AACCI Approved Methods Technical Committee Report: A New AACCI Approved Method (32-24.01) for Measuring Viscosity of β-Glucan in Cereal Products Using the Rapid Visco Analyzer

Tamer H. Gamel,[1,2] El-Sayed M. Abdel-Aal,[3] Nancy P. Ames,[4] Kelly Henderson,[5] Frédéric Prothon,[6] Teepakorn Kongraksawech,[7] and Susan M. Tosh[3]

Regulations allowing health claims linking the consumption of food products rich in β-glucan with reduced risk of coronary heart disease have increased the interest of food companies and consumers in such products as healthy food options. Oat and barley cereal products, in particular, have garnered more attention due to their high β-glucan contents. The mixed linkage β-glucan, (1→3)(1→4)-β-D-glucan, present in oat and barley has shown positive health benefits in many clinical trials, including reducing LDL cholesterol level (1,17) and glycemic response (2,5,14,16). These physiological effects have been highly correlated with β-glucan viscosity and molecular weight (7,16,18). Processing oat and barley into cereal food products may affect the physicochemical characteristics of β-glucan and subsequently its physiological effects. Changes in the weight-average molecular weight (M_w), solubility, and viscosity of β-glucan have been reported during extrusion of oat bran into a cereal food product as a result of severe shear rate (15), during breadmaking processes as a result of fermentation and preparation methods (3,6,8), and during freezing and freeze–thaw cycles due to the aggregation of β-glucan molecules and changes in solubility (10,11). Thus, it is important to monitor changes in the physicochemical characteristics of β-glucan throughout processing to preserve its positive physiological effects.

Need for a Rapid and Reliable Method to Determine β-Glucan Viscosity in Cereal Products

A β-glucan–specific in vitro extraction method designed to simulate the digestion process in the human gastrointestinal system and determine its physiological effects is currently being used (4). However, the length and complexity of the method limits the widespread use of this extraction technique, especially in industrial laboratories. Therefore, a simple and reliable method to determine the viscosity of β-glucan in cereal products and monitor changes during storage and processing would be extremely useful for industry and academia alike.

[1] Agriculture and Agri-Food Canada, Guelph Food Research Centre, Guelph, ON, Canada, and Alexandria University, Faculty of Agriculture, Food Science and Technology Department, Alexandria, Egypt.

[2] Corresponding author. E-mail: tamer.gamel@agr.gc.ca or tamer.gameal@alexu.edu.eg; Tel: +1.226.217.8102.

[3] Agriculture and Agri-Food Canada, Guelph Food Research Centre, Guelph, ON, Canada.

[4] Agriculture and Agri-Food Canada, Cereal Research Centre, Winnipeg, MB, Canada.

[5] Richardson Milling Limited, Portage la Prairie, MB, Canada.

[6] Swedish Oat Fiber, Bua, Sweden.

[7] Oregon State University, Corvallis, OR, U.S.A.

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At the 2011 AACC International (AACCI) Annual Meeting in Palm Springs, CA, the Oat and Barley Products Technical Committee specified the need for a standard method to measure the viscosity of β-glucan in oat and barley cereal products because it is the predominant factor in the health benefits of this compound. A rapid visco analyzer (RVA) method was developed to measure β-glucan viscosity in enzyme-digested cereal products (7,9). The RVA is an effective tool for dispersing and mixing β-glucan–rich products with digestive enzymes and directly monitoring the development of viscosity throughout the digestion test (2 hr). This method was highly correlated with the in vitro protocol established by Beer et al. (4), and the viscosity obtained by both techniques correlated well with the physiological effects of oat cereal foods (5,7,9,18).

Collaborative Study

To validate the new method and evaluate its performance, a mini-collaborative study with five laboratories (Table I) and five different cereals products was conducted in 2013. The data were discussed at the 2013 AACCI Annual Meeting in Albuquerque, NM, and the Oat and Barley Products Technical Committee agreed to proceed with a full-scale interlaboratory study, which was conducted in 2014. Ten research and industrial laboratories participated in the full-scale collaborative study (Table I). Each laboratory received 10 oat and barley products containing β-glucan (including two blind duplicates). Products, copies of the method and instructions, the sample weight and buffer for each product, and an adequate supply of enzymes, reagents, and β-glucan standards were shipped to the participating laboratories by overnight express courier under refrigerated conditions. The arrival time of the shipments varied among locations from 1 to 6 days. One shipment to the United States was delayed for 12 days due to border inspection

