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(54) Title: PROCESS FOR TREATING FRUIT– OR VEGETABLE–BASED PRODUCT WITH PECTINMETHYLESTERASE AND ADDED PECTIN

(57) Abstract

A process for preparing a fruit– or vegetable–based product comprising: adding pectin and pectinmethylesterase to a fruit or vegetable paste which is not sensitive to pectinmethylesterase; incubating the paste with the pectin and pectinmethylesterase; and optionally inactivating the pectinmethylesterase.

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PROCESS FOR TREATING FRUIT- OR VEGETABLE-BASED PRODUCT WITH PECTIN-METHYLESTERASE AND ADDED PECTIN

Field of the Invention

The present invention relates to a process for the preparation of fruit- or vegetable-based products of high quality and consistency and to products obtained by this process.

Background to the Invention

The texture of fruit- and vegetable-based products, such as purees and pastes, is usually considered to be a key determinant of their eating quality. Purees and pastes are prepared from whole fruit and vegetables by a combination of comminution and heating; the process conditions used are dependent on the raw material and the desired product attributes such as particle size, consistency and storage stability.

It is desirable to be able to produce fruit- or vegetable-based products having a high consistency and quality with a low solids content.

It is also desirable to be able to produce fruit- or vegetable-based products having a fresh, rather than a processed, flavour and/or aroma profile.

Fruits and vegetables contain pectins which are found in cell walls and comprise polygalacturonic acids esterified by methoxyl groups. Pectins are classified in accordance with the degree of esterification: pectins having greater than 50% esterification are called high-methoxylated pectins; pectins

having less than 50% esterification are called low-methoxylated pectins.

Enzymes, such as polygalacturonase (PG) and pectinmethylesterase (PME), are naturally-present in fruit and vegetables. When a fruit or vegetable is comminuted, these enzymes are released and PME catalyses the removal of methyl ester groups from pectin, thereby producing pectic and pectinic acids which can associate with each other via cation cross-links to form a gel-like structure. However, PG rapidly depolymerises these pectic and pectinic acids, thereby offsetting any viscosity increase resulting from gelling. Consequently, the resulting product has a thin and watery consistency.

Heating a fruit or vegetable rapidly to above 80°C (eg 90-95°C) whilst it is being comminuted inactivates PME and PG, thereby preventing any significant breakdown of pectin during processing, such that a good consistency is maintained. For the purpose of the present invention, products prepared in this way are termed >PME-sensitive= because subsequent addition of PME (in the absence of PG) results in an increase in viscosity.

EP 0624062 discloses adding PME to crude or pretreated fruits or vegetables containing high-methoxylated pectin; this demethoxylates the high-methoxylated pectin to form low-methoxylated pectin which gelifies with added or naturally present calcium ions.

The severe heat treatment used to inactivate PME and PG when preparing >PME-sensitive= pastes leads to increased rates of chemical reactions including the Maillard reaction, which leads to the browning of products and the generation of off-

flavours. Also, many important nutrient, colour and aroma compounds undergo chemical reactions or are driven off during heating.

In contrast, when a fruit or vegetable is comminuted at lower temperatures (eg 60-70°C), there is less heat damage and better retention of important nutrient, colour and aroma compounds. However, at these lower temperatures the enzymes PG and PME are active and break down pectin, resulting in a product having a poor consistency. For the purpose of the present invention, products prepared in this way are termed >PME-insensitive= because the subsequent addition of PME (in the absence or presence of PG) does not result in any significant thickening as the pectin has already been substantially demethoxylated.

The present invention seeks to provide a process for increasing the consistency and manipulating the texture of fruit- or vegetable-based products having a low solids content. Preferably, the products have flavour/aroma profiles and colours which are perceived as fresher than conventionally processed products with comparable consistencies.

Summary of the Invention

According to the present invention there is provided a process for preparing a fruit- or vegetable-based product comprising the following steps:

a) adding pectinmethylesterase and pectin to a pectinmethylesterase-insensitive fruit or vegetable paste;

b) incubating the paste with the pectinmethylesterase and the pectin;

c) optionally inactivating the pectin methylesterase.

The added pectin and pectinmethylesterase are preferably substantially free from pectin depolymerising enzymes such as pectin lyases and polygalacturonases.

A fruit- or vegetable-paste is, for example, chopped, diced, comminuted or macerated fruit or vegetables. It therefore includes fruit or vegetable pieces having a size in the range of from approximately 10 mm to approximately 3cm.

A PME-sensitive paste is one which shows an increase in viscosity of greater than 1000 Pa in G' (storage modulus) value at 12°Brix when about 265 units of PME activity are added to 100g of paste.

A PME-insensitive paste is one which shows an increase in viscosity of less than 1000 Pa in G' value at 12°Brix when about 265 units of PME activity are added to 100g of paste.

Preferably the process conditions are chosen such that the paste increases in consistency but does not substantially gel.

At the end of the process the G' value of the paste has preferably increased by more than 1000 Pa and less than 3000 Pa at 12° Brix.

The extent of consistency increase can be controlled by the amount of pectin added, the amount of PME added and the incubation conditions.

A final inactivation step is optional: for example, if a desired consistency is reached at the time that the reaction has reached effective completion then a final inactivation step is not required. This can be the case when the amount of pectin added limits the reaction. However, if the reaction has not reached effective completion at the time that the desired consistency has been reached, the PME is inactivated to prevent any further increase in consistency.

The controlled addition of PME may be achieved by titrating a solution of PME extract into the fruit- or vegetable-paste or adding PME extract in the form of a powder. Using an extract of PME allows control of the amount of PME added, since it is assayable. Also, it is easily dosed. Hence, the level of PME activity in the paste is easily pre-determined.

