STUDIES ON METHODOLOGY IN DIETARY FIBRE ANALYSIS: A NEUTRAL DETERGENT FIBRE METHOD USING GLUCOAMYLASE

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ABSTRACT

The dietary fibre content of foods is conveniently and rapidly determined by the neutral and acid detergent methods devised originally by Van Soest and associates. A serious disadvantage of the neutral detergent method relates to the interference caused by starch during filtration when the method is applied to cereals and cereal products. In these circumstances the results of neutral detergent fibre (NDF) measurements are variable and often over-estimated.

A study of the starch-lipid reaction which takes place when cereal products are heated in Van Soest's neutral detergent solution showed that although the precipitate derived from pure wheat starch and lipid is soluble in hot water this action is often far from complete when much fibrous cereal matter is present. Much of the starch appears to be occluded in the NDF residue which then takes on a gummy-like character and tends to clog the filter.

Southgate recently recommended purified amyloglucosidase from Aspergillus niger (Boehringer) for the purpose of hydrolysing starch in cereal samples before starting the neutral detergent extraction. Present studies have been concerned with the development of this enzymatic procedure with the aim of devising improved methodology and enhancing existing knowledge of the behavioural characteristics of amyloglucosidases from A. niger and from an alternative source, Rhizopus spp.

Preliminary investigations showed that amyloglucosidase from A. niger (Boehringer) was completely effective as a starch hydrolysing agent in the pretreatment of a cereal substrate but that in order

to use the enzyme economically it was necessary to use a semi-micro version of Van Soest's neutral detergent extraction procedure. The main features of the new method are as follows: preparation of a subsample of lipid-free food sample of fine particle size; gelatinization of starch before enzyme treatment; treatment with the minimum quantity of enzyme (2 mg); extraction with neutral detergent at half the normal rate; separation of detergent solution from the residue by means of centrifugation; dehydration of the residue with acetone before filtration; special techniques for filtration, drying and weighing procedures.

A table of NDF values for various cereal products determined by the semi micro procedure is presented. The results agree, for the most part, with the results of other workers in this field, the exceptions being for cornflakes, rolled oats and puffed wheat. The coefficients of variation for the NDF values compare favourably with those of other workers.

A semi micro version of Van Soest's acid detergent method of evaluating dietary fibre was devised and is described with supporting analytical data.

Tests performed with a low cost preparation of amyloglucosidase from Rhizopus spp (Sigma) showed that the crude enzyme was capable of fully hydrolysing the starch component of cereal products before commencing the neutral detergent extraction procedure but that it also seriously reduced the NDF values. In order to establish the cause of the discrepancies two approaches were made: an attempt was made to analyse the products of enzymatic hydrolysis; and a study of the effect of enzyme concentration on the yield of neutral detergent fibre was undertaken. The former approach proved

impracticable, the latter suggested that either impurities in the crude enzyme preparation were responsible or the amyloglucosidase itself was active towards one or more components of dietary fibre. In order to determine which of the alternative explanations was correct small amounts of the crude enzyme preparation were purified by means of anion exchange chromatography using DEAE cellulose and one of two buffer systems, one based on citrate-phosphate, the other on tris-HCl. The citrate-phosphate conditions reported by Pazur and Lineback et al for the column separation of amyloglucosidase of A. niger were found to be quite unsuitable for the enzyme from Rhizopus spp. and a new set of conditions had to be determined for this enzyme.

The activity of small amounts of the purified enzyme (< 1mg) was estimated by an improvised visual method using buffered 1% wheat starch, and the effect of the enzyme on cereal fibre was determined by means of the semi micro neutral detergent procedure using 0.08-0.2 g wholemeal flour as a substrate. It was found that both crude and purified forms of the enzyme caused a loss of ca 30% NDF from wholemeal flour, from which it was concluded that amyloglucosidase from Rhizopus spp was not a suitable enzyme for use in the neutral detergent method of measuring fibre.

A literature review of the known chemistry of the amyloglucosidases of A. niger and R. delemar showed that differences in molecular structure reported by Pazur and others could account for their different electrophoretic properties. In the light of the present work it appears that another important biochemical difference between these enzymes relates to the activity of the Rhizopus enzyme towards the dietary fibre component of cereals.

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CHAPTER 1

GENERAL INTRODUCTION

Dietary fibre is defined as the indigestible matter in the diet derived from the plant cell wall (69). The definition has been extensively discussed in the literature which is reviewed in Appendix 1.

Dietary fibre has a positive physiological role to play after ingestion in the form of foods. It is presently believed that adequate dietary fibre in the diet may function as a preventive measure against numerous Western civilization diseases (18). It has long been accepted that fibre serves as a natural laxative and is necessary to promote regular bowel habits (47).