Table I. Participants in mini- and full-scale collaborative studies

Participant	Country
Cereal Research Centre, Agriculture and Agri-Foods Canada[a]	Canada
General Mills[b]	USA
Guelph Food Research Centre, Agriculture and Agri-Foods Canada (leading team)[a]	Canada
Instituto de Agroquímica y Tecnología de Alimentos[b]	Spain
Oregon State University, Crop & Soil Science[a]	USA
PepsiCo Global[b]	USA
Richardson Milling Limited[a]	Canada
Swedish Oat Fiber[a]	Sweden
University of Helsinki, Food and Environmental Science[b]	Finland
VTT Technical Research Centre of Finland[b]	Finland

[a] Participated in both the mini- and full-scale collaborative studies.
[b] Participated in only the full-scale collaborative study.

issues. The products from this shipment were tested and compared with the data from the other labs; the data was within the average, indicating no deterioration occurred in the enzymes or reagents.

Products Tested

For the mini-collaborative study, five experimental and commercial oat and barley products (two extruded oat bran pellet products, pot barley, oat loops, and rolled oats) were selected, and two blind duplicates were included (extruded oat bran pellets and oat loops). For the full-scale collaborative study, eight commercial cereal products (oat flakes and clusters, oat and barley flakes and clusters, oat granola, extruded oat bran pellets, two shredded oat products, and two oat loop products) were tested, and two blind duplicates were included (shredded oats and oat loops).

Reagents

Sodium dihydrogen orthophosphate dihydrate and NaCl were purchased from Sigma/Aldrich. Enzymes were purchased from either Megazyme International or Sigma/Aldrich. For starch digestion, microbial α-amylase (*Bacillus licheniformis*) (Megazyme E-BLAAM100; EC.3.2.1.1; 3,000 U/mL) was used. For protein digestion, microbial protease (*B. licheniformis*) (Megazyme E-BSPRT100; EC.3.4.21.14; 300 U/mL) was used. Lichenase (endo-(1→3)(1→4)-β-D-glucan 4-glucanohydrolase) (Megazyme E-LICHN; EC.3.2.1.73; 1,000 U/mL) was used for β-glucan digestion.

Study Protocols and Analytical Test

The moisture and β-glucan contents of the products were determined according to AACCI Approved Methods 44-15.02 and 32-23.01, respectively (http://methods.aaccnet.org). These two parameters were used to calculate the amount of sample and buffer required for the test. Other β-glucan characteristics, including M_w and solubility, not required for the proposed method also were determined (15). The results are provided in Table II. All products were ground using a laboratory mill (M2 Universal, IKA-Werke) or similar type of mill to produce particle sizes able to pass through a 0.6 mm screen.

The amount of milled product and buffer (g) required to obtain a sample containing 1% β-glucan (as is basis) was calculated using the following equations:

$$S = (25/\beta\text{-glucan content of sample})\ (100/100 - M1)$$
$$W = 24.95 - M2$$

where S = corrected sample weight, W = corrected buffer weight, $M1$ = moisture content of product (%, as is), and $M2$ = moisture content in a gram of corrected sample weight (S).

The corrected weight of buffer (sodium phosphate buffer: 20 mmol/L + 10 mmol/L NaCl; pH 6.9) was dispensed into the RVA canister followed by the sample and digestive enzymes (microbial amylase and protease). Viscosity was recorded automatically throughout the 2 hr RVA run, and final viscosity (V_1) was reported. The digested sample was treated with lichenase for an additional 15 min under the same conditions (160 rpm and 37°C). This treatment was performed to determine whether other food constituents contribute to viscosity. The viscosity of the lichenase-treated sample was recorded automatically throughout the 15 min run, and final viscosity (V_2) was reported. The viscosity values provided in this report are the difference between V_1 and V_2, which represents the viscosity of the β-glucan. The method procedure is outlined in proposed AACCI Approved Method 32-24.01.

Statistical Treatment

The data from all the laboratories were collected and subjected to statistical analysis according to AACCI protocols and using a Microsoft Excel spreadsheet supplied by AOAC International. For the full-scale collaborative study, one lab had extremely biased values compared to all other participating laboratories, and the laboratory's results were excluded along with a few other outliers.

