freeze dryer (Model 3400, Edwards High Vacuum, Crawley, West Sussex, UK). Once dry, all the materials were milled to powders, the bulk samples in a hammer mill (Christy and Norris Ltd.), and the time-course samples in a Braun coffee grinder or with a pestle and mortar.

The processing parameters used to prepare the SHPs are summarised in Table IV. The reaction parameters used for the preparation of SHPs from Brazilian wheat were essentially very similar to those used for the manufacture of European wheat SHPs. The reaction parameters used for the preparation of yucca and corn SHPs from Latin America differed from those for wheat SHPs in that each starch substrate was pre-gelatinised prior to the addition of the enzyme. This

alteration in methodology was undertaken in light of previous results which showed poor enzyme activity with corn and tapioca substrates without prior pre-gelatinisation.

Table IV

Reaction parameters used in the preparation of SHPs

Example	Starch Substrate	Initial Starch concentration (%)	DE	Amount of enzyme (units/l)	Holding Time at 60°C(min)
10	Brazilian wheat	30	4.9	2,100	4.5
11	Brazilian wheat	30	10.7	2,100	14.5
12	Brazilian wheat	30	11.7	2,100	20.0
13	Mexican tapioca	30	4.6	2,100	8.0*
14	Mexican tapioca	30	6.8	2,100	20.0*
15	Brazilian corn	30	6.2	2,100	20.0**
16	Brazilian corn	10	7.3	2,100	6.0**
17	Brazilian corn	10	7.8	2,100	8.0**
18	Brazilian corn	10	6.3	2,100	10.0**

* Starch pregelatinised by heating to 70°C. Enzyme added on cooling to 60°C.

** Starch pregelatinised by heating to 75°C. Enzyme added on cooling to 60°C.

Table V

Viscosities of starch pastes measured in a Brabender Viscograph

Example	DE	Concentration (% wt/vol)	Starting viscosity (BU)	Pasting temp. (°C)	Peak viscosity Temp (BU) (°C)	Viscosity at 30°C (BU)	
native Brazilian wheat	0.4	10	0	67	385	97	1200
10	4.9	20	840*	35	1315	55	2080
11	10.7	20	370*	35	755	55	1130
native Mexican yucca	0.4	10	0	65	2015	77	1250
13	4.6	15	600	32	1910	54	1705
14	6.8	15	30	32	2290	54	2105
native Brazilian corn	0.4	10	0	70	1075	91	1950
18	6.3	20	615*	38	590	49	ND
17	7.8	15	58	37	63	55	ND

* viscosity decreased with initial stirring

Table VI shows the gel properties of the Latin American starches and their SHPs as measured in a Stevens/LFRA Texture Analyser. Again, the SHPs prepared from Brazilian wheat starch had broadly similar gelling properties to those obtained from European wheat starch. For example, SHP from Example 11 with a DE of 10.7 prepared from Brazilian wheat had a gel strength of 6.8 g/mm whilst SHP from Example 9 prepared from European wheat with a DE of 11.0 had a gel strength of 8.6 g/mm. Native Mexican tapioca starch, like its "European" counterpart (tapioca), failed to gel at the concentrations used in this study. However, SHPs from Example 13 (DE = 4.6) and Example 14 (DE = 6.8) prepared from Mexican tapioca produced gels with similar gel strengths as those prepared from either European or Brazilian wheat. The two SHPs prepared from Brazilian corn differed substantially in gel strength: SHP from Example 18 with a DE of 6.3 had a gel strength of 17.5 g/mm whilst SHP from Example 17 with a DE of 7.8 had a gel strength of 8.5 g/mm.

