Effective Extraction of Soluble Non-Starch Polysaccharides and Viscosity Determination of Aqueous Extracts from Wheat and Barley

Rodica Caprita, Adrian Caprita, and Iuliana Cretescu

***Abstract*—Cereal grains contain various amounts of non-starch polysaccharides (NSP), which are composed predominantly of arabinoxylans (pentosans), ß-glucans and cellulose. The detrimental effect of soluble NSP is mainly associated with the viscous nature of these polysaccharides and their physiological effects on the digestive medium. Experiments were conducted to investigate the influence of some extraction conditions on the viscosity of wheat and barley aqueous extracts. Water extract viscosities (WEV) appeared to be related to the particle size of the meals obtained after grinding. The study shows that viscosities of aqueous extracts of wheat and barley samples have maximum values at 0.5 mm size. The experiments carried out at different extraction temperatures revealed that the optimum temperature for extraction the soluble fraction of NSP is 40°C. At lower temperature (20°C) the extractability of NSP soluble fraction was lower, and at higher temperature (60°C) viscosity increased sharply due to the starch gelatinization The increase in WEV might be also explained by an aggegation of the polymers. WEV increased with the extraction time up to 60 minutes, as a result of the NSP slowly solubilizing. The results also indicate that the time elapsed after centrifugation to viscosity measurement allowed soluble NSP to be hydrolyzed by endogenous β-glucanases and consequently their molecular mass was reduced. The dynamic viscosity decreased with 22% for barley and with 17% for wheat when measured after 60 minutes from the extract isolation.**

***Index Terms*—arabinoxylans, barley, dynamic viscosity, β-glucans, non-starch polysaccharides, wheat**

I. Introduction

Cereal grains contain various amounts of non-starch polysaccharides (NSP), which are composed predominantly of arabinoxylans (pentosans), ß-glucans and cellulose [1]. The detrimental effect of soluble NSP is mainly associated with the viscous nature of these polysaccharides and their physiological effects on the digestive medium.

The NSP content and type differ among grains. The NSP content relative to dry matter is lower in wheat kernel (11.4%) than in rye (13.2%) and barley (16.7%). Arabinoxylans are the predominant NSP in wheat (6-8%) and rye (8.9%), while β-glucans are the predominant NSP in barley (7.6%) [2].

ß-Glucans are glucose polymers containing a mixture of ß1-3 and ß1-4 linkages that make their physicochemical properties totally different from cellulose that is a straight-chain glucose polymer with only ß1-4 linkages. Barley contains a high level of mixed-linked ß-glucans (3-4%) [3]. Barley also contains an appreciable amount of soluble NSP other than ß-glucans [4].

The structures of cereal pentosans (arabinoxylans) are composed predominantly of two pentoses, arabinose and xylose, and their molecular structure consists of a linear ß1-4-xylan backbone to which substituents are attached through O2 and O3 atoms of the xylosyl residues [5]. Most of the arabinoxylans in cereal grains are insoluble in water, but the arabinoxylans not bound to the cell walls can form highly viscous solutions and can absorb about ten times their weight of water.

Soluble NSP increases the viscosity of the small intestinal chime, generally hampering the digestion process, whereas insoluble NSP impedes the access of endogenous enzymes to their substrates by physical entrapping [6], [7].

The anti-nutritive effect of soluble NSP is manifested through inhibition of digestion of starch, lipid and protein in the foregut [8]. The mechanism of action of soluble NSP is thought to involve increased viscosity of digesta which limits contact between digestive enzymes and substrates, and contact between nutrients and absorption sites on the intestinal mucosa [9]-[11].

The retention time of digesta during the passage through the gastrointestinal tract under the presence of NSP is an important factor for digestion and absorption of nutrients. Soluble NSP lead to a prolonged passage rate in the preceacal digestive tract, while insoluble NSP mostly showed no or only slight influence [12]. Soluble NSP in the form of ß-glucans showed viscosity-elevating effects due to an increased stimulation of digestive juices secretion, which was also observed for insoluble NSP, and to their enormous water-binding capacity [13], [14]. While most experiments examine the influence of NSP on gastric emptying, there are only a few which consider physiological effects in the small intestine.

Besides changed retention times in the digestive tract, the intestine motility inhibiting effects of soluble NSP play an

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important role [15]-[17]. The formation of a water layer between digesta and mucosa, and decreased motility lead to a reduced contact between digestible substrate and the specific enzymes and might cause problems involving nutrient absorption due to a reduced contact between resorbable substrates and mucosa.

The degree of thickening when exposed to fluids depends on the chemical composition and concentration of the polysaccharide [2]. Concentration, conformation and molecular weight distribution of soluble NSP is important for rheological properties such as viscosity and gel formation.

Measurement of water extract viscosity (WEV) in cereals is an indirect means of estimation of their soluble non-starch polysaccharide content [18], [19].