Method Performance

The purpose of the new method was to measure the viscosity of β-glucan in cereal products subjected to enzyme digestion. The recorded viscosity would be attributable to the soluble β-glucan in a product following enzymatic hydrolysis of the starch

Table II. Moisture content and β-glucan characteristics (content, weight-average molecular weight [M_w], and solubility) of cereal products tested in the mini-collaborative and full-scale collaborative studies

Study Product Tested	Moisture (%)	β-Glucan Characteristic[a] Content (%, as is basis)	 M_w (kg/mol)	 Solubility (%)
Mini-collaborative				
Extruded oat bran pellets 1	4.9 ± 0.1	9.0 ± 0.12	2,570 ± 41	59.2 ± 0.6
Extruded oat bran pellets 2	4.6 ± 0.1	9.0 ± 0.44	2,630 ± 90	94.7 ± 0.6
Pot barley	12.0 ± 0.3	4.8 ± 0.14	1,220 ± 130	44.1 ± 0.9
Oat loops	5.2 ± 0.1	10.0 ± 0.5	2,170 ± 95	71.2 ± 0.7
Rolled oats	10.9 ± 0.2	4.0 ± 0.22	1,750 ± 45	37.6 ± 0.1
Full-scale collaborative				
Oat flakes and clusters	3.2 ± 0.06	3.6 ± 0.13	760 ± 45	34.1 ± 0.3
Shredded oats 1	3.5 ± 0.06	2.2 ± 0.10	1,280 ± 44	60.2 ± 1.5
Shredded oats 2	4.6 ± 0.10	2.1 ± 0.05	980 ± 15	52.0 ± 1.6
Oat and barley flakes and clusters	4.8 ± 0.02	3.2 ± 0.12	1,560 ± 53	56.9 ± 0.5
Oat loops 1	4.3 ± 0.21	4.3 ± 0.26	1,288 ± 175	36.8 ± 1.2
Oat loops 2	3.9 ± 0.22	3.6 ± 0.14	2,150 ± 156	39.6 ± 1.2
Oat granola	3.2 ± 0.09	3.5 ± 0.11	1,580 ± 177	49.7 ± 0.8
Extruded oat bran pellets 3	5.0 ± 0.16	8.0 ± 0.15	2,990 ± 111	85.0 ± 1.3

[a] Values are the average of triplicate measurements ± SD.

and proteins. A further treatment with lichenase (β-glucan–degrading enzyme) was included as an indirect control to help eliminate viscosity contributed by components other than β-glucan. By subtracting this value, the reported viscosity corresponded mainly to the physicochemical characteristics of the β-glucan. The viscosity of lichenase-treated samples for most of the products tested was low, suggesting β-glucan was the primary contributor to the developed viscosity. Because the β-glucan viscosity measurement obtained by this method was significantly correlated with blood glucose and cholesterol-lowering effects (7), RVA viscosity can be used as a quality control or screening tool for identifying cereal products that may have potential health benefits.

The statistical estimates for the mini-collaborative study results are summarized in Table III. In this study, no outliers were identified, and method performance within and between the five laboratories was promising, as indicated by the repeatability relative standard deviation (RSD_r < 5%) and reproducibility relative standard deviation (RSD_R < 10%). The results of the mini-collaborative study were discussed by the Oat and Barley Products Technical Committee members, who indicated there was a potential to standardize the method, and the decision was made to move ahead with a full-scale collaborative study. It should be noted that the HorRat value, a statistical parameter measuring method performance, is not applicable in this method due to the nature of viscosity measurement (12).

The products tested in the full-scale interlaboratory study were chosen to cover a wide range of viscosities (e.g., 225–3,613 cP) to ensure the effectiveness of the method for a variety of food products available in the market. This viscosity range represents large variations in M_w (760–2,990 kg/mol) and solubility (34–85%) among products (Table II). Differences among products also were found for the RVA viscosity profile developed over the course of the 2 hr digestion test (Fig. 1). This was due to variations in β-glucan M_w and solubilization rates among products and to differences in product structure matrices. Thus, samples behave differently when subjected to shearing and digestion treatment in the RVA canister.

