

FLAVOURING AGENTS ✓

FIELD OF THE INVENTION

- 5 The present invention relates to the production of flavouring agents, more particularly to a process for preparing flavour precursors from fruit pulp fibres.

BACKGROUND OF THE INVENTION

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- Fruit pulp fibers contain pectin and cellulosic materials such as hemicellulose. Pectin is present in the cell walls of all plant material and functions, in combination with cellulosic material, as an intercellular cementing material. The main component of pectin is a linear D-galacturonan, many of the carboxyl groups of which are esterified with methanol. Other components include L-arabinan and D-galactan which together may form up to 20% by weight of the pectin. The main raw materials for pectin and hemicellulose are the peel of citrus fruits such as lemon, lime, orange and grapefruit, as well as apple pomace which is the residue remaining after pressing of apple to obtain apple juice.

25 SUMMARY OF THE INVENTION

- Galacturonic acids and reducing sugars such as glucose, arabinose and galactose are known as flavour precursors and we have surprisingly found that such substances may be obtained by enzymatically hydrolysing fruit pulp fibres.

The present invention provides a process for preparing food flavour precursors which comprises enzymatically hydrolysing fruit pulp fibres with a pectolytic enzyme.



DETAILED DESCRIPTION OF THE INVENTION

5 The fruit pulp fibres used in the process of the present invention may be citrus pulp which may be dehydrated and ground, citrus pectin or apple pulp fibre which comprises primarily apple pomace which is the by-product of juice processing.

10 The pectolytic enzyme may be, for instance, polygalacturonase, pectinesterase, hemicellulase and pectintranseliminase or any mixture of two or more thereof, and more particularly a pectolytic enzyme preparation containing a mixture of the above enzymes.

15 Novo Pectinex 3X-L is a liquid pectolytic enzyme preparation with an enzyme activity of 3,000 FDU 55°C/ml (determined by measuring the depectinisation of apple juice) based on Novo standard activity determination. It is
20 a highly purified preparation produced from a selected strain of *Aspergillus niger*. The product mainly contains pectin-transeliminase, polygalacturonase, pectinesterase and hemicellulase. The optimum pH and temperature for pectinase activity are approximately 4.5 and 50°C,
25 respectively. The optimum pH for pectinase stability at 50°C is approximately 3 to 5.

Novo Pectinex Ultra SP-L is a highly active pectolytic enzyme preparation with an enzyme activity of 26,000 PG/ml
30 (determined by the measurement of the reduction of the viscosity of a solution of pectic acid at 20°C and at pH 3.5). It is produced from a selected strain of the *Aspergillus niger* group. This enzyme preparation contains pectolytic and a range of hemicellulytic activities.

The citrus pectin hydrolysate may be produced by hydrolysing citrus pectin, e.g. as an aqueous slurry having a dry solids content from 5-50%, preferably from 10-25% by weight with any one or more of the above pectolytic enzymes at a pH of from 4 to 6, a temperature of from 40° to 60°C for a period of time from 6 to 48 hours, e.g. the enzymatic hydrolysate of USP grade citrus pectin with Novo Pectinex or Novo Pectinex Ultra SP-L derived from a selected strain of *Aspergillus niger* at pH 4.5 and 50°C for 24 hours, to give mono-, di-, and tri-galacturonic acids as well as some L-arabinose and D-galactose.

The citrus pulp hydrolysate or apple pulp may be produced by hydrolysing citrus pulp with any one or more of the above pectolytic enzymes as well as a cellulase at a pH of from 4 to 6, a temperature of from 40° to 60°C for a period of time from 6 to 48 hours, e.g. the enzymatic hydrolysis of citrus pulp with Novo Pectinex or Novo Pectinex Ultra SP-L derived from *A.Niger* and the cellulase, Novo Celluclast 1.5L derived from *Trichoderma reesei* at pH 4.5 and 50°C for 24 hours gives products of mono-, di-, and tri-galacturonic acids as well as L-arabinose, D-glucose, D-galactose, and D-cellobiose. The citrus pulp may be used as an aqueous slurry having a dry solids content of from 2.5 to 25% and preferably from 5 to 20% by weight.

Celluclast 1.5L is a liquid cellulase preparation with an enzyme activity of 1,500 NCU/g. One Novo Cellulase Unit (NCU) is the amount of enzyme which, under standard conditions, degrades carboxymethylcellulose to reducing carbohydrates with a reduction power corresponding to 1 μ mol glucose per minute. It is made by submerged fermentation of a selected strain of the fungus *Trichoderma reesei*. The enzyme catalyzes the breakdown of cellulose into glucose, cellobiose and higher glucose polymers. The optimum pH and temperature for cellulase activity are

approximately 4.5-5.5 and 50-65°C, respectively. The influence of pH and temperature on the optimum stability of cellulase are 4-7 and up to 50°C respectively.

