Investigation of beta-glucan extracts from barley grain as ingredient in food formulations

Thesis submitted for the requirement for the degree of Doctor of Philosophy in Food and Nutritional Science

Dana Khaled Alsalem BSc, MSc

Department of Food and Nutritional Sciences

March 2021

Declaration

I confirm that this is my own work and the use of all materials from other sources have been properly and fully acknowledged.

Dana Khaled Alsalem

March 2021

ABSTRACT

Barley has been cultivated since antiquity and is the richest source of beta-glucans among all other cereals. Consumption of beta-glucans has been associated with positive health effects in humans. Specifically, the Federal Drug Administration (FDA) supports the claim that beta-glucans can reduce serum cholesterol and insulin levels and recommends consumption of three grams of beta-glucans per day for health benefits. Although barley is widely available, barley grain consumption is currently quite low, as rice and wheat are more popular types of cereals. The overarching purpose of this research project was to identify alternative ways for the utilisation of barley in the food industry and explore the technological properties of barley beta-glucans as a functional food ingredient. To this end, the main goal of the present study was to develop an efficient extraction process for beta-glucans from barley grains grown under different environmental conditions (Jordan and the UK) and incorporate these extracts into a food product to investigate the main physicochemical properties they impart in the food matrix. The experimental work of this thesis included the investigation of two extraction methods targeting beta-glucan extraction from barley flour. The first was conventional hot water extraction (HWE) method, in which different extraction times (90 min, 3 h and 4 h) and temperatures (50°C, 60°C and 70°C) were tested on defatted UK and Jordanian barley flour. The highest beta-glucan recoveries for the UK (9.3%, w/w) and the Jordanian barley (10.5%, w/w), respectively, were achieved at 60°C for 4 h and 50°C for 3h, respectively. Due to the low recovery yields of HWE, another method was investigated, that of ultrasound assisted extraction (UAE). Optimisation of the UAE conditions was followed by characterisation of the physicochemical and functional properties of the extracts. At the optimum extraction conditions for each barley cultivar, the highest beta-glucan recovery of 73.2% (w/w) was obtained for the Jordanian barley at a UAE amplitude

of 15 for 35 min, which provided extracts with beta-glucan purity of 12.9% (w/w). For the UK barley, 10 A ultrasonication for 20 min resulted in beta-glucan recovery of 55.5 % (w/w), containing 10% (w/w) beta-glucans. The extracts contained also low amounts of protein (less than 0.35%, w/w) but considerable amounts of starch (53.3% for Jordanian 22.4% for UK barley, respectively). Subsequently, the effect of barley flour substitution levels, as well as the addition of UAE extracts in cracker formulations was investigated. Six cracker formulations were prepared with barley flour inclusions of 10–60% (w/w). Two wheat cracker formulations enriched with beta-glucan extracts obtained via UAE were also developed. The resulting products were evaluated for their beta-glucan content, colour (L*, a* and b*), texture (hardness and crispness), water activity, moisture content, and dough penetration. Crackers produced with various proportions of barley flour demonstrated, as predicted, higher beta-glucan content than wheat crackers (control), ranging from 0.377 g/100 g for 10% UK barley flour to 1.542 g/100 g for 60% Jordanian barley. Wheat crackers with beta-glucan extracts demonstrated the highest beta-glucan content of 2.436 g/100 g and 2.673 g/100 g using the UK and Jordanian barley extracts, respectively. Barley flour cracker formulations exhibited darker colour, had greater redness values, were harder and less crispy than control wheat crackers. When comparing the replacement 60% wheat flour by barley flour with the addition of beta-glucan extract, a significant improvement in water activity and textural properties of the final product was observed. Moreover, UAE beta-glucan extracts were considered suitable to produce crackers with a high beta-glucan content that could meet the US FDA requirement.

Keywords: Barley, beta-glucan, hot water extraction, ultrasound-assisted extraction, crackers, texture, colour

ACKNOWLEDGEMENTS

I express my sincere gratitude to Dr Afroditi Chatzifragkou and Dr Julia Rodriguez Garcia for their supervision, guidance and continuous support during these years and especially these difficult times and uniquely challenging circumstances. I feel truly honoured to have worked with them, and I am forever grateful for having them as my supervisors.

I also would like to express my appreciation to all technical staff in the Food Bioscience (Harry Nursten) Building at the University of Reading for their assistance, cooperation and time in assisting me. To my colleagues in Lab 3.39, Room 2.01, thank you so much for your encouragement and continuous support you have given me during these years. A very special thanks to the Public Authority of Applied Education and Training in Kuwait for the opportunity to study in Reading.

I send very warm gratitude to all my family members, especially my dad Khaled and my mum Dr Lolwa for their endless support and encouragement throughout this PhD journey. The support that they provided helped me a great deal through this unforgettable journey. A very special thanks to my beloved son Aziz! I cannot imagine how I could accomplish this without your love, and seeing you brightens my days and nights.

A very warm and special thank you to my sister, coach and my support system Hadeel Almehri, for helping me overcome all the difficulties in the last 18 months, especially the quarantine life that I lived alone with my son. Changing my mindset helps me live in the moment, appreciate all the small things in my life and help me focus on the task at hand and find peace and pleasure in it.

Finally, To all my friends in Reading and Kuwait, the journey would not have been that beautiful without your love and support. Special thanks to my best friend Shaikha Alqemlas for always being there when I needed her and reassuring me during my difficult times, and taking care of my son for almost two years.

Thank you all.

Dana Khaled Alsalem

Table of Contents

List of Figures

List of Tables

Chapter 1: **INTRODUCTION**

Barley is one of the oldest cultivated cereals, emerging as a staple food before rice and wheat became popular. Currently as the fourth-largest grain crop, barley is garnering great interest, especially in the food industry. It is known to have the highest concentration of the mixed linkage $(1 \rightarrow 3)$, $(1 \rightarrow 4)$-β-D-glucan among cereals, known commonly as beta-glucan. Beta-glucan is a non-starch polysaccharide located in the cell walls of the endosperm of barley grains. Barley beta-glucan content varies from 2 to 11% and is influenced by genetic and environmental factors. Over the last two decades, beta-glucans have been considered bioactive ingredients due to their capacity to lower plasma cholesterol, improve lipid metabolism, and reduce glycaemic index (Lan-Pidhainy *et al*., 2007).

Beta-glucans has been determined by the US Food and Drug Administration (USFDA) and the European Food Safety Authority (EFSA) to have functional characteristics associated with the reduction in the risk for cardiovascular disease (Zielke *et al*., 2017). A variety of physiological functions of beta-glucan have been reported, such as reducing postprandial blood glucose (Singhal and Kaushik, 2016), lowering serum cholesterol (Mikkelsen *et al*., 2017a) and promoting intestinal health (Miyamoto *et al*., 2018). However, while barley possesses interesting nutritional properties because of its high beta-glucan availability, barley grains are mainly used worldwide for the manufacturing of alcoholic beverages (e.g. beer) and as feed for livestock (Mosele *et al*., 2018). Barley is not commonly used as food ingredient, in part due to its sensorial properties, as people prefer the texture of rice and wheat (Izydorczyk and Dexter, 2008b). Technological constraints may also be responsible, as the extraction process of complex beta-glucans is linked with higher costs (Zhu *et al*., 2016). Realising the full advantages of barley beta-glucans requires greater barley availability and the efficient extraction of its beta-glucans. Therefore, the purpose of this study

was to optimise the extraction of beta-glucans from barley grains grown under completely different environmental conditions (UK and Jordan). Beta-glucan were obtained by hot water and ultrasound-assisted extraction and the efficacy of those two extractions was evaluated. Furthermore, both UK and barley flours were incorporated as wheat flour substitutes in bakery formulations, to assess their technological impact on the dough and the final products. Additionally, extracts derived from ultrasonication assisted extraction process were also evaluated as ingredients in bakery formulations, with a view to develop beta-glucan-rich products.

1.1 Aims and Objectives

The aim of this study was to generate novel information on the impact of ultrasonication assisted extraction as means of beta-glucans extraction from barley and the implications of barley flour and extracts on product formulations. The specific objectives of the study were as follows:

- Identify differences in the composition of whole-grain barley cultivated in different environmental climates (namely, Jordan and the UK).
- Develop an ultrasound-assisted extraction process optimising beta-glucan extraction from barley grains in terms of yield and purity.
- Evaluate the effect of wheat flour replacement with barley flour on the physicochemical properties of bakery products.
- Develop novel food products with beta-glucan as a functional ingredient by understanding the link between barley extracts composition and their physicochemical properties as food ingredients.

1.2 Hypotheses of the Thesis

- Environmental conditions during barley cultivation would result in different cell wall polysaccharide composition, which could influence beta-glucan extraction (Chapter 3)
- UAE is an efficient extraction method to extract beta-glucan from barley grain flour (Chapter 4)
- Composition of barley flour influences the physicochemical properties of cracker formulations (Chapter 5)
- The addition of barley extracts would yield crackers with better quality parameters compared to wheat flour substitution approach (Chapter 5)

Chapter 2: **LITERATURE REVIEW**

2.1 Introduction

Whole grain cereals, such as oats, barley, wheat and rice, are considered staple foods worldwide because they are nutrient-dense, high in fibre, widely available and can grow in a diversity of climates. One of the important characteristics of whole-grain cereals is the naturally-occurring high dietary fibre content, which has been acknowledged for its critical role in a balanced diet (Collar and Angioloni, 2014). A diet rich in dietary fibre has been associated with multiple health benefits, such as a reduction in glycaemic index, weight control through increased satiety, relief of constipation, slowed gastric emptying, reduction of blood cholesterol levels and maintenance of insulin in the human body (Brennan, 2005, Behall et al., 2004). Another major health benefit is in reducing symptoms intensity of cancer, as several studies have shown that a diet rich in dietary fibre may reduce sympotoms of colon and breast cancer (Burkus, 1996, Behall et al., 2004, Anderson et al., 1994).

Beta-glucan are non-starch polysaccharides, mainly composed of glucose units linked with beta-glycosidic (1, 3) and (1, 4) bonds. The beta-glucan content in barley and oats is higher than in other cereal grains (Havrlentova et al., 2011). Beta-glucan plays a significant role in reducing blood cholesterol and maintaining insulin levels in humans, chickens and rats, as reported by multiple studies (Baik and Ullrich, 2008, Newman and Newman, 2006). The US FDA has supported the claim that beta-glucan can reduce serum cholesterol and insulin levels, and a daily intake of three grams of beta-glucan is recommended for the human body to derive benefit (Vasanthan and Temelli, 2008). One of the mechanisms by which beta-glucans reduces blood cholesterol levels may be related to their effect on the viscosity of the fluids in which they are

present (Marlett et al., 1994). When mixed with water, beta-glucans can form a gel-like network and accordingly improve the viscosity of the bolus throughout the human gastrointestinal system (Brennan, 2005). Moreover, due to the ability of beta-glucans to entrap bile acids in the intestine and eliminate them from interaction with the luminal membrane, the removal of bile acid in the faeces increases, resulting in significantly lower blood cholesterol levels (Chen and Huang, 2009).

Approximately two-thirds of worldwide barley production is used for animal feed, one-third for malting and 2% for human food products, such as baby food or pearled barley in soups (Baik and Ullrich, (2008). The main reason for the low percentage of barley in food production is that the perceived product quality and mouthfeel of barley products is lower than those of wheat and rice products (Newman and Newman, 2006). In addition, barley lacks various functionalities offered by other cereal products. For example, the quality of gluten proteins in barley is lower than in wheat, thereby creating low-quality breads due to decreased loaf volume (Zhen et al., 2015). Additionally, barley grains contain significant amounts of polyphenol oxidase, an enzyme known to interact with phenolic compounds and produce o-quinones, which, when reacting with other phenolic compounds or amino acids, cause discolouration in barley food products (Sharma and Kotari, 2017). Such discolouration is undesirable, further limiting the use of barley in food products (Sharma and Kotari, 2017). Since barley has the highest amount of beta-glucan of all other cereals (up to 11%) and due to the unique properties of these molecules, they can be extracted and used as novel ingredients in new product formulations. This extraction will add value to the barley grain since a fair amount of barley that is not used directly in food production could be used as starting material for beta-glucan extraction.

2.2 Barley

Barley (*Hordeum vulgare* L) is a cultivated grass categorised within the family Poaceae and was one of the first ancient cultivated crops (Nilan and Ullrich, 1993). Native barley cultivation has been investigated in the Middle East, specifically in Jordan (Badr et al., 2000, Nilan and Ullrich, 1993). Worldwide, barley ranks fourth in cereal crop production, and one of its unique characteristics is its ability to grow in almost all climates. Barley is recognised as one of the most genetically diverse cereal grains. It is classified as a spring or winter type, two-row or six-row type, malting or feed type or hulled or hulless depending on the presence or absence of a hull tightly attached to the grain (Baik and Ullrich, (2008). Moreover, based on grain composition, barley is classified as a normal, waxy or high amylose starch type, with high lysine, high beta-glucans and absence of proanthocyanidins.

The physical and compositional characteristics of barley differ, depending on the type (Baik and Ullrich, (2008). The primary type of barley cultivated is hulled barley with an attached fibrous husk. In contrast, hulless barley has a loose husk, which is easily removed during harvesting. This type of barley is not favourable in the malting industries due to the lack of hulls, which play a significant role in developing flavour. Therefore, hulled barley is favourable for malting, whereas hulless barley is commonly used in animal feed and, more recently, for incorporation into food products. One of the functions of the hull in malting barley is to protect the germinating embryo from any mechanical injury, thus providing greater uniformity during the germinating process. Hulless barley tends to be more efficiently used in the food industry since it has a higher beta-glucan content. The high beta-glucan content in hulless barley is mainly the result of the barley husk lacking beta-glucan and consisting mostly of cellulose, hemicellulose and lignin,

which are known to dilute the nutrient contents in hulled barley. In addition, the husk constitutes approximately 10%–13% of the barley grain dry weight (Fadel et al., 1989, Bhatty, 1999b).

Approximately 40%–50% of the barley crop in the UK is used for animal feed as barley is a rich source of carbohydrates and protein (Brennan and Cleary, 2005a). Although the main uses of barley are currently limited to malting and animal feed, the high concentration of beta-glucans in barley has a negative effect in both industries (Vis and Lorenz, 1997). Beta-glucans create a highly viscous solution, which causes problems in brewing industries, such as slowing of the filtration process, a problem resolved by using enzymes. Additionally, using barley as animal feed reduces feed digestibility and affects the animal's weight gain, which is why barley feed must be pre-treated with beta-glucan degrading enzymes.

However, concerning human consumption, there is a growing trend toward food products that are high in fibre, such as oats and barley. There is considerable customer demand to develop products that are palatable and have high nutritional value. This increase in demand is mainly due to health concerns regarding high cholesterol, obesity and cancer and the need to have healthy convenience foods suitable for working couples, single households and students (Fuentes-Zaragoza et al., 2010). Currently, food products high in fibre are mainly based on grains, such as oats. Granola, oatmeal, oat bars, oatcakes and flapjacks are among the plethora of oat products currently on the market. Oats have been used successfully in the food industry, and consumers might be open to expanding their diet to include other grains, such as barley.

2.2.1 Barley Grain Structure

A cereal grain, in general, produces a one-seeded fruit referred to as the 'kernel', also known as the caryopsis. The structural anatomy of the barley is composed of the husk, the pericarp-testa (bran) enclosing the germ (embryo) and the endosperm (Figure 1) (Kent, 1983). The endosperm consists of the starchy endosperm and the aleurone layers. It is considered the largest morphological portion of the barley grain, comprising 75%–80% (w/w) of the kernel and enriched in beta-glucans (Koehler and Wieser, 2013). The cell wall of the starchy endosperm contains 75% (w/w) beta-glucans and 20% (w/w) arabinoxylans, whereas the aleurone layer contains 26% beta-glucans and 71% arabinoxylans (Woodward *et al*., 1981). Therefore, beta-glucans and arabinoxylans are the primary components of the cell wall of the endosperm and aleurone, which act as a structural network. The husk is the second largest part of the grain and comprises approximately 23% (w/w) of the total weight of the grain. It is the outermost layer and covers the entire grain (Evers and Millar, 2002). In hulled barley, the husk is tightly attached to the kernel when the grain is threshed; in hulless barley, the kernel is threshed without the husk (Jadhav et al., 1998). Bran, composed primarily of the aleurone and subaleurone layers, is the fraction that surrounds the endosperm after the husk and acts as a protective cover for the whole kernel, forming approximately 8%–13% (w/w) of the total weight of the grain. The bran is a rich source of lipids, protein, phenolics, arabinoxylans and minerals (Macgregor and Fincher, 1993, Hoije et al., 2005). Predominately, the chemical components in cereals are not uniformly distributed in the grain. For instance, hull and bran are abundant in cellulose, pentosans and ash, whereas the endosperm is abundant in starch and protein. It is common for the cell wall of the barley bran to contain reasonably high levels of pentosans but low levels of beta-glucans, in contrast to the cell walls of the endosperm (Henry, 1986). The germ (embryo) accounts for 2%–4% of the grain and is located

at the attachment end of the kernel on its upper region. It is recognised as a rich source of protein (34%) and lipids (13%–17%), with most of the tocopherols located in this component (Jadhav *et al*., 1998).

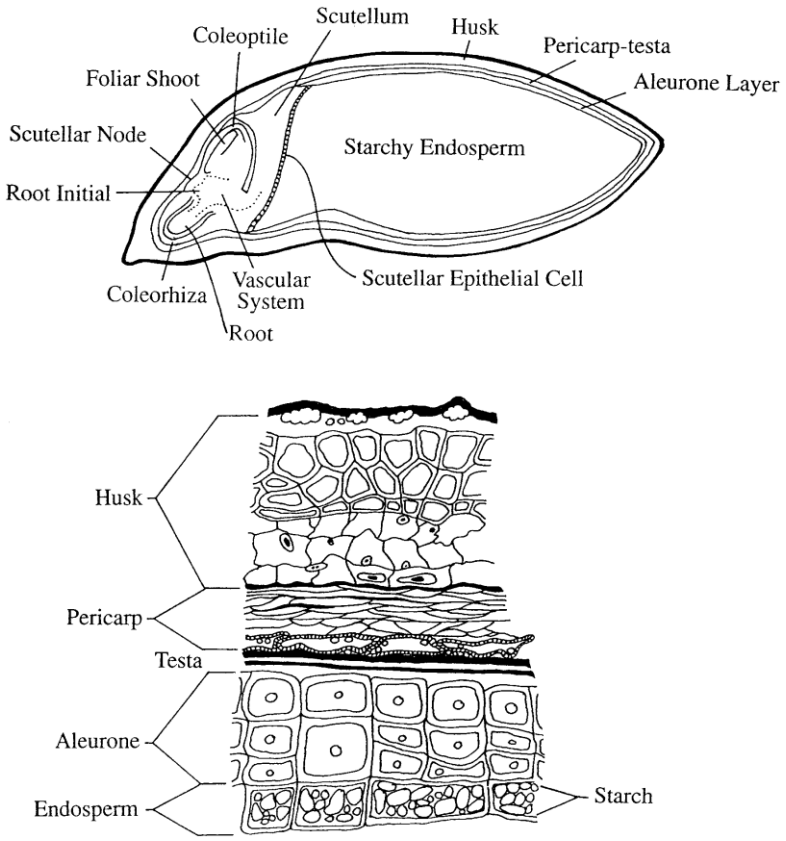

Figure 2.1: Anatomy of barley grain

Source: Kent (1983).

2.2.2 The Chemical Composition of Barley Grain

Barley grains are low in fat and contain an appropriate dietary balance of amino acids, complex carbohydrates that release energy slowly and vitamin E, which acts as an antioxidant. Starch, dietary fibre and protein are the major components of barley grain (Baik and Ullrich,

(2008). Andersson et al. (1999) analysed the chemical composition of multiple barley cultivars and reported that protein constitutes 8.7%–10.5%, starch 52.1%–63.8% and fat 2.2%–3.5%. However, Sullivan et al. (2010) reported total starch of 47.5% and 76.8% in hulled barley and barley flour, respectively. Ahmad et al. (2009) reported that in whole barley flour, the total starch content is 54.2%, fat 2.42% and protein 14.2%. Regardless of composition, environmental growth conditions and the genotypic characteristics of barley grain are the two main factors affecting its final composition.

2.2.2.1 Protein

Barley grain contains a considerable amount of protein, constituting 8% to 15% of the dry grain, whereas in barley grain, it may be as high as 20% (Newman and Newman, 1991, Macgregor and Fincher, 1993). The protein content in cereals depends mainly on the genotype, growing conditions and the rate and time of nitrogen supplied to the plant during growth (Friedman and Atsmon, 1988). Cereal storage proteins have been categorised into four fractions depending on their solubility: albumins, globulins, prolamins and glutelins. Prolamins, also known as hordeins, are soluble in aqueous alcohol and represent the main storage protein in barley, accounting for 35% (w/w) of the storage protein (Koehler and Wieser, 2013, Jadhav *et al*., 1998, Helm et *al*., 2004). Furthermore, barley has been reported to contain several essential amino acids, such as lysine, threonine, valine and arginine (Sullivan et al., 2010).

2.2.2.2 Lipids

The lipids in barley are concentrated mainly in the bran and the germ of the barley grain. Barley contains approximately 2%–4% lipids, in which the average values for neutral lipids, phospholipids and glycolipids are 70%, 20% and 9%, respectively. Most lipids (67%–78%) are non-polar. Linoleic acid is the major fatty acid in the barley grain, followed in decreasing amounts by palmitic, oleic, linolenic and stearic acid (Price and Parsons, 1979).

2.2.2.3 Phenolic acids

Phenolic acids are considered the major phenolic group in barley grain and are located in the bran. The phenolic acids in barley are categorised into benzoic acid and cinnamic acid and their derivatives (Figure 2.2) (Idehen et al., 2017b). The highest concentration of phenolic acids in barley is found in the bound form, followed by conjugated and free forms (Idehen et al., 2017a). Ferulic acid, a cinnamic acid derivative, is regarded as the primary phenolic acid in barley (Pham Van, 2016). Andersson et al. (2008) determined that the total concentration of ferulic acid is approximately 270 µg/g of dry matter; however, the average total concentrations of free, conjugated and bound ferulic acid for multiple barley cultivars are approximately 2.7 µg/g, 33.21 µg/g and 235 µg/g, respectively. The total phenolic acid concentration in barley is between 604 µg/g and 1346 µg/g (Idehen et al., 2017b). Since barley is considered a rich source of phenolic acids, it may serve as a superior dietary source of natural antioxidants with antiradical potential (Zhao and Moghadasian, 2008).

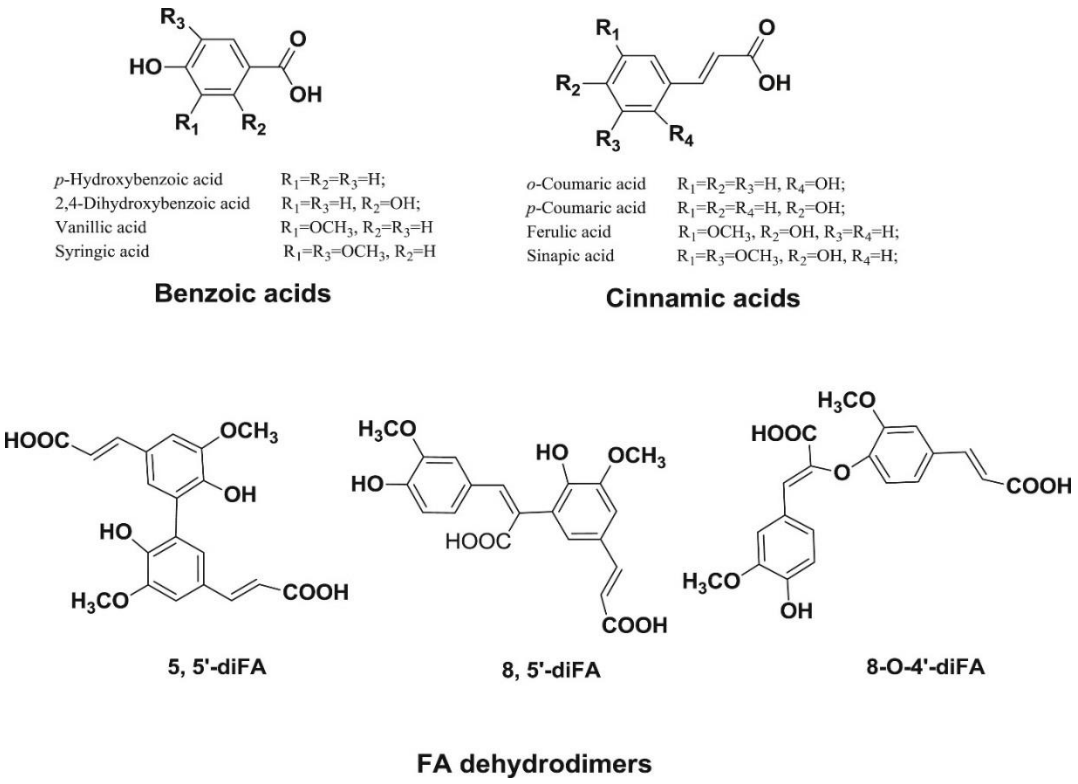

Figure 2.2: Structure of major phenolic acids in barley. FA = ferulic acid

Source: (Idehen et al., 2017a).

2.2.2.4 Other antioxidants

Compared with all other cereal grains, barley contains the highest amount of vitamin E, a fat-soluble vitamin derived from the tocols family. Approximately 97% of tocols in barley germ are tocopherols, whereas 80%–90% of the tocols in the endosperm are tocotrienols (Kerckhoffs et al., 2002). Tocols are acknowledged for their antioxidant and anticancer properties (Sen et al., 2007). Moreover, tocols found in cereals play a critical role in activating the immune system and reducing the risk of stroke by clearing atherosclerotic blockages in the carotid artery (Upadhyay and Misra, 2009). The structure of major tocols in barley is presented in Figure 2.3.

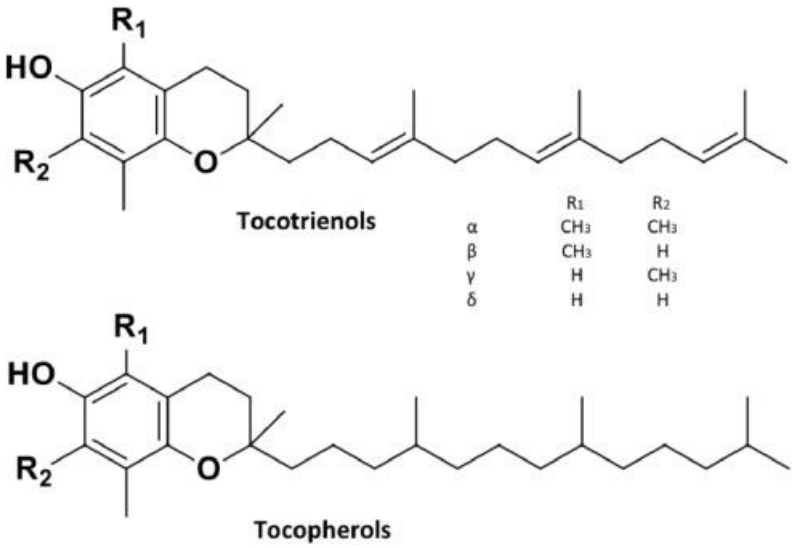

Figure 2.3: Structure of major tocols in barley

Source: Idehen et al. (2017b).

2.2.2.5 Minerals

The average ash content of barley grain usually ranges from 2% to 3%. Minerals in barley grain are concentrated mostly in the germ rather than the endosperm. Phosphorous, potassium and calcium are the main minerals present in barley, and chlorine, magnesium, sulphur and sodium are present in small amounts (Owen et al., 1977).

2.2.2.6 Starch

Starch is present in the endosperm portion of cereal grains and is considered the main energy reserve. The average starch content of barley grain ranges from 58% to 64%, and the amylose content varies from 20% to 30% of the starch content, although genetic and environmental

factors can cause significant variation. In waxy barley, amylose is as low as 1%, whereas it may be up to 45% in high-amylose barley (Macgregor and Fincher, 1993, Jadhav et al., 1998).

2.2.2.7 Dietary fibre

Dietary fibre is defined by the American Association of Cereal Chemists Expert Committee as 'cell wall polysaccharides, lignin and associated substances resistant to hydrolysis by the digestive enzymes of humans' (DeVries, 2003). Dietary fibre is concentrated in the bran and accounts for 27% of the barley grain. Cereal dietary fibre may be classified by solubility, either water-soluble or water-insoluble (Vitaglione et al., 2008). The major components of dietary fibre in barley are arabinoxylans, arabinogalactans, cellulose, beta-glucans, lignin and resistant starch. Cellulose is the most common insoluble dietary fibre, and beta-glucans and arabinoxylan may be distributed into the soluble and insoluble fractions.

There are numerous health benefits associated with dietary fibre intake, such as reducing bowel transit time, preventing constipation, regulating blood glucose and lowering plasma cholesterol levels, enhancing the production of short-chain fatty acids and stimulating the growth of beneficial gut microflora as a prebiotic (Feldheim and Wisker, 2000, Bornet et al., 1987, Karppinen et al., 2000, Crittenden et al., 2002). Much research has explored the mechanisms behind the reported effects of dietary fibre to ensure these effective outcomes in human health. For instance, the reduction in glycaemic response might depend on the amount and quality of fibre present, and the cholesterol-lowering effects of cereal dietary fibre seem to result from apparent activity in the upper gastrointestinal tract (Brennan, 2005). It seems that some physiological effects of dietary fibre may be related to the ability of cereal fibre to form a gel-like network and thus modify gastrointestinal viscosity (Thorburn et al., 1993, Nishimune et al., 1991).

2.3 Beta-Glucan Structure and Occurrence in Barley Grain

Beta-glucans are a cell wall component of cereal grains present in significant amounts in barley (3%–11%) and oats (3%–7%), yet only in minor quantities in wheat and rye (0.5%–1% and 1%–2%, respectively) (Skendi et al., 2003b). Beta-glucans are not limited to cereals. They are also found in various natural sources, such as mushrooms, and the cell walls of yeast, bacteria and algae (Zhu et al., 2015). They demonstrate a wide range of biological activities, including anti-tumour, anti-ageing and anti-inflammatory properties (Zhu et al., 2015, Newman and Newman, 2008a). The biological activities of beta-glucans depend on their sources and their molecular weights *(*Du et al., 2014a). For instance, beta-glucans in lichen (algae) consist of either (1→3), (1→6) or (1→3), (1→4) linkages, whereas the beta-glucan in cereals consist of (1→3), (1→4) linkages. Algal beta-glucans can boost the immune system and promote anti-tumour mechanisms. However, beta-glucans from cereals help lower blood glucose and plasma cholesterol levels (Du et al., 2015, Zhu et al., 2015). The molecular weight of beta-glucans span a broad range, from 20,000 to 3,000,000 daltons in oats and barley (Wood, 1991). The variation in molecular weights might be due to several factors, such as the type of cultivar, environmental conditions and extraction techniques (effects of solvent, pH and temperature, or presence of endogenous enzymes and shear during processing) (Izydorczyk and Biliaderis, 2000).

Cereal beta-glucans consist of β (1→3) and β (1→4) glycosidic bonds linkages. The β (1→3) linkages occur singly, whereas the β (1→4) linkages occur in groups of two to four. This structure is influenced by β (1→3)-linked cellotriosyl and cellotetraosyl units. The rest of the structure consists of longer blocks of (1→4)-linked β–D-glucopyranosyl units (Wood, 1991). The molecular structure of beta-glucans is presented in Figure 2.4. The range of the molecular weight of barley beta-glucan has been reported to be in the range of 31–2700 × 10^3 g/mol. The availability

of water during grain maturation is a major environmental factor known to affect beta-glucan content in barley. Dry conditions before harvest may enhance beta-glucan synthesis and increase concentration in the barley grain (Munck et al., 2004, Bendelow, 1975). Furthermore, the levels of beta-glucans in barley grains can vary between varieties, ranging between 2% and 11%, but are most commonly found to be between 2% and 6% of dry weight (Zhang et al., 2002).

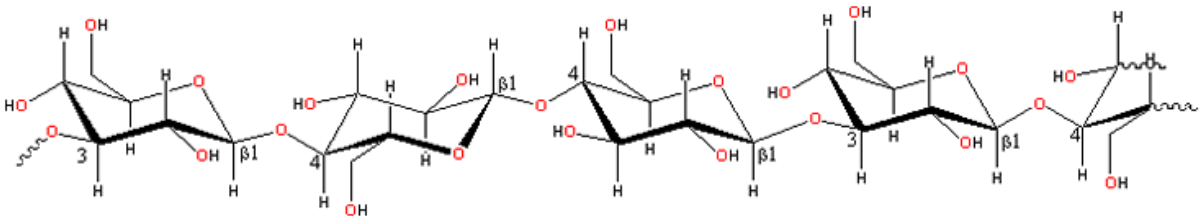

Figure 2.4: The molecular structure of barley beta-glucan.

2.3.1 Barley Beta-Glucan Extraction Methods

The extraction of beta-glucans is a complex process since the endosperm cells in barley contain starch, protein, fat and minerals, in addition to beta-glucans. Thus, a combination of methods is often required to achieve high beta-glucan purity and yield with only minor changes in its molecular structure. The extraction procedure may affect the yield, purity, rheology, viscosity, gel-formation and molecular weight of beta-glucans (Ahmad et al., 2010). Furthermore, the extraction process of beta-glucans ordinarily requires the inactivation of endogenous enzymes (β-glucanases and amylases), as intact enzymes may lead to degradation phenomena in beta-glucan products, leading to low molecular weight. Maintaining the molecular weight of beta-glucans is critical, as it determines its physicochemical properties, such as viscosity, which, in turn, influence the cholesterol-lowering potential of beta-glucans (Regand et al., 2011). The extraction

methodology of oats and barley beta-glucans include the application of several methods, such as hot water extraction, extraction in alkaline or acidic medium and enzymatic extraction (Asif et al., 2009, Benito-Roman et al., 2013).

Hot water extraction is straightforward: high-temperature water penetrates the cell wall structure and solubilises water-soluble contents, such as sugars and proteins. The optimum temperature of hot water extraction should be the highest practicable temperature below the starch gelatinisation temperature. As the temperature increases from 40 to 95°C, the recovery of beta-glucan increases from 20% (Storsley et al., 2003) to 75% (Beer et al., 1997b). Temperatures between 50°C and 55°C were found to extract the highest purity beta-glucan (Ahmad et al., 2010). Hot water extraction also presents clear disadvantages. For example, more starch gelatinisation occurs due to the high water temperatures, which tends to decrease the beta-glucan purity (Comino et al., 2013). Some water extraction methods are generally lengthy (up to 7 days) (Maheshwari et al., 2017), leading to low intrinsic viscosity and molecular weight of barley beta-glucans (Saulnier et al., 1994). Long extraction times might also lead to starch contamination (Ying et al., 2011). The extracted beta-glucan may be contaminated with considerable amounts of starch and protein unless α-amylase is used as pre-treatment to minimise the contamination and obtain a high yield (Benito-Roman et al., 2011, Asif et al., 2009).

The basic aqueous alkali extraction process, developed by Wood et al. (1989), involves four essential steps. First, flour is dispersed in aqueous alkali media because beta-glucan and proteins are solubilised under alkaline conditions. Subsequent centrifugation of the slurry separates the insoluble solid particles, such as starch and insoluble fibre, from the liquid phase that includes beta-glucan and proteins. The addition of acid then precipitates proteins, which are removed from the liquid phase by centrifugation. Finally, recovery of beta-glucan concentrate from the

liquid/aqueous phase is achieved by alcohol precipitation and centrifugation, followed by drying. The extraction process is summarised in Figure 2.5 (Vasanthan and Temelli, 2008). The precipitation of beta-glucans requires a significant amount of solvent for their concentration and recovery, a substantial hurdle to large-scale extraction.

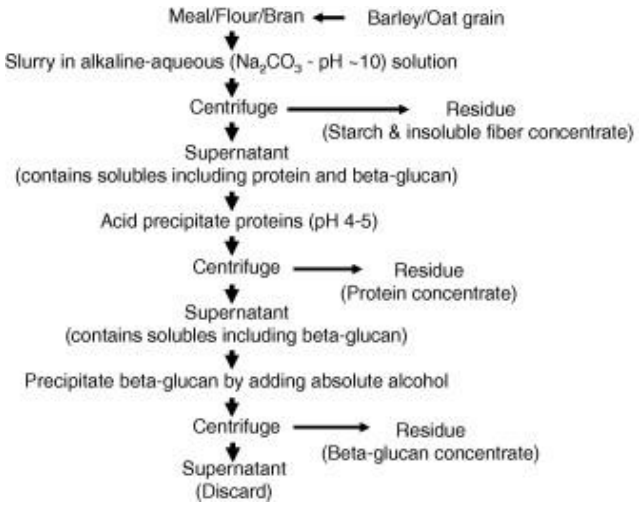

Figure 2.5: The aqueous alkali extraction process

Source: Wood et al. (1989).

Enzymatic extraction provides high yields of beta-glucan fractions due to the breakdown of starch, protein and pentosans during the extraction process utilising several enzymes, each with specific activities. α-Amylase and protease are responsible for starch and protein degradation, respectively, resulting in high chain length beta-glucan with less starch and fewer protein impurities (Asif et al., 2009, Ahmad et al., 2010, Wei et al., 2006, McCleary, 1988). Oat/barley flour is mixed with water and thermostable α-amylase, and the mixture is then heated to boiling. The solubilised beta-glucans are recovered by centrifugation and dried to a powder. At high temperatures, beta-glucan and starch will solubilise, and amylase will hydrolyse the starch into

dextrins (Vasanthan and Temelli, 2008). Porcine pancreas lipase is used for fat degradation, and lichenase enzymes are used for beta-glucan digestion (Gamel et al., 2014). Mikkelsen et al. (2017b) discovered lichenase side activity by combining a mixture of amylolytic enzymes that controlled the degradation of beta-glucan, which is favourable for achieving desirable chain length and increasing the extraction yield.

When combined with acid/alkali techniques, enzymatic extraction provides the highest yield and beta-glucan molecular weight (Ahmad et al., 2010, Maheshwari et al., 2017). The use of alkali can increase extraction levels from barley to 86–100% but degrade the beta-glucan molecules, which is undesirable (Beer et al., 1997b). Furthermore, the enzymatic extraction process is complex, harsh conditions and is costly. These extraction methods are difficult to industrialise and upscale, resulting in higher barley beta-glucan production costs (Maheshwari et al., 2017).

UAE represents a promising pathway to improve beta-glucan extraction by reducing the time, materials and energy consumed. Ultrasound is a unique type of soundwave, between 20 kHz and 100 MHz, exceeding the threshold of human hearing. Sound waves generate high and low pressure (compression and rarefaction) cycles (Benito-Roman et al., 2013). The continuous high and low pressure creates small bubbles in the liquid medium, which collapse when they can no longer consume energy. Cavitation occurs that produces, grows and collapses bubbles (Chemat et al., 2011). This phenomenon introduces strong shear forces and allows the solvent to penetrate deeper into the matrix, improving the diffusion rate of the required molecule into the solvent, one of the main advantages (Wang et al., 2008). The main principle in UAE is that the acoustic cavitation causes obstruction of the cell walls, decreases the particle size and increases contact between solvents and selected compounds (Ying et al., 2011). There are many advantages to the

UAE method, such as improved extraction efficiency, faster energy-transfer, higher yields and selectivity. Furthermore, it reduces extraction time, energy, solvent use and extraction temperature, requires fewer chemicals with lower physical risks and operates in an environmentally-friendly manner (Chemat et al., 2011, Huang et al., 2009). Previous studies have shown the efficiency of using ultrasound in the extraction process. Sun et al. (2004) found that the UAE method improved the extractability of hemicelluloses from sugar cane bagasse by destroying cell walls and linkages between lignin and hemicelluloses. The various extraction techniques of beta-glucan are reviewed in Table 2.1.

Table 2.1: Extraction methods of beta-glucan from various sources.

Extraction Method	Source	Purity (%, w/w)	Mass yield (%w/w)	Impurities/ Limitation	Reference
Hot water extraction	Barley	79.3%	5.1%	Beta-glucan molecular weight was relatively low – partial enzymatic hydrolysis.	(Burkus and Temelli, 1998)
Hot water extraction	Barley	83.1	5.4%	n/a	(Asif *et al.*, 2009)
Alkaline extraction	Barley	78.1 %	3.94 %	n/a	(Asif *et al.*, 2009)
Enzymatic extraction	Barley	81.4%	5.22%	Fewer starch and protein impurities were observed.	(Asif *et al.*, 2009)
Acidic extraction	Barley	80.4%	4.65%	Fewer starch and protein impurities were observed.	(Asif *et al.*, 2009)
Alkaline extraction	Hull-less barley and oat bran	n/a	n/a	High viscosity beta-glucan was obtained.	(Bhatty, 1995)
Alkaline extraction	Rolled oats	n/a	5.24%	n/a	(Dawkins and Nnanna, 1993b)
Ultrasonic - assisted extraction	Mushrooms	n/a	6.02%	n/a	(Tian *et al.*, 2012)

While the hot water extraction method is straightforward, it requires a large amount of energy and is not recommended for large-scale production. Moreover, the extracted beta-glucan may be contaminated with considerable starch and protein levels (Bhatty, 1995). Ahmad et al. (2010) reported that the enzymatic extraction method provided a higher yield and recovery of beta-glucans than acid and alkaline extraction. Two enzymes were used in the extraction method, namely a heat-stable α-amylase and a protease. High recovery by the enzymatic method is measured by protein and starch removal by specific enzymes (Ahmad et al., 2010, Xu et al., 2007). One advantage of this method is the minimum usage of solvents and heat (Puri et al., 2012). In the

alkaline extraction method, however, beta-glucans are dissolved in alkaline conditions then precipitated by alcohol or acetone, which results in high beta-glucan viscosity even at low concentrations. This outcome is undesirable since it affects its purity and recovery. Beta-glucan concentration and recovery via precipitation require significant solvent volumes, representing an obstacle to its application in large-scale extractions (Madacsi et al., 1983). The UAE method is considered a promising alternative since it enhances extraction efficiency by requiring a low amount of energy. Solvent consumption is also reduced, as the extraction time is much less than with conventional methods, which increases the yield and the quality of the extract (Maran and Priya, 2014, Zhang and Liu, 2008, Hromadkova et al., 1999b, Pico, 2013, Azmir et al., 2013). Many polysaccharides have been efficiently extracted using the UAE method without altering their molecular properties and biological activities (Fu et al., 2006, Hromadkova et al., 1999a). However, the main limitation of this approach is that the extraction yield is linked to the identity of the plant matrix. Therefore, the presence of a dispersed phase weakens the ultrasound wave and the active ultrasound portion inside the extractor is limited to a zone closest to the ultrasonic emitter (Wang and Weller, 2006). Moreover, the use of high ultrasound energy, notably equating to more than 20 kHz, may affect the extracted phytochemicals by forming free radicals (Kaufmann and atrice, 2002).

