Characterization and Functional Beverage Development using Coenzyme Q10-Impregnated Beta-glucan

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Food Science and Technology

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University of Alberta

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Abstract

Pressurized gas-expanded liquid (PGX) technology is a supercritical fluid technique used for producing micro/nano-particles, agglomerates and fibers from an aqueous solution of a high molecular weight biopolymer, which can also be employed for encapsulation or impregnation. PGX-processed beta-glucan (BG) and CoQ10-impregnated beta-glucan (iBG) were investigated, in terms of their physicochemical properties and potential as nutraceutical ingredients for functional beverages. Helium ion microscope, differential scanning calorimeter, X-ray diffractometer, AutoSorb iQ and rheometer were used to determine the particle morphology, thermal properties, crystallinity, surface area and viscosity, respectively. Results showed that both BG (7.7 μm) and iBG (6.1 μm) were produced as micrometer-scale particles, while CoQ10 nanoparticles were present in iBG with the average diameter of 92 nm; CoQ10 was successfully impregnated onto BG using the PGX process without interrupting the porous structure and viscosity of BG; and CoQ10 present in iBG was in its amorphous form adsorbed on the porous structure of BG. In addition, the effects of shear rate (1.29 s^{-1} to 129 s^{-1}), concentration (0.15%, 0.2%, 0.3% w/v) and temperature (0 ºC to 80 ºC) on the viscosity of BG and iBG solutions were investigated. As expected, viscosity increased with concentration, and decreased with temperature. However, shear rate had no effect on the viscosity of both BG and iBG solutions, demonstrating Newtonian behavior at these concentrations.

The formulation of an orange-flavored functional beverage containing BG or iBG and sweetened with stevia extract was developed and the quality of the beverages was evaluated by

sensory panels. There was no overall difference between the beverages prepared with BG and iBG. Ideal profile method (IPM) was applied to evaluate the overall consumer acceptance of the beverages prepared with 0.2% iBG. The effect of providing health information was also tested, and the positive effect of health information of ingredients including stevia extract, beta-glucan and CoQ10 on overall acceptance was demonstrated.

Determination of the physicochemical properties contributes to the fundamental understanding of the behavior of the bioactive combination, CoQ10 and BG in powder form. As well, the prototype functional beverage developed demonstrated the potential use of PGX-processed BG and iBG as nutraceutical ingredients in beverage products.

Preface

This thesis is an original work by Nian Liu under the supervision of Dr. Feral Temelli, comprised of five chapters: Chapter 1 provides background information and overall objectives of this thesis research; Chapter 2 is a literature review on several topics related to this research work; Chapter 3 is about the physicochemical characterization of beta-glucan and CoQ10-impregnated bet-glucan; Chapter 4 focuses on functional beverage development using these ingredients and sensory evaluation of the beverages; and Chapter 5 gives conclusions and recommendations. The research project reported in Chapter 4, received research ethics approval on October 18th, 2016, from the University of Alberta Research Ethics Board, "Sensory characteristics and consumer acceptance of orange-flavoured functional beverage sweetened with stevia extract and fortified with beta-glucan and CoQ10", No. Pro00065010. Dr. Wendy Wismer and Ha Nguyen provided guidance in experimental design and data analysis for the work reported in Chapter 4.

Acknowledgements

This research and thesis would not have been possible without the great support of my thesis supervisor, Dr. Feral Temelli who has been constantly providing insightful advice, knowledge, patience, encouragement, guidance and support throughout my graduate study. Thank you Dr.Temelli, I think you are the best supervisor. I highly appreciate your excellent advice for not only my research project but also personal development. You told me "Jump out of your comfort zone, you will figure out how much more you can do," and that will be my motto to encourage me to go far in my future research and development.

Thank you Dr. Wendy Wismer for being my supervisory committee member and giving great support and valuable guidance. Also, I appreciate Dr. Lynn McMullen to be an examining committee member and to give me great advice and feedback for this thesis.

Furthermore, I am grateful to Alberta Innovates Biosolutions and Ceapro Inc. for their financial support of this project.

Thanks to Drs. Paul Moquin and Bernhard Seifried, Ceapro Inc. for providing the beta-glucan and CoQ10-impregnated beta-glucan ingredients and their insights.

In addition, I thank Peng Li and Dr. Shihong Xu from nanoFAB for the technical support on helium ion microscopy. Thanks to Dr. Lingyun Chen for providing equipment for my particle size analysis. Thanks to Jun Gao for the help and support on beta-glucan content and viscosity determination. Thanks a lot to Dr. Ricardo Tomas Do Couto for his constant help with my experiments and enlightening discussions on my choices in life. Thanks to Dr. Ozan Nazim Ciftci

who was the first person to bring me into the research field. Thanks to Ha Nguyen for her help on statistical analysis. I also would like to thank my lab colleagues and friends, Jiayue Chen, Yujia Zhao, Carla Valdivieso, Eileen Santos, Yuanyao Chen, Rodrigo Araya Olguin, Karina Araus Sermiento, Robert Mukiibi, Guangyu Liu, Zhiqiang Jiu, Yussef Esparza and Eduardo Rodriguez Martinez for their motivation, help and support in many aspects throughout the past years.

Finally, I would like to thank my family and all my friends for giving me support, understanding, positive energy and love. Particularly to my parents, who always cheer me up when I need it. I would not be where I am today without their unconditional love and support.

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List of Abbreviations

A	Absorbance
a	Hunter color index a, red-green
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
b	Hunter color index b, blue -yellow
BG	Beta-glucan produced by the pressurized gas-expanded liquid technology
CO_2	Carbon dioxide
CoQ10	Coenzyme Q10
DSC	Differential scanning calorimeter
DP3	(1,4)-beta-linked cellotriosyl
DP4	(1,4)-beta-linked cellotetraosyl
EE	Entrapment efficiency
ESI	Electron spray ionization
FT-IR	Fourier Transform Infrared spectrophotometry
Glc	D-glucopyranosyl residues
HBG	High viscosity oat beta-glucan
HiM	Helium ion microscopy
HPLC	High-performance liquid chromatography
iBG	CoQ10 impregnated beta-glucan
IPM	Ideal profile method
L	Hunter color index L, lightness
m	Mass
MW	Molecular weight
PCA	Principal component analysis
PGX	Pressurized gas-expanded liquid
$SCCO_2$	Supercritical carbon dioxide
SCF	Supercritical fluid
SD	Standard deviation
T	Temperature
UV	Ultraviolet light
V	Volume
WI	Whiteness index
XRD	X-Ray diffraction
YI	Yellowness index
ρ	Density
η	Viscosity

Chapter 1: Introduction and objectives

Supercritical fluid (SCF) is a pure component (i.e. carbon dioxide, nitrogen, ethanol or others) that is heated above its critical temperature and compressed to a pressure above its critical pressure but below the pressure needed for solidification. Under these conditions, a SCF has no surface tension, enhanced mass transfer, liquid-like density and gas-like diffusivity and viscosity. SCF technology, known as an environmentally friendly process, has been studied over the past decades to extract high-value components at mild conditions, and to generate micro/nano-meter sized particles for pharmaceutical and food applications. Pressurized gas-expanded liquid (PGX) technology invented by Temelli and Seifried (2016) is a SCF technique to produce micro/nano-particles, agglomerates and fibers from an aqueous biopolymer solution of a high molecular weight (MW=70,000 Da to over 1,000,000 Da) biopolymer, and can encapsulate or impregnate bioactive components into the agglomerates or onto the micro/nano-particles, which includes the step of spraying a mixture of the aqueous solution, compressible gas and a water-soluble co-solvent/antisolvent (i.e. ethanol, acetone or isopropanol) into a pre-pressurized chamber.

The biopolymer investigated in this research was beta-glucan, which is a soluble dietary fiber component found in common cereals including oats (3-8 g per 100 g dry weight) and barley (2-20 g per 100 g dry weight) (Wood and Beer, 1998). This polysaccharide plays an important role in health promotion and prevention of diseases, focusing on the normalization of the gut microbial compositions, regulation of blood cholesterol levels and blood pressure, attenuation of

blood postprandial glycemic and insulinemic responses, prevention of central obesity, and promotion of skin health (Pins and Kaur, 2006; Aleixandre and Miguel, 2008; Khoury et al., 2012; Daou and Zhang, 2012; Du et al., 2014a). The daily amount of 3 g oat beta-glucan was recommended by Health Canada in 2010 to support healthy glucose metabolism and reduce the risk of coronary heart disease (Health Canada, 2010). However, earlier in 1997, the Food and Drug Administration (FDA) of the United States claimed that oat products containing 0.75 g of beta-glucan per serving may reduce the risk of heart disease (Federal Register, 1997).

The bioactive material studied in this research was Coenzyme Q10 (CoQ10), which is a natural antioxidant present in both animal and plant cellular membranes. Pure CoQ10 is an orange crystalline powder, which is sensitive to environmental conditions including light, oxygen and heat. It functions as an electron carrier and proton transporter to generate a proton gradient in the mitochondrial ATP synthesis, and consequently plays an important role in energy generation in the body (Mitchell, 1990; Turunen et al., 2004). The daily dosage of 30 to 100 mg recommended by Health Canada is claimed to maintain cardiovascular health (Health Canada, 2007). However, due to its lipophilicity and high molecular weight, CoQ10 is insoluble in water, which limits the fortification of aqueous foods. Recently, liposomal aggregation, κ-carrageenan coated oil-in-water emulsion, maize starch dispersion and nano-encapsulation with octenyl succinic anhydride modified starch have been successfully applied to increase the water solubility of CoQ10 (Xia et al., 2006; Chan et al., 2013; Yoon et al., 2014; Cheuk et al., 2015).

But these products were all generated in liquid form, which could lead to problems upon long-term storage, transportation and aqueous food applications.

PGX technology could be an alternative to impregnate CoQ10 on beta-glucan at mild conditions and generate ingredients in powder form. The properties of micro/nanoparticles or agglomerates produced by the PGX technology includes large surface area, low bulk density and high porosity, which may promote easier handling and dispersion, and faster dissolution in water, compared to the powder of the same material(s) produced by conventional methods like spray drying or freeze drying. However, the literature lacks information on the characteristics of CoQ10-impregnated beta-glucan particles and its potential product applications. Therefore, the overall goal of this thesis research was to: i) characterize the physicochemical properties of the beta-glucan powder (BG) and CoQ10-impregnated BG powder (iBG) produced by the PGX technology, and ii) to develop an appealing and nutritious prototype functional drink formulation based on iBG.

To achieve this main objective, the specific objectives were:

i) to determine the properties of BG powder and iBG powder generated by the PGX technology, in terms of morphology, impregnation yield, melting behavior, crystallinity, viscosity, surface area and pore size (Chapter 3); and

ii) to evaluate the overall acceptance of the functional beverage formulated with iBG using trained and consumer panels (Chapter 4).

Chapter 2: Literature review

2.1 Beta-glucan

2.1.1 Sources and structure

Beta-glucan is a non-digestible glucose polymer, originating from the cell walls of mushrooms, algae, bacteria and some commercially important cereals, such as oat, barley, rye and wheat (Lazaridou and Biliaderis, 2007; Iorio et al., 2008). The fungal and microbial glucans are mostly water-insoluble (Iorio et al., 2008). Mushroom is low in dry matter content, but a great source of beta-glucan. Beta-glucan content of mushrooms varies widely from 7.8 g to 60.8 g per 100 g dry weight, depending on the species (Sari et al., 2017). Beta-glucan content (g per 100 g dry weight) of common cereals generally ranges from 0.13 g in rice, 0.5-1.0 g in wheat, 0.8-1.7 g in maize, 1.3-2.7 g in rye and 1.1-6.2 g in sorghum, to 3-8 g (82% is water-soluble fraction) in oats and 2-20 g (65% is water-soluble fraction) in barley (Wood and Beer, 1998; Skendi et al., 2003; Ragaee et al., 2008; Bacic et al., 2009). Herrera et al. (2016) found that the beta-glucan content of oats was influenced by the oat cultivar, growing location and the interaction between cultivar and location, although the latter two factors had a minimal effect. They evaluated eight oat cultivars including Derby (4.37% w/w), Furlong (4.59% w/w), CDC Dancer (4.68% w/w), Morgan (4.71% w/w), Jordan (5.03% w/w), Leggett (5.25% w/w), SW Betania (5.39% w/w) and HiFi (5.82% w/w) grown in Canada, and indicated that growing location, oat cultivar, and their interaction also significantly affected the beta-glucan solubility and viscosity (Herrera et al., 2016).

In oats, beta-glucan is mainly present in the aleurone layers, while it is more uniformly distributed in the barley endosperm. On the contrary, the highest concentration of beta-glucan that can be found in wheat is located in the subaleurone layer, and the remaining small amount is distributed in the starchy endosperm (Izydorczyk et al., 2000; Lazaridou and Biliaderis, 2007). Beta-glucans from different sources have different structures. Beta-glucans derived from baker's yeast or mushrooms are polysaccharides of D-glucose residues linked via mixed beta-(1,3) and (1,6) linkages (Fig. 2-1). However, cereal beta-glucan is a linear homopolysaccharide comprised of D-glucopyranosyl residues (Glc) linked by a mixture of beta-(1,3) and beta-(1,4) linkages (Herrera et al., 2016). Unlike cellulose, which is made of beta-(1,4) linked D-glucose units, beta-glucan is composed of (1,3)-linked cellotriosyl (DP3) and cellotetraosyl (DP4) segments (Fig. 2-2). On average, the beta-(1,4) linkages are present in sequences of 2 or 3, resulting in the oligomeric cellulose units, whereas the beta-(1,3) linkages only occur singly in a molecule. Nevertheless, longer units linked via beta-(1,4) linkages of up to 14 glucosyl units still exist occasionally (Cui, 2001; Wood, 2001; Skendi et al., 2003). Particularly, some previous research point out that wheat (3.0-3.8) has the highest ratio of DP3 to DP4, followed by barley (2.8-3.4) and then oats (2.1-2.4), and they also predict that the longer sequences of beta-(1,4) linkages would decrease the water-solubility of beta-glucan due to the intermolecular interactions (Woodward et al., 1983; Cui and Wood, 2000; Wood, 2011).

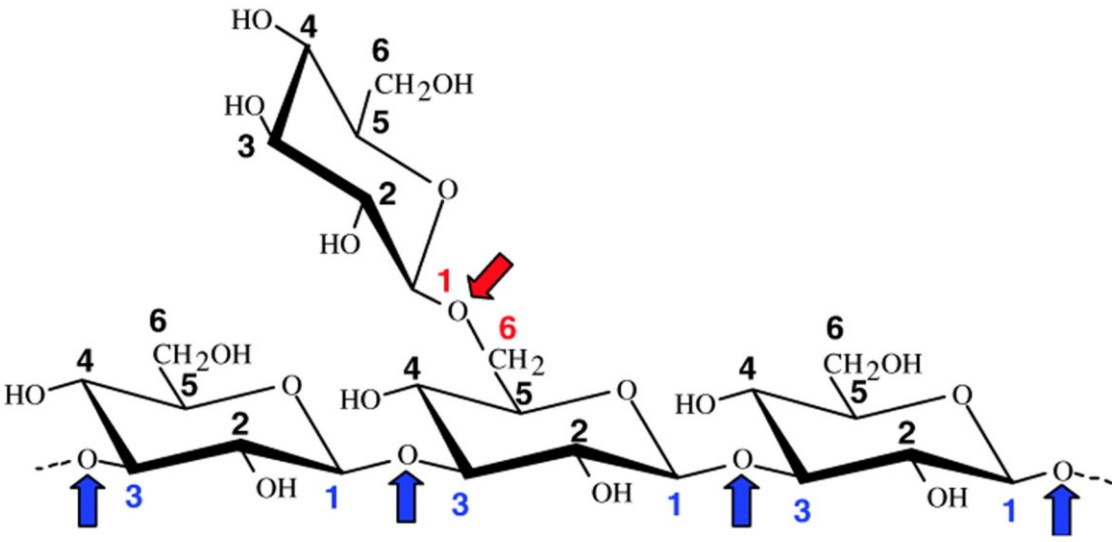


Figure 2-1. The structure of beta (1→3) (1→6) beta-glucan.

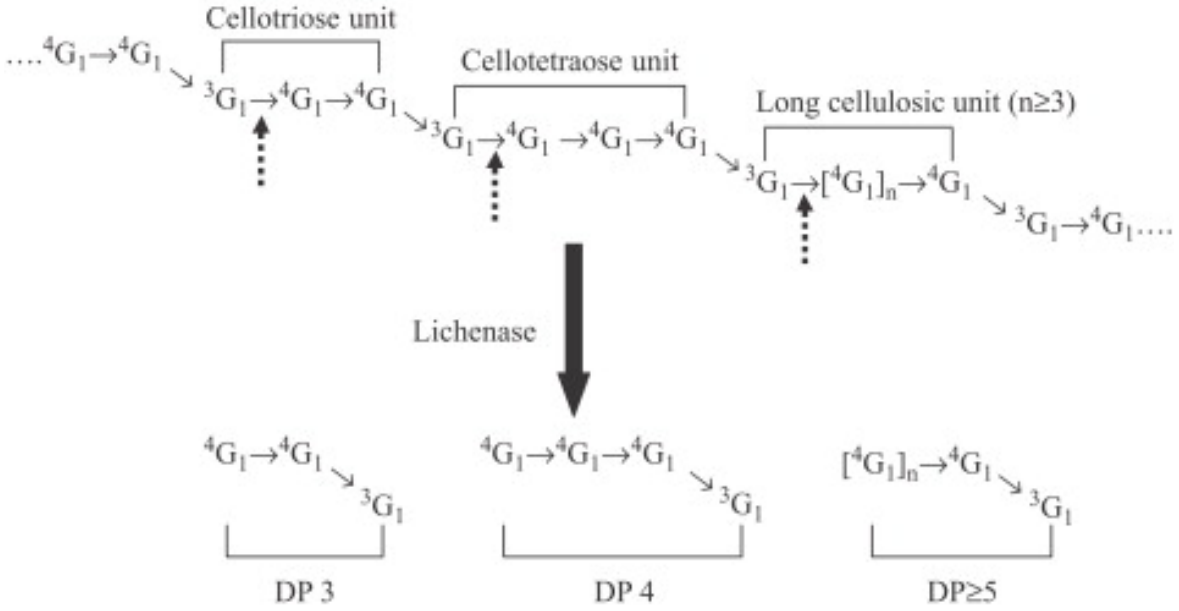


Figure 2-2. Generalised structure of cereal beta-glucans and their debranching with lichenase; dotted arrows indicate the lichenase hydrolysis sites on the polysaccharide chain. G: beta-D-glucopyranosyl unit; DP3: 3-O-beta-cellobiosyl-D-glucose; DP4: 3-O-beta-cellotriosyl-D-glucose; DP⩾5: cellodextrin-like oligosaccharides containing more than three consecutive 4-O-linked glucose residues. Reprinted from Lazaridou and Biliaderis (2007) with permission from Elsevier.