Experiments were conducted to investigate the influence of some extraction conditions: granulation, extraction temperature and time, on the viscosity of wheat and barley aqueous extracts, and to optimize the method for obtaining the soluble NSP extract.

II. Experimental

A. Apparatus

Dynamic viscosity was measured using a Wells Brookfield Cone/Plate Digital Viscometer Model DVIII Cone CP-40.

A LabTech LSB-015S water bath was used for the water extracting under constant shaking and temperature.

A Hettich 320R centrifuge was used for water extracts centrifugation.

A Kern ABJ 220-4M balance was used for precise weighing.

B. Procedures

The water-soluble fraction was obtained without endogenous enzyme inactivation, using a simple water extraction, with constant shaking, at three different temperatures: 20, 40 and 60°C. Incubation was performed for 15, 30 and 60 minutes for each temperature.

The grains were milled by a laboratory grinder. The extracts were obtained at a ratio of flour to deionised water of 1:2, by shaking the mixtures at 150 rpm in the water bath.

The extracts were centrifuged for 10 minutes at 5,000 rpm and 25°C.

Following the centrifugation, an aliquot of 0.5 mL supernatant was removed and assayed for dynamic viscosity at different times after the extract separation. Viscosity measurements were carried out at 25°C. All results were expressed in cP and calculated also as values relative to that of water.

III. Results and Discussion

First experiment studied the effect of granulation on WEV. Grinding grain samples breaks cell walls that contain NSP (cellulose, arabinoxylans, β-glucans) that are resistant to digestive enzymes [20], [21] and facilitates exposure of digestible components (starch, protein) to the action of digestive enzymes. Grinding increases the total surface relative to volume and thus exposure of nutrients to the action of digestive enzymes is improved [22]. Grinding contributes to digestive fluid, better mixing nutrients with digestive enzymes, and implicitly to more efficient use of cereals [23]. WEV appeared to be related to the particle size of the meals obtained after grinding. We studied the relationship between WEV and grain size for three granulations: 0.3, 0.5 and 1 mm. The results of experiments show that viscosities of aqueous extracts of wheat and barley samples have maximum values at 0.5 mm size. At lower or higher granulation the observed viscosities are lower and approximately equal (Fig. 1).

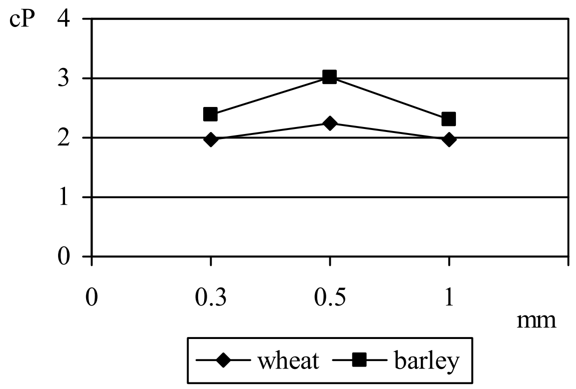

Fig. 1. The effect of particle size on the dynamic viscosity of the soluble fraction in wheat and barley.

Second experiment studied the effect of extraction temperature and time on the dynamic and relative viscosity of aqueous extracts. The influence of the time elapsed from the extract isolation until the viscosity measurement (time after centrifugation) on WEV was also studied. In order to observe better the variations in viscosity values, all experiments were carried out with wheat and barley grains milled by a laboratory grinder to pass through a 500 μm sieve.

The experimental results are presented in Tables I and II. WEV values for barley were higher than those for wheat, because of the presence of very high molecular weight β-glucans in barley.

Graphic representations of WEV obtained from wheat and barley at 20 and 40°C (Fig. 2 and Fig. 3) reveal higher dynamic viscosities for extraction at 40°C compared with extraction at 20°C, NSP solubility increasing with temperature in this range.

The viscosity of the extracts prepared from wheat and barley raised considerable when the extraction temperature was increased from 40 to 60°C. The significant increase of WEV at 60°C was probably caused by the gelatinization of the starch. The increase in WEV might be also explained by an aggegation of the polymers. Therefore, we consider that the optimum temperature for extraction of soluble NSP is 40°C.