Table VI

Gel properties of starch pastes measured in a Stevens/LFRA Texture Analyser

Example	DE	Concentration (% wt/vol)	Break load (g)	Elastic limit (mm)	Gel strength (g/mm)
native					
Brazilian	0.4	10	157.6	6.13	25.7
wheat					
10	4.9	20	71.6	5.25	13.6

	11	10.7	20	30.8	4.63	6.7
5	native Mexican yuca	0.4	10	no gel formed		
	13	4.6	15	37.0	6.25	5.9
	14	6.8	15	42.8	7.13	6.0
10	native Brazilian corn	0.4	10	182.4	9.25	19.7
	18	6.3	20	71.2	4.13	17.2
	17	7.8	15	20.8	4.38	4.7
15	Microscopy					

Both differential interference contrast and crossed polarised light showed typical ungelatinised starch grains in all three native starches. In addition, polarised light showed distinct Maltese crosses in the native starches. All the SHPs contained fragments of partially gelatinised starch of different sizes. Some wheat hairs and cell wall material were also observed in the SHPs prepared from Brazilian wheat. Under crossed polarised light, the corn SHPs showed slight birefringency (brightness) but no ungelatinised granules were observed. The SHPs prepared from Mexican tapioca showed slightly more birefringency than corn and so possibly contained some less gelatinised starch. The SHPs from Brazilian wheat showed the most birefringency of all the SHPs prepared in this study and Maltese crosses could be seen in some of the fragments. Overall, all the SHPs prepared in this study contained the sharp fragments of compacted and partially gelatinised starch granules

not found in most commercial maltodextrins.

Product Trials

5

Zero oil salad dressings were prepared using the SHPs obtained in Examples 10, 11, 13 and 14 as above.

10

The formulations of the salad dressings (control and 0% oil) were as follows:

	Control	Experimental
	32.5% oil	0% oil
	(Actual = 34% fat)	(Actual = 1.5% fat)
15	due to egg yolks	due to egg yolks
	Oil	0
	Water	76.4
20	Yolks	4.9
	Starch	4.6
	(National Purity HPC)	
	Sucrose	3.2
	Vinegar	3.1
25	(12% acetic acid)	
	Salt	1.5
	Mustard flour	0.1
	Potassium sorbate	0.1
	Xanthan gum	0.1
30	Fat mimetic	6.0

Method of Manufacture of the Salad Dressings

35

The following standard method of manufacture was used for all fat mimetics evaluated. Batch sizes of 1 kg were prepared.

- 5 i) All the dry ingredients were mixed together in
 a pan and the water and vinegar were added.
 The weight of the pan and contents was
 measured.
- ii) The mixture was heated to 90°C and held for 30
 seconds at this temperature.
- 10 iii) The mixture was then cooled to 20°C and the
 pan and contents were reweighed. Loss in
 weight by evaporation was corrected for, by
 the addition of water.
- iv) The egg yolk and oil (if used) was added
 gradually to the starch paste while the
 mixture was mixed for a total of 12 minutes
15 using a Silverson mixer.
- v) The dressing was distributed into 100-ml
 sterile jars and stored.

 During manufacture of the zero-oil dressings,
20 the SHPs prepared in this study formed pastes that
 were easily dispersed and smooth and that thickened on
 heating. The final SHP pastes were generally somewhat
 thinner than that of the control dressing. The
 organoleptic assessments of the dressings 24 h after
25 manufacture are shown in Table IV. Both Brazilian
 wheat and Mexican tapioca SHPs afforded acceptable (in
 terms of texture) zero-oil dressings when assessed 24
 h after manufacture although a very slight starchy
 off-flavour was occasionally detected.

 Following storage for 6 and 14 days, the
30 control dressing containing 32.5% oil had remained
 unchanged in terms of appearance, texture and flavour.
 However, the zero-oil dressings prepared with the
 Brazilian wheat SHPs developed a slightly more gel-
 like structure with time. Nevertheless, the dressings
35 were considered quite acceptable. Furthermore, the
 dressing prepared with the more hydrolysed Brazilian

wheat SHP (SHP from Example 11, DE = 10.7) appeared to benefit from the thickening effect over the 2-week storage period changing from a pourable dressing with starchy off-flavours at 24 h to a thick dressing similar in texture to the control and improved flavour after 14 days.

Slight improvements with time in texture, mouthfeel and flavour were also observed in the dressings prepared with Mexican tapioca SHPs.