2.1.2 Extraction of beta-glucan

To break the cell-wall structure and release the beta-glucan, there are two major techniques that are commonly employed: dry and wet separation processes. In dry methods, grain meal from dry milling or flour from dry milling and sieving is air-classified, based on the size and density differences between particles. The beta-glucan content of the fiber concentrate obtained was as high as 30% by adjusting the feed rate, air flow rate and classifier wheel speed (Vasanthan and Bhatty, 1995; Vasanthan and Temelli, 2008). Although dry separation processes are widely applied in the industry due to their simplicity, reasonable cost and avoidance of applying solvents, the beta-glucan yield of less than 30% (w/w, on dry weight basis) is relatively low (Vasanthan and Bhatty, 1995; Hu et al., 2015). The high lipid content of oat was believed to be the main reason for dry processes to be unable to yield highly concentrated beta-glucan fractions, because lipids could agglomerate oat meal and clog the screens during the sieving step (Vasanthan and Temelli, 2008). In addition, around 65% of the lipids is recovered in the bran fractions (Doehlert and Moore, 1997). In order to remove the lipid, Sibakov et al. (2011) used supercritical carbon dioxide ($SCCO_2$) to produce defatted oats, which were then used as the raw material for the subsequent fine milling and air classification operations. The highest beta-glucan concentration in the cell wall-enriched fraction was 33.9%, which was almost double that obtained without lipid removal (17.1%). The lipid removal with $SCCO_2$ performed by Sibakov et al. (2011) enhanced the separation of oat beta-glucan, but highly concentrated beta-glucan fractions still could not be obtained by conventional dry separation processes.

In order to increase the concentration of beta-glucan, wet separation processing was developed by Wood et al. (1989) who obtained an oat gum containing 78% beta-glucan by extracting it with sodium carbonate at pH 10. Generally, beta-glucan could be concentrated by solubilizing it in water or separating it in a solvent system that contains water and alcohol, which are also referred to as aqueous solvent system and semi-alcoholic solvent system, respectively. Unlike water in which beta-glucan is solubilized, alcohol concentration in the semi-alcoholic solvent system is usually kept high to make sure beta-glucan remains insoluble and stays within the cell wall, while other components, such as starch, protein and lipid, are removed. Extraction and purification techniques of beta-glucan from various sources, such as barley, oat and yeast, were reviewed by Vasanthan and Temelli (2008) and Ahmad et al. (2012). Also, US patents issued on beta-glucan production technologies were provided by Zhu et al. (2016).

Four basic steps are generally included in the wet separation processes: i) inactivation of endogenous enzymes (cellulase and beta-glucanase) by ethanol refluxing or thermal treatment; ii) extraction of beta-glucan by solubilizing in hot water or alkaline solutions; iii) separation of the dissolved proteins by adding acids to achieve isoelectric precipitation; iv) precipitation of the beta-glucan by ammonium sulfate, 2-propanol, ethanol, heating or freezing-thawing (Wood et al., 1978; Wood et al., 1989; Potter et al., 2016; Morgan, 2016; Vasanthan and Temelli, 2008; Zhu et al., 2016). Cellulase and beta-glucanase are responsible for the degradation of beta-glucan, resulting in the decrease of its molecular weight and in turn its viscosity, which is a critical parameter for beta-glucan health benefits. Additionally, lipase is naturally present within oats,

which leads to development of rancidity. Therefore, steaming and kiln drying are commonly applied in the industry to inactivate those endogenous enzymes. Refluxing with ethanol is also usually used to decrease enzyme activity, but such a step adds to the processing cost (Burkus and Temelli, 1998). However, endo-(1-3)-beta-D-glucanase is stable at 60 ^{o}C for 40 min, so the treatment temperature is usually set above 60 ^{o}C, which is the gelatinisation temperature of starch (Ballance and Meredith, 1976; Burkus and Temelli, 1998), and thus, starch polymers could be co-extracted with beta-glucan at temperatures above 60 ^{o}C. In order to minimize the amount of starch, mild extraction conditions are employed or thermostable alpha-amylase is applied to digest starch.

The effect of extraction conditions on beta-glucan recovery and functionality has been studied extensively. A positive relationship between temperature and beta-glucan content was found by Temelli (1997) who investigated the effect of temperature (40-55 ^{o}C) and pH (7-10) on beta-glucan recovery by alkaline extraction and its functional properties. The optimal extraction condition was found to be pH 7.0 and 55 ^{o}C, under which 86.5% of the beta-glucan was recovered with 89.1% purity, maximum emulsion stability and viscosity. The method described by Temelli (1997) was also studied by Limberger-Bayer et al. (2014); however, the maximum beta-glucan concentration obtained was 53.4% with mainly starch and protein as impurities. Other than temperature and pH, Benito-Román et al. (2011) also identified extraction time, particle size, stirring rate and solvent:flour ratio as the critical parameters that affect the extraction of beta-glucan from barley, and they obtained the highest extraction yield of 73.4% at

55 ºC, pH close to neutrality, particle size of 100 μm, solvent:flour ratio of 5, stirring rate at 1000 rpm and extraction time of 3 h.

In order to minimize the degradation of beta-glucan under the alkaline, acidic and hot water extraction conditions, a new fractionation technology based on the use of enzymes in aqueous ethanol medium was introduced by Vasanthan and Temelli (2009). This method concentrates beta-glucan by enzymatically digesting the other grain components, and thus beta-glucan with its native structural characteristics can be obtained (Ghotra et al., 2008). Enzymatic extraction methods were also studied by Irakli et al. (2004) to isolate beta-glucan from different Greek barley cultivars by applying termamyl and pancreatin. Beta-glucan with a purity of more than 93% on dry basis was obtained with water extraction at 47 ºC, enzymatic removal of protein and starch and precipitation with ammonium sulfate saturation. Compared to the alkaline and acidic extractions, enzymatic extraction was found to be the best method, resulting in the high yield, removal of more starch, fat and pentosans, and producing beta-glucan with good water binding capacity, viscosity and whippability properties (Ahmad et al., 2010). Relatively less starch and protein impurities remaining after enzymatic extraction was also observed by Ahmad et al. (2009). However, hot water extraction resulted in higher yield and recovery of beta-glucan, when it was included for comparison.

New fractionation methods for beta-glucan isolation have been under development over the past decade. Accelerated solvent extraction (ASE) was applied to extract beta-glucan from the bran of hull-less barley, and the extraction yield of 16% was obtained at 70 ºC and 10 MPa in

just 9 min (Du et al., 2014b). A high purity beta-glucan concentrate (greater than 70%) was extracted by the combination of ultrasound-assisted extraction, enzymatic hydrolysis and diafiltration (Benito-Román et al., 2014). Moreover, a high molecular weight beta-glucan (up to 500 kDa) with 52.4% yield was generated in a fixed-bed extractor using pressurized hot water as solvent at 155 ^{o}C, 2 MPa and a flow rate of 4 g/min (Benito-Román et al., 2013). Recently, Benito-Román et al. (2016) added supercritical carbon dioxide ($SCCO_2$) (8.5 MPa) to the pressurized hot water (155 ^{o}C; 1 mL/min water; a solvent-to-solid ratio of 40 mL/g) throughout the extraction of beta-glucan from *Ganoderma lucidum*. The addition of $SCCO_2$ enhanced the extraction yield of beta-glucan, which was calculated as beta-glucan content of liquid extract divided by that in *G. lucidum*, up to 72.5% (w/w).

2.1.3 Physicochemical properties

The physicochemical properties of cereal beta-glucans, in particular molecular weight, molecular structure (i.e. the ratio of DP3 to DP4) and rheological characteristics, including gelling capacity and viscosity in water solution have been investigated extensively in the past decades (Lazaridou and Biliaderis, 2007). The molecular weight (MW) of beta-glucan was found to be positively correlated with viscosity, which contributes to its physiological benefits like lowering of serum cholesterol or glucose (Varum and Smidsrod, 1988; Wood et al., 1994). Recently, beta-glucan with a higher molecular weight or increased viscosity was shown to have a better alleviating effect on hyperlipidemia and oxidative stress of diabetic mice (Zhao et al.,

2014). Therefore, investigation of the physicochemical properties of beta-glucan is needed to obtain a better understanding of its behavior under physiological conditions.

Beta-glucans from different sources apparently vary in terms of their molecular weight and structure. The average MW of beta-glucan extracted from oat, wheat and barley was reported as 172 kDa, 635 kDa and 742 kDa, respectively (Zhao et al., 2014). Due to the relatively higher MW, wheat and barley beta-glucan showed shear thinning behavior, and barley beta-glucan had the highest viscosity. Wheat beta-glucan with the highest value of DP3: DP4 ratio had no gelling property at the concentration of 5% (w/v) due to its lower MW, but barley beta-glucan at the same concentration formed a weak gel (Zhao et al., 2014). However, beta-glucan with a higher MW of up to 239 kDa did not show any tendency to form a gel, but lower MW beta-glucan with short chains had a better gelation property due to its higher mobility and greater possibility of forming junctions with neighboring chains (Doublier and Wood, 1995; Li et al., 2006). Also, shorter gelation time and higher gelation rate were observed for the low molecular weight oat beta-glucan (Skendi et al., 2003).

Shear-thinning behavior and gelling capacity are also highly dependent on the concentration of beta-glucan. As the concentration increases, the gelation rate increases (Skendi et al., 2003). Beta-glucan solution at the low concentration of 0.2-3% showed shear-thinning behavior, while at a higher concentration, it was more likely to form a gel (Cui, 2001; Lazaridou et al., 2003). The effect of beta-glucan concentration on gel behavior was also examined by Burkus and Temelli (1999), reporting that beta-glucan concentrates extracted from non-waxy Condor and

waxy barley gelled at the concentration of ≥ 5% (w/w) and gel strength increased as concentration increased. Furthermore, Mikkelsen et al. (2010) and Ryu et al. (2012) compared the properties of beta-glucans from barley and oat. Both of these studies reported that at the same concentration, the viscosity of oat beta-glucan was much higher (~100 fold) than that of barley beta-glucan due to the higher MW, and suggested that MW and molecular structure would be the major contributors to the rheological properties of beta-glucan. Different from what was observed by Zhao et al. (2014), Mikkelsen et al. (2010) characterized barley beta-glucan with the molecular weight of 126 kDa as a low-viscosity beta-glucan showing Newtonian flow behavior, while oat beta-glucan with the molecular weight of 355 kDa as a high-viscosity beta-glucan with shear-thinning flow behavior. However, identical lower molecular weight of 98.4-99.2 kDa was found for both barley and oat beta-glucan by Ryu et al. (2012), who also observed a strong shear-thinning behavior for all barley and oat beta-glucans. Within the oat group, cultivar played an important role on the rheological properties of beta-glucan, especially the rate of viscosity increase with concentration. Five new oat cultivars with increased beta-glucan concentrations (6-7.8% dry basis) and one traditional cultivar with 4.4% beta-glucan were evaluated by Colleoni-Sirghie et al. (2003). They found that beta-glucan from the traditional line had a low MW, leading to less entanglement with an increase in concentration, which consequently had the least sensitivity to the changes in concentration. In contrast, new cultivars with increased beta-glucan concentrations produced high-MW beta-glucan, which could form a more viscous solution than the traditional oat beta-glucan at the same concentration (Colleoni-Sirghie et al.,

2003). Therefore, evaluation of the viscosity of beta-glucans from different cultivars could be used as a criterion for selecting new oat cultivars.

Pre-preparation, extraction process and food production process would affect molecular, structural and functional properties (i.e. viscosity, water binding capacity and solubility) of beta-glucan, leading to changes in the sensory attributes of the food products it is incorporated into as well as the physiological benefits of beta-glucan. Firstly, the impact of extraction methods of beta-glucan including acidic, alkaline and enzymatic processes on the physicochemical properties needs to be considered. Ahmad et al. (2010) indicated that among the three methods, acidic extraction could produce beta-glucan with the highest water binding capacity, while the enzymatic method could result in an increase in viscosity. Both alkaline and acidic extraction methods reduced the whippability and viscosity of the extracted beta-glucan gum, because extreme pH can cause an unfavorable effect on the molecular structure, especially on the beta-(1,3) linkages. Panahi et al. (2007) compared the beta-glucans concentrated using aqueous and enzymatic methods, and reported that the viscosity of beta-glucan was highly preserved by the enzymatic method, which improved postprandial glycemic control when incorporated into a fiber drink.

Extrusion cooking of hull-less barley using a twin-screw extruder increased the water solubility index of beta-glucan, when the feed moisture was decreased or the extrusion temperature was increased. The highest water binding capacity was found after high temperature and high moisture extrusion (180 ^{o}C, 20% moisture) (Sharma and Gujral, 2013). Therefore, the

enhancement of the extractability of beta-glucan by extrusion cooking is mainly due to the significant increase in its water solubility. However, the main concern regarding extrusion cooking is the viscosity decrease caused by the shear force and the high extrusion temperature (150-180 ^{o}C). In bread baking, increased mixing and fermentation time could result in decreased molecular weight of beta-glucan without affecting the DP3-to-DP4 ratio (Andersson et al., 2004). However, molecular weight was not affected by the fermentation with lactic acid bacteria, but a decrease in the beta-glucan content and maximum viscosity were observed by Lambo et al. (2005). Also, solubilizing beta-glucan prior to dough formulation was found to decrease its solubility and viscosity by Moriartey et al. (2011), who also recommended to bake the dough at a temperature as high as possible to ensure adequate beta-glucan solubility to confer health benefits. Furthermore, the molecular weight, solubility and viscosity of beta-glucan in oat bran bread could be stable for 3 days at room temperature (Gamel et al., 2013). When the same bread was frozen at -80^{o}C, the viscosity of extracted beta-glucan significantly decreased, due to the decrease in solubility, but no change was observed on the molecular weight. The decline in the solubility of beta-glucan upon frozen storage was also observed by Beer et al. (1997) who evaluated the effects of cooking and storage on the amount and molecular weight of beta-glucan extracted from oat bran, oat bran muffins and oat porridge. Beer et al. (1997) explained that as the water crystallizes, beta-glucan is concentrated and retreats to the cell wall with extensive hydrogen bonding, resulting in the decrease in its solubility. Gamel et al. (2013) also suggested that liquid nitrogen could be used to freeze oat bran bread and porridge for shipping and storage

purposes, because this method did not significantly change the viscosity, so that the physiological benefits of beta-glucan would be maintained; however, the cost of such a treatment should also be taken into consideration.

2.1.4 Health benefits

Beta-glucans, particularly from barley and oat play an important role in health promotion and prevention of diseases. As a dietary fiber component, beta-glucan cannot be digested in the human body, so it can be used as a prebiotic helping the modulation of the gut microbial composition. Additionally, beta-glucan is able to lower blood cholesterol levels and blood pressure, attenuate blood postprandial glycemic and insulinemic responses, reduce the risk of cardiovascular diseases and cancer, stimulate immune functions, regulate appetite, prevent abdominal obesity, and promote skin health, and such health benefits of beta-glucan have been reviewed recently (Pins and Kaur, 2006; Aleixandre and Miguel, 2008; Khoury et al., 2012; Daou and Zhang, 2012; Du et al., 2014a).

Dietary beta-glucan could prevent central obesity, but the mechanism is still unclear. One possible mechanism could be associated with the effect of beta-glucan intake on appetite. After the intake of beta-glucan, the viscosity of the gastrointestinal chime would be increased, which delays gastric emptying and reduces enzymatic activity and mucosal absorption, thus slows down the absorption of glucose. These physiological effects could lead to early satiety sensations, which helps to decrease the overall energy intake (Jenkins et al., 1978; Isaksson et al., 1982;

Marciani et al., 2001). In addition, the fermentation of beta-glucan in caecum and colon could produce short-chain fatty acids, which are responsible for appetite regulation (Cummings et al., 1987; Topping and Clifton, 2001). The effect on appetite largely depends on the physicochemical properties of beta-glucan, intake dosage and carrier food form. Beverages with 2.5 g or 5 g of oat beta-glucan significantly increased the feeling of satiety (Lyly et al., 2009; Lyly et al., 2010). Increased postprandial satiety was also reported for the beverages with 1.2% w/v barley beta-glucan or 1.6% w/w oat bran (Lumaga et al., 2012; Pentikainen et al., 2014). However, no significant effect on satiety or gastric emptying was observed with beta-glucan enriched meal replacement bars, hot cereal with yogurt or muffins (Kim et al., 2006; Peters et al., 2009; Willis et al., 2009). Kirkmeyer and Mattes (2000) and Khoury et al. (2012) explained that solid or semi-solid foods may mask the satiating potential of beta-glucan, because compared to liquid foods, solid or semi-solid foods could decrease hunger and increase satiety more effectively.

Consumption of beta-glucan could lower fasting and postprandial glucose and insulin response, which was attributed to its viscosity and fermentability. Beta-glucan can increase the viscosity of digesta, which slows down enzyme diffusion and increasing the thickness of the unstirred water layer adjacent to the mucosa, thus decreasing glucose transport to enterocytes (Jenkins et al., 1978). On the other hand, short chain fatty acids produced by fermentation of beta-glucan in the colon are also responsible for a decrease in postprandial plasma glucose and insulin.

The dose of beta-glucan resulting in significant effects varies depending on the subjects. For example, on type 2 diabetic patients, addition of 6.2 g beta-glucan in a bar, 7.3 g in a breakfast cereal and 9.4 g in flour significantly decreased the peak and average increases in glucose and insulin after consumption (Jenkins et al., 2002; Tapola et al., 2005). However, for healthy individuals, incorporation of 1.5, 3 and 6 g of beta-glucan into snack bars did not show any additional glucose-lowering benefits, compared to the control snack bar containing 11.9 g total dietary fiber but 0 g beta-glucan (Panahi et al., 2014). In hypertensive individuals, consumption of oat cereals containing 7.7 g beta-glucan for 12 weeks significantly lowered fasting insulin, while no decrease was observed with oat cereals containing 5.5 g beta-glucan (Davy et al., 2002; Maki et al., 2007). Moreover, 5 g oat beta-glucan per day over a 5-week period and 10 g barley beta-glucan could improve postprandial glycemic and insulin responses in hypercholesterolemic individuals and obese women, respectively (Biorklund et al., 2005; Kim et al., 2009). However, only 4 g of oat beta-glucan was sufficient for healthy subjects to decrease their levels of glucose and insulin (Granfeldt et al., 2008; Hlebowicz et al., 2008). In addition, the carrier food form also plays an important role in glycemic regulation by beta-glucan. Low glycemic foods like rye bread or wheat pasta did not significantly lower postprandial glucose level, because these foods already have a very low glycemic response, which could attenuate the effect of beta-glucan on the postprandial glycemic response (Holm et al., 1992; Juntunen et al., 2002). Similar to the effect of beta-glucan on appetite regulation, beverages would be the best form for the delivery of beta-glucan to achieve the physiological benefits.

Beta-glucan is a dietary fiber component responsible for lowering serum cholesterol, and thus decreasing the risk of cardiovascular disease. Daily consumption of 5 g significantly decreases the serum total and low-density lipoprotein (LDL) cholesterol levels (Naumann et al., 2006; Theuwissen and Mensink, 2007). There are two main probable mechanisms: 1) increased viscosity of digesta could entrap whole micelles containing bile acids by forming a gel-like network, consequently increasing the bile acid excretion, which may have led to an induction of the cholesterol-7-alpha-hydrolase gene, thus lowering the reabsorption of bile acids and accelerating the transportation to the large intestine; and 2) propionate produced by the fermentation of beta-glucan in the colon could inhibit cholesterol synthesis (Zhang et al., 1992; Amaral et al., 1992; Goel et al., 1999; Khoury et al., 2012). Serum cholesterol reduction was reported with the consumption of muffins, cereals, shakes, beverage, bread and ready-meal soup containing 2 to 6 g beta-glucan (Davidson et al., 1991; Biorklund et al., 2005; Reyna-Villasmil et al., 2007; Biorklund and Holm, 2008). However, soup containing 3.5 g of oat beta-glucan and daily consumption of 8.1-11.9 g barley beta-glucan did not show any effect on lowering serum cholesterol levels (Keogh et al., 2003; Cugnet-Anceau et al., 2010). This could be due to the different physicochemical properties, especially MW of beta-glucan obtained from various sources.