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TABLE I
DYNAMIC AND RELATIVE VISCOSITIES OF WHEAT EXTRACTS

No.	Incubation temperature (°C)	Incubation time (min)	Time after centrifugation (min)	Dynamic viscosity (cP)	Relative viscosity (cP)
1.	20	15	0	1.94	2.20
2.	20	30	0	2.20	2.50
3.	20	60	0	2.30	2.61
			60	2.08	2.36
4.	40	15	0	2.34	2.65
			60	2.04	2.31
5.	40	30	0	2.36	2.68
			60	1.93	2.19
6.	40	60	0	2.46	2.79
			30	2.30	2.61
			60	2.04	2.31
7.	60	15	0	7.20	8.18

TABLE II
DYNAMIC AND RELATIVE VISCOSITIES OF BARLEY EXTRACTS

No.	Incubation temperature (°C)	Incubation time (min)	Time after centrifugation (min)	Dynamic viscosity (cP)	Relative viscosity (cP)
1.	20	15	0	2.44	2.77
2.	20	30	0	2.64	3
3.	20	60	0	2.74	3.11
			60	2.60	2.95
4.	40	15	0	2.80	3.18
			60	2.40	2.73
5.	40	30	0	3.11	3.53
			60	2.70	3.07
6.	40	60	0	3.32	3.77
			30	2.98	3.38
			60	2.60	2.95
7.	60	15	0	6.9	7.84

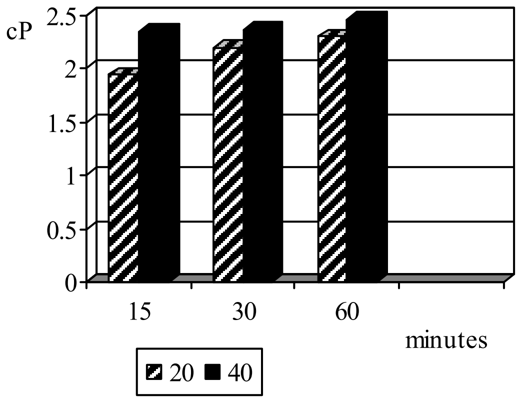

Fig. 2. Effect of temperature and extraction time on the dynamic viscosity in wheat.

The results demonstrated that the viscosities of the water extracts increased with the extraction time up to 60 minutes. The increase in viscosity with extraction time is the result of the NSP slowly solubilizing, so we recommend an incubation period of 60 minutes for a good soluble NSP extraction.

WEV values of aqueous extracts obtained at 40°C and 60 minutes extraction time show a decrease as the time elapsed from the extract isolation increased up to 60 minutes (Fig. 4). Soluble NSP were hydrolyzed by endogenous enzymes and consequently their molecular mass was reduced. The dynamic viscosity decreased with 22% for barley and 17% for wheat when measured after 60 minutes from the extract isolation.

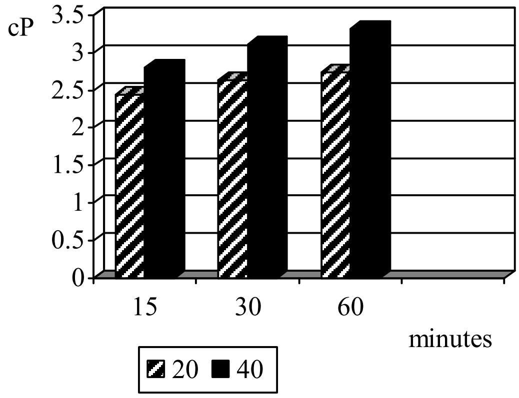

Fig. 3. Effect of temperature and extraction time on the dynamic viscosity in barley.

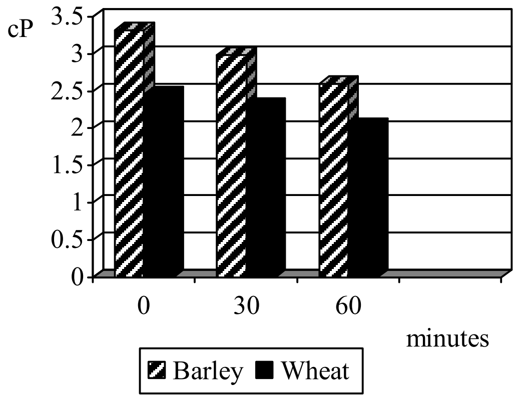

Fig. 4. Effect of the time elapsed after centrifugation on the dynamic viscosity in wheat and barley.

IV. CONCLUSION

The experiments revealed as optimum conditions for obtaining the soluble NSP extract from wheat and barley and for WEV determination:
- granulation of 0.5 mm size
- extraction temperature 40°C
- extraction time 60 minutes
- viscosity measurements immediately after extract isolation.

REFERENCES

[1] D. R. Lineback and V. F. Rasper, "Wheat carbohydrates", in: *Wheat: Chemistry and Technology*, vol. 1, Y. Pomeranz Ed. St. Paul: Am. Assoc. Cereal Chem., 1988, pp. 277-371.
[2] T. F. Schweizer and P. Würsch, "Analysis of dietary fiber", in: *The Analysis of Dietary Fiber in Food*, W. P. T. James and O. Theander Eds. New York: Marcel Dekker, 1981, pp. 203–216.

[3] G. S. Burnett, “Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation”, *British Poultry Science*, vol. 7, pp. 55-75, 1966.