Table VII

Informal organoleptic assessment of zero-oil dressings 24 h after manufacture

	<u>Appearance</u>	<u>Texture</u>	<u>Flavour</u>
20	<u>Control</u> (32.5% oil)	White, opaque, thick spoonable, smooth	Smooth, creamy mouth-coating
25	<u>Starch Substrate</u>		Creamy, well-rounded
	<u>Brazilian wheat:</u>		
30	SHP of Example 10 (DE = 4.9)	Thick, shiny, yellowish, spoonable, slightly gelled	Smooth, thick (but less than control) less acidic than control
35	SHP of Example 11 (DE = 10.7)	Smooth, shiny, pourable, not gelled	Bodiless, watery, not thick or creamy
			Very slight starchy off-flavour

Mexican tapioca:

5	SHP of Example 13 (DE = 4.6)	Smooth, shiny, spoonable, translucent, thinner than control	Thinner than control, a little watery	Acceptable, less acidic than control
10	SHP of Example 14 (DE = 6.8)	Smooth, shiny, thick, spoonable, slightly gelled	Smooth, thick but thinner than control	Not completely acceptable, starchy off- flavour

15 It is of interest to note that the results
obtained with the Latin American starches were
paralleled by those obtained with European sources of
starch. Thus, both European and Latin American SHPs
gave satisfactory dressings with a progressive
thickening effect over storage time. Slight
20 improvements with time were observed with "European"
tapioca SHPs, as with the Mexican tapioca SHPs.

Although certain dressings e.g., that
containing SHP from Example 10 (from Brazilian wheat),
were considered to have an acceptable texture, all
25 were different from the control in terms of "creamy
mouthfeel". Thus, the control dressing was considered
"creamy" in a slippery/oily sense whilst the best
zero-oil dressings were considered "creamy" on account
of their stickiness and slow breakdown in the mouth.

30 The results of rheological analyses of
dressings, summarised in Table VIII confirm the
organoleptic observations made in Table VII. Thus,
the dressings considered thinnest on sensory
assessment e.g., dressings containing Brazilian wheat
35 SHP from Example 11 and Mexican tapioca SHP from
Example 13, also had the lowest instrumental

Viscosities (PaS) of dressings containing Latin American SHPs following storage for 24 h, 6 days and 14 days

		STORAGE TIME								
		24 h			6 d			14 d		
		Shear rate (s^{-1})			Shear rate (s^{-1})			Shear rate (s^{-1})		
	Starch substrate	10	30	100	10	30	100	10	30	100
20	Control (32.5% oil)	13.9	5.8	2.5	12.9	5.5	2.4	12.5	5.3	2.3
25	Brazilian wheat: SHP Example 10 (DE = 4.9)	7.0	2.9	1.2	9.0	3.4	1.3	10.3	3.8	1.4
30	SHP Example 11 (DE = 10.7)	5.2	2.5	1.2	6.2	2.7	1.2	7.3	3.1	1.4
	Mexican tapioca SHP Example 13 (DE = 4.6)	5.6	2.5	1.2	6.9	2.9	1.3	7.9	3.2	1.3
35	SHP Example 14 DE = 6.8	6.6	3.1	1.4	8.1	3.5	1.5	8.7	3.7	1.6

EXAMPLES 19 - 22

5 Further samples of SHPs according to the
invention were prepared using wheat starch and potato
starch. The potato starch was obtained from Roquette
Freres (ref: 474095). The wheat starch was also
obtained from Roquette Freres (ref: 466539). The
enzyme used was porcine pancreatic α -amylase E.C.
10 3.2.1.1 Type VI-B (Sigma Chemia Co.) No. A-3176
(activity - 25 units/mg solid; 13% moisture).

The reaction was carried out in a Brabender
viscograph (801201) fitted with a 700 cm.g sensitivity
cartridge. The starch (approx. 30% dry starch
15 content) was suspended in 0.01 M sodium phosphate
buffer to a total weight of 498 g and transferred to
the Brabender vessel. The enzyme was added to the
slurry in the Brabender vessel as a dry powder.