The skin health effects of beta-glucan were recently reviewed by Du et al. (2014a). They found that beta-glucan could contribute to antioxidant activity, anti-wrinkle activity, anti-ultraviolet light, wound healing, moisturizing effect and skin permeation absorption.

However, beta-glucans with these functions were mostly from mushrooms, yeast and fungus. Only oat beta-glucan was shown to reduce wrinkle depth and height and overall skin roughness after an 8-week treatment (Pilai et al., 2005a; 2005b).

2.1.5 Applications in food

Concentrated beta-glucans from different cereals have been commercially produced, such as "Oatrim" (oat beta-glucan concentrate), "Nutrim" (barley beta-glucan concentrate) and "Ricetrim" (soluble fibers obtained by co-processing rice bran and barley flour) (Hallfrisch and Behall, 1997; Inglett, 2000; Hallfrisch et al., 2003; Inglett et al., 2004). Other commercially available beta-glucan products were reviewed by Zhu et al. (2016). A daily dose of 3 g oat beta-glucan is recommended by Health Canada to help reduce cholesterol, support healthy glucose metabolism and maintain a healthy digestive system (Health Canada, 2010). In order to reach the daily amount, beta-glucan has been incorporated into breakfast cereals, pasta, noodles and bakery products including muffins, bread, cake and cookies. Additionally, due to its functional properties such as thickening, stabilizing, emulsification and gelation, beta-glucan has been also added into reduced-fat dairy and meat products, beverages, salad dressings and soups. In bread making, incorporation of beta-glucan could result in reduced loaf volume and increased firmness, because large amounts of water could be bound by beta-glucan and the remaining amount of water is not enough for the development of gluten network (Pomeranz et al., 1977). However, this can be overcome by adding gluten and extra water into the formulation (Moriartey et al.,

2011). Moreover, beta-glucan can be used as a fat replacer owing to its high apparent viscosity, water holding capacity and emulsion stabilizing property. Barley beta-glucan was incorporated into reduced-fat breakfast sausages by Morin et al. (2002), who reported that the sausage product containing 0.3% (w/w) beta-glucan was comparable to the high-fat control, which is in agreement with Hughes et al. (1997), who studied the incorporation of oat beta-glucan into low-fat frankfurters. Thammakiti et al. (2004) used beta-glucan from brewer's yeast as a fat replacer in mayonnaise, and finally, they produced reduced fat mayonnaise with low energy content, high water content, but pale and dense appearance. However, such studies on cereal beta-glucans are limited. Recently, oat beta-glucan was used as a partial salt replacer in high pressure processed (400-600 MPa, 40 ºC) chicken breast meat to generate a gel-type product with desired structure (Omana et al., 2011). The color and gel hardness of samples with beta-glucan were comparable to those of NaCl containing samples, and chicken meat with beta-glucan had lower lipid oxidation levels, which were due to the water binding capacity of beta-glucan. In addition, an orange juice containing 0.25-2% (w/w) oat beta-glucan was developed by Lyly et al. (2003), and 0.3-0.7% (w/w) barley beta-glucan was incorporated into an orange-flavored beverage by Temelli et al. (2004). A reduced serum concentration of total and LDL cholesterol was observed by Naumanaa et al. (2006) who incorporated 5 g oat beta-glucan into an apple/pear fruit drink (250 mL). More examples of cereal beta-glucan applied in food and beverage products are listed in Table 2-1.

Table 2-1. Beta-glucan incorporation into various food and beverage products.

Type	Product	Effect along with health benefits	Reference
Oat	Chocolate breakfast flakes	Decrease water activity; Prolong durability; Protect a gut-friendly probiotic bacteria	Saarela et al. (2006)
Oat	Breakfast bar	No negative effect on sensory properties	Jenkins et al. (2002)
Barley	Bread	No negative effect on sensory properties; Reduced starch breakdown	Cavallero et al. (2002); Symons and Brennan (2004)
Oat	Bread	Improved crust color, softness and taste; Increased firmness of the bread crumb	Gormley and Morrissey (1999); Lazaridou et al. (2007)
Nutrim-5 (oat)	Pasta	Improved the overall strength	Inglett et al. (2005)
Nutrim (oat)	Low-fat cheddar cheeses	Softer texture; Decreased melting time; Lower sensory properties	Konuklar et al. (2004)
Oat	White low-fat cheeses	Improved texture; Negatively affected appearance, taste and odor	Volikakis et al. (2004)
Oat	Low-fat ice cream	Function as a fat replacer; Increased the viscosity;	Brennan et al. (2002)
-	Low-fat yogurt	Function as a fat replacer; Increased the viscosity; Decreased the separation of whey	Sahan et al. (2008)
Oat	Probiotic milk-based drinks	Increased stability	Angelov et al. (2006)
Oat	Milk beverage	No effect on sensory properties	Biorklund et al. (2005)
Oat	Beverage	Increased thickness	Lyly et al. (2003)
Barley	Orange-flavored beverage	Increased viscosity	Temelli et al. (2004)

Table 2-1. (cont'd).

Type	Product	Effect along with health benefits	Reference
Oat	Low-fat beef burgers	Improved cooking yield, moisture and fat retention; Better influence on emulsion stability, lightness and hardness	Troy et al. (1999); Pinero et al. (2008); Álvarez and Barbut (2013);
Oat	Low-fat sausages	Reduced cook loss; Increased both water holding capacity and emulsion stability	Hughes et al. (1997)
Barley	Reduced-fat (12%) breakfast sausages	Function as a fat replacer: No significant effect on product texture or flavor if added at the level of 0.3% (w/w)	Morin et al. (2002)
Oat	High pressure processed chicken breast	Function as a partial salt replacer; No effect on color characteristics; Lower lipid oxidation levels	Omana et al. (2011)
Oat	Beverages; Ready-to-eat shrimp soup	Thicker, more extensible, grainier and slimier; Thicker, more powdery and slimy	Lyly et al. (2007)
Oat	Yellow alkaline noodle	Decreased firmness, elasticity, surface smoothness and flavor; Inhibition of linoleic peroxidation in noodles	Choo and Aziz (2010)

2.2 Coenzyme Q10

2.2.1 Sources and structure

Coenzyme Q10 (CoQ10, 2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone) is a natural lipophilic compound that is present in all living cells in humans and most animals,

specifically located in the hydrophobic domain of the phospholipid bilayer of cellular membranes (Lenaz et al., 1999). CoQ10 is a 1,4-benzoquinone linked to a polyisoprenoid side chain consisting of 10 isoprenyl chemical subunits (MW=863.37 Da), as shown in Fig. 2-3. The melting point of CoQ10 at atmospheric pressure is 48-51.3 ^{o}C, and it can be spectrophotometrically measured at 275 nm (Nehilla et al., 2008; Yoon et al., 2014; Zhou et al., 2014; Tarate and Bansal, 2015). The redox active benzoquinone ring gives three alternate redox states of CoQ10: fully oxidized (ubiquinone), semiquinone (ubisemiquinone) and fully reduced (ubiquinol). Therefore, CoQ10 can act as a one-electron carrier between semiquinone and ubiquinone or ubiquinol, and as a two-electron carrier between the fully oxidized form and the fully reduced form. This capacity contributes to its primary biochemical function to participate in the mitochondrial electron-transfer process of cellular respiration and energy production. At the level of mitochondria (Fig. 2-4), CoQ10 works as a mobile electron carrier that shuttles electrons from complexes I and II to complex III, producing ubisemiquinone for the energy conservation at coupling site 2 of the respiratory chain (Mitchell, 1975). Meanwhile, CoQ10 can regulate the opening of transition pores on the mitochondrial membrane to counteract adenosine triphosphate (ATP) depletion, DNA fragmentation and release of cytochrome c into the cytosol (Papucci et al., 2003). In addition to its function in the mitochondria, other functions of CoQ10 have been summarized by Turunen et al. (2004) and Bentinger et al. (2010), including the improvement of endothelial dysfunction by stimulating endothelial release of nitric oxide, anti-atherosclerotic properties like regulation of the amount of beta-2-integrin CD11b (a multiligand macrophage

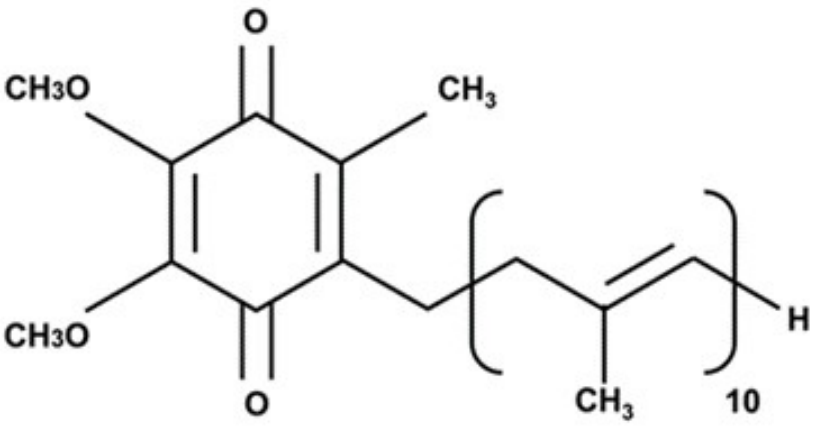


Figure 2-3. The molecular structure of coenzyme Q10, where Q refers to the quinone ring and 10 refers to the number of subunits in its side chain.

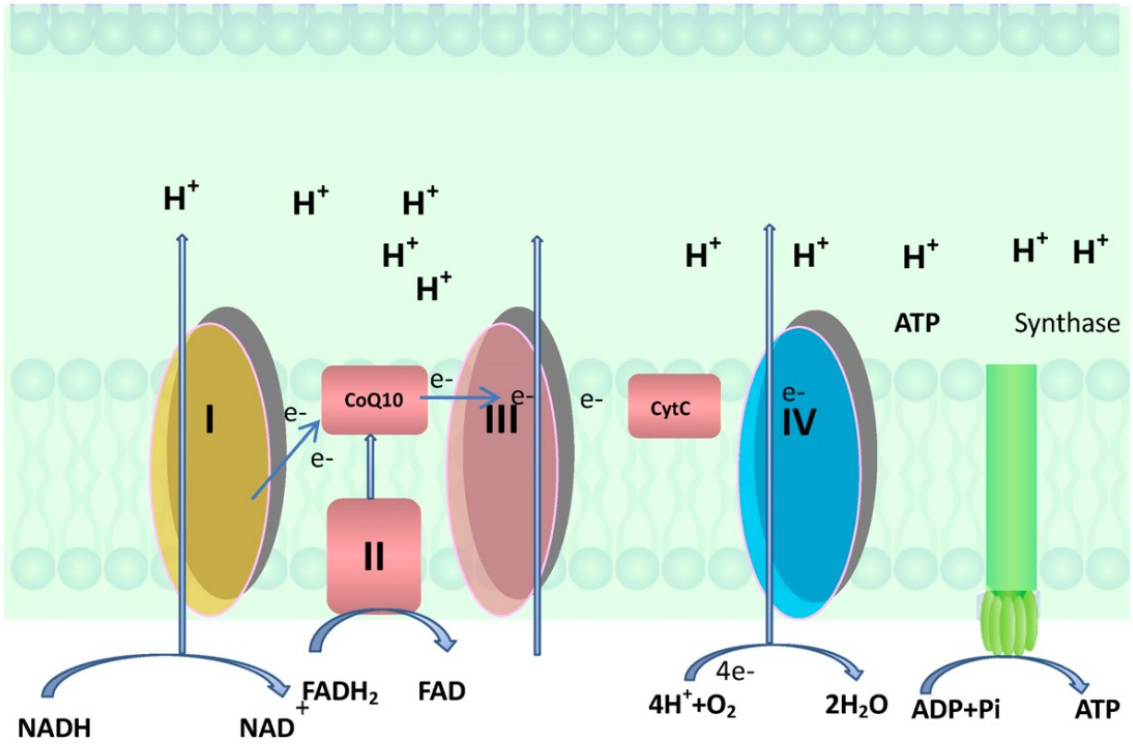


Figure 2-4. CoQ10 acts as an electron acceptor at the level of mitochondria. ADP, adenosine dipohosphate; Cyt c, cytochrome c; Pi, inorganic phosphate. Reprinted from Yang et al. (2015) with permission from Elsevier.

receptor with recognition specificity) on the surface of blood monocytes, anti-inflammatory effects achieved by influencing the expression of NFkB1-dependent genes and acting as endogenous enzyme cofactor for lipid-soluble antioxidant synthesis.

Since CoQ10 is an obligatory ingredient in the formation of ATP, it can be synthesized in the human body, and mainly found in the most active organs like heart (114 μg/g), kidney (67 μg/g), liver (55 μg/g), muscle (40 μg/g) and pancreas (33 μg/g) (Kalen et al., 1989; Aberg et al., 1992). More than 95% of the CoQ10 in the body is present in its reduced form (ubiquinol), which can be explained by its antioxidant property; however, CoQ10 present in the lungs and brain is predominantly in the oxidized form (Aberg et al., 1992; Challem, 2005). The amount of CoQ10 in the body increases with age until the age of 20 years, and then gradually decreases. Generally, the decrease would be more evident in mitochondria, rather than in the homogenate of human tissues (Sohal and Forster, 2007). In total, there are around 2 g CoQ10 present in an adult human body, but 0.5 g would be replaced daily by endogenous synthesis or food intake (Kalen et al., 1989).

Various amounts of CoQ10 can be found in meats and processed meat products, fish and shellfish, eggs, oils, cereals, pulses and their processed products like tofu and some vegetables like parsley, but it is rarely found in most fruits and berries. An overview of the CoQ10 content of various foods was provided by Pravst et al. (2010). CoQ10 is high in oils, meats and fishes, due to its lipophilic property. In oils, CoQ10 amount ranges from 109 to 279 mg/kg in commercially available oils like soybean, corn and olive oils. In meats, the highest CoQ10

amount could be found in pork heart (118.1-282 mg/kg), and then chicken heart (92.3-192 mg/kg), followed by beef heart (113.3 mg/kg), since heart is one of the most active organs. In fish and shellfish, the CoO10 content is in the range of 0.3 to 148.4 mg/kg depending on the species. Additionally, CoQ10 can be found in most dairy products like cheese, butter, yogurt and milk, but in very low amounts ranging from 0.3 to 7.1 mg/kg. Among these dairy products, butter is the richest source of CoQ10 (7.1 mg/kg), which is probably due to its high fat content. For the same reason, avocado is high in CoQ10, 9.5 mg/kg, but most fruits have very low concentration of CoQ10. Therefore, foods with high levels of fat and mitochondria can be a great source of CoQ10. However, geographical origin should be also taken into consideration when determining the CoQ10 content of various foods (Pravst et al., 2010).

To obtain more precise values on the CoQ10 content of various sources, analytical methods have been under development over the past decade. In 2004, a reversed-phase high performance liquid chromatography (HPLC) with coulometric detection method was developed for measuring mouse tissue concentrations of reduced and oxidized CoQ10 (Tang et al., 2004). This method was accurate, sensitive and reproducible with the detection limit from 15 μg/L to 20 mg/L and the analytical recoveries of 90-104%. Using this method, Tang et al. (2004) found that the reduced form of CoQ10 was mostly present in mouse liver, heart and muscle tissues, while the oxidized form was mainly present in the brain, which is comparable to previous studies. Later, a liquid chromatography tandem mass spectrometry with electron spray ionization (ESI) method was studied by Ruiz-Jimenez et al. (2007) to rapidly and simultaneously determine the reduced

and oxidized forms of CoQ10 in human serum without any alteration. This method had very low quantification limits for oxidized (5.49 ng/mL) and reduced (15.80 ng/mL) CoQ10. By applying this quantification method, it was shown that the ratio of reduced CoQ10 to oxidized CoQ10 could be used as a marker to evaluate the oxidative stress in healthy middle-aged women (Ruiz-Jimenez et al., 2007). Recently, HPLC with electrochemical detection and Fourier Transform Infrared (FT-IR) spectrophotometry were also applied to determine the CoQ10 status in swine tissue and CoQ10 pharmaceutical formulations, respectively (Niklowitz et al., 2013; Bunaciu et al., 2015).

2.2.2 Health benefits

CoQ10 is recommended by Health Canada for maintaining cardiovascular health and migraine prophylaxis with the daily consumptions of 30-100 mg and 75-100 mg, respectively (Health Canada, 2007). The physiological benefits of CoQ10 are based primarily on its antioxidant property, which continues to attract research interest. Cardiovascular disorder is generally considered to be a result of impaired mitochondrial function and oxidative stress. CoQ10, an electron carrier at the level of mitochondria, could act as an antioxidant to slow down the progression of this disease.

Oxidative stress is the damage on the basic cellular constituents like lipids, proteins and DNA, caused by reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$), where ROS are produced by the reduction of

oxygen via enzymatic processes or non-enzymatic pathways (Ernster, 1993; Bentinger et al., 2007). Generally, three main steps are involved in lipid oxidation: 1) initiation with the production of alkyl radical ($L^{\bullet}$) and peroxyl radical ($LOO^{\bullet}$); 2) propagation with the production of alkyl radical ($L^{\bullet}$) and lipid hydroperoxide (LOOH), which can regenerate alkoxyl radical ($LO^{\bullet}$) and peroxyl radical ($LOO^{\bullet}$); and 3) termination with the production of a stable lipid alcohol (LOH) (Ernster, 1993). CoQ10 in fully reduced form could effectively inhibit the formation of lipid peroxyl radicals during initiation, producing ubisemiquinone and H_2O_2. Similarly, in protein oxidation, CoQ10 works as a chain-breaking antioxidant to prevent propagation by quenching the initiating perferryl radicals (Bentinger et al., 2007). Furthermore, CoQ10 is also effective in preventing DNA oxidation, particularly DNA in mitochondria, by decreasing the amount of ADP-Fe^{3+}, which is responsible for DNA strand breaks (Ernster and Dallner, 1995; Bentinger et al., 2007).

Oxidative stress has a significant effect on the development of myocardial structural and functional abnormalities, and consequently influences cardiac function, by producing renin-angiotensin-aldosterone system (RAAS) via the interaction between ROS and protein, DNA and cell membranes (Bergamini et al., 2009; Yang et al., 2015). However, due to the effect of the antioxidant activity on the initiation process, CoQ10 can effectively prevent the development of diastolic dysfunction and the activation of apoptosis, and subsequently has an alleviating effect on cardiovascular disorder (Rocha et al., 2002; Groneberg et al., 2005). In addition, since chronic cardiovascular disease is usually associated with chronic inflammation,

the alleviating effect on the disease indicates the anti-inflammatory effect of CoQ10. This effect was explained as the result of decreasing the level of nitric oxide by Jun et al. (2009). Moreover, nitric oxide also contributes to the development of arterial hypertension, so CoQ10 also plays an important role in the regulation of blood pressure (Lyamina et al., 2011; Yang et al., 2015). Furthermore, CoQ10 was found to enhance bone regeneration through transcription factor activity and have an inhibitory effect on osteoclastogenesis by scavenging the intracellular ROS, indicating the potential for its application in the treatment of osteoporosis and other bone diseases associated with excessive bone resorption (Moon et al., 2013). Despite all these benefits, the amount of CoQ10 in the human body declines with aging and the average daily intake from the diet is just around 3-5 mg, which is substantially lower than the recommended daily amount of 30-100 mg (Weber et al., 1997).

