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Optimisation of yield and molecular weight of β-glucan from barley flour using response surface methodology

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ABSTRACT

The optimum conditions to maximize yield and molecular weight of β-glucan from Irish grown barley were determined using response surface methodology. The independent variables investigated were temperature, pH and their interaction on the extraction of β-glucan. Preliminary experiments indicated the best solid to liquid ratio and extraction time to be 1:5 and 4 h respectively. The model predicted that a temperature of 55.7 °C and pH 6.6 were optimal for maximising the yield and molecular weight of β-glucan. The reliability of the method was confirmed by performing experiments under optimal conditions and the experimental values were found to be in close agreement with the values predicted from the developed quadratic polynomial equation (average mean deviation (E) of 1.8% for extraction yield and 1.59% for molecular weight).

1. Introduction

Barley (*Hordeum vulgare*) is an important cereal crop grown throughout the developing world. Worldwide, the total production of barley in 2012 was 132 million tons and it was the fourth most abundantly produced crop after maize, rice and wheat (FAO, 2012). Approximately two-thirds of the global barley crop is used for animal feed, one third for malting and brewing of beer and whiskey and about 2% for food directly (Baik and Ullrich, 2008). However in recent years, barley in its pearled, whole or flaked form has increasingly been used in the manufacture of breakfast cereals, soups, porridge, baby foods etc (Bhatty, 1993a). In addition, the increased prevalence of obesity in the western countries has driven a trend in the use of barley and its components in breads and other baked products.

The favourable nutritive profile of barley is in part related to the presence of a water-soluble fibre component, which is a linear polysaccharide consisting of β(1 → 4) and β(1 → 3) glycosidic linkages, known as β-glucan. β-glucans comprise approximately 75% of the barley endosperm cell wall and are distributed uniformly throughout it (Miller and Fulcher, 1994). Interest in β-glucan has arisen because of its ability to lower the level of total and low-density lipoprotein (LDL) cholesterol (Brown et al., 1999) and also attenuate the postprandial glucose and insulin levels in the body (Wood et al., 1994). In fact, health claims linking β-glucan soluble fibre consumption to a hypocholesterolemic effect and reduced risk of coronary heart disease in the human body have been approved by FDA and more recently by EFSA (FDA, 2005; EFSA Panel on Dietetic products, Nutrition and Allergies, 2011). The mechanism of action of β-glucan is considered to result from the ability of this cereal fibre to form a gel-like network thus increasing the gastrointestinal viscosity (Brennan and Cleary, 2005). This ability is in turn a function of the molecular weight (MW) and concentration (c) of β-glucan in the solution (Lazaridou et al., 2003). In fact a direct relationship has been established between the log (MW × c) of β-glucan and log (viscosity) of the extract (Regand et al., 2011). Thus the parameter MW × c is a key indicator of the health promoting potential of extracted β-glucan.

Thus, a cost effective method for the extraction and enrichment of β-glucan from barley which maximises yield and MW would have immediate industrial applications. Reductions in MW of β-glucan during extraction can be brought about by a number of factors such as latent activity of β-glucanase (the major in-situ enzyme responsible for its degradation), acid/alkali depolymerisation or simply thermal degradation (Beer et al., 1997). In fact the

Abbreviations: MW, molecular weight; c, concentration; PBGR, peak blood glucose response; RSM, response surface methodology; CCD, central composite design; S:F, solvent to flour; HPLC, High performance liquid chromatography; SEC, size exclusion chromatography; analysis of variance, ANOVA.

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factor that most importantly dictates the MW is the enzyme β-glucanase and de-activation of this enzyme is crucial to the production of a high MW β-glucan product. Prior reflux with aqueous ethanol (Bhatty, 1993b) or extraction using sodium hydroxide has been used for achieving de-activation of the enzyme, but the yield of β-glucan drastically decreases in these procedures, with the added risk of degradation of the polymer (Burkus and Temelli, 1998; Wood et al., 1977).

The effect of multiple parameters such as solvent composition, temperature, pH, stirring rate on the efficiency of extraction of β-glucan has been studied (Bhatty, 1993b; Benito-Román et al., 2011; Dawkins and Nnanna, 1993). However, a systematic study evaluating the effects of the two crucial factors, temperature and pH on both the yield and MW of β-glucan has not yet been reported. The conventional approach for optimisation of a multivariable system is usually one variable at a time, which is time consuming. Response surface methodology (RSM) is a very useful and time efficient tool for this purpose as it reduces the number of required experiments. As a package of statistical and mathematical techniques employed for developing, improving and optimising process, RSM can be effectively used to evaluate the effects of multiple factors and their interaction on one or more response variables (Myers et al., 2009).

