Chemical Methods for the Determination of Soluble and Insoluble Non-Starch Polysaccharides - Review

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Abstract

Polysaccharides are macromolecules of monosaccharides linked by glycosidic bonds. Non-starch polysaccharides (NSP) are principally non-α-glucan polysaccharides of the plant cell wall. They are a heterogeneous group of polysaccharides with varying degrees of water solubility, size, and structure. The water insoluble fiber fraction include cellulose, galactomannans, xylans, xyloglucans, and lignin, while the water-soluble fibers are the pectins, arabinogalactans, arabinoxylans, and β-(1,3)(1,4)-D-glucans (β-glucans). Both the enzymatic-gravimetric and enzymatic-chemical methods used for the determination of soluble and insoluble non-starch polysaccharides have undergone a number of modifications and improvements, most occurring over the last 20 years.

Keywords: enzymatic chemical methods, enzymatic gravimetric methods, non-starch polysaccharides.

Introduction

In animal nutrition, as "non-starch-polysaccharides" (NSP) are summarized polysaccharides which cannot be degraded by endogenous enzymes, and therefore reach the colon almost indigested. Individual NSP groups have different chemical and physical characteristics that result in various effects on physiology of intestine, and on organism in general.

Non-starch polysaccharides are principally non-α-glucan polysaccharides of the plant cell wall. The water insoluble fiber fraction include cellulose, galactomannans, xylans, xyloglucans, while the water-soluble fibers are the pectins, arabinogalactans, arabinoxylans, and β-(1,3)(1,4)-D-glucans (β-glucans) [1].

The NSP comprise 700-900 g kg^{-1} of the plant cell wall, with the remaining being lignin, protein, fatty acids, waxes, etc. Plant cell wall NSP is a diverse group of molecules with varying degrees of water solubility, size and structure, which may influence the rheological properties of the gastrointestinal contents, flow of digesta and the digestion and absorption process to a variable degree.

A large amount of the cell wall components in barley and oats consist of ß-glucans, whereas arabinoxylans are common in wheat, rye and triticale.

The main polysaccharides constituents of wheat endosperm cell walls are arabinoxylans [2, 3, 4], whereas arabinoxylans and β-glucans predominate in wheat aleurone layers, and arabinoxylans and cellulose predominate in cell walls of pericarp/testa [5, 6, 7].

The NSP content of plants varies not only in accordance to the plant species, but also varies between genotype, or cultivar of the same species. Furthermore, the agronomic cultivation conditions, such as environmental factors prior to harvest and storage conditions after harvest, can influence NSP content.

The classification of NSP was based originally on the methodology used for extraction and isolation of polysaccharides.

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Enzymatic gravimetric methods

The methods developed for analyzing feed in the 19th century did not contain specific techniques for the determination of carbohydrates, which are considered remaining material after the analysis of protein, fat, ash and moisture [8].

The oldest and most commonly used method for analysis of fiber in feedstuff is the crude fiber method [9].

This method measures only a fraction of the fiber components because it can solubilize some of the structural polysaccharides and lignin. The crude fiber method uses successive acid and alkaline digestion to isolate the insoluble fraction. The crude fiber method continues to be used in some regions of the world as well as in the animal feed industry. However, its usefulness is severely limited by the loss of all soluble polysaccharides, some insoluble polysaccharides, and some lignin, and the inclusion of some nitrogenous material in the remaining residue.

In the 1960s, Van Soest and colleagues [10, 11] introduced the use of detergents to solubilize protein. The more commonly used *fiber* analysis *methods* for concentrate feeds are *detergent methods,* although these methods were originally developed for forage fiber.

The Acid Detergent Fiber (ADF) method, which was adopted for animal feeds, utilizes strong acid to hydrolyze all polysaccharides, except cellulose and lignin, which are therefore the only components in ADF [11]. Other cell wall polysaccharides are not included in this method, limiting its usefulness for human nutrition in the same way as crude fiber method does. Recognizing the need to describe and include other cell wall constituents, Van Soest [12, 13] developed the Neutral Detergent Fiber (NDF) method, which measures all insoluble cell wall material. This proved to be a better predictor of the nutritional value of dietary fiber in animal feeds than crude fiber method. In the 1970s, the use of the NDF method spread to human nutrition, but its utility remained limited because it did not include soluble fiber components, nor did it remove all starch.

