Symbiosis, 2 (1986)
Balaban Publishers, Philadelphia/Rehovot

Pectinases and Other Cell-Wall Degrading Enzymes of Industrial Importance

F.M. ROMBOUTS* and W. PILNIK

Agricultural University, Department of Food Science, De Dreijen 12
6703 BC Wageningen, The Netherlands
Tel. 08370-82233 Telex 45015

Abstract

Pectinases have been introduced in fruit juice technology initially to facilitate apple juice clarification and are now widely used for extraction of juices, clarification of juices and wines, and maceration and liquefaction of fruits, vegetables and other plant material. There appear on the market preparations with a broad spectrum of enzymes designed for liquefaction and saccharification purposes, and specialty products designed to achieve more specific effects. Examples of the latter group are polygalacturonase for maceration of fruits and vegetables, pectinesterase for clarification of cider, "pentosanase" for improvements in baking technology and β-glucanase and xylanase in the brewing industry.

Keywords: Pectinase, polygalacturonase, β-glucanase, pentosanase, maceration, clarification, fruit juices, beer, wines

1. Introduction

There is a steadily growing market for industrially produced bulk enzymes. Although this commodity occupies a relatively modest position among the products obtained by fermentation (Table 1), its world-wide production value stood at US$ 265 million in 1981. The largest single use of enzymes is that of the detergent proteases, followed by the group of enzymes used in starch conversion. Pectinases and other plant cell wall degrading enzymes (pentosanases, β-glucanases) represent a market value of US$ 20–25 million.

* Invited in the framework of the Biomolecular Engineering Program of the Commission of the European Communities. Invited lecture

0334-5114/86/$03.00

Table 1. World-wide production of enzymes in 1981 in relation to other fermentation products*

Commodity	Value (millions in US$
Antibiotics	6300
Ethanol	2100
Organic acids	500
Amino acids	500
Enzymes, industrial (bulk)	265
Enzymes, analytical and therapeutic	35

* Source: Gist-Brocades, Delft, The Netherlands

These enzymes are applied in fruit and vegetable juice technology (pectinases mainly), in wine making (pectinases and β-glucanases), and in brewing and baking (β-glucanases, pentosanases). Continuous research efforts are directed towards improving existing enzyme preparations. Developments seem to go into two directions. There appear on the market preparations with a broad spectrum of enzymes (pectic enzymes, hemicellulases, cellulases) designed for liquefaction and saccharification purposes, and specialty products designed to achieve more specific effects.

2. Pectins and Pectic Enzymes

The cells in fruit and vegetable tissues are connected through the middle lamella which is pectinous in nature. The primary cell walls are composed of cellulose microfibrils embedded in a matrix of pectins, arabinans, galactans and proteins (McNeil et al., 1984), while in these tissues lignin is virtually absent. In secondary walls, which usually occur, cellulose is preponderant.

Pectins are composed of a rhamnogalacturonan backbone in which 1,4-linked α,D-galacturonan chains are interrupted at intervals with α,L-rhamnopyranose residues. Side chains occur, in which arabinose, galactose, xylose, mannose and glucose are the predominant sugar residues (Aspinall, 1980). Part of the galacturonic acid residues carry methyl ester groups. Acetyl groups may occur as substituents to hydroxyl groups.

Pectic enzymes are active towards the galacturonan part of the pectins (Rombouts and Pilnik, 1980). Pectin methylesterase (pectinesterase) splits methanol off from pectin, transforming it gradually into pectic acid. Three groups of pectin depolymerases exist: polygalacturonases, pectate lyases and pectin lyases (Fig. 1). Polygalacturonases (galacturonanases) split the glycosidic linkages in the galacturonan chain by hydrolysis. Pectate lyases degrade galacturonosyl bonds by β-elimination (Fig. 1). For both types of enzymes pectate and low methoxyl pectins are the preferred substrates, while highly methylated pectin is hardly attacked at all. In contrast, pectin lyases, another group of eliminatively splitting enzymes, are specific for highly methylated pectins. Naturally occurring pectins can be efficiently degraded by the combination of pectin methylesterase and polygalacturonase or pectate lyase, or else, if they are sufficiently highly methylated, by pectin lyase alone.