In order to respond to the recommended amount, CoQ10 supplements and CoQ10-enriched food products are on the rise in the market. Most commercial formulations are in the form of soft gel, hard capsules, tablets and powder, while some are in syrup form. Schulz et al. (2006) evaluated five supplements where CoQ10 is in crystalline powder, oil dispersions and solubilizates encapsulated in hard gelatin or soft gel capsules. They found that all formulations resulted in higher CoQ10 plasma level, but a novel Solu^{TM} Q10 solubilizate had the best bioavailability and absorption characteristics. Additionally, in order to optimize the functionality of CoQ10, Evans et al. (2009) encapsulated reduced CoQ10 (CoQH-CF) in soft gel and compared the plasma concentration in individuals over 60 years of age, with those who were

treated with a commercial formulation (CoQ10 hard capsule). As a result, significantly higher plasma concentration of CoQ10 was found in the group who took CoQH-CF soft gels. Since CoQ10 was well solubilized in the soft gel, the rate of absorption in the intestine significantly increased, consequently resulting in greater bioavailability. Therefore, solubilized reduced-CoQ10 in soft gel would be the best source for CoQ10 supplements to meet the daily demand.

CoQ10 can be also incorporated into food and beverage products to enhance the intake. Ercan and El (2012) developed CoQ10-enriched yogurt where CoQ10 was in the form of emulsified CoQ10, γ-cyclodextrin/CoQ10 complex or nanoparticles, and found that the yogurt fortified with CoQ10 nanoparticles had the highest bioavailability (73.81%), indicating the potential application of nano-technology in food product development. Furthermore, grapefruit juice was reported to inhibit the activity of P-glycoprotein which works as a medium for efflux transportation of CoQ10 in the human intestinal cell line; therefore, the combination of CoQ10 and grapefruit juice could increase the uptake of CoQ10 (Itagaki et al., 2010).

2.3 Delivery systems involving CoQ10 and beta-glucan

In pure form, CoQ10 is a thermosensitive crystalline powder with low aqueous solubility (< 4 ng/mL) and high molecular weight (863 Da), which limits its incorporation into foods and presents challenges for its oral delivery (Swarnakar et al., 2011). In order to increase its water solubility and protect CoQ10 from degradative effects, a number of different approaches have

been developed, including oily solution, emulsion, cyclodextrin complexes, solid dispersions, micro/nano-encapsulation (bioactive coated by a polymer), impregnation (bioactive loaded onto a polymer) and a self-emulsifying drug delivery system and others (Kommuru et al., 1999; Terao et al., 2006; Bhandari et al., 2007; Onoue et al., 2012). Initially, various oils, surfactants and co-surfactant were used to synthesize self-emulsifying drug delivery systems of CoQ10 to improve its bioavailability. However, there are safety concerns associated with the use of some surfactants. Therefore, Zhou et al. (2014) developed a novel lipid-free nano-formulation to minimize the surfactant content. In this study, CoQ10 powder was melted and dissolved into glycerol aqueous solution with 1 g surfactant (Kollidon 30) to produce a CoQ10-suspension, which was then characterized in terms of particle size, zeta potential, particle morphology, physical state of CoQ10 in the particle and *in vivo* bioavailability of CoQ10 in rats. In the end, nano-scale CoQ10 was obtained with the particle size of 66-93 nm, and the CoQ10 in the particle was in a supercooled state, which provided physicochemical stability without crystallization. The results obtained by Zhou et al. (2014) indicated that this novel lipid-free system significantly enhanced the plasma concentration, and could be used for improving oral bioavailability of CoQ10. Although the above lipid-free nano-formulation succeeded in bioavailability improvement, the presence of 1 g surfactant raises concerns about potential toxicity, and thus polymeric nanoparticles without surfactant have been investigated. Among various polymers, starch and dextrin have been successfully applied for stabilizing dispersions of CoQ10 in water, due to their hydrophilic property (Moldenhauer and Cully, 2003). In addition, poly

(lactic-co-glycolic acid) (PLGA) draws increasing attention due to its biocompatibility, biodegradation and non-toxicity. Nehilla et al. (2008) produced CoQ10-loaded PLGA nanoparticles with the particle size of sub-200 nm and 50% entrapment efficiency (EE) by a nano-precipitation method. These nanoparticles were stable for 2 months with a 2-week steady CoQ10 release, which indicates the potential implication as a sustained drug delivery system. Moreover, CoQ10-loaded PLGA particles (CoQ10-NP) with smaller size (~100 nm) and higher EE (~92%) were obtained using the emulsion-diffusion-evaporation technique by Swarnakar et al. (2011). CoQ10 inside the CoQ10-NP was present in amorphous form rather than the crystalline form based on the characteristic peaks of pure CoQ10, PLGA, CoQ10-NP and a physical mixture of PLGA and CoQ10 in X-ray diffraction (XRD) patterns (Swarnakar et al., 2011). In an animal study, CoQ10-NP showed improved oral bioavailability and higher anti-inflammatory activity as compared to the free CoQ10.

Considering the high manufacturing cost and poor industrial scalability, alternative cost-effective liposomal formulations have been successfully developed by Xia et al. (2006; 2012). Liposomes are hydrophilic vesicles with phospholipid bilayers surrounding an aqueous core. The unique structure enhanced the absorption of water-insoluble nutrients from the intestinal lumen fluids into enteric cells (Keller, 2001). By applying ethanol injection and sonication, CoQ10 nano-liposomes were produced with a size of 68 nm and greater than 95% EE, which was relatively stable at 4 ^{o}C in the dark for 90 days. Also, this method has been scaled up to a pilot plant level to produce CoQ10 nano-liposomes in a large quantity with the same quality

and stability obtained in the laboratory (Xia et al., 2006). Another method that was used to produce CoQ10 proliposomes was supercritical anti-solvent (SAS) technique, which is known as a non-toxic and environmentally friendly process. This technique was used to obtain CoQ10 loaded liposomes in powder form at mild conditions (35 ^{o}C, 8 MPa), so that particles would be produced with good quality (particle size of 50 nm; 82.28% EE) and less thermal damage (Xia et al., 2012).

Overall, liposomal aggregation, κ-carrageenan coated oil-in-water emulsion, maize starch dispersion and nano-encapsulation with octenyl succinic anhydride modified starch have been successfully applied to increase the water-dispersibility of CoQ10 as summarized in Table 2-2. However, only a limited number of studies evaluated the use of beta-glucan as a carrier (Table 2-2). As discussed previously, beta-glucan is a linear polysaccharide with a large molecular weight, providing health benefits mainly on the regulation of blood glucose level, serum cholesterol level and blood pressure. All these properties indicate the great potential of beta-glucan for use as an encapsulating material in particle formation. Generally, previous studies have modified cereal beta-glucans into aerogels or micelles, before using them for encapsulation or impregnation, owing to their unique structures. Micelles, also called nano-scale core-shell self-aggregates, whose structure is like liposomes, consists of a lipophilic core for capturing lipophilic ingredients and a hydrophilic shell for dispersing in aqueous media (Owen et al., 2012). Liu et al. (2013) generated octenylsuccinate oat beta-glucan (OSG) micelles by

Table 2-2. Particle formation studies with CoQ10 as the bioactive or beta-glucan as the carrier in the past decade.

Carrier	Bioactive	Technique	Particle size	EE[a]	Ref.
Glyceryl monooleate; phytantriol	CoQ10	Modified solvent diffusion-evaporation method	140.45 nm; 238.42 nm	>90%	Swarnakar et al. (2014)
PLGA	CoQ10	Nano-precipitation method	>200 nm	50%	Nehilla et al. (2008)
PLGA	CoQ10	Emulsion-diffusion-evaporation technique	<100 nm	91.97%-92.43%	Swarnakar et al. (2011)
Gum Arabic (GA); Modified starch (MS); Maltodextrin (MD)	CoQ10	Emulsion and spray drying	14.05-20.69 μm	46.22%-85.06%	Bule et al. (2010)
Lipid (glycerol monolaurate: acetylated mono- and diglycerides: octyl and decyl glycerate=3:2:3)	CoQ10	Hot high-pressure homogenization	190 nm	98.4%	Liu et al. (2012)
Glycerol	CoQ10	Hot high-pressure homogenization	66.3-92.7 nm	-	Zhou et al. (2014)
Amylomaize starch; Dextrin	CoQ10	Dispersion	5-7 μm; 80-250 nm after ultrasonication	-	Kim et al. (2012)
Maize starch	CoQ10	Dispersion	<150 nm	9.8%-57.3%	Yoon et al. (2014)
Phospholipid and cholesterol	CoQ10	Ethanol injection and sonication techniques	67 nm	>95%	Xia et al. (2006)

Table 2-2. (cont'd)

Carrier	Bioactive	Technique	Particle size	EE[a]	Ref.
Cholesterol and soya bean phosphatidylcholine	CoQ10	Supercritical anti-solvent (SAS) technique	50 nm	82.28%	Xia et al. (2012)
Octenyl succinic anhydride modified starch	CoQ10	High pressure homogenisation	200-300 nm	98.2%	Cheuk et al. (2015)
Octenylsuccinate oat beta-glucan (OSG) micelles	Curcumin	Suspension and homogenization	308 nm	4.21μg/mg[b]	Liu et al. (2014)
Octenylsuccinate oat beta-glucan (OSG) micelles	Beta-carotene	Suspension and homogenization	~400 nm	>85%	Ma et al. (2016)
Barley beta-glucan	Probiotic bacterial cultures[c]	Emulsion	-	74.01%	Shah et al. (2016)
Barley beta-glucan; lecithin	Resveratrol; Tebuconazole	Emulsion with spray drying, particle from gas saturated solutions-drying (PGSS-drying) or supercritical anti-solvent (SAS)	10-100 μm	57-94%	Salgado et al. (2015)
Barley beta-glucan aerogels	Flax oil	Supercritical CO_2 drying	-	47.79%-60.96%	Comin et al. (2012)

[a] $Entrapment\ Efficiency\ (EE) = (Entraped\ CoQ10/Total\ CoQ10) \times 100\%$;

[b] $Curumin\ loading\ capacity\ (\mu g/mg) = mass\ of\ loaded\ curcumin\ in\ 1\ mL\ OSG\ solution\ (\mu g)/mass\ of\ used\ OSG\ in\ 1\ mL\ OSG\ solution\ (mg)$;

[c] Probiotic bacterial cultures *Lactobacillus plantarum* (NCDC 012), *Lactobacillus casei* (NCDC 297), and *Lactobacillus brevis* (NCDC 021)

incubating oat beta-glucan with 2-octen-1-ylsuccinic anhydride at 45 ºC for 2 h. By adjusting the volume of 2-octen-1-ylsuccinic anhydride, OSG with different degrees of substitution was obtained. Then, this micelle was used for impregnating curcumin and beta-carotene by homogenization (Liu et al., 2014; Ma et al., 2016). Both of these studies indicated that molecular weight and the degree of substitution of OSG as well as the input power during stirring were the key parameters for determining the solubility and loading capacity of the bioactive. Moreover, barley beta-glucan aerogels were investigated for carrying flax oil via $SCCO_2$ drying by Comin et al. (2012). In this study, hydrogels were first turned into alcogels, and then ethanol was removed by $SCCO_2$ to form aerogels. Due to the rapid removal of ethanol under supercritical conditions, aerogels maintained the network structure of the original hydrogels, and were high in porosity but very low in density (Mehling et al., 2009; Haimer et al., 2010; Comin et al., 2012). In the end, flax oil impregnated beta-glucan aerogel was obtained with the loading capacity of 65.39% via a dynamic $SCCO_2$ impregnation process at 40 ºC and 15 MPa. Other than optimizing the pressure, temperature and process type (dynamic or static), oil loading could be also increased by adjusting the flow rate and the point of oil addition during the process (Comin et al., 2012).

Recently, barley beta-glucan was applied to encapsulate probiotic bacterial cultures (74.01% EE) by emulsion to protect probiotics from gastrointestinal tract conditions (Shah et al., 2016). An improvement on tolerance to heat treatment and simulated gastrointestinal conditions and storage was consequently found with the encapsulated probiotics (Shah et al., 2016). In addition,

linear beta-1,3-glucan from seaweeds was used to encapsulate resveratrol for the improvement of its anti-fungal property (Salgado et al., 2015). The effect of three different drying methods including spray drying, particle from gas saturated solutions-drying (PGSS-drying) and SAS drying, on particle characteristics was determined. PGSS-drying and SAS drying both are based on supercritical fluid technology, which are performed at a lower temperature than spray drying. In PGSS-drying, a suspension containing a bioactive and a polymer is firstly saturated with $SCCO_2$ until equilibrium is reached. Then, particles are formed upon sudden decompression through a nozzle. In SAS, polymer and bioactive are firstly solubilized or dispersed into an organic solvent, which should be highly soluble in $SCCO_2$. When the solution or suspension is sprayed via a nozzle together with $SCCO_2$, the organic solvent gets saturated with $SCCO_2$, and consequently the solute precipitates to form particles (Rodríguez-Rojo, 2012). However, SAS did not result in particle formation, while encapsulated particles in amorphous state were obtained with both spray drying and PGSS-drying (Salgado et al., 2015). In addition, resveratrol in amorphous state was identified as having enhanced antifungal activity against *Botrytis cinerea*. Therefore, beta-glucan could be utilized as an encapsulating agent to protect bioactives from conditional stress, improve the functionality and enhance their bioavailability.

Pressurized gas-expanded liquid (PGX) technology, invented by Temelli and Seifried (2016), is a supercritical fluid technique used for producing micro-/nano-particles, agglomerates and fibers from an aqueous solution of a high molecular weight biopolymer, which could also be applied for encapsulation or impregnation. Briefly, the process is based on spraying a mixture of

the aqueous biopolymer solution, compressible gas (i.e. carbon dioxide) and a water soluble co-solvent/antisolvent (i.e. ethanol, acetone or isopropanol) into a pre-pressurized chamber. Beta-glucan obtained by PGX drying maintains its native linear structure and high molecular weight, and shows high water-solubility, which could be used as an encapsulating material to carry CoQ10 for protection from the environmental conditions such as heat, light and oxygen and improvement of bioavailability. Moreover, impregnation with PGX technology could be performed at mild conditions (32-80 ºC, 8-40 MPa) and generate products in powder form, so that impregnated particles would have lower risk of degradation upon long-term storage, transportation and thermo-degradation, compared to other particle formation methods like spray drying or emulsion. Furthermore, to the author's knowledge, there are no studies that use oat beta-glucan as the carrier to impregnate CoQ10. Therefore, PGX beta-glucan and PGX-produced CoQ10 impregnated beta-glucan are the focus of investigation in the following chapters in terms of their physicochemical properties and their potential utilization in functional beverage development.

Chapter 3: Characterization of beta-glucan and coenzyme Q10-impregnated beta-glucan generated by pressurized gas-expanded liquid (PGX) technology

3.1 Introduction

Coenzyme Q10 (CoQ10) is an ubiquinone, known as a natural antioxidant present in both animal and plant cellular membranes (Lenaz et al., 1999). Pure CoQ10 is an orange colored crystalline powder, which is sensitive to environmental conditions like light, oxygen and heat. CoQ10 is recommended by Health Canada for maintaining cardiovascular health and migraine prophylaxis with the daily consumption of 30-100 mg and 75-100 mg, respectively (Health Canada, 2007). However, the amount of CoQ10 in the human body declines with aging, while the daily intake from the diet is only around 3-5 mg, which is much less than the recommended daily amount (Weber et al., 1997). Therefore, CoQ10 supplements in the form of soft gel, hard capsules and powder, as well as CoQ10-enriched foods like CoQ10-enriched yoghurt and grapefruit juice have been introduced in the market. However, due to its lipophilicity and high molecular weight, CoQ10 is insoluble in water, which when combined with its crystalline nature decreases its bioavailability and limits the fortification in aqueous foods. Alternatively, a water-soluble biopolymer can be used as a carrier to impregnate or encapsulate CoQ10 to increase its dispersibility in water. Recently, liposomal aggregation, κ-carrageenan coated oil-in-water emulsion, maize starch dispersion and nano-encapsulation with octenyl succinic anhydride modified starch have been successfully applied to increase the water solubility of CoQ10, but these products were in liquid form, which would create limitations upon long-term

storage, transportation and food applications (Xia et al., 2006; Chan et al., 2013; Yoon et al., 2014; Cheuk et al., 2015). An alternative approach is to impregnate CoQ10 onto beta-glucan by using pressurized gas-expanded liquid (PGX) technology, which was invented by Temelli and Seifried (2016).

Beta-glucan is a soluble dietary fiber mainly found in oat and barley, which plays an important role in health promotion and prevention of diseases, as discussed in Chapter 2. Recently, oat beta-glucan micelles and barley beta-glucan aerogels have been used as a delivery system for curcumin, beta-carotene, probiotic bacterial cultures and flax oil (Comin et al., 2012; Liu et al., 2014; Ma et al., 2016; Shah et al., 2016). However, the literature lacks information on the use of oat beta-glucan as a carrier for the delivery of CoQ10. Such a combination will provide the health benefits of both beta-glucan and CoQ10. For successful delivery of such an ingredient, it is essential to characterize its properties. Better understanding of the properties and functionality will lead to its incorporation into various product formulations. Therefore, the objective of this study was to determine the physicochemical properties of beta-glucan powder (BG) and CoQ10-impregnated beta-glucan (iBG) obtained using the PGX process, in terms of its proximate composition, morphology, impregnation yield, melting behavior, crystallinity, viscosity, surface area and pore size.

3.2 Materials and Methods

3.2.1 Materials

Samples of BG and iBG were provided by Ceapro Inc (Edmonton, AB, Canada). BG (batch no. 15-E1) was food-grade, which was extracted from oat and dried using the PGX process. iBG (batch no. 15-D1) was obtained by impregnating the BG with CoQ10 in a second step using supercritical carbon dioxide ($SCCO_2$). Hexane, purchased from Fisher Scientific (Ottawa, ON, Canada), was used as the solvent in CoQ10 loading and lipid content determinations. Anhydrous ethanol (Sigma-Aldrich, St. Louis, MO, USA) was used to disperse particles in beta-glucan content determination. High viscosity oat beta-glucan (HBG, >94% purity, molecular weight (MW) of 361,000 Da, Megazyme Pty. Ltd., Chicago, IL, USA) was used as the reference for comparison in the viscosity measurements. Beta-glucan assay kit (mixed linkage) including lichenase, beta-glucosidase, GOPOD reagent buffer, GOPOD reagent enzymes and D-glucose standard solution, was purchased from Megazyme Inc. (Chicago, IL, USA) to determine the purity of BG. Nitrogen with a purity of 99.998% that was used in lipid content determination was purchased from Praxair (Edmonton, AB, Canada). CoQ10 powder (98.34% purity) was purchased from PureBulk (Roseburg, OR, USA). Sodium azide ($\geq 99.5\%$ purity) from Sigma-Aldrich (Oakville, ON, Canada) was used to prevent microbial growth in beta-glucan solutions.