A suitable source for PME is any vegetable or fruit from which it can be extracted. For example, green beans, snap beans, potatoes, cauliflowers, sour cherries, green tomatoes, green peppers, oranges, apples, bananas, pears, turnips, onions, garlic and mixtures thereof. Immature fruit and vegetables are preferred. Particularly preferred sources are green tomatoes, green peppers, green beans and snap beans.

The most preferred source of PME is green tomatoes, as they contain high levels of PME and insignificant (ie undetectable) levels of PG.

PME may be extracted from green tomatoes by stabilising green tomatoes or pieces thereof and extracting pectin methylesterase therefrom. The stabilised green tomatoes and pieces thereof are ambient stable and convenient to use.

Preferably, the extract of PME is a solution or powder for practical and controlled dosing into paste.

The pectin may be sourced from fruit, vegetables and mixtures thereof. It is preferably sourced from tomatoes, apples or citrus fruits. It may be added in the form of a serum extracted from a fruit or vegetable paste which is PME-sensitive. It may also be added as a solid or a solution of pre-extracted pectin obtained from commercial suppliers.

The pectin and the PME extract are added to the paste, optionally with any other ingredients. The mixture is then incubated to activate the enzyme and allow it to demethoxylate the added pectin, resulting in an increase in viscosity. Different pastes take different lengths of time to respond to the same amount of PME activity at the same temperature of incubation. Once the desired consistency (thickness) has been achieved, the PME is optionally inactivated by, for example, heating, adjusting pH, and/or adding inhibitors. If heating is used, the temperature is typically raised to 90°C for 10 minutes.
The paste treated in accordance with the present invention is preferably a tomato paste.

A tomato paste which is PME-insensitive is one which may be prepared using a >cold-break= process. In one example of a cold-break process, raw tomatoes are chopped and fed into a vessel provided with steam coils (or steam jackets) and a rotating blade assembly for agitating the material in the vessel, such that good heat transfer is obtained. The cold-break process is typically conducted at a temperature of less than 80°C, preferably from 60 to 70°C.

A tomato paste which is PME-sensitive is one which may be prepared using a >hot-break= process. In one example of a hot-break process, the cold break process described above is used, except that the hot-break process is typically conducted at a temperature greater than 80°C, preferably from 85 to 95°C.

The fruit- or vegetable-paste prepared using the process of the present invention can be sold as it is, or can be used as the ingredient of products, such as sauces, ketchup, pizza toppings and soups.

By selecting the right conditions for steps a, b and c of the present invention, it is possible to improve the viscosity and/or texture of fruit- or vegetable-pastes, whilst preferably retaining their fresh colour, flavour and aroma.

Examples of the products and processes of the invention will now be described to illustrate, but not to limit, the invention, with reference to the accompanying figures, in which:

figure 1 is a graph of time (mins) (x axis) against G'(Pa) (y axis) for five pastes;

figure 2 is a graph of time (mins) (x axis) against G'(Pa) (y axis) for three pastes; and

figure 3 is a graph of time (mins) (x axis) against G'(Pa) (y axis) for three pastes.

figure 4 is a graph of Bostwick value and G' value for a hot break tomato paste incubated with PME.

figure 5 shows the Bostwick and blotter results over time on the paste of figure 4.

figure 6 is a 2-hour strain response curve.

figure 7 gives the strain dependence of the paste of figure 4.

In all these figures, the G' values are expressed as an increase in G' from the start of incubation.

Detailed Description of the Invention

Preparation of PME-containing extracts:

Mature green tomatoes (without any signs of colour change) were harvested. The tomatoes were quartered and the seeds and locular contents removed. The pericarp tissue was either used immediately or frozen in liquid nitrogen and stored at -20/C until required for use. A known weight of pericarp was taken and an equal volume of 2M NaCl was added (i.e. if 100g pericarp was taken, then 100ml of salt solution was used). The pericarp was homogenised in a blender, stirred at 4/C for 2 hours and centrifuged (27,000 x *g*, 30 minutes, 4/C). The supernatant was collected and strained through muslin. The supernatant (ie pericarp salt extract) was stored at -20/C in small aliquots until required. The PME activity of the pericarp salt extract was determined by titration of a 1% pectin solution with 25 mM NaOH at pH 7.5 and 30/C. Units of activity are expressed as :moles COO^- generated/min.

Preparation of PME-sensitive paste:

Tomato paste which was sensitive to PME addition was prepared in the laboratory using a domestic microwave oven. Two tomatoes were heated for 3.5 mins, comminuted and heat-pasteurised. The resulting paste was then separated via centrifugation into serum and pellet. Paste of a suitable consistency was prepared by recombining the pellet with a suitable volume of the serum.

Preparation of PME-insensitive paste:

This was prepared in the same way as for PME-sensitive paste, except that the two tomatoes were heated for 1.5 mins in a microwave oven.

General measurement techniques:

Small deformation rheology: a small amount (approx 3g) of paste was placed between parallel plates on a rheometer, model RDA2 available from Rheometrics, the plates being 5cm in diameter and roughened by attachment of emery paper to the plate surfaces to reduce slippage or surface friction phenomena. Time sweep measurements were made at 0.5% strain, 10 $rads^{-1}$ at 30/C. All samples were sealed with liquid paraffin to avoid desiccation or water exchange.

Bostwick measurements: these were made in a standard Bostwick viscometer. Paste was placed in the chamber of the levelled Bostwick, the shutter opened and the timing clock started. The distance (in centimetres) over which the paste flowed in 30 seconds was recorded. Pastes with a low Bostwick value have a high viscosity and *vice versa*.

Serum:pellet ratios: these were determined by placing a weighed amount of each paste into a centrifuge tube,

centrifuging at 5,000 - 10,000 x *g* for 30 minutes, pouring off the serum, and recording the weights of the serum and the pellet. From these values the serum to pellet ratio was calculated.