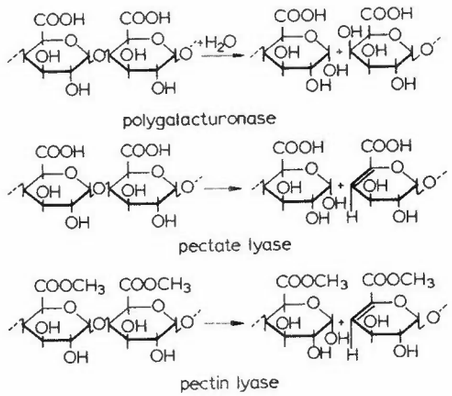

Figure 1. Substrate specificities and modes of attack of pectin depolymerases.

In broad-spectrum technical enzyme preparations ("pectinases"), which are commonly derived from *Aspergillus niger* or related fungi, pectin methylesterase, polygalacturonases and pectin lyases occur in varying amounts, along with other enzymes, such as arabinanases, galactanases, xylanases, cellulases, glycosidases and proteases.

3. Fruit and Vegetable Products

Pectinases have originally been introduced for the clarification of the turbid juice which is obtained upon pressing of pulped apples. Through the single action of pectin lyase, or the combined activity of pectin methylesterase and polygalacturonase the pectin, dissolved in the juice, as well as the negatively charged pectin coating of the suspended particles are degraded. Subsequently the destabilized particles flocculate through electrostatic interaction, and precipitate. A clear juice is then obtained by filtration or centrifugation.

The depectinized juice can be concentrated easily to a high soluble-solids content, to reduce the cost of cold storage and transport.

A variation of this enzymic clarification process has been introduced in the French farm-based cider industry. Traditionally clarification of the must was a spontaneous process, ill controlled as it was dependent on pectin methylesterase activity, naturally occurring in widely different quantities in various apple varieties. Fungal pectinesterase is now added to the must, in order to achieve partial demethylation of the pectin so that it coagulates with added calcium salts, resulting in the formation of the "chapeau brun" on top of the clear must. For this process, the production of a pectin methylesterase without polygalacturonase and pectin lyase was required (Baron and Drilleau, 1982).

The pulp of most soft fruits, such as black currants, strawberries and raspberries is by no means easy to press. Treatment of these pulps with pectinases ("Maischefermentierung") to facilitate pressing and to ensure high yields of juice and pigments has been common practice for a few decades already. In the early seventies enzymic juice extraction was also introduced for apples. Even for easy-pressing apples, pressing characteristics were improved, as well as juice yields, which may rise above 80% (Rombouts and Pilnik, 1978).

A further development was the introduction of the enzymatic liquefaction process for fruit and vegetable pulps (Pilnik et al., 1975). Through the use of pectic and cellulolytic enzymes (both endo- and exocellulases) the cell walls of fruit pulps are degraded to the stage of almost complete liquefaction. Juice yields are further increased to over 90%, and as a result of the solubilization of the cell-wall polysaccharides the dry-matter content of the juices is also sensibly increased. The use of expensive presses is greatly reduced, since most of the juice can be separated in vacuum filters, centrifuges or decanters. The introduction of this new process induced the leading enzyme companies to develop new brands of enzymes which, as indicated above, contain pectic enzymes, hemicellulases and cellulases. A problem was the development of a haze in concentrates of apple juice, produced with the new technology. It appeared that the pectinases involved contained arabinofuranosidase activity, which "linearizes" the branched arabinans occurring in apple juice, inducing a precipitation of arabinan, reminiscent of the retrogradation of amylose (Voragen et al., 1982). Evidently, formation of this type of haze can be avoided by either using enzyme preparations without arabinofuranosidase activity, or formulations which contain additional endoarabinanase to depolymerize the linearized arabinans (Fig. 2).

The pulp liquefaction technology holds promises for the processing of tropical fruits, because of the high yields of juice which are obtained without the need for expensive process equipment.