3.2.2 Proximate analysis

Proximate analysis was performed on the BG sample using standard methods to determine its moisture, protein, lipid, ash and carbohydrate contents. All measurements were done in triplicate.

3.2.2.1 Moisture content

The moisture content was determined according to the AACC method 44-16 (AACC International, 2000a). A sample of BG (around 1 g) was dried in an oven (Model 655G, Isotemp Oven, Fisher Scientific, Ottawa, ON) at 105 ^{o}C for 2 days, and the moisture content was calculated using the following equation:

$$Moisture\ \% = \left(\frac{Fresh\ sample\ weight - Dried\ sample\ weight}{Fresh\ sample\ weight}\right) \times 100\% \quad (3.1)$$

3.2.2.2 Protein content

The protein content was determined according to the AACC method 46-09 (AACC International, 2000b). A sample of BG (around 0.1 g) was made into a pellet, and then its nitrogen content was determined using the Leco TruSpec N/C analyzer (Leco Corp., St. Joseph, MI, USA). Protein content was calculated by using a conversion factor of 5.8 (i.e., nitrogen conversion factor for wheat and most cereals) (Merrill and Watt, 1973).

3.2.2.3 Lipid content

The method of determining lipid content of BG was modified from Baümler et al. (2010). A sample of BG (2-3 g) and hexane (40 mL) were mixed in a 50-mL test tube with a cap. Test tubes were stored in the dark for 1-2 days with occasional shaking to extract the lipids. Then, test tubes containing the sample and hexane were centrifuged (J-6M centrifuge, Beckman Coulter, Mississauga, ON, Canada) at 1500 rpm for 10 min. Around 20 mL of supernatant was transferred into a pre-weighed vial, and dried under nitrogen. The weight difference between the empty vial and the vial after nitrogen drying was recorded as the lipid content. Hexane (40 mL) without sample was used as the blank.

3.2.2.4 Ash content

The ash content was determined according to the AACC method 08-01 (AACC International, 2000c). A sample of BG (around 0.5 g) was burnt in a muffle furnace (Barnstead/Thermolyne® Muffle Furnaces, Waltham, MA, USA) at 600 ºC for 7 days, and the ash content was calculated according to the following equation:

$$Ash\ \% = \left(\frac{Burnt\ sample\ weight}{Fresh\ sample\ weight}\right) \times 100\% \quad (3.2)$$

3.2.2.5 Carbohydrate content

Carbohydrate content was determined by difference according to the following equation:

$$Carbohydrate\ \% = 100\% - Ash\% - Moisture\% - Lipid\% - Protein\% \quad (3.3)$$

3.2.3 Beta-glucan content

The purity of BG was determined according to the AACC method 32-23.01 (AACC International, 2000d), using the beta-glucan assay kit (Megazyme Inc., Chicago, IL, USA). Around 0.03 g BG was mixed with 1 mL 50% (v/v) ethanol and 5 mL sodium phosphate buffer (pH=6.5) and incubated in boiling water for 10 min. The cooled mixture was mixed with 4 mL water and 0.2 mL lichenase. After one-hour digestion at 50 ºC in a water bath (WNB 29, Memmert, Schwabach, FRN, Germany), 20 mL water was added to adjust the total volume to 30 mL. An aliquot of 2 mL was centrifuged (accuSpin^{TM} Micro, Fisher Scientific, Ottawa, ON, Canada) at 7500 rpm for 5 min. Then, 100 µL of supernatant was mixed with 100 µL beta-glucanase in sodium acetate buffer (pH=4.0, 50 mM) and incubated at 50 ºC for 20 min. Another 20-min incubation at 50 ºC was applied after adding 3 mL GOPOD reagent. Absorbance of the solution was determined using a spectrophotometer (Model 6305, Jenway, Stone, SFD, UK) at 510 nm. The sample was prepared in quadruplicate, and the sample prepared without beta-glucanase was treated as the blank. The beta-glucan content (% w/w) was calculated according to the following equation:

$$Beta-glucan\ (\%\ w/w) = \Delta A \times \frac{F}{W} \times 27 \quad (3.4)$$

where, ΔA is the absorbance after beta-glucanase treatment (reaction) minus reaction blank absorbance, W is the weight of the sample in mg and F is the factor for the conversion of absorbance values to µg of glucose (100 µg D-glucose / absorbance of 100 µg D-glucose), which is 1.065 in this study.

3.2.4 Particle size and morphology

Particle size of both BG and iBG was determined using the Mastersizer 3000 laser diffraction particle size analyzer (Malvern, UK) equipped with an Aero S dry powder dispersing system. Results were expressed as D(50) and span that is defined as [D(90)-D(10)]/D(50), where D(10), D(50) and D(90) indicate diameters at the 10^{th}, 50^{th} and 90^{th} percentiles of the particle size distribution, respectively. Helium ion microscopy (HiM) analysis of BG and iBG particles was performed on a Zeiss Orion NanoFab Helium Ion Microscope (Ostalbkreis, BW, Germany). Secondary Electron (SE) images were collected at 30 kV accelerating voltage and 1.5 pA beam current. An electron flood gun was utilized to neutralize positive charges accumulated on the sample surfaces, which enables direct imaging of insulating materials.

3.2.5 Surface area and pore size analysis

The surface area and pore size of both BG and iBG were determined using AutoSorb iQ (Quantachrome Instruments, Boynton Beach, FL, USA) based on nitrogen adsorption/desorption isotherm. Samples (around 0.2 g) were degassed at room temperature (around 25 ^{o}C). Measurements were done in duplicate. Data were analyzed by AutoSorb iQ software using DFT/Monte-Carlo method.

3.2.6 Differential scanning calorimetry (DSC) analysis

DSC analysis of the BG, CoQ10, iBG and a physical mixture (PM) of CoQ10 (4% w/w) and BG was performed using a DSC Q2000 system (TA Instruments, Mississauga, ON, USA) calibrated with indium standards. Around 3-4 mg sample was weighed into an aluminum pan. Empty pans were treated as blank. Measurements were carried out at the heating rate of 10 ºC/min from 0 to 200 ºC. Data interpretation was done using Advantage software.

3.2.7 X-ray diffraction (XRD) analysis

The XRD patterns of BG, CoQ10, iBG and the 4% physical mixture (PM) were investigated using a Rigaku Geigerflex Powder Diffractometer (Ultima IV, Rigaku, Tokyo, Japan) equipped with a cobalt tube, graphite monochromator and scintillation detector. Measurements were carried out at a voltage of 38 kV and 38 mA. Samples were continuously scanned from 5 to 90 degrees at 2 degrees 2θ per minute with a step size of 0.02. Data interpretation was done using JADE 9.6 software.

3.2.8 CoQ10 loading content

iBG (20 mg) was mixed with 10 mL hexane and then incubated in a water bath at 58 ºC for 15 min. The mixture was then centrifuged (accuSpin^{TM} 400, Fisher Scientific, Ottawa, ON, Canada) at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 270 nm using a UV spectrophotometer (Model 6305, Jenway, Stone, SFD, UK) to determine the

concentration of CoQ10 based on the 9-point standard curve (0.008, 0.012, 0.02, 0.035, 0.06, 0.07, 0.098, 0.12, 0.14 mg/mL) established using pure CoQ10. Measurements were done in quadruplicate. The R^2 value of the standard curve was 0.99556. Absorbance maximum for CoQ10 in hexane was determined to be at 270 nm after scanning the range from 200 to 700 nm using a UV spectrophotometer (SpectraMax®M3 Multi-Mode Microplate Reader, Molecular Devices, Sunnyvale, CA, USA). The CoQ10 loading percentage was calculated as follows:

$$CoQ10\ loading\ \% = \frac{CoQ10\ amount\ (mg)}{iBG\ amount\ (mg)} \times\ 100\% \quad (3.5)$$

3.2.9 Viscosity

The viscosity of BG, iBG and HBG solutions were determined using a rheometer (Discovery HR-1, TA Instrument, Mississauga, ON, USA) with a cup (Peltier Concentric Cylinder) and rotor attachment, over a shear rate (γ) range of 1.29-129 s^{-1} at temperatures of 4 ºC and 25 ºC. The shear rate range was selected according to Ghotra et al. (2009). The temperature effect on viscosity was evaluated at a fixed shear rate of 50 s^{-1} over a temperature range of approximately 0-80 ºC. Solutions (25 mL) were prepared in duplicate at different concentrations as listed in Table 3-1. HBG, BG and iBG samples required different extents of boiling at 97 ºC with stirring on a hot plate (Model SP131325, Barnstead/Thermolyne Cimarec® Digital Hot Plates, Thermo Fisher Scientific, Ottawa, ON, Canada) to ensure complete dissolution as indicated in Table 3-1. The weight of solution was adjusted back to the initial level by adding water to make up for evaporative losses during the heating step. Then, 0.02% (w/w) sodium azide was added to

prevent microbial growth. Prior to viscosity measurements, a two-piece cover was placed on the cup edge to prevent evaporative loses during measurement.

Table 3-1. Solution preparation for viscosity measurements

Sample	Mass (g)	Water (mL)	Solution solids conc. (%, w/v)	Actual beta-glucan conc. (%, w/v)*	Heating time (min)
HBG	0.0375	25	0.15	0.14	0.5
	0.05	25	0.2	0.19	0.5
	0.075	25	0.3	0.28	0.5
BG	0.0375	25	0.15	0.13	10
	0.05	25	0.2	0.17	10
	0.075	25	0.3	0.25	10
iBG	0.0375	25	0.15	0.13	1
	0.05	25	0.2	0.17	1
	0.075	25	0.3	0.25	1

* Actual beta-glucan concentration was calculated by multiplying the solids concentration in solution by the purity of the sample, which is 83.6% for BG and iBG, and 94% for HBG.

3.2.10 Color

BG and iBG powder and 0.2% (w/v) solutions of each were used for color measurement, according to the ASTM D2244 method (ASTM, 2011). A Hunter Lab colorimeter (CR-400/CR-410, Konica Minolta, Ramsey, NJ, USA) with a D65 illuminant with an opening of 14 mm and a 10° standard observer was used to determine the values of L (0 = black, 100 = white), a (negative = green, positive = red) and b (negative = blue, positive = yellow). The colorimeter was calibrated with a standard white plate (L^* = 97.31, a^* = 0.05, b^* =4.38). For

powder samples, the color measurements were performed by placing a Petri dish (35×10 mm Style, Corning®, Corning, NY, USA), containing enough sample to cover the bottom of the Petri dish, under the colorimeter. The tube-like end of the colorimeter created the dark area by placing the sensor lens directly over the area of the specimen to be measured. Measurements were performed in triplicate by recording the values of 3 portions from one powder sample. Solution samples were prepared as described for viscosity measurements, but without sodium azide. An aliquot of solution (around 5 mL) enough to cover the bottom of the dish was transferred and the color measurement was performed by placing the dish under the colorimeter. Total color difference (ΔE), yellowness index (YI), and whiteness index (WI) were calculated according to Boun and Huxsoll (1991):

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (3.6)$$

$$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{0.5} \quad (3.7)$$

$$YI = 142.86\, b/L \quad (3.8)$$

where, L^*, a^* and b^* are the color values of the white standard tile and L, a and b are the measured color parameters of the BG and iBG samples.

3.2.11 Statistical analysis

Statistical analyses were performed using R 3.2.3 with the car and agricolae packages (R Core Team, 2015; Mendiburu, 2015; Fox and Weisberg, 2011). Analysis of variance (ANOVA)

was performed and means were compared by paired t-test of least square means with significance defined at $p<0.05$.

3.3 Results and discussion

3.3.1 Chemical composition and particle characteristics

The proximate composition of BG is presented in Table 3-2. On wet basis, the beta-glucan content of the BG sample was 83.6%, which was around 4% (w/w) lower than the total carbohydrate content, indicating the presence of other carbohydrates, anticipated to be mostly starch. In addition, the BG sample contained 1.5% (w/w) protein and 2.9% (w/w) ash. Furthermore, the moisture content of the BG sample was around 7.4% (w/w). Therefore, to preserve the water-soluble polymer, storing in a dry place with desiccant is necessary.

Table 3-2. Proximate composition of BG sample.

Proximate composition (%, w/w, as is basis)*	
Moisture	7.4±0.1
Protein	1.5±0.1
Fat	0
Carbohydrate	88.0
Beta-glucan	83.6±1.1
Ash	2.9±0.3

* Data were presented as mean ± standard deviation based on triplicate measurements.

Visually, BG is white fine fibrils, while iBG is a yellowish powder (Fig. 3-1). The loading percentage of CoQ10 in iBG was 1.4% (w/w). The particle morphology of both BG and iBG was investigated using a helium ion microscope that can directly image insulating materials, eliminating the need for any coating material like gold, typically used in scanning electron microscopy. The delicate porous structures of both particles were apparent in the images obtained at two magnifications depicted in Fig. 3-2, which confirmed that the PGX process could create highly porous structures. Pores on the fibrils of BG and iBG as well as the distinct CoQ10 particles on iBG could be clearly observed. Based on the microscopic images, CoQ10 nanoparticles were spherical shaped with the average diameter of 92 nm (range of 35-160 nm), dispersed uniformly throughout the BG fibrils.

Figure 3-1. Visual appearance of (A) BG and (B) iBG samples.

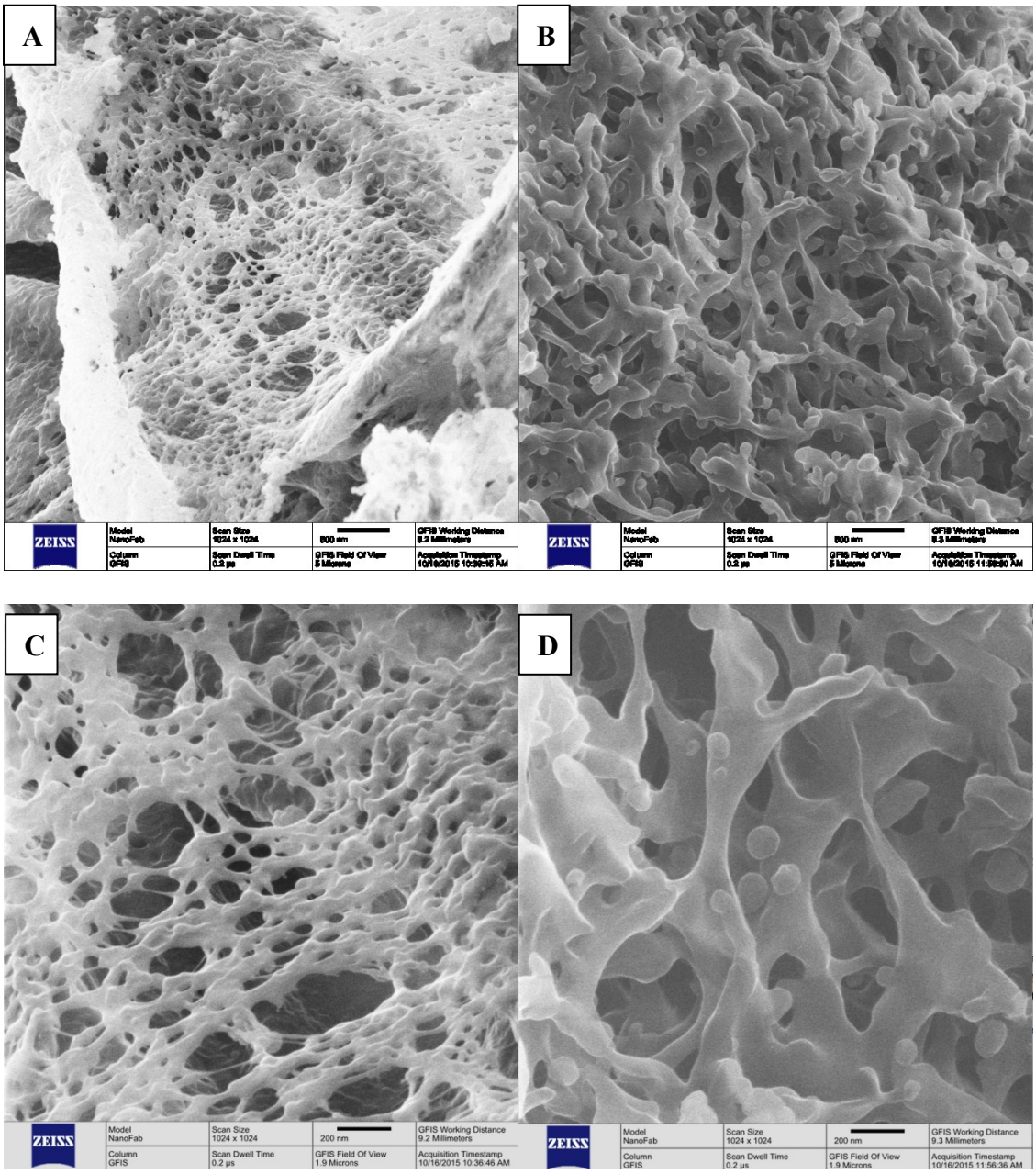


Figure 3-2. Helium ion microscopy images of (A), (C) BG and (B), (D) iBG. Scale bars represent 500 nm in (A) and (B), and 200 nm in (C) and (D).

Surface area and pore size of BG and iBG were also analyzed. Samples were firstly outgassed overnight under vacuum at room temperature (approximately 25 ºC) to avoid melting

of CoQ10. Then, after a 4-hour N_2 adsorption/desorption analysis, it was found that both BG and iBG had similar pore sizes (half pore width = 27.9 nm), which indicates that CoQ10 was impregnated onto BG without damaging its porous structure. Generally, larger surface area is associated with smaller particle size. By comparing their visual appearance, iBG, yellowish powder, was predicted to have smaller particle size and larger surface area than BG, which was white fine fibrils (Fig. 3-1). In fact, the average particle size of BG was larger than that of iBG (7.7 vs. 6.1 μm), which confirmed the above prediction. However, the surface area of BG (24.1 m^2/g) was almost twice as large as that of iBG (12.9 m^2/g). The unexpected smaller surface area of iBG could be explained by the presence of CoQ10 nanoparticles that occupied some surface.