Serum viscosity: a Low Shear 30 Rheometer obtained from Contraves in Switzerland was used to measure the serum viscosity. A small amount of serum was prepared in the same way as for measuring serum:pellet ratios. The serum was placed in a cup-and-bob apparatus of the rheometer and a range of shear rates was used to determine the viscosity at zero shear.

Dry weight: a sample of the paste or serum was placed into a pre-weighed glass vial and the filled vial was weighed again. Three vials were set up for each sample. The filled vials (without lids) were placed in a vacuum oven at 80/C for three days and were then weighed again. After subtraction of the weight of the vial, the total weight and dry weight of the sample was calculated, and then the dry weight expressed as a percentage of the total weight was calculated.

/Brix measurements: these values reflect the content of soluble sugars in the serum fraction by determination of refractive index. The measurements were made using a RFM 320 Refractometer (from Bellingham and Stanley Ltd), calibrated against distilled water. A sample of tomato paste was squeezed through filter paper and two or three drops of serum placed on the measurement surface of the refractometer. The value measured by the Refractometer was recorded.

Comparative Example A.

2.5% (v/w) of salt extract from green tomato pericarp (which contains 265 Units of PME activity) was added to PME-

sensitive paste. The paste was incubated with the PME for 250 mins at 30°C by placing it between two plates of a rheometer. Structuring was monitored by small deformation rheology.

The results are plotted (line A) in Figure 1 from which it can be seen that this paste rapidly increased in G= values over the time of incubation.

Comparative Example B

PME-sensitive paste was incubated for 250 minutes at 30°C in the absence of added PME by placing it between two plates of a rheometer. Structuring was monitored by small deformation rheology.

The results are plotted (line B) in Figure 1 from which it can be seen that this paste did not show any significant changes in G= values over the time of incubation.

Comparative Example C

The serum from PME-sensitive paste was removed from the paste and replaced by water. The serum was removed by placing a weighed amount of paste into a centrifuge tube, centrifuging at 5,000 - 10,000 x *g* for 30 minutes, pouring off the serum, resuspending the pellet in water and re-centrifuging twice to ensure complete removal of all serum. The resulting washed paste was incubated for 250 minutes at 30°C in the absence of added PME by placing it between two plates of a rheometer. Structuring was monitored by small deformation rheology.

The results are plotted (line C) in Figure 1 from which it can be seen that the washed paste did not show any significant changes in G= values over the time of incubation.

Comparative Example D

2.5% (v/w) of salt extract from green tomato pericarp (which contains 265 Units of PME activity) was added to the washed paste of comparative example C. The paste was incubated with the PME for 250 mins at 30°C by placing it between two plates of a rheometer. Structuring was monitored by small deformation rheology.

The results are plotted (line D) in Figure 1 from which it can be seen that the addition of PME to the washed paste did not show any significant changes in G= values over the time of incubation.

Comparative Example E

The method of comparative example D was repeated, but with the extra step of adding calcium (6mM) to the washed paste.

The results are plotted (line E) in Figure 1 from which it can be seen that the addition of PME and calcium to the washed paste did not show any significant changes in G= values over the time of incubation

Conclusions

These comparative examples illustrate that adding PME to PME-sensitive pastes increases the viscosity of the pastes. They also show that it is the serum polymers which are critical in permitting this PME-mediated structuring.

Example 1

265 units of PME activity per 100g of paste were added to a PME-insensitive paste comprising 10g of PME-insensitive pellet and 10ml of PME-insensitive serum. The paste was incubated with the PME for up to 800 mins at 30°C. Structuring was monitored by a time sweep performed on an RDA2 Rheometrics system.

The results are plotted (line 1) in Figure 2 from which it can be seen that this PME-insensitive paste showed no significant increase in G= values (ie less than 1000 Pa) over the time of incubation.

Example 2

The method of example 1 was repeated except that the PME-insensitive serum was replaced by PME-sensitive serum.

The results are plotted (line 2) in Figure 2 from which it can be seen that there was a significant increase in G= values over the time of incubation, showing that the paste was sensitive to the action of PME.

At the end of the incubation period, although consistency had increased, no gel had been formed.

Example 3

The method of example 2 was repeated, except that the PME-sensitive serum used was 8 times more concentrated than the PME-sensitive serum of example.2.

The results are plotted (line 3) in Figure 2 from which it can be seen that there was a significant increase in G= values over the time of incubation and that the extent of structuring (maximum G= value) was greater than in example 2.

The material produced was a weak gel. This indicates that it is possible to control the level of structuring by the amount of PME-sensitive serum added to the system.

Example 4

The method of example 1 was repeated using a different batch of PME-insensitive paste.

The results are plotted (line 4) in Figure 3 from which it can be seen that this paste showed an increase in G= values of less than 1000 Pa over the time of incubation.

Example 5

The method of example 4 was repeated except that the PME-insensitive serum was replaced by a 0.5% solution of commerical pectin (with a degree of esterification of 37%).

The results are plotted (line 5) in Figure 3 from which it can be seen that there was a significant increase in G= values over the time of incubation.

Example 6

The method of example 4 was repeated except that the PME-insensitive serum was replaced by a 1.0% solution of commerical pectin (with a degree of esterification of 37%).

The results are plotted (line 6) in Figure 3 from which it can be seen that there was a significant increase in G= values over the time of incubation and that the extent of structuring (maximum G= value) was greater than for example 5.

This indicates that the addition of pectin permits PME-mediated structuring and that it is possible to control the level of structuring by the amount of pectin added to the system.

Example 7

Differentiation between thickened and gelled tomato paste after PME treatment.

Blotter, Bostwick and rheological time sweep measurements: as above.