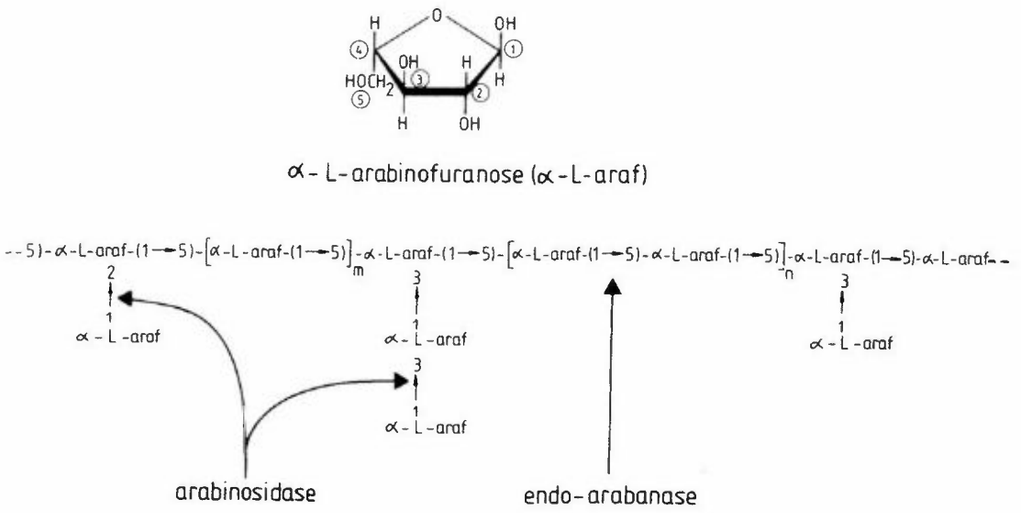

Figure 2. Points of attack of arabinan-degrading enzymes (Voragen et al., 1982).

Quasi-complete liquefaction is not desirable in the production of nectars. These comminuted fruit juices are viscous, pulpy drinks prepared from a variety of fruits, such as pears, peaches, apricots, berries, guava, papaya and passion fruit. They are usually prepared by a mechanical-thermal dispersion process. However, the desirable cloud stability is not always achieved and with firmflesh fruits, such as apples, a smooth consistency is hard to realize. For that purpose pectinases have been introduced which produce suspensions of loose cells from fruit and vegetable tissues. These macerating pectinases have an activity towards the middle lamella only and leave the walls of loose cells rather well intact. One of the successful enzyme preparations in this field is predominantly an endopolygacturonase with no pectin methylesterase activity and limited amounts of other polysaccharide-degrading enzymes, such as endocellulase (Grampp, 1969). The potentials of enzymic maceration are evident when carrots are considered. Very finely dispersed, stable purees with high contents in soluble solids, pigments and vitamins (β-carotene) are

obtained, which may be used in baby foods and cloudy vegetable juices. In a study of apricot nectar it appeared that highly branched methylated pectin played a crucial role in cloud destabilization (Siliha, 1985).

Citrus juices are another category of cloudy products. In citrus-juice manufacture a rather pulpy juice is pressed out of the fruit. Coarse pulp and seeds are removed in a finisher operation. This pulp contains considerable amounts of juice which may be recovered in a counter-current washing operation, a so-called pulp wash. The use of pectinases in this process results in increased soluble-solids content of the pulp-wash, and decreased viscosity, so that concentration is possible.

Further, polygalacturonase may be used as a remedy to cloud instability in citrus juices. Destabilization of the cloud is caused by the uncontrolled activity of pectin methylesterase, naturally present in cell walls of citrus fruits. This enzyme may demethylate the juice pectin to an extent that it starts to coagulate with calcium ions from the juice. Timely addition of polygalacturonase may cure this quality defect. Again in this case the enzyme preparation should be devoid of significant quantities of other enzymes, if a cloud-stable pigmented juice, such as orange juice is wished (Krop and Pilnik, 1974). In contrast, in the case of lemon juice, where complete removal of pulp is desired to obtain an opalescent juice, pectinases of the polygalacturonase-pectinesterase type are required (Grampp, 1980).

4. Grape Juice and Wines

In the USA the extraction of red grape juice from Concord grapes (which have a slimy consistency upon crushing) is routinely carried out with pectinases. The crushed grapes are heated to 60–65°C, to ensure maximum extraction of the pigments, and enzymic depectinization of the juice is realized at this temperature.