2.3.2 Beta-Glucan Rheological and Physiological Properties

According to Temelli et al. (2004b), the solubility of beta-glucan is particularly important in the nutritional, sensory, and rheological suitability of such molecules. Other authors have indicated that when beta-glucan is to be utilised as a thickener in products such as salad dressings, dairy products, and drinks, thickness is an essential consideration (Kaur and Riar, 2020, Vaikousi

et al., 2004). Vaikousi et al. (2004) stated that the complicated organisation of long-chain beta-glucans is facilitated by such chains possessing a high MW as this means they can create pseudo-plastic solutions and gels. Such products could then be utilised as thickening solutions within food products. Notably, highly concentrated and soft gels are frequently produced by degraded/modified beta-glucan since this possesses a low MW. This result is in contrast to the antinutritional results sometimes obtained by using thick beta-gluten in food products. Similarly, Adams et al. (2018) noted that selective dietary fibres could minimise nutrient bioavailability which, according to Parada and Aguilera (2007), is not impacted by the food's microstructure. Interestingly, food products possessing the desired viscosity and rheology could be created by exploiting key beta-glucan properties, accomplished by utilising processing approaches that alter beta-glucan molecular weight, as well as the extent to which it branches. This practice could be of particular use when considering that a low viscosity beta-glucan is typically the most sought after since this discourages phase separation. The chain length, temperature, and concentration of beta-glucan affect its overall thickness, and it could prove useful as a stabilising agent while minimising insulin and plasma cholesterol.

According to Lazaridou and Biliaderis (2007), beta-glucans can be employed as a) fat replacements during the processing of calorie-reduced foods, or b) thickening agents, since the rheological properties of beta-glucans (e.g., abilities to increase the thickness of aqueous solutions and create gels) lead to their utility in the food industry as hydrocolloids. Other authors have found that purified beta-glucans or beta-glucan-rich fractions from cereals have been mixed successfully into bread, dairy products, cereal, muffins, and meat products (Hudson et al., 1992, Brennan et al., 2002). In addition to gelling, beta-glucans have excellent water-holding characteristics, meaning they can form a thick solution when dissolved in water. Indeed, their solubility in water is one of

their main and most recognisable physiological functions, coupled with their functioning in the small intestine.

2.4 The Role of Barley Beta-Glucan as a Functional Food Ingredient

According to Brennan and Cleary (2005a), functional foods can be defined as those which provide health-related advantages in addition to their direct nutritional values; or, more precisely, those which improve at least one bodily function in such a way that it directly enhances the individual's health or contributes toward the prevention of disease in the body (Anon, 1999). Providing additional functional characteristics or eradicating certain disadvantageous characteristics of food are two ways in which functional foods may contribute to one's health (Anon, 2004). Research interest regarding the benefits of consuming such functional foods is growing exponentially. Minerals, fibre, lignans, and other components are just some of the bioactive ingredients of cereal that make it a functional food. Ingredients such as these have great potential in either significantly enhancing health or reducing the symptoms of chronic disease (Madhujith and Shahidi, 2007), with both the EFSA (2010) and the FDA (2006) stating that coronary heart disease could reduce the symptoms by consuming three grams of beta-glucans a day.

Food passes through the colon more quickly when high amounts of fibre are consumed. Barley beta-glucans facilitate weight loss, and beta-glucans as a whole can also minimise blood cholesterol and glucose, thus reducing the symptoms of type 2 diabetes (Delaney et al., 2003, Braaten et al., 1994, Baik and Ullrich, 2008). Furthermore, some researchers have theorised that increased thickness of the contents within the small intestine and stomach is the result of the drastic increase in insulin and glucose that occurs after the ingesting of food with high amounts of thick

soluble fibre (Edwards et al., 1987). It has also been noted that the ingestion of food with thick polysaccharides results in minimised postprandial insulin concentration and increased blood glucose (Jenkins et al., 1978; Wood et al., 1994). Charalampopoulos et al. (2002) noted that the prebiotic concept is followed by the water-soluble fibre (e.g., oligosaccharides and beta-glucan). Moreover, the growth of bifidobacteria and lactobacilli within the colon can be encouraged by the nondigestible carbohydrates commonly found in cereal.

The large intestine's bile acids are absorbed by thick beta-glucan solutions (which stall the absorption of such acids within the small intestine), and the resulting acid reduction may reveal how beta-glucans reduce blood cholesterol. Moreover, as Malkki (2004) noted, weight loss and satiety result from beta-glucan consumption since they stall gastric emptying. Furthermore, at least some authors assert that liver cholesterol synthesis is minimised by viscous-soluble fibre since it minimises insulin secretion (De Schrijver et al., 1992). Finally, (Hill and Fernandez, 1990) maintain that beta-glucans reduce bacterial creation of carcinogens, and increase the colon's transit rate, thereby reducing the colon's carcinogen density.

2.4.1 The Application of Beta-Glucans in Food Products

Beta-glucans possess a wide range of advantageous characteristics besides their nutrition and health benefits, including minimising calories in fat mimetics and thickening of ice cream and salad dressings, among other products (Lazaridou et al., 2004). Furthermore, jams, soups, dairy products, jellies, and meat products often require food processors to utilise beta-glucan for gelling, water-holding, emulsification, and oil-holding purposes (Ahmad et al., 2012a). Notably, barley beta-glucan is particularly useful for cereal beta-glucan because it possesses adequate fibre and

physical smoothness (Du et al., 2014b). Giese (1992) proposed that beta-glucan could substitute for thickening agents such as xanthan gum, Arabic gum, carboxymethylcellulose, alginates, and pectin due to its viscosity. For example, barley fibre was added to muffins to prevent the dough from being too sticky (Hudson et al., 1992) and added to both biscuits and muffins to increase their fibre (Newman et al., 1998). Temelli et al. (2004a) found that as the extracts concentration and thickness increased, and the flavour maintained when barley beta-glucan was added to an orange-flavoured beverage, leading to the conclusion that beta-glucans are smooth enough to be included in drinks. Brennan et al. (2002) noted that the addition of beta-glucan to low-fat ice cream and yoghurt enhanced the texture of such food products. There is a wealth of potential for beta-glucans to be used in soups, drinks, sauces, and similar foods due to their properties (Burkus and Temelli, 2000).

The effect of roasting barley and oat flakes on beta-glucan was investigated with the overall aim of establishing the usefulness of beta-glucan in cereal-based food (Schlörmann et al., 2019, Schlörmann et al., 2020). These studies concluded that roasting up to 160°C enhanced the sensory characteristics of the cereal but did not visibly change the beta-glucan, although the extract thickness was considerably minimised. Błaszczyk et al. (2015) found that using physiologically active beta-glucan for its water-holding, gel-forming, and high-thickness characteristics occasionally resulted in problems in baked goods. To resolve this issue, Kurek et al. (2018b) explored the impact of multiple treatments on beta-glucan fortified bread. The authors concluded that the breads dried before being frozen and then boiled were the most springy, had the best crumb robustness and colour, and provided the highest beta-glucan levels. Another comparable study found beta-glucan minimised the bread's glycaemic index (occurring as a result of the decreased starch digestion rate) and enhanced the volume of the bread (Jayachandran et al., 2018). Zhao et

al. (2020) noted at the conclusion of their study that the physiology of beta-glucan can be greatly affected by intermolecular aggregation and depolymerisation.

With regards to type 2 diabetes, Tessari and Lante (2017) noted that long-term metabolic control was significantly enhanced with the ingestion of bread high in fibre and low in starch with an increased ratio of starch to beta-glucan. The nutrition, bread quality and dough rheology are all enhanced by adding oat-derived beta-glucan to gluten-free bread. Hager et al. (2011), and Skendi et al. (2010a) similarly noted that bread quality tends to improve when the beta-glucan molecular weight increased. Interestingly, β-glucanase thinner texture and lower solubility result in its limited nutraceutical activity (Moriartey et al., 2010). Skendi et al. (2010a) noted that endogenous β-glucanase's activity compromises the versatility of wheat bread. A related study concluded that, due to the enzyme-induced depolymerisation, oat bread stored for three days experienced a drastic reduction in viscosity (Gamel *et al*., 2013). Notably, this reduction was prevented in a subsequent phase of the same study by freezing the bread at -18 °C, maintaining the bread's nutraceutical and physicochemical characteristics. (Lan-Pidhainy et al., 2007) found that repeated freezing and defrosting can result in the product's reduced viscosity and solubility and recommend further research to identify a more suitable solution. A similar study noted that substituting 30% semolina flour for high beta-glucan barley flour in pasta provided the health benefits associated with beta-glucan (De Paula et al., 2017). These researchers also concluded that the increased viscosity occurring due to beta-glucan addition meant that its dryness and extruding properties were both counter balanced. Messia et al. (2019) found that beta-glucan rich barley flour had the same quality as semolina when substituted in couscous.

Andersen et al. (2017) studied consumer opinion of an experimental beta-glucan fruit drink compared to a standard control fruit drink. Participants rated the drink smell, aftertaste, and texture, and there were no differences in the overall ratings of the drinks. These results support the well-studied notion that to be approved by consumers, the beta-glucan product's sensory traits must be comparable to the original product. In a similar study, Yu et al. (2007) observed no differences in overall sensory quality between a sponge cake fortified with 1%–4% beta-glucan and the non-fortified version. In contrast, Choo and Aziz (2010) found that adding oat beta-glucan to yellow alkaline noodles reduced the smoothness, firmness, and overall quality of the product, although the taste was unaffected. Finally, after adding 0.5% barley beta-glucan to yoghurt, (Brennan and Tudorica, 2008) found that the texture of the yoghurt and its other sensory characteristics were satisfactory and thereby concluded that it would be suitable as a substitute for fat in such products. Unfortunately, they failed to examine the effect on the yoghurt's sensory qualities when a substantial amount of beta-glucan was added. Indeed, from all of the examined literature in this field, it seems there is a substantial gap in the current research concerning a) the effects of beta-glucan foods after processing and storage and b) the effect of beta-glucan on food products that are not baked goods or drinks.

Barley and oat grain, comprised of approximately 18% beta-glucan, are current sources of beta-glucan as a food additive. (Zhu et al., 2016) posit that there are a wealth of approaches for creating a food product with enhanced quality via the addition of beta-glucan. Sarteshnizi et al. (2015) incorporated both resistant starch and beta-glucan into prebiotic sausage and found that the sausage's sensory and physical characteristics were considerably impacted by beta-glucan, which was added alongside the resistant starch using the *D*-optimal mixture design method.

Upon adding beta-glucan and pectin to yoghurts, Rinaldi et al. (2015) concluded that the perceived quickened proteolysis occurred due to segregation between the diary proteins and the beta-glucan. This segregation occurred because of high protein and enzyme concentrations during separation. In addition to this proteolysis, more free amino acids were observed than those with beta-glucan or starch, and fewer large peptides were also observed. Finally, Brennan et al. (2013) found that the inclusion of mushroom and barley fractions in snacks facilitated the manipulation of such foods' glycaemic responses.

2.4.2 Beta-Glucan as a Baking Ingredient

Beta-glucans could be utilised to fortify bread to ameliorate common health problems resulting from the consumption of standard bread. (Flander et al., 2007) found that bread was fresher for longer with the incorporation of oat flour since it possessed sufficient water retention. Similarly, according to Forssell et al. (1998), bread staling could be hindered by including lecithin or oat starch, although Gormley and Morrissey (1993) point out that this has the potential to compromise the quality of the overall bake. Furthermore, moisture can be maintained within bread for a longer duration via the addition of barley beta-glucan since this leads to additional rounded gas cells, whose strength results in maintaining moisture (Foschia *et al.*, 2013). In this way, the issue of weight loss in bread during storage can be managed.

Numerous studies (Ahmad et al., 2008) have sought to produce bread containing significantly more fibre than observed in typical bread to improve its beneficial effects (e.g. minimised risk of colon cancer). Notably, due to the enhanced gas retention, beta-glucan enhances bread’s volume and maintains such volume for up to two days, although according to Skendi et al.

(2010b), the bread's firmness is compromised. Crowley et al. (2000) concluded that the water absorption of bread increases with the incorporation of beta-glucan. Finally, it enhances bread thickness and uniformity, indicating that the crust's thickness and robustness are also improved. Furthermore, beta-glucan does not impact the bread's expected symmetry. Ahmad et al. (2008) concluded that the baking industry would greatly benefit from including it in bread dough.

In the same vein, crumb, texture, and colour have been positively impacted by foods high in fibre (e.g. resistant starch, locust bean gum and wheat bran), although this is notably dependent on the amount and type of such foods included. Consumers of baked goods typically prefer to see fibre and bran particles in their bread, which also means that when such consumers rated bread in terms of colour and appearance, those with added fibre and bran are always preferred (Almeida et al., 2013). Increased crumb chroma and moisture are attained when bran fibre is added and high-speed mixing used to combine the bread dough. Flander et al. (2007) found that the sensory crumb characteristics of a loaf of oat bread is impacted when 1 g/100g of oat flour and 49 g/100g of wheat flour are added to the dough. They also concluded that the flavour and other characteristics of the crumbs are impacted solely by the conditions in which they were baked. Furthermore, two slices of the resulting loaf possessed 0.78g of beta-glucan—0.03g more than the FDA's recommended dose per portion. Thus, the bread possessed notable health benefits. (Iranshahi et al., 2014) found that a loaf of Barbari bread with both beta-glucan and inulin added had a later expiry date and appropriate sensory characteristics. After preparing beta-glucan rusk via a breadmaking process, (Izydorczyk et al., 2014) concluded that the beta-glucan had high solubility during the fermentation and mixing processes. The researchers additionally noted that, as with bread's porous structure, rusk structure fares best in a warm temperature.

Skendi et al. (2010b) concluded that maintaining the bread's original form, the darkening of bread colour, and the increased development time and stability of the bread were all accomplished with the incorporation of beta-glucan.

2.4.3 The Addition of Barley Flour/Beta-Glucan Extract to Crackers

There has been little research on the addition of barley flour to cracker formulations, but there is a wealth of research concerning the addition of barley flour to bread. Crackers can be categorised as snack/sprayed crackers, flavoured/savoury crackers, and soda/saltine/cream crackers (Shukla, 1994). Crackers can also be defined as a thin, crispy baked unsweetened dough. Crackers are appropriate for the inclusion of bioactive ingredients and are extremely popular on a global scale. Consistent with this popular demand, there have been numerous studies aiming to enhance crackers' nutritional value in recent years, mainly via the inclusion of bioactive ingredients. Beta-glucan extract and barley flour could both potentially be effective bioactive ingredients for incorporation into crackers, achieving both objectives of increasing the nutritional value of this snack and using bioactive ingredients in a common food product. Studies have also added black currant pomace (Schmidt et al., 2018), Bambara groundnut (Yeboah-Awudzi et al., 2018) and pulse flour (Millar et al., 2017) to crackers.

The term 'cracker' refers to a baked food with 1%–5% moisture and a wheat/oat/barley cereal base in which large air pockets are prevented during the baking stage by cutting small holes in the dough (Zydenbos and Humphrey-Taylor, 2003). Katz and Labuza (1981) considered the crispiness of crackers a result of their low water-holding aptitude, which also prevents mould production during baking (Han et al., 2010). Snack/sprayed crackers are sprayed with either

sodium bicarbonate or ammonium, whilst flavoured/savoury crackers are baked similarly to soda/cream crackers (for which the ingredients are combined and then allowed to ferment for approximately 18 hours before baking). Soda/cream crackers are by far the most popular globally, and both cream and savoury crackers are baked between 200 and 300 °C (Zydenbos & Humphrey-Taylor, 2003; Han *et al.*, 2010). Savoury crackers are also often served with savoury toppings, including herbs, cheeses, and seeds.

2.4.4 The Effect of Food Processing on Beta-Glucan Availability

The molecular (chemical structure and degree of polymerisation), structural (molecular interaction) and functional (viscosity, water binding capacity and solubility) properties of beta-glucans are vulnerable to change under specific processing conditions. The molecular weight of beta-glucans can be reduced during extraction, as well as through the effect of enzymatic or chemical hydrolysis, heat treatment or mechanical shear. Reduction in the molecular weight of barley beta-glucan contents during bread-making and ready-to-eat barley cereals has been reported in multiple studies (Klamczynski et al., 2004, Sundberg et al., 1996, Andersson et al., 2004). The molecular weight degradation in bread-making occurs during mixing and fermentation by endogenous enzymes within flour or yeast. However, Andersson et al. (2004) stated that no significant change in the beta-glucan structure in barley occurred during the stages of mixing, fermenting or baking bread dough.

Water-holding capacity (WHC), water-absorption capacity (WAC) and water solubility index are the main physicochemical properties of beta-glucans. The WAC of barley flour depends on the presence of beta-glucans and insoluble fibre. WHC plays a critical role in the final product's

textural properties since it affects the baking time and shelf life of food products. High water-retention is associated with the lower staling rate of bread and chapatti (Paras and Kotari, 2017). WAC mostly depends on the composition, particle size, extraction method, presence and type of dietary fibre (Elleuch et al., 2011).

2.5 References

Adams, S., Sello, C.T., Qin, G.-X., Che, D. & Han, R. (2018) Does dietary fiber affect the levels of nutritional components after feed formulation? *Fibers,* **6,** 29.

Ahmad, A., Anjum, F., Zahoor, T., Chatha, Z. & Nawaz, H. (2008) Effect of barley β-glucan on sensory characteristics of bread. *Pakistan Journal of Agricultural Sciences,* **45,** 88-94.

Ahmad, A., Anjum, F.M., Zahoor, T., Nawaz, H. & Ahmed, Z. (2010) Extraction and characterization of beta-D-glucan from oat for industrial utilization. *International Journal of Biological Macromolecules,* **46,** 304-309.

Ahmad, A., Anjum, F.M., Zahoor, T., Nawaz, H. & Dilshad, S.M.R. (2012) Beta glucan: a valuable functional ingredient in foods. *Critical Reviews in Food Science and Nutrition,* **52,** 201-212.

Almeida, E.L., Chang, Y.K. & Steel, C.J. (2013) Dietary fibre sources in bread: Influence on technological quality. *LWT-Food Science and Technology,* **50,** 545-553.

Andersen, B.V., Mielby, L.H., Viemose, I., Bredie, W.L. & Hyldig, G. (2017) Integration of the sensory experience and post-ingestive measures for understanding food satisfaction. A case study on sucrose replacement by *Stevia rebaudiana* and addition of beta glucan in fruit drinks. *Food Quality and Preference,* **58,** 76-84.

Anderson, J.W., Smith, B.M. & Gustafson, N.J. (1994) Health benefits and practical aspects of high-fiber diets. *American Journal of Clinical Nutrition,* **59,** 1242S-1247S.

Andersson, A.A.M., Elfverson, C., Andersson, R., Regner, S. & Aman, P. (1999) Chemical and physical characteristics of different barley samples. *Journal of the Science of Food and Agriculture,* **79,** 979-986.

Andersson, A.A.M., Armo, E., Grangeon, E., Fredriksson, H., Andersson, R. & Aman, P. (2004) Molecular weight and structure units of (1 -> 3, 1 -> 4)-beta-glucans in dough and bread made from hull-less barley milling fractions. *Journal of Cereal Science,* **40,** 195-204.

Andersson, A.A.M., Lampi, A.-M., Nystrom, L., Piironen, V., Li, L., Ward, J.L., Gebruers, K., Courtin, C.M., Delcour, J.A., Boros, D., Fras, A., Dynkowska, W., Rakszegi, M., Bedo, Z., Shewry, P.R. & Aman, P. (2008) Phytochemical and Dietary Fiber Components in Barley Varieties in the HEALTHGRAIN Diversity Screen. *Journal of Agricultural and Food Chemistry,* **56,** 9767-9776.

Anon 2004. Commercialising science: the Finnish functional food innovations. *Innovations in Food Technology,* **48**, 48-46.

Asif, A., Muhammad Anjum, F., Tahir, Z., Haq, N. & Ahmad, D. (2009) Physicochemical and functional properties of barley beta-glucan as affected by different extraction procedures. *International Journal of Food Science & Technology,* **44,** 181-187.

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H. A., Ghafoor, K., Norulaini, N.A.N. & Omar, A.K.M. (2013) Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering,* **117,** 426-436.

Badr, A., Muller, K., Schafer-Pregl, R., El Rabey, H., Effgen, S., Ibrahim, H. H., Pozzi, C., Rohde, W. & Salamini, F. (2000) On the origin and domestication history of barley (*Hordeum vulgare*). *Molecular Biology and Evolution,* **17,** 499-510.

Baik, B.-K. & Ullrich, S. E. (2008) Barley for food: Characteristics, improvement, and renewed interest. *Journal of Cereal Science,* **48,** 233-242.

Beer, M.U., Wood, P.J., Weisz, J. & Fillion, N. (1997) Effect of cooking and storage on the amount and molecular weight of (1→ 3)(1→ 4)-β-D-glucan extracted from oat products by an in vitro digestion system. *Cereal Chemistry,* **74,** 705-709.

Behall, K.M., Scholfield, D.J. & Hallfrisch, J. (2004) Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. *American Journal of Clinical Nutrition,* **80,** 1185-1193.

Bendelow, V.M. (1975) Determination of non-starch polysaccharides in barley breeding programs. *Journal of the Institute of Brewing,* **81,** 127-130.

Benito-Román, O., Alonso, E. & Lucas, S. (2011) Optimization of the beta-glucan extraction conditions from different waxy barley cultivars. *Journal of Cereal Science,* **53,** 271-276.

Benito-Román, O., Alonso, E. & Cocero, M.J. (2013) Ultrasound-assisted extraction of beta-glucans from barley. *Lwt-Food Science and Technology,* **50,** 57-63.

Bhatty, R.S. (1995) Hull-less barley bran: A potential new product from an old grain. *Cereal Foods World,* **40,** 819-824.

Bhatty, R.S. (1999) The potential of hull-less barley. *Cereal Chemistry,* **76,** 589-599.

Błaszczyk, K., Wilczak, J., Harasym, J., Gudej, S., Suchecka, D., Królikowski, T., Lange, E. & Gromadzka-Ostrowska, J. (2015) Impact of low and high molecular weight oat beta-glucan on oxidative stress and antioxidant defense in spleen of rats with LPS induced enteritis. *Food Hydrocolloids,* **51,** 272-280.

Bornet, F.R.J., Costagliola, D., Rizkalla, S.W., Blayo, A., Fontvieille, A.M., Haardt, M.J., Letanoux, M., Tchobroutsky, G. & Slama, G. (1987) Insulinemic and glycemic indexes of 6 starch-rich foods taken alone and in a mixed meal by type-2 diabetics. *American Journal of Clinical Nutrition,* **45,** 588-595.

Braaten, J.T., Scott, F.W., Wood, P.J., Riedel, K.D., Wolynetz, M.S., Brule, D. & Collins, M.W. (1994) High beta-glucan oat bran and oat gum reduce postprandial blood-glucose and insulin in subjects with and without type-2 diabetes. *Diabetic Medicine,* **11,** 312-318.

Brennan, C.S., Tudorica, C.M. & Kuri, V. (2002) Soluble and insoluble dietary fibres (non-starch polysaccharides) and their effects on food structure and nutrition. *Food Industry Journal,* **5,** 261-272.

Brennan, C.S. (2005) Dietary fibre, glycaemic response, and diabetes. *Molecular Nutrition & Food Research,* **49,** 560-570.

Brennan, C.S. & Cleary, L.J. (2005) The potential use of cereal (1 -> 3,1 -> 4)-beta-D-glucans as functional food ingredients. *Journal of Cereal Science,* **42,** 1-13.

Brennan, C.S. & Tudorica, C.M. (2008) Carbohydrate-based fat replacers in the modification of the rheological, textural and sensory quality of yoghurt: comparative study of the utilisation of barley beta-glucan, guar gum and inulin. *International Journal of Food Science & Technology,* **43,** 824-833.

Brennan, M.A., Derbyshire, E., Tiwari, B.K. & Brennan, C.S. (2013) Integration of β-glucan fibre rich fractions from barley and mushrooms to form healthy extruded snacks. *Plant Foods for Human nutrition,* **68,** 78-82.

Burkus, Z. (1996) *Barley beta-glucan: Extraction, functional properties and interactions with food components.* PhD Dissertation/Thesis. University of Alberta: Canada.

Burkus, Z. & Temelli, F. (1998) Effect of extraction conditions on yield, composition, and viscosity stability of barley beta-glucan gum. *Cereal Chemistry,* **75,** 805-809.

Burkus, Z. & Temelli, F. (2000) Stabilization of emulsions and foams using barley beta-glucan. *Food Research International,* **33,** 27-33.

Charalampopoulos, D., Wang, R., Pandiella, S.S. & Webb, C. (2002) Application of cereals and cereal components in functional foods: a review. *International Journal of Food Microbiology,* **79,** 131-141.

Chemat, F., Zill E.,H. & Khan, M.K. (2011) Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry,* **18,** 813-835.

Chen, J. & Huang, X.-F. (2009) The effects of diets enriched in beta-glucans on blood lipoprotein concentrations. *Journal of Clinical Lipidology,* **3,** 154-158.

Choo, C.L. & Aziz, N.A.A. (2010) Effects of banana flour and β-glucan on the nutritional and sensory evaluation of noodles. *Food Chemistry,* **119,** 34-40.

Collar, C. & Angioloni, A. (2014) Nutritional and functional performance of high β-glucan barley flours in breadmaking: mixed breads versus wheat breads. *European Food Research and Technology,* **238,** 459-469.

Comino, P., Shelat, K., Collins, H., Lahnstein, J. & Gidley, M.J. (2013) Separation and purification of soluble polymers and cell wall fractions from wheat, rye and hull less barley endosperm flours for structure-nutrition studies. *Journal of Agricultural and Food Chemistry,* **61,** 12111-12122.

Crittenden, R., Karppinen, S., Ojanen, S., Tenkanen, M., Fagerstrom, R., Matto, J., Saarela, M., Mattila-Sandholm, T. & Poutanen, K. 2002. In vitro fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. *Journal of the Science of Food and Agriculture,* **82,** 781-789.

Crowley, P., Grau, H. & Arendt, E. 2000. Influence of additives and mixing time on crumb grain characteristics of wheat bread. *Cereal Chemistry,* **77,** 370-375.

Dawkins, N. L. & Nnanna, I. A. 1993. Oat gum and beta-glucan extraction from oat bran and rolled oats - temperature and pH effects. *Journal of Food Science,* **58,** 562-566.

De Paula, R., Abdel-Aal, E.-S. M., Messia, M.C., Rabalski, I. & Marconi, E. (2017) Effect of processing on the beta-glucan physicochemical properties in barley and semolina pasta. *Journal of Cereal Science,* **75,** 124-131.

De Schrijver, R., Fremaut, D. & Verheyen, A. (1992) cholesterol-lowering effects and utilization of protein lipid fiber and energy in rats fed unprocessed and baked oat bran. *Journal of Nutrition,* **122,** 1318-1324.

Delaney, B., Carlson, T., Frazer, S., Zheng, T., Hess, R., Ostergren, K., Kierzek, K., Haworth, J., Knutson, N., Junker, K. & Jonker, D. (2003) Evaluation of the toxicity of concentrated barley beta-glucan in a 28-day feeding study in Wistar rats. *Food and Chemical Toxicology,* **41,** 477-487.

Devries, J. . (2003) On defining dietary fibre. *Proceedings of the Nutrition Society,* **62,** 37-43.

Du, B., Bian, Z. & Xu, B. (2014a) Skin Health Promotion Effects of Natural Beta-Glucan Derived from Cereals and Microorganisms: A Review. *Phytotherapy Research,* 28**,** 159-166.

Du, B., Zhu, F. & Xu, B. (2014b) Beta-glucan extraction from bran of hull-less barley by accelerated solvent extraction combined with response surface methodology. *Journal of Cereal Science,* **59,** 95-100.

Du, B., Lin, C., Bian, Z. & Xu, B. (2015). An insight into anti-inflammatory effects of fungal beta-glucans. *Trends in Food Science & Technology,* **41,** 49-59.

Edwards, C.A., Blackburn, N. A., Craigen, L., Davison, P., Tomlin, J., Sugden, K., Johnson, I.T. & Read, N.W. (1987) Viscosity of food gums determined invitro related to their hypoglycemic actions. *American Journal of Clinical Nutrition,* **46,** 72-77.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. & Attia, H. (2011) Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry,* **124,** 411-421.

Evers, T. & Millar, S. (2002) Cereal grain structure and development: Some implications for quality. *Journal of Cereal Science,* **36,** 261-284.

Fadel, J.G., Newman, R.K., Newman, C.W. & Graham, H. (1989) Effects of baking hulless barley on the digestibility of dietary-components as measured at the ileum and in the feces in pigs. *Journal of Nutrition,* **119,** 722-726.

Feldheim, W. & Wisker, E. (2000) Studies on the improvement of dietary fibre intake. *Deutsche Lebensmittel-Rundschau,* **96,** 327-330.

Flander, L., Salmenkallio-Marttila, M., Suortti, T. & Autio, K. (2007) Optimization of ingredients and baking process for improved wholemeal oat bread quality. *LWT-Food Science and Technology,* **40,** 860-870.

Forssell, P., Shamekh, S., Härkönen, H. & Poutanen, K. (1998) Effects of native and enzymatically hydrolysed soya and oat lecithins in starch phase transitions and bread baking. *Journal of the Science of Food and Agriculture,* **76,** 31-38.

Foschia, M., Peressini, D., Sensidoni, A. & Brennan, C.S. (2013) The effects of dietary fibre addition on the quality of common cereal products. *Journal of Cereal Science,* **58,** 216-227.

Friedman, M. & Atsmon, D. (1988) Comparison of grain composition and nutritional quality in wild barley (hordeum-spontaneum) and in a standard cultivar. *Journal of Agricultural and Food Chemistry,* **36,** 1167-1172.

Fu, C., Tian, H., Li, Q., Cai, T. & Du, W. (2006) Ultrasound-assisted extraction of xyloglucan from apple pomace. *Ultrasonics Sonochemistry,* **13,** 511-516.

Fuentes-Zaragoza, E., Riquelme-Navarrete, M.J., Sanchez-Zapata, E. & Perez-Alvarez, J. A. (2010) Resistant starch as functional ingredient: A review. *Food Research International,* **43,** 931-942.

Gamel, T.H., Badali, K. & Tosh, S.M. (2013) Changes of β-glucan physicochemical characteristics in frozen and freeze dried oat bran bread and porridge. *Journal of Cereal Science,* **58,** 104-109.

Gamel, T.H., Abdel-Aal, E.-S.M., Ames, N.P., Duss, R. & Tosh, S.M. (2014) Enzymatic extraction of beta-glucan from oat bran cereals and oat crackers and optimization of viscosity measurement. *Journal of Cereal Science,* **59,** 33-40.

Gormley, T.R. & Morrissey, A. (1993) A note on the evaluation of wheaten breads containing oat flour or oat flakes. *Irish Journal of Agricultural and Food Research,* 205-209.

Havrlentova, M., Petrulakova, Z., Burgarova, A., Gago, F., Hlinkova, A. & Sturdik, E. (2011) Cereal beta-glucans and their Significance for the Preparation of Functional Foods - A Review. *Czech Journal of Food Sciences,* **29,** 1-14.

Helm, C.V., De Francisco, A., Gaziola, S.A., Fornazier, R.F., Pompeu, G.B. & Azevedo, R.A. (2004) Hull-less barley varieties: Storage proteins and amino acid distribution in relation to nutritional quality. *Food Biotechnology,* **18,** 327-341.

Hill, M.J. & Fernandez, F. (1990) Bacterial metabolism, fiber, and colorectal cancer. In: Kritchevsky, D., Bonfield, C.T., & Anderson, J.W. (eds) *Dietary Fiber: Chemistry, Physiology, and Health Effects.* Boston: Springer.

Hoije, A., Grondahl, M., Tommeraas, K. & Gatenholm, P. (2005) Isolation and characterization of physicochemical and material properties of arabinoxylans from barley husks. *Carbohydrate Polymers,* **61,** 266-275.

Hromadkova, Z., Ebringerova, A. & Valachovic, P. (1999a). Comparison of classical and ultrasound-assisted extraction of polysaccharides from Salvia officinalis L. *Ultrasonics Sonochemistry,* **5,** 163-168.

Hromadkova, Z., Kovacikova, J. & Ebringerova, A. (1999b) Study of the classical and ultrasound-assisted extraction of the corn cob xylan. *Industrial Crops and Products,* **9,** 101-109.

Huang, W., Xue, A., Niu, H., Jia, Z. & Wang, J. (2009) Optimised ultrasonic-assisted extraction of flavonoids from Folium eucommiae and evaluation of antioxidant activity in multi-test systems in vitro. *Food Chemistry,* **114,** 1147-1154.

Hudson, C.A., Chiu, M.M. & Knuckles, B.E. (1992) Development and characteristics of high-fiber muffins with oat bran, rice bran, or barley fiber fractions. *Cereal Foods World,* **37,** 373-376.

Idehen, E., Tang, Y. & Sang, S. (2017a) Bioactive phytochemicals in barley. *Journal of Food and Drug Analysis,* **25,** 148-161.

Idehen, E., Tang, Y. & Sang, S.M. (2017b) Bioactive phytochemicals in barley. *Journal of Food and Drug Analysis,* **25,** 148-161.

Iranshahi, M., Mahdi, S., Ardebili, S. & Yasini, S. (2014) Effect of inulin and β-glucan on the physicochemical, rheological, and sensory properties barbari bread. *Journal of Engineering Res and Application,* **4,** 90-97.

Izydorczyk, M.S. & Biliaderis, C.G. (2000) Structural and functional aspects of cereal arabinoxylans and beta-glucans. *Novel Macromolecules in Food Systems,* **41,** 361-384.

Izydorczyk, M.S. & Dexter, J.E. (2008) Barley β-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products–a Review. *Food Research International,* **41,** 850-868.

Izydorczyk, M.S., Miller, S.S. & Beattie, A.D. (2014) Milling Food Barley: Production of Functional Fractions Enriched with beta-Glucans and Other Dietary Fiber Components. *Cereal Foods World,* **59,** 277-285.

Jadhav, S. J., Lutz, S.E., Ghorpade, V.M. & Salunkhe, D.K. (1998) Barley: Chemistry and value-added processing. *Critical Reviews in Food Science and Nutrition,* **38,** 123-171.

Jayachandran, M., Chen, J., Chung, S.S. M. & Xu, B. (2018). A critical review on the impacts of β-glucans on gut microbiota and human health. *The Journal of Nutritional Biochemistry,* **61,** 101-110.

Jenkins, D.J., Wolever, T.M., Leeds, A.R., Gassull, M.A., Haisman, P., Dilawari, J., Goff, D.V., Metz, G.L. & Alberti, K.G. (1978) Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *British Journal of Medicine,* **1**, 1392-1394.

Karppinen, S., Liukkonen, K., Aura, A.M., Forssell, P. & Poutanen, K. (2000) In vitro fermentation of polysaccharides of rye, wheat and oat brans and inulin by human faecal bacteria. *Journal of the Science of Food and Agriculture,* **80,** 1469-1476.

Katz, E. & Labuza, T.P. (1981) Effect of water activity on the sensory crispness and mechanical deformation of snack food products. *Journal of Food Science,* **46,** 403-409.

Kaufmann, B. & Atrice (2002) Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. *Phytochemical Analysis,* **13,** 105-113.

Kaur, R. & Riar, C.S. (2020) Sensory, rheological and chemical characteristics during storage of set type full fat yoghurt fortified with barley β-glucan. *Journal of Food Science and Technology,* **57,** 41-51.

Kent, N.L. (1983) *Technology of Cereals*. Oxford: Pergamon Press.

Kerckhoffs, D., Brouns, F., Hornstra, G. & Mensink, R.P. (2002) Effects on the human serum lipoprotein profile of beta-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. *Journal of Nutrition,* **132,** 2494-2505.

Klamczynski, A.P., Knuckles, B.E. & Glenn, G.M. (2004) Puffing and jet cooking affect solubility and molecular weight of barley beta-glucans. *Journal of Food Processing and Preservation,* **28,** 433-441.

Koehler, P. & Wieser, H. (2013) Chemistry of Cereal Grains. In: Gobbetti, M. & Gänzle, M. (eds.) *Handbook on Sourdough Biotechnology.* Boston, MA: Springer.

Kurek, M. A., Wyrwisz, J., Brzeska, M., Moczkowska, M., Karp, S. & Wierzbicka, A. (2018) Effect of different beta-glucan preparation pretreatments on fortified bread quality. *Food Science and Technology*, **38**, Available at https://www.researchgate.net/publication /327827650_Effect_of_different_beta-glucan_preparation_pretreatments_on_fortified _bread_quality

Lan-Pidhainy, X., Brummer, Y., Tosh, S.M., Wolever, T.M. & Wood, P.J. (2007) Reducing beta-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect. *Cereal Chemistry,* **84,** 512-517.

Lazaridou, A., Biliaderis, C., Micha-Screttas, M. & Steele, B.R. (2004) A comparative study on structure–function relations of mixed-linkage (1→ 3),(1→ 4) linear β-d-glucans. *Food Hydrocolloids,* **18,** 837-855.

Lazaridou, A. & Biliaderis, C.G. (2007) Molecular aspects of cereal beta-glucan functionality: Physical properties, technological applications and physiological effects. *Journal of Cereal Science,* **46,** 101-118.

MacGregor, A.W. & Fincher, G.B. (1993). *Carbohydrates of the barley grain*. In: Henry, R.J. and Kettlewell, P.S. (eds) Cereal Grain Quality, Dodrecht: Springer.

Madacsi, J.P., Parrish, F.W. & Roberts, E.J. (1983) Nonenzymic method for determination of beta-glucan in the presence of starch. *Journal of the American Society of Brewing Chemists,* **41,** 161-162.

Madhujith, T. & Shahidi, F. (2007) Antioxidative and antiproliferative properties of selected barley (*Hordeum vulgarae* l.) cultivars and their potential for inhibition of low-density lipoprotein (LDL) cholesterol oxidation. *Journal of Agricultural and Food Chemistry,* **55,** 5018-5024.

Maheshwari, G., Sowrirajan, S. & Joseph, B. (2017) Extraction and Isolation of β-Glucan from Grain Sources—A Review. *Journal of Food Science,* **82,** 1535-1545.

Malkki, Y. (2004) Trends in dietary fibre research and development - A review. *Acta Alimentaria,* **33,** 39-62.

Maran, J.P. & Priya, B. (2014) Ultrasound-assisted extraction of polysaccharide from Nephelium lappaceum L. fruit peel. *International Journal of Biological Macromolecules,* **70,** 530-536.

Marlett, J.A., Hosig, K.B., Vollendorf, N.W., Shinnick, F.L., Haack, V.S. & Story, J.A. (1994) Mechanism of serum-cholesterol reduction by oat bran. *Hepatology,* **20,** 1450-1457.

McCleary, B.V. (1988) Purification of (1- 3),(1- 4)-beta-d-glucan from barley flour. *Methods in Enzymology,* **160,** 511-514.

Messia, M.C., Oriente, M., Angelicola, M., De Arcangelis, E. & Marconi, E. (2019) Development of functional couscous enriched in barley β-glucans. *Journal of Cereal Science,* **85,** 137-142.

Mikkelsen, M. S., Jensen, M. G. & Nielsen, T. S. (2017a) Barley beta-glucans varying in molecular mass and oligomer structure affect cecal fermentation and microbial composition but not blood lipid profiles in hypercholesterolemic rats. *Food & Function,* **8,** 4723-4732.

Mikkelsen, M. S., Meier, S., Jensen, M. G., Qin, F., Stoica, I.-M., Martens, H. J., Blennow, A. & Jespersen, B. M. (2017b) Barley genotypic beta-glucan variation combined with enzymatic modifications direct its potential as a natural ingredient in a high fiber extract. *Journal of Cereal Science,* **75,** 45-53.

Miyamoto, J., Watanabe, K., Taira, S., Kasubuchi, M., Li, X., Irie, J., Itoh, H. & Kimura, I. (2018) Barley β-glucan improves metabolic condition via short-chain fatty acids produced by gut microbial fermentation in high fat diet fed mice. *PLoS One,* **13,** e0196579.

Moriartey, S., Temelli, F. & Vasanthan, T. (2010) Effect of Formulation and Processing Treatments on Viscosity and Solubility of Extractable Barley β-Glucan in Bread Dough Evaluated Under In Vitro Conditions. *Cereal Chemistry,* **87,** 65-72.