The detergent method provides a more descriptive measurement of the fiber that is insoluble in neutral detergent (NDF), and in acid detergent (ADF). NDF measures hemicellulose, cellulose and lignin, while ADF measures cellulose and lignin allowing calculation of hemicellulose by difference. This calculation does not give the exact measurement of NSP because water-soluble and water-insoluble NSP may be lost in the NDF procedure, starch and protein may contaminate the NDF residue, and hemicellulose may be left in the ADF fraction [14, 15, 16]. NSP can be estimated by difference between total dietary fiber and NDF [17].

German researchers introduced, during the nineteenth century, the use of enzymes to remove available carbohydrate.

Schaller [18] introduced the treatment with amylase in the neutral detergent method of Van Soest & Wine, to solve problems in applying the method to products with high content in starch, which starch is not completely solubilized. By modifying the method, Mongeau & Brassard [19,20], by determination of a soluble fiber fraction, developed a rapid gravimetric method, of high precision, but which had the inconvenience that it could not completely remove the starch and / or protein in some samples.

More precise procedures of NSP analysis, including enzymatic-chemical method, or Englyst method, [21, 22] and the non-enzymatic gravimetric method of Prosky [23], have shown to be the main approaches for the NSP measurement [24, 25, 17]. The enzymatic-chemical method has been shown to be easier and quicker to perform than the non-enzymatic gravimetric method of Prosky [25].

Knudsen [17] described that the enzymatic-chemical method yields information on the monomeric composition of the NSP divided into soluble and insoluble fractions. This method gives a general view of the functional properties of the fiber, in particular when working with identifiable cell wall material.

The first gravimetric methods for measuring soluble and insoluble components of NSP were developed independently by Furda [26,27], Schweizer and Würsch [28,29], and Asp and Johansson [30]. These authors, along with DeVries, Prosky and Harland, have developed the first version of the enzymatic gravimetric method of AOAC [31]. The method was later adapted for soluble and insoluble fractions, and then was simplified using 4-morpholine-ethanesulfonic acid-TRIS buffer [32, 33].

The method of Prosky and Asp [34] was adapted by AOAC [35].

The method provides a measure of total dietary fiber by enzymatic removal of available starch and solubilization and extraction of a portion of the protein; the remaining residue is dried, weighed, and corrected for crude protein and ash contents. An initial step is added to remove fat if it is present at concentrations of 10 percent or more. The method is relatively rapid and easy to perform and has been automated to enable a large number of samples to be assessed. It has been adopted as an official method for dietary fiber analysis by many countries.

This method digested the gelatinized sample with thermostable amylase, amyloglucosidase and protease. Undigested fraction is precipitated with ethanol. Correction of the residue is made with Nx6.25 and ash. AOAC method has the disadvantage that hydrolyzed byproducts remain in solution. Na and Ca salts in the buffers and in the samples are insoluble in alcohol and increase the amount of ash. Loss of volatile components at 525^{o}C contributes to an overestimation of polysaccharides.

Because of these problems, the method was modified, including dialysis with urea, which avoids the heat treatment and removes byproducts by dialysis. The principle of the method consists in the special activity of thermostable enzyme in 8M urea solution [36].

Asp [37] developed a method for the analysis and characterization of food polysaccharides factions, which was adapted for feed by Southgate [38] and Bach Knudsen [39].

Enzymatic-Chemical Methods

Research conducted in the past aimed at determining the structural polysaccharides and lignin in roughage feeds [40, 41, 42]. Only a few analyses of combined feeds and by-compound were made [43, 44].