Conventional pectinases are also used in wine technology. With white wine, the yields of free-run juice and pressjuice can be raised by pectinases added to the grapes during or after crushing. Also, the must can be largely clarified before fermentation. In the process of "thermovinification" extraction of red grapes is done at temperatures of 55 to 70°C, in the presence of pectinases. Fermentation of the clear juice is then possible, rather than having to ferment "on the skins".

The clarification and filtration problems, sometimes encountered in wine making are not always due to gums from the grapes. They are often closely associated with wines which have been produced from grapes infected with

Botrytis cinerea (pourriture noble; pourriture grise). The fungus produces a β-glucan with an extremely high molecular weight (around a million daltons), which effuses from the grapes into the must, and causes filtration problems in concentrations as low as 5 mg per litre. The glucan is composed of a linear backbone of β-D-1,3-glucan with β-D-1,6 glucosyl substituents (Fig. 3). Elimination of the problem was possible through the introduction of a β1,3/β1,6-glucanase preparation derived from *Trichoderma* (Dubourdieu et al., 1981). The precise composition and specificity of the enzyme (s) are as yet unknown.

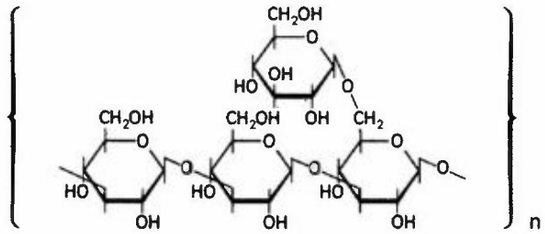

Figure 3. Tentative structure of the β1,3/β1,6 glucan produced by *Botrytis cinerea* in grapes.

5. Beer and Bakery Products

These products have in common that they are derived from cereals. The cell walls of cereal grains are different from those of fruit and vegetable tissues. They are composed of linear glucans with β1,3/β1,4 mixed linkages (Fig. 4), and arabinoxylans (Fig. 5). Enzymes from microbial origin to degrade these polysaccharides have been introduced in brewing and baking for various reasons.

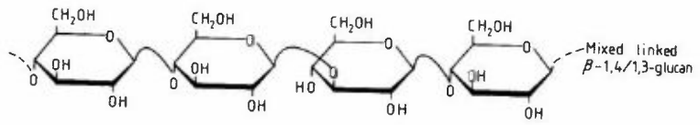

Figure 4. Tentative structure of barley glucan (Salomonsson, 1985).

obtained, which may be used in baby foods and cloudy vegetable juices. In a study of apricot nectar it appeared that highly branched methylated pectin played a crucial role in cloud destabilization (Siliha, 1985).

Citrus juices are another category of cloudy products. In citrus-juice manufacture a rather pulpy juice is pressed out of the fruit. Coarse pulp and seeds are removed in a finisher operation. This pulp contains considerable amounts of juice which may be recovered in a counter-current washing operation, a so-called pulp wash. The use of pectinases in this process results in increased soluble-solids content of the pulp-wash, and decreased viscosity, so that concentration is possible.

Further, polygalacturonase may be used as a remedy to cloud instability in citrus juices. Destabilization of the cloud is caused by the uncontrolled activity of pectin methylesterase, naturally present in cell walls of citrus fruits. This enzyme may demethylate the juice pectin to an extent that it starts to coagulate with calcium ions from the juice. Timely addition of polygalacturonase may cure this quality defect. Again in this case the enzyme preparation should be devoid of significant quantities of other enzymes, if a cloud-stable pigmented juice, such as orange juice is wished (Krop and Pilnik, 1974). In contrast, in the case of lemon juice, where complete removal of pulp is desired to obtain an opalescent juice, pectinases of the polygalacturonase-pectinesterase type are required (Grampp, 1980).

4. Grape Juice and Wines

In the USA the extraction of red grape juice from Concord grapes (which have a slimy consistency upon crushing) is routinely carried out with pectinases. The crushed grapes are heated to 60–65°C, to ensure maximum extraction of the pigments, and enzymic depectinization of the juice is realized at this temperature.

