The Megazyme story

How a world class test and reagent technology specialist, Megazyme, emerged from a garage in Australia . . .

By Barry V. McCleary, Megazyme International Ireland Limited

The Biocon Days:
In 1983-84, in response to direct requests from barley breeders in NSW, together with Malcolm Glennie-Holmes, I researched and developed a procedure for the measurement of β-glucan in barley grain (mixed-linkage β-glucan).

The method (Figure 1) worked well and was subjected to interlaboratory evaluation within Australia. On successful evaluation, the Cereal Chemistry Division of the Royal Australian Chemical Institute accepted the method. Since the procedure required the use of pure enzymes for it to be generally adopted, it was obvious that the reagents needed to be commercially available. Since the NSW Department of Agriculture had no interested in patenting the procedure, the method was published.

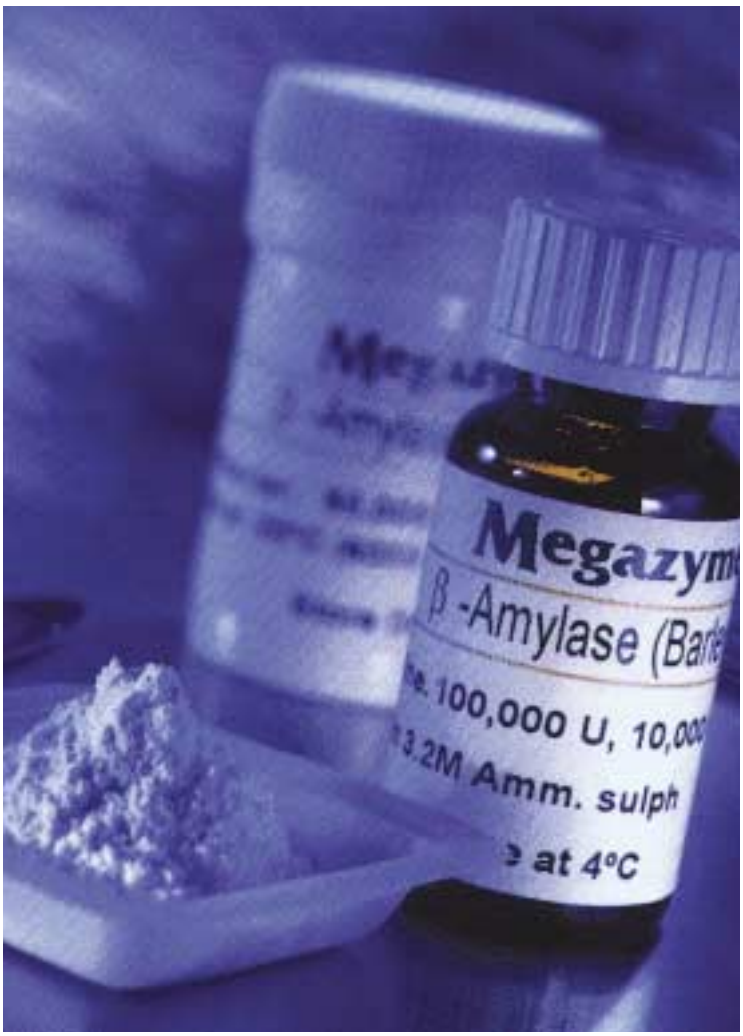
Commercialised
Subsequently, the method was commercialised by Biocon Australia. I worked with Colin Dowzer, Director of Biocon Australia, to ensure that the commercialisation proceeded smoothly. I was delighted with Colin's enthusiasm, and out of this project developed a great business relationship and a life-long friendship.

Later in 1984, I met Les Auchincloss, the owner of Biocon, and was invited to visit Biocon Biochemicals, Cork. This I did in July 1985, spending 4 weeks there. The place was electric, and Les, as always, was dynamic. I revisited Biocon Biochemicals the following year, and stayed for "a year or so". I arrived as a visiting scientist, and was soon promoted to the position of Research Director.

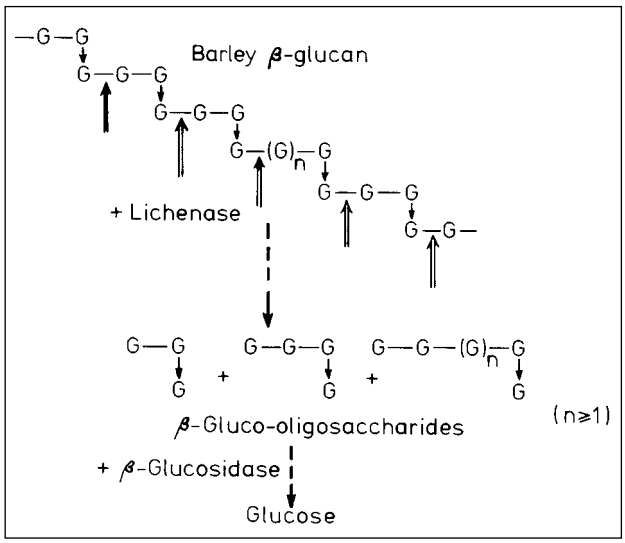
This gave me an opportunity to directly converse with the brilliant team of managers and directors that Les had assembled – Declan McFadden, Frank Ollington, Roland Cocker, Ann Francis, Mary O'Callaghan and Richie Piggott.