Mosele, J. I., Motilva, M. A.-J. & Ludwig, I. A. (2018) Beta-glucan and phenolic compounds: their concentration and behavior during in vitro gastrointestinal digestion and colonic fermentation of different barley-based food products. *Journal of Agricultural and Food Chemistry,* **66,** 8966-8975.

Munck, L., Moller, B., Jacobsen, S. & Sondergaard, I. (2004) Near infrared spectra indicate specific mutant endosperm genes and reveal a new mechanism for substituting starch with (1 -> 3,1 -> 4)-beta-glucan in barley. *Journal of Cereal Science,* **40,** 213-222.

Newman, R. K. & Newman, C. W. (1991) Barley as a food grain. *Cereal Foods World,* **36,** 800-805.

Newman, C. W. & Newman, R. K. (2006) A brief history of barley foods. *Cereal Foods World,* **51,** 4-7.

Newman, R. K. & Newman, C. W. (2008) *Barley for Food and Health: Science, Technology, and Products*.

Nilan, R. A. & Ullrich, S. E. (1993) *Barley: Taxonomy, origin, distribution, production, genetics, and breeding*. Hoboken, NJ: Wiley.

Nishimune, T., Yakushiji, T., Sumimoto, T., Taguchi, S., Konishi, Y., Nakahara, S., Ichikawa, T. & Kunita, N. (1991) Glycemic response and fiber content of some foods. *American Journal of Clinical Nutrition,* **54,** 414-419.

Owen, B.D., Sosulski, F., Wu, K.K. & Farmer, M.J. (1977) Variation in mineral-content of saskatchewan feed grains. *Canadian Journal of Animal Science,* **57,** 679-687.

Parada, J. & Aguilera, J. (2007) Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science,* **72,** R21-R32.

Paras, S. & Kotari, S.I. (2017) Barley: impact of processing on physicochemical and thermal properties-a review. *Food Reviews International,* **33,** 359-381.

Pham Van, H. (2016) Phenolic compounds of cereals and their antioxidant capacity. *Critical Reviews in Food Science and Nutrition,* **56,** 25-35.

Pico, Y. (2013) Ultrasound-assisted extraction for food and environmental samples. *Trac-Trends in Analytical Chemistry,* **43,** 84-99.

Price, P. B. & Parsons, J. (1979) Distribution of lipids in embryonic axis, bran-endosperm, and hull fractions of hulless barley and hulless oat grain. *Journal of Agricultural and Food Chemistry,* **27,** 813-815.

Puri, M., Sharma, D. & Barrow, C. J. (2012) Enzyme-assisted extraction of bioactives from plants. *Trends in Biotechnology,* **30,** 37-44.

Regand, A., Chowdhury, Z., Tosh, S.M., Wolever, T.M.S. & Wood, P. (2011) The molecular weight, solubility and viscosity of oat beta-glucan affect human glycemic response by modifying starch digestibility. *Food Chemistry,* **129,** 297-304.

Rinaldi, L., Rioux, L.-E., Britten, M. & Turgeon, S.L. (2015) In vitro bioaccessibility of peptides and amino acids from yogurt made with starch, pectin, or β-glucan. *International Dairy Journal,* **46,** 39-45.

Sarteshnizi, R.A., Hosseini, H., Bondarianzadeh, D. & Colmenero, F.J. (2015) Optimization of prebiotic sausage formulation: Effect of using β-glucan and resistant starch by D-optimal mixture design approach. *LWT-Food Science and Technology,* **62,** 704-710.

Saulnier, L., Gévaudan, S. & Thibault, J.-F. (1994) Extraction and partial characterisation of β-glucan from the endosperms of two barley cultivars. *Journal of Cereal Science,* **19,** 171-178.

Schlörmann, W., Zetzmann, S., Wiege, B., Haase, N., Greiling, A., Lorkowski, S., Dawczynski, C. & Glei, M. (2019) Impact of different roasting conditions on chemical composition, sensory quality and physicochemical properties of waxy-barley products. *Food & Function,* **10,** 5436-5445.

Schlörmann, W., Zetzmann, S., Wiege, B., Haase, N., Greiling, A., Lorkowski, S., Dawczynski, C. & Glei, M. (2020) Impact of different roasting conditions on sensory properties and health-related compounds of oat products. *Food Chemistry,* **307,** 125548.

Schmidt, C., Geweke, I., Struck, S., Zahn, S. & Rohm, H. (2018) Blackcurrant pomace from juice processing as partial flour substitute in savoury crackers: dough characteristics and product properties. *International Journal of Food Science & Technology,* **53,** 237-245.

Sen, C.K., Khanna, S. & Roy, S. 2007. Tocotrienols in health and disease: The other half of the natural vitamin E family. *Molecular Aspects of Medicine,* **28,** 692-728.

Sharma, P. & Kotari, S.L. (2017) Barley: Impact of processing on physicochemical and thermal properties-A review. *Food Reviews International,* **33,** 359-381.

Shukla, T. (1994) Future snacks and snack food technology. *Cereal Foods World (USA).* Washington, D.C.: Food and Agricultural Organization of the UN.

Singhal, P. & Kaushik, G. (2016) Therapeutic effect of cereal grains: a review. *Critical Reviews in Food Science and Nutrition,* **56,** 748-759.

Skendi, A., Biliaderis, C.G., Lazaridou, A. & Izydorczyk, M.S. (2003) Structure and rheological properties of water soluble beta-glucans from oat cultivars of Avena sativa and Avena bysantina. *Journal of Cereal Science,* **38,** 15-31.

Skendi, A., Biliaderis, C., Papageorgiou, M. & Izydorczyk, M. (2010a) Effects of two barley β-glucan isolates on wheat flour dough and bread properties. *Food Chemistry,* **119,** 1159-1167.

Skendi, A., Biliaderis, C. G., Papageorgiou, M. & Izydorczyk, M. S. (2010b) Effects of two barley β-glucan isolates on wheat flour dough and bread properties. *Food Chemistry,* **119,** 1159-1167.

Storsley, J., Izydorczyk, M., You, S., Biliaderis, C. & Rossnagel, B. (2003) Structure and physicochemical properties of β-glucans and arabinoxylans isolated from hull-less barley. *Food Hydrocolloids,* **17,** 831-844.

Sullivan, P., O'Flaherty, J., Brunton, N., Gee, V.L., Arendt, E. & Gallagher, E. (2010) Chemical composition and microstructure of milled barley fractions. *European Food Research and Technology,* **230,** 579-595.

Sun, J. X., Sun, X. F., Zhao, H. & Sun, R. C. (2004) Isolation and characterization of cellulose from sugarcane bagasse. *Polymer Degradation and Stability,* **84,** 331-339.

Sundberg, B., Wood, P., Lia, A., Andersson, H., Sandberg, A. S., Hallmans, G. & Aman, P. (1996) Mixed-linked beta-glucan from breads of different cereals is partly degraded in the human ileostomy model. *American Journal of Clinical Nutrition,* **64,** 878-885.

Temelli, F., Bansema, C. & Stobbe, K. (2004a) Development of an orange-flavored barley beta-glucan beverage. *Cereal Chemistry,* **81,** 499-503.

Temelli, F., Bansema, C. & Stobbe, K. (2004b) Development of an orange-flavored barley β-glucan beverage. *Cereal Chemistry,* **81,** 499-503.

Tessari, P. & Lante, A. (2017) A multifunctional bread rich in beta glucans and low in starch improves metabolic control in type 2 diabetes: a controlled trial. *Nutrients,* **9,** 297.

Thorburn, A., Muir, J. & Proietto, J. (1993) Carbohydrate fermentation decreases hepatic glucose output in healthy-subjects. *Metabolism-Clinical and Experimental,* **42,** 780-785.

Tian, Y., Zeng, H., Xu, Z., Zheng, B., Lin, Y., Gan, C. & Lo, Y. M. (2012) Ultrasonic-assisted extraction and antioxidant activity of polysaccharides recovered from white button mushroom (*Agaricus bisporus*). *Carbohydrate Polymers,* **88,** 522-529.

Upadhyay, J. & Misra, K. (2009) Towards the interaction mechanism of tocopherols and tocotrienols (vitamin E) with selected metabolizing enzymes. *Bioinformation,* **3,** 326-31.

Vaikousi, H., Biliaderis, C. & Izydorczyk, M. (2004) Solution flow behavior and gelling properties of water-soluble barley (1→ 3, 1→ 4)-β-glucans varying in molecular size. *Journal of Cereal Science,* **39,** 119-137.

Vasanthan, T. & Temelli, F. (2008) Grain fractionation technologies for cereal beta-glucan concentration. *Food Research International,* **41,** 876-881.

Vis, R. B. & Lorenz, K. (1997) beta-glucans: Importance in brewing and methods of analysis. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie,* **30,** 331-336.

Vitaglione, P., Napolitano, A. & Fogliano, V. (2008) Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science & Technology,* **19,** 451-463.

Wang, L.J. & Weller, C.L. (2006) Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology,* **17,** 300-312.

Wang, J., Sun, B., Cao, Y., Tian, Y. & Li, X. (2008) Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry,* **106,** 804-810.

Wei, L., Cui, S.W. & Kakuda, Y. (2006) Extraction, fractionation, structural and physical characterization of wheat beta-D-glucans. *Carbohydrate Polymers,* **63,** 408-416.

Wood, P.J., Weisz, J., Fedec, P. & Burrows, V.D. (1989) Large-scale preparation and properties of oat fractions enriched in (1- 3)(1- 4)-beta-d-glucan. *Cereal Chemistry,* **66,** 97-103.

Wood, P.J. (1991) Physicochemical characterization of barley beta glucans. *Cereal Foods World,* **36,** 679-679.

Woodward, J.R., Phillips, D.R., Fincher, G.B. & Stone, B.A. (1981) Fine structure and physiochemical properties of water soluble barley 1 3 1 4-BETA GLUCAN. *Proceedings of the International Botanical Congress,* **13,** 45-45.

Xu, F., Sun, J. X., Geng, Z. C., Liu, C. F., Ren, J. L., Sun, R. C., Fowler, P. & Baird, M. S. (2007) Comparative study of water-soluble and alkali-soluble hemicelluloses from perennial ryegrass leaves (Lolium peree). *Carbohydrate Polymers,* **67,** 56-65.

Ying, Z., Han, X. & Li, J. (2011) Ultrasound-assisted extraction of polysaccharides from mulberry leaves. *Food Chemistry,* **127,** 1273-1279.

Zhang, G., Chen, J. & Wang, J. (2002) Variation in barley endosperm beta-glucan content in three barley cultivars as a function of spike number and within-spike position. *Journal of Cereal Science,* **35,** 99-101.

Zhang, L. & Liu, Z. (2008) Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. *Ultrasonics Sonochemistry,* **15,** 731-737.

Zhao, Y., Zhou, H.-M., Huang, Z.-H. & Zhao, R.-Y. (2020) Different aggregation states of barley β-glucan molecules affects their solution behavior: A comparative analysis. *Food Hydrocolloids,* **101,** 105543.

Zhao, Z. & Moghadasian, M.H. (2008) Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. *Food Chemistry,* **109,** 691-702.

Zhen, L., Ying, D., Lai-Ting, Y. & La-Ni, S. (2015) Effect of whole barley flour addition on dough properties and quality of bread. *Modern Food Science and Technology***,** 197-202.

Zhu, F., Du, B., Bian, Z. & Xu, B. (2015) Beta-glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. *Journal of Food Composition and Analysis,* **41,** 165-173.

Zhu, F., Du, B. & Xu, B. (2016) A critical review on production and industrial applications of beta-glucans. *Food Hydrocolloids,* **52,** 275-288.

Zielke, C., Kosik, O., Ainalem, M.-L., Lovegrove, A., Stradner, A. & Nilsson, L. (2017) Characterization of cereal β-glucan extracts from oat and barley and quantification of proteinaceous matter. *PLoS One,* **12,** e0172034.

Zydenbos, S. & Humphrey-Taylor, V. (2003) *Biscuits, Cookies, and Crackers: Nature of the Products*. In: Caballero, B (ed), Encyclopedia of Food Sciences and Nutrition. 3rd ed. Amsterdam: Elsevi

Chapter 3: **HOT WATER EXTRACTION OF BETA-GLUCAN FROM UK AND JORDANIAN BARLEY**

Abstract

This study aimed to evaluate the use of hot water extraction as a process for obtaining beta-glucan from the flour of two types of barley grain grown under different environmental conditions (UK and Jordan). Proximate analysis was carried out for both barley grain flours. Variable hot water extraction times (90 min, 3 h and 4 h) and temperatures (50 °C, 60 °C and 70 °C) were tested for both grains. The highest mass yields were obtained at 60°C for 4 h for the UK barley and 50 °C for 3 h for the Jordanian barley. The highest beta-glucan recovery, 9.35 (%, w/w) and 10.58 (%, w/w) for the UK and Jordanian barley, respectively, was obtained using the same conditions listed above**.** The starch content in the extraction residue was constant for both the UK and Jordanian barley, ranging from 32–37% (%, w/w) for the UK grains and 40–43% (%, w/w) for the Jordanian barley. The protein content in the extract was quite low, approximately 2% (%, w/w).

Keywords: Hot water extraction, beta-glucan, purity, recovery, barley flour

3.1 Introduction

Beta-glucans are complex carbohydrates made of linear glucose polymers joined together by 70% β-(1–4) and 30% β-(1–3) glycosidic bonds (Du *et al*., 2014a). Together with cellulose, hemicelluloses, and other carbohydrates, beta-glucans are common components of cell walls in cereal grains (Doblin *et al*., 2010). The presence of β-(1–3) bonds renders beta-glucans more flexible, soluble and viscous than the rigid and insoluble polymers that contain only β-(1–4) bonds (e.g., cellulose) (Burton *et al*., 2010). Beta-glucans in barley occur mainly in the endosperm cell wall, together with other non-starch polysaccharides, which enclose the grain's starch, protein, and lipid reserves. Differences in beta-glucan content within barley grains have been attributed to variability in environmental growing conditions. The beta-glucan content may be related to the cultivar itself and the season and location of harvesting. The availability of water during grain maturation seems to be one of the main environmental factors influencing beta-glucan levels in the grain. Dry conditions before harvest result in high levels of beta-glucan, and the reverse is true under moist conditions (Fastnaught *et al*., 1996; Böhm & Kulicke, 1999). Furthermore, it has been shown that a higher growth temperature favours an increase in beta-glucan content in both barley and oat (Saastamoinen *et al*., 1992; Fastnaught *et al*., 1996; Colleoni-Sirghie *et al*., 2004,).

The localization of beta-glucans in cereal grains influences the procedures required to extract and purify them (Fulcher & Rooney Duke, 2002). Thus, the extractability and characteristics of beta-glucans vary greatly, depending on the extraction procedure performed. This variability has resulted in the development of multiple technologies for obtaining beta-glucans from cereals. These extraction processes can be classified into two categories: dry and wet. Dry extraction involves the use of dry milling and sieving to reduce the particle size of the grain. Since beta-glucans are mainly concentrated in the endosperm, they can be separated from

other components based on their size, yielding a flour fraction rich in beta-glucans. Dry milled barley grain is usually used as a starting material for subsequent aqueous extraction methods to obtain a product with a higher beta-glucan concentration. Wet extraction uses a solvent to obtain an aqueous extract, and a variety of such techniques have been employed by researchers to extract beta-glucans from cereals, including hot-water extraction, solvent extraction, enzymatic extraction, and alkali extraction. These processes could be highly complex since they usually involve multiple isolation and purification steps.

The extraction of cereal beta-glucans is often challenging, causing beta-glucan extracted from cereal to be more expensive than that obtained from other natural sources such as yeast, mushrooms, bacteria and algae (Daou & Zhang, 2012; Zhu *et al*., 2015). The most common method used to obtain beta-glucans from different sources is hot water extraction (HWE). This method is made possible by the solubility of beta-glucans in hot water. It involves separating co-extracted water-soluble proteins and utilizing isoelectric precipitation and ethanol-induced precipitation of beta-glucans (Saulnier *et al*., 1994). The principles behind the HWE process are simple and straightforward: water at a high temperature penetrates the cell wall and dissolves water-soluble components such as sugars and proteins. However, the high temperature and long extraction time may lead to starch contamination, thereby decreasing the purity of beta-glucans (Ying *et al*., 2011). HWE should be performed at the highest practicable temperature below that of starch gelatinisation to ensure optimal results. Temperatures ranging from 50 °C to 55 °C were found to be the most conducive for extracting the highest-purity beta-glucans (Ahmad *et al*., 2010). Moreover, once extracted, beta-glucans may be contaminated with considerable amounts of starch and protein unless alpha-amylase pre-treatment is used to minimise starch contamination (Asif *et al*., 2009; Benito-Román *et al*., 2011). A combination of methods, such as milling and defatting

the grain, is often required to achieve high purity and yield with minimal changes to their molecular structure. Beta-glucan extraction must be preceded by milling and reducing particle size to increase extraction efficiency (Wood *et al*., 1978). Beta-glucan extraction from cereal grains generally involves three essential steps: (1) inactivation of endogenous enzymes, (2) extraction, and (3) beta-glucan precipitation. Before extraction, endogenous enzymes (glucanases and amylases) must be deactivated. In the case of endogenous glucanases, inactivation is necessary because these enzymes are responsible for beta-glucan degradation and would cause a decrease in molecular weight that would ultimately affect the functional properties of extracted beta-glucans (Irakli *et al*., 2004). Inactivation is usually achieved by refluxing the barley with aqueous ethanol or treating the barley flour with dilute aqueous ethanol at temperatures above 60°C to aid in the process (Brennan & Cleary, 2005).

Although HWE is the most common method used to extract beta-glucans, other methods include alkali extraction (Kao *et al*., 2012), microwave-assisted extraction (Routray & Orsat, 2012), and acidic extraction (Park *et al*., 2014). However, some of these methods have drawbacks, such as long extraction times (Routray & Orsat, 2012), high process costs, and low environmental sustainability. Some of these issues have been addressed by newer methods such as ultrasound-assisted extraction (Du *et al*., 2014b), which has also been shown to provide increased yields. In a comparative study of beta-glucan extraction methods, Ahmad *et al*. (2009) found that HWE (90 min) resulted in the highest production yields and recovery of barley beta-glucans (5.4% and 83.1%, respectively). One reason for the high recovery of beta-glucans using HWE is that preliminary treatment (reflux with 80% ethanol) inactivated the β-glucanase enzyme, while increasing the starch gelatinisation and protein solubilisation.

The objective of this study was to identify differences in the composition of whole-grain barley cultivated in different environmental climates (UK and Jordan). The study also aimed to assess the efficiency of HWE extraction on purity and recovery of beta-glucans from the two different barley cultivars. The hypothesis was that barley grains cultivated in dry environmental conditions would have higher beta-glucan levels than barley grown in wet conditions, promoting greater beta-glucan extraction.

3.2 Materials and Methods

3.2.1 Raw Materials

Hulled barley grain samples were obtained from the UK and Jordan. The Jordanian barley was purchased from the Figs and Olive bakery in Kuwait as a food product (produced in 2017), and the UK barley was purchased from Heygates Ltd. as an animal feed product (produced in 2017). Barley grains were ground into flour in the laboratory using a coffee grinder (De'Longhi; Type KG46; 20 mesh size, 840 microns). The resulting barley flour was stored in glass bottles at room temperature (20 °C) and used in all experiments.

3.2.2 Proximate Analysis

Proximate analysis was carried out for the UK and Jordanian barley flours. The volumes of starch, protein, fat, moisture, ash, and structural carbohydrates were determined as described below.

3.2.2.1 Starch Analysis

The starch content of the barley flours was enzymatically determined using the total starch test kit from Megazyme (Megazyme, Ireland), which includes thermostable α-amylase and amyloglucosidase. This method is based on the AOAC (Official Method 996.11) and AACC (Official Method 76.13.01) methods. Thermostable α-amylase hydrolyses starch into soluble branched and unbranched maltodextrins, whereas amyloglucosidase (AMG) hydrolyses maltodextrins into D-glucose. Subsequently, D-glucose is oxidised into D-gluconate with the release of one mole of hydrogen peroxide (H_2O_2), quantitatively measured in a colourimetric reaction employing peroxidase and the production of a quinonimine dye. Absorbance was measured at 510 nm. The quantification of starch was calculated using Equation 3.1.

$$Total\ starch\ (\%) = \Delta A \times \frac{F}{W} \times FV \times 0.9 \qquad \textit{Equation 3.1}$$

where: ΔA = absorbance difference between sample and blank

F = factor for the conversion of absorbance values to μg of glucose

$$= \left(\frac{100\ (\mu g\ of\ D-glucose)}{absorbance\ of\ 100\ \mu g\ of\ D-glucose}\right)$$

W = the weight of the barley extracts analysed (mg)

FV = final volume (100 ml)

3.2.2.2 Protein Analysis

The protein content of barley flours was determined using the Kjeldahl method (AOAC, 2005). The barley flours (UK and Jordanian) were digested in concentrated sulfuric acid, H_2SO_4,

followed by distillation and titration with 0.1N H_2SO_4. Protein was calculated by multiplying the nitrogen (N) content by 6.25 (nitrogen conversion factor for barley).

3.2.2.3 Moisture Analysis

The moisture content was determined by heating 1 g of sample in a halogen moisture analyser (Mettler Toledo HE53, China) at 105 °C until a constant weight was achieved.

3.2.2.4 Fat Analysis

Lipid content was gravimetrically determined according to the Soxhlet method (AOAC, 2005). Briefly, 1 g of sample was placed into a weighed pre-dried extraction thimble closed using a fat-free piece of cotton. The thimbles were then placed into the Soxhlet extractor apparatus, and the lipids were extracted by petroleum ether for 8 hours. Upon completion of the extraction, petroleum ether was removed under vacuum using a rotary evaporator. The round-bottom flask was dried in the oven at 100 °C for 1 hour and cooled in the desiccator until it reached a constant weight. Equation 3.2 was used to calculate crude fat.

$$Crude\ lipid\ content\ (\%) = 100\ \times \frac{B-A}{C} \qquad \textit{Equation 3.2}$$

where: A = Weight of clean dry round-bottom flask (g)

B = Weight of round-bottom flask with fat (g)

C = Weight of samples (g)

3.2.2.5 Ash Analysis

Ash content was measured by weighing 5 g of barley flour in a pre-weighed crucible and placing it into a furnace at 600 °C for 4 hours. Samples were cooled in a desiccator before weighing on an analytical balance to calculate the ash content.

3.2.2.6 Structural Carbohydrates and Lignin Determination

Structural carbohydrates and lignin (Klason and acid-soluble) were determined using the NREL procedure (Sluiter *et al*., 2008). Briefly, 300 mg of barley flour was pre-hydrolysed with 3 ml of 72 % (v/v) H_2SO_4 at 30 °C for 1 h. Subsequently, 84 ml of distilled water were added to the mixture to dilute the sulphuric acid content to 4 % (v/v), and samples were placed in an autoclave; hydrolysis was carried out at 121 °C for 30 mins. On the completion of hydrolysis, the mixtures were neutralised with calcium carbonate to pH 5–6. Samples were then filtered, and the filtrate was measured for acid-soluble lignin spectrophotometrically at 240 nm. Acid-soluble lignin (ASL) was calculated according to Equation 3.3. The washed residue was dried at 100 °C for 18 hrs. Subsequently, the dried samples were placed in a furnace (500 °C; 5 h), and the ash weight was classified as ASL. Total lignin was calculated as the sum of acid-soluble and acid-insoluble lignin, based on the equation below:

$$ASL = \frac{Absorbance \times Dilution\ factor \times filtrate\ volume\ (ml)}{Extinction\ coefficient \times Weight\ of\ sample\ (g)} \qquad \textit{Equation 3.3}$$

An aliquot of the filtrate (1 ml) was used to determine monosaccharides (basic unit of carbohydrates in barley flour). Monosaccharides were determined by HPLC analysis (Agilent 1260 Infinity) with an Aminex HPX-87H (Biorad, UK) column coupled to a differential

refractometer and a diode array detector. The operating conditions were as follows: sample volume, 20 µl; mobile phase, 5 mM H_2SO_4; flow rate, 0.6 ml/ min; column temperature, 65 °C. Monosaccharides (glucose, galactose, xylose and arabinose) and uronic acids were quantified based on standard curves constructed using standard solutions.

3.2.3 Chemical Analysis

The beta-glucan content in barley flours and the HWE extracts was determined using the mixed-linkage beta-glucan enzymatic kit by Megazyme (Megazyme, Ireland) and Equation 3.4. Briefly, samples were suspended and hydrated in a pH 6.5 buffer solution, incubated with purified lichenase and filtered. An aliquot of the filtrate was then hydrolysed to completion with purified beta-glucosidase. The D-glucose produced was determined using a glucose oxidase/peroxidase reagent and by measuring the absorbance of the aliquot at 510 nm. The protein content in the extracts from the HWE method was measured using the Bradford protocol (Kruger, 2009).

$$Beta-glucans\ (\%, w/w) = \Delta A \times \frac{F}{W} \times FV \times 0.9 \qquad \textit{Equation 3.4}$$

where: ΔA = absorbance difference between sample and blank

F = factor for the conversion of absorbance values to µg of glucose

$$\left(= \frac{100\ (\mu g\ of\ D-glucose)}{absorbance\ of\ 100\ \mu g\ of\ D-glucose}\right)$$

FV = final volume of sample (9.4 ml)

W = weight in mg of barley flour (100 mg)

3.2.4 Extraction of Beta-glucans

3.2.4.1 Hot Water Extraction

Hot water extraction (HWE) was carried out according to the method described by Vriesmann *et al*. (2011) with some modifications. The individual protocol is described in (Figure 3.1). Briefly, barley flour was refluxed with ethanol (80%, v/v) for 6 h to defat the sample and deactivate endogenous glucanases, then oven-dried at 40 °C overnight. The defatted milled barley flour (5 g) was refluxed in 125 ml of water for different extraction times (90 min, 3 h and 4 h) and temperatures (50 °C, 60 °C and 70 °C). Upon cooling, the mixture was centrifuged at 15,000 × *g* for 30 min, and the solid residue was dried for 24 hours in the oven at 50 °C. The supernatant was centrifuged at 15,000 × *g* for 20 min to purify the extract. The pH of the supernatant was adjusted to 4 with 0.1 HCl, and the mixture was again centrifuged at 15,000 × *g* for 25 min to precipitate any water-soluble proteins with different isoelectric points. The separated precipitated proteins were then discarded. After centrifugation, the supernatant was transferred to a new centrifuge tube, and to that filtrate, 99% ethanol was added (1:1, v/v) to precipitate beta-glucans. The suspension obtained after the addition of ethanol was stored in a refrigerator for 24 h (4 °C) to permit the precipitation of beta-glucans. The suspension was centrifuged (4000 × *g* for 15 min). The pellets obtained were frozen at -20°C overnight and then freeze-dried for 24 h under vacuum at -45°C (Virtis SP Scientific, UK).

Two parameters were introduced to demonstrate the effectiveness of the extraction numerically. The first referred to the mass yield of extraction and was expressed as

$$Mass\ yield\ (\%) = \frac{mass\ of\ extract\ (g)}{initial\ mass\ of\ sample\ (g)} \times 100 \qquad \textit{Equation 3.5}$$

Beta-glucan recovery corresponds to the percentage ratio of the weight of beta-glucans in the extracts to the weight of beta-glucans in the initial sample. The efficiency of the method in terms of beta-glucan extraction was expressed as

$$\textit{Beta-glucan recovery (\%)} = \frac{amount\ of\ beta{-}glucans\ in\ extract\ (g)}{amount\ of\ beta{-}glucans\ in\ initial\ sample\ (g)} \times 100 \quad \textit{Equation 3.6}$$

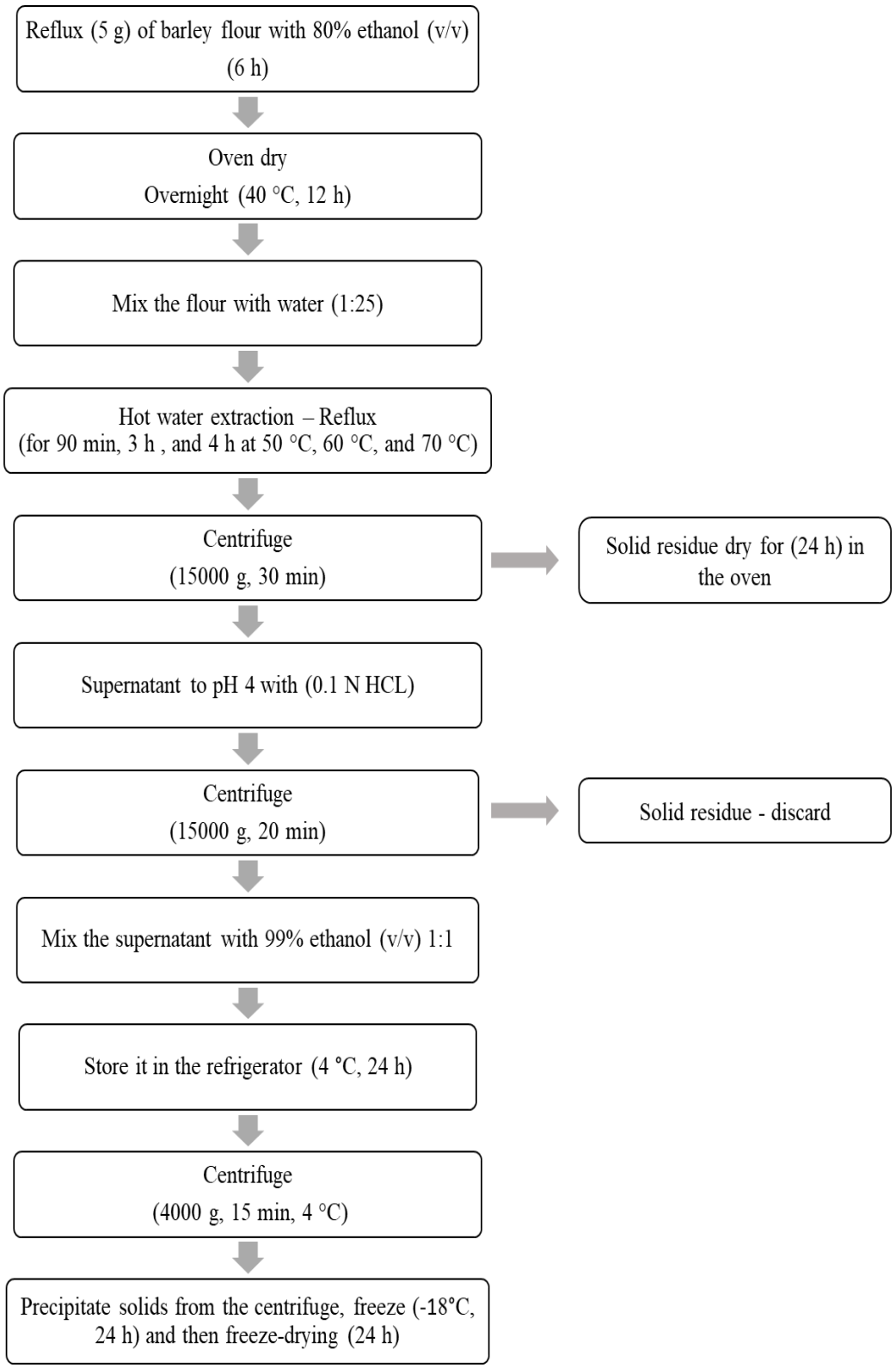

Figure 3.1: Hot water extraction of beta-glucans from barley flour.

3.2.5 Compositional Analysis of the Residue

Starch and beta-glucan content of the HWE barley residues were measured using the total starch test kit and the mixed-linkage beta-glucan enzymatic kit, respectively, both obtained from Megazyme (Megazyme, Ireland), as described earlier in this chapter (sections 3.2.2.1 and 3.2.5.1, respectively).

3.3 Statistical Analysis

All experiments were repeated at least twice, and data were represented as mean ± standard deviation. Statistical analysis was conducted using Minitab statistical analysis software version 17.1.0. One-way analysis of variance (ANOVA) with Tukey's multiple comparison test was used to determine significant differences between treatments, at a confidence level of 95% ($p<0.05$).

3.4 Results and Discussion

3.4.1 Proximate Analysis

The first step of the experimental work included analyses of the chemical composition of barley flours (UK and Jordanian). A summary of their macronutrient composition is given in (Table 3.1). The starch content in the UK barley (52.3% w/w) was higher than in the Jordanian barley (40.3% w/w). Both grains had similar levels of total protein (11% w/w for the UK barley and 12.7% w/w for the Jordanian barley). The protein content of barley may vary from 8% to 15% (w/w) (Paras & Kotari, 2017) and is mainly affected by the genotype of the cultivar, the growing conditions, and the rate and timing of nitrogen supply to the plant during growth (Friedman & Atsmon, 1988). Total lipid content in the UK barley was 3.96% (w/w), and it was lower in the

Jordanian barley (2.94% w/w). The average lipid content in barley grain ranges from 2% to 4% (w/w), most of which is concentrated in the bran and germ (Aman & Newman, 1986). Barley lipids contain a variety of fatty acids, including linoleic, linolenic, and oleic acid, as well as lecithin and encephalin. Most of the lipids in barley are non-polar, and linoleic acid is the main fatty acid found in this grain (Bhatty, 1993b). Small differences in total lignin were noted between the two barley grains (1.02% w/w for the UK barley and 1.48% for the Jordanian barley).

Table 3.1: Proximate analysis of the UK and Jordanian barley flours.

Chemical Composition (%, w/w)	**Barley Flour (UK)**	**Barley Flour (Jordan)**
Moisture	8.00 ± 0.37	6.03 ± 0.36
Protein	11.00 ± 0.36	12.71 ± 0.16
Fat	3.96 ± 0.01	2.94 ± 0.03
Starch	52.32 ± 1.81	40.32 ± 2.32
Beta-glucans	2.65 ± 0.36	3.61 ± 0.20
Ash	2.65 ± 0.01	3.50 ± 0.14
Total lignin	1.02 ± 0.06	1.48 ± 0.12
Structural carbohydrates	16.4 ± 1.10	29.41 ± 2.50
Hemicellulose	14.98 ± 2.10	12.88 ± 1.81
Cellulose	3.42 ± 1.80	13.40 ± 2.54

There were notable differences in the structural carbohydrate content and composition of the barley grains (Table 3.1). The UK barley grain contained 16.4% (w/w) non-starch carbohydrates, of which ~15% (w/w) were hemicelluloses (primarily arabinoxylans). In Jordanian barley, structural carbohydrates accounted for 29.4% (w/w), of which 12.8% (w/w) was hemicelluloses. The xylose to arabinose ratio for both grains was similar (2.2 and 2.3 for UK and

Jordanian barley, respectively), indicating a low degree of substitution. Generally, arabinoxylans of the starchy endosperm and aleurone of barley have relatively low ratios of xylose to arabinose residues, which means the (1→4)-β-xylan backbone has a relatively high degree of substitution with arabinose residues. In contrast, the arabinoxylans from the outer pericarp-seed coat layers of the barley grain have a lower degree of substitution with arabinose residues and therefore have a higher xylose-arabinose ratio (Trafford & Fincher, 2014). In terms of cellulose content, notable differences were seen between the two cultivars (Table 3.1). In the aleurone cell walls and the starch endosperm of barley, cellulose accounts for ~2% of total polysaccharides, but the hull usually contains most of the cellulose and hemicellulose content in the grain. Notably, both grains were hulled; the UK barley was destined for animal feed purposes and the Jordanian for human food applications.

Among all nutrients in barley grain, starch accounts for the largest fraction of the kernel. It is a soluble polysaccharide and the main source of energy for the nourishment and growth of the new plant after germination. The starch in the endosperm accounts for up to 70% of the total dry weight of barley (Asare *et al*., 2011). Amylose and amylopectin are the main carbohydrate components of starch granules. Barley starch consists of 25–30% amylose and 70–75% amylopectin (Morrison *et al*., 1984). Both polysaccharides are built up of 1,4-linked α-D-glucose. Previous studies have found that the average starch content of barley grain ranges from 58% to 64% (w/w), and the differences in starch content among barley grains can be mainly attributed to environmental and soil conditions affecting plant growth, as well as the genetic features of the cultivar (MacGregor & Fincher, 1993; Jadhav *et al*., 1998). This was observed in the current study, as the Jordanian barley (cultivated and harvested in much drier climatic conditions compared to the UK) had much less starch content (40%, w/w). Any reductions in the level of starch are usually

accompanied by a small increase in fibre components such as beta-glucans and simple sugars, including fructose, glucose, and sucrose (Xue *et al*., 1997), which was also observed in the current study. Variations in the content of non-starch carbohydrates such as beta-glucans are mainly attributed to factors such as the genotype of the barley grain and the environmental conditions during grain growth, including climate, water availability, and season (Baik and Ullrich, (2008)). Barley grown in Jordan experiences drought stress during the grain-filling period. Prolonged periods of drought stress may result in major losses in the grain yield of rainfed crops in Jordan, subjected to sparse and irregularly distributed rain, with patterns that may vary between years (Samarah, 2005). Studies have also shown that, as a general rule, a wet harvest, such as is likely to be encountered in the UK, results in barley grains providing flour characterised by low extract viscosity and beta-glucan content. In conttrast, a dry harvest, (as often observed in Jordan), results in barley flour that has high extract viscosity and beta-glucan content (Aastrup Steem, 1979). Izydorczyk *et al*. (2000) investigated the variations in total beta-glucan content in hull-less barley from 29 experimental genotypes and found significant differences among many of these specimens (normal, high amylose, waxy and zero amylose waxy). Specifically, the highest average beta-glucan content was observed in high-amylose barley, with an average content of 7.49% (w/w); this was followed by waxy barley (6.86% w/w), zero-amylose waxy barley (6.30% w/w), and normal barley (4.38% w/w). The greatest variations in beta-glucan content were found in normal barley, in which values ranged from 3.30% (w/w) to 6.28% (w/w). It is worth noting that, although a high beta-glucan content in barley is indicative of high levels of dietary fibre, consumption of the soluble component of beta-glucan is associated with beneficial health effects, including lower serum cholesterol and blood glucose levels (Whitehead *et al*., 2014).

Beta-glucans are divided into soluble and insoluble forms depending on the degree of polymerization (DP). Beta-glucans with DP over 100 are usually completely insoluble in water (Du *et al*., 2014a). Previous studies found that the insoluble beta-glucan content is significantly higher in hulled barley varieties, and as such, it can be concluded that insoluble beta-glucans are found mainly in the seed coat of the grain (Jiang & Vasanthan, 2000). Insoluble beta-glucans in grain cell walls encapsulate easily available nutrients such as starch, intracellular proteins, and fats, acting as a physical barrier to nutrient hydrolysis and utilization. On the other hand, soluble beta-glucans are located in the inner parts of the grain and yield viscous solutions, which may also interfere with nutrient availability (Hesselman & Åman, 1986; Gajdošová *et al*., 2007).

3.4.2 Hot Water Extraction (HWE)

3.4.2.1 Effect of HWE Conditions on Mass Yield

HWE was applied to investigate the extractability of beta-glucans from barley flours. To this end, barley grains were ground into finer particles to increase the surface area of the sample. Barley flour was refluxed with ethanol (80%, v/v) to defat the flour and deactivate inherent enzymes, such as b-glucanases, responsible for the breakdown of beta-glucans within the grain.

The effects of extraction time (90 min, 3 h and 4 h) and temperature (50 °C, 60 °C and 70 °C) on the mass yields of the extracts from the defatted UK and the Jordanian barley flours were studied. The results of UK barley HWE show that the temperature increase from 50 °C to 60 °C did not impact the extract mass yields significantly (Figure 3.2) for extractions of 90 min and 3 h (mass yields of 1.2–1.8%, w/w). However, at both temperatures, a higher mass yield was observed when the extraction time was extended to 4 h (2.4%, w/w at 50 °C and 4%, w/w at 60 °C). A contrasting trend was observed when HWE extraction was carried out at 70 °C. Specifically, a

shorter extraction duration (90 min) was capable of yielding 3% (w/w) of mass in the extract, whereas a prolonged duration of extraction led to lower mass yields (approximately 1.4% w/w for both 3 and 4 h).