Conventional pectinases are also used in wine technology. With white wine, the yields of free-run juice and pressjuice can be raised by pectinases added to the grapes during or after crushing. Also, the must can be largely clarified before fermentation. In the process of "thermovinification" extraction of red grapes is done at temperatures of 55 to 70°C, in the presence of pectinases. Fermentation of the clear juice is then possible, rather than having to ferment "on the skins".

The clarification and filtration problems, sometimes encountered in wine making are not always due to gums from the grapes. They are often closely associated with wines which have been produced from grapes infected with

Botrytis cinerea (pourriture noble; pourriture grise). The fungus produces a β-glucan with an extremely high molecular weight (around a million daltons), which effuses from the grapes into the must, and causes filtration problems in concentrations as low as 5 mg per litre. The glucan is composed of a linear backbone of β-D-1,3-glucan with β-D-1,6 glucosyl substituents (Fig. 3). Elimination of the problem was possible through the introduction of a β1,3/β1,6-glucanase preparation derived from *Trichoderma* (Dubourdieu et al., 1981). The precise composition and specificity of the enzyme (s) are as yet unknown.

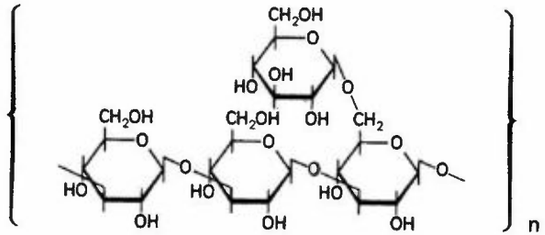

Figure 3. Tentative structure of the β1,3/β1,6 glucan produced by *Botrytis cinerea* in grapes.

5. Beer and Bakery Products

These products have in common that they are derived from cereals. The cell walls of cereal grains are different from those of fruit and vegetable tissues. They are composed of linear glucans with β1,3/β1,4 mixed linkages (Fig. 4), and arabinoxylans (Fig. 5). Enzymes from microbial origin to degrade these polysaccharides have been introduced in brewing and baking for various reasons.

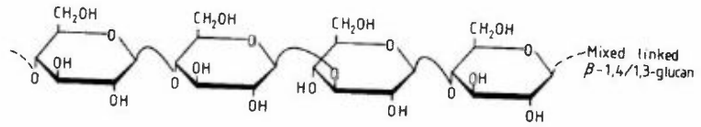

Figure 4. Tentative structure of barley glucan (Salomonsson, 1985).

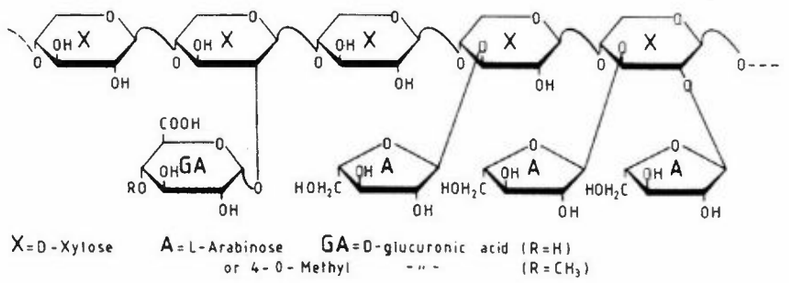

Figure 5. Tentative structure of an arabinoxylan (Salomonsson, 1985).

During malting of barley a number of enzymes are produced, including α-amylase, proteases, β-xylanase and β-glucanase. In addition, the latter two enzymes degrade the endosperm cell walls, so that starch and proteins are easily liberated during mashing, and largely hydrolysed by the corresponding enzymes. Due to time reduction in the malting process, β-glucanase and β-xylanase are not always produced in sufficient amounts. Also, in many countries part of the malt is replaced by so-called adjuncts (unmalted barley, maize, rice, sorghum). In these cases the malt enzymes are no longer sufficient and undegraded glucans and xylans may remain in wort and beer, causing serious filtration problems. Enzymes of microbial origin have to be supplemented: α-amylase, β-glucanase, β-xylanase. Particularly suitable are β-glucanases from *Bacillus subtilis*, as the substrate specificity of these enzymes is very similar to that of the malt enzyme (Ducroo and Delecourt, 1971).