- 5 The enzymes may be used in varying concentrations with respect to the citrus pectin or citrus pulp, e.g. from 0.5 to 2.0% (v/w).

- 10 If desired, the citrus pulp may be hydrolysed with an acid e.g. HCl, before the enzymatic hydrolysis. The acid hydrolysis may be carried out at a temperature from 80° to 100°C, preferably 85° to 95°C for a period of from 1 to 10 hours, preferably from 4 to 8 hours using an aqueous slurry of the citrus pulp having a dry solids content of from 2.5
15 to 25% and preferably from 5 to 20% by weight. After the acid hydrolysis, the citrus pulp may be neutralised to from pH 4 to 6, preferably from 4.5 to 5.5 with sodium hydroxide.

- 20 After the enzymatic hydrolysis, the hydrolysed materials may be treated to inactivate the enzymes, e.g. by heating to a temperature from 80° to 100°C for a period of time from 5 to 30 minutes, conveniently from 10 to 20 minutes.

- 25 The fruit pulp fibres are preferably used in a powdered form for the hydrolysis, preferably a fine powder having an average particle size of from 45 to 2000 microns.

- After the enzymatic hydrolysis, the hydrolysates may be
30 dried, e.g. by spray drying to give a powder. Preferably, an anti-caking or anti-sticking agent such as silicon dioxide or a maltodextrin, e.g. maltodextrin 10DE (10 dextrose equivalents) may be added for the spray-drying.

- 35 The fruit pulp hydrolysates prepared in accordance with the process of the present invention may be used as flavour

precursors for reaction flavour development such as meat flavours, coffee, chocolate and other food flavours as well as pet food flavours.

- 5 The present invention, therefore, also provides a food flavour comprising an effective flavouring amount of a fruit pulp hydrolysate prepared in accordance with the process of the present invention, e.g. an amount of from 1 to 5%, preferably from 2 to 4 % and especially from 2.5 to 10 3.5% by weight based on the total weight of the food flavour.

EXAMPLES

- 15 The following Examples further illustrate the present invention. Parts are given by weight unless otherwise indicated. Parts by weight bear the same relation to parts by volume as do kilograms to litres.

20 **EXAMPLE 1**

To a mixture of 24,000 parts of water, 120 parts by volume of Pectinex 3X-L and 120 parts by volume of Pectinex Ultra SP-L are added stepwise 6000 parts of citrus pectin. 1,530 25 parts of a 50% solution of NaOH are added to control the pH at 4.5 and enzymatic hydrolysis takes place at 50°C for 24 hours.

After the hydrolysis, the enzymes are inactivated by 30 heating the mixture at 95°C for 15 minutes followed by cooling to 25°C. The mixture is then spray dried in a Niro atomizer at an inlet temperature of 150°C and an outlet temperature of 90°C to give a powdered flavour precursor. The liquid chromatographic analysis of the flavour 35 precursor is given in Table 1.

EXAMPLE 2

To a mixture of 27000 parts of water, 60 parts by volume of Pectinex 3X-L, 60 parts by volume of Pectinex Ultra SP-L and 60 parts by volume of Celluclast 1.5L are added stepwise 3000 parts of citrus pulp cells obtained from Freeman Industries Inc. prepared from the albedo which is the white spongy, inner part of the peel and which has been washed, dehydrated and ground. 480 parts of a 50% solution of NaOH are added to control the pH at 4.5 and enzymatic hydrolysis takes place at 50°C for 24 hours.

After the hydrolysis, the enzymes are inactivated by heating the mixture at 95°C for 15 minutes followed by cooling to 25°C. The mixture is then spray dried in a Niro atomizer at an inlet temperature of 150°C and an outlet temperature of 90°C to give a powdered flavour precursor. The liquid chromatographic analysis of the flavour precursor is given in Table 1.

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EXAMPLE 3

27000 parts 0.1N HCl are added to 3000 parts of citrus pulp cells (similar to that used in Example 2 and hydrolysed at pH 2.0 at 90°C for 6 hours. 50% NaOH is added to the hydrolysed product to adjust the pH to 4.5 and then 60 parts by volume of Pectinex 3X-L, 60 parts by volume of Pectinex Ultra SP-L and 60 parts by volume of Celluclast 1.5L are added. Enzymatic hydrolysis takes place at 50°C for 24 hours while 50% NaOH is added to control the pH at 4.5.

After the hydrolysis, the enzymes are inactivated at by heating the mixture at 95°C for 15 minutes followed by cooling to 25°C. The mixture is then spray dried in a Niro atomizer at an inlet temperature of 150°C and an outlet

temperature of 90°C to give a powdered flavour precursor. The liquid chromatographic analysis of the flavour precursor is given in Table 1.