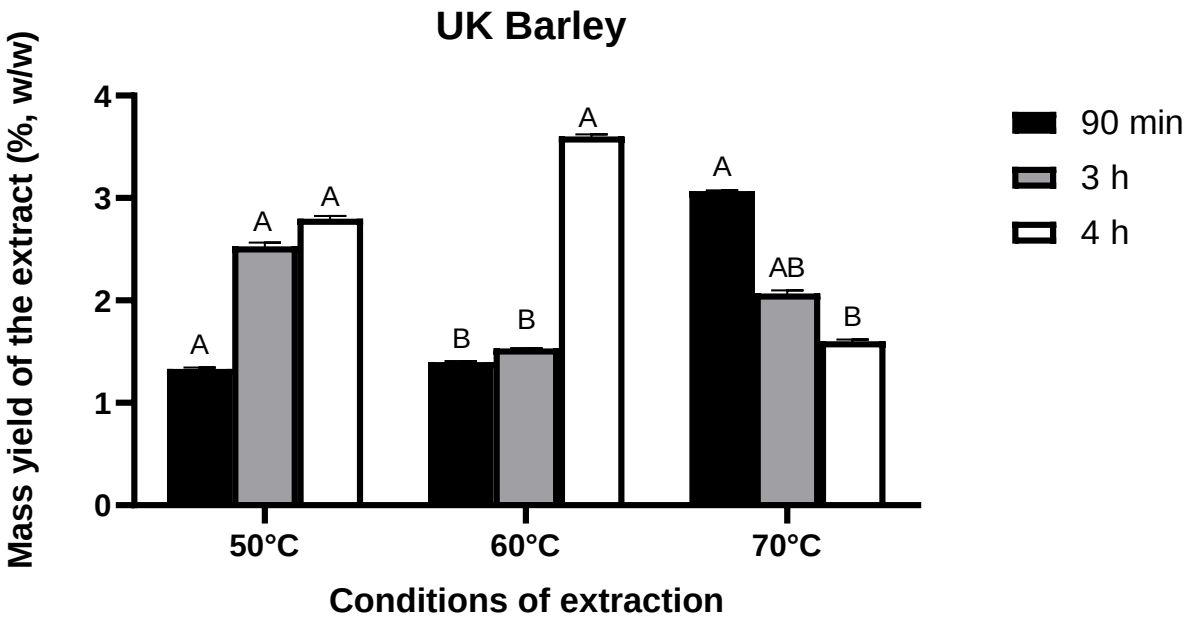

Figure 3.2: Mass yields (%, w/w) of the extracts for the UK barley via HWE. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

For Jordanian barley flour, the effect of extraction temperature was less pronounced, although not insignificant. Specifically, in extractions carried out at 50 °C, the best results were achieved for extraction duration of 3 h (mass yield 3.3% w/w), whereas a prolonged extraction time decreased the extraction mass yield (Figure 3.3). The same trend was observed at an extraction temperature of 60 °C, with the highest mass yield obtained at 3 h (2.9%, w/w) and a lower yield noted at 4 h extraction (2.3%, w/w). At an extraction temperature of 70 °C, the Jordanian barley flour extraction results were similar to the UK barley, whereby the highest mass

yield was achieved at 90 min of extraction (2.6%, w/w). Longer extraction times led to a lower mass yield. Results showed no significant differences between reactions conducted at 50 °C for the UK barley and 70 °C for the Jordanian barley over all extraction times. However, the mass yields did differ between extractions conducted at 60 °C and 70 °C for the UK barley and 50 °C and 60 °C for Jordanian barley. Dawkins and Nnanna (1993) reported HWE mass yields of 2.99–6.28% from oat bran and 1.83–5.24% from rolled oats at 50–70 °C, whereas Wood *et al*. (1977) reported mass yields of 0.63–3.5%.

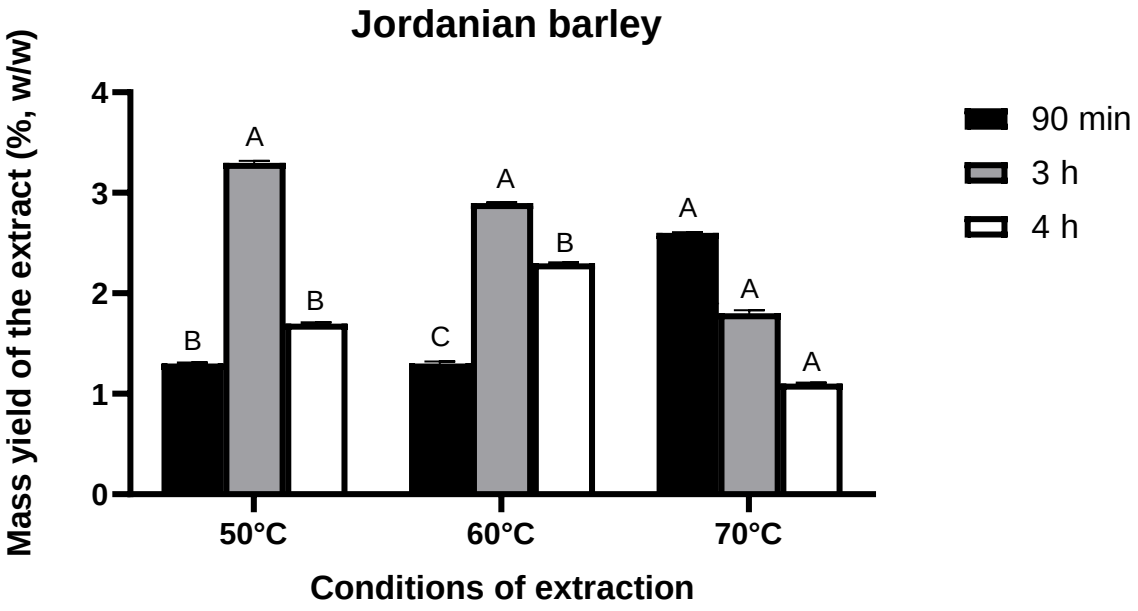

Figure 3.3: Mass yields (%, w/w) of the extracts for the Jordanian barley via HWE. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

The mass yield is the first indication of the efficiency of an extraction process and allows for the subsequent characterisation of the obtained extracts. HWE targets the extraction of water-soluble components, and in the case of cereals, starch solubilisation may also occur. Benito-Román

et al. (2011) achieved maximum solubilisation of β-glucans at 55 °C, and additional temperature increases did not significantly increase the maximum amount of β-glucan dissolved. At temperatures above 55 °C, the maximum solubility of β-glucans in water does not increase significantly. Furthermore, above that temperature, the co-extraction of starch contaminates the extracts and hinders the stirring process and, ultimately, the solid–liquid separation and purification processes. As such, in this study, the increase in mass yield was likely to be associated with starch co-extraction rather than a selective increase in the beta-glucan content of the extracts.

3.4.2.2 Purity and Recovery of Beta-glucans in HWE Extracts

A key objective of this work was to evaluate the effectiveness of HWE on the extraction of beta-glucans from barley flours. As such, the next step was to assess the quantity of beta-glucans in the HWE extracts, which is an indication of their beta-glucan purity. Calculations of the recovery of beta-glucans in the extracts provide insights into the effectiveness of HWE on beta-glucan extraction from the UK and Jordanian barley flours.

With regards to the UK barley flour extracts, as shown in Figure 3.4, at 50 °C and 60 °C, HWE extracts were richer in beta-glucans after 90 min. The same was not observed in extractions at 70 °C, at which temperature the extracts became richer in beta-glucans after 4 hours of extraction (Figure 3.4 a). The temperature seemed to affect the beta-glucan recovery pattern in different ways. Specifically, in extracts obtained at 50 °C, a prolonged extraction time did not positively affect beta-glucan recovery; approximately 9% (w/w) of beta-glucans were extracted after 3 and 4 h. At 60 °C, the recovery of beta-glucans increased as extraction time increased, reaching 9.5% (w/w) after 4 h. At a higher extraction temperature (70 °C), the highest recovery was achieved at 90 min

(approximately 9.5%, w/w), whereas extending the extraction time led to decreasing beta-glucan recovery (Figure 3.4.b).

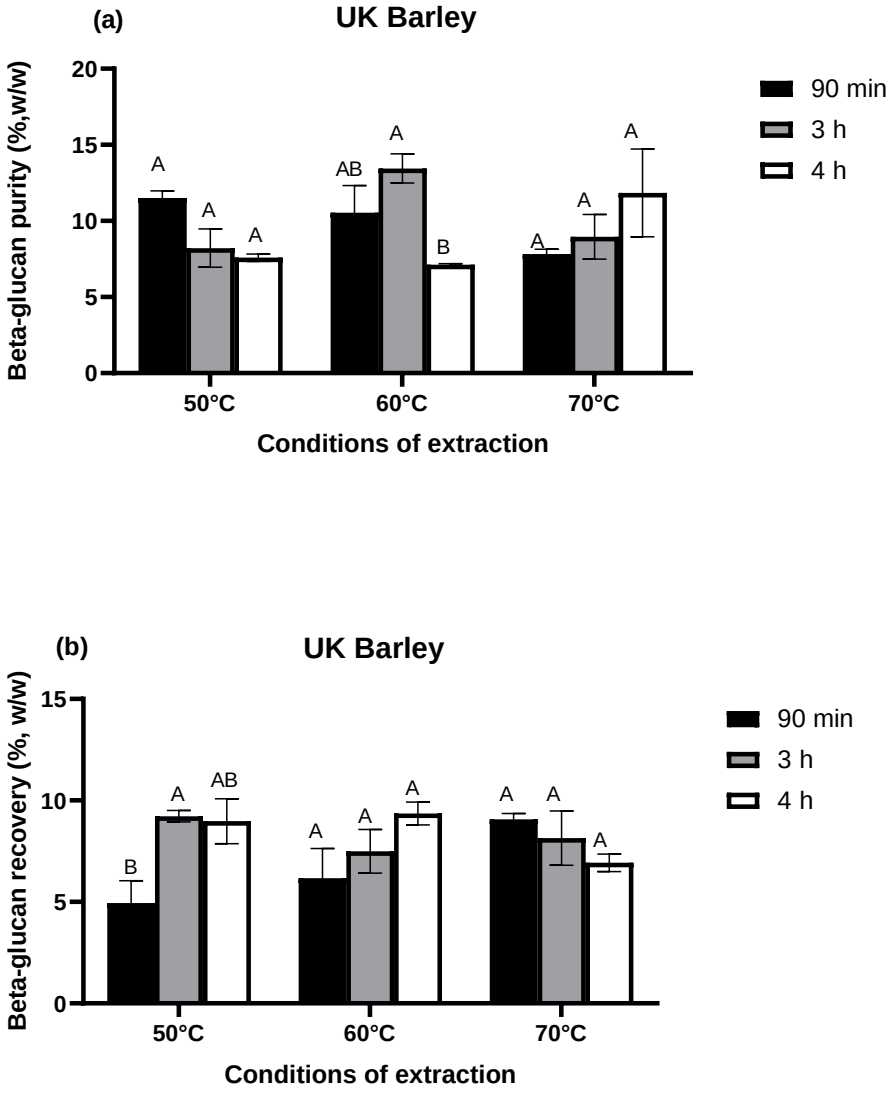

Figure 3.4: (a) Beta-glucan purity (%, w/w) and (b) recovery (%, w/w) of UK barley via HWE. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

For Jordanian barley flour, the trend was quite different (Figure 3.5). The purity of beta-glucan in the extracts was not greatly impacted by temperature or duration of extraction, ranging between 10.6–13.4%, w/w, across all test conditions (Figure 3.5 a). Recovery of beta-glucans in the Jordanian extracts seemed to follow the same pattern as the mass yield: 3 h of extraction favoured higher recovery yields at both 50 °C and 60 °C, equal to 10.58% and 9.15 % (w/w), respectively. At 70 °C, the highest beta-glucan recovery was achieved at 90 min of extraction (9.68%, w/w) and was followed by a declining trend as extraction times increased (Figure 3.5 b).

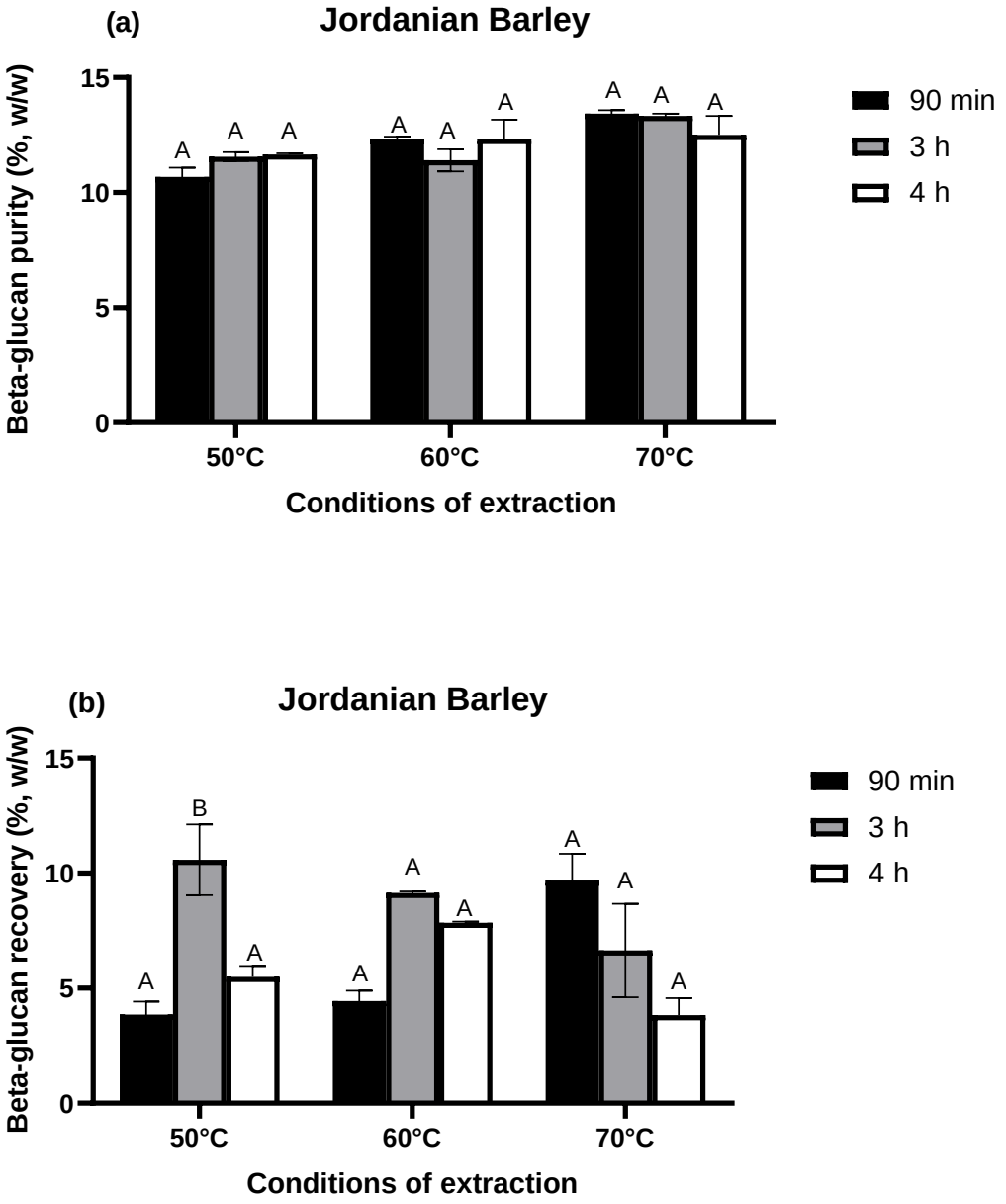

Figure 3.5: (a) Beta-glucan purity (%, w/w) and (b) recovery for the Jordanian barley via HWE. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

Beta-glucan is an unbranched polysaccharide composed of long linear chains of glucose with both β-(1 → 3) and β-(1 → 4) linkages (Bacic & Stone, 1981). These linkages are arranged in a specific pattern, with (1 → 4) links occurring in groups of two to four and (1 → 3) links occurring singly (Skendi *et al*., 2003). These molecular and structural features are important for solubility. The presence of β-(1 → 3) links breaks up the regularity of β-(1 → 4)-link sequences, resulting in increased flexibility and allowing water to penetrate the molecular chains and solubilize the fibre. Meanwhile, adjacent β-(1 → 4) links may exhibit interchain aggregation via strong hydrogen bonds, reducing the solubility of beta-glucan (Gomez *et al*., 1997). Temelli (1997) indicated that the recovery rate of beta-glucans was positively correlated with temperature. The author found that the recovery of beta-glucan increased linearly with temperature for both oat bran and rolled oats, and under the conditions tested, beta-glucan yield increased with increasing reaction time but then decreased for longer extraction times. This observation is due to the increase in thermal degradation when beta-glucan is exposed to high temperatures for longer times. A similar trend was reported by Benito-Román *et al*. (2014) in a study of HWE of beta-glucans from barley. However, Benito-Román *et al*. (2011) found that the optimal extraction time for beta-glucans from waxy barley was 3 h at 55 °C, while Gangopadhyay *et al*. (2015) recommended extraction for 4 h at 55.7 °C to obtain the maximum amount of beta-glucan. The increase in the extractability of beta-glucans with increasing temperature or ionic strength of the solvent might be explained by differences in the proportion of β-(1→3) and β-(1→4) linkages in the polymeric chains; in linkage sequences on the chain; or in the DP, which could lead to increased physical intermolecular associations (Izydorczyk et al., 1998). In many early studies, oat and barley beta-glucans were extracted using water at 47 °C to 52 °C to minimise starch solubilisation (Skendi *et al*., 2003; Irakli *et al*., 2004; Lazaridou *et al*., 2004; Vaikousi *et al*., 2004). The choice of extraction

temperature was related to the solubility of beta-glucans. Starting the treatments at a low temperature (e.g. 50 °C) can allow for solubilisation of water-soluble beta-glucans in the aqueous phase and help prevent the solubility and subsequent gelatinisation of starch. If there is a considerable amount of starch present in the flour, the application of higher temperatures may also lead to starch gelatinisation, which, in turn, can contaminate the extract obtained and lead to lower recovery efficiency (Limberger-Bayer *et al*., 2014). Several researchers reported that in addition to beta-glucan extracts, there were small amounts of fat, protein, starch, pentosans, and mineral (ash) matter also present in flour. They were extracted along with beta-glucans as impurities, reducing the recovery of beta-glucans.

Apart from structure, many factors could affect the extractability of beta-glucans, including particle size, particle size distribution and cultivar, stage of kernel development, and growing conditions (Lazaridou & Biliaderis, 2004). Furthermore, factors such as pH, temperature, extraction time, solvent, and flour ratio could also influence extraction performance, so that substantial differences in extractability might be expected with changes to any of these parameters (Benito-Román *et al*., 2011). Decreasing particle size and increasing temperature result in increased extraction efficiency. Defatting does not significantly affect beta-glucan yield from oats, whereas enzyme deactivation with hot alcohol treatment was reported to decrease the extraction yield of barley and oat beta-glucan (Wood, 1986). The amount of beta-glucans extracted from oats and barley by HWE varied from 50 to 70% (Beer *et al*., 1997, Colleoni-Sirghie *et al*., 2003). Successive treatments with increasing water temperatures, starting from room temperature and rising to the point of boiling, resulted in extraction yields of 72 to 90% for oat and barley beta-glucans (Bhatty, 1993a). Henry (1985) reported that 36% of rye grain beta-glucans could be extracted using boiling water, while Härkönen *et al*. (1997) found that 30, 25, and 45% of the total

beta-glucan content could be extracted from the bran, short, and flour fractions of rye, respectively, at 30 °C.

Previous studies on HWE reported a beta-glucan recovery of 57.8–88.4% (w/w) for pH values of 7, 8, 9 and 10, adjusted with sodium carbonate (20%, w/v), and temperatures of 40, 45, 50, 55 °C (Burkus & Temelli, 1998; Temelli, 1997). The highest beta-glucan extraction yield was achieved at 55 °C. Symons and Brennan (2004) observed a lower HWE efficiency due to beta-glucan cleavage by glucanases resulting from thermal degradation or starch contamination. In the current study, preliminary treatment via refluxing with 80% ethanol was carried out to inactivate endogenous glucanases and remove the lipid content of the barley flour that could interfere with extraction efficiency. However, the recovery yield obtained was still quite low (no higher than 9.6% for Jordanian flour).

Notably, the amount of protein in the Jordanian extracts ranged between 1.5% and 2.1% (w/w), and in the UK extracts, between 1.2% and 1.5% (w/w) (Figure 3.6). The initial protein percentage in the Jordanian and the UK barley grains was equal to 12.71% and 11% (w/w), respectively. Protein content in the hot water extracts was minimised by adjusting their pH to 4 and removing precipitated proteins through centrifugation. Many researchers have shown that the protein contents of preparations obtained after adjusting the pH to 4 results were from less than 1% to 3.8% (w/w) of protein in the final extracts (Ahluwalia & Ellis, 1985; Bhatty, 1999; Skendi *et al*., 2003).

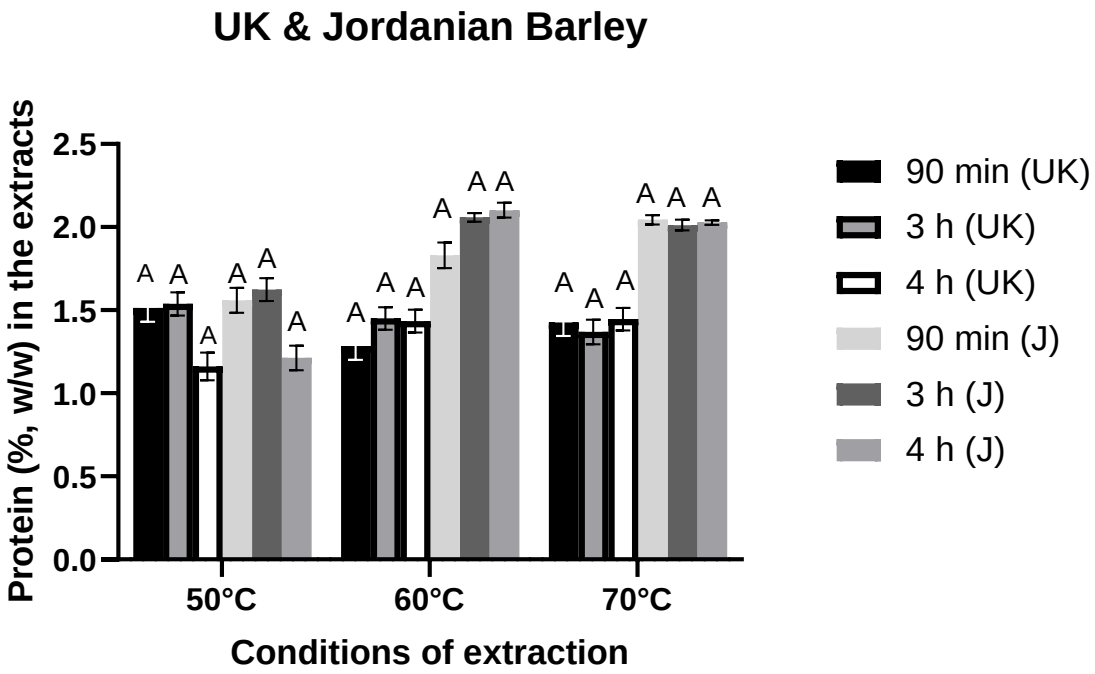

Figure 3.6: Protein content (%, w/w) in the UK & the Jordanian barley extracts. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

3.4.2.3 Composition of barley flour residues after HWE

Starch analyses were performed on the UK and the Jordanian barley flour residues (as shown in Figure 3.7) to monitor the key macronutrient composition of the extraction residues after HWE.

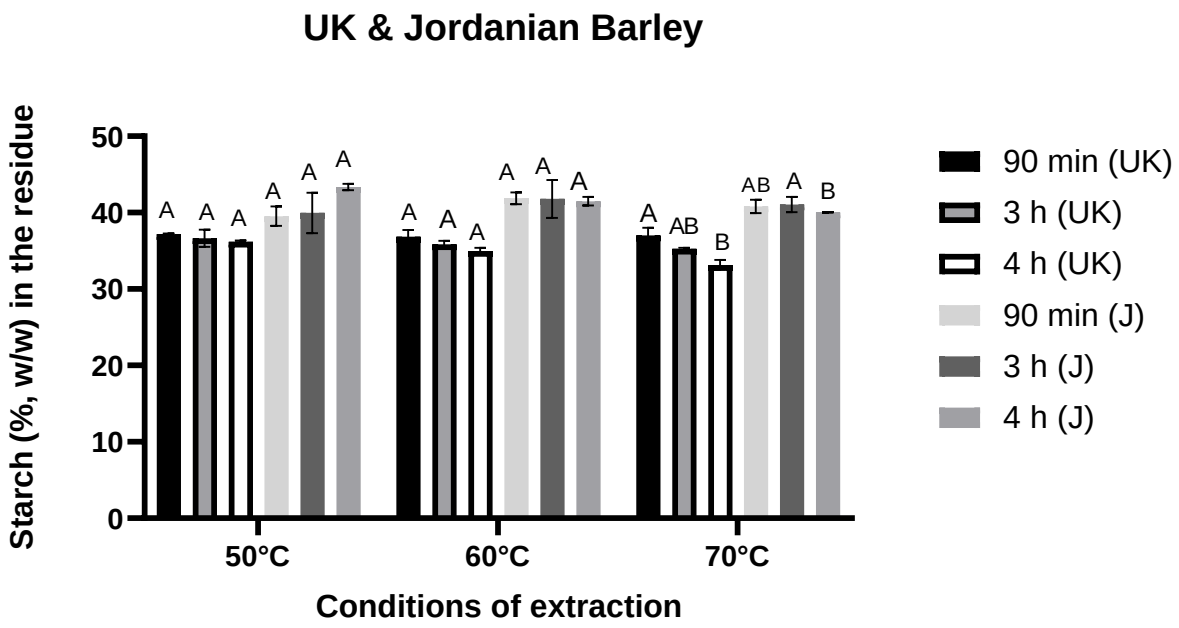

Figure 3.7: Starch (%, w/w) in the residue for the UK & Jordanian barley. Values are presented as means ± standard deviation of duplicate samples; recovery of the extracts with different letters are significantly different ($p<0.05$).

For the UK barley, there was no significant difference in starch content between 50 °C and 60 °C for the entire time range. However, when the temperature increased to 70 °C, starch content in the residue decreased as the extraction time increased, indicating greater starch gelatinisation and co-extraction during HWE (Figure 3.7). For Jordanian barley, the starch content remained almost constant (40-42%, w/w) regardless of temperature or extraction duration.

No significant differences were observed for both the UK and Jordanian residues in beta-glucan content in the residue, equal to 2.2–2.3% (w/w) in all samples (Figure 3.8). The presence of beta-glucans in the residue was expected, as only 10% of the original beta-glucan content of the flours was recovered in the HWE extracts.

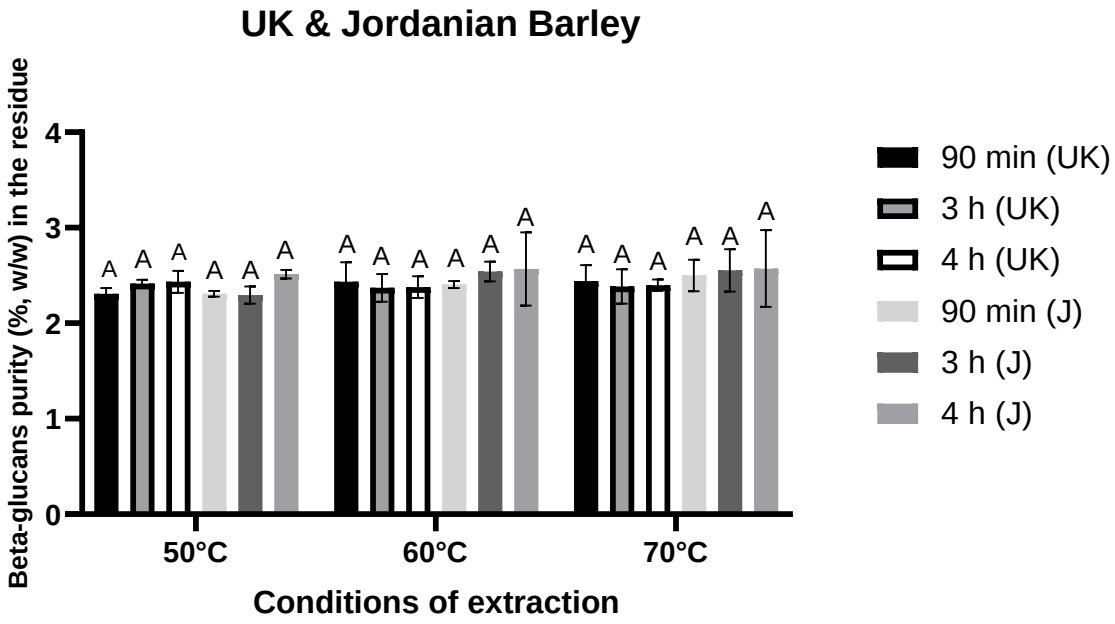

Figure 3.8: Beta-glucans (%, w/w) in the residue of the UK and the Jordanian barley. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

It has been reported that insoluble beta-glucan content is significantly higher in hulled barley varieties, indicating that insoluble β-glucans are found mainly in the coat parts of the grain, yet the soluble glucans are located in the inner parts of the grain (Gajdošová *et al*., 2007). Moreover, structural variations of beta-glucans are associated with their differential solubility and extractability properties. It has been suggested that alkali extractable β-glucans in barley appear to have a slightly higher ratio of DP3/DP4 fragments and a greater amount of long, continuously linked β-(1 → 4) glucose residues (DP ≥ 5) than their water-extractable counterparts (Izydorczyk & Dexter, 2008). In addition, insoluble β-glucans can be non-covalently bound to arabinoxylans, enabling them to remain insoluble despite their low molar mass (Johansson *et al*., 2004).

3.5 Conclusion

The main advantage of hot water extraction is that it is an environmentally friendly process, as the extraction medium is water, and it is often carried out in relatively mild temperatures. In the current study, the highest beta-glucan recovery for the UK and the Jordanian barley were at 4 h, 60 °C and 3 h, 50 °C respectively, at approximately 10% (w/w). In the present study, HWE did not appear to be a highly efficient extraction method for barley beta-glucans, possibly due to the inefficiency of the mixing and heat transfer in the HWE system, but also due to differences between the two barley cultivars in terms of cell wall structure and location of beta-glucans. As such, it seems that HWE extraction alone cannot result in highly pure beta-glucan extracts, and further processing steps are required to purify HWE fractions (e.g. enzymatic digestion of starch, dialysis to remove low molecular weight impurities) and increase beta-glucan content in the final extracts.

3.6 References

Aastrup, S. (1979) The effect of rain on β-glucan content in barley grains. *Carlsberg Research Communications,* **44,** 381-393.

Ahluwalia, B. & Ellis, E. (1985) Studies of beta-glucan in barley, malt and endosperm cell walls. In: Hill, R.D. (ed.) *New Approaches to Research on Cereal Carbohydrates* (*Progress in biotechnology 1).* Amsterdam: Elsevier.

Ahmad, A., Anjum, F.M., Zahoor, T., Nawaz, H. & Din, A. (2009) Physicochemical and functional properties of barley β-glucan as affected by different extraction procedures. *International Journal of Food Science & Technology,* **44**, 181-187.

Ahmad, A., Anjum, F.M., Zahoor, T., Nawaz, H. & Ahmed, Z. (2010) Extraction and characterization of beta-D-glucan from oats for industrial utilization. *International Journal of Biological Macromolecules,* **46**, 304-309.

Aman, P. & Newman, C.W. (1986) Chemical-composition of some different types of barley grown in Montana, USA. *Journal of Cereal Science,* **4**, 133-141.

AOAC (ed.) 2005. *Official methods of analysis of AOAC International*

Asare, E.K., Jaiswal, S., Maley, J., Båga, M., Sammynaiken, R., Rossnagel, B.G. & Chibbar, R.N. (2011) Barley grain constituents, starch composition, and structure affect starch in vitro enzymatic hydrolysis. *Journal of Agricultural and Food Chemistry,* **59**, 4743-4754.

Asif, A., Muhammad A.F., Tahir, Z., Haq, N. & Ahmad, D. (2009) Physicochemical and functional properties of barley beta-glucan as affected by different extraction procedures. *International Journal of Food Science & Technology,* **44**, 181-187.

Bacic, A. & Stone, B. (1981) Chemistry and organization of aleurone cell wall components from wheat and barley. *Functional Plant Biology,* **8**, 475-495.

Baik, B.-K. & Ullrich, S.E. (2008) Barley for food: Characteristics, improvement, and renewed interest. *Journal of Cereal Science,* **48**, 233-242.

Beer, M.U., Wood, P.J. & Weisz, J. (1997) Molecular weight distribution and (1→ 3)(1→ 4)-β-D-glucan content of consecutive extracts of various oat and barley cultivars. *Cereal Chemistry,* **74**, 476-480.

Benito-Román, O., Alonso, E. & Lucas, S. (2011) Optimization of the beta-glucan extraction conditions from different waxy barley cultivars. *Journal of Cereal Science,* **53**, 271-276.

Benito-Román, O., Alonso, E., Palacio, L., Pradanos, P. & Cocero, M.J. (2014) Purification and isolation of beta-glucans from barley: Downstream process intensification. *Chemical Engineering and Processing,* **84**, 90-97.

Bhatty, R. (1993a) Extraction and enrichment (1 leads to 3),(1 leads to 4)-beta-D-glucan from barley and oat brans. *Cereal Chemistry,* **70**, 73-77.

Bhatty, R. (1993b) Physicochemical properties of roller-milled barley bran and flour. *Cereal Chemistry,* **70,** 397-402.

Bhatty, R. (1999) The potential of hull-less barley. *Cereal Chemistry,* **76,** 589-599.

Böhm, N. & Kulicke, W.-M. (1999) Rheological studies of barley (1→ 3)(1→ 4)-β-glucan in concentrated solution: mechanistic and kinetic investigation of the gel formation. *Carbohydrate Research,* **315**, 302-311.

Brennan, C.S. & Cleary, L.J. (2005) The potential use of cereal (1→ 3, 1→ 4)-β-D-glucans as functional food ingredients. *Journal of Cereal Science,* **42**, 1-13.

Burkus, Z. & Temelli, F. (1998) Effect of extraction conditions on yield, composition, and viscosity stability of barley beta-glucan gum. *Cereal Chemistry,* **75,** 805-809.

Burton, R.A., Gidely, M.J. & Fincher, G.B. (2010) Heterogeneity in the chemistry, structure and function of plant cell walls. *Nature Chemical Biology,* **6,** 724-732.

Colleoni-Sirghie, M., Kovalenko, I.V., Briggs, J.L., Fulton, B. & White, P.J. (2003) Rheological and molecular properties of water soluble (1, 3)(1, 4)-β-D-glucans from high-β-glucan and traditional oat lines. *Carbohydrate Polymers,* **52,** 439-447.

Colleoni-Sirghie, M., Jannink, J.L., Kovalenko, I.V., Briggs, J.L. & White, P.J. (2004) Prediction of β-glucan concentration based on viscosity evaluations of raw oat flours from high β-glucan and traditional oat lines. *Cereal Chemistry,* **81,** 434-443.

Daou, C. & Zhang, H. (2012) Oat beta-glucan: its role in health promotion and prevention of diseases. *Comprehensive Reviews in Food Science and Food Safety,* **11,** 355-365.

Dawkins, N. & Nnanna, I. (1993) Oat gum and β-glucan extraction from oat bran and rolled oats: Temperature and pH effects. *Journal of Food Science,* **58,** 562-566.

Doblin, M. S., Pettolino, F. & Bacic, A. (2010) Plant cell walls: the skeleton of the plant world. *Functional Plant Biology,* **37,** 357-381.

Du, B., Zhu, F. & Xu, B. (2014a) Beta-glucan extraction from bran of hull-less barley by accelerated solvent extraction combined with response surface methodology. *Journal of Cereal Science,* **59,** 95-100.

Du, B., Zhu, F. & Xu, B. (2014b) β-Glucan extraction from bran of hull-less barley by accelerated solvent extraction combined with response surface methodology. *Journal of Cereal Science,* **59,** 95-100.

Fastnaught, C., Berglund, P., Holm, E. & Fox, G. (1996) Genetic and environmental variation in β-glucan content and quality parameters of barley for food. *Crop Science,* **36,** 941-946.

Friedman, M. & Atsmon, D. (1988) Comparison of grain composition and nutritional quality in wild barley (*Hordeum spontaneum*) and in a standard cultivar. *Journal of Agricultural and Food Chemistry,* **36,** 1167-1172.

Fulcher, R. & Rooney Duke, T. (2002) Whole-grain structure and organization: implications for nutritionists and processors. In: Marquart, L., Slavin, J.L., & Fulcher, R.G. (eds.) *Whole-grain Foods in Health and Disease*. St. Paul, MN: American Association of Cereal Chemists.

Gajdošová, A., Petruláková, Z., Havrlentová, M., Červená, V., Hozová, B., Šturdík, E. & Kogan, G. (2007) The content of water-soluble and water-insoluble β-D-glucans in selected oats and barley varieties. *Carbohydrate Polymers,* **70,** 46-52.

Gangopadhyay, N., Hossain, M.B., Rai, D.K. & Brunton, N.P. (2015) Optimisation of yield and molecular weight of β-glucan from barley flour using response surface methodology. *Journal of Cereal Science,* **62,** 38-44.

Gomez, C., Navarro, A., Manzanares, P., Horta, A. & Carbonell, J. (1997) Physical and structural properties of barley (1→ 3),(1→ 4)-β-d-glucan. Part II. Viscosity, chain stiffness and macromolecular dimensions. *Carbohydrate Polymers,* **32,** 17-22.

Härkönen, H., Pessa, E., Suortti, T. & Poutanen, K. (1997) Distribution and some properties of cell wall polysaccharides in rye milling fractions. *Journal of Cereal Science,* **26,** 95-104.

Henry, R. J. (1985) A comparison of the non-starch carbohydrates in cereal grains. *Journal of the Science of Food and Agriculture,* **36,** 1243-1253.

Hesselman, K. & Åman, P. (1986) The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low-or high-viscosity. *Animal Feed Science and Technology,* **15,** 83-93.

Irakli, M., Biliaderis, C.G., Izydorczyk, M.S. & Papadoyannis, I.N. (2004). Isolation, structural features and rheological properties of water-extractable β-glucans from different Greek barley cultivars. *Journal of the Science of Food and Agriculture,* **84,** 1170-1178.

Izydorczyk, M. & Dexter, J. (2008) Barley β-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products–a Review. *Food Research International,* **41,** 850-868.

Izydorczyk, M., Macri, L. & MacGregor, A. (1998) Structure and physicochemical properties of barley non-starch polysaccharides—I. Water-extractable β-glucans and arabinoxylans. *Carbohydrate Polymers,* **35,** 249-258.

Jadhav, S.J., Lutz, S.E., Ghorpade, V.M. & Salunkhe, D.K. (1998) Barley: Chemistry and value-added processing. *Critical Reviews in Food Science and Nutrition,* **38,** 123-171.

Jiang, G. & Vasanthan, T. (2000) MALDI-MS and HPLC quantification of oligosaccharides of lichenase-hydrolyzed water-soluble β-glucan from ten barley varieties. *Journal of Agricultural and Food Chemistry,* **48,** 3305-3310.

Johansson, L., Tuomainen, P., Ylinen, M., Ekholm, P. & Virkki, L. (2004) Structural analysis of water-soluble and-insoluble β-glucans of whole-grain oats and barley. *Carbohydrate Polymers,* **58,** 267-274.

Kao, P.-F., Wang, S.-H., Hung, W.-T., Liao, Y.-H., Lin, C.-M. & Yang, W.-B. (2012) Structural characterization and antioxidative activity of low-molecular-weights beta-1, 3-glucan from the residue of extracted *Ganoderma lucidum* fruiting bodies. *Journal of Biomedicine and Biotechnology,* 673764.

Kruger, N.J. (2009). The Bradford method for protein quantitation. In: Walker, J.M. (ed.) 3rd ed. *The Protein Protocols Handbook.* Berlin: Springer.

Lazaridou, A., Biliaderis, C.G., Micha-Screttas, M. & Steele, B.R. (2004) A comparative study on structure–function relations of mixed-linkage (1→ 3),(1→ 4) linear β-d-glucans. *Food Hydrocolloids,* **18,** 837-855.

Lazaridou, A. & Biliaderis, C.G. (2004) Cryogelation of cereal beta-glucans: structure and molecular size effects. *Food Hydrocolloids,* **18,** 933-947.

Limberger-Bayer, V.M., De Francisco, A., Chan, A., Oro, T., Ogliari, P.J. & Barreto, P.L. M. (2014) Barley beta-glucans extraction and partial characterization. *Food Chemistry,* **154,** 84-89.

MacGregor, A.W. & Fincher, G.B. (1993) Carbohydrates of the barley grain. In: MacGregor, A.W. & Bhatty, R.S. (eds.) Barley Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Morrison, W.R., Milligan, T.P. & Azudin, M. (1984) A relationship between the amylose and lipid contents of starches from diploid cereals. *Journal of Cereal Science,* **2,** 257-271.

Paras, S. & Kotari, S.I. (2017) Barley: impact of processing on physicochemical and thermal properties-a review. *Food Reviews International,* **33,** 359-381.

Park, H., Ka, K.-H. & Ryu, S.-R. (2014) Enhancement of β-glucan content in the cultivation of cauliflower mushroom (*Sparassis latifolia*) by elicitation. *Mycobiology,* **42**, 41-45.

Routray, W. & Orsat, V. (2012) Microwave-assisted extraction of flavonoids: a review. *Food and Bioprocess Technology,* **5,** 409-424.

Saastamoinen, M., Plaami, S. & Kumpulainen, J. (1992) Genetic and environmental variation in β-glucan content of oats cultivated or tested in Finland. *Journal of Cereal Science,* **16,** 279-290.

Samarah, N.H. (2005) Effects of drought stress on growth and yield of barley. *Agronomy for Sustainable Development*, **25,** 145-149.

Saulnier, L., Gévaudan, S. & Thibault, J.-F. (1994) Extraction and partial characterisation of β-glucan from the endosperms of two barley cultivars. *Journal of Cereal Science,* **19,** 171-178.

Skendi, A., Biliaderis, C., Lazaridou, A. & Izydorczyk, M. (2003) Structure and rheological properties of water soluble β-glucans from oat cultivars of *Avena sativa* and *Avena bysantina*. *Journal of Cereal Science,* **38,** 15-31.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. & Crocker, D. (2008) Determination of structural carbohydrates and lignin in biomass. *Laboratory Analytical Procedure,* **1617,** 1-16.

Temelli, F. (1997) Extraction and functional properties of barley beta-glucan as affected by temperature and pH. *Journal of Food Science,* **62,** 1194-1201.

Trafford, K. & Fincher, G.B. (2014) Barley grain carbohydrates: starch and cell walls. In: Shewry, P.R. & Ulrich, S.E. (2nd ed.) *Barley: Chemistry and Technology*. St. Paul, MN: American Association of Cereal Chemists.

Vaikousi, H., Biliaderis, C. & Izydorczyk, M. (2004) Solution flow behavior and gelling properties of water-soluble barley (1→ 3, 1→ 4)-β-glucans varying in molecular size. *Journal of Cereal Science,* **39,** 119-137.