At Biocon, it was possible to be involved in every element of the business; from product development and production, through QC, marketing, sales, technical support and customer feedback. One could see the whole picture.

When customers had problems with products, there would be immediate feedback, though not always polite. But, the exciting aspect of this was that the customers were helping us to clearly define their problem and thus help define



The product range is divided into Diagnostic Kits, Reagents, Carbohydrase Test Tablets, Soluble and Insoluble Chromogenic Substrates, Enzymes, Polysaccharides and Oligosaccharides. Each of the products developed and supplied was produced, either in response to a specific request from a customer, or as a consequence of the company's perception of what a particular industry may need.



BELOW: Figure 1. The original method for measurement of β-glucan in barley grain (mixed-linkage β-glucan).

ABOVE LEFT: May 2000. Dr McCleary (right) with Les Auchincloss, the owner of Biocon. He was invited to visit Biocon Biochemicals, Cork in July 1985, eventually becoming Research Director for the company.

ABOVE RIGHT: Angela Kennedy and An Taoiseach (Prime Minister) Bertie Ahern, at the conference Dietary Fibre 2000, Dublin.

the target. I really appreciated this aspect of the job. In trying to apply biotechnology to solving food-related problems, the biggest single challenge was in trying to define the "real problems".

After 18 months at Biocon Biochemicals, my attitude to science and technology had been changed forever. My days with the Department of Agriculture were numbered.

Megazyme Australia

In April 1989, I resigned from my position as Principal Research Scientist within the Department of Agriculture to take up my new position as Technical Director, and co-owner of Megazyme Australia Pty. Ltd., located in two garages off the side of our home. In hindsight, this was a fairly brave move. Half of my professional colleagues thought that I was "mad" and the others suggested that "Microzyme" might be a more fitting name for the company.

Angela conceived the company name, and, as always, she projects forward. Her attitude is, that the name does not reflect where we are, or where we have come from, but rather where we aspire to be.

Garages

The garages at 6, Altona Place, North Rocks, NSW, were the best equipped garages in the street. In fact, they were the best equipped garages in Australia. The first garage was fully equipped as a lab, with benches, fume hood, gas liquid chromatograph and most of the usual small equipment found in a lab.

The second garage housed a high-speed centrifuge and large cold cabinets which housed fraction collectors and chromatographic columns – and was also used as the packaging area.

In establishing Megazyme, our aim was to develop a range of products for sale. However, we realised that in the first few years of business, we would need to generate cash to buy equipment and pay the bills. Thus, initially, time was divided evenly between consultancy and product development. I took up consultancy agreements with several companies in the areas of methods development for enzyme and carbohydrate measurement.

We also offered an analytical service for measurement of specific components such as β-glucan, starch and a range of enzymes. I also initiated an interesting project with British Sugar plc. in the area of enzymic modification of sugar beet pulp constituents, particularly arabinan. Julian Cooper and Ted Williams were great colleagues and became great friends. The project developed well and products were patented.

By the end of our first year of business, Angela gave up her career in real estate and assumed a full time sales and marketing role within Megazyme.

Since Biocon Australia marketed the β-Glucan kit that I had developed some years earlier, we decided to extend this arrangement to include all of the kits developed by Megazyme. Megazyme would then just market and directly sell the other products; namely enzymes, polysaccharides, oligosaccharides and chromogenic substrates.

This arrangement changed in 1990 when Quest International purchased Biocon Biochemicals. After many discussions on marketing and marketing arrangements, we decided that if we wished to maintain the separate identity of Megazyme, we would need to make a break.

Direct marketing

From that time on, Megazyme directly marketed and supplied its full range of products including the kits directly to the customer, or through agents; but always in the Megazyme name. Although there were a few ripples at the time of the separation, all of the old associations and friendships were soon reformed. Since the relocation of Megazyme to Ireland, Deltagen Australia, formerly Biocon Australia, handles all Megazyme business within Australia.

In 1990, I attended my first annual meeting of the American Association of Cereal Chemists (AACC) and quickly saw the value of this Association to myself professionally, and also to Megazyme – from both business and marketing aspects.

I soon became involved in Technical Committee meetings and realised the value of having Megazyme methods subjected to interlaboratory evaluation through AACC. Over the following years I also became involved with ICC (International Association for Cereal Science and Technology), AOAC International, the Institute of Brewing and the European Brewing Convention.

Most years we held a trade display at the annual AACC and ICC meetings and because of the distance from Australia, Angela has tended to deal more with ICC, and myself with AACC. At present I am the International Director of AACC.

In 1991, Quest International decided that they would no longer manufacture β-glucan, as it was fringe to their product range. Megazyme was invited to pick up this product, which we were delighted to do, particularly since I had worked on this process during my time with Biocon. We purchased the required equipment and set up pilot production facilities in the backyard of our home. Fortunately, we had very understanding neighbours.