The starch was heated from 25°C to 60°C at a
20 rate of 1.5°C/minute and the temperature was held at
60°C for 20 minutes (15 minutes in one experiment).
The starch was heated to 90°C at 1.5°C/minute and then
boiled for 15 minutes in a water bath to inactivate
the enzyme. The resulting SHPs were allowed to cool
25 and were then frozen and lyophilised in an Edwards MFD
0.5 Pilot scale freeze-drier. The dried SHPs were
ground to a fine powder in a coffee grinder.

The reducing sugar content of SHP samples was
determined using the dinitrosalicylic acid (DNSA)
30 method (Miller G.L. (1959) "Use of dinitrosalicylic
acid reagent for determination of reducing sugar".
Analyt. Chem., 31, 426). Results were quoted in
dextrose equivalents (DE).

35 The process conditions are set out in the
following Table IX.

Table IX

Conditions used for the production of SHPs
and the resultant DEs

5

		Enzyme concentration units/g dry starch)			
		Starch	Wet starch	Dry starch	Porcine DE
10	Ex No.	source	content (%)	content (%)	α -amylase
	19	Potato	30	24	8 8
	20	Potato	36	28.8	7.3 7
	21	Wheat	30	26.1	8.1 9.7
15	22	Potato	32.6	26.1	8 7.1

It was observed that all samples of SHP prepared as described above were not pourable at 90°C but were semi-solid and had to be transferred to a
20 beaker with a ladle.

EXAMPLES 23 - 25

Three SHPs were prepared according to the
25 present invention using wheat starch.

Porcine α -amylase was obtained from Sigma Chemical Company Ltd. The enzyme preparation (No. A-6880, Type VI-A) contained sodium chloride and lactose as extenders and had a reported activity of 14 units/
30 mg solid at pH 6.9. One unit was defined as liberating 1 mg of maltose from starch in 3 min at 20°C and pH 6.9. Reported β -amylase activity was 3.3 units/mg solid at pH 4.8. The reported temperature optimum for porcine α -amylase was 37°C.

35 Wheat starch (1.2 kg) was dispersed in 2.8 litres of 0.06 M sodium phosphate buffer (pH 7) to

5 give a final concentration of 30% starch and a final
volume of 4 litres, in a five-litre round-bottom flask
on a heating mantle. The enzyme was dissolved in the
same buffer and added to the starch slurry to give the
appropriate activity units at time zero. The reaction
mixture was stirred using a top-loaded paddle mixer
until the start of gelatinisation. Following this,
10 the mix was stirred manually and the temperature of
the mix was raised.

The enzyme was allowed to hydrolyse the starch
at the appropriate temperature for varying lengths of
time (holding time) to produce different DEs. The
reaction was terminated by rapid heating of the starch
15 mix to 70° - 85°C to inactivate the enzyme. The hot
SHP was poured into metal trays, allowed to cool and
blast frozen at -30°C prior to freeze-drying (Edwards
Mini-Fast Model 3400). Once dry, the material was
ground in a hammer mill (Christy and Norris Ltd) to
20 obtain a powder.

The course of selected hydrolysis reactions
was monitored by periodic removal of 5 ml of the
starch mixture and their addition to 10 ml of methanol
to inactivate the enzyme and precipitate the starch.
25 These samples were dried by rotary evaporation.

Details of the processing parameters used to
prepare the SHPs are shown below in Table X.

Table X

30

Example No	DE	Amount of		Holding time (min)	Holding temperature (°C)	
		enzyme				
		(g)	units/l			
35	23	3.8	0.20	700	11	58
	24	4.5	0.20	700	15	59
	25	9.0	0.60	2100	11	60

The SHPs were evaluated as fat mimetics in salad dressings containing 15% oil and 0% oil. The formulations for these dressings and also for a control dressing were as follows:

	<u>Ingredients</u>	<u>Control (32.5% oil)</u>	<u>15% oil</u>	<u>0% oil</u>
		(%)	(%)	(%)
10	Hot soluble starch (Purity HPC, National Starch)	4.6	4.6	4.6
	Egg yolk	4.9	4.9	4.9
	Vinegar	3.1	3.1	3.1
15	(12% acetic acid)			
	Salt	1.5	1.5	1.5
	Sucrose	3.2	3.2	3.2
	Mustard flour	0.1	0.1	0.1
	Potassium sorbate	0.1	0.1	0.1
20	Xanthan gum	0.1	0.1	0.1
	Water	49.9	63.6	76.4
	Oil	32.5	15.0	0
	SHP	0	3.8	6.0

25 Note:

- 1) The total fat content of the dressings was 1 - 2% higher than suggested by the level of oil added, owing to the fat content of the egg yolks.

The dressings prepared were as follows (Table XI)

35

Table XI

	<u>Dressing</u>	<u>Oil Content</u>	<u>SHP Used</u>
5	A	15%	Ex 23
	B	15%	Ex 24
	C	15%	Ex 25
	D	0%	Ex 23
10	E	0%	Ex 24
	F	0%	Ex 25

The dressings were prepared according to the procedure described above in Examples 10 - 18. Following storage, at 4°C, the dressings were allowed to equilibrate to room temperature for 2 hours and were then subjected to organoleptic assessment. The results are shown in Tables XII - XIV below.

Table XII

**Organoleptic Assessment of Dressings (15% oil)
Following Short-Term Storage at 4°C**

	<u>Dressing</u>	<u>Appearance</u>	<u>Taste</u>
25	Control (32.5% oil) (Age 6 days)	Smooth, white, shiny, holds shape well.	Very smooth, bland, sticky, pleasant tang, leaves film.
30	A (Age 1 day)	Shiny, smooth, slightly thinner than control.	Very smooth, bland, slightly vinegary, not lumpy.
35	B (Age 1 day)	Shiny, thixotropic, holds shape well.	Very smooth, sticky, bland, slightly

starchy flavour
but well-liked.

5	C (Age 1 day)	Spoonable, shiny, thinner than B or control.	Very smooth, not grainy, tangy, clear favourite.
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Table XIII

10

**Organoleptic Assessment of Dressings (15% oil)
Following Longer-Term Storage at 4°C**

	<u>Dressing</u>	<u>Appearance</u>	<u>Taste</u>
15	Control (32.5% oil) (Age 109 days)	White, opaque, thick, spoonable.	Bland with a vinegary 'kick', filmy, smooth, slightly gummy.
20	A (Age 100 days)	Spoonable, thick, like control, slightly yellow.	Very smooth, slightly vinegary, not grainy, well- liked, second favourite.
25			
30	B (Age 100 days)	Spoonable, very like control	Closest in texture to control, pleasant flavour, first favourite.
35	C (Age 100 days)	Spoonable, a suggestion of 'mousse-like' consistency.	Smooth, slightly thicker than control, vinegar after-kick, third favourite.

Table XIV

Organoleptic Assessment of Dressings (0% oil)
Following 24 Hour Storage at 4°C

	<u>Dressing</u>	<u>Appearance</u>	<u>Texture</u>	<u>Taste</u>
5				
10	D	Translucent, slightly yellow.	Spoonable, smooth, slightly more gel-like than control.	Sweet, vinegary, pleasant.
15	E	Translucent, almost trans- parent, slightly yellow.	Thick, smooth, spoonable and slightly gel- like.	Acceptable sweet, tangy, no off flavours.
20				
25	F	Translucent and creamy, slightly yellow.	Spoonable, thick-bodied, creamy, smooth.	Sharp, acceptable no off flavours.

In addition to the above; 0% oil dressings were prepared according to the above formulation and procedure but using prior art SHPs. These were "Instant N-Oil" obtained from National Starch & Chemical Company (believed to be a product according to US 4,510,166) and potato SHP, produced according to GB 1,423,780 by converting potato starch using bacterial α -amylase and obtained directly from the proprietor of that patent. The results of an organoleptic assessment of the dressings prepared using these SHPs are shown below in Table XV.