A summary of the statistical analysis for the study results is presented in Table IV. Among the 10 laboratories participating in the full-scale collaborative study, the results from 1 of the labs were excluded from the statistical analysis. The viscosity values received from this lab were significantly lower compared with the average values for all participating laboratories, perhaps due to lack of proper RVA calibration. Additionally, a few scattered values among the laboratories were considered outliers based on statistical analysis. After excluding these outliers (2.4%), the statistical estimates, including Cochran, single Grubbs, and double Grubbs, were all below critical levels.

It has been reported that the precision of a method is mostly reflected by the standard deviations and that the quantitative measures of precision depend on the applied conditions, such as laboratory, operator, time between measurements, instrument calibration, and reagent batch (13). In this new method, two β-glucan standards were used to determine the variation within and between laboratories. The RSD_r values for the two standards were 5.3 and 7.1 (Table IV), which indicates the method was precise within laboratories. However, the RSD_R values were quite high (13.8 and 13.7) for the two standards. The high hygroscopic nature of pure β-glucan could result in lump formation during solubilization, which could affect viscosity development. Addition of a few drops of 95% ethanol (≈1 mL) prior to the addition of buffer improves solubilization of β-glucan. Proper solubilization of the β-glucan standard and regular RVA calibration are crucial to obtain a consistent viscosity measurement and achieve high reproducibility between lab-

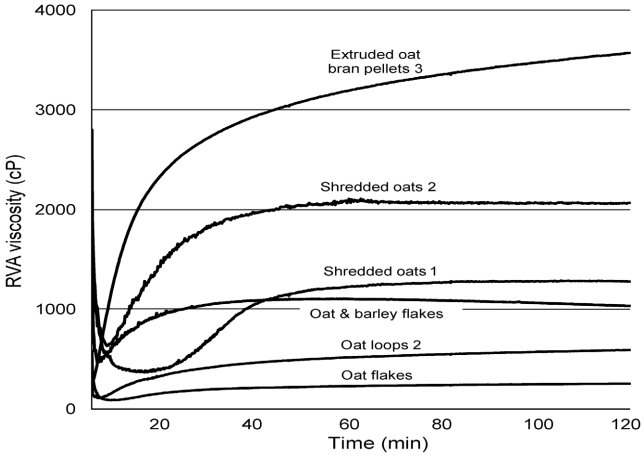

Fig. 1. Rapid visco analyzer (RVA) viscosity profiles for selected products tested in the full-scale collaborative study. Viscosity development over the test period (2 hr) is shown.

Table III. Summary of overall means and statistics for determination of the β-glucan viscosity measurement in cereal products using the rapid visco analyzer (mini-collaborative study)

	β-Glucan–Containing Cereal Product					β-Glucan Standard	
Statistical Parameter[a]	Extruded Oat Bran Pellets 1	Extruded Oat Bran Pellets 2	Pot Barley	Oat Loops	Rolled Oats	STD 1	STD 2
Total number of labs	5	5	5	5	5	5	5
Total number of replicates	15	15	15	15	15	15	15
Total number of outliers	0	0	0	0	0	0	0
Overall mean (cP)	2,834	3,012	510	802	693	206	447
Largest average lab result (cP)	2,933	3,138	544	862	737	229	474
Smallest average lab result (cP)	2,741	2,940	487	759	635	181	399
Repeatability S_r	48.7	36	24.3	30	28	9	9
Reproducibility S_R	82.9	87	30.2	47	55	19	30
Repeatability RSD_r	1.7	1.2	4.8	3.7	4.1	4.3	2.1
Reproducibility RSD_R	2.9	2.9	5.9	5.8	7.9	9.3	6.7

[a] S_r: within laboratory variability; S_R: between laboratory variability; RSD_r: within laboratory relative variability; RSD_R: between laboratory relative variability.

oratories. For all tested cereal products, RSD_r and RSD_R values were <10%, except for two products with regard to reproducibility. Method performance and lab consistency also were monitored using blind duplicates, which had an overall difference from their corresponding samples of <10%. It is important to mention that some laboratories were not able to use the recommended laboratory mill (M2 Universal, IKA-Werke) for milling the samples. Grinding using a 600 µm sieve also was not an easy task in some labs, in which case a 500 µm sieve was used instead. Additionally, the latest version of the RVA apparatus (RVA 4500) has a viscosity sensitivity limit of up to 10 cP, while older versions of the RVA, such as RVA-4, have a higher sensitivity limit (50 cP). Thus, achieving good repeatability of viscosity measurements (<10% variation) in low-viscosity products (≤200 cP) may not be straightforward. Consistent viscosity readings can be achieved for products with viscosity values of ≤300 cP. Despite all the factors that may affect viscosity and the repeatability of results, the method precision was statistically acceptable.