An extensive contribution to our knowledge of the role played by dietary fibre in human nutrition has been made by Burkitt and Trowell (7) based on 30 years of medical experience in East Africa. They noticed that a great number of non-infective diseases common in western man were rare in rural Africans. The largest coherent group of these diseases was and still is associated with the colon (70). It was concluded that fibre might play a part in the diseases of the colon - relating to constipation, diverticular disease, irritable colon, appendicitis, haemorrhoids, ulcerative colitis; also cancer of the large bowel (70). Other diseases considered related to fibre intake are heart disease, gallstones and obesity (66). Evidence in support of the contention that heart disease and the intake of dietary fibre are related comes from Morris et al (34) who studied a daily individual weighed dietary survey of 337 healthy middle-aged men in London and South-east

England during 1956-66. By the end of 1976 45 of them had developed clinical coronary heart disease (CHD) which showed two main relationships with diet. Men with a high energy intake (reflecting physical activity) had a lower rate of disease than the rest, and independently of this men with a high intake of dietary fibre from cereals also had a lower incidence of CHD.

Certain types of fibre, particularly pectin, have the property of binding bile salts, cholesterol and other sterols which may account for their ability to reduce blood cholesterol (48).

Fibre has water binding properties which increases the rate and volume of faecal elimination. Furthermore the satiating capabilities of fibre in food (e.g. wholemeal bread) may prevent over-ingestion of fat and sugar in the diet and reduce the potential for obesity (48).

Recently Oakenfull (36) has claimed that saponins rather than fibre lead to the reduction in blood cholesterol levels. Thus if high levels of blood cholesterol do contribute to coronary heart disease only saponin-containing fibre will reduce the risk of heart attack. The evidence is based on feeding trials with rats but also on an Italian experiment with 20 human patients carried out by Sirtori et al (53) who found that a low lipid diet containing soybean protein (a food particularly rich in saponins) considerably lowered blood cholesterol levels.

Dramatic advances have been made during the past decade or two in our understanding of the biological functions of dietary fibre in human nutrition but impressive advances have also occurred in the

analytical methodology of fibre measurement in foods - although not all authorities are yet satisfied. For example, the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition (25) has complained that the results of feeding high fibre diets differ from researcher to researcher, one explanation being the relatively poor analytical methods available for establishing fibre data.

The methodology of fibre analysis has its beginnings in the early nineteenth century with the development of the Weende method for determining crude fibre in animal forages and feeds. This procedure was eventually standardised by the AOAC in 1887 (76).

Remy (46) in 1931 was the first to advocate the use of enzymes in the measurement of plant fibre in fruits, vegetables and grain. His method gave values roughly twice those obtained by the classic Weende method. Remy's enzymatic method was improved upon by Williams and Olmsted (83) and later by other workers. The basic studies of McCance and Laurence (31) in 1929 directed attention to unavailable carbohydrates in foods and led ultimately to the analytical schemes put forward by Southgate (70).

One important difficulty encountered by the pioneers of fibre analytical methodology was the lack of a generally acceptable term and definition for this type of fibre. Hipsley (22) in 1953 was the first to use the term 'dietary fibre' but 20 years were to elapse before a new definition of dietary fibre based on physiological considerations, proposed by Trowell (69), made the term widely popular (70).

Even at the present time (1979) there is still lack of agreement on

the meaning of dietary fibre and the search for a more appropriate term continues. In a recent review paper by Spiller et al (65) 14 different terms for fibre are listed which have been proposed by various authorities over many years. Spiller et al (65) favour 'plantix' so as to avoid confusion with fibre of animal origin. Yet, as Trowell (70) points out, indigestible amino polysaccharides in animal connective tissues eaten by carnivores and Eskimos (19) should be included when defining fibre. For the time being the term dietary fibre is likely to be widely used despite certain minor anomalies. One may also expect abbreviations such as NDF and ADF (for neutral detergent fibre and acid detergent fibre, respectively) to be used in appropriate circumstances to denote the methodology of fibre measurement.

A problem that concerns the fibre analyst at the present time is the difficulty experienced in measuring accurately the dietary fibre content of starch-rich foods by the rapid neutral detergent method devised by Van Soest. Even if this problem can be resolved, however, the fundamental objection remains that the neutral detergent method will underestimate total dietary fibre because the water insoluble polysaccharides are lost during extraction, these losses being quite substantial with some materials (54).

Nevertheless, any step towards the improvement of the method must be considered worthwhile. The present study reveals some of the basic problems associated with the use of starch hydrolysing enzymes when applied to the measurement of dietary fibre by Van Soest's neutral detergent method. The study also reveals how these enzymes may be exploited in a new semi micro modification of Van Soest's method. A close study of one of the enzymes investigated, amyloglucosidase

from Rhizopus spp, provides an opportunity of recording certain hitherto unreported biochemical characteristics relating to its effect on dietary fibre components.