Strain sweep: hot break tomato paste (Copais) at 13° Brixwas treated with 2.5% PME extract and placed onto a Rheometrics RDA II strain controlled rheometer equiped with a 50 mm diameter parallel plates. Emery paper was attached to the plates with cyanoacrylate glue to reduce sample slippage. Samples were sealed in with liquid paraffin to stop water exchange. Experiments were carried out at an incubation temperature of 30°C. For all experiments, except time zero, the structuring of the treated sample was followed with a rheological time sweep. The conditions of this measurement were: frequency 1Hz, temperature 30°C and a strain range of 0.5-200%. The value for strain dependence was determined from the slope intersects of a plot of stress to strain (figure 6).

Figure 5 shows the Bostwick and blotter values for 13° Brix hot break paste (Copais) as a function of time of incubation. Paste which is thickened shows a gradual decrease in Bostwick and Blotter. For this particular example the maximum thickenening was achieved at 2 hours. Beyond this time the blotter rapidly increases indicating gross syneresis in the product and the Bostwick becomes effectively zero. At this poit the paste has become a continuous gel. If the Bostwick behaviour is plotted relative to the rheological time sweep for the same PME treatment it can be seen that the estimated gel point corresponds to a G' value of around 3000 Pa. Gel formation is occurring during the rapid structuring phase of the time sweep. The strain dependence value also shows a distinct step change between 2 and 3 hours (figure 7). Up to 2 hours the linear region of the strain response is relatively short (figure 6) and the dependence values do not exceed 8%. Between 2 and 3 hours the onset of gel formation is accompanied by an increase in strain dependence to 14%, and the strain response plots have increased linar regions indicating a greatly increased resistance to breadown and flow. It is therefore possible to define physical and instrumental characteristics which differentiate between a gel network (strain dependence of >8%, as defined in figure 6) and a thickened system of increased consistency (starin dependence <8%.

Claims

1. A process for preparing a fruit- or vegetable-based product comprising the following steps:

   a) adding pectinmethylesterase and pectin to a pectinmethylesterase-insensitive fruit or vegetable paste;

   b) incubating the paste with the pectinmethylesterase and the pectin;

   c) optionally inactivating the pectin methylesterase.

2. A process as claimed in claim 1 wherein the added pectin and pectinmethylesterase are substantially free from pectin depolymerising enzymes.

3. A process as claimed in any preceding claim wherein, at the end of the process, the paste has increased in consistency but has not substantially gelled.

4. A process as claimed in any preceding claim wherein, at the end of the process, the G' value of the paste has increased by more than 1000 Pa at 12° Brix.

5. A process as claimed in any preceding claim wherein, at the end of the process, the G' value of the paste has increased by less than 3000 Pa at 12° Brix.

6. A process as claimed in any preceding claim wherein the paste is a tomato paste.

7. A process as claimed in claim 6, wherein the pectinmethylesterase-insensitive paste is prepared using a cold break process.

8. A process as claimed in any preceding claim wherein the pectinmethylesterase is sourced from a vegetable, fruit or mixture thereof.

9. A process as claimed in any preceding claim wherein the pectin is sourced from a vegetable, fruit or mixture thereof.

10. A process as claimed in claim 9 wherein the pectin is in the form of a serum extracted from a pectinmethylesterase-sensitive fruit or vegetable paste.

11. A fruit- or vegetable-based product prepared in accordance with the process claimed in any one of the preceding claims.

Fig.1.

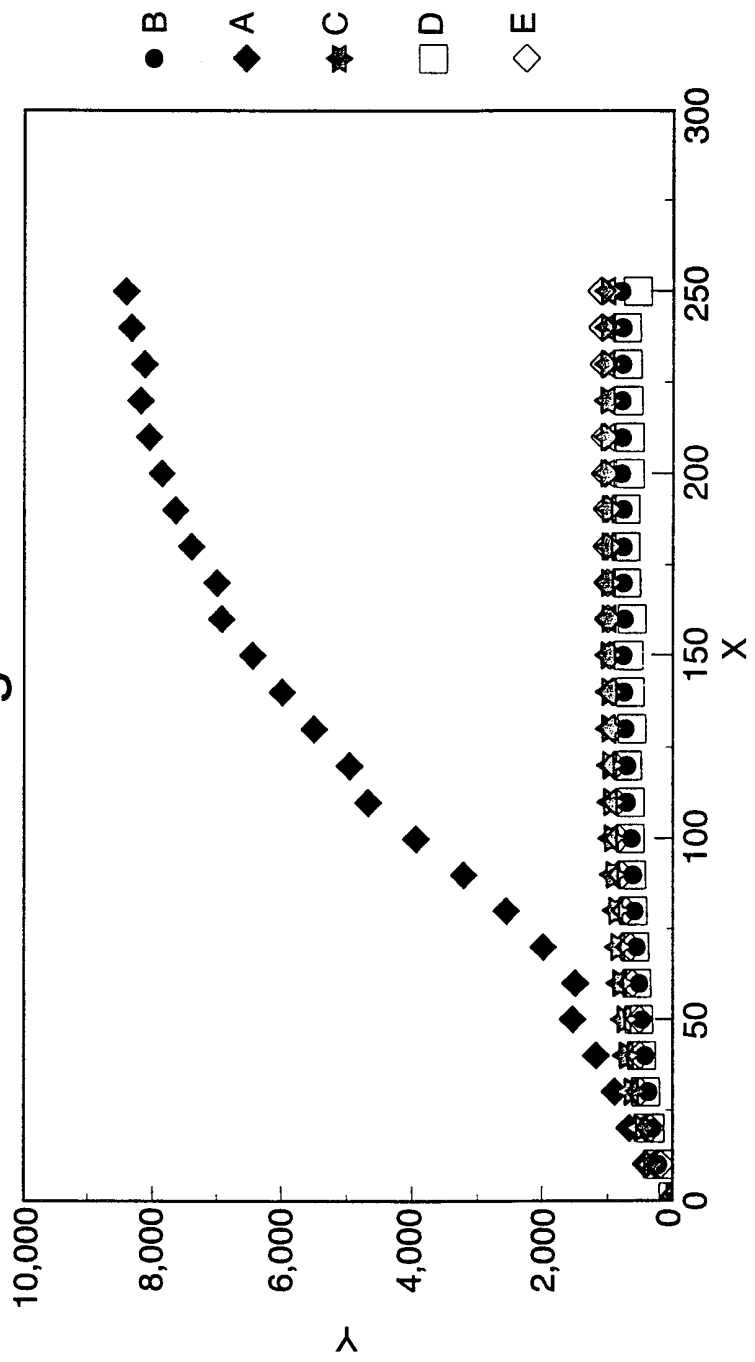


Fig.2.