During the 1950s, methods [45] were improved by introducing a series of extraction steps followed by hydrolysis of polysaccharides and subsequent colorimetric analysis of monosaccharides component. Southgate [45] recognized that a crucial step was the complete removal of starch, since incomplete removal would result in overestimation of glucose-based dietary fiber. The Southgate method was modified for human nutrition during the 1970s and became incorporated in the United Kingdom nutrient tables in the Fourth Edition of McCance and Widdowson's *The Composition of Foods* [46]

Although the method provided considerable information on monosaccharide groups (hexoses, pentoses, and uronic acids), Southgate recognized that a colorimetric assay did not distinguish individual monosaccharides, and recommended that gas chromatography (GC) or high-performance liquid chromatography (HPLC) be employed [45]. In addition, there remained difficulties with the removal of starch, which gave inflated values for many individual food items with high starch content, such as starchy vegetables, legumes, and grains.

Englyst and coworkers [47] published a procedure extending Southgate's work for the measurement of nonstarch polysaccharides, using GC. The method involved more complete removal of available starch and allowed for determination of the different monosaccharides present as constituents of dietary fiber in food products. It also allowed separation of cellulose from noncellulosic polysaccharides, and soluble from insoluble polysaccharides. Hence, the method provided considerable detail on the polysaccharide components of human foods.

Englyst method is an enzymatic-chemical method for the determination of NSP and it is an improved method of McCance [48] and Southgate [45]. Starch is removed completely by the enzyme, and NSP are determined as the sum of the constituent sugars released by acid hydrolysis. Carbohydrates can be determined by gas-liquid chromatography (GLC), or by HPLC to obtain values for the constituent monosaccharides, or colorimetric to obtain the total amount of reducing monosaccharides. Values for total NSP, soluble and insoluble NSP can be obtained, and by a slight modification of the method, cellulose can be separately determined. Englyst method determines total, soluble and insoluble NSP in 8 hours with the colorimetric version, or in one and a half day with chromatographic procedures. By applying GLC, NSP is determined as the sum of neutral carbohydrates, and uronic acids are determined separately. HPLC measures NSP as the amount of neutral sugars and uronic acids.

Enzymatic and chemical methods for determining the NSP involves treating the sample to remove free sugars and starch by enzymatic hydrolysis. NSP are then recovered by precipitation with 80% ethanol, then washed and dried. NSP are

hydrolyzed, either with dilute acid, to hydrolyze the majority of non-cellulose polysaccharides and with 12M sulfuric acid, which hydrolyze cellulose, or with 12M sulfuric acid, which completely hydrolyzes NSP.
Monosaccharides are analyzed by GLC after derivatization (as alditol acetate), by HPLC [47], or colorimetric [22].
Several modifications have been made to the Englyst method [47]. One of these was the removal of resistant starch, which was identified in the early 1980s [49]. Resistant starch consists of: (1) starch that is not physically accessible to digestive enzymatic hydrolysis; (2) retrograded starch that has been rendered resistant to hydrolysis by processing, or by cooking and cooling; and (3) uncooked starch in granules that is not accessible to enzymatic hydrolysis unless it is gelatinized by heating [50]. Englyst and Cummings [49] removed resistant starch from the nonstarch polysaccharide component in a method using dimethyl sulfoxide. Since resistant starch is created by cooking and processing, the method ensured that foods could be assessed using nonstarch polysaccharide values of ingredients by the use of recipes, and that each food product did not have to be individually measured to obtain an accurate value.
Englyst and Hudson [22] developed an alternative colorimetric method for the measurement of the monosaccharides component. Englyst made the procedure faster in another modification, with a more rapid procedure for the removal of starch [51]. HPLC methods were developed for the measurement of uronic acids [52,53].

Components included in each method of analysis

Non-Starch Polysaccharides
All the current methods include all NSP that precipitate in 78-80% ethanol. Polysaccharides that do not precipitate in ethanol are not included in any of the existing methods.
Polysaccharides that are excluded by ethanol precipitation include inulin, other fructans, modified cellulose, and some arabinogalactans.