[4] M. Choct and A. Kocher, “Non-starch carbohydrates: Digestion and its secondary effects in monogastrics”, *Proceedings of the Nutrition Society*, vol. 24,pp. 31-38, 2000.

[5] A. K. Sinha, V. Kumar, H. P. S. Makkar, G. De Boeck, and K. Becker, “Non-starch polysaccharides and their role in fish nutrition – A review”, *Food Chemistry*, vol. 127, pp. 1409-1426, 2011.

[6] M. R. Bedford, “Mechanism of action and potential environmental benefits from the use of feed enzymes”, *Animal Feed Science and Technology*, vol. 53, pp. 145-155, 1995.

[7] S. Steenfeldt, K. E. Knuden, C. F. Boring, and B. O. Eggum, “The nutritive value of decorticated mill fractions of wheat. 2. Evaluation with raw and enzyme treated fractions using adult cockerels”, *Animal Feed Science and Technology*, vol. 54, pp. 249–265, 1995.

[8] M. Choct and G. Annison, “The inhibition of nutrient digestion by wheat pentosans”, *British Journal of Nutrition*, vol. 67, pp. 123-132, 1992.

[9] G. Annison, “The role of wheat non-starch polysaccharides in broiler nutrition”, *Australian Journal of Agricultural Research*, vol. 44, pp. 405-422, 1993.

[10] M. R. Bedford and A. J. Morgan, “The use of enzymes in poultry diets”, *World's Poultry Science Journal*, vol. 52, pp. 61-68, 1996.

[11] H. M. Smits, A. Veldman, M. W. A. Verstegen, and A. C. Beynen, “Dietary carboxymethyl cellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens”, *Journal of Nutrition*, vol. 127, pp. 483-487, 1997.

[12] A. G. Low, “Nutritional regulation of gastric secretion, digestion and emptying”, *Nutritional Research Reviews*, vol. 3, pp. 229-252, 1990.

[13] H. N. Johansen, K. E. Bach Knudsen, B. Sandström, and F. Skjøth, “Effects of varying content of soluble dietary fibre from wheat flour and oat milling fractions on gastric emptying in pigs”, *British Journal of Nutrition*, vol. 75, pp. 339-351, 1996.

[14] N. Miquel, K. E. Bach Knudsen, and H. Jørgensen, “Impacts of diets varying in dietary fibre characteristics on gastric emptying in pregnant sows”, *Archiv Tierernährung*, vol. 55, pp. 121-145, 2001.

[15] C. Edwards, “Mechanisms of action of dietary fibre on small intestine adsorption and motility”, *Advances in Experimental Medicine and Biology*, vol. 270, pp. 95-104, 1990.

[16] B. Flourié, “Influence of dietary fibre on carbohydrate digestion and adsorption”, in: *Dietary fibre-A component of food: Nutritional function in health and disease*, T. F. Schweizer and C. A. Edwards Eds. London: Springer-Verlag, 1992, pp. 181-196.

[17] I. Furda, “Interaction of dietary fibre with lipids-mechanistic theories and their limitations”, in: *New developments in dietary fibre: physiological, physiochemical and analytical aspects*, I. Furda and C. J. Brine Eds. New York: Plenum Press, 1990, pp. 67-82.

[18] D. Boros, R. R. Marquardt, B. A. Slominski, and W. Guenter, “Extract viscosity as an indirect assay for water-soluble pentosan content in rye”, *Cereal Chemistry*, vol. 70, pp. 575-580, 1993.

[19] L. Saulnier, N. Peneau and J. F. Thibault, “Variability in grain extract viscosity and water-soluble arabinoxylan content in wheat”, *Journal of Cereal Science,* vol. 22, pp. 259-264, 1995.

[20] H. Jørgensen, X.-Q. Zhao, and B. O. Eggum, “The influence of dietary fibre and environmental temperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hind gut and energy metabolism in pigs”, *British Journal of Nutrition*, vol. 75, pp. 365-378, 1996.

[21] C. F. M. De Lange, “Characterisation of nonstarch polysaccharides in animal feeds”, in: *New Developments in Feed Evaluation*, International Postgraduate Seminar, Wageningen University, The Netherlands, 2000, pp. 77–92.

[22] K. J. Wondra, J. D. Hancock, G. A. Kennedy, R. H., Hines, and K. C. Behnke, “Reducing particle size of corn in lactation diets from 1,200 to 400 micrometers improves sow and litter performance”, *Journal of Animal Science*, vol. 73, pp. 421-426, 1995.

[23] S. J. Ohh, G. L. Allee, K. C. Behnke, and C. W. Deyoe, “Effect of particle size of corn and sorghum grain on performance and digestibility of nutrients for weaned pigs”, *Journal of Animal Science*, vol. 57(Suppl. 1), p. 260, 1983.

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