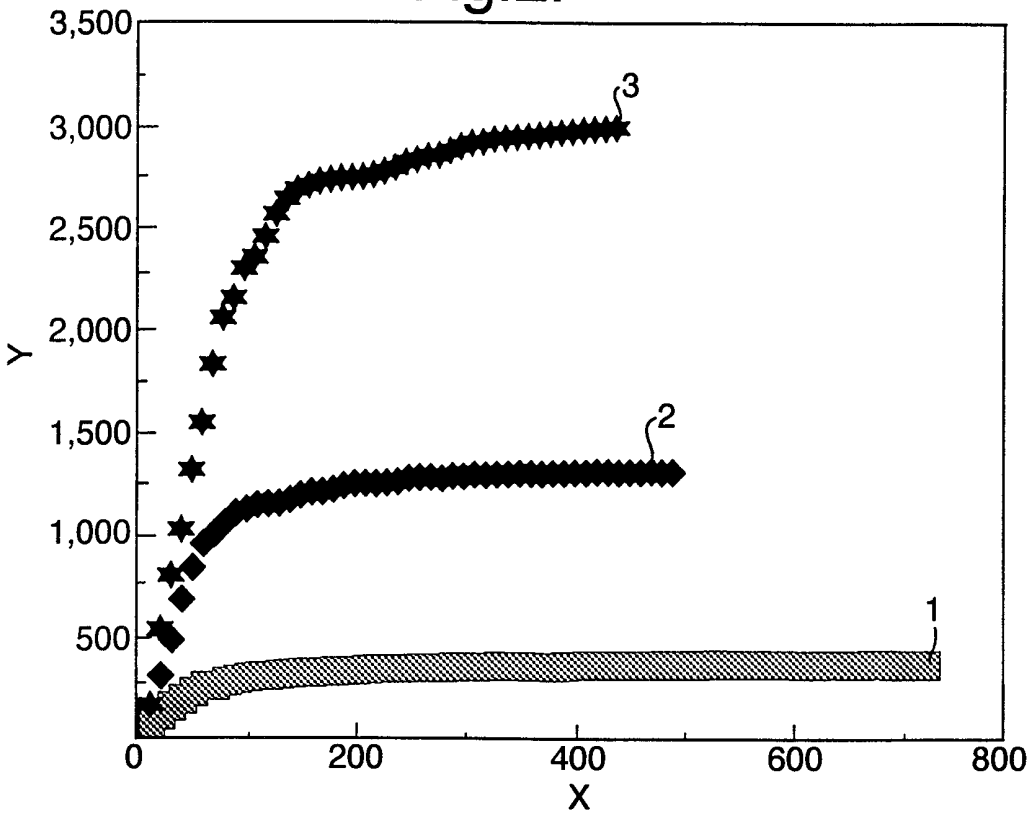


Fig.3.

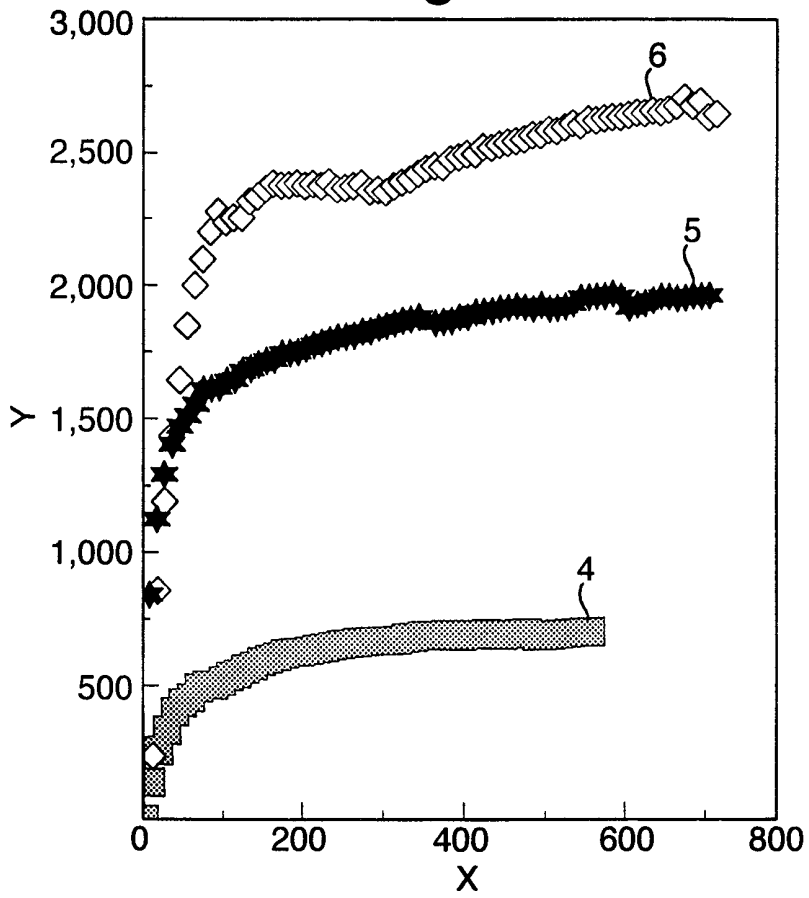


Fig.4.