3.3.2 Crystallinity and thermal analysis

The thermal behavior of BG, CoQ10, iBG and their physical mixture were analyzed using DSC (Fig. 3-3). Pure CoQ10 gave a sharp endothermic peak at around 50 ºC in the DSC thermogram, indicating melting of crystals. Such a peak was also present for the physical mixture but absent for iBG, which indicated the possibility that the CoQ10 impregnated onto the BG particles may be in the amorphous form. The same four samples were also analyzed by XRD to investigate the extent of crystallinity. The characteristic peaks of the pure CoQ10 were not present for the iBG sample in the XRD spectra, shown in Fig. 3-4. This finding seemed to confirm the possibility that CoQ10 was present inside the iBG particles in amorphous form rather than crystalline form. However, it is also possible that the CoQ10 peaks may be hidden

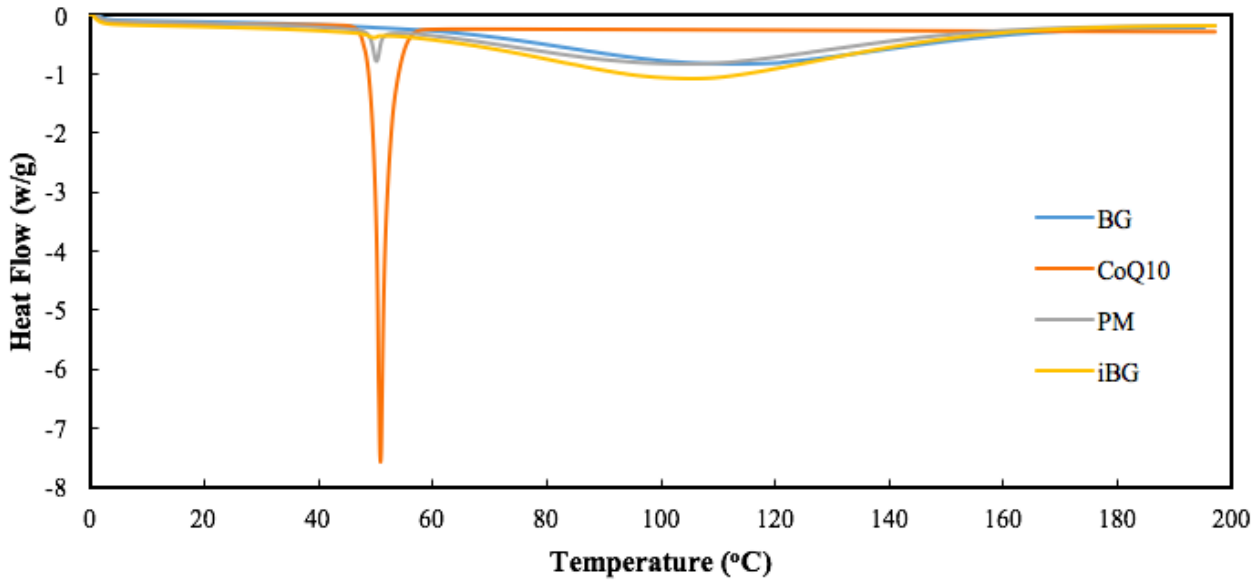


Figure 3-3. DSC thermograms of BG, CoQ10, iBG and physical mixture (PM, 4% CoQ10) at the heating rate of 10 ºC/min.

under the broad peak of beta-glucan due to the low concentration of CoQ10. In order to further confirm the absence of the characteristic peaks of CoQ10, the iBG sample was digested with the lichenase enzyme to hydrolyze beta-glucan. In addition, physical mixtures of lower CoQ10 content (1% and 2%) were included for comparison. In this case, some CoQ10 peaks became apparent as depicted in Fig. 3-5. Thus, it is concluded that the crystalline nature of CoQ10 is preserved to a small extent in the iBG particles. A similar conclusion was reached by Swarnakar et al. (2011) who loaded CoQ10 on poly (lactic-co-glycolic acid) (PLGA) particles using an emulsion-diffusion-evaporation method. Swarnakar et al. (2011) also suggested that having CoQ10 in its amorphous form on the polymer confirmed its formulation excipients for pharmaceutical applications, because amorphous solids have higher solubility, dissolution rate and thus better bioavailability, compared to corresponding crystals (Yu, 2001).

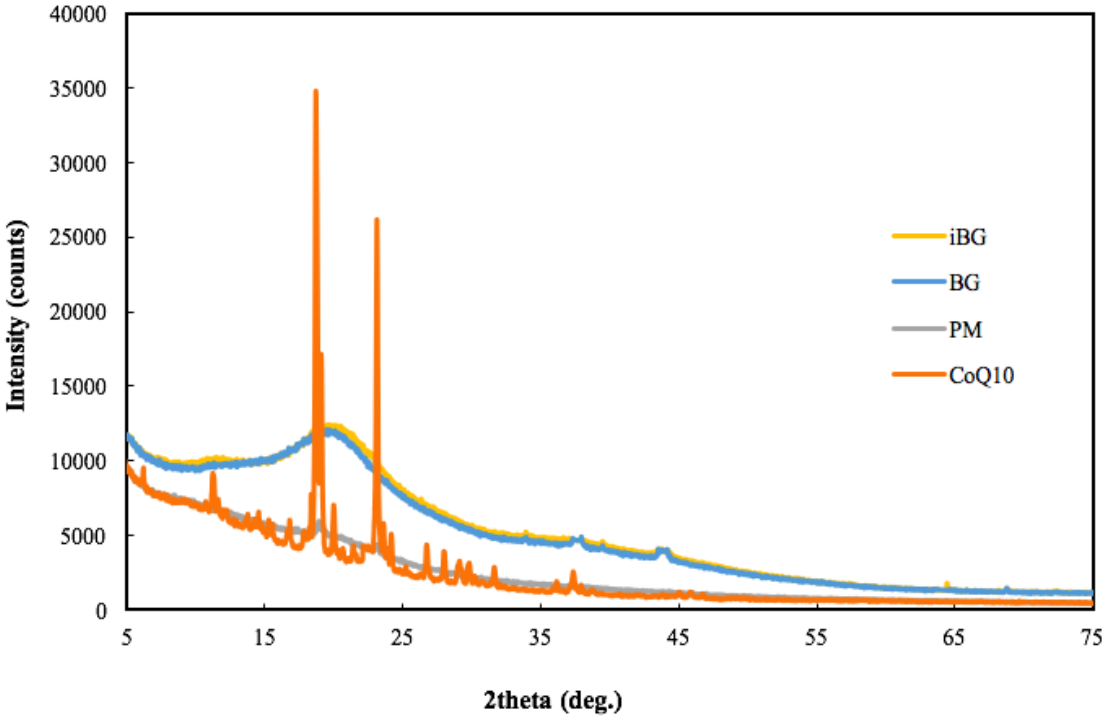


Figure 3-4. XRD spectra of BG, CoQ10, iBG and physical mixture (PM, 4% CoQ10).

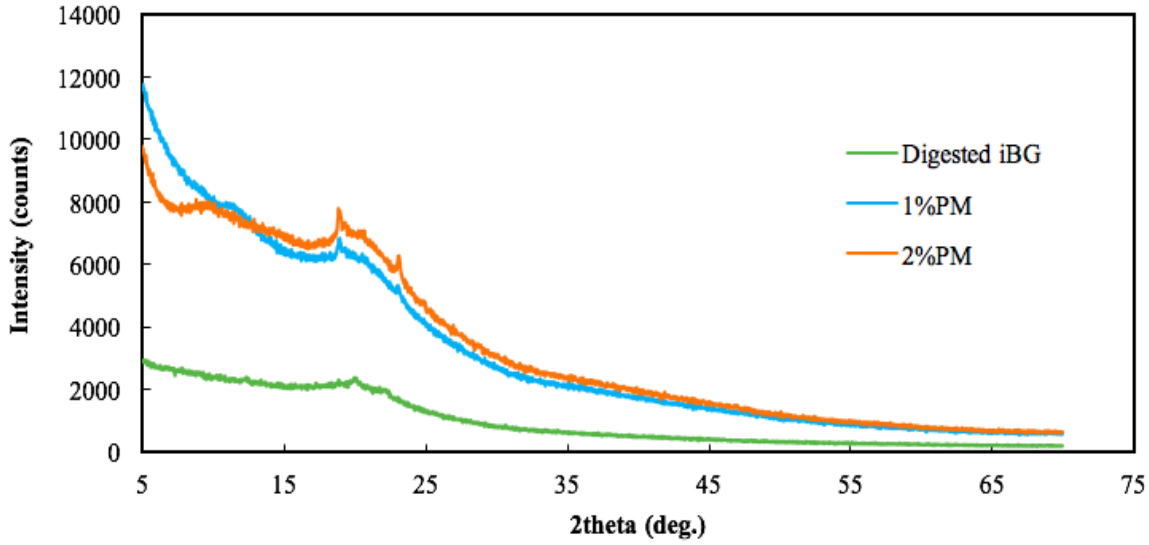


Figure 3-5. XRD spectra of 1% and 2% physical mixtures (PM) and lichenase-digested iBG.

3.3.3 Color

The data for the color of BG and iBG are presented in Fig. 3-6. The total color difference was observed for both powder and solution samples. Specifically, iBG in powder form has a significantly higher yellowness index, which indicates the successful impregnation of CoQ10. Additionally, the yellowness of 0.2% (w/v) iBG solution indicates the presence of CoQ10 after boiling, which suggested that BG could protect CoQ10 from severe conditions. In a previous study, 64% of CoQ10 in the CoQ10 + cyclodextrin complex remained unchanged after 120 min of exposure to heat at 80 ºC and UV light (Fir et al., 2009). In the present study, the total time from adding iBG into 25 mL water at room temperature to complete dissolution was around 1 min, which means CoQ10 was exposed to boiling temperature for less than 1 min, and it is expected that most of CoQ10 in iBG was preserved. Additionally, while evaluating the CoQ10 content of the average Danish diet, Weber et al. (1997) found that 14-32% of CoQ10 in food would be degraded by frying, but no degradation was detected upon boiling. To confirm the preservation of CoQ10 in the iBG after the boiling step employed in this study, it is recommended to perform high performance liquid chromatography (HPLC) analysis.

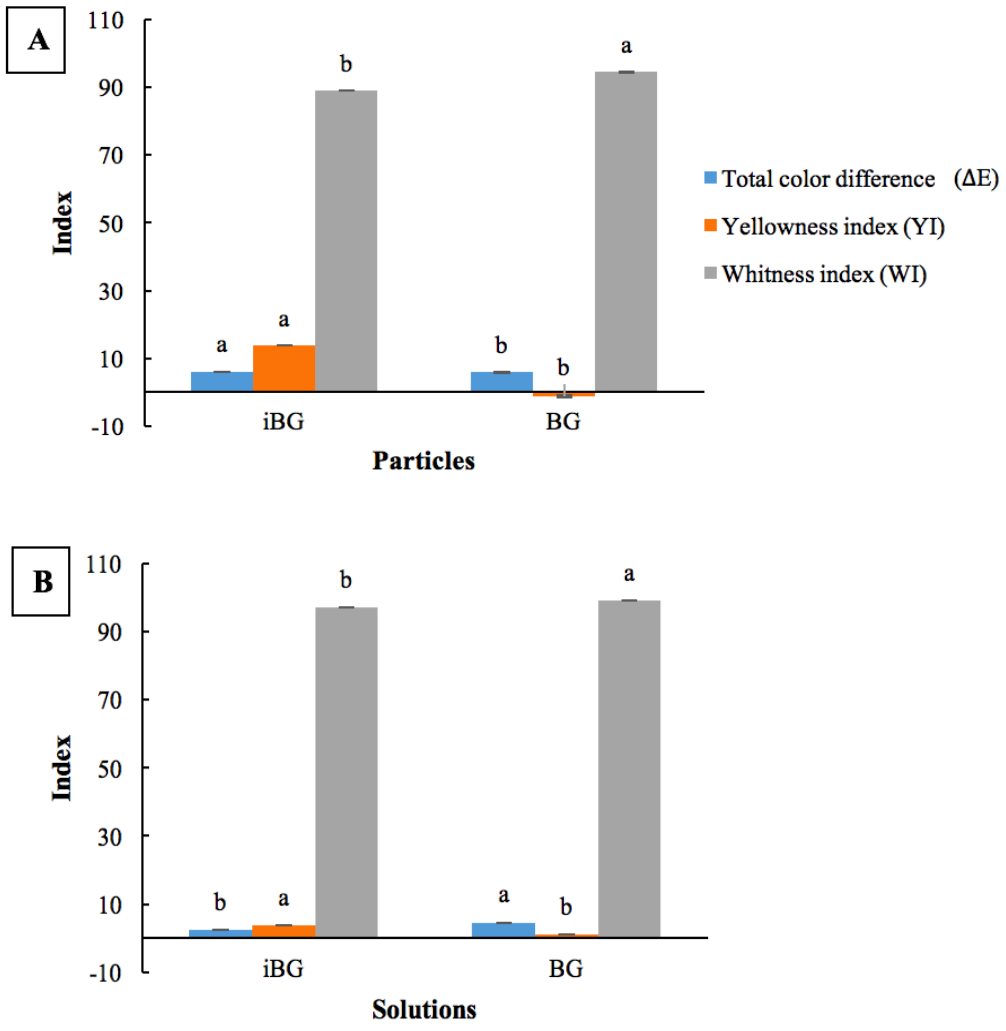


Figure 3-6. Total color difference (ΔE), yellowness index (YI) and whiteness index (WI) of BG and iBG in (A) powder form and (B) 0.2% (w/v) solution. Bars for each parameter with different letters are significantly different at $p<0.05$.

Furthermore, lower YI of solution samples, compared to that of powder samples, could be explained by the low concentration of CoQ10, because when placing the sample on the dish, more powder sample was needed to cover the bottom of the dish, while around 5 mL solution

was enough to do so. For the same reason, WI(s) of BG and iBG solutions were closer, compared with that of BG and iBG powders, even though they were still statistically different.

3.3.4 Viscosity

The viscosity of BG and iBG solutions prepared at different concentrations (0.15%, 0.2% and 0.3% w/v) as a function of shear rate at temperatures of 4 ºC and 25 ºC is shown in Figs. 3-7 and 3-8, respectively. The shear rate was increased logarithmically from 1.29 to 129 s^{-1}. The instability at very low shear rates (1.29-5.13 s^{-1}) was due to the limitations of the instrument. These two temperatures were chosen for potential application of BG and iBG in beverage products. For ease of comparison, the viscosity at 51 s^{-1} is presented in Table 3-3, because the oral perception of liquid and semi-solid foods has been found to correlate to viscosity measurements performed at shear rates around 50 s^{-1} (Wood, 1968). For the same reason, the temperature effect on viscosity behavior was determined at the fixed shear rate of 50 s^{-1}. Based on the health claims approved by the US Food and Drug Administration and Health Canada, the three concentrations adopted in this study were calculated to provide 0.75 g beta-glucan per serving sizes of 500 mL, 375 mL and 250 mL of a potential beverage product.

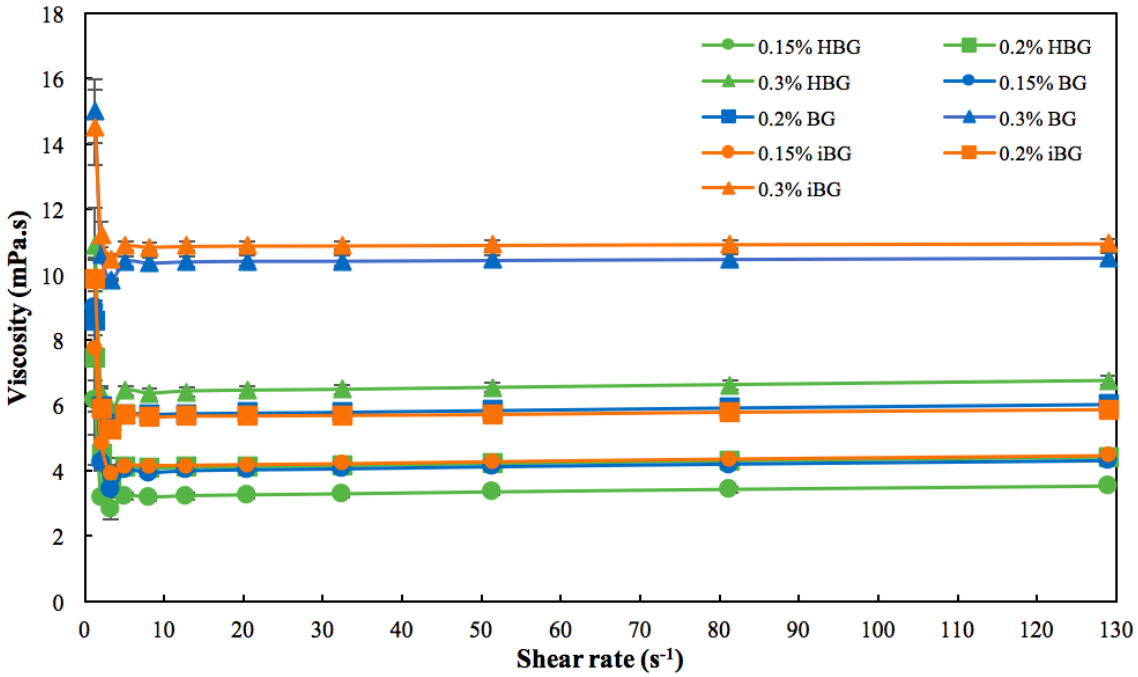


Figure 3-7. Viscosity at 4 ºC as a function of shear rate. Data were presented as mean values and error bars based on duplicate measurements.

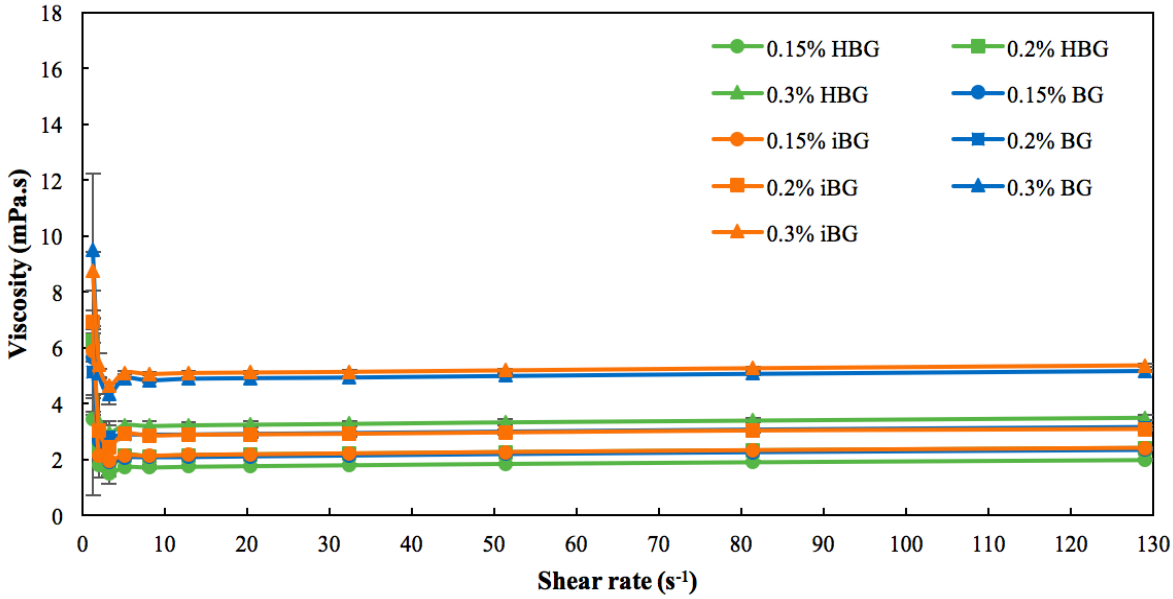


Figure 3-8. Viscosity at 25 ºC as a function of shear rate. Data were presented as mean values and error bars based on duplicate measurements.

Table 3-3. Viscosity* of HBG, BG and iBG solutions at 51 s^{-1}.