5 **TABLE 1**

COMPONENT	EXAMPLE 1	EXAMPLE 2	EXAMPLE 3
Mono-galacturonic acid	27.11	10.27	16.30
Di-galacturonic acid	3.62	0.16	0.50
Tri-galacturonic acid	43.00	22.60	20.10
D-Glucose		4.90	5.05
L-Arabinose	1.32	1.20	11.05
D-Galactose	1.36	4.30	5.18
D-Cellobiose		13.38	6.58
Moisture*	3.70	3.74	3.00
Insoluble Matters*	19.89	39.45	32.24
Total	100.00	100.00	100.00

*All samples were extracted by adding 100 ml water to 1 gram of sample for the HPLC analysis and insoluble matters
 10 are calculated by the difference.

EXAMPLES 4, 5 & 6 & COMPARATIVE EXAMPLES A & B

Beef flavours are prepared from the ingredients shown in
 15 the following Table 2.

TABLE 2

Ingredients	EXAMPLES				
	A	B	4	5	6
HPP 4BE Powder	30.0	30.0	30.0	30.0	30.0
Special Light Yeast Powder	5.0	5.0	5.0	5.0	5.0
Monosodium Glutamate	2.7	2.7	2.7	2.7	2.7
Thiamine Hydrochloride	0.5	0.5	0.5	0.5	0.5
L-cysteine Hydrochloride	0.8	0.8	0.8	0.8	0.8
Dextrose	1.2	1.2	1.2	1.2	1.2
Xylose	1.2	--	--	--	--
DPP	--	3.0	--	--	--
Pectin Hydrolysate of Example 1	--	--	3.0	--	--
Pulp Hydrolysate of Example 2	--	--	--	3.0	--
Pulp Hydrolysate of Example 3	--	--	--	--	3.0
Water	100.0	100.0	100.0	100.0	100.0
Maltodextrin	60.0	60.0	60.0	60.0	60.0
Total	201.4	203.2	203.2	203.2	203.2

All the above ingredients except maltodextrin are added in
 5 a pressure vessel and mixed to ensure maximum solubility.
 The mixture is heated to 120°C and maintained at 120°C for
 2 hours. The mixture is then cooled to 50°C and homogenised
 with maltodextrin and then finally spray dried in a Niro
 atomizer at an inlet temperature of 150°C and an outlet
 10 temperature of 90°C to give powdered beef flavours.

The beef flavours of Examples 4, 5 and 6 containing flavour precursors of Examples 1, 2 and 3 of the present invention respectively were more intense than the beef flavour of Comparative Example A where xylose was used as flavour precursor and were similar to the beef flavour of Comparative Example B using DPP - a commercially available depolymerised pectin.



CLAIMS

1. A process for preparing food flavour precursors which comprises enzymatically hydrolysing fruit pulp fibres with
5 a pectolytic enzyme.
2. A process according to claim 1 wherein the fruit pulp fibre used is citrus pulp, citrus pectin or apple pulp fibre.
- 10 3. A process according to claim 1 wherein the pectolytic enzyme is one or more of polygalacturonase, pectinesterase, hemicellulase or pectintranseliminase.
- 15 4. A process according to claim 2 wherein the hydrolysed citrus pectin is produced by hydrolysing citrus pectin with a pectolytic enzyme at a pH from 4 to 6, at a temperature from 40° to 60°C for a period of time from 6 to 48 hours.
- 20 5. A process according to claim 4 wherein the citrus pectin is used as an aqueous slurry having a dry solids content of 5 to 50% by weight.
- 25 6. A process according to claim 2 wherein the hydrolysed citrus pulp or apple pulp is produced by hydrolysing the citrus pulp or apple pulp with a pectolytic enzyme as well as with a cellulase at a pH of from 4 to 6, at a temperature of 40° to 60°C for a period of time from 6 to 48 hours.
- 30 7. A process according to claim 6 wherein the citrus pulp or apple pulp is used as an aqueous slurry having a dry solids content of from 2.5 to 25% by weight.

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8. A process according to claim 1 wherein the pectolytic enzyme is used in an amount of from 0.5 to 2.0% volume/weight with respect to the fruit pulp fibre.
- 5 9. A process according to claim 2 wherein the citrus pulp is hydrolysed with an acid before the enzymatic hydrolysis.
10. A process according to claim 1 wherein after enzymatic hydrolysis, the pectolytic enzyme is inactivated.
- 10 11. A process according to claim 1 wherein after enzymatic hydrolysis, the hydrolysate is dried.
12. A process according to claim 11 wherein the hydrolysate is dried to a powder.
- 15 13. A food flavour precursor whenever produced by a process according to any of the preceding claims.
- 20 14. A process for the preparation of a food flavour which comprises mixing the ingredients of the food flavour with from 1 to 5% of a food flavour precursor according to claim 1.
- 25 15. A food flavour whenever prepared by a process according to claim 14.

