

**MacRae J C & Armstrong D G.** Enzyme method for determination of  $\alpha$ -linked glucose polymers in biological materials. *J. Sci. Food Agr.* 19:578-81, 1968.  
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Classical methods for estimation of starch are not satisfactory for the determination of  $\alpha$ -linked glucose polymers in biological materials. A method is described in which hydrolysis of starch completely to glucose is achieved using amyloglucosidase. Glucose is estimated using the oxidase method. [The *SCI*® indicates that this paper has been cited in over 130 publications since 1968, making it the 5th most-cited paper published in this journal.]

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"My PhD project, supervised by David Armstrong in the department of agricultural biochemistry, concerned the measurement of starch-like material escaping fermentation in the forestomachs of sheep. One major problem was the lack of a precise method for the determination of starch-like material in the intestinal contents obtained from sheep.

"The so-called 'direct' methods of starch estimation based on the physicochemical properties of the polymers seemed unsuitable for the mixed dietary and microbial polysaccharides of post-ruminal digesta and so my first task was to look for an alternative technique. Acid hydrolysis looked hopeful, particularly when combined with the specific glucose oxidase method of end-product estimation, but there was a problem with the duration of the hydrolysis if complete breakdown of the polymer was to be achieved without degradation of the product. Early attempts to circumvent this problem by stimulating the carbohydrase system of an animal were frustrated by the fact that oligo 1:6 glucosidase was not commercially available. Without it,  $\alpha$ -amylase can only liberate approximately 80 percent of the theoretical

recovery of glucose from pure starch, the actual recovery depending on the ratio of amylose to amylopectin chains in the starch. Then Cuthbertson of Glaxo Laboratories, Greenford, Middlesex, England, referred me to a commercial enzyme used for converting starch slurries into glucose in the confectionery industry. This purified form of amyloglucosidase, prepared from *Aspergillus niger* fermentation broths (trade name AGIDEX), liberated the theoretical glucose recoveries from gelatinised starches but had no effect on cellulosic materials (e.g., powdered cellulose, filter paper, or cotton wool).

"Incorporation of AGIDEX into the cited method provided the specificity needed in my animal nutrition experiments<sup>1</sup> (i.e., it determined the amounts of starch-like material as opposed to hemicellulose and cellulose which entered the lower gastrointestinal tract of sheep). Unfortunately, as published, the method did not determine exclusively ' $\alpha$ -linked' polymers, a fact which was not appreciated at the time and is probably of little consequence to most nutrition studies anyway. This error was realised when, on graduating, I took up an appointment in the Applied Biochemistry Division of the New Zealand DSIR. I was asked to modify the cited method for use in plant physiology experiments (i.e., with very small amounts of leaf tissue). During this modification, I tested AGIDEX against a range of soluble  $\beta$ -glucans not normally found on the shelves of animal biochemistry laboratories, but prepared over a number of years by Ray Bailey, a carbohydrate chemist. The commercial preparation hydrolysed nearly all these polymers because it contained a  $\beta$ -glucanase. The latter could be separated out on Sephadex and the subsequent description of the 'micro-method'<sup>2</sup> detailed the purification. This modified technique<sup>2</sup> was later included in a collaborative study, conducted in New Zealand and the US, which compared six methods for the measurement of starch in leaf tissue."<sup>3</sup>

1. MacRae J C & Armstrong D G. Studies on intestinal digestion in the sheep. 2. Digestion of some carbohydrate constituents in hay, cereal and hay-cereal rations. *Brit. J. Nutr.* 23:377-87, 1969.
2. MacRae J C. Quantitative measurement of starch in very small amounts of leaf tissue. *Planta* 96:101-8, 1971.
3. MacRae J C, Smith D & McCready R M. Starch estimation in leaf tissue—a comparison of results using six methods. *J. Sci. Food Agr.* 25:1465-9, 1974.