Method Recommendations

In general, the new method is robust and easy for food companies, food ingredient suppliers, and food research laboratories to use. Good precision can be achieved when the method recommendations are followed:

- The RVA calibration should be checked regularly. Calibration can be performed by the manufacturer and authorized agents. Verification of RVA calibration and performance over time can be conducted using standard materials and procedures available from the manufacturer or suppliers.
- The recommended laboratory mill (M2 Universal, IKA-Werke) or similar mill should be used. The milled sample must pass through a U.S. standard screen (No. 30, 600 µm). It is advisable to use a dry product or sample (moisture content ≤14%) that can be easily milled into fine particles.
- The method is recommended for testing low-moisture (<10%) products containing ≥2.0% (db) β-glucan or for products that contain 10–14% moisture and ≥3.0% (db) β-glucan. Low β-glucan content results in a high amount of sample in the canister, and effective mixing with buffer and enzymes is hard to achieve.
- Proper mixing of sample, buffer, and enzymes is essential to avoid the formation of lumps, which will result in artificially lower viscosity values than is typical. If lumps persist, the analysis should be repeated.

Summary

A new proposed AACCI Approved Method (32-24.01) for the measurement of β-glucan viscosity in cereal products using RVA has been developed and validated. The objective of this method is to determine the viscosity of β-glucan that is soluble under simulated digestion conditions without requirements for pretreatments (e.g., predigestion or extraction of β-glucan) of cereal products. This viscosity measurement is obtained by mixing an aqueous suspension of ground sample with digestive enzymes in a disposable canister in the RVA. The method can be used by industrial and research laboratories as a screening tool to identify products that may have positive physiological effects. It can also be used as a quality assurance method to ensure consistency of β-glucan characteristics in production.

Ten laboratories participated in the collaborative study to evaluate method performance, using eight cereal products, two β-glucan standards, and two blind samples. The method showed acceptable precision, with RSD_r values between 2.3 and 7.1 and RSD_R values between 4.5 and 18.6. A calibrated RVA and proper mixing of samples with buffer and digestive enzymes are keys to obtaining consistent results. In general, the results of the mini- and full-scale collaborative studies indicate the potential of this method to become an AACCI Approved Method.

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Table IV. Summary of overall means and statistics for determination of the β-glucan viscosity measurement in cereal products using the rapid visco analyzer (full-scale collaborative study)

	β-Glucan–Containing Cereal Product								β-Glucan Standard	
Statistical Parameter[a]	Oat Flakes and Clusters	Shredded Oats 1	Shredded Oats 2	Oat and Barley Flakes	Oat Loops 1	Oat Loops 2	Oat Granola	Extruded Oat Bran Pellets 3	STD 1	STD 2
Total number of labs	9	8[b]	9	9	9	9	9	8[b]	9	9
Total number of replicates	27	24	27	27	27	27	27	24	27	27
Total number of outliers	0	3	0	0	0	0	0	3	0	0
Overall mean (cP)	225	1,267	1,903	1,074	885	564	673	3,613	307	170
Largest average lab result (cP)	244	1,309	2,056	1,348	944	613	807	3,647	358	198
Smallest average lab result (cP)	184	1,190	1,736	808	809	473	375	3,567	236	130
Repeatability S_r	9.5	52.3	116.1	67.2	43.8	17.4	24.3	84.0	16.3	12.0
Reproducibility S_R	21.6	56.6	147.3	200.1	59.3	45.1	144.6	84.0	42.3	23.2
Repeatability RSD_r	4.2	4.1	6.1	6.3	4.9	3.1	3.6	2.3	5.3	7.1
Reproducibility RSD_R	9.6	4.5	7.7	18.6	6.7	8.0	21.7	2.3	13.8	13.7

[a] S_r: within laboratory variability; S_R: between laboratory variability; RSD_r: within laboratory relative variability; RSD_R: between laboratory relative variability.
[b] Outliers from one lab were excluded from the statistical analysis.

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