Enzymes have also been introduced in milling and baking. Examples are fungal α-amylase, which may be added to improve baking quality by increasing loaf volume and crust color, and fungal protease, to influence the rheology of dough by limited degradation of gluten proteins. A more recent application is that of so-called pentosanases. These enzymes are active towards the arabinoxylans in the flour. By limited degradation of the water-insoluble pentosans the swelling properties of these flour components can be regulated, so that delayed swelling, resulting in toughening of bread can be controlled (Sproessler, 1977).

6. Biomass Liquefaction and Saccharification

Large quantities of plant cell-wall material become available as by-products in the beet-sugar and potato-starch industries. For instance, in The

Netherlands 4.7×10^5 tons (dry matter) of beet pulp and 0.6×10^5 tons (dry matter) of potato fiber are produced annually. Most of these products are used as cattle feed. They are potentially rich sources of fermentable sugars. The fermentation industry can also be seen as a possible outlet for agricultural surplusses, such as whole sugar beet, sugar cane, potatoes, maize and wheat. These solid primary products must then be liquefied, and their polysaccharides converted into simple sugars. This process has been the subject of study in our laboratory (Beldman et al., 1984). A large spectrum of polysaccharide-degrading enzymes is needed to achieve a quasi-complete liquefaction and saccharification. For that purpose we used a technical cellulase preparation from *Trichoderma viride* in combination with a pectinase/hemicellulase from *Aspergillus niger*. Hydrolysis limits of well over 90% of the polysaccharides in sugar-beet pulp and potato fiber could be reached. Important savings on enzymes could be realized in a packed-column reactor connected to a hollow-fiber ultrafiltration module (Fig. 6).

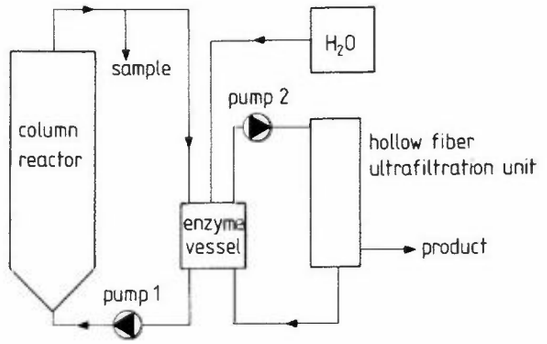

Figure 6. Saccharification of beet pulp in a packed column, connected to an ultrafiltration module (Beldman et al., 1984).

7. Application of Pectate Lyase

One group of enzymes which has not been mentioned in relation to any application is the group of bacterial pectate lyases, but has been very well studied in relation to bacterial plant diseases. Because of their low activity at pH values commonly encountered in the enzymatic processes described above (pH 3 to 5) they are not very suitable. However, some bacteria such as *Bacillus subtilis, Erwinia carotovora* and *Pseudomonas fluorescens* excrete

large quantities of these enzymes, and some potential uses should be mentioned.

In 1981 a patent was taken out by Bock et al. (1981), describing the treatment of pulses with pectate lyase from *E. carotovora* as an alternative to the hydrothermal treatment applied to obtain dried instant, so-called Tempo-products. Indeed the macerating properties of pectate lyases are very pronounced and these enzymes may eventually be introduced in other processes, such as the production of potato puree and powder.

A long-standing utilization of bacterial pectic enzymes is in anaerobic retting carried out in concrete tanks, and there the enzymes are produced *in situ* by *Clostridium felsineum*. A variation to this practice is the recently described biochemical pulping process to liberate paper fibers from woody plants, such as mulberry shoots (Kobayashi and Matsuo, 1984). *E. carotovora* was selected as producing the most effective retting enzymes.

8. Outlook

There are legal limitations to the application of microbial enzymes in food technology. Only enzymes derived from microorganisms involved in the fermentation of traditionally known foods are accepted. It has been established that these organisms are not pathogenic or toxic to humans and they have so-called GRAS status (generally recognized as safe). This is the reason why not more of the plant-pathogenic microorganisms are used for enzyme production. This barrier will soon be overcome thanks to the possibility of transmitting genes into organisms with GRAS status. It is anticipated that the first microbially produced calf rennin preparations will appear on the market within the next few years.