Megazyme Australia, Pty. Ltd, (1989-1992), 6 Altona Place, North Rocks, Sydney. Probably the best equipped garages in Australia...

Leaving the garage

However, it immediately became obvious that we had outgrown our garage facilities. In the years 1989-1992, the company had spread like a fungus through our home. One room became an office, bags of celite accumulated under the house, and data booklets filled the linen cupboards. It was time to move. By the end of 1992 we had purchased a factory unit in Warriewood, and following internal modifications, we relocated in July 1993.

In mid-1992, we decided to nominate our company for the "NSW Small Business of the Year" awards. We were notified that we were one of the finalists, and since one of the categories was for "Business with up to 5 Employees", we felt that we had a chance. On the night of the awards, Megazyme was named as winner of the category "Manufacturer of the Year with up to 50 Employees", and then went on to win the overall award of "NSW Small Business of the Year". We still remain amazed by this achievement and can only conclude that anything is possible if one is truly committed to their dreams. As winner of the "NSW Small Business of the Year" award, Megazyme was automatically a finalist in the "Australian Small Business of the Year" awards. Later that year, the company went on to win the award of "Australian Small Business of the Year – Manufacturer with up to 100 Employees". By that stage our staff numbers had doubled to four.

To coin a phrase, Megazyme was "born global". From the outset we knew that most of our business would come from Europe and America, so we had to develop an international, as well as a local, marketing strategy.

In 1992, we placed our first advertisement in *Cereal Foods World*. Working with a commercial advertising company, Angela had a lot of work to do to upgrade the company image and to produce a top quality advertisement. I went pale at the costs involved, but that one advertisement contributed very significantly to the 300% increase in Megazyme sales in the following year.

Needless to say, Angela did convince me that "it pays to advertise."

A lot happened in 1993-94. First of all, Angela took it on herself to computerise all of our accounts. To our great good fortune, the consultant who helped Angela with the accountancy package (MYOB; Mind Your Own Business) had experience with the internet. After a few short discussions, we realised that what Megazyme really needed was a website. Within months we were on-line. The site at that stage was used solely for marketing purposes.

In 1997 we introduced purchasing on-line, and in 1998 the "Product Data Booklets" were included on the website. In 1999, a Federal Express tracking system (for shipped packages) was included, and the most recent updates include Credit Card

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The founders

The co-directors and co-owners of Megazyme International Ireland Limited are Dr. Barry McCleary and Angela Kennedy. Dr McCleary had a traditional education, beginning in a small country town, Harden in inland NSW, Australia, and completing at Marist Brothers High School, Parramatta. He completed an undergraduate degree and PhD in Agricultural Chemistry at the University of Sydney and took up employment with the NSW Department of Agriculture in 1975. He remained in their employment until 1988, until he resigned to found Megazyme. While with the Department of Agriculture he was fortunate in having the opportunity to study at the University of Miami (Bill Whelan's group: on starch hydrolysing enzymes), Swiss Federal Institute of Technology (Hans Neukom's group: galactomannans, organic synthesis), Unilever Research, Bedford, U.K. (Dai Rees' group: gelling interaction of polysaccharides) and Biocon Biochemicals, Cork, Ireland (industrial application of enzymes). In the NSW Department of Agriculture, he researched numerous areas including enzymic modification and measurement of polysaccharides, thiamine deficiency in sheep and rubber production by guayule. In 1994 he was honoured by being awarded the F B Guthrie Medal by the Cereal Chemistry Division of the Royal Australian Chemical Institute for "meritorious service to cereal chemistry in Australia, in the broadest sense".

ANGELA KENNEDY started her career as Science Technologist, working in Experimental Medicine, Pathology and Anatomy Departments at University College, Galway. She then progressed into the marketing of scientific, medical and diagnostic reagents. In 1985, she was appointed to the position of Marketing Manager Diagnostics for Biocon Biochemicals, Cork, Ireland. In 1987, she relocated to Australia with her sister Maeve. In Sydney, she decided to look at a new area of marketing, and became a licensed Real Estate Agent, after completing courses at the University of Western Sydney. In 1999 Angela was elected as ICC National Delegate for Ireland. She is currently completing an MBA Degree at the Michael Smurfit Graduate School of Business, University College Dublin.

RIGHT: Megazyme International Ireland Ltd. (1997 – to present).

INSET: Megazyme International Ireland Ltd. (August 1996 – May 1997).

payment on-line, and inclusion of "Material Safety Data Sheets" (MSDS) and sections on "Frequently Asked Questions" (FAQ's) and "Publications".

Megazyme International Ireland Limited

In 1994, Megazyme won the DHL sponsored, NSW Small Business Exporter of the Year" award, and in 1996, Megazyme exported itself to Ireland. This move was made for several reasons, some personal, some business.

Running a small international company from Australia was difficult, and the Irish Government had created a very positive business environment with many incentives and tax breaks.