Vriesmann, L.C., Amboni, R. & Petkowicz, C.L.D. (2011) Cacao pod husks (*Theobroma cacao* L.): Composition and hot-water-soluble pectins. *Industrial Crops and Products,* **34,** 1173-1181.

Wood, P. (1986) Oat β-glucan: structure, location and properties. In: Webster, F.H. (ed.) *Oats: Chemistry and Technology*. St. Paul, MN: American Association of Cereal Chemists.

Wood, P., Paton, D. & Siddiqui, I. (1977) Determination of beta-glucan in oats and barley. *Cereal Chemistry*, **54**, 524-533.

Wood, P., Siddiqui, I. & Paton, D. (1978) Extraction of high-viscosity gums from oats. Cereal Chemistry, **55**, 1038-1049.

Xue, Q., Wang, L., Newman, R., Newman, C. & Graham, H. (1997). Influence of the hulless, waxy starch and short-awn genes on the composition of barleys. *Journal of Cereal Science,* **26**, 251-257.

Ying, Z., Han, X. & Li, J. (2011). Ultrasound-assisted extraction of polysaccharides from mulberry leaves. *Food Chemistry,* **127**, 1273-1279.

Zhu, F., Du, B., Bian, Z. & Xu, B. (2015) Beta-glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. *Journal of Food Composition and Analysis,* **41,** 165-173.

Chapter 4: **ULTRASOUND-ASSISTED EXTRACTION OF BETA-GLUCAN FROM UK AND JORDANIAN BARLEY**

Abstract

Cereal grains such as barley and oats are important sources of dietary fibre, such as beta-glucan, which can regulate sugar and lower cholesterol levels in blood. Beta-glucan is a soluble fibre found in the endosperm cell wall of barley and is used as a food ingredient. This study explored the potential of ultrasonication as a process targeting beta-glucan extraction from barley flour. To this end, two different barley flours (from the UK and Jordan) were used as starting materials. For the Jordanian barley, the highest beta-glucan recovery of 73.2% (w/w) was obtained during ultrasonication-assisted extraction (UAE) at amplitude of 15 (A) for 35 min, which provided a beta-glucan purity of 12.9% (w/w). For the UK barley, 10 A ultrasonication for 20 min resulted in the highest beta-glucan recovery of 55.5 % (w/w) containing 9% (w/w) beta-glucans. The rheological properties of 1% (w/v) extracts showed shear thinning behaviour at low shear rates. The barley extracts showed promising properties for increasing the viscosity in food formulations such as sauces or soups.

Keywords: Barley, flour, ultrasound-assisted extraction, amplitude, beta-glucans, mass yields, recovery, purity

4.1 Introduction

Beta-glucans, together with arabinoxylans, are the major structural constituents of the cell wall in various barley grain tissues. Their interaction with other components (proteins, starch, lignin and lipids) affects the isolation and purification procedures targeting fractions enriched in beta-glucans (Izydorczyk & Dexter, 2008). In the starchy endosperm of barley grains, beta-glucans and arabinoxylans could represent up to 85% of total cell wall polysaccharides. The distribution of beta-glucans in the barley endosperm is more uniform than in oats, in which greater concentrations of beta-glucans are found in the subaleurone (the region just below the aleurone layer) (Izydorczyk & Dexter, 2008).

Beta-glucans are divided into soluble and insoluble forms depending on the degree of polymerisation (DP). Beta-glucans with greater than 100 DP are usually completely insoluble in water (Du *et al*., 2014). Previous studies demonstrated that the insoluble beta-glucan content is significantly higher in hulled barley varieties, indicating that insoluble beta-glucans are found mainly in the seed coat of the grain (Jiang & Vasanthan, 2000). Insoluble beta-glucans in grain cell walls encapsulate easily available nutrients such as starch, intracellular proteins, and fats, acting as a physical barrier to nutrient hydrolysis and utilisation. In contrast, soluble beta-glucans are located in the inner parts of the grain and give rise to viscous solutions, which may also interfere with nutrient availability (Hesselman & Åman, 1986; Gajdošová *et al*., 2007). The viscosity of beta-glucans is also important because it is related to the functional properties during food processing and is linked to the physiological benefits. This quality makes them potentially valuable ingredients in food applications. Due to their viscosity, they are considered a non-caloric thickener for multiple foods. Moreover, beta-glucans are used as a stabilising agent in foams and

emulsions and as a fat substitute. Given that beta-glucans act as hydrocolloids, they can manipulate many food products' rheological and textural properties.

A combination of methods is often required to achieve both high purity and high yield of beta-glucan extracts with only minor changes in their molecular structure. Previous studies have investigated the extraction of beta-glucans from cereal grains, mainly barley and oats (Ahmad *et al*., 2012; Kaur *et al*., 2020). There are many parameters to consider, and the main ones include the choice of specific cultivar, pH and ionic strength of solvent, temperature and duration of extraction, liquid–solid ratio, pre-treatments (such as heating and drying), and presence of enzymes (endogenous or from contaminating microorganisms). The milling method and particle size are also important, and substantial differences in extractability might be expected with changes in any of these parameters (Biliaderis & Izydorczyk, 2006). Controlling the main parameters has been shown to significantly affect extract recovery and the functional properties of the beta-glucans extracted (Ahmad *et al*., 2010).

Most methods currently employed to extract beta-glucans are focused on removing proteins and starch molecules from the beta-glucans to increase their purity and maximise their functionality. This strategy results in the beta-glucan structure having a lower molecular weight, with reduced functional properties, which may be further degraded during processing and incorporation into food (Asif *et al*., 2009).

Ultrasonic-assisted extraction (UAE) is considered a green extraction method (Tao & Sun, 2015). UAE uses acoustic cavitation to disrupt plant cell walls, reduce particle size, and enhance the contact between solvents and targeted compounds (Zhang *et al*., 2016). Ultrasound is a unique type of soundwave that ranges from 20 kHz to 100 MHz, exceeding the threshold of human hearing. The mechanism of sound waves in a medium involves high and low pressure

(compression and rarefaction) cycles. This technique induces cavitation, which promotes the production, growth, and collapse of bubbles (Chemat *et al*., 2011). The cavitation process produces strong shear forces and allows the solvent to penetrate deeper into the matrix. The advantage of this is an improvement in the diffusion rate of the desired molecule to the solvent (Wang *et al*., 2008). UAE offers several advantages over other extraction methods, such as substantially higher beta-glucan yields, shorter extraction time, moderate solvent requirements, lower environmental impact, and potential for industrial upscaling.

The aim of this chapter was to assess the effect of ultrasound-assisted extraction (UAE) on beta-glucans from barley grain flour. To this end, two barley flours were investigated, originating from the UK and Jordan respectively. The obtained extracts were characterised for their beta-glucan, starch and protein content and their physicochemical properties were evaluated, with a view to provide information of their technological value as ingredients in food formulations.

4.2 Materials and Methods

4.2.1 Raw Materials

Hulled barley grain samples were obtained from the UK and Jordan. The Jordanian barley was purchased from the Figs and Olive bakery in Kuwait as a food product (produced in 2017); meanwhile, the UK barley was purchased from Heygates Ltd. as an animal feed product (produced in 2017). Barley grains were ground into finer particles in the laboratory using a coffee grinder (De'Longhi; Type KG46). The resulting barley flour was stored in glass bottles at room temperature (20°C) and used in all subsequent experiments.

4.2.2 Ultrasound Assisted Extraction of beta-glucans

The ultrasonic system used was a high intensity ultrasonic process system (P100/6-20, Celbius Ltd, UK) with a typical titanium process horn configuration operated at a nominal frequency of 20 KHz. The diameter of the transducer was 34 mm. The probe was attached to a clamp to allow it to be submerged to a depth of 2.0 cm in the sample contained in a water jacketed vessel (total volume 80 mL) through which water at 50 ^{o}C was circulated, to maintain the extraction temperature constant. The effects of various amplitude levels (10, 15) and treatment times (5, 10, 20, 30 min for the UK; 5, 10, 20, 30, 35, 40 min for Jordanian barley) were studied.

The extraction process is depicted in (Figure 4.1). Briefly, 10 g of barley flour was refluxed with ethanol (80%, v/v) for 6 hours to defat the sample and deactivate endogenous glucanases. The flour was then dried overnight in an oven at 40 °C. Five grams of the defatted milled barley flour was mixed with distilled water pre-heated to 50 °C at a ratio of 1 to 10 (w/v). The suspension pH was adjusted to 5.0 with 0.1 M HCl to increase the extraction yields of beta-glucans: lower pH destroys other polysaccharides and insoluble fibre and converts them into smaller, water-soluble components (Hematian Sourki *et al*., 2017b). The ultrasonication was conducted at 50 °C. Upon cooling, the mixture was centrifuged at 15,000 × g for 10 min, and the solid residue was dried for 24 hours in the oven at 50 °C. Meanwhile, the supernatant pH was adjusted to 4.0 with 0.1 N HCL to precipitate any water-soluble proteins, and the mixture was again centrifuged at 15,000 × g for 20 min. The separated precipitated proteins were then discarded. The pH of the supernatant was adjusted to 7.0 with 1 M NaOH to solubilise hemicellulose. After centrifugation, the supernatant was transferred to a new centrifuge tube, and to that filtrate, 99% ethanol was added (1:1 v/v) to precipitate beta-glucans. The suspension obtained after the addition of ethanol was stored in a refrigerator for 24 hours (4 °C) to facilitate beta-glucan precipitation. This precipitation was

followed by centrifugation (at 4000 g for 15 min). The pellets obtained were frozen at -20°C overnight and then freeze-dried for 24 h under vacuum at -45°C (VirTis, SP Scientific, Ipswich, UK).

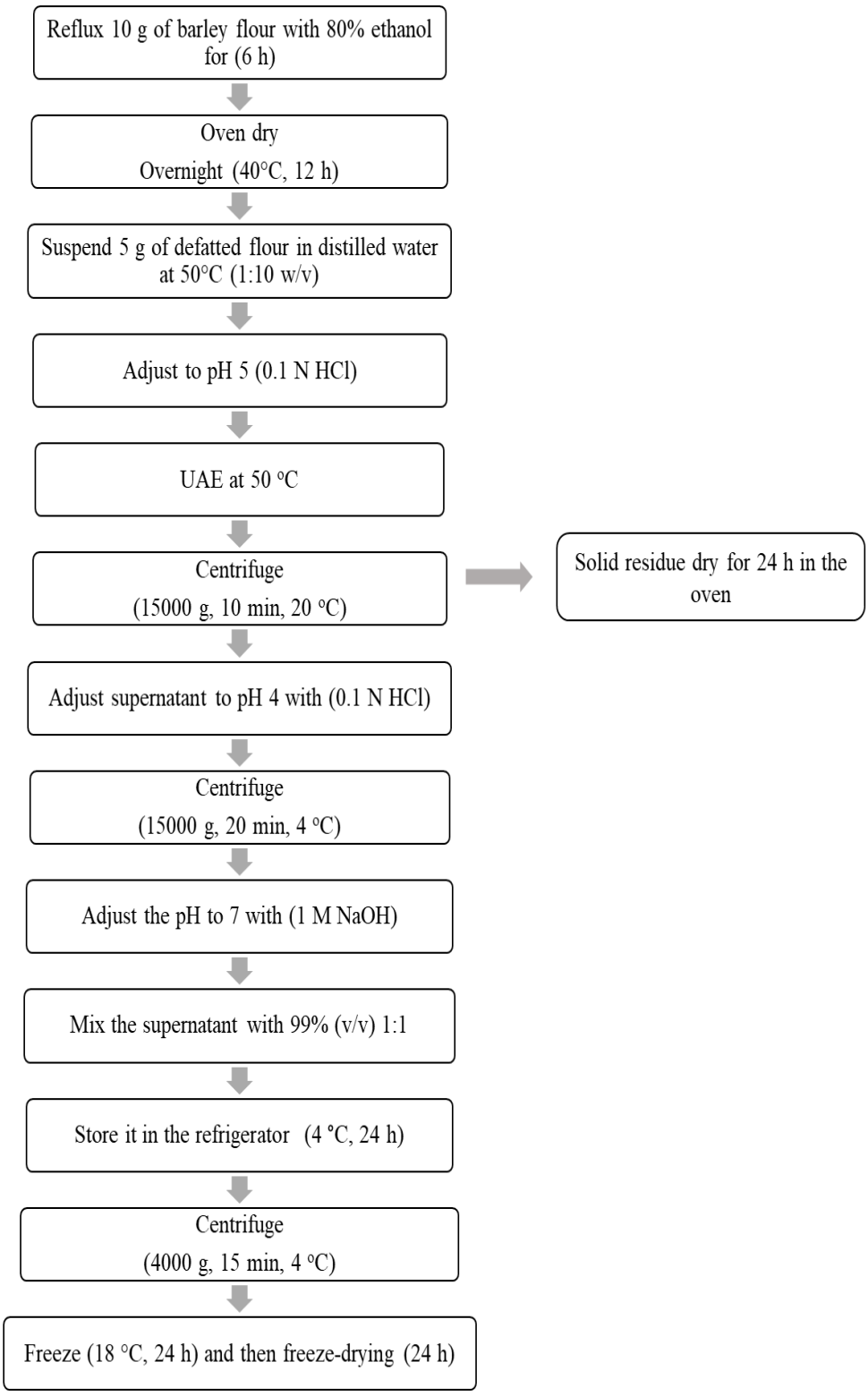

Figure 4.1: Scheme for the extraction of beta-glucans from barley grain via ultrasound-assisted extraction.

4.2.3 Compositional Analysis of the Extracts and Residues

4.2.3.1 Mass Yield of Extracts

The mass yield of the extract (%, w/w) was calculated as the amount of extract obtained from the barley flour divided by the total barley amount in the sample, according to the following Equation 4.1:

$$Mass\ yield\ (\%, w/w) = \frac{mass\ of\ freeze\ dried\ extract}{mass\ of\ barley\ flour\ in\ sample} \times\ 100 \qquad \textit{Equation 4.1}$$

where:

Mass of freeze-dried extract = the weighed mass of extracts after drying (g)

Mass of barley flour = Mass of defatted barley flour used at start of UAE extraction (5 g)

4.2.3.2 Beta-Glucan purity in extracts and residues

The beta-glucan content in the dried extracts, as well as in the solid residues of the extraction, was determined based on the mixed-linkage beta-glucan enzymatic kit (Megazyme, Ireland). Briefly, samples were suspended and hydrated in a buffer solution of pH 6.5 and then incubated with purified lichenase and filtered. Thereafter, an aliquot of the filtrate was hydrolysed to completion with purified β-glucosidase. The produced D-glucose was quantified using a glucose oxidase/peroxidase reagent by measuring the absorbance of the aliquot at 510 nm, according to Equation 4.2:

$$Beta - glucans\ purity\ (\%, w/w)\ =\ \Delta A\ \times \frac{F}{W} \times FV \times 0.9 \qquad \textit{Equation 4.2}$$

where: ΔA = absorbance of analysed sample (Abs)

F = factor for the conversion of absorbance values to μg of glucose

$$(= \frac{100\ (\mu g\ \text{of D}-\text{glucose})}{\text{absorbance of } 100\ \mu g\ \text{of D}-\text{glucose}})$$

FV = final volume of sample (9.4 ml)

W = weight in mg of barley flour (100 mg)

4.2.3.3 Beta-Glucan Recovery in the Extracts

Beta-glucan recovery in the extracts was calculated as a percentage ratio (%, w/w) of the amount of beta-glucans in the extracts to the amount of beta-glucans in the initial sample, as expressed in the following Equation (4.3):

$$Beta-glucan\ recovery\ (\%) = \frac{amount\ of\ beta-glucans\ in\ extract\ (g)}{amount\ of\ beta-glucans\ in\ the\ inital\ sample\ (g)} \times 100 \quad \textit{Equation 4.3}$$

4.2.3.4 Protein content in UAE extracts

The content of protein in the extracts was measured using the Bradford protocol (Kruger, 2009). A standard stock solution of bovine serum albumin (BSA), with a concentration of 10 mg/ml, was used to make solutions of 0.1–0.4 mg/ml. For the UAE extracts, 5 mg of dried barley extracts were weighed and mixed with 1 ml of distilled water to make a concentration of 5 mg/ml. The mixture was mixed for 30 min at 45 °C until completely dissolved. A total of 0.05 ml of the standards and barley extract solutions was mixed with 1.5 ml of Bradford reagent and allowed to stand for 5 min before the absorbance was measured at 595 nm; a blank solution containing 0.05 ml of distilled water and 1.5 ml of Bradford reagent was included in the assay. A calibration plot was formulated using the absorbance and the concentration of BSA standard solutions. The protein

content in the beta-glucan extracts was expressed as mg of protein per g of extract mass and then converted to a percentage.

4.2.3.5 Starch content in the extracts and residues

The start content of the barley extracts and residues was analysed according to the total starch Megazyme kit (amyloglucosidase/α-amylase method). This method is based on AOAC methods (Official Method 996.11and Official Method 76.13.01). In brief, thermostable α-amylase hydrolyses starch into soluble branched and unbranched maltodextrins, whereas amyloglucosidase (AMG) hydrolyses maltodextrins into D-glucose. Thereafter, D-glucose is oxidised into D-gluconate with the release of one mole of hydrogen peroxide (H_2O_2), which is quantitatively measured in a colorimetric reaction employing peroxidase and the production of a quinonimine dye; absorbance was measured at 510 nm. The quantification of starch is calculated as the Equation 4.4 as described below.

$$Total\ starch\ (\%) = \Delta A \times F/W \times FV \times 0.9 \qquad \textit{Equation 4.4}$$

where: ΔA = absorbance read against reagent blank

F = factor for the conversion of absorbance values to μg of glucose

$$(= \frac{100\ (\mu g\ of\ D-glucose)}{absorbance\ of\ 100\ \mu g\ of\ D-glucose})$$

W = the weight in mg of the barley extracts analysed

FV = final volume (100 ml)

4.2.4 Physical Properties

4.2.4.1 Water Holding Capacity

The water holding capacity (WHC) was determined based on the method used by Liu *et al*. (2015) with minor modifications. Briefly, 0.1 g of barley extract was mixed with 25 ml of water in a pre-weighed centrifuge tube, followed by thorough agitation in a vortex mixer. The mixture was stored at 4 °C for 1 h and then centrifuged for 30 min at 3,000 × *g*. The supernatant (unbound water) was discarded, and the tube and wet pellet were weighed together in an analytical balance. The WHC was calculated as per the Equation 4.5:

$$WHC\ (g/g)\ = \frac{wet\ extract\ weight - dry\ extract\ weight}{dry\ extract\ weight} \qquad \textit{Equation 4.5}$$

where,

wet extract weight = mass of extract in centrifuge tube (g)

Dry extract weight = mass of extract use (0.1 g)

4.2.4.2 Viscosity of the Extracts

The rheological properties of the barley extracts were analysed using a rheometer (Anton Paar Modular Compact Rheometer; MCR 102) equipped with a concentric cylinder (CC27, Anton Paar, UK). A 1% (w/v) solution was prepared using 0.2 g of the barley extracts mixed with 20 ml of sodium phosphate buffer (pH 6.5; 20 mM) into a beaker. The preparation of the solution was done following Kurek *et al*. (2018) protocol with some modifications. Samples were heated to 80°C with constant stirring and the temperature was held at 80 °C for 10 min, before cooling to room temperature with constant stirring. The volume was adjusted back to 20 ml using distilled

water, and the samples were stored at 4 °C for 24 h before measurements. Viscosity was measured in a shear range of 0.01 to 100 s^{-1} The temperature was controlled at 20 °C.

4.3 Statistical Analysis

All experiments were repeated at least twice, and data were represented as means ± standard deviations. Statistical analysis was conducted using Minitab statistical analysis software version 17.1.0. One-way analysis of variance (ANOVA) with a Tukey's multiple comparison test was used to determine significant differences between treatments among samples from each of the barley flours, at a confidence level of 95% ($p<0.05$).

4.4 Results and Discussion

4.4.1 Effect of ultrasonication on beta-glucan extraction from barley flour

Defatted barley flours, originating from the UK and Jordan, were subjected to ultrasound assisted extraction at varying amplitudes and time, aiming to assess the effect of the ultrasonication on the extraction of beta-glucans. The main goal of extraction was to provide the highest mass of targeted compounds with few contaminants. In UAE, the temperature was controlled at 50 °C and standardised for all the extraction conditions, to avoid starch gelatinization.

4.4.1.1 Mass yield of extraction

In the case of the Jordanian barley flour, as shown in Figure 4.2, at 10 amplitude, extending the duration of the treatment from 5 min to 30 min did not enhance the mass yield of the extract

(mass yield remained around 5%, w/w). On the contrary, a positive correlation between the mass yield and the duration of extraction was seen at 15 amplitude. As such, it was deemed necessary to extend the time of extraction beyond 30 min in this occasion up to 40 min (Figure. 4.2). The optimum extraction time of the Jordanian barley at 15 A was 35 min, reaching the highest mass yield of 21.5% (w/w).

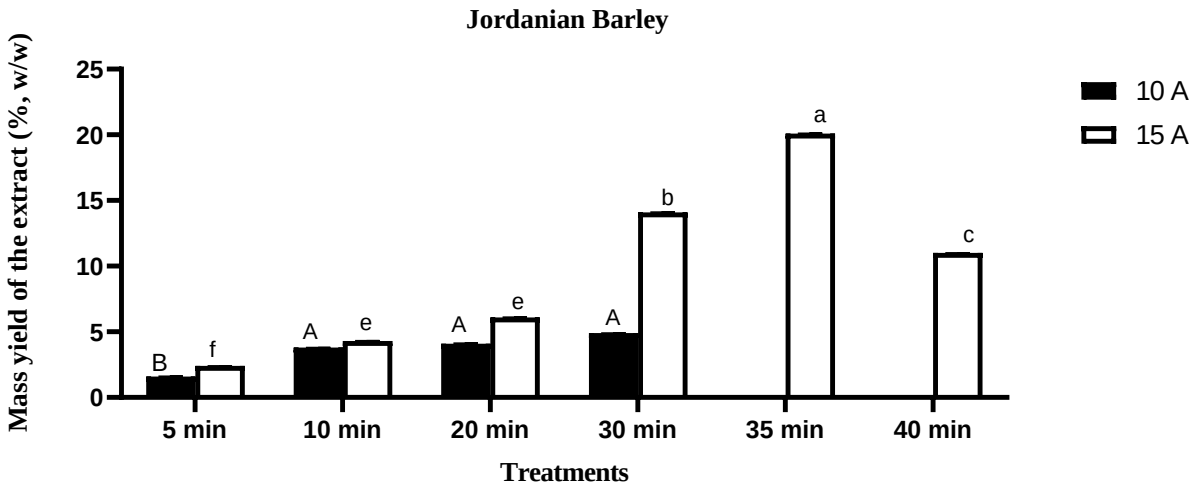

Figure 4.2: Mass yields (%, w/w) of the Jordanian barley extracts obtained via UAE. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

In the case of the UK barley flour, the effect of amplitude was more pronounced. Specifically, at 10 amplitude, 5 and 10 min of extraction did not seem to have a positive effect on mass yield (Figure 4.3); however, at 20 min of extraction mass yield reached ~15% (w/w), whereas the extension of the extraction duration to 30 min resulted in decreased mass yields (~ 7%, w/w). At 15 amplitude, the mass yield pattern was similar, with 20 min of extraction resulting in the highest mass yield (23.6%, w/w) (Figure 4.3).

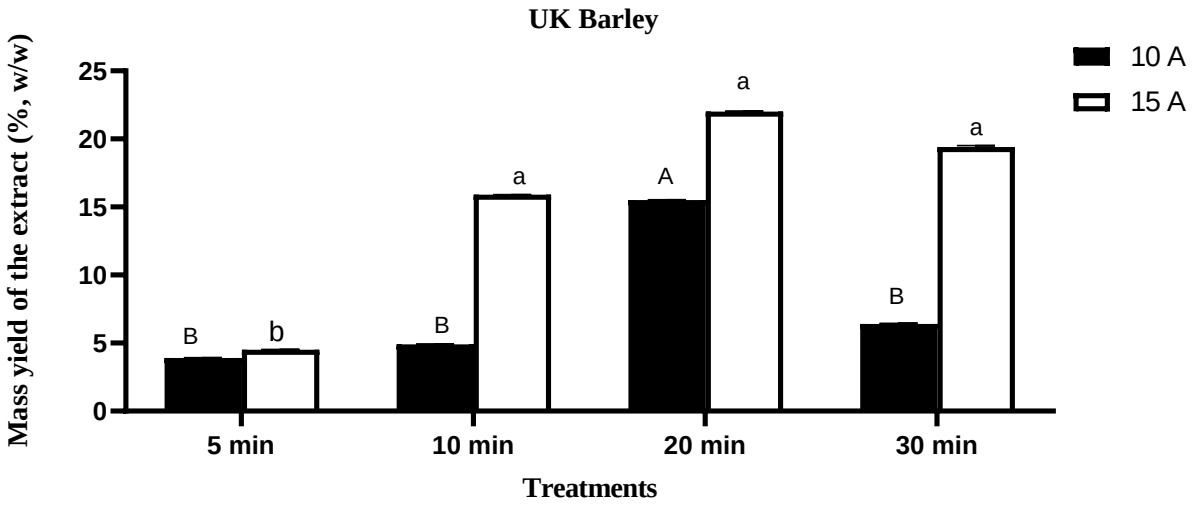

Figure 4.3: Mass yields (%, w/w) of the UK barley extracts obtained via UAE. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

It can be clearly seen that in UAE, the mass yield is mainly affected by the extraction time and the amplitude of oscillation. Except for 10 amplitude in the Jordanian barley, in all other cases, it was observed that the longer the extraction time, the higher the extraction yield. However, the effect of extraction time on the mass yield for the experimental range tested in this study, did not follow a linear trend. After 20 min (for UK barley) or 35 min (for Jordanian barley), a notable decrease in the mass yield was seen, indicating possibly degradation phenomena (Du *et al*., 2014). The amplitude of oscillation controls the intensity of the cavitation, which helps to release intracellular components from the matrix (polysaccharides, proteins); therefore, the higher the amplitude the higher the mass yield. Other studies have demonstrated similar results, indicating that at higher amplitude values, the cell wall components decompose rapidly and a notable amount of solid material can be extracted and dissolved in the aqueous liquid phase (Hematian Sourki *et al*., 2017a).

4.4.1.2 Composition of UAE barley extracts

Since the evaluation of the mass yield provides only a preliminary assessment of the effect of UAE on barley flour extractions, the next step in the experimental process involved the compositional characterisation of the extracts. Special attention was given to the purity of the extracts in beta-glucans and their recovery, together with their starch and protein content.

In Jordanian barley extracts at 10 amplitude, no statistically significant changes were observed in terms of their purity in beta-glucans (11–12%, w/w) ($p<0.05$) in extractions between 5 and 20 min (Figure 4.4a). However, a slight increase was noted when the extraction was extended to 30 min (15%, w/w). When the intensity of oscillation was increased (15 amplitude), maximum purity was achieved after 5 min of extraction (15%, w/w), whereas prolongation of the treatment did not seem to impact positively the purity of extraction, ranging between 11–13% (w/w). In terms of beta-glucan recovery, the trend was different (Figure 4.4b). For both oscillation intensities, a longer extraction time resulted in increased recovery of beta-glucans [up until 30 min for 10 A, equal to 20% (w/w) and at 35 min for 15 A, reaching ~73% (w/w)], with a notable drop in the recovery after 40 min of extraction in the case of 15 amplitude. It seems evident that for the Jordanian barley, higher amplitude at 15 A and 35 min of extraction, resulted in maximum beta-glucan recovery.

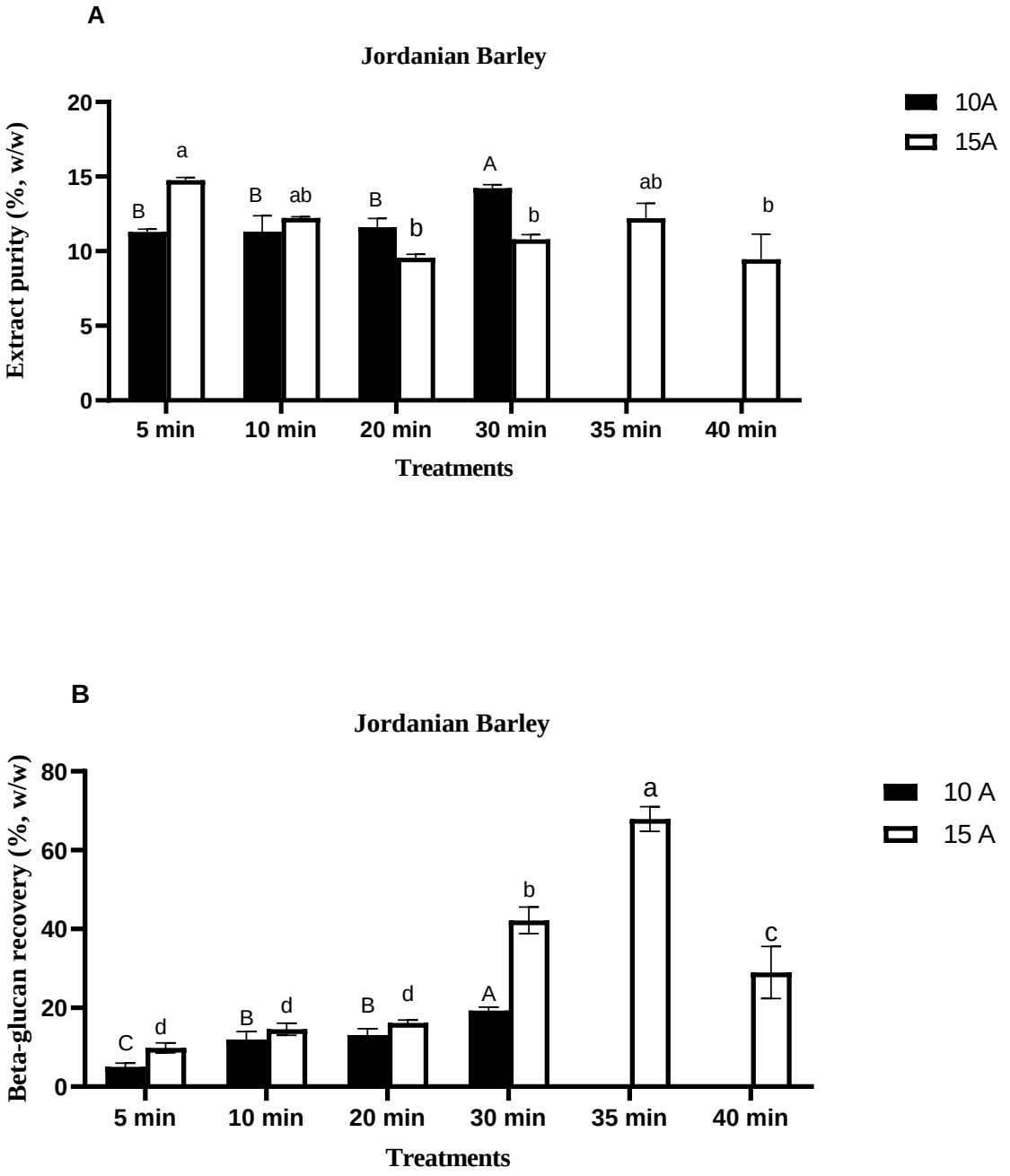

Figure 4.4: (A) Extract purity (%, w/w) and (B) beta-glucan recovery from Jordanian barley using UAE. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

For the UK barley, the UAE treatment which led to the highest purity of beta-glucans in the extract was at 10 A for 5 min (~15%, w/w) as shown in (Figure 4.5 A); after that point, extended extraction times led to similar purity results (10–12%, w/w). In terms of beta-glucan recovery, the lower oscillation intensity (10 A) gave the highest beta-glucan recovery for the UK barley after 20 min of treatment (~55%, w/w). Around 50% (w/w) of recovery was noted at 15 A after 10 min, whereas for both intensities, an extension of the treatment to 30 min resulted in decreased recovery of beta-glucans in the extracts. These results showed that the highest purity of beta-glucans was achieved by reducing the time, as a longer extraction time is associated with greater energy generated from the ultrasound waves, and possibly leading to starch gelatinization and leak out of other "impurities". Hematian Sourki *et al.* (2017) noted a rise in energy due to cavitation as extraction time increases; this energy later caused a disruption in beta-glucans structure and increased cell disruption to allow more water-soluble polysaccharides to be extracted.

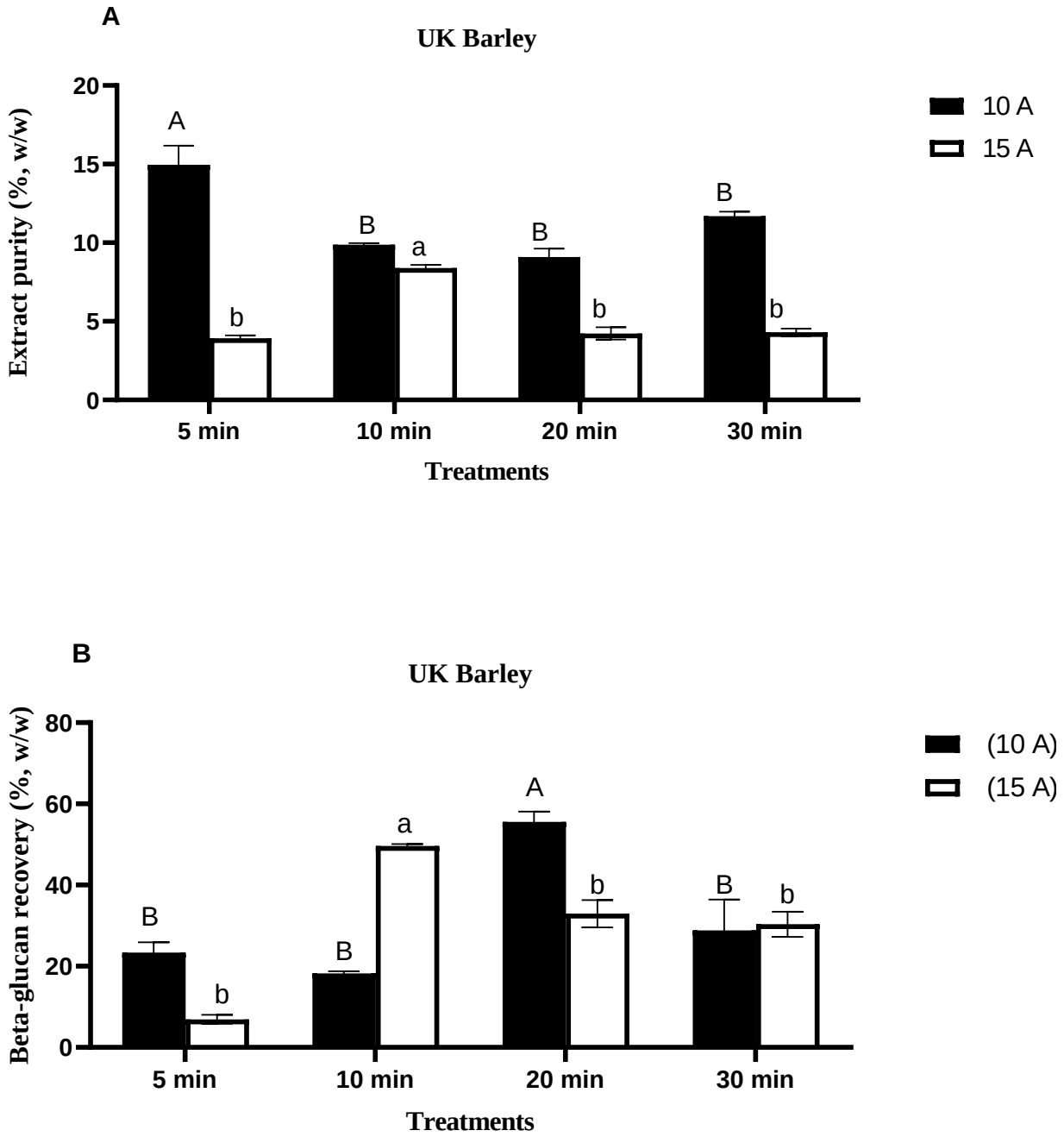

Figure 4.5: (A) Extract purity (%, w/w) and (B) beta-glucan recovery from UK barley using UAE. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

A comparison of the two UAE approaches revealed that the cavitation phenomenon was more effective when a high intensity of extraction was applied rather than simply prolonging the time of extraction. The purity of beta-glucans decreased with increasing ultrasound amplitude. This is apparently due to the degradation of cell wall components at high amplitudes, which increases the release of polysaccharides other than beta-glucans into the aqueous phase as impurities. Due to the prolonged exposure of the barley flour to the ultrasonic waves, which caused more water-soluble extracts to accumulate in the water, more starch was extracted as part of the extracts. For the UK barley, treatment at 15 A for 20 min achieved the highest mass yield (23.6%, w/w), but a lower beta-glucan recovery was obtained compared to treatment at 10 A for 20 min. The highest recovery of the beta-glucans was at 10 A for 20 min (55.57%) for the UK barley and at 15 A for 35 min (73.2%) for the Jordanian barley.

The beta-glucan content decreased as the extraction time increased, which shows that the shorter the sonication time, the more selective ultrasonication process can be towards beta-glucans. The use of shorter extraction times to obtain higher amounts of beta-glucan was noted by Benito-Román *et al.* (2013), who used extraction times less than 10 min and observed a marked decrease in beta-glucan amounts as the extraction time increased. In the current study, Jordanian barley tended to need a higher power (15 A) and a longer extraction time compared to the UK barley. The solubility of beta-glucans in water depends on several factors, mainly their structure, which is associated with their origin. Their beta-glucan solubility increases with increasing temperature. Protein-bound glucans are insoluble, but after partial hydrolysis, their molecules can produce gels (Rop *et al*., 2009). The beta-glucan fractions in barley grains differ in protein content. One possible explanation for less extractable beta-glucans could be their binding onto the cell walls with other components. Some evidence for covalent or physical associations between beta-glucans and

proteins has been reported that might affect the extractability of the polysaccharide (Robertson *et al*., 1997; Izydorczyk *et al*., 2000). A high frequency of long blocks of adjacent (1–4) linkages could increase the possibility of interactions and junction zone formation with other glucan molecules or with the heteroxylan and cellulose chains in the cell wall, resulting in decreased water extractability. The insoluble fractions are likely held in the wall matrix by entanglement and hydrogen bonding with the other wall components rather than covalent bonding (Biliaderis and Izydorczyk, 2006).

Consistent with previously published research, UAE in this study increased the yield of extracted components, decreased the extraction time and required lower temperatures than HWE. Tian *et al*. (2012) showed that extraction of polysaccharides from white button mushrooms (*Agaricus bisporus*) via UAE gave a higher yield than the HWE method, with the UAE resulting in relative increases of 155 %.

4.4.1.3 Protein Content in the Extracts

In terms of other plant cell wall components that could be co-extracted during UAE, the results show that the protein content in beta-glucan extracts ranged between 0.10–2.17% (w/w) (Figures 4.6 and 4.7). In Jordanian barley extracts, at 10 A protein content decreased when the extraction time was increased but was kept low in any case (less than 0.2 %, w/w). When oscillation was increased (15 A), extended extraction times led to greater protein content in the extracts, reaching 0.4% (w/w) at 40 min (Figure 4.6). On the contrary, in UK barley extracts, although 10 A did not seem to influence protein extraction, at increased oscillation intensity (15 A) protein extraction increased linearly with time, reaching 2.17% (w/w) at 30 min ($p<0.05$) (Figure 4.7).