Lignin
All methods except those of Englyst [22,49,53] for NSP include lignin. In the enzymatic-gravimetric methods, lignin is included as part of the residue after filtration. In the enzymatic-chemical methods of Theander and coworkers [54] and Southgate [45], lignin is analyzed as a separate component, using the Klason lignin method [55]. This method measures native lignin, but can also include tannins, cutins, and Maillard reaction products [56].

Resistant Starch
Resistant starch is not included in the Englyst methods for NSP [22,49,53], since it is removed using dimethyl sulfoxide. In all the other methods, a proportion of resistant starch is included in the analysis for dietary fiber, largely as retrograded amylose. However, this proportion of resistant starch is not constant for different foods made from the same ingredients, as retrograded amylose is created by cooking and cooling food and through food processing. Since resistant starch has many physiological properties similar to those of dietary fiber, there is a need for a uniform method for its analysis.
There are currently a number of methods available for measurement of resistant starch, although none have been submitted for evaluation by the approved methods process of the AOAC. Englyst and colleagues [50] calculated resistant starch as the difference between available starch and total starch.
A list of potential components of fiber included in each analysis is provided in Table 1.

Table 1. Methods of Fiber Analysis

Procedure Type	Measures	Reference (Method)
Enzymatic-gravimetric	Soluble dietary fiber Insoluble dietary fiber Total dietary fiber	Asp et al., 1983 [30]
Enzymatic-gas chromatographic	Total NSP Individual constituent sugars	Englyst and Cummings, 1984 [49]
Enzymatic-colorimetric	Soluble nonstarch polysaccharides, by difference Insoluble nonstarch polysaccharides Total nonstarch polysaccharides	Englyst and Hudson, 1987 [22]
Enzymatic-gravimetric liquid chromatographic	Total dietary fiber including low molecular weight resistant maltodextrins	Gordon and Ohkuma, (AOAC 2001.03) [57]
Enzymatic-gravimetric using MES-TRIS buffer	Soluble dietary fiber Insoluble dietary fiber Total dietary fiber	Lee et al., 1992 (AOAC 991.43) [32]
Enzymatic-gravimetric (for foods and food products with ≤ 2% starch)	Total dietary fiber	Li and Cardozo, 1994 (AOAC 993.21) [58]
Enzymatic-spectrophotometric	Fructans	McCleary et al., 2000 (AOAC 999.03) [59]
Enzymatic-gravimetric	Soluble dietary fiber Insoluble dietary fiber Total dietary fiber	Mongeau and Brassard, 1993 (AOAC 992.16) [60]
Enzymatic-gravimetric	Total dietary fiber	Prosky et al., 1985 [23]
Enzymatic-gravimetric	Insoluble dietary fiber	Prosky et al., 1992 (AOAC 991.42) [61]
Enzymatic-gravimetric	Soluble dietary fiber	Prosky et al., 1994 (AOAC 993.19) [62]
Enzymatic-high performance liquid chromatographic	Soluble nonstarch polysaccharides, by difference Insoluble nonstarch polysaccharides Total nonstarch polysaccharides Individual constituent sugars	Quigley and Englyst, 1992 [63]
Enzymatic-gravimetric	Soluble dietary fiber Insoluble dietary fiber Total dietary fiber	Schweizer and Würsch, 1979 [28]
Enzymatic-colorimetric	Soluble dietary fiber Insoluble dietary fiber Total dietary fiber	Southgate, 1969 [45]
Enzymatic-gas chromatographic	Insoluble neutral polysaccharides Soluble neutral polysaccharides Insoluble uronic acids Soluble uronic acids Klason lignin Total dietary fiber	Theander and Åman, 1979 [64]
Enzymatic-gas chromatographic	Insoluble neutral polysaccharides Soluble neutral polysaccharides Insoluble uronic acids Soluble uronic acids Klason lignin Total dietary fiber	Theander and Westerlund, 1986 [65]
Enzymatic-gas chromatographic	Neutral polysaccharides Uronic acids Klason lignin Total dietary fiber	Uppsala Method of Theander et al., 1995 (AOAC 994.13) [56]

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