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ABSTRACT:

5 A process for preparing food flavour precursors which comprises enzymatically hydrolysing fruit pulp fibres with a pectolytic enzyme and a process for the preparation of a food flavour which comprises mixing the ingredients of the food flavour with from 1 to 5% of the food flavour precursor thus prepared.

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flavouring agents, more particularly to a process for
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15 material. The main component of pectin is a linear D-
galacturonan, many of the carboxyl groups of which are
esterified with methanol. Other components include L-
arabinan and D-galactan which together may form up to 20%
by weight of the pectin. The main raw materials for pectin
20 and hemicellulose are the peel of citrus fruits such as
lemon, lime, orange and grapefruit, as well as apple pomace
which is the residue remaining after pressing of apple to
obtain apple juice.

25 **SUMMARY OF THE INVENTION**

Galacturonic acids and reducing sugars such as glucose,
arabinose and galactose are known as flavour precursors and
we have surprisingly found that such substances may be
30 obtained by enzymatically hydrolysing fruit pulp fibres.

The present invention provides a process for preparing food
flavour precursors which comprises enzymatically
hydrolysing fruit pulp fibres with a pectolytic enzyme.

REPLACEMENT SHEET

30 AUG 1997

FLAVOURING AGENTS

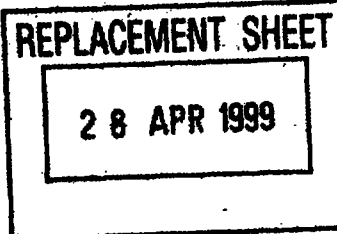
ABSTRACT:

5 A process for preparing food flavour precursors which comprises enzymatically hydrolysing fruit pulp fibres with a pectolytic enzyme and a process for the preparation of a food flavour which comprises mixing the ingredients of the food flavour with from 1 to 5% of the food flavour precursor thus prepared.

NO FIGURE TO BE SUGGESTED

REPLACEMENT SHEET

20 AUG 1997



CLAIMS

1. A process for preparing food flavour precursors, which comprises enzymatically hydrolysing fruit pulp fibres with a pectolytic enzyme at a pH from 4 to 6 and a temperature from 40° to 60°C for a period of time from 6 to 48 hours to form the flavour precursor.
2. The process according to claim 1 wherein the hydrolysis is conducted with a mixture of pectolytic enzymes.
3. The process according to claim 1 wherein the hydrolysis is conducted with the pectolytic enzyme in combination with a cellulase.
4. The process according to claim 1 wherein the fruit pulp fibre is citrus pulp, citrus pectin, or apple pulp fibre.
5. The process according to claim 1 wherein the pectolytic enzyme is polygalacturonase, pectinesterase, hemicellulase, or pectintranseliminase.
6. The process according to claim 4 wherein the citrus pectin is used as an aqueous slurry having a dry solids content of 5 to 50 weight percent.
7. The process according to claim 4 wherein the fruit pulp fibres are used as an aqueous slurry having a dry solids content of from 2.5 to 25 weight percent.
8. The process according to claim 1 wherein the pectolytic enzyme is used in an amount of from 0.5 to 2 percent volume/weight with respect to the fruit pulp fibre.
9. The process according to claim 1 which further comprises inactivating the pectolytic enzyme after the enzymatic hydrolysis.
10. The process according to claim 1 which further comprises drying the food flavour precursors after enzymatic hydrolysis.
11. The process according to claim 10 wherein the food flavour precursors are dried to a powder.
12. A process for preparing food flavour precursors, which comprises:
hydrolysing citrus pulp fibre with an acid; and
enzymatically hydrolysing the citrus pulp fibre after the acid hydrolysis with a pectolytic enzyme at a pH from 4 to 6 and a temperature from 40° to 60°C for a period of time from 6 to 48 hours.
13. The process according to claim 12 wherein the acid hydrolysis occurs at a temperature from 80° to 100°C for a period of from 1 to 10 hours.



14. The process according to claim 12 wherein the citrus pulp fibres are used in an aqueous slurry having a dry solids content of from 2.5 to 25 weight percent.
15. The process according to claim 12 which further comprises neutralising the citrus pulp fibres to a pH of from 4 to 6 after the acid hydrolysis.
16. The process according to claim 12 wherein the enzymatic hydrolysis is conducted with a mixture of pectolytic enzymes.
17. The process according to claim 12 wherein the enzymatic hydrolysis is conducted with the pectolytic enzyme in combination with a cellulase.
18. The process according to claim 12 which further comprises inactivating the pectolytic enzyme after the enzymatic hydrolysis.
19. The process according to claim 12 which further comprises drying the food flavour precursors after enzymatic hydrolysis.
20. The process according to claim 19 wherein the food flavour precursors are dried to a powder.