The aim of the present study was to optimise conditions for maximising the amount and MW of β-glucan in a single step, by altering the two very important extraction parameters, temperature and pH. With a view to maintaining the food friendliness of the procedure, water was used as the extraction solvent and extraction was carried out using mild conditions with minimal use of chemicals. Response surface methodology (RSM) based on a five level, two variable central composite design (CCD) was used for the optimisation of yield and MW and to generate insights in to the effect of the independent variables under investigation on these factors.

2. Materials and methods

2.1. Materials

Nine cultivars of Irish spring barley from the 2012 harvest were provided by Seedtech and Glanbia (Waterford, Ireland). Whole barley grains were milled using a Perten Lab mill 3100 (Perten Instruments, AB, Kungens Kurva, Sweden). Moisture content of the milled cultivars was determined based on the ICC method 110/1 (1976) using a Brabender moisture oven (Brabender, Duisberg, Germany). The mixed-linked β-glucan assay kit and five pure (>99% purity specified) MW standards of β-glucan were obtained from Megazyme International Ltd. Wicklow, Ireland. Tris (tris-(hydroxymethyl)-aminomethane) used in the preparation of HPLC mobile phase, sodium hydroxide (NaOH), hydrochloric acid (HCl), α-amylase and papain were purchased from Sigma, Wicklow, Ireland. Distilled water was used for the extractions.

2.2. Determination of β-glucan content

β-glucan content of the nine cultivars was determined according to McCleary and Glennie-Holmes (1985) using the β-D-glucan enzymatic assay kit. The concentration of β-glucans is reported in g of β-glucan/100 g of sample. The cultivar with the highest amount of β-glucan was used for further experiments.

2.3. Selection of the solvent to flour (S:F) ratio and extraction time

A preliminary study was conducted to determine the optimal solvent (water) to flour ratio to extract β-glucan from barley. The extraction was carried out using 10 g of milled barley flour and five different volumes of water: 20, 50, 100, 200, 250 ml giving S:F ratios of 2:1, 5:1, 10:1, 20:1 and 25:1 respectively. The temperature, time and pH were maintained constant at 50 °C, 3 h and 7.0 respectively and the samples were agitated at 200 rpm. A second set of experiments, aimed at determining the best time for extraction of β-glucan from barley was also conducted. The optimal S:F ratio selected from the previous test (5:1 v/m) and identical extraction conditions were employed. The extractions were carried out at one hour intervals ranging from one to five hours. Only the extraction yield was evaluated for optimisation of these parameters. The β-glucan content of the barley residue recovered after extraction was determined. The method of Benito- Roman et al. (2011) was used for calculation of β-glucan yield, using the following equation (Eq. (1)):

$$\textit{Extraction yield}\ (\%) = \frac{\%\ \beta-\textit{glucan in raw barley} - \%\ \beta-\textit{glucan in exhausted barley}}{\%\ \beta-\textit{glucan in raw barley}} \times 100 \quad (1)$$

2.4. Single factor experiments for determining the lower, middle and upper levels of extraction temperature and pH

A second preliminary series of single factor experiments to determine the range to be used for the two variables (temperature and pH) in the RSM model were carried out. Milled barley grain (10 g) was extracted at three temperatures (30 °C, 60 °C and 90 °C) and three pHs (4.0, 8.0 and 12.0) at the S:F ratio (5:1) and time (4 h) optimised in the previous experiment. The pH of water was manipulated using 0.1 N HCl or 1 M NaOH. In this case, the effect of these two variables on both the extraction yield (Eq. (1)) and MW of β-glucan were investigated.

2.5. Determination of MW of barley β-glucans

Prior to determination of molecular weight, the extracts were centrifuged for removal of the exhausted barley and starch residue. Further, removal of impurities was carried out using an enzymatic method (Ahmad et al., 2010). The extract was first treated for removal of starch with α-amylase at 50 °C, pH 6.0 for 1 h, followed by centrifugation at 8000 g for 10 min. The supernatant was then treated with the protease enzyme papain, at 60 °C, pH 6.0 for 2 h, for removal of proteins. After centrifugation of the extract, the enzymes were de-activated by incubating the extracts at 100 °C for 10 min. It has been shown in a previous study that the purification (including α-amylase and heat treatment at 100 °C) does not lead to any significant degradation of the β-glucan molecule (Mikkelsen et al., 2010).

The molecular weight of β-glucan in the liquid extracts was determined using size exclusion chromatography (HPLC–SEC) on an Agilent 1100 series chromatography system consisting of a G 1311A pump, a G1313A automatic sample injector, guard column (SB-G 6; Shodex), a gas permeation (GPC) column (SB-804HQ; Shodex) and a differential refractive index detector G1362A (Agilent 1200 series). Samples were filtered through syringe