The relocation started in April 1996. We visited Ireland and discussed opportunities in Ireland with Dan Flinter of Forbairt. Our minds were made up, and over a period of two weeks we located the land for the factory site in Bray, and met with architects to outline the first basic draft of the building. Within weeks, tenders had gone out and a builder had been selected. Foundations were laid in July.

"After six months in Ireland, friends asked Angela how I had settling in. Her reply was "fairly well, he now threatens to leave just twice a day".

Angela relocated to Ireland in August and organised two portacabins on the land beside the factory site; fully equipped with phone, fax and e-mail facilities. I remained in Australia to dismantle the factory there which took three months. Orders were filled from Warriewood until Friday, 30th August, when this function was switched totally to the Bray facility over a single weekend. All phone, fax and e-mail lines were re-directed and it took several months before some customers realised that Megazyme had relocated.

The transfer was seamless, with not even the loss of one day of business. To ensure uninterrupted supply of products, a good stock of packaged goods were airfreighted to Ireland. The rest of the goods and equipment were shipped by sea, a total of three forty-foot containers.

The Megazyme factory in Bray Business Park was ready for occupancy in May 1997. Not before time! For me, the winter of 1996 was long and cold. Portacabins are not great in retaining heat. We had icicles inside the windows, and it wasn't until midday that my hands had thawed adequately for me to write. After six months in Ireland, friends asked Angela how I had settling in. Her reply was "fairly well, he now threatens to leave just twice a day". I am now used to the weather in Ireland; which means that I complain about it no more than anyone else.

The factory in Bray, being purpose built, really serves us well. Megazyme is flourishing and we are finally completing some of our ongoing research projects and are now ready to release a range of new products. The internet is an integral part of our business and contributed significantly to the company recently winning the Eircom/Irish Independent "Irish E-Business of the Year" award.

Megazyme has won a lot of awards and has made a lot of relocations, but you may well ask, "what exactly do they do". Basically, we are a specialised, world-class manufacturer of high quality and innovative test technology and reagents for the cereals, food, feed and fermentation industries.

Our product range is divided into Diagnostic Kits, Reagents, Carbohydrase Test Tablets, Soluble and Insoluble Chromogenic Substrates, Enzymes, Polysaccharides and Oligosaccharides. Each of the products developed and supplied was produced, either in response to a specific request from a customer, or as a consequence of our perception of what a particular industry may need.

In trying to define exactly what type of products Megazyme would make and supply, we set as our target "the development of methods/kits/substrates for the measurement of ***all*** enzymes, polysaccharides and oligosaccharides that are of industrial significance in the utilisation and processing of plant products."

Thus, for starch, for example, the aim was to develop methods for the measurement of total starch, starch damage, resistant starch, amylose/amylopectin ratios, α-amylase, β-amylase, limit dextrinase and amyloglucosidase. For β-glucan, the aim was to develop good methods for β-glucan and β-glucanase; for arabinoxylan – methods for arabinoxylan and *endo*-β-xylanase; for pectin – methods for pectin/pectinase, polygalacturonanase, the lyases, *endo*-arabinanase, *endo*-galactanase and rhamnogalacturonanase – and so on for other polysaccharides.

To date, we have realised our goals for starch, with a kit for Resistant Starch (the last of the target methods) currently being released. The goals for β-glucan have been achieved, but those for arabinoxylan have been only partly realised; we are still struggling with the development of a good method for arabinoxylan. For pectin, we have had some success, but some areas remain to be resolved.

Marketing Megazyme and its products

As a scientist, with a traditional scientific background, I had a lot to learn about marketing of products. I certainly realised that this exercise was necessary if you wished to sell products and stay in business, but I had no real idea of how to go about it, or what it would cost.

Fortunately, Angela's background was in sales and marketing, so she completely assumed this role within the company. It has been her job to decide on and organise journal advertisements and trade displays. She also has assumed the dominant role in the development of our web site; however, the scientific input into this was, of course, my responsibility.

With technical products, as with all products, it pays to advertise. However, with technical products it is even more important to convince the customer that the product actually does work, and that it will give results that can be related to data from a method currently in use. Even more importantly, if the method is to be used routinely in analytical laboratories, it is essential that the method be validated. The best avenue open to scientists is to organise an interlaboratory evaluation of the procedure through AOAC International, AACC, ICC, EBC or IOB.

If there is a burning need for a particular method, this is usually easy to organise as it is relatively easy to find collaborators who wish to be involved. If however, you are offering a method which you perceive to be an advance on currently available methods, finding collaborators, or even getting approval to perform the study under the auspices of a particular scientific or industry association may be more difficult.

For an interlaboratory study to succeed, it is essential to have the participation of collaborators who really want the study to succeed. This usually happens if they have a vested interest in the success of the method, i.e. they need a validated method for in-house use, or they need a more reliable and robust method to replace a tedious method, which they are currently obliged to use. Fortunately, through AACC/AOAC International we have found no problems in getting the participation of many interested scientists.