Another promising development is the so-called protein engineering. Through small changes in a gene coding for a particular enzyme, slightly modified enzymes are obtained which may have improved properties from the point of view of application. Finally, the advent of cheap and effective affinity-based methods for enzyme purification will enable the selective isolation of particular enzymes and cut down enzyme isolation costs. These developments are a guarantee for a steady growth of enzyme applications, also in the field of plant cell walls.

REFERENCES

Aspinall, G.O. 1980. Chemistry of cell wall polysaccharides. In: *The biochemistry of Plants, a Comprehensive Treatise.* Vol. 3. J. Preiss, ed. Academic Press, New York, pp. 473–500.

Baron, A. and Drilleau, J.-F. 1982. Utilisation de la pectinestérase dans les industries cidricoles. In: *Utilisation des Enzymes en Technologie Alimentaire.* P. Dupuy, ed. Lavoisier, Paris, pp. 471–476.

Beldman, G., Rombouts, F.M., Voragen, A.G.J., and Pilnik, W. 1984. Application of cellulase and pectinase from fungal origin for the liquefaction and saccharification of biomass. *Enzyme Microb. Technol.* **6**, 503–507.

Bock, W., Krause, M., Friedrich, E., and Henniger, H. 1981. Verfahren zur enzymatischen Vorbehandlung von Hülsenfrüchten und stückigem Trockengemüse. GDR Patent (11) 158610.

Dubourdieu, D., Villetaz, J.C., Desplanques, C., and Ribereau-Gayon, P. 1981. Dégradation enzymatique du glucane de *Botrytis cinerea.* Application à l'amélioration de la clarification des vins issus de raisins pourris. *Connaissance Vigne Vin* **15**, 161–177.

Ducroo, P. and Delecourt, R. 1971. Hydrolyse enzymatique des beta-glucanes de l'orge. *Bios* **10(10)**, 1–7.

Grampp, E. 1969. Die Verwendung von Enzymen in der Lebensmittelindustrie. *Dtsche Lebensm. Rundschau* **65**, 343–347.

Grampp, E. 1980. Methods for making flocculation-resistant citrus juices. United States Patent 4211799.

Kobayashi, Y. and Matsuo, R. 1984. Rapid retting of Kôzo (paper mulberry) bast with *Erwinia carotovora. Agric. Biol. Chem.* **48**, 1333–1335.

Krop, J.J.P. and Pilnik, W. 1974. Cloud-loss studies in citrus juices: cloud stabilization by a yeast polygalacturonase. *Lebensm.-Wiss. Technol.* **7**, 121–124.

McNeil, M., Darvill, A.G., Fry, S.C., and Albersheim, P. 1984. Structure and function of the primary cell walls of plants. *Ann. Rev. Biochem.* **53**, 625–663.

Pilnik, W., Voragen, A.G.J., and de Vos, L. 1975. Enzymatische Verflüssigung von Obst und Gemüse. *Flüssiges Obst* **42**, 448–451.

Rombouts, F.M. and Pilnik, W. 1978. Enzymes in fruit and vegetable juice technology. *Process Biochem.* **13(8)**, 9–13.

Rombouts, F.M. and Pilnik, W. 1980. Pectic enzymes. In: *Economic Microbiology. Vol. 5, Microbial Enzymes and Bioconversions.* A.H. Rose, ed. Academic Press, London, pp. 227–282.

Salomonsson, A.-C. 1985. Studies on carbohydrates and phenolic acids in grains of normal and high-lysine barley genotypes. Doctoral Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Siliha, H.A.I. 1985. Studies on cloud stability of apricot nectar. Doctoral Thesis, Agricultural University, Wageningen, The Netherlands.

Sproessler, B. 1977. Enzymatische Behandlung von starken Weizenmehlen und hochamylogrammigen Roggenmehlen. *Mühle und Mischfuttertechnik* **114**, 235–236.

Voragen, A.G.J., Geerst, F., and Pilnik, W. 1982. Hemicellulases in enzymic fruit processing. In: *Utilisation des Enzymes en Technologie Alimentaire.* Lavoisier, Paris, pp. 497–502.