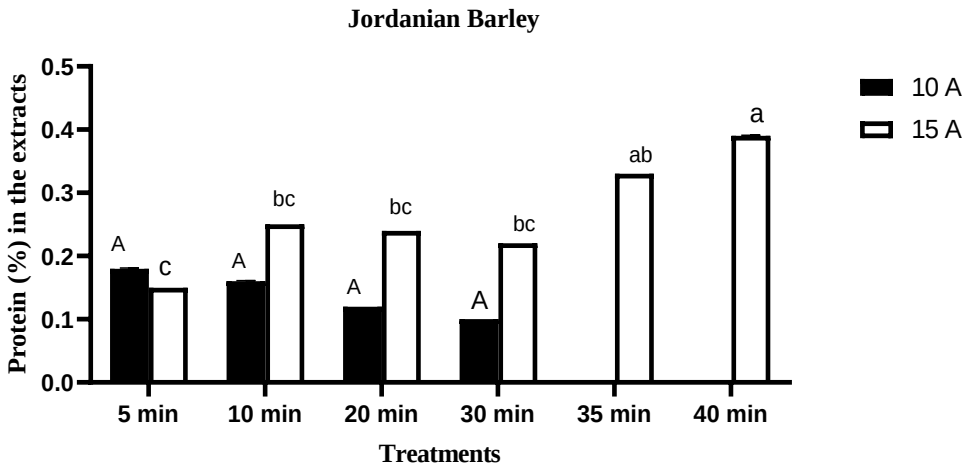

Figure 4.6: Protein (%) in the extracts obtained from the Jordanian barley. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

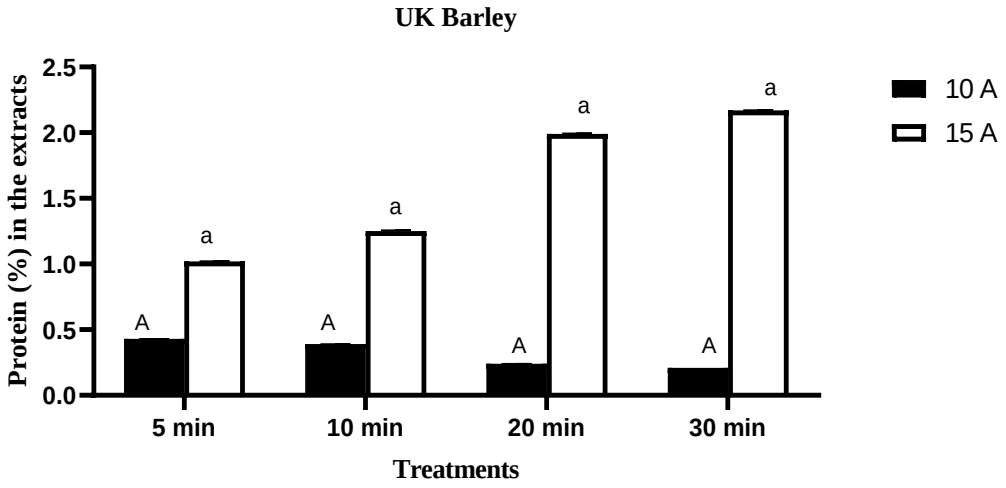

Figure 4.7: Protein (%) in the extracts obtained from the UK barley. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

The initial protein content of the Jordanian and UK barley grain was 12.7% (w/w) and 11.0 % (w/w), respectively. Barley grains that are grown in more arid conditions are expected to have a higher protein content compared to barley grown with more water (Newman and Newman, 2008). Carbohydrates are chemically bound to protein in barley, and they are classified as glycoproteins. Prolamins, known as hordeins in barley, are the major storage proteins in the endosperm, accounting for 34–50% of the total nitrogen content (Kirkman *et al*., 1982). From a mechanism point of view, UAE involves cavitation generated in the extraction medium, by the passage of ultrasonic waves circulating through the cell wall. The waves are significantly affected by the ultrasonic temperature and sonication power. Studies have shown that a high temperature or high sonication power leads to protein contamination in the polysaccharides due to an increase in the number of cavitation bubbles formed and a lower yield in the extracted material (Li *et al*., 2007). One method for removing protein contamination in the extracts is by maintaining a pH at 4.5 and removing precipitated proteins by centrifugation; the protein content of preparations using this treatment may range from 1 to 3.8% (Bhatty, 1999; Skendi *et al*., 2003). In the current study, this pH adjustment was part of the experimental process, and resulted in only a small amount of protein left in the final extracts. This could be an indication of the presence of barley proteins in UAE extracts with different isoelectric points than the one applied (pH 4), as well as the extraction of proteins that are linked to other macromolecules (beta-glucans or other polysaccharides).

4.4.1.4 Starch Content in the Extracts

The obtained UAE extracts were also characterised for their starch content. For both barley flours, it was seen that as the extraction time increased, starch content in the extracts also increased (Figures 4.8 and 4.19). The highest starch content was found for both barley flours in extracts

obtained at the highest intensity at prolonged extraction times. In terms of comparison, the Jordanian barley flour extracts contained more starch 57.9%, w/w) than the UK barley flour ones (33.8%, w/w). Worth noticing is the fact that originally the UK flour contained more starch (52.3%, w/w) compared to the Jordanian barley one (40.3%, w/w). Patist and Bates (2008) reported that the energy generated by cavitation destroyed the cell walls and enhanced the release of cellular components such as polysaccharides. Moreover, other studies have shown that with increased UAE time, the diffusion of polysaccharides and other molecules also increases (Skenderidis *et al*., 2017).

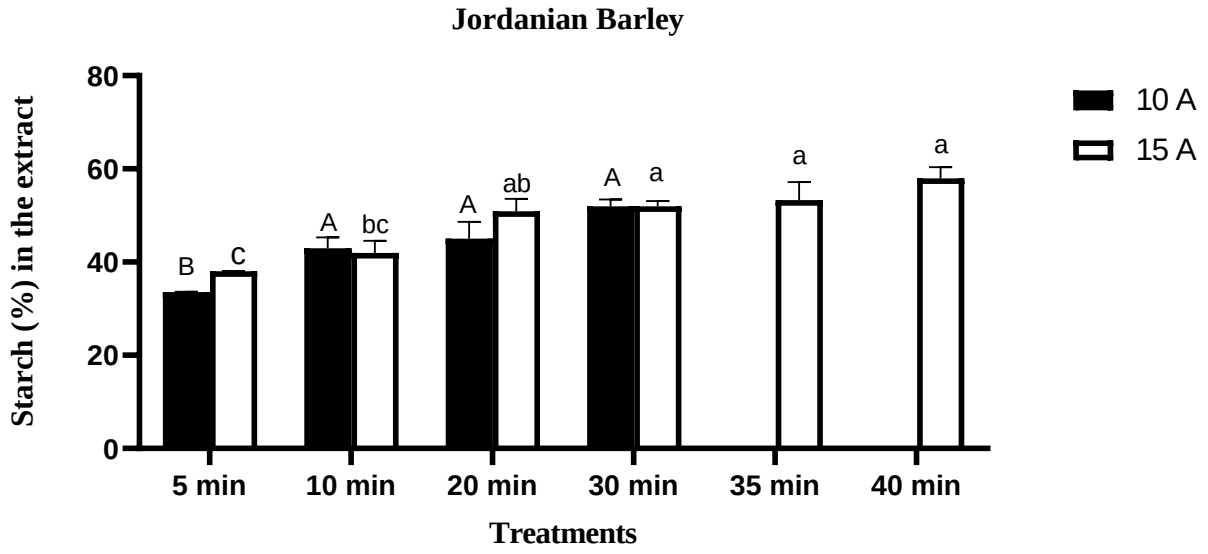

Figure 4.8: Starch (%) in the extracts obtained from the Jordanian barley. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

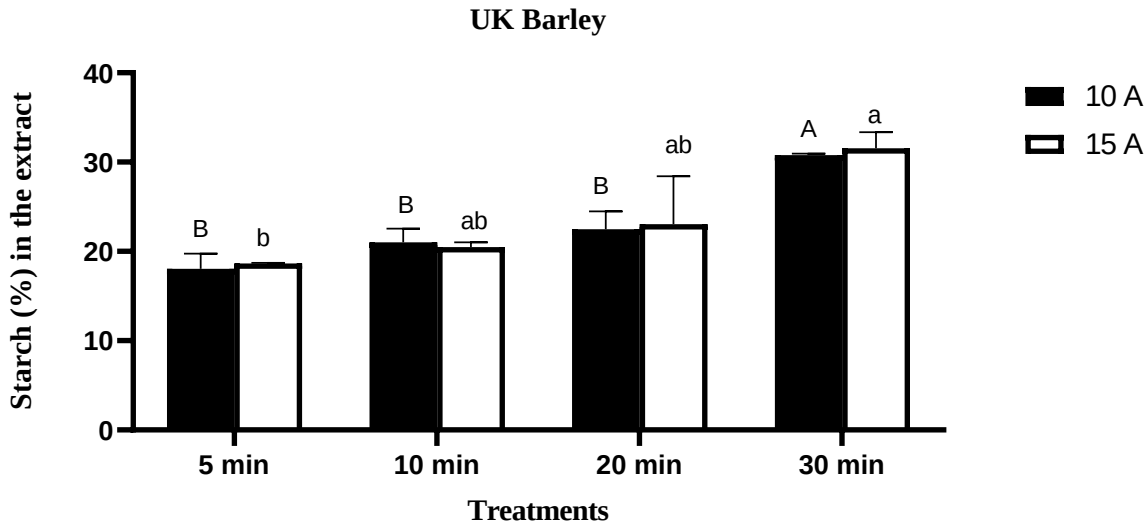

Figure 4.9: Starch (%) in the extracts obtained from the UK barley. Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

The amount of starch in the obtained extracts revealed that UAE process is not selective and caused other molecules in the barley flour to co-extract apart from beta-glucans. According to the findings of this work, for the Jordanian barley, UAE extracts with the highest beta-glucan recovery (73% after 30 min at 15 A) contained 13% (w/w) beta glucans, 55% (w/w) starch and 0.3% (w/w) protein. On the contrary, the UK barley extracts obtained at UAE conditions with the highest beta glucan recovery (55% after 20 min at 10 A) contained 10% (w/w) beta-glucans, 23% (w/w) starch and 0.3% (w/w) protein. Linking these findings with the compositional characterisation of the two flours (Chapter 3), it seems that the reason for the high oscillation intensity and time needed for beta glucans extraction in the Jordanian barley could be the presence of more hemicelluloses in the grain (almost 2-fold compared to the UK grain). Insoluble β-glucans have usually low molar mass and can be non-covalently bound to arabinoxylans (Johansson *et al*. 2004). It is likely than in our case, hemicelluloses such as arabinoxylans, were also extracted via UAE under high oscillation intensity and extended extraction time and part of them would have been removed during the post-extraction steps of the process (pH of extracts adjusted to 7 to solubilise hemicelluloses).

4.4.2 Compositional Analysis of UAE Residues

The remaining solids after UAE were further characterised compositionally, in order to assess changes in the macronutrient content of both barley flours, which are linked with the applied UAE extraction conditions. The results are shown in the following Table 4.1. The original composition of both flours is also included in the Table for comparison reasons.

Table 4.1: Compositional analysis of the UAE residues of UK and Jordanian barley, obtained at various intensities and extraction times

Compound (%, w/w)	Jordanian barley (control)	UK barley (control)	Amplitude (A)	Extraction time (min)									
				Jordanian barley residue						UK barley residue			
				5	10	20	30	35	40	5	10	20	30
Moisture	6.03 ±0.36	10.00 ± 0.37	10	2.12^{c}±0.02	3.31^{a}±0.01	2.95^{b}±0.09	2.73^{b}±0.05	-	-	4.98^{A}±0.23	4.78^{A}±0.2	4.46^{A}±0.07	4.24^{A}±0.15
			15	3.14^{ab}±0.12	2.91^{ab}±0.18	2.66^{b}±0.10	3.33^{a}±0.14	2.78^{b}±0.07	2.85^{b}±0.04	2.73^{A}±0.05	2.35^{B}±0.31	2.44^{B}±0.08	2.38^{B}±0.02
Lipids	2.94±0.035	3.96±0.018	10	0.51^{a}±0.07	0.52^{a}±0.05	0.46^{a}±0.09	0.61^{a}±0.07	-	-	0.44^{B}±0.02	0.49^{AB}±0.02	0.51^{AB}±0.02	0.6^{A}±0.07
			15	0.52^{a}±0.09	0.56^{a}±0.04	0.55^{a}±0.04	0.71^{a}±0.04	0.78^{a}±0.02	0.78^{a}±0.06	0.57^{A}±0.04	0.52^{A}±0.02	0.51^{A}±0.07	0.7^{A}±0.07
Ash	3.50± 0.14	2.65 ±0.01	10	1.24^{a}±0.04	1.33^{a}±0.05	1.49^{a}±0.04	1.49^{a}±0.03	-	-	1.26^{B}±0.02	1.28^{AB}±0.14	1.34^{AB}±0.01	1.46^{A}±0.05
			15	1.29^{b}±0.02	1.38^{b}±0.04	1.41^{b}±0.01	1.82^{a}±0.10	1.82^{a}±0.09	1.86^{a}±0.04	1.33^{C}±0.03	1.54^{BC}±0.1	1.78^{AB}±0.08	1.81^{A}±0.09
Protein	12.71 ±0.16	11.00 ± 0.36	10	13.15^{a}±0.12	12.81^{a}±0.42	12.89^{a}±0.16	13.92^{a}±0.72	-	-	10.05^{C}±0.09	11.07^{BC}±0.13	12.49^{A}±0.31	12.14^{AB}±0.40
			15	15.32^{b}±0.42	13.34^{b}±0.52	13.61^{b}±0.43	15.23^{b}±0.39	18.95^{a}±0.62	14.67^{b}±0.49	10.42^{C}±0.03	12.01^{B}±0.21	14.8^{A}±0.61	13.49^{AB}±0.42
Starch	40.32 ± 2.32	52.32±1.81	10	34.51^{a}±0.41	30.54^{b}±0.81	30.17^{b}±0.21	28.25^{b}±1.85	-	-	47.02^{A}±0.22	46.02^{A}±0.63	42.71^{A}±1.60	31.68^{B}±0.98
			15	29.6^{a}±0.89	28.82^{ab}±0.74	27.64^{abc}±0.81	23.98^{bcd}±1.93	23.68^{cd}±0.65	21.55^{d}±0.85	45.06^{A}±0.10	42.02^{A}±0.12	35.99^{B}±0.64	31.8^{B}±2.16
β-Glucans	3.61± 0.20	2.65±0.36	10	3.33^{a}±0.13	3.21^{a}±0.04	3.08^{a}±0.07	3.11^{a}±0.09	-	-	2.21^{AB}±0.02	2.28^{AB}±0.10	2.12^{B}±0.03	2.44^{A}±0.07
			15	3.42^{ab}±0.28	3.07^{b}±0.05	3.41^{ab}±0.08	3.33^{ab}±0.09	3.87^{a}±3.87	3.66^{ab}±0.0	2.76^{B}±0.15	2.19^{C}±0.14	3.24^{AB}±0.15	3.39^{A}±0.05

Values are presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

In terms of the Jordanian barley residues, it is evident that as the extraction time increased, most components were also concentrated in the residues (lipids, protein, ash). The only exception to this trend was that of starch, which seemed to decrease in concentration, indicating its gelatinisation and co-extraction during UAE. It was also noted that the increase in the intensity of the extraction led to more pronounced differences in the macromolecules' concentration in the residues. The only compound that seemed not to be majorly affected by the extraction point or intensity was beta-glucans (Table 3.1). Similar trends were observed for the UK residues, whereas absolute concentration values of the major components quantified were similar to those in the Jordanian residues at the same time points. Worth also mentioning is the fact that the lipid content in both residues is much lower than that of the original grains, a fact that reflects the ethanol reflux step that was carried out prior to UAE to defat the flour.

4.4.3 Physical Properties of UAE barley extracts

4.4.3.1 Water Holding Capacity

Water holding capacity (WHC) is one of the main physical properties that appears to be related to beta-glucans in the extracts. In this study, it was decided to assess the effect of UAE extraction time on the WHC of the extracts. As such, the optimum amplitude was chosen for each barley flour, on the basis of beta-glucan recovery: 10 A for the UK barley and 15 A for the Jordanian barley. The results of the WHC determination are shown in (Figure 4.10). The WHC of the extracts ranged from 6.05–8.21 g/g. For the Jordanian extracts, the WHC was 6.4–8.21 g/g, and there were no significant differences due to treatment duration. The WHC values gradually increased with increasing extraction time, until 15 A and 35 min, and then decreased slightly; however, this fluctuation was not significant. It is important to note that in these extracts, the beta-

glucan content did not change significantly (10–15%, w/w) but a more pronounced difference was that of starch content (from 30%, w/w at 5 min to ~55% at 40 min).

The WHC of UK extracts ranged between 5.8–8.2 (g/g). although WHC values did not change significantly between treatments, suggesting that as starch content increased in the extracts (as UAE treatment time increased), WHC also slightly increased. Beta-glucan contents in the extracts were lower than starch content; thus, beta-glucans may have had less influence than starch content on extracts' WHC. Moreover, differences in WHC among UK and Jordanian barley extracts were also driven by starch content, as samples did not differ substantially in terms of beta-glucan content. At the same time point (5 min of UAE treatment), the Jordanian extract contained the same amount of beta-glucans (15%. w/w) but higher starch content (~38%, w/w). In fact, WHC is reportedly influenced by strongly bonded micellar networks and amylopectin molecular structures (Kratz *et al*., 2013).

The WHC results are consistent with Kurek *et al*. (2018), who applied the same WHC protocol used in this study to examine WHC for beta-glucans extracted from barley and oats. The WHC values were 4.56–7.42 g/g. However, all samples indicated that the beta-glucan WHC was quite high. This range of results is similar to that of other studies (Ahmad *et al*., 2010, Liu *et al*., 2015). The WHC values of all beta-glucan extracts were higher than those reported in other studies (Kurek, 2018). These results indicate that the extracts could be used in the food industry as a thickener.

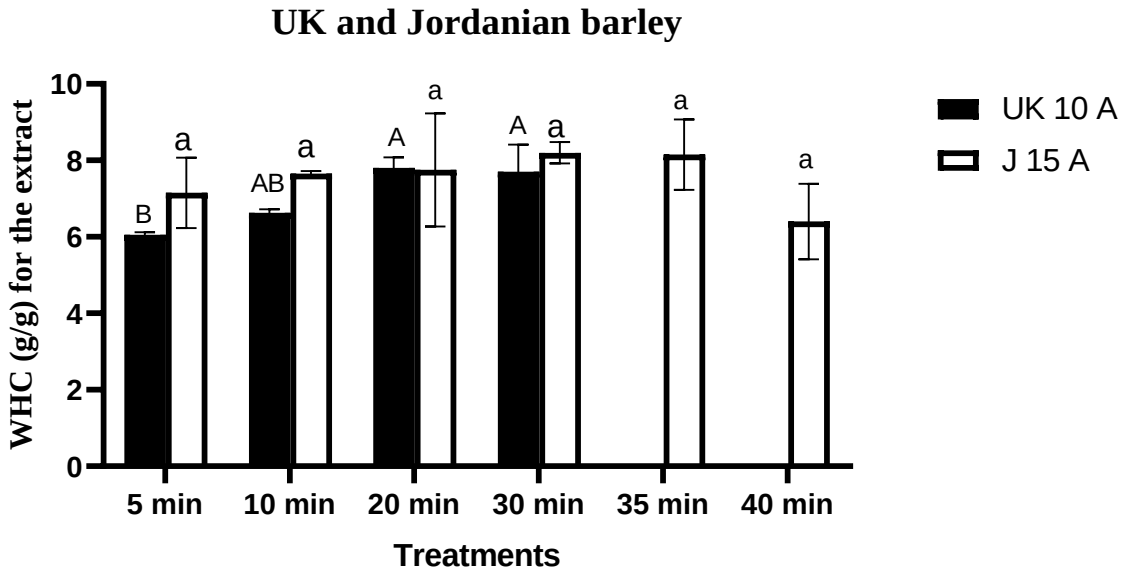

Figure 4.10: Water holding capacity (g/g) of extracts obtained from UK and Jordanian barley, presented as means ± standard deviation of duplicate samples; values with different letters are significantly different ($p<0.05$).

4.4.4 Viscosity of UAE Barley Extracts

The extracts with the highest beta-glucan recovery (Jordanian barley extracts obtained at 15 A and 35 min of treatment, and UK extracts obtained at 10 A and 20 min of treatment) were selected and compared to the Jordanian and UK extracts obtained via treatments with the minimum extraction time (5 min). The extracts showed a shear thinning behaviour at lower applied shear rates (0.01–1 s^{-1}) (Figure 4.11). In general, starch dispersions at low concentration behave as shear thinning products due to a change in starch granule orientation during shear into a more ordered structure that shows less resistance to flow. However, all extracts showed Newtonian behaviour from 1 to 100 s^{-1}. UK extracts obtained after 5 min of UAE demonstrated the lowest viscosities through the entire range of shear rates, followed by the Jordan extract obtained after 5 min of UAE.

Both extracts obtained at 5 min were composed of 15% beta-glucans, but Jordanian extracts were 38% starch while UK extracts were 18%. These results suggest that starch played a major role in defining the viscosity behaviour of the extracts. The UK extract obtained at 10 A and 20 min showed higher viscosity values than the Jordanian extract obtained at 15 A and 35 min through the entire range of shear rate evaluated. The UK extract (10 A, 20 min) was 13 (%, w/w) beta-glucans and 30%, (w/w) starch, while the Jordanian extract (15 A, 35 min) was 10 (%, w/w) beta-glucans and 55% (w/w) starch. Thus, although both extracts were very similar in the final beta-glucan content and the Jordanian extract had higher starch content, the effect of the UAE on beta-glucan structure could have defined the final viscosity of the extracts. A previous study reported that 'although ultrasound could increase beta-glucan extraction yield, it could also have a negative effect on the thickening effect of this polymer' (Hematian Souki *et al*., 2017). Increasing ultrasound amplitude and time increased destruction of the polymer molecular structure, reducing its molecular weight and thus its viscosity.

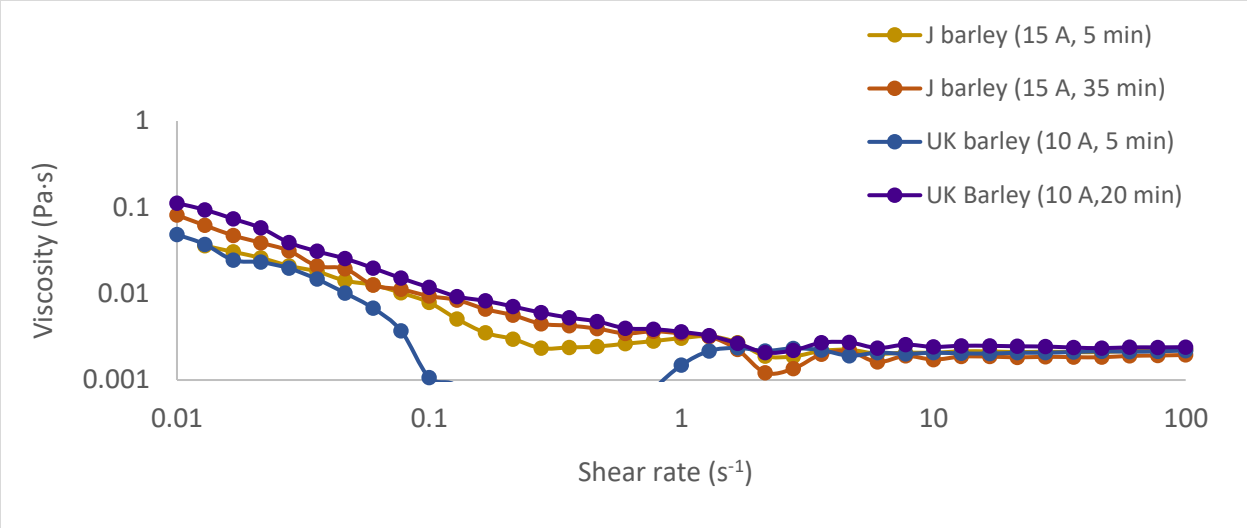

Figure 4.11: Viscosity profile of barley extracts obtained via UAE as function of shear rate. The data presented are representative viscosity values for each sample.

4.5 Conclusion

Ultrasound-assisted extraction was evaluated as a process for the extraction of beta-glucans from barely. In Jordanian barley extracts, maximum beta-glucan recovery was 73.2% and in UK extracts 55.57%. The UAE process was not selective, as both Jordanian and UK extracts contained notable amounts of starch (30-55%) and up to 2% protein. UAE could increase the yield of extracted components, decrease the extraction time and require lower temperatures. UAE barley extracts with high beta-glucan yields showed higher viscosity values, indicating the extracts could be applied as non-calorific thickeners, stabilizing agents for foams and emulsions and fat substitutes in food formulation.

4.6 References

Ahluwalia, B. & Ellis, E. (1985) Studies of beta-glucan in barley, malt and endosperm cell walls. In: Hill, R.D. (ed.) *New Approaches to Research on Cereal Carbohydrates* (*Progress in biotechnology 1)*. Amsterdam: Elsevier.

Ahmad, A., Anjum, F.M., Zahoor, T., Nawaz, H. & Ahmed, Z. (2010) Extraction and characterization of beta-D-glucan from oat for industrial utilization. *International Journal of Biological Macromolecules,* **46**, 304-309.

Aman, P. & Newman, C.W. (1986) Chemical-composition of some different types of barley grown in Montana, USA. *Journal of Cereal Science,* **4**, 133-141.

Anderson, J.W., Smith, B.M. & Gustafson, N.J. (1994) Health benefits and practical aspects of high-fiber diets. *American Journal of Clinical Nutrition,* **59**, 1242S-1247S.

Andersson, A.A.M., Elfverson, C., Andersson, R., Regner, S. & Åman, P. (1999a) Chemical and physical characteristics of different barley samples. *Journal of the Science of Food and Agriculture,* **79**, 979-986.

Andersson, A.A.M., Elfverson, C., Andersson, R., Regnér, S. & Åman, P. (1999b) Chemical and physical characteristics of different barley samples. *Journal of the Science of Food and Agriculture,* **79**, 979-986.

Andersson, A.A.M., Armo, E., Grangeon, E., Fredriksson, H., Andersson, R. & Aman, P. (2004) Molecular weight and structure units of (1 -> 3, 1 -> 4)-beta-glucans in dough and bread made from hull-less barley milling fractions. *Journal of Cereal Science,* **40**, 195-204.

Anon (1999) Functional foods - A trend with a future. *Food Marketing and Technology,* **13**, 10-10.

Anon (2004) Commercialising science: the Finnish functional food innovations. *Innovations in Food Technology,* **48**, 48-46.

Asif, A., Muhammad Anjum, F., Tahir, Z., Haq, N. & Ahmad, D. (2009) Physicochemical and functional properties of barley beta-glucan as affected by different extraction procedures. *International Journal of Food Science and Technology,* **44**, 181-187.

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H. A., Ghafoor, K., Norulaini, N.A.N. & Omar, A.K.M. (2013) Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering,* **117**, 426-436.

Badr, A., Muller, K., Schafer-Pregl, R., El Rabey, H., Effgen, S., Ibrahim, H.H., Pozzi, C., Rohde, W. & Salamini, F. (2000) On the origin and domestication history of barley (Hordeum vulgare). *Molecular Biology and Evolution,* **17**, 499-510.

Baik, B.-K. & Ullrich, S.E. (2008) Barley for food: Characteristics, improvement, and renewed interest. *Journal of Cereal Science,* **48**, 233-242.

Behall, K.M., Scholfield, D.J. & Hallfrisch, J. (2004) Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. *American Journal of Clinical Nutrition,* **80**, 1185-1193.

Bendelow, V.M. (1975) Determination of non-starch polysaccharides in barley breeding programs.. *Journal of the Institute of Brewing,* **81**, 127-130.

Benito-Román, O., Alonso, E. & Cocero, M.J. (2013) Ultrasound-assisted extraction of beta-glucans from barley. *Lwt-Food Science and Technology,* **50**, 57-63.

Benito-Román, O., Alonso, E. & Lucas, S. (2011) Optimization of the β-glucan extraction conditions from different waxy barley cultivars. *Journal of Cereal science,* **53,** 271-276.

Beresford, G. & Stone, B.A. (1983) (1→ 3),(1→ 4)-β-D-glucan content of Triticum grains. *Journal of Cereal Science,* **1,** 111-114.

Bhatty, R.S. (1995) Hull-less barley bran: A potential new product from an old grain. *Cereal Foods World,* **40**, 819-824.

Bhatty, R. S. (1999b). The potential of hull-less barley. *Cereal Chemistry,* 76**,** 589-599.

Bornet, F.R.J., Costagliola, D., Rizkalla, S.W., Blayo, A., Fontvieille, A.M., Haardt, M.J., Letanoux, M., Tchobroutsky, G. & Slama, G. (1987) Insulinemic and glycemic indexes of 6 starch-rich foods taken alone and in a mixed meal by type-2 diabetics. *American Journal of Clinical Nutrition,* **45**, 588-595.

Braaten, J.T., Scott, F.W., Wood, P.J., Riedel, K.D., Wolynetz, M.S., Brule, D. & Collins, M. W. (1994) High beta-glucan oat bran and oat gum reduce postprandial blood-glucose and insulin in subjects with and without type-2 diabetes. *Diabetic Medicine,* **11,** 312-318.

Brennan, C.S. & Cleary, L.J. (2005a) The potential use of cereal (1 -> 3,1 -> 4)-beta-D-glucans as functional food ingredients. *Journal of Cereal Science,* **42,** 1-13.

Brennan, C.S. & Cleary, L.J. (2005b) The potential use of cereal (1→ 3, 1→ 4)-β-D-glucans as functional food ingredients. *Journal of Cereal Science,* **42,** 1-13.

Brennan, C.S., Tudorica, C.M. & Kuri, V. (2002) Soluble and insoluble dietary fibres (non-starch polysaccharides) and their effects on food structure and nutrition. *Food Industry Journal,* **5,** 261-272.

Brennan, C.S. (2005) Dietary fibre, glycaemic response, and diabetes. *Molecular Nutrition and Food Research,* **49,** 560-570.

Burkus, Z. (1996). *Barley beta-glucan: Extraction, functional properties and interactions with food components.* PhD Dissertation/Thesis. University of Alberta: Canada.

Burkus, Z. & Temelli, F. (1998) Effect of extraction conditions on yield, composition, and viscosity stability of barley beta-glucan gum. *Cereal Chemistry,* **75,** 805-809.

Burkus, Z. & Temelli, F. (2000) Stabilization of emulsions and foams using barley beta-glucan. *Food Research International,* **33,** 27-33.

Charalampopoulos, D., Wang, R., Pandiella, S.S. & Webb, C. (2002) Application of cereals and cereal components in functional foods: a review. *International Journal of Food Microbiology,* **79,** 131-141.

Chemat, F., Zill E.H. & Khan, M.K. (2011) Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry,* **18,** 813-835.

Chemat, S., Aissa, A., Boumechhour, A., Arous, O. & Ait-Amar, H. (2017) Extraction mechanism of ultrasound assisted extraction and its effect on higher yielding and purity of artemisinin crystals from *Artemisia annua* L. leaves. *Ultrasonics Sonochemistry,* **34,** 310-316.

Chen, J. & Huang, X.-F. (2009) The effects of diets enriched in beta-glucans on blood lipoprotein concentrations. *Journal of Clinical Lipidology,* **3,** 154-158.

Collar, C. & Angioloni, A. (2014) Nutritional and functional performance of high β-glucan barley flours in breadmaking: mixed breads versus wheat breads. *European Food Research and Technology,* **238,** 459-469.

Colleoni-Sirghie, M., Kovalenko, I.V., Briggs, J.L., Fulton, B. & White, P.J. (2003) Rheological and molecular properties of water soluble (1, 3)(1, 4)-β-D-glucans from high-β-glucan and traditional oat lines. *Carbohydrate Polymers,* **52,** 439-447.

Conry, M.J. (1994) Comparative effect of 6 cultivars at 4 rates of nitrogen on the grain-yield and grain quality of spring-sown malting barley in Ireland. *Journal of Agricultural Science,* **122,** 343-350.

Crittenden, R., Karppinen, S., Ojanen, S., Tenkanen, M., Fagerstrom, R., Matto, J., Saarela, M., Mattila-Sandholm, T. & Poutanen, K. (2002) In vitro fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. *Journal of the Science of Food and Agriculture,* **82,** 781-789.

Daou, C. & Zhang, H. (2012) Oat beta-glucan: its role in health promotion and prevention of diseases. *Comprehensive Reviews in Food Science and Food Safety,* **11,** 355-365.

Dawkins, N.L. & Nnanna, I.A. (1993) Oat gum and beta-glucan extraction from oat bran and rolled oats - temperature and pH effects. *Journal of Food Science,* **58,** 562-566.

De Schrijver, R., Fremaut, D. & Verheyen, A. (1992) Cholesterol-lowering effects and utilization of protein lipid fiber and energy in rats fed unprocessed and baked oat bran. *Journal of Nutrition,* **122,** 1318-1324.

Delaney, B., Carlson, T., Frazer, S., Zheng, T., Hess, R., Ostergren, K., Kierzek, K., Haworth, J., Knutson, N., Junker, K. & Jonker, D. (2003) Evaluation of the toxicity of concentrated barley beta-glucan in a 28-day feeding study in Wistar rats. *Food and Chemical Toxicology,* **41,** 477-487.

Devries, J.W. (2003) On defining dietary fibre. *Proceedings of the Nutrition Society,* **62,** 37-43.

Du, B., Bian, Z. & Xu, B. (2014a) Skin health promotion effects of natural beta-glucan derived from cereals and microorganisms: A review. *Phytotherapy Research,* 28**,** 159-166.

Du, B., Zhu, F. & Xu, B. (2014b) Beta-Glucan extraction from bran of hull-less barley by accelerated solvent extraction combined with response surface methodology. *Journal of Cereal Science,* 59**,** 95-100.

Du, B., Lin, C., Bian, Z. & Xu, B. (2015) An insight into anti-inflammatory effects of fungal beta-glucans. *Trends in Food Science and Technology,* **41,** 49-59.

Edwards, C.A., Blackburn, N.A., Craigen, L., Davison, P., Tomlin, J., Sugden, K., Johnson, I.T. & Read, N.W. (1987) Viscosity of food gums determined invitro related to their hypoglycemic actions. *American Journal of Clinical Nutrition,* **46,** 72-77.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. & Attia, H. (2011) Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry,* **124,** 411-421.

Evers, T. & Millar, S. (2002) Cereal grain structure and development: Some implications for quality. *Journal of Cereal Science,* **36,** 261-284.

Fadel, J.G., Newman, R.K., Newman, C.W. & Graham, H. (1989) Effects of baking hulless barley on the digestibility of dietary-components as measured at the ileum and in the feces in pigs. *Journal of Nutrition,* **119,** 722-726.

Feldheim, W. & Wisker, E. (2000) Studies on the improvement of dietary fibre intake. *Deutsche Lebensmittel-Rundschau,* **96,** 327-330.

Forrest, I. & Wainwright, T. (1977) The mode of binding of β-glucans and pentosans in barley endosperm cell walls. *Journal of the Institute of Brewing,* **83,** 279-286.

Friedman, M. & Atsmon, D. (1988) Comparison of grain composition and nutritional quality in wild barley (*Hordeum spontaneum*) and in a standard cultivar. *Journal of Agricultural and Food Chemistry,* **36,** 1167-1172.

Fu, C., Tian, H., Li, Q., Cai, T. & Du, W. (2006) Ultrasound-assisted extraction of xyloglucan from apple pomace. *Ultrasonics Sonochemistry,* **13,** 511-516.

Fuentes-Zaragoza, E., Riquelme-Navarrete, M.J., Sanchez-Zapata, E. & Perez-Alvarez, J.A. (2010) Resistant starch as functional ingredient: A review. *Food Research International,* 43**,** 931-942.

Gamel, T.H., Abdel-Aal, E.-S. M., Ames, N.P., Duss, R. & Tosh, S.M. (2014) Enzymatic extraction of beta-glucan from oat bran cereals and oat crackers and optimization of viscosity measurement. *Journal of Cereal Science,* **59,** 33-40.

Hames, B., Ruiz, R., Scarlata, C., Sluiter, A., Sluiter, J. & Templeton, D. (2008) Preparation of samples for compositional analysis. *Laboratory Analytical Procedure (LAP),* 1617.

Havrlentova, M., Petrulakova, Z., Burgarova, A., Gago, F., Hlinkova, A. & Sturdik, E. (2011) Cereal beta-glucans and their Significance for the Preparation of Functional Foods - A Review. *Czech Journal of Food Sciences,* **29,** 1-14.

He, J.-L., Guo, H., Wei, S.-Y., Zhou, J., Xiang, P.-Y., Liu, L., Zhao, L., Qin, W., Gan, R.-Y. & Wu, D.-T. (2020) Effects of different extraction methods on the structural properties and bioactivities of polysaccharides extracted from Qingke (Tibetan hulless barley). *Journal of Cereal Science,* 92.

Helm, C.V., De Francisco, A., Gaziola, S.A., Fornazier, R.F., Pompeu, G.B. & Azevedo, R.A. (2004) Hull-less barley varieties: Storage proteins and amino acid distribution in relation to nutritional quality. *Food Biotechnology,* **18,** 327-341.

Hematian Sourki, A., Koocheki, A. & Elahi, M. (2017) Ultrasound-assisted extraction of beta-d-glucan from hull-less barley: Assessment of physicochemical and functional properties. *International Journal of Biological Macromolecules,* **95,** 462-475.

Hoije, A., Grondahl, M., Tommeraas, K. & Gatenholm, P. (2005) Isolation and characterization of physicochemical and material properties of arabinoxylans from barley husks. *Carbohydrate Polymers,* **61,** 266-275.

Hromadkova, Z., Ebringerova, A. & Valachovic, P. (1999a) Comparison of classical and ultrasound-assisted extraction of polysaccharides from Salvia officinalis L. *Ultrasonics Sonochemistry,* **5,** 163-168.

Hromadkova, Z., Kovacikova, J. & Ebringerova, A. (1999b) Study of the classical and ultrasound-assisted extraction of the corn cob xylan. *Industrial Crops and Products,* **9,** 101-109.

Huang, W., Xue, A., Niu, H., Jia, Z. & Wang, J. (2009) Optimised ultrasonic-assisted extraction of flavonoids from *Folium eucommiae* and evaluation of antioxidant activity in multi-test systems in vitro. *Food Chemistry,* **114,** 1147-1154.

Idehen, E., Tang, Y. & Sang, S. (2017a) Bioactive phytochemicals in barley. *Journal of Food and Drug Analysis,* **25,** 148-161.

Idehen, E., Tang, Y. & Sang, S.M. (2017b) Bioactive phytochemicals in barley. *Journal of Food and Drug Analysis,* **25,** 148-161.

Irakli, M., Biliaderis, C.G., Izydorczyk, M.S. & Papadoyannis, I.N. (2004) Isolation, structural features and rheological properties of water-extractable β-glucans from different Greek barley cultivars. *Journal of the Science of Food and Agriculture,* **84,** 1170-1178.

Izydorczyk, M.S. & Biliaderis, C.G. (2000) Structural and functional aspects of cereal arabinoxylans and beta-glucans. *Novel Macromolecules in Food Systems,* **41,** 361-384.

Jadhav, S. J., Lutz, S.E., Ghorpade, V.M. & Salunkhe, D.K. (1998) Barley: Chemistry and value-added processing. *Critical Reviews in Food Science and Nutrition,* **38,** 123-171.

Karppinen, S., Liukkonen, K., Aura, A.M., Forssell, P. & Poutanen, K. (2000) In vitro fermentation of polysaccharides of rye, wheat and oat brans and inulin by human faecal bacteria. *Journal of the Science of Food and Agriculture,* **80,** 1469-1476.

Kerckhoffs, D., Brouns, F., Hornstra, G. & Mensink, R.P. (2002) Effects on the human serum lipoprotein profile of beta-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. *Journal of Nutrition,* **132,** 2494-2505.

Klamczynski, A.P., Knuckles, B.E. & Glenn, G.M. (2004) Puffing and jet cooking affect solubility and molecular weight of barley beta-glucans. *Journal of Food Processing and Preservation,* **28,** 433-441.

Koehler, P. & Wieser, H. (2013) Chemistry of Cereal Grains. In: Gobbetti, M. and Gänzle, M. (eds.) *Handbook on Sourdough Biotechnology.* Boston, MA: Springer US.

Kruger, N.J. (2009) The Bradford method for protein quantitation. In: Walker, J.M. (ed.) 3rd ed. *The Protein Protocols Handbook.* Berlin: Springer.

Kurek, M.A., Karp, S., Stelmasiak, A., Pieczykolan, E., Juszczyk, K. & Rieder, A. (2018) Effect of natural flocculants on purity and properties of beta-glucan extracted from barley and oat. *Carbohydrate Polymers,* **188,** 60-67.

Lavoie, J.M. & Stevanovic, T. (2007) Selective ultrasound-assisted extractions of lipophilic constituents from *Betula alleghaniensis* and *B. papyrifera* wood at low temperatures. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques,* **18,** 291-299.

Lazaridou, A. & Biliaderis, C.G. (2004) Cryogelation of cereal beta-glucans: structure and molecular size effects. *Food Hydrocolloids,* **18,** 933-947.

Lazaridou, A. & Biliaderis, C.G. (2007) Molecular aspects of cereal beta-glucan functionality: Physical properties, technological applications and physiological effects. *Journal of Cereal Science,* **46,** 101-118.

Lazaridou, A., Biliaderis, C., Micha-Screttas, M. & Steele, B.R. (2004) A comparative study on structure–function relations of mixed-linkage (1→ 3),(1→ 4) linear β-d-glucans. *Food Hydrocolloids,* **18,** 837-855.

Lee, C.J., Horsley, R., Manthey, F. & Schwarz, P. (1997) Comparisons of β-glucan content of barley and oat. *Cereal Chemistry,* **74,** 571-575.

Li, J.-W., Ding, S.-D. & Ding, X.-L. (2007) Optimization of the ultrasonically assisted extraction of polysaccharides from *Zizyphus jujuba* cv. jinsixiaozao. *Journal of Food Engineering,* **80,** 176-183.

Limberger-Bayer, V.M., De Francisco, A., Chan, A., Oro, T., Ogliari, P.J. & Barreto, P.L.M. (2014) Barley beta-glucans extraction and partial characterization. *Food Chemistry,* **154,** 84-89.

MacGregor, A.W. & Fincher, G.B. (1993) *Carbohydrates of the barley grain*. In: MacGregor, A.W. & Bhatty, R.S. (eds.) Barley Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Madacsi, J.P., Parrish, F.W. & Roberts, E.J. 1983 Nonenzymic method for determination of beta-glucan in the presence of starch. *Journal of the American Society of Brewing Chemists,* **41,** 161-162.

Madhujith, T. & Shahidi, F. (2007) Antioxidative and antiproliferative properties of selected barley (Hordeum vulgarae l.) cultivars and their potential for inhibition of low-density lipoprotein (LDL) cholesterol oxidation. *Journal of Agricultural and Food Chemistry,* **55,** 5018-5024.

Maheshwari, G., Sowrirajan, S. & Joseph, B. (2017) Extraction and Isolation of β-Glucan from Grain Sources—A Review. *Journal of Food Science,* **82,** 1535-1545.