Planning and co-ordinating an interlaboratory study is a considerable undertaking, but if planned carefully, it can be quite rewarding in terms of both a successful study, and from a company point-of-view, the

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acceptance of one of its methods. In the period 1991-2001 we have co-ordinated AACC interlaboratory evaluations of a number of Megazyme methods, namely those for Beta-Glucan, Total Starch, Starch Damage, Fructan, Amylazyme (α-amylase) and Ceralpha: α-amylase (currently underway). To date, all of the evaluations have been successful. In many cases, these interlaboratory studies were joint AACC/AOAC International studies, so several of the methods also have AOAC International validation (Table 1).

Later this year, we plan to organise an interlaboratory evaluation of a modified procedure that we have developed and evaluated, for the measurement of Resistant Starch.

In the mid-1990's, interest in dietary fibre and new dietary fibre ingredients was booming. At Megazyme we realised that there was a need for an International Conference on this topic. Angela, as Irish National Delegate for ICC, was invited to host the ICC Annual meeting in Ireland, so we realised that this was an ideal opportunity to organise a dietary fibre meeting in Dublin.

We invited Dr. Leon Prosky, a world expert in this area, to co-chair the technical aspects of the conference, and AOAC International to co-sponsor it. Both accepted. The result was a resounding success thanks to the untiring efforts of Angela, Shirley Delaney and Una MacLeod.

Dietary Fibre 2000

At the conference "Dietary Fibre 2000", 42 world authorities presented the state-of-the-art knowledge on the topic, and this was subsequently published by Blackwell Science as "Advanced Dietary Fibre Technology." The conference was opened by the Taoiseach, Mr. Bertie Ahern.

In world terms, Megazyme is still a small company, but we have not lost sight of why we formed the company. Our mission statement was, and still is, "Setting New Standards in Test Technology for the cereals, food, feed, fermentation and allied industries."

We feel that we have made a contribution, but there is still a lot more to be done. Megazyme is essentially, completely funded by product sales. Our success is largely due to the support and loyalty of you, our customers. We are always looking for new problems to solve and new products to make.

To improve our service to you, our customers, we have recently updated our website (www.megazyme.com) to include answers to frequently asked questions, MSDS, abstracts of relevant publications, and very importantly, the facility for you to pay on-line by credit card.

TABLE 1. ACCEPTANCE OF MEGAZYME METHODS

Mixed-linkage β-glucan	AACC Method 32-23 EBC Methods 3.11.1, 4.16.1 and 8.11.1 AOAC Method 995.16 ICC Standard No. 166 RACI Standard Method
Total starch	AOAC Method 996.11 AACC Method 76-13 ICC Standard Method no. 168 RACI Standard Method
Starch Damage	AACC Method 76-31 ICC Standard No. 164 RACI Standard Method
Fructan	AOAC Method 999.03 AACC Method 32-32
Amylazyme (α-amylase)	AACC Method 22.05 RACI Standard Method
Ceralpha (α-amylase)	ICC Standard No. 303 RACI Standard Method CCFRA Flour Testing Working Group 0018 AOAC and AACC – under evaluation
Method	
Total Dietary Fibre	Megazyme is a recognised supplier of enzymes of the required purity.
ASBC/International method for α-amylase	Megazyme is a recognised supplier of β- amylase of the required purity.
Betamyl (β-amylase)	RACI Standard Method
Limit Dextrizyme	RACI Standard Method
Beta-Glucanase (Azo-Barley Glucan)	RACI Standard Method
Xylazyme AX (xylanase)	Xylazyme AX Tablets have been widely adopted in the fermentation and feeds industries for the measurement of xylanase.

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Megazyme: development and applications

TO give a more in-depth view of the development and application of some of our products and methods, I will now discuss some of these in more detail.

1. Barley β-Glucan

The reasons for developing this method were described earlier in this article. In the early to mid-1980s, several methods were developed for the measurement of β-glucan. In most cases, impure cellulase was used, which resulted in overestimation of the β-glucan content (Martin & Bamforth, 1981[7]), through hydrolysis of both starch and amorphous cellulose.

We (McCleary and Glennie-Holmes, 1985[8]), and another group (Henry, 1984[4]), decided to use highly purified lichenase (an enzyme specific for 1,3;1,4-β-D-glucan) to depolymerise the β-glucan. The difference with our format was that the β-gluco-oligosaccharides were specifically and completely hydrolysed to glucose using a highly purified β-glucosidase, and the released glucose was measured with high-purity glucose oxidase/peroxidase reagent. The method is specific, quantitative and reliable and has been successfully evaluated by EBC (Methods 3.11.1, 4.16.1 and 8.11.1 for barley, malt, wort and beer), AACC (Method 32-23), AOAC International (Method 995.16) and ICC (Method No. 166).

The principle of the assay is shown in Figure 1. This method has stood the test of time and after 16 years is still the method of choice for quantitative measurement of β-glucan in cereal grains and food products.

2. Total Starch

In 1993/94 we decided to develop a method for the measurement of the total starch content of cereal grains, plant products and "pure" starch samples. Initially, we simply intended to copy the then "AOAC International" standard method (AOAC Method 979.10), and simply supply it in kit form.

