Analytical Aspects of Total Starch Polarimetric Determination in Some Cereals

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Abstract

Starch is the most important digestible polysaccharide present in foods and feeds. The starch concentration in cereals cannot be determined directly, because the starch is contained within a structurally and chemically complex matrix. Fine grinding and boiling in dilute HCl are preparative steps necessary for complete release of the starch granules from the protein matrix. Starch can be determined using simple and inexpensive physical methods, such as density, refractive index or optical rotation assessment. The polarimetric method allows the determination even of small starch contents due to its extremely high specific rotation. For more accurate results, the contribution of free sugars is eliminated by dissolution in 40% (V/V) ethanol. The influence of other optically active substances, which might interfere, is removed by filtration/clarification prior to the optical rotation measurement.

Keywords: polarimetry, starch, cereals

1. Introduction

The chemical composition of cereal grains is characterized by the high content of carbohydrates [1,2]: available carbohydrates, mainly starch deposited in the endosperm (56–74%) and fiber, mainly located in the bran (2–13%). Cereal grains contain 66–76% carbohydrates. The major carbohydrate is starch (55–70%) followed by minor constituents such as arabinoxylans (1.5–8%), β-glucans (0.5–7%), sugars (~3%), cellulose (~2.5%), and glucofructans (~1%) [3,4].

Starch is the most important digestible polysaccharide that is found in foods and feeds. It is also the most abundant storage polysaccharide in plants, present in high amounts in roots, tubers, cereal grains and legumes [5]. Starch is digested primarily in the small intestine by enzymatic degradation, but some can escape digestion and be fermented in the large bowel [6]. Starch is a homopolysaccharide built of glucose molecules linked together by α-D-(1-4) and/or α-D-(1-6) glycosidic bonds. About 70% of the starch granule mass is amorphous, and 30% crystalline. The amorphous region contains almost all amount of amylase, and a part of amylopectin molecules. The crystalline region is mainly composed of amylopectin [7].

The ratio of starch to non-starch polysaccharides (NSP) in cereals may exceed 40:1 so that for accurate analysis it is essential to complete separation of NSP.

Various physical, chemical and biochemical methods have been reported in the last decades for the analysis of total starch [8,9].

Starch can be determined using simple and inexpensive physical methods, such as density, refractive index or optical rotation assessment.

Starch molecules contain asymmetric carbon atoms that have the ability to rotate plane polarized light. The extent of polarization is related to the concentration of the optically active molecules in solution. The angle of rotation depends on temperature, wavelength of light and the concentration of optically active molecules in solution.

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The starch content in various cereals cannot be determined directly because the starch is contained within a structurally and chemically complex matrix [10]. Therefore fine grinding and boiling in dilute HCl are necessary preparative steps to break down the endosperm tissue and for complete release of the starch granules from the protein matrix [11,12].
The influence of other optically active substances, which might interfere, is removed by filtration/clarification prior to the optical rotation measurement. The clearing agents remove turbid materials and coloring substances. The most used clarifying agents are heavy metal salts (such as lead acetate) which form insoluble complexes with the interfering substances.

2. Materials and methods

Cereal grains were milled by a laboratory grinder to 500 μm granulation. 2.5 g of crushed sample were analyzed for total starch (TS) by polarimetry. A compound is considered to be optically active if linearly polarized light is rotated when passing through it. The amount of optical rotation is determined by the molecular structure and concentration of chiral molecules in the substance. Each optically active substance has its own specific rotation as defined in Biots law:

$[\alpha]_D^{25} = \frac{\alpha}{l \cdot d}$, where α is the measured angle of rotation, [α] is the optical activity (which is a constant for each type of molecule), *l* is the optical path length in dm, and *c* is the concentration in g/100 mL.
Two experiments were conducted. Experiment I: The soluble, optically active compounds accompanying the substance under examination are extracted with 10 % ethanol and removed by centrifugation [13]. Following the extraction of soluble sugars, the residue can then be used for the extraction of insoluble sugars such as starch. The starch remaining in the residue is dissolved by boiling in 50 mL dilute hydrochloric acid (1.125% HCl), dissolved proteinanceous substances are precipitated with 5 mL Carrez I solution and with 5 mL Carrez II solution and then filtered.
The optical rotation in angle degrees of the filtrate is measured and the starch content is calculated. The extent of polarization is related to the concentration of the optically active molecules in solution by the equation:

$$TS\% = \frac{2000 \cdot \alpha}{[\alpha]_D^{20}}$$

The angle of rotation was measured with a Carl Zeiss Jena polarimeter at standardized temperature (20°C) and wavelength (589.3 nm), using a sample cell of 200 mm optical path length.
The specific rotation is taken from the literature for the type of carbohydrates present [14].

Starch	$[\alpha]_D^{20}$ (°)
Wheat	182.7
Barley	181.5
Rice	185.9
Corn	184.6

Experiment II consists of two determinations [15,16]. In the first, the sample is boiled with dilute hydrochloric acid. The influence of other optically active substances, which might interfere, is removed by filtration/clarification prior to the optical rotation measurement. In the second determination, free sugars are extracted from the sample with 40% and 80% ethanol solutions. After acidifying the filtrate with hydrochloric acid, clarifying and filtering, the optical rotation is measured as in the first determination. The difference between the two measurements, multiplied by a factor, gives the starch content of the sample.

$$TS\% = \frac{2000\,(\alpha_1 - \alpha_2)}{[\alpha]_D^{20}}$$

The optical rotation in the clarified filtrate of all samples was measured at 20°C by using a sample cell of 200 mm optical path length. All samples were analyzed in duplicate and results are presented as the mean values.
All chemicals used were of analytical reagent grade. Double distilled deionised water was used for all experiments.

3. Results and discussion

Extraction with aqueous ethanol provides a convenient way of isolating the free sugars. The monosaccharides and oligosaccharides are soluble in the ethanol solution, while the starch is insoluble [17]. The solubilities of

monosaccharides are decreased as the concentration of ethanol in the solvent is increased over the range of concentrations investigated in the study [18].
Hence, the starch can be separated from the sugars by filtering or centrifuging the solution.
Although free sugars are soluble in water, extraction must be performed in ethanol solution, to avoid the co-extraction of proteins and other soluble polysaccharides. The final concentration of ethanol must be 80% to precipitate all polysaccharides. As some sugars have low solubility at this strength of ethanol, it is preferable to use 40% ethanol solution for extraction and to adjust strength later.
The results of both experiments are presented in Table 1.

Table 1. Total starch content (TS%)

Cereal/ethanol	Experiment I	Experiment II	
	10%	40%	80%
Wheat	74.50	70.38	71.40
Barley	63.22	62.26	63.20
Rice	94.25	90.10	90.40
Corn	76.05	75.67	76.22

The results obtained when using 40% ethanol solution are comparable with those obtained whit 80% ethanol (Fig. 1). The greater values obtained than in the enzymatic method (E), can be attributed to an effect of the hydrochloric acid hydrolysis that is susceptible to transforming constituents other than starch into optically active products.

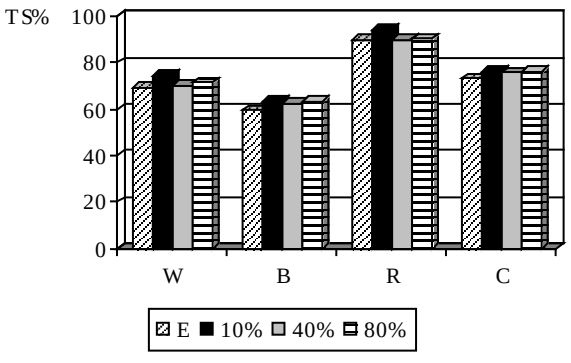

Figure 1. TS in cereals determined with different ethanol concentrations

4. Conclusions

The polarimetric method allows the determination even of small starch contents due to its extremely high specific rotation. For more accurate results, the contribution of free sugars is eliminated by dissolution in 40% (V/V) ethanol. The influence of other optically active substances, which might interfere, is removed by filtration/clarification prior to the optical rotation measurement.

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