Malkki, Y. (2004) Trends in dietary fibre research and development - A review. *Acta Alimentaria,* **33,** 39-62.
Maran, J.P. & Priya, B. (2014) Ultrasound-assisted extraction of polysaccharide from *Nephelium lappaceum* L. fruit peel. *International Journal of Biological Macromolecules,* **70,** 530-536.
Marlett, J.A., Hosig, K.B., Vollendorf, N.W., Shinnick, F.L., Haack, V.S. & Story, J.A. (1994) Mechanism Of Serum-Cholesterol Reduction By Oat Bran. *Hepatology,* **20,** 1450-1457.
Mccleary, B.V. (1988) Purification of (1- 3),(1- 4)-beta-D-glucan from barley flour. *Methods in Enzymology,* **160,** 511-514.
Mikkelsen, M.S., Meier, S., Jensen, M.G., Qin, F., Stoica, I.-M., Martens, H.J., Blennow, A. & Jespersen, B.M. (2017) Barley genotypic beta-glucan variation combined with enzymatic modifications direct its potential as a natural ingredient in a high fiber extract. *Journal of Cereal Science,* **75,** 45-53.
Munck, L., Moller, B., Jacobsen, S. & Sondergaard, I. (2004) Near infrared spectra indicate specific mutant endosperm genes and reveal a new mechanism for substituting starch with (1 -> 3,1 -> 4)-beta-glucan in barley. *Journal of Cereal Science,* **40,** 213-222.
Newman, C.W. & Newman, R.K. (2006) A brief history of barley foods. *Cereal Foods World,* **51,** 4-7.
Newman, R. K. & Newman, C.W. (1991) Barley as a food grain. *Cereal Foods World,* **36,** 800-805.
Newman, R. K. & Newman, C.W. (2008) *Barley for food and health. Science, technology, and products*. New Jersey: Wiley.
Newman, R., Klopfenstein, C., Newman, C., Guritno, N. & Hofer, P. (1992) Comparison of the cholesterol-lowering properties of whole barley, oat bran, and wheat red dog in chicks and rats. *Cereal Chemistry,* **69,** 240-244.
Nishimune, T., Yakushiji, T., Sumimoto, T., Taguchi, S., Konishi, Y., Nakahara, S., Ichikawa, T. & Kunita, N. (1991) Glycemic response and fiber content of some foods. *American Journal of Clinical Nutrition,* **54,** 414-419.
Owen, B.D., Sosulski, F., Wu, K.K. & Farmer, M.J. (1977) Variation in mineral-content of saskatchewan feed grains. *Canadian Journal of Animal Science,* **57,** 679-687.
Paras, S. & Kotari, S.I. (2017) Barley: impact of processing on physicochemical and thermal properties-a review. *Food Reviews International,* **33,** 359-381.
Pham Van, H. (2016) Phenolic compounds of cereals and their antioxidant capacity. *Critical Reviews in Food Science and Nutrition,* **56,** 25-35.
Pico, Y. (2013) Ultrasound-assisted extraction for food and environmental samples. *Trac-Trends in Analytical Chemistry,* **43,** 84-99.
Price, P.B. & Parsons, J. (1979) Distribution of lipids in embryonic axis, bran-endosperm, and hull fractions of hulless barley and hulless oat grain. *Journal of Agricultural and Food Chemistry,* **27,** 813-815.
Puri, M., Sharma, D. & Barrow, C.J. (2012) Enzyme-assisted extraction of bioactives from plants. *Trends in Biotechnology,* **30,** 37-44.
Regand, A., Chowdhury, Z., Tosh, S.M., Wolever, T.M.S. & Wood, P. (2011) The molecular weight, solubility and viscosity of oat beta-glucan affect human glycemic response by modifying starch digestibility. *Food Chemistry,* **129,** 297-304.
Rieder, A., Knutsen, S.H. & Ballance, S. (2017) In vitro digestion of beta-glucan rich cereal products results in extracts with physicochemical and rheological behavior like pure beta-

glucan solutions–A basis for increased understanding of in vivo effects. *Food Hydrocolloids,* **67,** 74-84.
Saulnier, L., Gévaudan, S. & Thibault, J.-F. (1994) Extraction and partial characterisation of β-glucan from the endosperms of two barley cultivars. *Journal of Cereal Science,* 19**,** 171-178.
Sen, C.K., Khanna, S. & Roy, S. (2007) Tocotrienols in health and disease: The other half of the natural vitamin E family. *Molecular Aspects of Medicine,* **28,** 692-728.
Sharma, P. & Kotari, S.L. (2017) Barley: Impact of processing on physicochemical and thermal properties-A review. *Food Reviews International,* **33,** 359-381.
Skendi, A., Biliaderis, C.G., Lazaridou, A. & Izydorczyk, M.S. (2003b) Structure and rheological properties of water soluble beta-glucans from oat cultivars of *Avena sativa* and *Avena bysantina*. *Journal of Cereal Science,* **38**, 15-31.
Sourki, A. H., Koocheki, A. & Elahi, M. (2017) Ultrasound-assisted extraction of beta-D-glucan from hull-less barley: Assessment of physicochemical and functional properties. *International Journal of Biological Macromolecules,* **95**, 462-475.
Sullivan, P., O'Flaherty, J., Brunton, N., Gee, V.L., Arendt, E. & Gallagher, E. (2010) Chemical composition and microstructure of milled barley fractions. *European Food Research and Technology*, **230**, 579-595.
Sun, J.X., Sun, X.F., Zhao, H. & Sun, R.C. (2004) Isolation and characterization of cellulose from sugarcane bagasse. *Polymer Degradation and Stability,* **84,** 331-339.
Sundberg, B., Wood, P., Lia, A., Andersson, H., Sandberg, A.S., Hallmans, G. & Aman, P. (1996) Mixed-linked beta-glucan from breads of different cereals is partly degraded in the human ileostomy model. *American Journal of Clinical Nutrition,* 64**,** 878-885.
Symons, L. & Brennan, C. (2004) The effect of barley β-glucan fiber fractions on starch gelatinization and pasting characteristics. *Journal of Food Science,* **69,** FCT257-FCT261.
Tao, Y. & Sun, D.-W. (2015) Enhancement of food processes by ultrasound: a review. *Critical Reviews in Food Science and Nutrition,* 55**,** 570-594.
Temelli, F. (1997) Extraction and functional properties of barley beta-glucan as affected by temperature and pH. *Journal of Food Science,* **62,** 1194-1201.
Temelli, F., Bansema, C. & Stobbe, K. (2004) Development of an orange-flavored barley beta-glucan beverage. *Cereal Chemistry*, **81,** 499-503.
Thorburn, A., Muir, J. & Proietto, J. (1993) Carbohydrate fermentation decreases hepatic glucose output in healthy-subjects. *Metabolism-Clinical and Experimental,* **42,** 780-785.
Tian, Y., Zeng, H., Xu, Z., Zheng, B., Lin, Y., Gan, C. & Lo, Y.M. (2012) Ultrasonic-assisted extraction and antioxidant activity of polysaccharides recovered from white button mushroom (*Agaricus bisporus*). *Carbohydrate Polymers,* **88,** 522-529.
Upadhyay, J. & Misra, K. (2009) Towards the interaction mechanism of tocopherols and tocotrienols (vitamin E) with selected metabolizing enzymes. *Bioinformation,* **3,** 326-31.
Vaikousi, H., Biliaderis, C. & Izydorczyk, M. (2004) Solution flow behavior and gelling properties of water-soluble barley (1→ 3, 1→ 4)-β-glucans varying in molecular size. *Journal of Cereal Science,* **39,** 119-137.
Vasanthan, T. & Temelli, F. (2008) Grain fractionation technologies for cereal beta-glucan concentration. *Food Research International,* **41,** 876-881.
Vis, R. B. & Lorenz, K. (1997) Beta-glucans: Importance in brewing and methods of analysis. *Food Science and Technology-Lebensmittel-Wissenschaft and Technologie,* **30,** 331-336.

Vitaglione, P., Napolitano, A. & Fogliano, V. (2008) Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science and Technology,* **19,** 451-463.

Vriesmann, L.C., Amboni, R. & Petkowicz, C.L.D. (2011) Cacao pod husks (*Theobroma cacao* L.): Composition and hot-water-soluble pectins. *Industrial Crops and Products,* **34,** 1173-1181.

Wang, J., Sun, B., Cao, Y., Tian, Y. & Li, X. (2008) Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry,* **106,** 804-810.

Wang, L.J. & Weller, C.L. (2006) Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science and Technology,* **17,** 300-312.

Wang, W. & Liu, J. (2020) Efficient extraction, antioxidant activities and anti-inflammation of polysaccharides from *Notopterygium franchetii* Boiss. *Carbohydrate Polymers*, 248.

Wei, L., Cui, S.W. & Kakuda, Y. (2006) Extraction, fractionation, structural and physical characterization of wheat beta-D-glucans. *Carbohydrate Polymers,* **63,** 408-416.

Wood, P. & Fulcher, R.G. (1978) Interaction of some dyes with cereal beta-glucans. *Cereal Chemistry*, **55**, 952-966.

Wood, P., Siddiqui, I. & Paton, D. (1978) Extraction of high-viscosity gums from oats. Cereal *Chemistry*, **55**, 1038-1049.

Wood, P.J., Weisz, J., Fedec, P. & Burrows, V.D. (1989) Large-scale preparation and properties of oat fractions enriched in (1-3)(1-4)- beta-D-glucan. *Cereal Chemistry,* **66,** 97-103.

Wood, P.J. (1991) Physicochemical characterization of barley beta glucans. *Cereal Foods World,* **36,** 679-679.

Wood, P., Weisz, J. & Mahn, W. (1991) Molecular characterization of cereal β-glucans. II, Size-exclusion chromatography for comparison of molecular weight. *Cereal Chemistry,* **68,** 530-536.

Woodward, J.R., Phillips, D.R., Fincher, G.B. & Stone, B.A. (1981) Fine structure and physiochemical properties of water soluble barley 1-3 1-4 beta glucan. *Proceedings of the International Botanical Congress,* **13,** 45-45.

Xu, F., Sun, J.X., Geng, Z.C., Liu, C.F., Ren, J.L., Sun, R.C., Fowler, P. & Baird, M.S. (2007) Comparative study of water-soluble and alkali-soluble hemicelluloses from perennial ryegrass leaves (*Lolium peree*). *Carbohydrate Polymers*, **67,** 56-65.

Ying, Z., Han, X. & Li, J. (2011) Ultrasound-assisted extraction of polysaccharides from mulberry leaves. *Food Chemistry,* **127,** 1273-1279.

Zhang, D., Doehlert, D. & Moore, W. (1998) Rheological properties of (1→ 3),(1→ 4)-β-D-glucans from raw, roasted, and steamed oat groats. *Cereal Chemistry,* **75,** 433-438.

Zhang, D.-Y., Wan, Y., Xu, J.-Y., Wu, G.-H., Li, L. & Yao, X.-H. (2016). Ultrasound extraction of polysaccharides from mulberry leaves and their effect on enhancing antioxidant activity. *Carbohydrate Polymers,* **137,** 473-479.

Zhang, G., Chen, J. & Wang, J. (2002) Variation in barley endosperm beta-glucan content in three barley cultivars as a function of spike number and within-spike position. *Journal of Cereal Science,* **35,** 99-101.

Zhang, L. and Liu, Z. (2008) Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. *Ultrasonics Sonochemistry,* **15,** 731-737.

Zhao, Z. & Moghadasian, M.H. (2008) Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: A review. *Food Chemistry,* **109,** 691-702.

Zhen, L., Ying, D., Lai-Ting, Y. & La-Ni, S. (2015) Effect of whole barley flour addition on dough properties and quality of bread. *Modern Food Science and Technology***, 52**, 197-202.

Zhu, F., Du, B., Bian, Z. & Xu, B. (2015) Beta-glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. *Journal of Food Composition and Analysis,* **41,** 165-173.

Zielke, C., Lu, Y., Poinsot, R. & Nilsson, L. (2018) Interaction between cereal β-glucan and proteins in solution and at interfaces. *Colloids and Surfaces B Biointerfaces,* **162**, 256-264.

Chapter 5: **DEVELOPMENT OF CRACKERS ENRICHED IN BETA-GLUCAN CONTENT USING BARLEY FLOUR AND BETA-GLUCANS EXTRACTS**

Abstract

Whole grains are processed prior to consumption and added to other foods to improve their nutritional profile. Increasing consumer health awareness has led to the demand for the development of high-fibre products, which have been associated with delivering specific nutritional benefits. The objective of this study was to develop crackers with increased beta-glucan content using barley flour and beta-glucan extracts. Barley flour samples were produced from barley grain from the UK and Jordan, and six cracker formulations were prepared by replacing wheat flour with barley flour at increasing proportions (10, 20, 30, 40, 50 and 60%). Beta-glucan extracts obtained via ultrasonication were added to achieve the same beta-glucan content as in the crackers with 60% barley flour. The resulting products were evaluated for their beta-glucan content, dough hardness, water activity, moisture content, texture, and colour (L*, a* and b*). Crackers produced with various proportions of barley flour demonstrated, as predicted, higher beta-glucan content than wheat crackers (control), ranging from 0.377 g/100 g for 10% UK barley flour-based crackers to 1.542 g/100 g for 60% Jordanian barley flour-based crackers. Wheat crackers with beta-glucan extracts demonstrated the highest beta-glucan content of 2.436 g/100 g and 2.673 g/100 g using extracts from the UK and Jordanian barley grains, respectively. With an increasing proportion of barley flour, crackers were harder and less crispy, had a darker colour and greater redness values than control wheat crackers. However, when using the beta-glucan extracts in cracker formulations, the textural properties and water activity values were similar to the control

cracker, suggesting that the extracts could be successfully used as a functional ingredient in crackers.

Keywords: Barley, beta-glucans, crackers, whole grain, high-fibre products, texture, colour

5.1 Introduction

The health benefits of dietary fibre have led to the development of a large market for fibre-rich products and ingredients, and researchers have identified new dietary fibre sources in recent years (Chau and Huang, 2003). Fruits, vegetables and cereals provide some of these dietary sources, including hemicellulose, beta-glucans, pectin and gums. Barley (*Hordeum vulgare* L.) is an ancient cereal grain crop, historically consumed by humans as a source of nutrition; however, the rising popularity of other cereal crops, such as rice and wheat, has decreased barley utilisation (Baik and Ullrich, (2008)). Today, one-third of all barley produced is used in the malting and brewing industry, and approximately two-thirds are used as animal feed. Only 2% of total barley grain production worldwide is used in products intended for human consumption (Baik & Ullrich, 2008).

Among the variety of cereals available, barley has been studied extensively as a fibre source, especially because of its naturally high content in soluble dietary fibre, such as beta-glucans, which are cell wall components of cereal grains. They are present in greater proportions in barley (3–11%) and oats (3–7%) than wheat and rye (0.5–1% and 1–2%, respectively) (Skendi et al., 2003b). Health-promoting effects attributed to the consumption of beta-glucans include blood cholesterol reduction, blood sugar regulation, and weight management (Kinner *et al*., 2011). Reasons for adding dietary fibre to food products are not just nutritionally focused. Dietary fibre can alter a food's functional properties by increasing water and oil holding capacity, forming emulsions and foams, modifying texture and eating properties, and stabilising structure and extension of shelf-life (Yilmaz and Karaman, 2017). Substantial research has been devoted to understanding the functional and technological properties of barley flour and its related ingredients. Whole grain barley flour has been successfully incorporated into bread at varying

proportions: 15% and 20% of wheat flour replacement by barley flour (Collar and Angioloni, 2014); 20–26% of wheat flour replacement by barley flour (Al-Attabi et al., 2017, Blandino et al., 2015), and 40–100% barley flour (Mariotti et al., 2014, Kinner et al., 2011, Rieder et al., 2012, Trogh et al., 2004). Kinner *et al*. (2011) demonstrated that sourdough bread composed of 100% naked barley had acceptable sensory and technological properties. Mariotti *et al*. (2014) also developed bread composed of 100% hull-less barley flour.

The main challenge in enriching a bakery product with fibre is its undesirable effects on end-product quality parameters such as texture, colour and shelf life. As a result, such products generally have lower consumer acceptability (Ktenioudaki and Gallagher, 2012). Bakery products in which wheat flour is replaced with barley flour have a significantly decreased volume/height/spread ratio, rough texture (increased crumb hardness and loss of crispness), dull dark colour, altered density and surface properties, and bitter flavour (Ktenioudaki & Gallagher, 2012). The extent to which these attributes are observed in the final baked product depends on the barley variety, supplementation level, and processing parameters, such as milling conditions and extraction rate (Gill *et al*., 2002).

The snack market is ever-expanding, and consumers continue to investigate broader and increasingly nutritive snack options as these foods constitute a large portion of their daily diets (Bord Bia, 2014). Crackers currently represent a substantial share of the snack market and provide key opportunities for novel product development, particularly among functional foods. They are a popular snack food due to their varied flavours, long shelf life, and relatively low cost (Ahmed and Abozed, 2015). The term ‘cracker’ describes a baked product with a cereal base (e.g. wheat, oat, barley) and low moisture (1–5%) and a crispy texture (Katz and Labuza, 1981).

Some researchers have investigated the addition of fibre to crackers. Results suggested that crackers with pulse flour, new fibre sources (orange seed fibres) or rice bran were accepted and sometimes preferred by consumers; thus, fibre could be used to develop functional foods by enhancing the nutritional quality of wheat-based snacks (Millar et al., 2017, Ranok et al., 2021, Yilmaz and Karaman, 2017). Very few studies have focused on the use of barley flour as a dietary fibre source in crackers. Gangopadhyay et al. (2019) worked with barley flour and barley bran to enhance the health-salutary components of crackers: beta-glucan, phenolic content and in vitro antioxidant capacities. O'Shea et al. (2017) used three different barley fractions (bran, middlings and endosperm) to successfully develop a crispier cracker with increased total dietary fibre content. Research on the addition of barley grown under completely different environmental conditions in crackers is limited. Barley flour originating from different countries varies in its nutritional composition, and therefore in its technological functionalities. In this project, it is hypothesised that barley flour from Jordan with higher levels of protein, beta-glucan and fibre would yield crackers with different physicochemical properties than barley flour from the UK. Moreover, different inclusion levels of barley flour (10–60%) were assessed to evaluate the impact of barley flour addition on beta-glucan content and cracker physicochemical properties. Finally, beta-glucan extracts obtained through an ultrasound extraction method were added to crackers as a source of beta-glucans to compare them with raw barley flour incorporation. It was hypothesised that the addition of beta-glucans through the extract would yield crackers with quality parameters closer to those of the control cracker than the addition of barley flour as gluten will be less diluted.

5.2 Materials and Methods

5.2.1 Preliminary tests and formulation development

During the preliminary phase of this experiment, five cracker formulations were prepared to examine the effect of substituting wheat flour with barley flour on dough quality, processing parameters (baking time and temperature), and cracker properties. Control crackers composed of 100% wheat flour were prepared, along with other four cracker types with varying levels of wheat flour replacement by barley flour: 25%, 50%, 75%, and 100%. Cracker doughs composed of 75% and 100% barley flour were very difficult to mix and roll into a sheet, and the resulting baked products were harder and darker. Thus, six cracker formulations in which wheat flour was replaced by barley flour (10%, 20%, 30%, 40%, 50% and 60%) were chosen for the main experimental design of this chapter and subsequent processing and analyses.

Crackers with barley flour required more water for dough formation than the control cracker. Thus, for the preparation of the crackers with barley flours and beta-glucan extracts, 25% more water was added in the formulations to allow for hydration of the added fibre without competition with other flour components (starch and gluten).

5.2.2 Ingredients

The ingredients used in the preparation of the crackers were: soft wheat flour (fat 0.9 g/100g, carbohydrates 70.8 g/100 g, protein 9.73 g/100g; Asda, UK), vegetable oil (fat 91.7 g/100g; Asda, UK), dry yeast, salt, sodium carbonate, tap water. Barley flours from both the UK and Jordan were obtained by grinding whole barley grains in a multi-mill (Kenwood Chef, UK). The flour was then passed through two sieves with diameters of 20 and 22 mesh. The flours'

proximate composition was analysed following the procedure described in Chapter 3, section 3.2.2. Extracts from barley grown in the UK and Jordan were obtained following the ultrasound extraction method described in Chapter 4, section 4.2.2. The proximate composition of the final Jordanian (15 A, 35 min) and UK extracts (10 A, 20 min) was analysed. Jordanian barley extracts contained 53.32 g/100 g of starch, 11.08 g/100 g of beta glucans and 0.33 g /100 g of protein and the UK barley extracts contained 22.48 g/100 g of starch, 9.09 g/100 g of beta glucans and 0.24 g /100 g of protein were also calculated following the procedures described in Chapter 4.

5.2.3 Cracker preparation

Six UK/Jordanian barley flour-based crackers were prepared according to Table 5.1. A control formulation with 100% wheat flour (control) and six formulations in which wheat flour was replaced with barley flour at 10% (UK/J10), 20% (UK/J20), 30% (UK/J30), 40% (UK/J40), 50% (UK/J50) and 60% (UK/J60). Two more crackers with the beta-glucan extract from barley grown in the UK (UKE) or Jordan (JE) were prepared according to Table 5.2. The quantities of extracts to be added were calculated to match the beta-glucan content in the highest barley flour-based cracker: 0.094 g of beta-glucans per cracker in UK60 and 0.113 g of beta-glucans per cracker in J60.

For the preparation of cracker doughs, all dry ingredients were mixed with a Kenwood attachment at high speed for 30 s (Kenwood Chef, model A901, UK). The liquid ingredients (water and oil) were then added and mixed for an additional 10 min at high speed to form a homogenous dough. The dough was allowed to rest for 10–15 min at 19 °C. The dough was then rolled into a sheet using a Rondo machine (Rondo LTD, Chessington, Surrey, UK) to achieve a 1.25 mm-thick dough. Rectangular crackers (40 × 10 × 1.25 mm) were cut. The dough pieces were packed in

sealable plastic bags and assessed in the hour following preparation in all cases. Three replicates of the same flour-based cracker dough formulation were prepared on different days. Three replicates of the beta-glucan extract-based cracker dough formulation were prepared.

The same procedure was followed for cracker preparation, but once the dough pieces were cut, they were immediately transferred to a baking tray with greaseproof baking paper. A fork was used to create holes across the entire cracker surface to ensure homogenous baking and avoid bubble formation. The crackers were baked in an air-forced convection oven (Kwik-co, Salva, Spain) at 250 °C for 4 min. Crackers were kept at room temperature for 30 min to cool. Crackers were packed in heat-sealed polypropylene bags (Protective Packaging Ltd., UK) and stored at 19 °C. The crackers were evaluated within the following 24 h in all cases. Three batches of each UK/Jordanian barley-flour based cracker formulation were prepared.

The same procedure was performed for crackers with beta-glucan extracts. The extract was dispersed in water and added to the mixed dry ingredients to distribute the extract in the dough evenly. Two batches of each beta-glucan extract-based cracker formulation were prepared.

Table 5.1: Cracker formulations with varying proportions (10, 20, 30, 40, 50, 60%) of UK or Jordanian (J) barley flour.

Dough Ingredients	Control	UK/J10	UK/J20	UK/J30	UK/J40	UK/J50	UK/J60
Wheat flour (g)	64.87	54.82	48.73	42.64	36.55	30.46	24.37
Barley flour (g)	0	6.09	12.18	18.27	24.37	30.46	36.55
Water (g)	25.95	30.46	30.46	30.46	30.46	30.46	30.46
Vegetable oil (g)	8.11	7.61	7.61	7.61	7.61	7.61	7.61
Salt (g)	0.91	0.86	0.86	0.86	0.86	0.86	0.86
Sodium bicarbonate (g)	0.16	0.15	0.15	0.15	0.15	0.15	0.15
Total (g)	100	100	100	100	100	100	100

Table 5.2: Crackers with beta-glucan extract (E) from barley obtained from the UK or Jordanian (J) barley

Dough Ingredients (UK)	Control	UKE	JE
Wheat flour (%)	64.87	50.27	49.75
Barley flour extract (g)	0.00	10.65	11.17
Water (g)	25.95	30.46	30.46
Vegetable oil (g)	8.11	7.61	7.61
Salt (g)	0.91	0.85	0.85
Sodium bicarbonate (g)	0.16	0.15	0.15
Total (g)	100.00	100.00	100.00

5.2.4 Determination of mixed-linkage beta-glucan content in the crackers

Total and water-extractable beta-glucan content in barley flour-based crackers and extract-based crackers was determined using a mixed-linkage beta-glucan enzymatic procedure for

cooked, toasted, or extruded cereal products (Megazyme, Ireland). The method is described in Chapter 3, section 3.2.3.

Five crackers from each batch were weighed, and the average cracker weight was calculated (7.1 g). The average cracker weight was multiplied by the β-D-glucan content (%, w/w) calculated per 100 grams of cracker to determine the beta-glucan content per cracker, as described in Chapter 3, section 3.2.3.

5.2.5 Dough texture

Dough penetration analyses were conducted using a TA.XTPlus Texture Analyser (Stable Micro Systems, UK) equipped with Texture Exponent software (version 2.0.7.0.). Dough pieces (40 × 10 × 1.25 mm) were compressed to 3.25 mm with a stainless-steel spherical probe (P/0.5S). The load cell used was 5 kg, the strain was 60%, and the test speed was 1 mm s^{-1}. Ten dough pieces from each batch were analysed.

5.2.6 Moisture content (%) and water activity (a_w)

Four crackers milled from each batch were used for moisture and water activity analyses. Samples (2 g) were placed in a moisture analyser (Mettler Toledo HE53, China) at 105 °C until a constant weight was achieved. The moisture content (%) was measured in triplicate for each cracker batch. Approximately 2 g of ground sample was placed in the water activity metre (Decagon AquaLab meter, Pullman, USA), and water activity measurements were performed in duplicate for each cracker batch.

5.2.7 Cracker textural properties

The hardness and crispiness of cracker samples were measured using a texture analyser described above. The cracker was placed on a heavy-duty platform (HDP/90) with a holed plate, and the penetration test was conducted using a 2 mm cylinder probe (P/2) that penetrated the sample to 3 mm. The following were the experimental mode conditions: force was measured by compression with a pre-test speed of 1.00 mm/s, a test speed of 0.5 mm/s, and a post-test speed of 10.00 mm/s. The distance was set at 3 mm (average cracker thickness). For the texture analysis, a 2 mm cylinder probe (SMS P/2) using a 5 kg load cell and a heavy-duty platform (HDP/90) with a holed plate were used. Two textural parameters were measured: hardness, calculated as the area under the curve (N·s), and crispiness, calculated as the number of peaks. Ten crackers per batch were assessed at two different points.

5.2.8 Colour measurements of cracker prototypes

Four crackers were milled (Kenwood glass multi-mill, UK) for 30 s, and the colour of the ground sample was evaluated by chromameter (CR-400, Minolta Co., Japan). The results were expressed according to the CIELAB system (illuminant C and 10° viewing angle). Colour was measured using an 8 mm diameter diaphragm inset with optical glass. The parameters measured were L* (L* = 0 [black], L* = 100 [white]), a* (+a* = red) and b* (+b* = yellow). Measurements were performed in triplicate for each cracker batch. The total colour difference (ΔE*) between the control sample and each cracker type was calculated as follows (Francis and Clydesdale, 1975):

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \qquad \textit{Equation 5.1}$$

The following values were used to determine whether the total colour difference was visually obvious: $\Delta E^* < 1$, for colour differences not obvious to the human eye, $1 < \Delta E^* < 3$, minor colour differences possibly detected by the human eye depending on the hue, and Chroma, $\Delta E^* > 3$, colour differences are obvious to the human eye (Bodart *et al*., 2008).

5.2.9 Statistical analyses

Two-way analysis of variance (ANOVA) was used to determine the effects of barley flour used (from the UK or Jordan) and the percentage of wheat flour replaced by barley flour (10%, 20%, 30%, 40%, 50% or 60%) on cracker beta-glucan content, dough texture, cracker water activity, moisture, texture and colour. One-way ANOVA was used to determine the effect of the addition of barley flour (60%) or beta-glucan extract on cracker beta-glucan content, dough texture, cracker water activity, moisture, texture and colour. Using Tukey's Honest Significant Difference test (HSD), multiple pairwise comparisons were performed to evaluate mean value differences. These analyses were performed using XLSTAT (2021.1.1; Addinsoft, France), and $p<0.05$ was considered statistically significant.

5.3 Results and Discussion

5.3.1 Determination of mixed-linkage beta-glucan content in the crackers

No significant interactions ($p>0.05$) were observed between the type of barley flour used and the proportion of wheat flour replacement by barley flour. The percentage of wheat flour replacement had a significant ($p<0.05$) effect on the beta-glucan content in the crackers, as shown

in Figure 5.1A. As wheat flour replacement increased, beta-glucan content in crackers increased significantly ($p<0.05$). The content of beta-glucans in crackers with various barley flour inclusion levels ranged from 0.377 (%, w/w) to 1.542 (%, w/w) for UK10 and J60 samples, respectively. Among all cracker types, control crackers with 100% wheat flour had the lowest beta-glucan content along with UK10 and UK20. UK60 crackers had 5.5 times more beta-glucan than the control. These results were expected, as beta-glucan content in barley flours was higher (2.65% w/w UK barley flour, 3.50% w/w Jordanian barley flour) than in wheat flour (1% w/w) (Chapter 3, section 3.4.1, Table 3.1). Pejcz *et al*. (2017) also showed that when replacing increasing amounts of wheat flour with wholemeal barley flour in bread formulations, the end products contained increased concentrations of beta-glucans. The inclusion of 20%, 30% and 40% barley flour caused a 10, 13 and 17-fold increase in beta-glucans, respectively (Pejcz *et al*., 2017). Collar and Angioloni (2014) observed a beta-glucan content of 1.5% in bread that consisted of 40% barley flour, which was 15 times higher than in bread with only wheat flour.

There was no significant difference ($p>0.05$) between formulations with different barley flours (UK or J) at the same proportion of wheat flour replacement (Figure 5.1B). Thus, the type of flour did not affect the beta-glucan content in crackers significantly.

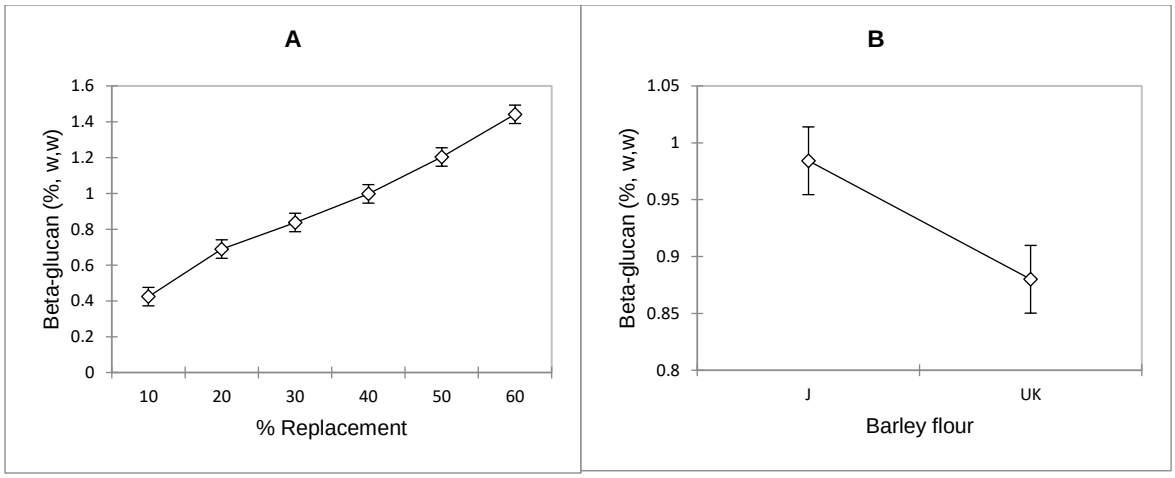

Figure 5.1. Mean plots with standard error as error bars. A: mean values of beta-glucan content in crackers according to the percentage of wheat flour replaced by barley flour. B: mean values of the beta-glucan content in crackers according to the type of barley flour used (from Jordan [J] or the UK).

Significant differences ($p<0.05$) in beta-glucan content were found between samples of 60% barley flour-based crackers and beta-glucan extract-based crackers (Tables 5.3 and 5.4). Although the amounts of beta-glucan in the doughs were very similar, the beta-glucan content in the extract-based crackers was significantly higher than in the flour-based crackers for both groups of samples (UK and J). These results could be due to the lower ($p<0.05$) moisture content of extract-based crackers than flour-based crackers (Table 5.1, section 5.2.1).

Table 5.3. Beta-glucan content in crackers: Control (100% wheat flour), UK60 (60% UK barley flour), UKE (wheat flour with 8.7% of beta-glucan extract from UK barley).

Sample	Beta-Glucans (%)
Control	$0.210^{c} \pm 0.016$
UK60	$1.338^{b} \pm 0.110$
UKE	$2.437^{a} \pm 0.219$

Table 5.4. Beta-glucan content in crackers: Control (100% wheat flour), J60 (60% Jordanian barley flour), JE (wheat flour with 9.2% of beta-glucan extract from Jordanian barley flour).

Sample	Beta-Glucans (%)
Control	$0.210^{c} \pm 0.016$
J60	$1.543^{b} \pm 0.047$
JE	$2.677^{a} \pm 0.084$

The beta-glucan content per serving (eight crackers) was calculated (Table 5.5). Suggested serving sizes of products already on the market are eight crackers (approximately 56 g of product) consumed as a snack between meals. Both the US FDA (2005) and the European Food Safety Authority (2009) require that for a food product to be called 'health promoting', it should provide at least 3 g/day of beta-glucans. The dosage of 3 g of beta-glucans per day is suggested to be fulfilled by four portions, each consisting of 0.75 g beta-glucans (Kinner *et al*., 2011). The results of the current study suggest that only UK60 and J60 Jordanian and UKE and JE crackers met the recommended minimum requirement of beta-glucan content per serving.

Table 5.5: Beta-glucan content in crackers per serving (8 crackers). Cracker formulations are Control (100% wheat flour), UK10 (10% UK barley flour), UK20 (20% UK barley flour), UK30 (30% UK barley flour), UK40 (40% UK barley flour), UK 50 (50% UK barley flour), UK 60 (60% UK barley flour), J10 (10% Jordanian barley flour), J20 (20% Jordanian barley flour), J30 (30% Jordanian barley flour), J40 (40% Jordanian barley flour), J50 (50% Jordanian barley flour), J60 (60% UK barley flour), UKE (wheat flour with 8.7% of beta-glucan extract from UK barley flour) and JE (wheat flour with 9.2% of beta-glucan extract from Jordanian barley flour).

Sample	Beta-glucans per serving (g)
C	0.132
UK10	0.211
UK20	0.366
UK30	0.424
UK40	0.530
UK50	0.679
UK60	0.750
J10	0.276
J20	0.429
J30	0.570
J40	0.579
J50	0.641
J60	0.903
UKE	1.218
JE	1.337

5.3.2 Dough Texture

Significant interactions ($p>0.05$) were observed between barley flour type and the proportion of wheat flour replacement. Among crackers with different levels of wheat flour

replacement by barley flour (UK or Jordanian), there were no significant differences, except for J30 that was the softest ($p<0.05$) dough. UK barley flour-based doughs showed significantly higher hardness values ($p<0.05$) than Jordanian barley flour-based doughs, as shown in Figure 5.3. These results could be explained by the higher dietary fibre (lignin, cellulose and hemicellulose) content of Jordanian barley flour Jordanian (chapter 3, section 3.4.1, Table 3.1). The UK barley grain contained 16.4% (w/w) non-starch carbohydrates, of which ~15% (w/w) were hemicelluloses (primarily arabinoxylans). In Jordanian barley, structural carbohydrates accounted for 29.4% (w/w), of which 12.8% (w/w) were hemicelluloses. Previous studies also showed that increased addition of fibre (such as cereal brans or tea fibre) in biscuit dough resulted in weaker doughs (Sudha *et al*., 2007, Soma *et al*., 2016).

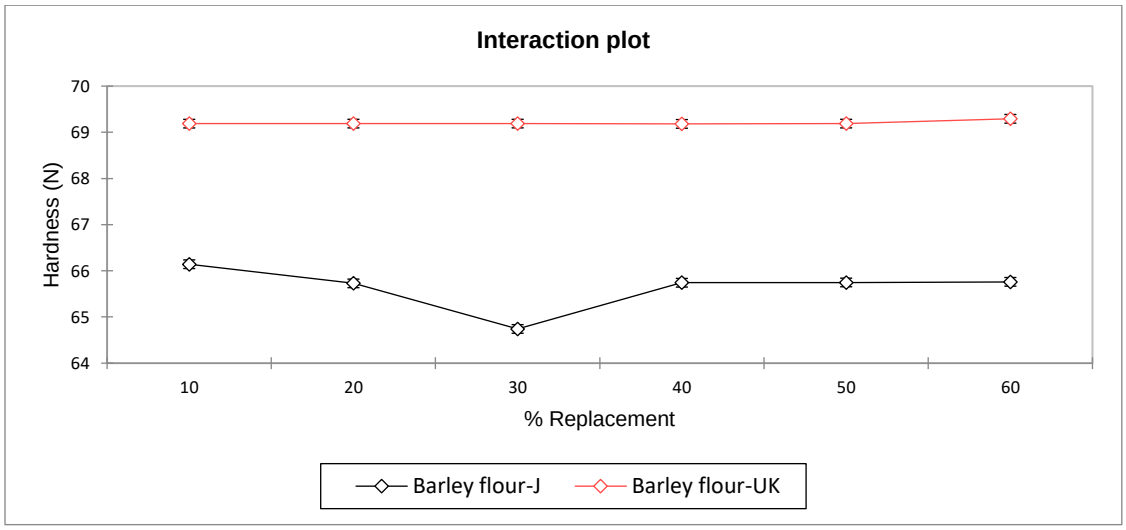

Figure 5.2: Interactions between the type of flour and the wheat flour replacement level for cracker dough hardness. Error bars represent standard errors.

Both sets of barley crackers (UK60, UKE and J60, JE) showed significantly higher dough hardness than control crackers (Tables 5.6 and 5.7). These results were unexpected, as doughs with barley flours and beta-glucan extracts had higher fibre content and lower gluten content than control doughs; thus, they were expected to be softer than the control dough. These doughs were crumblier and showed brittle behaviour during the preparation procedure.

Table 5.6: Dough hardness in crackers. Control (100% wheat flour), UK 60 (60% wheat flour replacement by UK barley flour), UKE (wheat flour with 8.7% beta-glucan extract from UK barley).

Sample	Dough Hardness (N)
Control	$65.596^{c} \pm 0.129$
UK60	$69.293^{a} \pm 0.100$
UKE	$68.370^{b} \pm 0.037$

Table 5.7: Dough hardness in crackers. Control (100% wheat flour), J60 (60% wheat flour replacement by Jordanian barley flour), JE (wheat flour with 9.2% beta-glucan extract from Jordanian barley flour).

Sample	Dough Hardness (N)
Control	$65.596^{c} \pm 0.129$
J60	$65.762^{b} \pm 0.067$
JE	$68.468^{a} \pm 0.058$

5.3.3 Moisture content and water activity (a_w)

No significant interactions ($p>0.05$) were observed in cracker moisture or water activity between the type of barley flour and the proportion of wheat flour replaced by barley flour. However, both factors individually had a significant effect on these parameters, as seen in (Figure 5.3). The moisture and water activity decreased significantly ($p<0.05$) when the barley flour proportion increased in crackers. These results could be explained by the differences in composition between wheat and barley flours. A higher amount of gluten plays a key role in water retention and the formation of cohesive and elastic structures in bakery products (Ranok et al., 2021, Yeboah-Awudzi et al., 2018), and fibre competes with gluten for hydration and does not retain moisture as tightly as gluten. Thus cracker with higher amounts of fibre showed lower moisture and water activity values than samples with higher amounts of gluten (control group crackers).

A similar trend was observed when comparing the control crackers with the 60% barley flour-based and extract-based crackers (Tables 5.8 and 5.9). Extract-based crackers had significantly smaller ($p<0.05$) moisture and water activity values than the other samples, as these samples had a higher amount of fibre than the others.

Typically, crackers contain less moisture than other baked products. During baking, the maximum amount of moisture evaporates, making crackers light and crunchy with low moisture content. Generally, excess moisture tends to degrade the finished product's quality. Thus, for both microbiological safety and sensory acceptability, dry snack products such as crackers require an a_w below 0.5 (Smith *et al*., 2004). The a_w ranged from 0.288 to 0.551, indicating a reduced possibility of microbial growth and a long product shelf life. These results indicate that crackers

with greater proportions of barley (40% and higher for the UK barley) might be more stable and have a longer shelf life than control crackers.