However, after evaluating this method on a range of starch samples, we noticed that for many starches, particularly high amylose starches, the starch content was seriously underestimated. We then evaluated all of the literature methods for starch measurement, and eventually combined the best elements from several methods.

The initial method we offered was based on the use of four enzymes, thermostable α-amylase, β-amylase, pullulanase and amyloglucosidase and was successfully evaluated in an AACC interlaboratory study.

It was subsequently accepted as AACC Method 76-12. However, we noticed that the method was not routinely used in research and analytical laboratories. We decided to survey customers for their comments. We focused particularly on customers who purchased once, but not again. The general consensus was that the method did work, but was too tedious. Immediately, we re-researched the method and developed a new format based on the use of thermostable a-amylase and amyloglucosidase, with DMSO pre-treatment of samples that contained high-amylose starches or resistant starches.

This method also, was successfully evaluated in a joint AACC/AOAC International study, and was subsequently adopted (AACC Method 76-13; AOAC Method 996.11) (McCleary et al., 1997[11]).

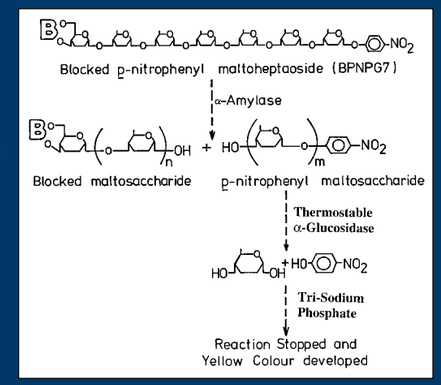


Figure 2

3. Malt β-Glucanase

The traditional method for the measurement of β-glucanase in malt is a viscometric procedure employing pure barley β-glucan of defined viscosity (as supplied by Megazyme since 1991). An alternative procedure, developed by Megazyme, employs a soluble dye-labelled β-glucan (Azo-Barley Glucan)(McCleary & Shameer, 1987[9]). Malt extract is incubated with the substrate and after a defined time, the reaction is terminated and high molecular weight (MW) dyed β-glucan is precipitated by addition of a solvent.

Low MW, dyed material remains in solution, and the colour of the solution, following centrifugation, is a direct measure of β-glucanase activity. There is a linear correlation between IRV Units (viscometric assay) and International Units of activity obtained using the Azo-Barley Glucan method. The major advantage of the Azo-Barley Glucan method is ease of use and rapidity (Buch, 1986[1]; Gill & Haselmore, 1987[3]). Accuracy and reliability are similar to that of the viscometric method in the hands of a good analyst. (Buckee & Baker, 1988[2]).

Similar soluble dyed substrates have been made for a range of other enzymes. The Azo-CM-Cellulose substrate for cellulase and the Azo-Wheat Arabinoxylan substrate for endo-xylanase have been adopted by the UK silage industry for the standardisation of enzyme preparations used in silage additive mixtures.

4. Pure Polysaccharides

Megazyme is the sole world manufacturer of a range of highly purified polysaccharides used as analytical reagents. These polysaccharides include barley and oat β-glucans, wheat arabinoxylan, potato galactan and linear arabinan from sugar beet.

More recently, the company was approached by representatives of the UK malting industry, with a request to produce and supply β-limit dextrin for use in the Scalar Flow Injection analysis systems for measurement of α-amylase in malt. Up to 1998, a β-limit dextrin product was produced and supplied by Rank Hovis for the U.K. flour millers and bakers (for use in the Farrand β-limit dextrin/iodine procedure for measurement of α-amylase).

In 1998/99 the flour millers and bakers decided to change from the Farrand α-amylase method to the Megazyme Ceralpha method for α-amylase (Figure 2). Since Rank Hovis had ceased manufacture of β-limit dextrin, we were pleased to take up the challenge to produce a similar material.

Using a pure β-amylase available in-house, we produced β-limit dextrin from a range of starches, and eventually identified a starch that gave a β-limit dextrin with the desired properties. The process for the production of β-limit dextrin was scaled-up, and the material we supply appears to satisfy the needs of the maltsters.

β-Limit dextrin (as suggested by the name) is the resistant dextrin produced on extensive hydrolysis (i.e. to the limit) by pure β-amylase. The final reaction mixture contains approximately 50% β-limit dextrin and 50% maltose.

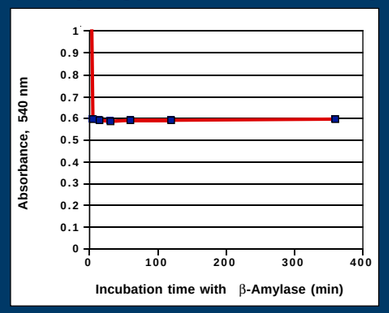
The β-limit dextrin supplied by Rank Hovis still contained the maltose, and thus was extremely hydroscopic. If not properly stored in a dessicator, this material rapidly absorbed moisture from the atmosphere, eventually forming a "toffee-like" material. We realised that this type of product would cause Megazyme no end of problems and technical queries, so we decided to remove the maltose. The product supplied by Megazyme contains less than 1% of maltose and is not

In depth view *(continued from previous page)*

hydroscopic. As such, it is simple to handle and to use.

