Establishment and Evaluation of a Barley Starch Isolation Method with Focus on Representability

Åsa Martén

Supervisor: Anna Källman, Department of Food Science, SLU

Examiner: Kristine Koch, Department of Food Science, SLU

Credits: 30 hec
Level: Advanced A1E
Course title: Independent Project in Food Science
Course code: EX0427
Programme/education: Agronomy Program

Place of publication: Uppsala
Year of publication: 2012
Cover picture: Salla Marttila, SLU, Alnarp
Title of series: Publikation/Sveriges lantbruksuniversitet, Institutionen för livsmedelsvetenskap
Serie no: 388
Online publication: http://stud.epsilon.slu.se

Keywords: Cereal starch isolation; Barley starch; Starch recovery; Starch purity; Amylose-Amylopectin ratio; B granules; Chemical deproteinisation; Toluene shaking; Proteinase K

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Food Science

Abstract

The high viscosity of barley material makes starch isolation problematical using regular methods established for cereals. An adjusted starch isolation method has been set up for barley, based on fractionation and purification. The focus is on attaining truly representative isolates of six flour samples selected for widely differing characteristics within the research program *BarleyFunFood (BFF)*. Beside establishment of the method, this diploma work aspires to serve the BFF with isolated material of sufficient yield valid for further starch characterisation. A pre study was conducted evaluating available wet mixing equipment, experimental conditions and mode of procedure. The yield and purity of isolates of the *BFF* flours were determined to 55.7-72.2% and 61.0-76.4% respectively. Due to an error in the procedure, that partly explains the poor results, one of the samples was isolated de novo resulting in an increase of the yield from 59.7 to 96.2%. Additionally, the amylose-amylopectin ratios were determined to approximately 2/99 in a waxy type, 41/59 in one high-amylose type and 30/70 in remaining varieties. New isolations are suggested to be carried out prior to further analysis of the remaining flours to achieve higher representability.

Keywords*: Cereal starch isolation; Barley starch; Starch recovery; Starch purity; Amylose-Amylopectin ratio; B granules; Chemical deproteinisation; Toluene shaking; Proteinase K*

Sammanfattning

Hög viskositet i kornmaterial gör tillämpning av befintliga stärkelseisoleringsmetoder för spannmål problematiskt. En isoleringsmetod, baserad på fraktionering och rening, har etablerats för korn med fokus på att uppnå högt representativa stärkelseisolat. En förstudie genomfördes för att utvärdera tillgänglig utrustning för våtmixning, experimentella betingelser och generellt tillvägagångssätt. Stärkelse isolerades från sex mjölprover utvalda för sina vitt skilda egenskaper inom forskningsprogrammet *BarleyFunFood* (*BFF*). Utöver etablering av metoden, strävar detta examensarbete till att tjäna *BFF* med isolerat material med tillräckligt högt utbyte för vidare analys. *BFF*-isolatens utbyte och renhet bestämdes till 55,7-72,2% respektive 61,0-76,4%. De låga resultaten berodde delvis på ett inställningsfel under isoleringsproceduren. Ett av proverna (0120) isolerades därför på nytt under korrekta betingelser. Utbytet ökade då från 59,7 till 96,2%. Dessutom bestämdes amylos-amylopektin ratiot till ungefär 2/98 i en vaxig sort, 41/59 i en högamylossort och cirka 30/70 i övriga sorter. För att uppnå högre representativitet på resterande prover föreslås nya isoleringsförsök för vidare analys av stärkelsen i *BFF*-materialet.

Nyckelord: *Cereal Stärkelseisolering; Kornstärkelse; Stärkelseutbyte; renhet; Amylos-amylopektin ratio; B-granuler; Kemisk proteinavlägsning; Toluenskakning; Proteinas K*

Table of contents

INTRODUCTION **5**

THE BARLEYFUNFOOD PROJECT 5

BARLEY STARCH 6

BARLEY STARCH ISOLATION 6

MATERIAL & METHODS **7**

MATERIALS 7

METHOD 7

Pre study *7*

Isolation procedure 8

Starch determination 8

Dry matter content determination 9

Scanning electron microscopy (SEM) of granules 9

Major study *9*

Isolation, starch content analysis and dry matter determination 9

Amylose-Amylopectin analysis 10

RESULTS **12**

PRE-STUDY 12

Yield and purity *12*

Degree of damaged starch granules *12*

MAJOR STUDY 14

Yield and purity *14*

Amylose and amylopectin ratio *15*

DISCUSSION **16**

CONCLUSIONS **19**

ACKNOWLEDGEMENTS 20

LIST OF REFERENCES 21

Introduction

Barley (*Hordeum Vulgare L.*) has historically been the main bread cereal in Europe and is still of global importance as alimentation, being the fourth largest cereal produced worldwide. Nowadays barley is used mainly as feed and in malting and brewing, making it the second most commonly grown cereal in Scandinavia with a year production of 1,44 million tons (The Swedish Board of Agriculture, 2007). The very small use of barley for food prevails in the form of groats, soup thickener or as ingredient in breakfast cereals, bakery goods and pasta. Barley as part of the human diet has lately been shown increased interest regarding its nutritional aspects. A high content of soluble dietary fibres, as β-glucans is the main contributor to the so called *hypocholesterolemic effect* (lowering of serum cholesterol) imparting health claims to barley consumption.