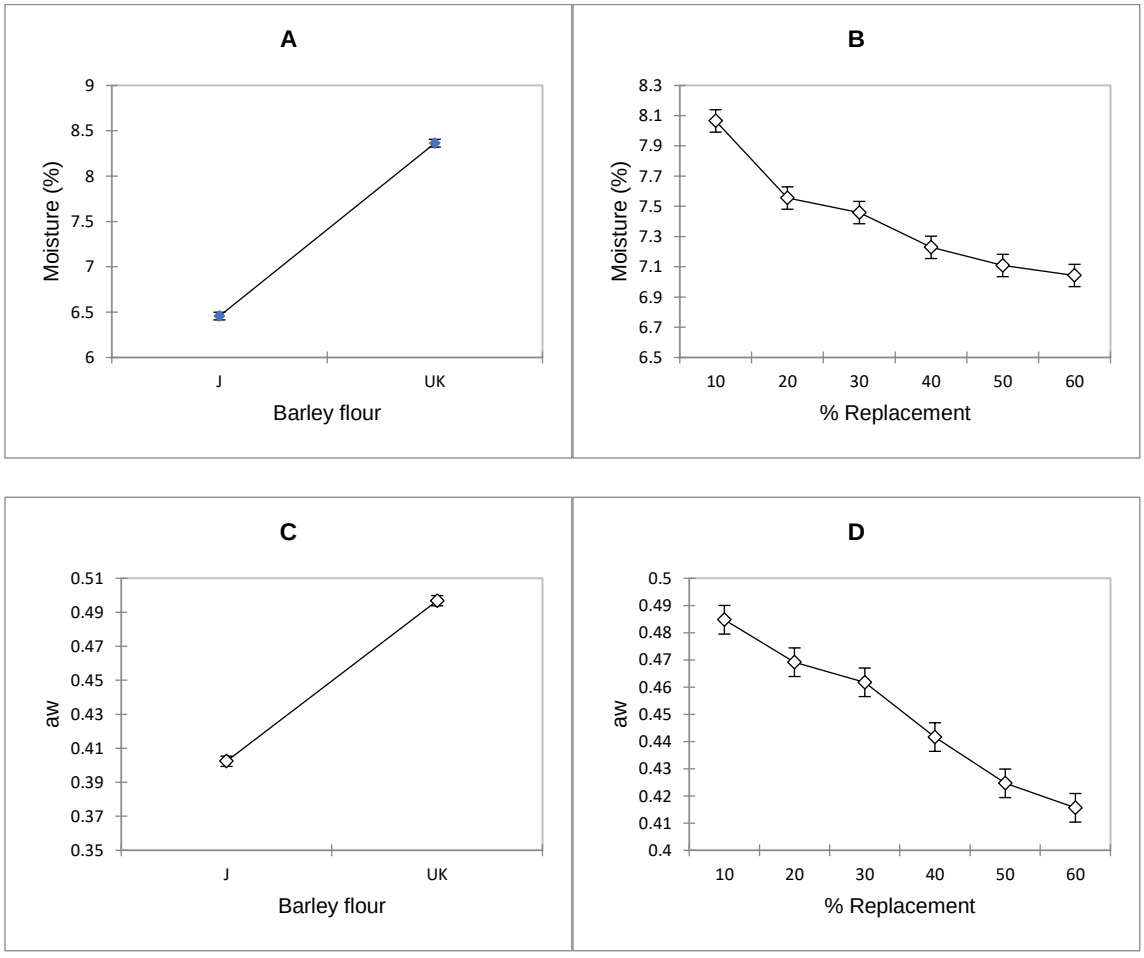

Figure 5.3. Mean plots with standard error represented by error bars. A: mean values of moisture content in crackers according to the type of barley flour used (from Jordan [J] or the UK); B: mean values of moisture content in crackers according to the percentage of wheat flour replaced by barley flour; C: mean values of water activity in crackers according to the type of barley flour used (from Jordan [J] or the UK); D: mean values of water activity in crackers according to the percentage of wheat flour replaced by barley flour.

Table 5.8: Moisture content (%) and water activity (aw) in the control sample (100% wheat flour), UK60 (60% UK barley flour), UKE (wheat flour with 8.7% beta-glucan extract from UK barley flour).

Sample	Moisture Content (%)	Water Activity (Aw)
Control	$7.153^{b} \pm 0.082$	$0.358^{b} \pm 0.040$
UK60	$8.020^{a} \pm 0.280$	$0.464^{a} \pm 0.004$
UKE	$5.190^{c} \pm 0.117$	$0.288^{c} \pm 0.004$

Table 5.9: Moisture content (%) and water activity (aw) in the control sample (100% wheat flour), J60 (60% Jordanian barley flour), JE (wheat flour with 9.2% beta-glucan extract from Jordanian barley flour).

Sample	Moisture Content (%)	Water Activity (Aw)
Control	$7.153^{a} \pm 0.082$	$0.358^{a} \pm 0.040$
J60	$6.065^{b} \pm 0.0135$	$0.367^{a} \pm 0.014$
JE	$5.788^{c} \pm 0.0124$	$0.318^{a} \pm 0.002$

5.3.4 Texture profile analysis of cracker prototypes and dough penetration

Crispness is associated with the rupture of air cells or cavities in food products (Vickers and Bourne, 1976). Crispness is associated with small fracture events, so the higher the number of peaks recorded when assessing the texture of a cracker, the crispier the product will be perceived. Crispness is a desirable property for crackers, which most probably obtained their name from the characteristic sound made when eaten. Hardness is measured as the force needed to penetrate the sample.

No significant interaction between factors was observed for these two textural properties. The type of barley flour used to replace wheat flour did not significantly affect the textural

characteristics of the crackers (Figure 5.4 A). However, the level of replacement of wheat flour by barley flour did have a significant effect ($p<0.05$) in both the number of peaks (Figure 5.4 B) and cracker hardness (Figure 5.5). As the proportion of barley flour increased, the number of fracturability points decreased and cracker hardness significantly increased ($p<0.05$). These results could be due to the decrease in gluten proteins and the increase in protein and fibre from barley flour in the reformulated crackers. The resulting protein network (gluten and non-gluten proteins) in crackers with wheat and barley flours was less developed than in crackers with higher proportions of wheat flour; the presence of fibre components also caused mechanical interference with the gluten network (Collar and Angioloni, 2014), yielding a cracker less able to hold air during baking, and thus fewer fracturability peaks (crispness). Soluble dietary fibre, such as beta-glucans, can act as a thickener, stabiliser, and texturiser in processed food products. However, the addition of dietary fibre to baked products generally causes undesirable alterations to their texture and consistency, leading to increased crumb hardness, as well as a loss of crispness (Ktenioudaki and Gallagher, 2012; Yilmaz and Karaman, 2017).

The lower expansion of the cracker and the higher proportion of fibre yielded a more compact and harder cracker (when higher levels of wheat flour were replaced by barley flour). The hard texture of barley bakery products is mainly attributed to gluten dilution, leaving less gluten available in the dough to bind water due to the competition for water between dietary fibre and flour components (Gill *et al*., 2002). Similarly, previous studies suggested a negative correlation between hardness and moisture and a positive correlation between crackers' hardness and total fibre and protein content (Millar *et al*., 2017). Multiple studies have examined texture attributes, such as hardness and fracturability, of baked products enriched with fibre. Yilmaz and Karaman, 2017 showed that crackers enriched with 2.9% dietary fibre extracted from wheat grain,

orange seeds and grapefruit had significantly greater hardness and fracturability values than control samples containing only wheat flour. Blandino *et al*. (2015) observed increased hardness with increasing barley flour substitution, with a significant increase observed for barley flour supplementation of 15–25%. Gill *et al*. (2002) showed that on day one of storage, breads supplemented with barley flour (5–15%) were firmer than a control bread sample and that, as substitution level increased, the firmness values continued to increase.

Crackers with 60% wheat flour replaced by barley flours (from the UK or Jordan) showed the lowest number of ($p<0.05$) fracturability peaks and the highest ($p<0.05$) hardness values, compared to control crackers and crackers made with beta-glucan extracts from both countries (Table 5.10 and 5.11, for the UK and Jordan, respectively).

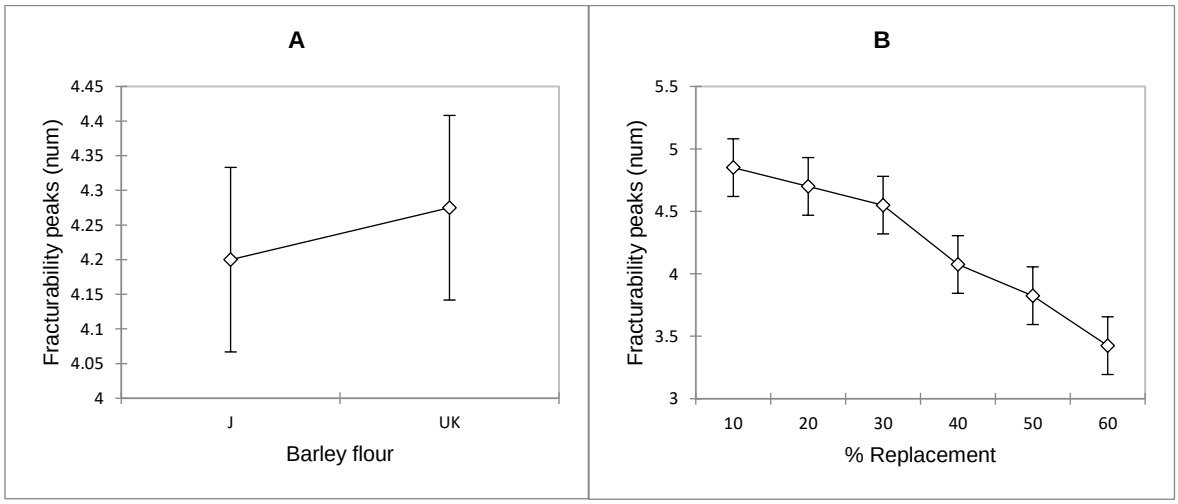

Figure 5.4. Mean plots with standard error represented by error bars. A: mean values of fracturability peaks in crackers according to the type of barley flour used (from Jordan [J] or the UK); B: mean values of fracturability peaks in crackers according to the percentage of wheat flour replaced by barley flour.

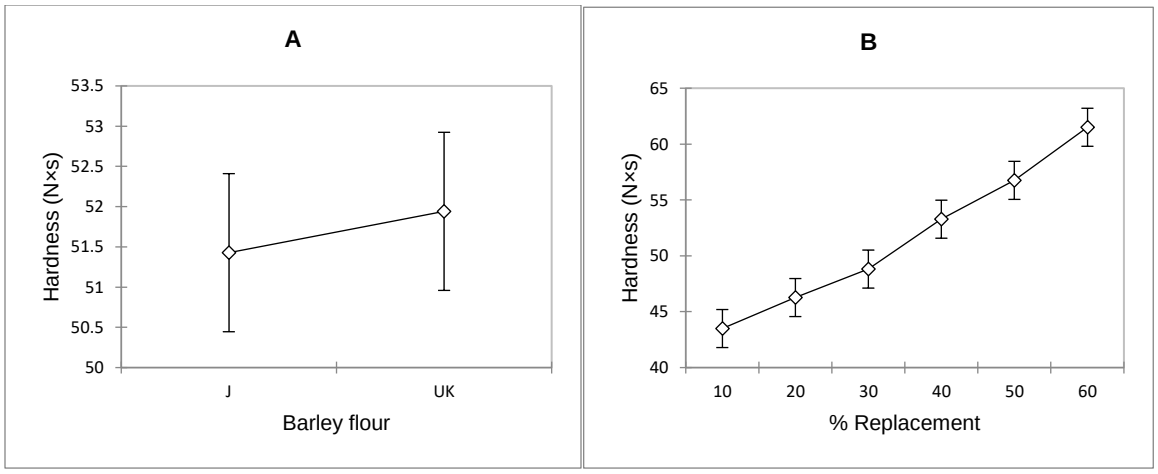

Figure 5.5: Mean plots with standard error represented by error bars. A: mean values cracker hardness according to the type of barley flour used (from Jordan [J] or the UK); B: mean values of cracker hardness according to the percentage of wheat flour replaced by barley flour.

Table 5.10: The number of fracturability peaks and the hardness (N-s) of the crackers in the control sample (100% wheat flour), UK60 (60% UK barley flour), UKE (wheat flour with 8.7% beta-glucan extract from UK barley flour).

	Number of Fracturability peaks	Hardness (N·s)
Sample		
Control	$4.750^{a} \pm 0.942$	$47.275^{b} \pm 7.748$
UK60	$3.450^{b} \pm 1.396$	$60.981^{a} \pm 9.344$
UKE	$3.680^{b} \pm 1.211$	$50.685^{b} \pm 7.505$

Table 5.11: The number of fracturability peaks and the hardness of the crackers in the control sample (100% wheat flour), J60 (60% Jordanian barley flour), JE (wheat flour with 9.2% beta-glucan extract from Jordanian barley flour).

Sample	Number of Fracturability peaks	Hardness (N·s)
Control	$4.750^{a} \pm 0.842$	$47.275^{b} \pm 7.748$
J60	$3.400^{b} \pm 1.594$	$62.025^{a} \pm 10.161$
JE	$4.200^{ab} \pm 0.678$	$51.413^{b} \pm 6.091$

5.3.5 Colour measurements of cracker prototypes

There was no significant interaction between factors for L* and a* values. However, the level of replacement of wheat flour by barley flour had a significant effect on the cracker colour parameters L* and a* (Figures 5.6). There was a significant interaction between factors for b* values (Figure 5.6). Brightness (L*) decreased significantly ($p<0.05$) as the wheat flour replacement level by barley flour increased (Figure 5.6 B). The darker colour of crackers containing a higher proportion of barley flour could be partly attributed to the darker colour of the raw material (barley flour) compared to wheat flour, resulting from the higher proportion of natural fibres and phenolic compounds in barley flour. Barley grains have higher amounts of phenolics (0.2–0.4%) than other cereal grains. A negative relationship between total polyphenolic levels and the brightness of grain products has been observed (Baik & Urlich, 2008). The enzymatic reaction responsible for the darker colour is polyphenol oxidation to o-quinones by polyphenol oxidase. Enzymatic reaction products react with amino acids or other phenolic compounds to discolour the flour. The development of barley grain genotypes lacking polyphenolics and demonstrating

minimum enzyme activity could minimise dark discolouration in the final product (Baik and Ullrich, (2008)). Moreover, the replacement of wheat flour by barley flours increased protein content in the final cracker, which could increase Maillard-browning reactions (García-Baños *et al*., 2004), resulting in higher concentrations of melanoidins in the final products.

Positive a* values (redness) significantly increased ($p<0.05$) as wheat flour replacement by barley flour increased. UK barley flour-based crackers showed significantly higher ($p<0.05$) a* values that Jordanian barley flour-based crackers. b* values (yellowish) were higher in UK barley flour-based crackers ($p<0.05$) than in Jordanian barley flour-based crackers, except when wheat flour was replaced at 50% and 60% when J50 and J60 crackers showed similar yellowness as UK50 and UK60 crackers.

Previous studies have reported a significant change in colour attributes of bakery products supplemented with barley flour. Pejcz *et al*. (2017) prepared breads with 10%, 30% and 40% barley flour substitution, and bread crumbs with barley flour had significantly lower L* values than the control crumb (wheat flour bread), while a* and b* values were lower.

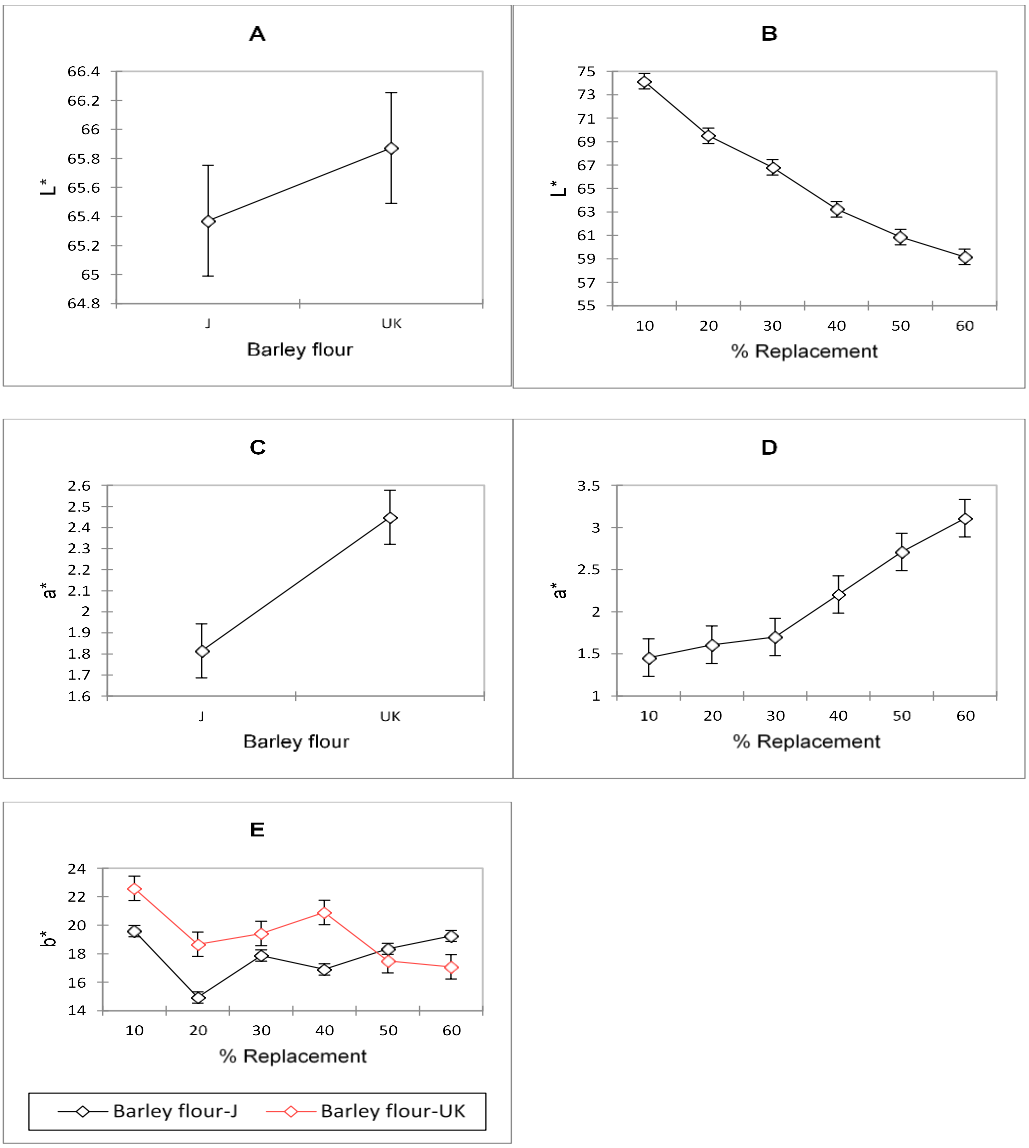

Figure 5.6: Mean and interaction plots with standard errors represented by error bars. A: mean values of lightness (L*) in crackers according to the type of barley flour used (from Jordan [J] or the UK); B: mean values of lightness (L*) in crackers according to the percentage of wheat flour replaced by barley flour; C: mean values of a* in crackers according to the type of barley flour used (from Jordan [J] or the UK); D: mean values of a* in crackers according to the percentage of wheat flour replaced by barley flour; E: Interactions between the type of barley flour and the percentage of wheat flour replacement for barley flour.

Control crackers made from wheat flour had the highest *L** (Table 5.13) value, demonstrating that the wheat flour was lighter than barley flour or flour with beta-glucan extracts. UKE crackers showed similar a* and b* values to control crackers. This colour similarity to wheat flour might be because the flour with beta-glucan extract was paler than barley flour, and the extract-based crackers contained mainly wheat flour. Both beta-glucan extract-based crackers (UKE and JE) demonstrated lower a* values (less redness) than the barley flour-based crackers (UK60 and J60) (Tables 5.12 and 5.13, respectively). For all flour-based and extract-based crackers, the ΔE* values were higher than 3, implying that their colour difference from the control was obvious to the human eye.

Table 5.12: Colour values of crackers. The control sample (100% wheat flour), J60 (60% Jordanian barley flour), JE (wheat flour with 9.2% beta-glucan extract from Jordanian barley flour).

Sample	L*	a*	b*	ΔE*
Control	$74.865^{a} \pm 0.065$	$0.860^{b} \pm 0.210$	$20.875^{a} \pm 0.385$	0
UK60	$57.750^{c} \pm 0.620$	$3.185^{a} \pm 0.145$	$17.080^{b} \pm 0.460$	17.68
UKE	$70.330^{b} \pm 0.310$	$1.285^{b} \pm 0.255$	$19.945^{a} \pm 0.025$	4.65

Table 5.13: Colour values of crackers. The control sample (100% wheat flour), J60 (60% Jordanian barley flour), JE (wheat flour with 9.2% beta-glucan extract from Jordanian barley flour)

Sample	L*	a*	b*	ΔE*
Control	$74.865^{a} \pm 0.065$	$0.860^{b} \pm 0.210$	$20.875^{a} \pm 0.385$	0
J60	$60.595^{c} \pm 0.275$	$3.035^{a} \pm 0.115$	$19.245^{ab} \pm 0.405$	14.53
JE	$69.820^{b} \pm 0.060$	$0.620^{b} \pm 0.010$	$18.505^{b} \pm 0.085$	5.58

5.4 Conclusions

The results of this study suggest that the proportion of flour replacement by beta-glucan flour is the main factor determining cracker properties (texture, water activity, colour). Moreover, the composition of the barley flour significantly affected some of the properties of the final crackers, such as dough hardness, cracker moisture and water activity.

Cracker formulations made from a mixture of wheat and barley flour had 2–6 times higher beta-glucan concentrations than wheat-based crackers. By increasing the replacement levels of wheat flour by barley flour, there was an increase of fibre from the barley flours and dilution of the gluten content in the final crackers. The main effects of these compositional changes were a decrease in moisture, increased hardness and reduced number of fracture peaks in the final crackers. The incorporation of barley flour yielded significantly darker and redder crackers; the total colour differences between control and crackers with barley flour was obvious to the human eye at all levels of substitution. Crackers with added beta-glucan extract demonstrated superior water activity and textural properties to crackers made with 60% substitution of wheat flour by barley flour. Moreover, crackers with beta-glucan extracts may be considered suitable as a high beta-glucan content food that could meet US FDA requirements.

Further studies evaluating the development, water absorption and cracker properties of dough with added beta-glucan extract could be conducted to optimise the properties of the final crackers. Future research should be conducted to define sensory attributes and evaluate consumer acceptance of the cracker formulations. The introduction of barley flour-supplemented crackers has the potential to allow consumers to increase their daily intake of the soluble fibre beta-glucan, which imparts many health benefits, such as reducing total and LDL cholesterol. The findings in

this work are expected to increase the consumption of crackers enriched with barley flour and beta-glucans.

5.5 References

Ahmed, Z.S. & Abozed, S.S. (2015) Functional and antioxidant properties of novel snack crackers incorporated with *Hibiscus sabdariffa* by-product. *Journal of advanced research,* **6**, 79-87.

Al-Attabi, Z.H., Merghani, T.M., Ali, A. & Rahman, M.S. (2017) Effect of barley flour addition on the physico-chemical properties of dough and structure of bread. *Journal of Cereal Science,* **75**, 61-68.

Baik, B.-K. & Ullrich, S.E. (2008) Barley for food: Characteristics, improvement, and renewed interest. *Journal of Cereal Science,* **48**, 233-242.

Blandino, M., Locatelli, M., Gazzola, A., Coïsson, J.D., Giacosa, S., Travaglia, F., Bordiga, M., reyneri, A., Rolle, L. & Arlorio, M. (2015) Hull-less barley pearling fractions: Nutritional properties and their effect on the functional and technological quality in bread-making. *Journal of Cereal Science,* **65**, 48-56.

Bodart, M., De Peñaranda, R., Deneyer, A. & Flamant, G. (2008) Photometry and colorimetry characterisation of materials in daylighting evaluation tools. *Building and Environment,* **43**, 2046-2058.

Chau, C.-F. & Huang, Y.-L. (2003) Comparison of the chemical composition and physicochemical properties of different fibers prepared from the peel of *Citrus sinensis* L. Cv. Liucheng. *Journal of Agricultural and food chemistry,* **51**, 2615-2618.

Collar, C. & Angioloni, A. (2014) Nutritional and functional performance of high β-glucan barley flours in breadmaking: mixed breads versus wheat breads. *European Food Research and Technology*, **238**, 459-469.

Francis, F.J. & Clydesdale, F.M. (1975) *Food colorimetry: theory and applications*. Westport: AVI Publishing Co. Inc.

Gangopadhyay, N., O'Shea, N., Brunton, N.P., Gallagher, E., Harrison, S. M. & Rai, D.K. (2019) Fate of beta-glucan, polyphenols and lipophilic compounds in baked crackers fortified with different barley-milled fractions. *LWT - Food Science and Technology*, **114**, 108413.

García-Baños, J.L., Villamiel, M., Olano, A.N. & Rada-Mendoza, M. (2004) Study on nonenzymatic browning in cookies, crackers and breakfast cereals by maltulose and furosine determination. *Journal of Cereal Science*, **39**, 167-173.

Gill, S., Vasanthan, T., Ooraikul, B. & Rossnagel, B. (2002) Wheat bread quality as influenced by the substitution of waxy and regular barley flours in their native and extruded forms. *Journal of Cereal Science*, **36**, 219-237.

Katz, E. & Labuza, T.P. (1981) Effect of water activity on the sensory crispness and mechanical deformation of snack food products. *Journal of Food Science*, **46**, 403-409.

Kinner, M., Nitschko, S., Sommeregger, J., Petrasch, A., Linsberger-Martin, G., Grausgruber, H., Berghofer, E. & Siebenhandl-Ehn, S. (2011) Naked barley—optimized recipe for pure barley bread with sufficient beta-glucan according to the EFSA health claims. *Journal of Cereal Science,* **53**, 225-230.

Ktenioudaki, A. & Gallagher, E. (2012) Recent advances in the development of high-fibre baked products. *Trends in Food Science & Technology*, **28**, 4-14.

Mariotti, M., Garofalo, C., Aquilanti, L., Osimani, A., Fongaro, L., Tavoletti, S., Hager, A.-S. & Clementi, F. (2014) Barley flour exploitation in sourdough bread-making: A technological, nutritional and sensory evaluation. *LWT-Food Science and Technology,* **59,** 973-980.

Millar, K. A., Barry-Ryan, C., Burke, R., Hussey, K., Mccarthy, S. & Gallagher, E. (2017) Effect of pulse flours on the physiochemical characteristics and sensory acceptance of baked crackers. *International Journal of Food Science & Technology,* **52**, 1155-1163.

Mohebbi, Z., Homayouni, A., Azizi, M.H. & Hosseini, S.J. (2018) Effects of beta-glucan and resistant starch on wheat dough and prebiotic bread properties. *Journal of Food Science and Technology,* **55**, 101-110.

O'Shea, N., Kilcawley, K.N. & Gallagher, E. (2017) Aromatic composition and physicochemical characteristics of crackers containing barley fractions. *Cereal Chemistry,* **94,** 611-618.

Pejcz, E., Czaja, A., Wojciechowicz-Budzisz, A., Gil, Z. & Spychaj, R. (2017) The potential of naked barley sourdough to improve the quality and dietary fibre content of barley enriched wheat bread. *Journal of Cereal Science,* **77,** 97-101.

Ranok, A., Dissamal, P., Kupradit, C., Khongla, C., Musika, S. & Mangkalanan, S. 2021. Physicochemical properties and antioxidant activity of gluten-free riceberry-cheese cracker under simulated gastrointestinal transit. *Journal of Food Science and Technology***,** 1-9.

Rieder, A., Holtekjølen, A.K., Sahlstrøm, S. & Moldestad, A. (2012) Effect of barley and oat flour types and sourdoughs on dough rheology and bread quality of composite wheat bread. *Journal of Cereal Science,* **55,** 44-52.

Skendi, A., Biliaderis, C.G., Lazaridou, A. & Izydorczyk, M.S. (2003) Structure and rheological properties of water soluble beta-glucans from oat cultivars of Avena sativa and Avena bysantina. *Journal of Cereal Science,* **38,** 15-31.

Smith, J.P., Daifas, D.P., El-Khoury, W., Koukoutsis, J. & El-Khoury, A. (2004) Shelf life and safety concerns of bakery products—a review. *Critical Reviews in Food Science and Nutrition,* **44,** 19-55.

Soma, G., Mahadevamma, S. & Sudha, M. (2016) Characterisation of tea fiber and its utilisation as a functional ingredient in the preparation of biscuits. *International Food Research Journal*, 23.

Sudha, M., Vetrimani, R. & Leelavathi, K. (2007) Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. *Food Chemistry,* **100,** 1365-1370.

Trogh, I., Courtin, C., Andersson, A., Åman, P., Sørensen, J. & Delcour, J. (2004) The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1→ 3, 1→ 4)-β-D-glucan levels. *Journal of Cereal Science,* **40,** 257-267.

Vickers, Z. & Bourne, M.C. (1976) A psychoacoustical theory of crispness. *Journal of Food Science,* **41,** 1158-1164.

Yeboah-Awudzi, M., Lutterodt, H.E., Kyereh, E., Reyes, V., Sathivel, S., Manful, J. & King, J.M. (2018) Effect of bambara groundnut supplementation on the physicochemical properties of rice flour and crackers. *Journal of Food Science and Technology*, **55,** 3556-3563.

Yilmaz, E. & Karaman, E. (2017) Functional crackers: incorporation of the dietary fibers extracted from citrus seeds. *Journal of Food Science and Technology,* **54,** 3208-3217.

Chapter 6: **GENERAL DISCUSSION AND RECOMMENDATIONS FOR FUTURE STUDIES**

6.1 General Discussion

Barley grain is the richest source of beta-glucans among all cereals. Barley grains have the proven ability to ameliorate diet-related health problems, including obesity, type-2 diabetes and high cholesterol. Although barley is widely available, the current consumption of barley grains in the diet is quite low compared to other cereals; rice and wheat have become more popular since they possess more attractive technological and sensorial characteristics. The overarching purpose of this research project was to identify alternative ways for the utilisation of barley in the food industry and explore the technological properties of barley beta-glucans as a functional food ingredient. To this end, the main goal of the present study was to develop an efficient extraction process for beta-glucans from barley grains grown under different environmental conditions – in Jordan and the UK – and incorporate these extracts into a food product to investigate the main physicochemical properties they impart in the food matrix. Besides the potential missed opportunity of the underutilisation of barley as human food in the UK for public health, the development of a health food market for barley could also benefit UK barley growers as it could strengthen the UK barley supply chain via the introduction of new food applications.

In Chapter 3, compositional analysis of Jordanian and UK barley flours, both originating from hulled grains, showed that beta-glucan concentrations were greater in Jordanian barley grain than in the UK grains. Jordanian barley had higher beta-glucans, protein and ash and less starch and fat compared to the UK barley. The variation in the chemical composition of barley grains seems to be firstly determined by the genotype of cultivar, and secondly by environmental

conditions during grain growth (including climate, water availability, season) (Baik and Ullrich, 2008). Specifically, it is a general observation that a wet harvest (e.g. in the UK) results in barley grains whose flour is characterised by low extract viscosity and beta-glucan content, while a dry harvest (e.g. in Jordan) results in grains whose flour has high extract viscosity and beta-glucan content (Aastrup Steem, 1979). It has been also suggested that environmental conditions could modify the expression of genes associated with beta glucan content (Molina-Cano *et al*., 2007). As such, a breeding strategy could lead to development and selection of cultivars that have high beta-glucan content and could be channelled towards human consumption, whereas cultivars with low beta-glucan content could be used for malting.

Key objective of the experimental work in Chapter 3 was to assess whether the compositional variations of the two barley grains could affect the extraction of beta glucans. As such, hot water extraction (HWE) was implemented, as an established process for the extraction of beta glucans from cereals (oats, barley). The main advantage of HWE is that it is an environmentally friendly process, as it uses water as the extraction medium and is often carried out at relatively low temperatures. The highest recovery in beta-glucans in UK and Jordanian HWE extracts was approximately 10% (w/w), occurring at 4 h, 60 °C and 3 h, 50 °C, respectively. HWE did not appear to be a highly efficient extraction method for barley beta-glucans. This is likely due to the fact that HWE is capable of extracting mainly water-soluble beta-glucans. Water-soluble and water-insoluble beta-glucan content in different barley cultivars has been reported to range from 3.7 to 7.9% and 10.8 to 21.7%, respectively (Gajdošová *et al*., 2007). Insoluble beta-glucan content is significantly higher in hulled barely varieties (as is the case for the barley grains tested in this study), indicating that insoluble beta-glucans are found mainly in the coat parts of the grain, whereas the soluble glucans are located in the inner parts of the grain (Gajdošová *et al*. 2007). The

basis of variation in extractability and solubility of beta-glucans is not clear yet. The differences in proportion of water-extractable beta-glucan among various barley cultivars had been considered as a heritable trait. Genotypic variation in extractability of beta-glucans may be due to variation in the thickness of cell walls, exhibiting a greater resistance to extraction (Lazaridou *et al*., 2007). Worth also mentioning is the fact that insoluble beta-glucans can be non-covalently bound to arabinoxylans, a fact which enables them to remain insoluble despite their low molar mass (Johansson *et al*., 2004). As such, HWE extraction alone cannot provide highly pure beta-glucan extracts, and further processing steps are required to purify HWE fractions (e.g. enzymatic digestion of starch, dialysis to remove low molecular weight impurities) and increase beta-glucan content in the final extracts.

Following on from these observations, Chapter 4 focused on the investigation of ultrasonication as an alternative process for the extraction of beta-glucans from barley grains. Recently, there has been increased attention paid to greener and more sustainable and environmentally friendly approaches in the extraction of specific compounds. Ultrasound assisted extraction (UAE) offers several advantages over other conventional extraction methods, such as shorter extraction time, moderate solvent requirements, lower environmental impact, and the potential for industrial upscaling. UAE uses acoustic cavitation to disrupt plant cell walls, reduce particle size, and enhance the contact between solvents and targeted compounds (Zhu *et al*., 2016). The mechanism of sound waves in a medium involves high and low pressure (compression and rarefaction) cycles. This technique induces cavitation, which promotes the production, growth, and collapse of bubbles (Chemat *et al*., 2011). The cavitation process produces strong shear forces and allows the solvent to penetrate deeper into the matrix. The advantage of this is an improvement in the diffusion rate of the desired molecule to the solvent (Wang *et al*., 2008).

In the present study, UAE was more effective in the extraction of beta-glucans. For the Jordanian barley, the highest beta-glucan recovery by UAE was 73.2 % (w/w) compared to only 10.58% (w/w) achieved in HWE. The same trend was observed for the UK barley, with highest beta-glucan recovery in UAE reaching 55.57 % (w/w) compared to 9.74 % (w/w) in HWE. This study showed that the cavitation phenomenon was more efficient for beta glucan extraction when high oscillation intensity was applied rather than simply prolonging the time of extraction, further supporting the observation that UAE was capable of extracting water-insoluble beta-glucans. However, the purity of beta-glucans in the extracts decreased in high oscillation intensity. This could be attributed to the degradation of cell wall components at high amplitudes, which enhanced the release of polysaccharides other than beta-glucans into the aqueous phase as impurities. Due to the prolonged exposure of the barley flour to the ultrasonic waves, which caused more water-soluble extracts to accumulate in the water, more starch was extracted as part of the extracts. Generally, ultrasonication breaks polymer chains at the centre, which is the structurally weakest point. Furthermore, linear polymer chains (as in the case of beta-glucans) are more easily sonolysed compared to branched ones. Worth noticing here is the fact that the starch content in the extracts of the UK and Jordanian barley was significantly different (up to ~30% in the UK and ~60% in the Jordanian extract). This could indicate that in the UK barley grain, starch was more branched, containing lower amylose to amylopectin ratio, compared to the Jordanian barley grain. It is worth noting that longer-chained molecules also lead to higher solution viscosity which also affects the rate of cavitation (Ogutu *et al*., 2015). It is evident that although cavitation as an extraction mechanism renders UAE a non-selective extraction process. If the purpose of the UAE is to obtain highly enriched extracts, downstream steps targeting the removal of cell wall co-extracts is deemed essential. However, in the case of the current research, the UAE extracts were

investigated as ingredients in food formulations, and in such applications, a highly pure extract is not always required.

The last experimental chapter of this work (Chapter 5) focused on investigating the addition of barley flour and UAE extracts in bakery products. Specifically, barley flour, originating from grains grown under two very different conditions, was added as a replacement at different inclusion levels (10%–60%) in cracker recipes to examine its effect on the quality and the highest beta-glucans levels were found, as expected, in formulations with the highest inclusion level of barley flour. Cracker formulations made from a mixture of wheat and barley flour increased beta-glucan concentrations by 2 to 6-fold, compared to wheat-based crackers. By increasing the replacement levels of wheat flour by barley flour, an increase of fibre from the barley flours was observed (marked as an increase in beta glucan content) and a dilution of the gluten content in the final crackers. The main effects of these compositional changes were a decrease in moisture, increased hardness and reduced number of fracture peaks in the final crackers. Incorporation of barley flour gave place to significantly darker and redder crackers. The addition of UAE extracts in the crackers formulation resulted in end products with higher beta-glucan level than those with 60% inclusion of barley flour. When comparing the replacement 60% wheat flour by barley flour with the addition of beta-glucan extract (aiming to achieve the same concentration of beta-glucans in the final cracker), a significant improvement in water activity and textural properties of the end product was noted. Moreover, beta-glucan extracts were considered suitable for the production of crackers with a high beta-glucan content that could meet US FDA dietary requirement. The development of crackers with UAE barley extracts has the potential to allow consumers to increase their daily intake of beta-glucans, which impart many health benefits, such as reducing total and LDL

cholesterol. These results bridge a current knowledge gap in the field, as this study is the first to investigate the inclusion of UAE barley extracts in bakery products.

6.2 Considerations for future work

Although the study presented in this thesis has established the potential for utilising ultrasonication as a process for the extraction of beta-glucans, it only represents a preliminary investigation. The research findings of this work could form the basis for future work, aiming to further advance different aspects of the topic with potential applications for the food industry. Firstly, the effect of ultrasonication on barley flour polysaccharides is worth exploring in much more depth. The combination of oscillation intensity and time of extraction could lead to substantial degradation effects on major barley carbohydrates, namely starch, arabinoxylans and beta glucans. Currently, there are no systematic studies investigating the mechanism of action during UAE on the degradation of cereal carbohydrates. There is also the possibility of structural conformation changes occurring during UAE cavitation phenomena. These conformation changes could relate to the molecular weight and apparent viscosity of the polymers and could form the basis for their directional degradation and modification, leading into altered rheological and functional properties of the obtained UAE extracts.

Another angle of the ultrasonication extraction is the investigation of strategies to improve the purity of barley extracts in beta-glucans. In this study, it was evident that starch was the major impurity in the UAE extracts, regardless of the extraction time or oscillation intensity. One approach could be the addition of a thermostable α-amylase in the extraction process. The enzyme could be protected from the denaturation effect of the sonication by changing the extraction setup and allowing for constant circulation of the extraction medium in the system and the use of

intermitted sonication. The addition of the α-amylase could breakdown co-extracted starch molecules, which could subsequently be removed either via dialysis or via precipitation of beta-glucans from the extraction medium with ethanol. If successful, this strategy would decrease post-extraction processing steps and could lead to increased purity of UAE extracts in beta-glucans. Worth mentioning is the fact that any studies targeting detailed structural investigation of extracted beta-glucans would require further processing steps to remove contaminants and allow for a better chromatographic (e.g. ion exchange chromatography) or spectrographic (^{1}NMR, C-13 NMR) resolution of the polymers.

Another aspect worth investigating is the sensory evaluation and consumer acceptance of the developed crackers. Substitution of wheat flour with barley flour in bakery products has been associated with greater dietary fibre and polyphenol content in the end products (mainly bread). However, from a sensorial point of view, wheat flour substitution up to 20% (w/w) is usually recommended; higher barley flour levels have been reported to lead to reduction in sensory quality, due to the presence of proanthocyanidins and phenolic acids which give a bitter and astringent taste to the end products. This study showed that the use of barley extracts significantly enriched the crackers with beta glucan and improved the texture of the final products compared to formulations with 60% barley flour inclusion. It would be of interest to determine whether this physicochemical improvement is also validated from a sensory and consumer acceptance point of view too.

Finally, the effect of processing and storage conditions on the quality and stability of the developed crackers would complement the findings of the study. Although the amount of protein in the extracts was low (not higher than 2% in the UK barley extract), the formation of acrylamide during baking should be monitored. Additionally, the low water activity of the crackers prepared

with UAE extracts indicated that they could exhibit a longer shelf life, compared to the control ones (wheat and barley inclusion at 60%). A storage trial of a couple of months would be necessary, to assess colour stability, moisture uptake and changes in hardness in the developed crackers over time.

6.3 References

Aastrup, S. (1979) The effect of rain on β-glucan content in barley grains. *Carlsberg Research Communications,* **44,** 381-393.

Baik, B.-K. & Ullrich, S. E. (2008) Barley for food: Characteristics, improvement, and renewed interest. *Journal of Cereal Science,* **48,** 233-242.

Chemat, F., Zill E, H. & Khan, M. K. (2011) Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry,* **18,** 813-835.

Gajdošová, A., Petruláková, Z., Havrlentová, M., Červená, V., Hozová, B., Šturdík, E. & Kogan, G. (2007) The content of water-soluble and water-insoluble β-D-glucans in selected oats and barley varieties. *Carbohydrate polymers,* **70,** 46-52.

Johansson, L., Tuomainen, P., Ylinen, M., Ekholm, P. & Virkkl, L. 2004. Structural analysis of water-soluble and-insoluble β-glucans of whole-grain oats and barley. *Carbohydrate polymers,* **58,** 267-274.

Lazaridou, A., Biliaderis, C. G. & Izydorczyk, M. S. (2007) Cereal beta-glucans: structures, physical properties, and physiological functions. *Functional food carbohydrates***,** 1-72.

Molina-cano,J.-L., Moralejo,M., Elia, M., Munoz, P., Russell, J., Perez-vendrell , A. & Swanston, J. (2007) QTL analysis of a cross between European and North American malting barleys reveals a putative candidate gene for β-glucan content on chromosome 1H. *Molecular Breeding***, 19,** 275-284.

Ogutu, F. O., Mu, T.-H., ElahiI, R., Zhang, M. & Sun, H.-N. (2015) Ultrasonic modification of selected polysaccharides-review. *Journal of Food Processing & Technology,* 6**,** 1.

Wang, J., Sun, B., Cao, Y., Tian, Y. & Li, X. (2008) Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry,* **106,** 804-810.

Zhu, F., Du, B. & Xu, B. (2016) A critical review on production and industrial applications of beta-glucans. *Food Hydrocolloids,* **52,** 275-288.