5. Pure Enzymes

Megazyme produces a range of highly purified enzymes, and many of its analytical procedures are based on the use of these enzymes. In 1998/99 we were notified by the American Society for Brewing Chemists that they were evaluating β-amylase enzymes produced and supplied by several companies in an attempt to find one of suitable purity to replace the enzyme previously supplied by Boehringer Ingleheim, but which was no longer available.



	1 Time	2 Iodine colour
1	0	1.48
2	5	0.598
3	15	0.591
4	30	0.589
5	60	0.593
6	120	0.59
7	360	0.597
8	1200	0.57

Figure 3. Change in iodine colour on incubation of ASBC special starch with Megazyme β-amylase at a β-amylase/starch ratio of 30-times that used in the standard procedure for the preparation of β-limit dextrin.

In 2000 we were notified that of the preparations evaluated, only the Megazyme β-amylase was of suitable purity, and thus was being adopted and recommended by ASBC.

The Megazyme β-amylase is of barley origin and is purified by a combination of chromatographic procedures and crystallisation. The degree of contamination by α-amylase is extremely low, less than one part (by activity) per 2 million parts of β-amylase. α-Amylase, if present, results in the production of an unsuitable β-limit dextrin substrate, or a substrate which is unstable (i.e. shows a diminishing starch/iodine colour over the expected period of use).

To critically evaluate the purity of the β-amylase that we produce, we perform incubations of starch with β-amylase using a β-amylase/starch ratio 30-times higher than that used in the standard ASBC method. We then monitor the reaction by the traditional starch/iodine procedure, and by the measurement of released maltose (measured using a maltase/glucose oxidase-peroxidase assay procedure).

The results of one such experiment are shown in Figure 3. The iodine colour reaction stabilises after 20 min and then remains the same for the next 16 hr (1,000 min).

This shows that the β-amylolysis reaction is complete within the 20 min and that α-amylase levels are so low that they have no effect on the substrate, even after 16 hours of incubation of the starch with 30-times the recommended concentration of enzyme (Figure 3).

This result is supported by the hydrolysis values (not shown), which reach an end point after 20 min, and remain the same for the next 20 hours.

6. Oligosaccharides

With pure enzymes and pure polysaccharides available in-house, it seemed a natural extension that Megazyme should produce a range of pure oligosaccharides. Megazyme is the sole world manufacturer of most of the oligosaccharides in its product range. These find widespread use in research projects as reference compounds.

7. Test Tablets for the Measurement of Carbohydrase Activities.

a. Amylazyme.

In 1989, I attended a Technical Committee meeting of the American Association of Cereal Chemists in Dallas, Texas. Dr. Paul Mathewson stated that there was a real need for a product to replace Amylochrome tablets for the measurement of α-amylase, which were no longer produced by Roche Chemicals. These tablets were required in one of the AACC standard methods for α-amylase.

I felt that Megazyme could, with a bit of research input, fill this need. After just a few months of research we had resolved the chemical problems, and had made the material AZCL-Amylose (azurine, crosslinked amylose). Then, we entered into the foreign world of "tabletting technology". Initially it was hard to find a company that would make trial batches of tablets for us, and certainly, nobody would let me near their tabletting machines. A whole new technology (for me) was involved, with discussions on excipients, flow agents, swelling agents and tablet disintegrating agents. Finally, I met an owner of a small production company in the north of Sydney, who would not only make the trial batches of tablets, but would allow me into the factory area to learn the skills of the trade.

Finally we developed the optimal formulation. Sadly, about a year later, the owner of this company, Mr. Des Thompson (truly one of Nature's gentlemen) was shot to death in his factory during a break-in and robbery.

Having developed the chemistry for the tablets to measure α-amylase (Amylazyme Tablets), we went on to apply a similar chemistry to the development of tablets for the measurement of a range of other enzymes, including xylanase, cellulase, endo-arabinanase, limit-dextrinase and many others. The Amylazyme tablets were subjected to interlaboratory evaluation (by AACC) and adopted as AACC Method 22-05.

b. Limit-Dextrazyme

Limit-Dextrazyme tablets were relatively simple to make (McCleary, 1992[10]). The required polysaccharide, pullulan, was commercially available, and the chemistry used to dye and crosslink this turned out to be very similar to that used in preparing AZCL-Amylose.

Although this product has not been subjected to interlaboratory evaluation, it gained instant acceptance by the major groups researching limit-dextrinase in Canada (Macri, et al, 1993[6]), Finland (Stenholm & Home, 1999[13]), Scotland (Longstaff & Bryce, 1993[5]) and Australia (Sissons, 1996[12]). For progress in research on the properties and industrial significance of limit-dextrinase, there was an absolute need for a simple assay procedure, this product filled this need.

Following these research applications, this product has found more widespread application in monitoring production processes Walker, et al.[14]).

c. Beta-Glucazyme, Beta-Mannazyme, Cellazyme C and Cellazyme T

The preparation of tablets for the measurement of β-glucanase, β-mannanase and cellulase was also relatively straightforward. We had already developed procedures for the purification of barley β-glucan and carob galactomannan and soon identified the correct cellulose derivative to use. All these tablets find industrial application, but the one most widely used is Cellazyme C.