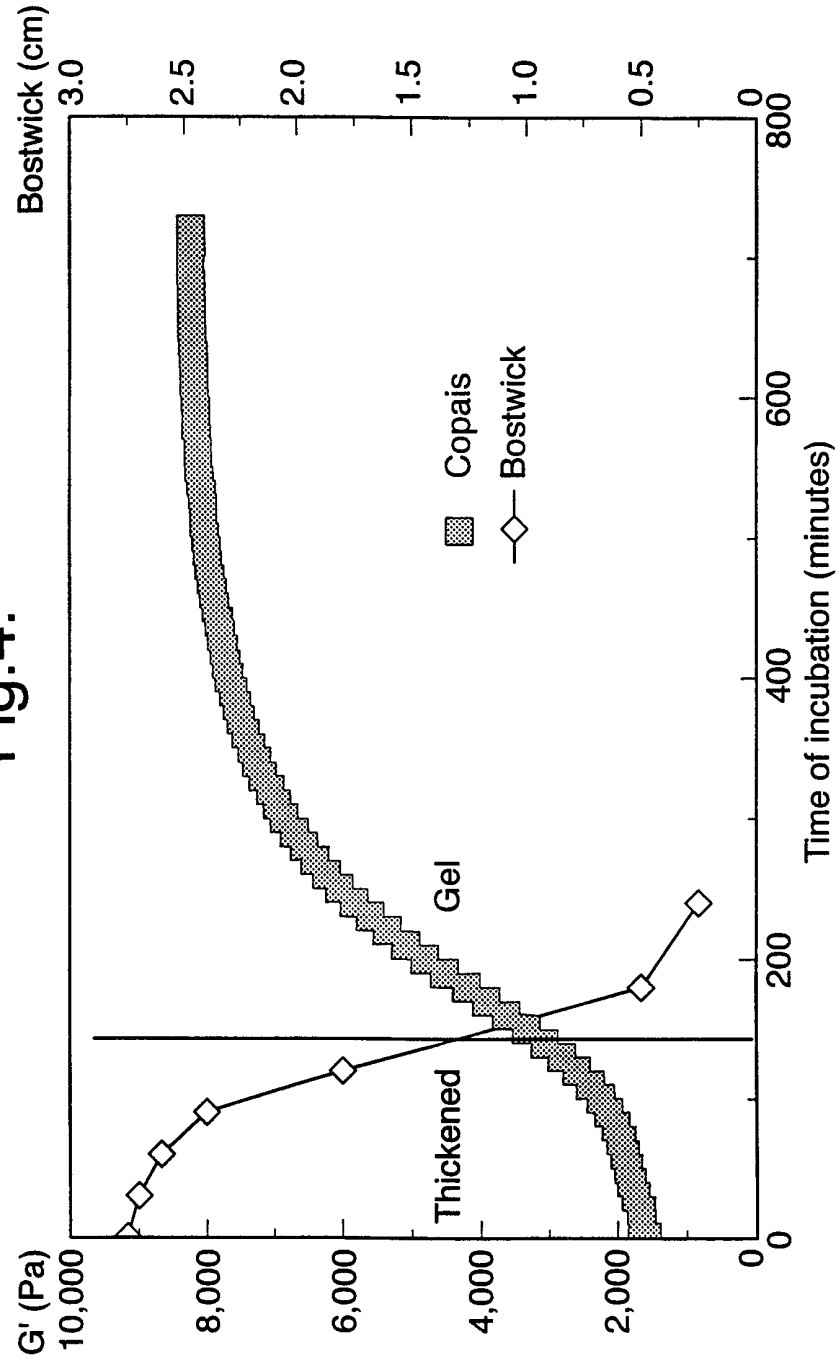

Bostwick (cm)
3.0
2.5
2.0
1.5
1.0
0.5
0
800
600
400
200
0
Time of incubation (minutes)
G' (Pa)
10,000
8,000
6,000
4,000
2,000
0
Copais
Bostwick
Gel
Thickened

Fig.5.