Sample	Concentration (%, w/v)	Viscosity at 4 °C (mPa.s)	Viscosity at 25 °C (mPa.s)
HBG	0.15	3.3 ± 0.1	1.8 ± 0.0
	0.2	4.2 ± 0.1	2.3 ± 0.1
	0.3	6.5 ± 0.1	3.3 ± 0.1
BG	0.15	4.1 ± 0.0	2.2 ± 0.0
	0.2	5.9 ± 0.2	3.0 ± 0.1
	0.3	10.5 ± 0.2	5.0 ± 0.1
iBG	0.15	4.3 ± 0.0	2.3 ± 0.0
	0.2	5.7 ± 0.1	3.0 ± 0.1
	0.3	10.9 ± 0.1	5.2 ± 0.1

* Data were presented as mean ± standard deviation based on duplicate measurements.

As expected, at both temperatures of 4 °C and 25 °C, as the concentration increased, the viscosity of all solutions increased (Table 3-3). In addition, at the concentration of 0.3%, the viscosity of iBG was slightly higher than that of BG. Except that, BG and iBG had similar viscosities at the concentrations of 0.15% and 0.2%, which further confirmed that the BG polymer structure was not affected during the CoQ10 impregnation step. The viscosity of BG and iBG solutions was higher than that of HBG (MW= 361,000 Da) at the same solids concentration. In addition, the actual beta-glucan concentration of HBG solution was higher than that of the BG and iBG solutions (Table 3-1), when the purity of the powders was taken into account. Ryu et al. (2012) and Mikkelsen et al. (2010) suggested that molecular weight and concentration are major contributors to the viscosity of beta-glucan. Therefore, it can be

concluded that the production of BG and iBG by the PGX process preserved the native structure of beta-glucan, having a molecular weight higher than 361,000 Da. Furthermore, with an increase in shear rate, the viscosity of all solutions stayed almost constant, which indicates their Newtonian behavior at the concentrations tested. A similar result was obtained by Temelli (1997) who worked with 1% (w/v) barley beta-glucan dispersions over the shear rate range of 600 to 5000 s^{-1}. Differently, Dongowski et al. (2005) observed shear-thinning flow behavior of 2% and 4% beta-glucan solutions prepared from oat bran. Also, they indicated that the shear-thinning behavior was dependent on the molecular weight and concentration of the polymer, which was in agreement with the conclusions reached by Lazaridou et al. (2003) and Skendi et al. (2003). The reason for the Newtonian behaviors observed in the present study is the low concentration of beta-glucan (0.13%-0.25%) in the solutions tested. However, it was found that low concentration (0.2-3%) solutions of oat beta-glucan with the molecular weight of 355,000 Da exhibited shear-thinning flow behavior (Mikkelsen et al. 2010; Cui, 2001).

Viscosity behavior of BG and iBG solutions at the shear rate of 50 s^{-1} over the temperature range of 0 ºC and 80 ºC is shown in Fig. 3-9. The temperature range of 0 ºC and 80 ºC was chosen to predict the viscosity behavior of beverages potentially prepared with BG and iBG and consumed as hot or cold. Viscosity increase of all solutions with concentration was also observed. Additionally, viscosity decrease with increasing temperature was demonstrated as expected, because as temperature rises, hydrogen bonds between beta-glucan molecules would be broken. Moreover, the viscosities of BG and iBG showed similar decreasing trends, but were still higher

than that of HBG. After reaching 70 ºC, the viscosity curves of all solutions approached each other, and at 80 ºC, they almost overlapped. The decreasing trend with temperature was also observed by Burkus and Temelli (2005) who investigated the viscosity behavior of barley beta-glucan gum over the temperature range of 0.1 ºC and 75 ºC.

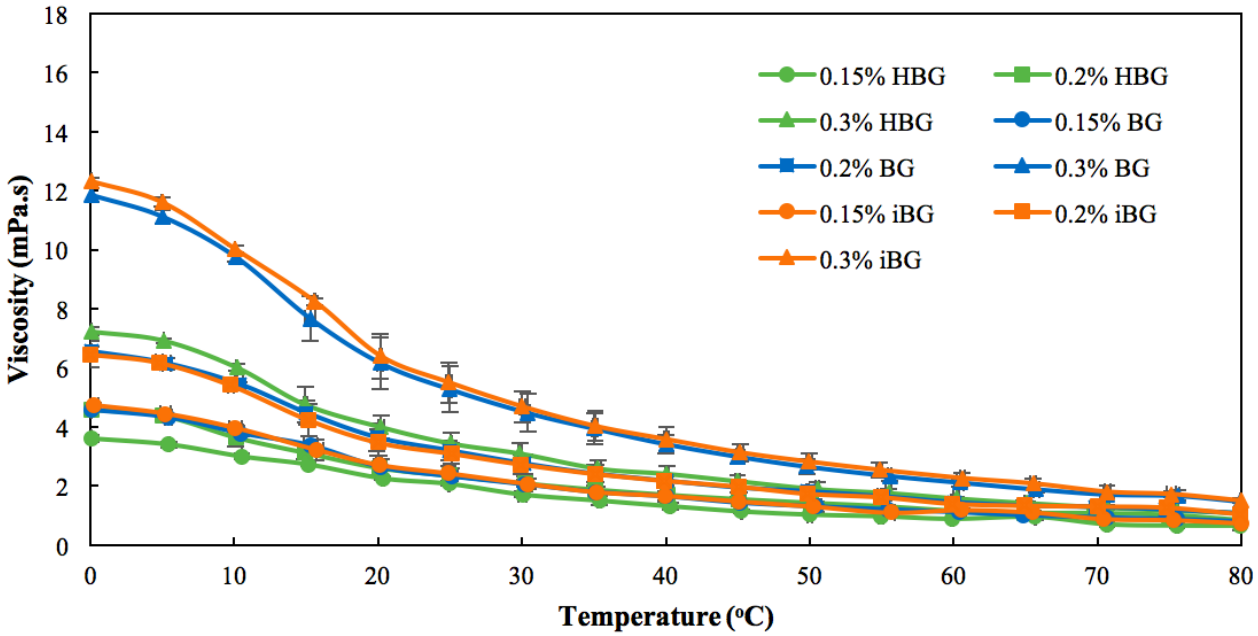


Figure 3-9. Viscosity at 50 s^{-1} as a function of temperature. Data were presented as mean values and error bars based on duplicate measurements.

3.4 Conclusions

BG (83.56% purity) dried using the PGX process is white fibrils with high molecular weight (>> 361,000 Da), containing 7.4% (w/w) water, 1.5% (w/w) protein and 2.9% (w/w) ash. CoQ10 was also successfully impregnated into BG using the PGX process, without interrupting the

porous structure and viscosity of BG. iBG is a yellowish powder with 1.4% (w/w) CoQ10 mainly in its amorphous form. Both BG (7.7 μm) and iBG (6.1 μm) were produced in micrometer sized particles, while CoQ10 nanoparticles present in iBG had an average diameter of 92 nm. After a quick boiling step to prepare solutions, the yellowness of iBG was still present, indicating that CoQ10 was well dispersed in the water. The viscosity of BG and iBG solutions increased as the concentration increased from 0.15% to 0.3% (w/v), while exhibiting Newtonian behavior with a constant viscosity through the shear rate range of 1.29 s^{-1} to 129 s^{-1}. Also, a decrease in the viscosity of BG and iBG solutions was observed at the shear rate of 50 s^{-1} when temperature was increased from 0 ºC to 80 ºC. iBG had a similar viscosity behavior to BG, indicating that the impregnation of BG particles with CoQ10 using the PGX process did not affect the viscosity of BG. Overall, the physicochemical properties determined in this study would provide feedback for the fine-tuning of the operating conditions of the PGX process, and guidance for the development of a functional beverage formulation in the next chapter.

Chapter 4: Development of an orange-flavored functional beverage formulated with beta-glucan and coenzyme Q10-impregnated beta-glucan

4.1 Introduction

Beta-glucan is a soluble dietary fiber component that is mainly present in the starchy endosperm with the rest in the aleurone layers of oats and barley (Izydorczyk and Biliaderis, 2000; Herrera et al., 2016). After extraction from the cereal grains, beta-glucan is usually obtained in liquid or suspension form, which leads to issues in terms of storage, transportation and potential applications and therefore drying is needed to resolve these issues. But, as known, beta-glucan forms highly viscous solutions and therefore it is challenging to use conventional drying methods like freeze drying or spray drying to obtain beta-glucan powder. Alternatively, beta-glucan solution could be dried by the pressurized gas-expanded liquid (PGX) technology (Temelli and Seifried, 2016). This technology was designed to produce dry particles, agglomerates and/or fibers from highly viscous aqueous solutions of high molecular weight biopolymers. The produced micro- or nanoparticles are generally low in bulk density, high in porosity and large in surface area, which facilitate 99% reduction in shipping weights and ease of handling and dispersion in water. Additionally, PGX-produced beta-glucan (BG) could maintain its high molecular weight, resulting in a high viscosity, which contributes to its physiological benefits like serum cholesterol or glucose lowering (Varum and Smidsrod, 1988; Wood et al., 1994).

The PGX process can also be used to impregnate bioactive materials onto the dried particles to not only protect the bioactive components from atmospheric conditions such as light, heat and oxygen, but also enhance their dispersibility in water and consequently bioavailability. In this study, Coenzyme Q10 (CoQ10) was the bioactive material impregnated onto the PGX-dried beta-glucan (iBG). CoQ10 is a natural antioxidant present in both animal and plant cellular membranes, which has anti-inflammatory effect, regulates blood pressure and enhances bone regeneration (Jun et al., 2009; Lyamina et al., 2011; Yang et al., 2015). Due to its hydrophobicity, application of CoQ10 in food and beverage products is limited. Recently, CoQ10 has been incorporated into a dairy product like yoghurt, due to its high fat content (Ercan and El, 2012). However, functional beverage products containing CoQ10 are not available.

With the growing attention to body health and lifestyle, functional beverages, which contain health-benefiting ingredients, have been receiving increased demand since the early 21th century (Szakály et al., 2012). In the present study, a functional beverage formulated with iBG was targeted, where the health benefits of both beta-glucan and CoQ10 would be provided. The basic formulation was adopted from a previous study by Temelli et al. (2004), who developed an orange-flavored barley beta-glucan beverage. In addition, stevia extract (steviol glycosides) and ground annatto seeds were used as the sweetener and colorant, respectively, to meet the consumer demand for “natural ingredients” and “low/no sugar content” in the functional beverage market. However, different from the conventional sugar, beverages sweetened with stevia extract are usually associated with a bitter taste and off flavour (Cadena et al., 2013). On

the other hand, an increasing addition of oat and barley beta-glucans was found to decrease the perception of saltiness and sharpness of tomato soup, and sourness of fruit beverages (Lyly et al., 2004; Mielby et al., 2016). However, reports on the impact of beta-glucan on the bitterness and aftertaste of stevia are lacking. Furthermore, health claims or nutrition information labels are established to communicate the benefits of products to consumers to help them make better-informed choices. A positive increase was found in the overall liking of açaí fruit juices by providing such information (Sabbe et al., 2009). Thus, more information is needed on the effect of providing health information on the overall acceptance of functional beverages.

Therefore, the objectives of this study were: i) to develop a formulation of a functional beverage incorporating BG or iBG, ii) to study the effect of BG concentration and storage temperature on the sensory attributes of the functional beverage using a trained panel, ii) to determine if there is a difference between beverages prepared with BG and iBG, iv) to evaluate the sensory quality and acceptability of the beverage formulated with iBG in comparison to a commercial product using a consumer panel, and v) to evaluate the effect of health information on the overall acceptance of the beverage.

4.2 Materials and methods

4.2.1 Materials

Food-grade BG (batch no. 15-E1, 83.6%, w/w, as is basis) extracted from oat, iBG (batch no. 15-D1, 1.4%, w/w CoQ10) and food-grade iBG (batch no. 16-J3) were provided by Ceapro Inc

(Edmonton, AB, Canada). Citric acid (anhydrous) and stevia extract (Truvia®, *Stevia rebaudiana*) were provided by Cargill Inc. (Wayzata, MN, USA). Orange flavor powder was provided by Gold Coast Ingredients Inc. (Commerce, CA, USA). Orange color powder (Annatto seeds) was purchased from Natures Flavors (Orange, CA, USA). Serving containers (2.5 oz., Conex® Complements™ Portion Containers) were purchased from Dart Inc. (Mississauga, ON, Canada). Commercial products used for sensory panels were purchased from a local grocery store. Chemicals involved in characterization were similar to those specified in Chapter 3 (Section 3.2.1).

4.2.2 Beverage formulation development

Beverage formulations (Table 4-1) were initially developed without the addition of BG or iBG to obtain a base product with color and flavor similar to that of Vitaminwater® (Orange flavor, Rise Zero, Glacéau, New York City, NY, USA), using laboratory scale trials. Colleagues in the food lab were invited to evaluate the trial products to help with formulation development.

Table 4-1. Beverage formulations.

Ingredients	Amount (g/mL, w/v)
BG or iBG	0%, 0.15%, 0.2%, or 0.3%
Citric acid	0.2%
Stevia extract	0.015%
Orange flavor powder	0.1%
Orange food colorant powder	0.05%

After the initial base formulation was developed, BG was added while keeping the concentration of other ingredients constant. Beverages were formulated with 0.15%, 0.2% and 0.3% (w/v) BG targeting to meet the FDA recommendation of providing 0.75 g beta-glucan per serving sizes of 500 mL, 375 mL and 250 mL, respectively. However, considering the purity of beta-glucan in BG, the final actual concentrations were 0.13%, 0.17% and 0.25% (w/v), requiring serving sizes of 576 mL, 441 mL and 300 mL, respectively, to ensure the delivery of 0.75 g beta-glucan per serving. The protocol for the preparation of 1 L of beverage containing, for example, 0.2% (w/v) BG or iBG is shown in Fig. 4-1. This protocol was adopted after trying different approaches to blending of ingredients. Water was added slowly while stirring manually and vigorously into the beaker containing BG or iBG. It took up to 1 h of heating for the complete dissolution of BG, while around 15 min was sufficient for the iBG.

4.2.3 Determination of physicochemical properties

Physicochemical properties of food-grade iBG (batch no. 16-J3) were determined, in terms of particle morphology, surface area and CoQ10 loading amount, according to the protocols described in Chapter 3 (Sections 3.2.4, 3.2.5, 3.2.8)

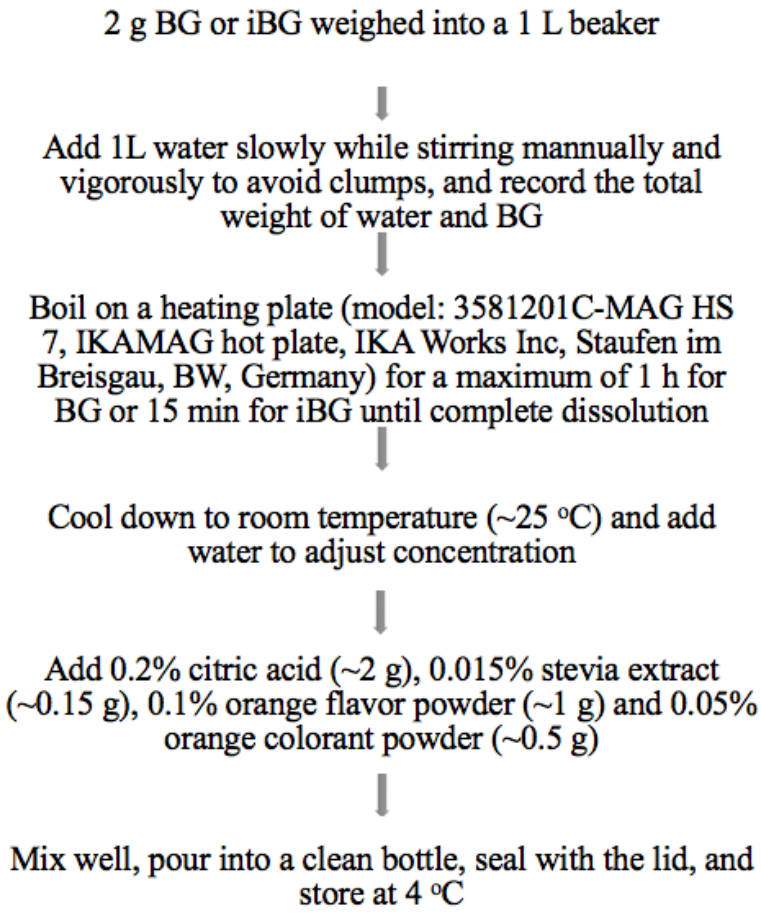


Figure 4-1. Protocol for the laboratory scale preparation of 1 L orange-flavored functional beverage containing 0.2% (w/v) BG or iBG.

4.2.3.1 Viscosity

Prior to receiving the food-grade iBG (batch no. 16-J3), non-food grade iBG (batch no. 15-D1) and food-grade BG (batch no. 15-E1) were used to formulate preliminary beverages. The effects of concentration (0.15%, 0.2% and 0.3%, w/v), temperature (4 °C and 25 °C), shear rate (1.29-129 s^{-1}) and the presence of citric acid on the viscosity of beverages prepared with BG or iBG were evaluated. Sample preparation for viscosity measurements was similar to that

described in Chapter 3 (Section 3.2.9). After BG or iBG solutions were cooled, water was added to make up for evaporation losses and to adjust the concentration. Stevia extract, orange flavor powder and colorant were added. Citric acid was added, depending on the treatment. In addition, the viscosity of the beverage containing 0.2% food-grade iBG was evaluated to determine if the two batches of iBG were comparable.

4.2.3.2 Color

Beverages prepared with 0.2% (w/v) BG and iBG powder were used for color measurement as described in Chapter 3 (Section 3.2.10) and the total color difference (ΔE) and yellowness index (YI) were calculated according to Equations (3.6) and (3.8).

4.2.4 Sensory evaluation

4.2.4.1 Experimental design

The sensory attributes of orange-flavored beverages prepared at three concentrations (0.15%, 0.2% and 0.3%, w/v) of BG, served at room temperature (~25 ºC) and refrigerator temperature (~4 ºC) were evaluated by a trained panel. Based on the feedback of participants in the trained panel, one concentration and one temperature (0.2%, 4 ºC) were chosen to prepare the beverages containing iBG for the consumer panel to evaluate the overall acceptance. In between, a triangle test was performed to determine if there was an overall difference between beverages formulated with BG and iBG. All samples were served in 30 mL portions in plastic containers with sealable

lids and coded with randomized three-digit numbers. All participants recruited for the three panels were students and staff of the University of Alberta. The experimental protocols were approved by the Research Ethics Board of the University of Alberta.

4.2.4.2 Trained panel

Descriptive analysis was conducted according to the "General guidance for the selection, training and monitoring of selected and expert assessors" (BS ISO 8589, 2014). One screening session was held first to determine impairment and sensory acuity, and also to evaluate a candidate's potential for describing and communicating sensory perceptions (Appendix A.1). Based on the screening results, 12 participants (11 women, age 18-29 years) were recruited for training, prior to the evaluation of beverages. Seven training sessions were held over two weeks to train the panelists on the assessment of 7 attributes, including orange flavor, sweetness, sourness, aftertaste, metallic taste, bitterness and viscosity. In each session, participants were trained on one attribute. The next session started with a refresher of the attributes previously trained before moving on to a new attribute. Participants were trained on how to evaluate samples on a 15-point category scale during the first session. Water was used as the reference sample to represent "None", and solutions prepared with different concentrations of ingredients were used as standard solutions (Table 4-2) for the training in the use of scales for the different attributes.