It is used to measure cellulase in fermentation broths and various agricultural applications, but the major use is in standardising and monitoring cellulases used in the "stone washing" of jeans. Cellulase treatment is used to produce the "well-worn" look of denim jeans and jackets, and it replaces the traditional washing of the fabrics with volcanic pumice stone to create the same effect.

A small amount of fabric modification is the target; too much and the jeans fall to bits. The Cellazyme C tablets form the basis of a very simple test, which can be used on the factory floor.

d. Xylazyme and Xylazyme AX

The preparation of the active component for these tablets proved to be a real challenge. Initially, we simply used xylan from birchwood, which was commercially available.

However, when we got to the stage of scaling-up we found that the quality of the commercial birchwood xylan had declined, and that the material did not yield a stable dyed, crosslinked product. Basically, to produce stable AZCL-polysaccharides with the desired hydration and swelling properties, the polysaccharide has to have a relatively high molecular weight so that there will be sufficient crosslinking to give insolubility and stability.

The available birchwood xylan no longer had the desired molecular weight. Consequently, there was an immediate need to locate an alternative xylan-type polysaccharide, which had the desired molecular weight and would be readily hydrolysed by xylanase. In Australia, the only potential candidate was wheat flour arabinoxylan.

This had the desired properties, but published methods for its purification were tedious and yielded, at best, only gram quantities of product. Following research efforts over several months, we developed a process, which allowed production of kilogram quantities of this polysaccharide at an acceptable cost and in a realistic time frame.

In preparing dyed polysaccharide substrates, or dyed, crosslinked polysaccharide substrates (as are incorporated into the tablets) it is impossible to obtain the exact level of dyeing/crosslinking every time.

Thus, for each batch of tablets, the standard curve relating enzyme activity to release of dyed fragments will vary slightly (+/- 10-20%).

This is not acceptable to the customer. The only solution to the problem is to prepare large batches of dyed products. Typically, we produce product batches that we consider will satisfy the world demand for 5-10 years. This means that Megazyme must hold large stocks of products and thus must ensure that they are held under conditions in which they are stable.

To fill this requirement for Xylazyme and Xylazyme AX tablets, we needed to purify 30 kg of wheat arabinoxylan for the first large production run of AZCL-Arabinoxylan (wheat). This was a major task, and extended over several months. However, our customers receive a consistent product, and, we believe they are quite pleased with it.

e. Arabinazyme and Galactazyme Tablets

These tablets are used for the measurement of *endo*-arabinanase and *endo*-galactanase enzymes (enzymes important in the degradation of pectin fragments, i.e. of no relevance to brewing). I mention these simply to demonstrate how difficult it has been to produce some of the required polysaccharides to make the AZCL-substrates.

In the production of apple and pear juice, pectinase enzymes are usually added to destroy the gelatinous nature of the pulp (cell-wall structure) and give better juice yield. Traditionally, pectinase preparations have had adequate levels of arabinofuranosidase enzyme (an *exo*-acting enzyme) but were deficient in *endo*-arabinanase. Consequently, the pectinase enzyme complex would release highly branched arabinan from pectin, and this arabinan would be de-branched by arabinofuranosidase to produce a linear 1,5-α-L-arabinan.

This linear arabinan self-associated to produce a crystalline type material which formed an undesirable haze in the juice. Clearly, this problem could be "dissolved" by ensuring an adequate level of *endo*-arabinanase was present in the pectinase enzyme preparation. However, the main problem was that there was no readily available substrate for measurement of *endo*-arabinanase.

Mr. Heinz Meisenzahl of Novo Ferment, Basle, Switzerland, first introduced me to this problem in 1986. He stated that their only source of substrate was the arabinan haze material in the juice. This was recovered by micro-filtration of large volumes of hazy juice. I suggested the use of sugar-beet arabinan as an alternative, but they had already tried this material and found little enzyme activity on it.

Clearly, there was a need to produce and evaluate a de-branched arabinan (i.e. sugar-beet arabinan which has been treated with arabinofuranosidase to remove all of the 1,3-α-L-arabinosyl branches, to produce a de-branched, or liner 1,5-α-L-arabinan). Eventually, produced some of this and compared it to a sample of "haze" arabinan supplied by Novo. Both products behaved the same and each were good substrates for *endo*-1,5-α-L-arabinanase.

To improve the substrate properties of the linear arabinan, I subjected it to light carboxymethylation (to improve solubility) and used this in a reducing-sugar assay. The next was to produce a soluble, dye-labelled substrate (Red Debranched Arabinan).

Finally, dyed, crosslinked de-branched arabinan was produced and incorporated into Arabinazyme tablets. These are now the industry standard for measurement of endo-arabinanase and are employed by both enzyme and fruit juice manufacturers. ■

Angela Kennedy, Barry McCleary and Mr. Charlie MacCreevy at the official opening of Megazyme International Ireland Limited,