Covered barley, as originally occurring, with husk tightly cemented to the pericarp are the types always used in preparation of malt. Through breeding development barley also exist in form of several hull-less varieties, suitable for food use, since abrasion causes nutrient loss. The extensive variation of barley genotypes expressing different grain- and spike morphology (two- and poly-row) starch quality and protein characteristics is a result from both induced and spontaneous mutations and offers the opportunity to select particular genotypes for specific uses.

Barley as a source of starch for either food- or nonfood applications is uncommon but has commercial potential, possessing high swelling power, good freeze-thaw stability and unique film-forming properties. Production of ethanol, extraction of β-glucan and preparation of native and modified starch are other applications of barley. Naturally, isolated barley starch also applies to basic research, aiming to elucidate the detailed molecular structure and chemical properties of the starch components.

The over-all goal for this diploma work is to establish and evaluate a laboratory scale starch isolation method based on fractionation and purification of six barley flours, to facilitate future work within the research program *BarleyFunFood (described below)*. Apart from the method set-up, this work aspires to generate starch isolates of adequate yield for further molecular characterisation of starch. Focus has been on reaching the requirements of the *BarleyFunFood* project by acquiring quantitative isolates that highly represents the true distribution of granule sizes. Representability correlates to a quantitative yield, why high yield rather than purity is desired in this isolation trial. Furthermore, a quantitative analysis of amylose and amylopectin of isolates is also included in the study.

The BarleyFunFood project

BarleyFunFood is one of four present multidisciplinary research programs at the Faculty of Natural Resources and Agricultural Sciences at the Swedish University of Agricultural Sciences (SLU). The project was initiated during the 80's and is a collaboration involving SLU, Lantmännen R&D and SW Seed, Svalöf Weibull AB. Research work within plant biology and breeding, food science, chemistry, microbiology and nutrition is exploring the biology of barley and nutritional effects of cereal carbohydrates.

A collection of 250 barley varieties with a wide genetic background have been screened regarding composition using near infrared reflectance techniques. Among the material were both naked and covered, round-shaped and elongated kernels as well as waxy and high-amylose types

represented. Out of the collected varieties, 20 lines were selected for amplification based on phenotype descriptions as well as for interesting carbohydrate composition, interpreted from spectral data. For the possibility to achieve double generations in one year, cultivation was located to the south hemisphere in the village Vilcún, in Chile where the average temperature is around 15˚ C and winters are mild, somewhat resembling Swedish climate conditions. The 20 lines were grown in the winter of 2010. The starch content in these varieties varies between 30 and 60 % and among them are four low-amylose mutants. The selection was narrowed down to five with large differences in carbohydrate composition. The Swedish feed barley variety *Gustav* was also included, resulting in totally six samples.

Barley starch

As in other types of cereal and higher plants, starch constitutes the major energy storage component of barley. Starch molecules are organised in highly ordered layers stored as granules of various shapes formed in amyloplasts in the plant cell. The starch content of barley varies between 47-67 % (Oscarsson et al., 1996; Li et al., 2001; Åman et al., 1985; Frégeau-Reid et al., 2001). As most common starches, the two distinct polymer fractions amylopectin and amylose, with clearly different molecular weight and structural organisation, constitute barley starch. Amylopectin is a large polymer built up of several α-(1→4)-linked D-glucose chains connected to each other through α-(1→6)-linkages arranged in clusters. Three types of unit chains, arranged in clusters, constitute the molecule; the outermost unbranched A-chains connected through their reducing group to B-chains, carrying either A-chains and/or other B-chains; and C-chains carrying all other chains and containing the only reducing group (Eliasson et al., 1987). Amylose is an essentially linear polymer comprising the same residues and type of linkages as amylopectin but is only branched at a few locations. The amylose content is normally 19-30 % of total starch but varies from 0 % in waxy types up to 44 % in high-amylose varieties (Andersson, A, 1999; Fredriksson et al. 1997; Zheng & Batty, 1998). Degradation of starch results in glucose moieties, valid as fuel in all biological systems including the human body.