Table XV

	<u>Dressing</u>	<u>Appearance</u>	<u>Texture</u>	<u>Taste</u>
5	Using	Orangy-yellow,	Just pourable,	Slight
	"Instant	translucent.	not smooth	off-flavours
	N-Oil"		flowing, not	detected.
10			spoonable	
			reasonable	
			body.	
	Potato	Creamy, pale	Thick and	Starchy,
	SHP	yellow, shiny,	spoonable,	floury,
15	(Academie	translucent.	strongly	very acidic.
	der		gelled.	
	Wissenschaften			
	DDR)			

20 Rheological analyses confirmed the sensory observations. Figure 1 shows the flow curves of shear stress versus shear rate for the control dressing (32.5% oil), and three 0% oil dressings (E and F and the prior art potato SHP) shows that the

25 flow properties of the dressings containing wheat SHPs of the invention were very similar to those of the control dressing; dressing F was a little thinner and dressing E was a little thicker than the control. The shape of the flow curve for the dressing F most

30 closely matched the shape of the control curve. On the other hand, the flow curve for the dressing containing the prior art potato SHP was completely different.

35 The results in Figure 1 can also be shown in tabular form. Table XVI below shows the dynamic yields and viscosities at three different shear rates.

Again, the results show that the rheological properties of the dressing containing the prior art SHP were very different from those of the control or of the dressings containing SHPs of the invention.

During rheological analysis, it was noted that the dressing containing the prior art potato SHP behaved quite differently in that it tended to break up and fall apart like a gel whilst the other dressings remained smooth and paste-like under shear. Whilst all starch containing dressings tended to thicken with age, the problem was most severe in the dressing containing the prior art potato SHP and least extensive in the dressing F of the invention.

Table XVI

Rheological characteristics of salad dressings
(All dressings were evaluated after 23 days of storage)

<u>Sample</u>	<u>Dynamic Yield</u> (NM ⁻²)	<u>Viscosity (NM^{-2.5})</u> <u>at shear rates</u>		
		0.5s ⁻¹	2.5s ⁻¹	10s ⁻¹
Control (32.5% oil)	77.2	186	44.1	14.0
Dressing E	60.4	201.0	49.1	15.2
Dressing F	38.9	130.0	32.4	10.2
Prior art potato SHP 0% oil (GB 1,423,780)	93.4	326.7	73.2	20.4

Molecular Weight Profiling

5 The molecular weight profile of the water soluble fraction of the products of the invention was investigated for comparison with prior art products.

10 All samples were made up as 0.1% solutions in 0.1M sodium sulphate by heating to 85°C and filtering through 45 μ m membranes. They were eluted from a TSK PW column set (5000/4000/4000) at 45°C at 0.5ml/min. The amount of any insoluble fraction was determined after boiling a 1% mixture of the product in water for 5 minutes by isolating and drying the insoluble material. A range of pullulan standards was made up in cold sodium sulphate solution and eluted under the same conditions.

15 The results of the molecular weight profile investigations are shown in Figure 2 in which the amount of fraction in the eluate is plotted against time, with the time axis converted to a molecular weight scale by reference to the results for the pullulan standards. The plots on the graph are as follows.

20 -

25 Dashed line A represents the results for a starch hydrolysis product of the present invention made from potato starch in accordance with the method of Examples 1 to 9 but using the porcine α -amylase used in Examples 23 to 25.

30 -

 Continuous line B represents the product of Example 7.

 -

 Dotted line C represents the prior art product Paselli SA2.

35 -

 Dotted and dashed line D represents a prior art product which is believed to have been produced in accordance with the method

disclosed in GB 1,423,780.

5 All samples contain a salt peak (sodium sulphate) at the end of the run and this coincides in some cases with SHP material of low molecular weight. Since it was not possible to completely resolve the low molecular weight material, namely tri-, di- and monosaccharides from the salt peak, it was not possible to quantify the data to produce molecular weight distributions and averages. Instead, the molecular weight scale, as determined by reference to pullulan standards, is given.