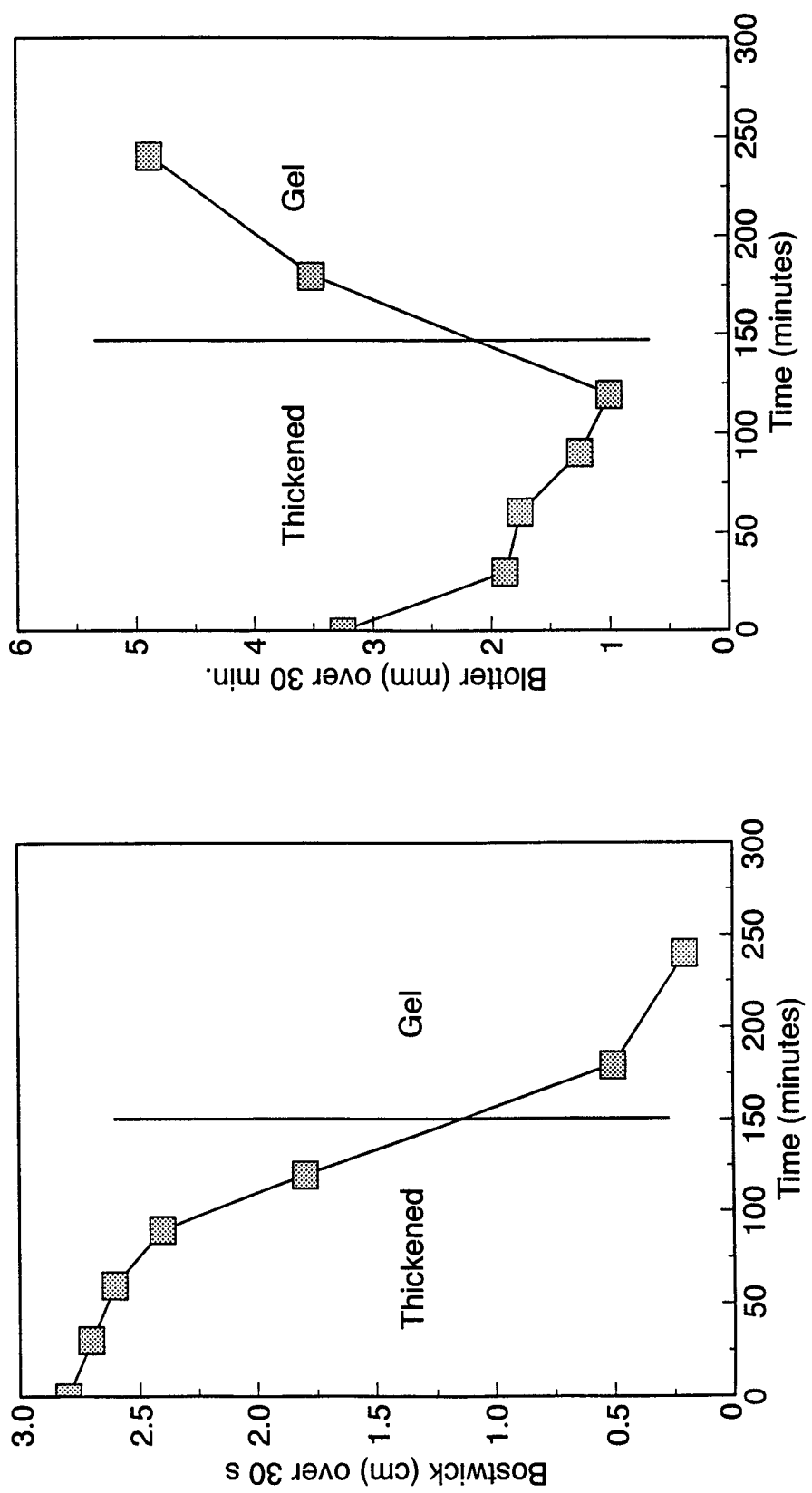

Bostwick (cm) over 30 s
3.0
2.5
2.0
1.5
1.0
0.5
0
Thickened
Gel
0
50
100
150
200
250
300
Time (minutes)
Blotter (mm) over 30 min.
6
5
4
3
2
1
0
Thickened
Gel
0
50
100
150
200
250
300
Time (minutes)

Fig.6.