Barley starch expresses a distinct bimodal distribution of granule sizes with both large lenticular/disc-shaped A-granules (10-30 μm) and small spherical B-granules (<6 μm), similar to wheat and rye (Delcour & Hoseney 2010, 1993; Bathgate & Palmer 1972; Stoddard, 1999). The number of A-granules is lower (10% of total number) than that of B-granules, but these on the other hand represent the major mass (90%) of the starch (Stoddard, 1999; Bathgate & Palmer, 1972) with variations among genotypes, as investigated by Li et al. (2001).

Amylose and amylopectin are not equally distributed in A- and B-granules, as stated by Bathgate and Palmer (1972), there is a higher amylose content of B-granules than of A-granules. Ao & Jane (2006) reported an amylose content of 28.1% in A- and 23% in B-granules. Ao & Jane also proposed structure models of the amylopectins of the A- and B-granule starches based on results from chain length (CL) analysis. A higher degree of polymerisation was found in A-granule amylopectin chains (26.7 units compared to 24.9 units in B granules), which was also verified by Salman et al. (2008), who also report larger lamellar repeat distance of A-granules. The amylose-amylopectin ratio strongly influences the physical properties such as gelatinization temperature, retrogradation rate etc. of starch, as investigated by Fredriksson et al. (1997) and Bathgate & Palmer (1972).

Barley starch isolation

The harder texture and lower water contents of cereals compared to other botanical materials, such as potatoes, makes isolation of cereal starch in general challenging. Cracking and milling of the starchy endosperm, which naturally is a crucial treat, unavoidably results in partial physical

damage of starch granules. Available isolation procedures suitable for wheat and other cereals have not shown to be satisfactory applicable to barley. The major endosperm cell wall component β-D-glucan and the generally high dietary fiber content (14-24% according to Andersson et al., 1999) contribute to high viscosity, which obstructs starch extraction. This adds to the cereal associated difficulty with starch isolation from barley in particular, which has led to the need of developing a customised isolation method.

In Sweden, commercial starch is isolated through alkali extraction, where starch is purified through stepwise fractionation based on size and solubility properties of the different components in increased pH. Generally, starch isolation methods include several centrifugation steps in order to separate the sample into fractions based on mass. On centrifugation, a white layer is produced at the bottom, mainly comprising starch, overlaid by a darker grey-purple coloured layer (generally referred to as the 'brown layer'), containing basically protein. Already reported by Bathgate & Palmer in 1972 there is a tendency for small starch granules to associate with this protein fraction with a content of 1.5 % associated protein in B-granules versus 0.2% in large ones. Hence, there is a considerable loss of small granules on discarding the proteinous layer, which in turn implies a poor representability of the isolated starch. Available isolation methods have been investigated by McDonald & Stark (1988), stressing the importance of retrieving small granules trapped within the proteinaceous layer to obtain representative isolates. The industry on the other hand, employ isolation methods providing high yields to lowest possible cost, where retrieval of B-granules is not prioritised.

Material & Methods

Materials

Barley flours and reference materials- The covered barley (*Hordeum Vulgare*) cultivar *Golf*, with a starch content of 63.8% was used for isolation trials within the pre study. The Major study included six barley types grown in Vilcún (Chile) within the project *BarleyFunFood*; one with shrunken endosperm (0155), one antocyanin-rich (0120), one high-amylose (0228), one lysine-rich (0181), one waxy (0224) variety and one feed variety (0249). All samples had been crushed in a Cemotec 1090 Sample Mill (Tecator) and subsequently milled to flour with a particle size of 500 μm, using a Retsch Type ZM 1. Native starch and commercial potato flour from Lyckeby Stärkelsen (Kristianstad, Sweden) as well as Defatted Barley Starch from ALKO ltd (Helsinki, Finland) were used as reference samples in starch content analysis.

Chemicals - Proteinase K (origin: *Tritirachium album;* activity: 30 U/mg), was purchased from Sigma-Aldrich **(**St.Louis, USA**).** Pullulanase M1 (2.00 U; *origin: Klebsiella planticola;* activity: 699 U/mL), Isoamylase (activity: 520 U/mL), thermostable α-amylase (activity: 3.000 U/mL) and amyloglucosidase (activity: 3.260 U/ml) and Glucose standard solution (100 μg/ 0.1 ml) were obtained from Megazyme (Wicklow, Ireland)

Method

Pre study

A pre-study was conducted in order to find optimal experimental conditions such as sample size, equipment for wetmixing and mixing time as well as to become familiar with the behaviour of the material. Among isolation procedures previously described in the literature, the method by McDonald & Stark (1988) was selected as a basis for this study in order to get a representatitive starch isolate. Some modifications were done in order to adapt for the current sample material.