10 The elution profiles C and D indicate that the prior art materials have quite different molecular weight distributions. Both of these SHPs are completely soluble in water. C has two major components whilst D shows a broad distribution increasing in content as the molecular weight decreases. D also shows a peak at low molecular weight, around 6000 daltons, which coincides with the lower molecular weight component of C. The product illustrated by line D also contains a higher proportion of lower molecular weight material which merges with the salt peak.

20 The SHPs of the present invention are not completely soluble in water even when heated and the analysis applies to the soluble fraction only. The elution profiles show a broad distribution of molecular weight, increasing in content as the molecular weight decreases and producing a peak at around 12000 daltons in both cases. This peak occurs at a significantly higher molecular weight than the peak in D and there is much less material of lower molecular weight in the products of the invention.

35

CLAIMS

1 A process for the production of a starch
5 hydrolysis product which contains partly gelatinized
starch granules comprising the steps of

- (i) preparing an aqueous starch suspension
 containing from 10 to 30% by weight (dry
 substance) of starch;
- 10 (ii) heating the aqueous starch suspension to a
 temperature in the range of from 58°C to 80°C
 being not significantly higher than the
 gelatinisation temperature of the starch;
- 15 (iii) treating the aqueous starch suspension with a
 porcine pancreatic α -amylase at a temperature
 in the range of from 58° to 63°C for a
 suitable period of time to achieve the
 required DE and/or molecular weight for the
 water soluble fraction of the product; and
- 20 (iv) deactivating the enzyme.

2 A process according to claim 1, wherein the
temperature in step (iii) is about 60°C.

3 A process according to either claim 1 or claim
2, wherein the period of time in step (iii) is from 5
25 to 30 minutes.

4 A process according to any one of claims 1 to
3, wherein the starch is corn starch, wheat starch,
barley starch, tapioca starch, potato starch, rice
starch or sago starch.

30 5 A process according to claim 4, wherein the
starch is wheat starch.

6 A process according to claim 5, wherein the
temperature to which the aqueous suspension of starch
is heated in step (ii) in claim 1 is in the range of
35 from 58° to 63°C.

7 A process according to any one of claims 1 to



6, wherein the enzyme is deactivated by heating the suspension to a temperature of from 90° to 95°C.

5 8 A process according to any one of claims 1 to 7, wherein the step (iii) of treating the aqueous starch suspension with the α -amylase enzyme is carried out for a period of time to give a product having a water soluble fraction which has a peak in its molecular weight profile in the range of from 6 to 10 30 kDa.

9 A process according to any one of claims 1 to 8, wherein the step (iii) of treating the aqueous starch suspension with the α -amylase enzyme is carried out for a period of time to achieve a DE in the range 15 of from 3 to 12.

10 A process according to any one of claims 1 to 9 which comprises the further step of subjecting the suspension, after the enzyme has been deactivated, to drying.

20 11 A starch hydrolysis product suitable for use as a fat and/or oil substitute in food compositions characterised in that it is obtainable by a process according to any one of claims 1 to 10, said product being partly soluble in cold water, being not pourable 25 at 90°C, containing partly gelatinized starch granules and exhibiting birefringence under crossed-polarised light.

12 A starch hydrolysis product according to claim 11 capable of forming aqueous gels at a concentration 30 of 20% (wt/v) having a gel strength of not more than 25 g/mm in 24 hours at 4°C.

13 A starch hydrolysis product according to claim 11 or claim 12 having a DE in the range of from 3 to 12.

35 14 A starch hydrolysis product suitable for use as a fat and/or oil substitute in food compositions



characterised in that it is partly soluble in cold water and its water soluble fraction has a peak in its molecular weight profile in the range of from 6 to 30 kDa.

15 Product as claimed in claim 14, wherein the peak in the molecular weight profile is in the range of from 8 to 20 kDa.

16 A food composition containing one or more food ingredients and a body-imparting and/or consistency-imparting carrier characterised in that the body-imparting and/or consistency-imparting carrier comprises a starch hydrolysis product according to any one of claims 11 to 15.

17 A food composition according to claim 16 which contains no oil.

18 A food composition according to claim 16 or claim 17 which is a salad dressing.