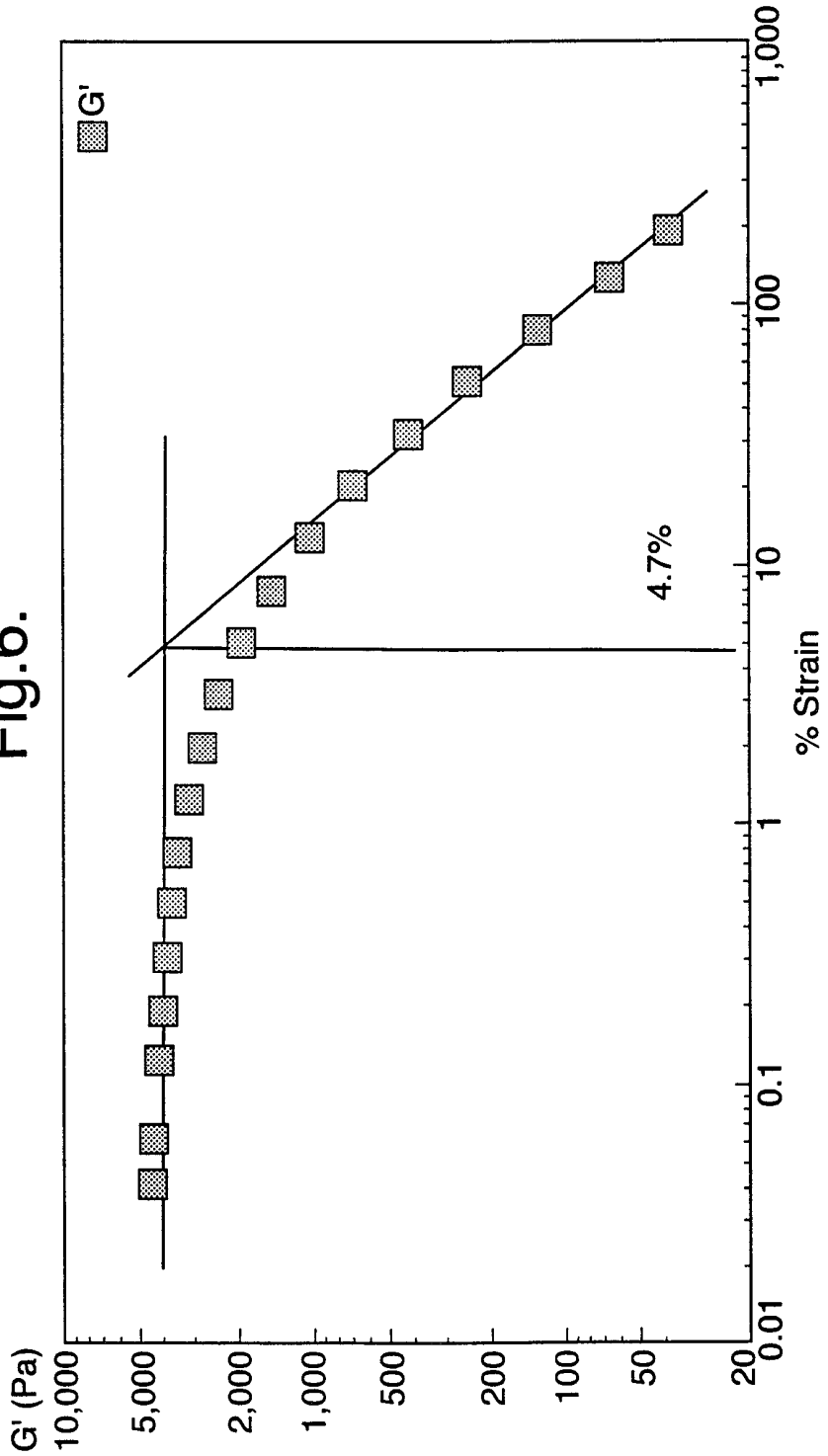
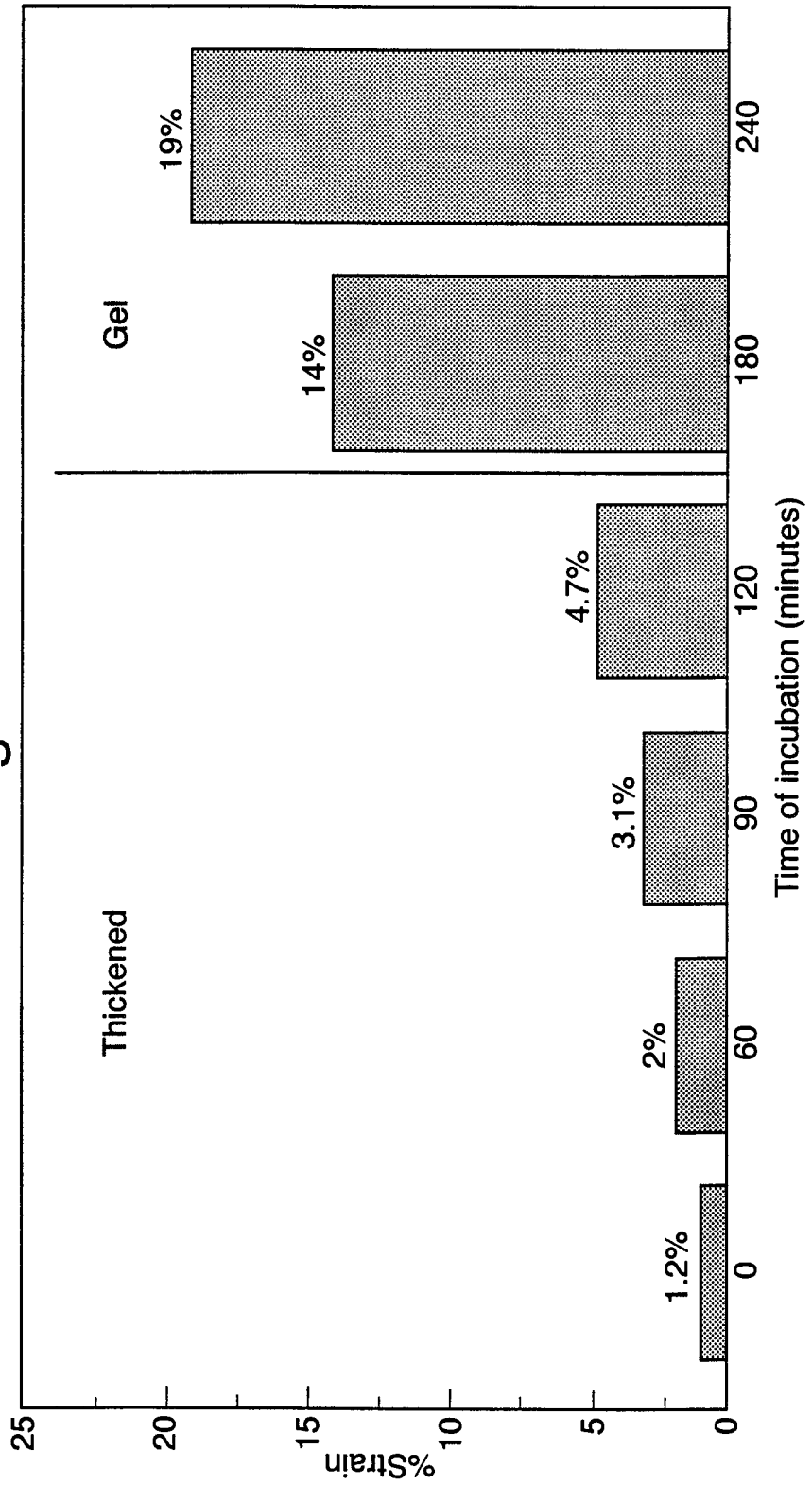

G'
G' (Pa)
10,000
5,000
2,000
1,000
500
200
100
50
20
0.01
0.1
1
10
100
1,000
% Strain
4.7%

Fig.7.


%Strain
25
20
15
10
5
0
Thickened
Gel
1.2%
2%
3.1%
4.7%
14%
19%
0
60
90
120
180
240
Time of incubation (minutes)

INTERNATIONAL SEARCH REPORT

Intern ıal Application No
PCT/EP 98/05278

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/212 A23L1/0524

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 12055 A (GIST BROCADES NV ;GRASSIN CATHERINE MARIE THERES (FR); FAUQUEMBERG) 9 June 1994 see page 4, line 32 - line 35 see page 5, line 12 - line 38 see page 6, line 24 - line 26 see page 3, line 28 - line 29 & EP 0 624 062 A cited in the application	1,2,8,9, 11
X	WO 96 11588 A (UNILEVER PLC ;UNILEVER NV (NL)) 25 April 1996 see the whole document	11
X,P	WO 97 38592 A (UNILEVER PLC ;UNILEVER NV (NL)) 23 October 1997 see page 2, line 35 - page 5, line 36	11

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 December 1998	11/12/1998

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Interı nal Application No
PCT/EP 98/05278

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern ıal Application No
PCT/EP 98/05278

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WO 9847391 A	29-10-1998	NONE	