The sensory evaluation of the functional beverages was carried out in three different sessions on three separate days. On each day, the participants were provided with one set of beverages served at 25 ^{o}C and the other set at 4 ^{o}C. Each set consisted of 4 beverages prepared with 0%, 0.15%, 0.2% and 0.3% BG. Samples were presented in a random order on an off-white fiberglass tray. Room temperature distilled water and two pieces of unsalted crackers were provided for cleansing the palate, together with a napkin, a pencil and a questionnaire (Appendix A.2). Between the two sets, participants were asked to take a 15-20 min break to avoid fatigue. The evaluation was performed in standard sensory panel booths compliant with international standards (ASTM, 1986). Panelists were rewarded for participation after all training sessions and sample evaluations.

Table 4-2. Standard solutions used for training in the use of scales for the different attributes evaluated.

Attribute	**Ingredient**	**Concentrations**		
Sweetness	Stevia extract	0.01%	0.15%	0.3%
Aftertaste	Stevia extract	0.01%	0.15%	0.3%
Metallic taste	$FeSO_4.7H_2O$	0.000025%	0.00005%	0.0001%
Sourness	Citric acid	0.05%	0.15%	0.3%
Orange flavor	Orange flavor powder	0.05%	0.1%	0.2%
Bitterness	Caffeine	0.05%	0.1%	0.2%
Viscosity	BG	-	0.5%	1%

4.2.4.3 Triangle test

Triangle test was conducted according to ISO 4120 (2004) to determine if there is an overall difference between the beverages prepared with 0.2% (w/v) iBG and BG. The panel consisted of 30 assessors (18 women, aged 18-49 years). The three beverages presented were either two iBG beverages and one BG beverage, or two BG beverages and one iBG beverage. They were served at 4 ºC and presented in a balanced order with 5 blocks. Participants were also provided with room temperature distilled water, two pieces of unsalted crackers, a napkin, a pencil and a questionnaire (Appendix A.3). The evaluation was performed in standard sensory panel booths with red light to avoid interference due to beverage color. The proportion distinguished (p_d) was calculated as follows:

$$p_d = [1.5(^x/_n) - 0.5] = 1.5z_\beta\sqrt{(nx - x^2)/n^3} \quad (4.1)$$

where x is the number of correct answers, n is the number of assessors and z_β varies as follows: 1.28 for $\beta = 0.10$, 1.64 for $\beta = 0.05$, 2.33 for $\beta = 0.01$, and 3.09 for $\beta = 0.001$.

4.2.4.4 Consumer panel

Ideal profile method (IPM) was applied for the consumer panel. In this method, panelists were asked to rate not only the perceived intensity of a list of attributes for each product tested, but also the ideal intensity of those attributes, which could help with improving the existing products (Worch et al., 2012). A total of 102 participants (60 women, 94% aged 18-39 years and

6% aged 40-59 years) were recruited at the Agriculture/Forestry Centre, University of Alberta. General information about the project was explained to the potential participants. Once they agreed to participate, panelists were guided into a sensory panel booth, where an off-white fiberglass tray containing beverages, a glass of room temperature distilled water, two pieces of unsalted crackers, a napkin, a pencil and a questionnaire (Appendix A.4) were provided. Beverages were provided in two sets served at 4ºC. The first set included one beverage without iBG, one beverage with 0.2% iBG and one Vitaminwater®. Participants informed one of the panel coordinators after they finished the first set, and then the second set including one beverage with 0.2% iBG and one beverage with 0% iBG was provided. Beverages served in the second set were identical to those in the first set, but the panelists had to read the provided health benefits information regarding beta-glucan, CoQ10 and stevia extract prior to evaluation (Table 4-3). The overall acceptance of beverages scored by participants without any health information in the first set was treated as "pre-information acceptance", while that scored after health information was provided was referred to as "post-information acceptance". The difference in the overall acceptance was calculated as "post-information acceptance" minus "pre-information acceptance", with positive values identified as "like more", zero as "no change" and negative values as "like less". The order of beverages was balanced according to Williams square design (Williams, 1949). Participants evaluated the intensity of orange flavor, sweetness, sourness, aftertaste, bitterness, metallic taste and thickness on a nine-point scale with "Weak" scored as 1 and "Strong" scored as 9. In addition, a nine-point hedonic scale (1=Dislike extremely, 2=Dislike

Table 4-3. The provided health benefits information regarding beta-glucan, CoQ10 and stevia extract.

Beverage containing 0.2% iBG	Beverage containing 0% iBG
Stevia Extract (Rebaudioside A): • non-sugar sweetener • natural sweetener • zero calories Beta-glucan: • soluble dietary fiber • 0.75g per serving beta-glucan helps reduce/lower cholesterol, a risk factor for heart disease • health claim approved by Health Canada Coenzyme Q10: • vitamin-like antioxidant • help maintain cardiovascular health	Stevia Extract (Rebaudioside A): • non-sugar sweetener • natural sweetener • zero calories

very much, 3=Dislike moderately, 4=Dislike slightly, 5=Neither like nor like, 6=Like slightly, 7=Like moderately, 8=Like very much, 9=Like extremely) was used to evaluate the overall acceptance of each beverage. Participants were also asked to score their ideal intensity for all of the above attributes at the end. All consumer panelists were offered sweet treats after participation.

4.2.5 Statistical analysis

Evaluation of each beverage by the trained panel was performed in triplicate. Panelists participating in the triangle test and consumer panel performed a single evaluation. Color of the 0.2% iBG and 0.2% BG beverages, and viscosity of all treatments were measured in duplicate.

CoQ10 loading amount was determined in triplicate. Analyses were performed in R 3.2.3 using the car, agricolae, lsmeans and SensoMineR packages (R Core Team, 2015; Mendiburu, 2015; Fox and Weisberg, 2011; Lenth 2016; Husson et al., 2014). For the results of the trained and consumer panels, analysis of variance (ANOVA) was conducted. Tukey's test was performed for the multiple comparison of the means with significance defined at $p<0.05$. Specifically, for the trained panel data, a two-way ANOVA was performed for each attribute by specifying serving temperature, BG concentration and their interaction as the fixed effects and participants as the random effect. However, for the consumer panel data, a one-way ANOVA was performed for each attribute by specifying beverages as the fixed effect and both participants and sample order as the random effects. Principal component analysis (PCA) was also performed on the consumer panel data to evaluate the product space among 0% iBG beverage, 0.2% iBG beverage, Vitaminwater® and ideal product. For the results of the triangle test, means were compared by paired t-test of least square means with significance defined at $p<0.05$.

4.3 Results and discussion

4.3.1 Beverage formulation development

With the increased awareness of the link between diet and health, the functional foods market is continually growing since it was first introduced in the mid-1980s (Ozen et al., 2012). The functional beverages sector is a sub-sector of the functional foods, where the beverages not only hydrate one's body, but also provide overall nutritional well-being. It remains the first choice for

the functional foods category among consumers, due to its convenience and the possibility to meet consumer demand for health-benefiting ingredients, size and appearance (Corbo et al., 2014). In addition, fiber, vitamins, antioxidants, omega-3s, green tea extract and probiotics have been the most popular ingredients in functional beverages in the 2010s (Sloan and Hutt, 2012). Meanwhile, with the fast spreading condition of obesity and diabetes, artificial sweeteners such as non-caloric sweeteners or low glycaemic index sweeteners have been used as an alternative to decrease the intake of sugar. In 2005, around 50% of the new food and drink products were manufactured with sucralose, while 32% and 20% contained aspartame and Acesulfame K, respectively (Mintel Group I, 2005). However, there is ongoing debate over whether such extensive usage of these artificial sweetener could lead to health problems (i.e., dysregulation of food intake) (Swithers et al., 2006). Recently, a zero-calorie natural sweetener, which is an extract from *Stevia rebaudiana* leaves, received much attention. Vitaminwater® (Zero) is one of the popular functional beverages in the market, which is sweetened with stevia and provides vitamins and minerals; therefore, it was selected as the reference in this study to develop the prototype functional beverage formulated with iBG and stevia. Also, orange flavor still remains a highly popular beverage flavor, according to "2016 flavor trends for food and beverage" (Grebow, 2016). Therefore, an orange-flavored functional beverage sweetened with stevia was the first prototype in the current study to explore the potential of iBG as a functional ingredient in beverage products.

4.3.2 Physicochemical properties of beverages

The beverage formulation developed in laboratory scale is shown in Table 4-1. The beverage base prepared with 0% BG or iBG was similar in the appearance to Vitaminwater® (Fig. 4-2). All beverages containing BG or iBG were prepared according to the procedure described in Fig. 4-1. Water added after the cooling step was to adjust the concentration of BG or iBG, which was affected by water loss during the boiling step. Other ingredients (i.e., citric acid, stevia extract, colorant and orange flavor powder) were added after cooling and concentration adjustment, because the viscosity of beta-glucan was found to decrease when heated in the presence of citric acid (Kivelä et al., 2009b). As discussed in Chapter 2, the viscosity of beta-glucan plays a very important role for its functionality and health benefits, and therefore it was necessary to determine how the addition of other beverage ingredients, especially citric acid would affect the

Figure 4-2. Visual comparison between the laboratory-scale orange-flavored beverage without BG/iBG and Vitaminwater®.

viscosity of the beverage, which is also responsible for mouthfeel. In addition, the effects of BG or iBG concentration (0.15%, 0.2% and 0.3%, w/v), temperature (0-80 ºC) and shear rate (1.29-129 s^{-1}) on beverage viscosity were evaluated. The beverages used for viscosity measurements were prepared with non-food-grade iBG (batch no. 15-D1).

The results of viscosity measurements are presented in Figs. 4-3 and 4-4. The viscosity of iBG beverages was similar to that of BG beverages under all conditions tested, which indicated that neither impregnation using the PGX process nor the addition of CoQ10 induced any effect on the structure of BG. In addition, the presence of citric acid had no effect on the viscosity of beverages, which was in agreement with Kivelä et al. (2009b) who reported that only ascorbic acid caused an immediate decrease in the viscosity of beta-glucan, compared with citric and malic acids, because ascorbic acid can participate in oxidative cleavage by reducing dissolved oxygen to produce OH-radicals, which induced depolymerisation of beta-glucan (Kivelä et al., 2009a). Furthermore, as expected, the effects of temperature and shear rate on beverage viscosity were similar to those found in Chapter 3 for BG and iBG solutions: i) increased temperature caused a decrease in viscosity, and ii) viscosity did not change with increased shear rate.

After receiving the food-grade iBG (batch no. 16-J3), all viscosity measurements were repeated for the beverage containing 0.2% food-grade iBG, and the results indicated that these two iBG samples had similar viscosity behavior and, thus, the viscosity data generated with non-food grade iBG could be used to predict the behavior of the beverages prepared with food-grade iBG. Additionally, food-grade iBG had a surface area of 21.8 m^2/g. Its particle morphology was also

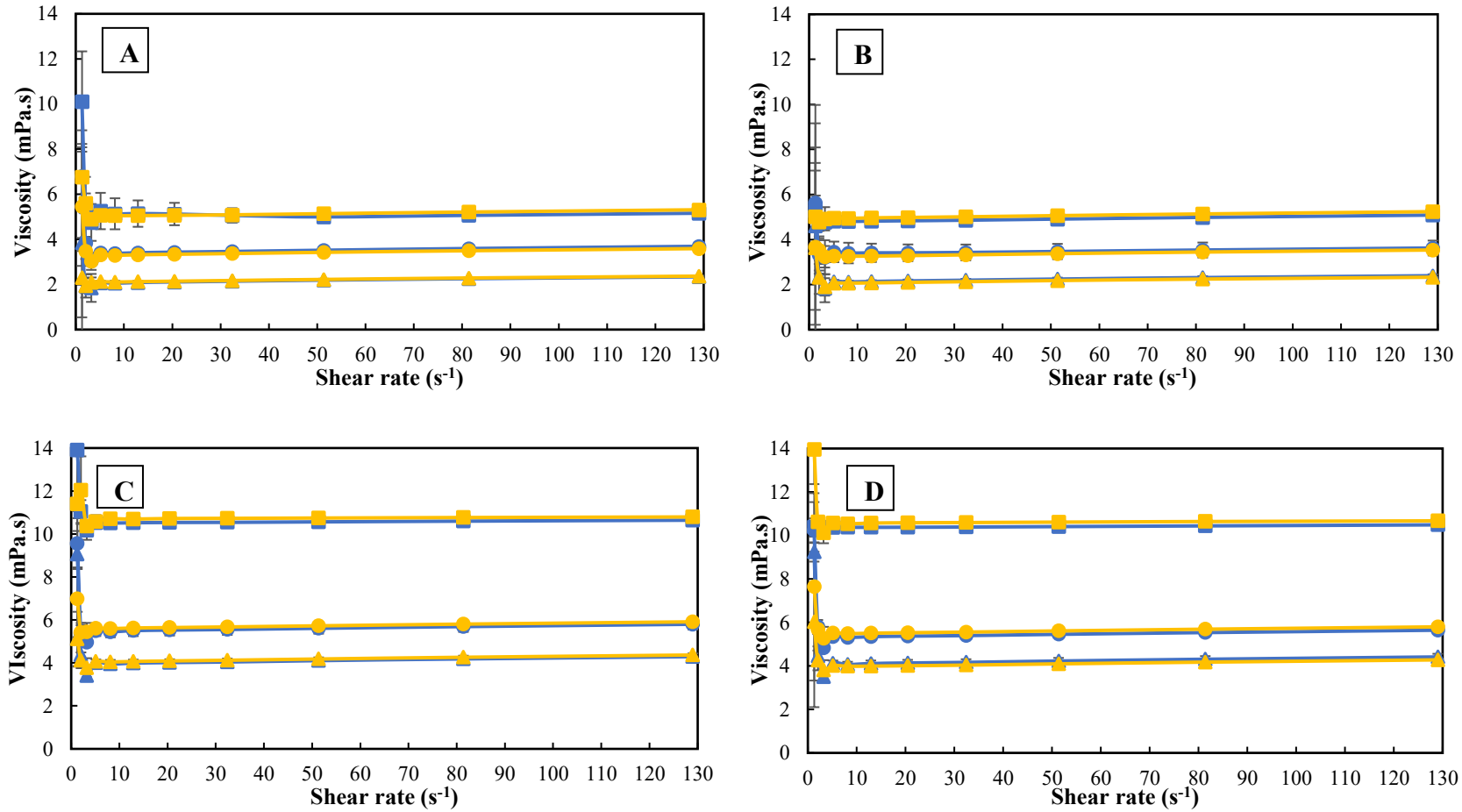


Figure 4-3. Viscosity of beverages prepared with 0.15% (Δ), 0.2% (O) and 0.3% (□) BG (blue) or iBG (yellow) as a function of shear rate at (A) 25 ºC, with citric acid; (B) 25 ºC, without citric acid; (C) 4 ºC, with citric acid; (D) 4 ºC, without citric acid. Data were presented as mean values and error bars based on duplicate measurements.

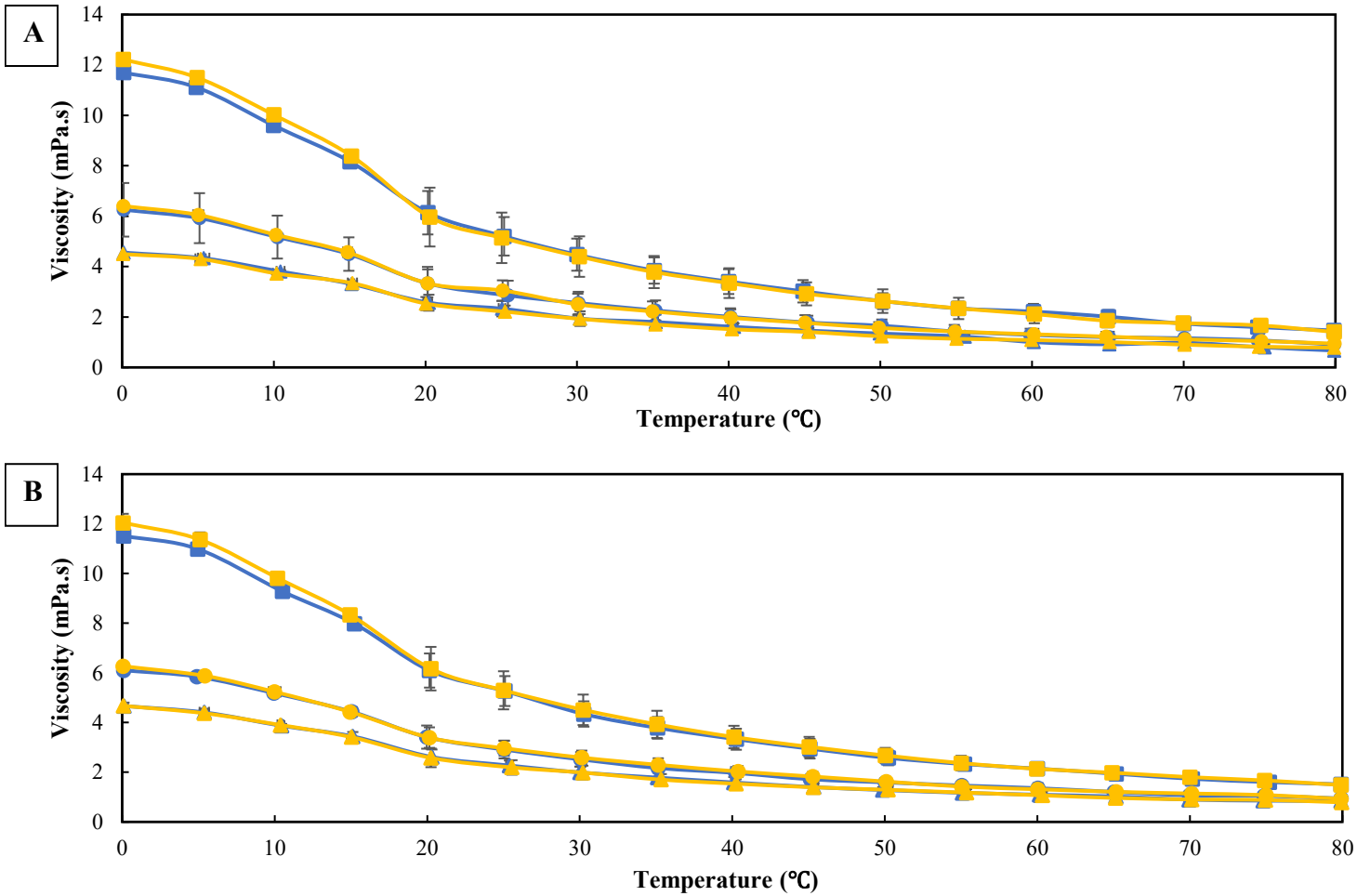


Figure 4-4. Viscosity of beverages prepared with 0.15% (Δ), 0.2% (O) and 0.3% (□) BG (blue) or iBG (yellow) as a function of temperature, at a shear rate of 50 s^{-1} (A) with citric acid; (B) without citric acid. Data were presented as mean values and error bars based on duplicate measurements.

investigated using a helium ion microscope as described in Chapter 3. Porous structures and spherical shaped CoQ10 particles were observed as shown in Fig. 4-5. However, compared with the particle morphology of the non-food grade iBG shown in Chapter 3 (Fig. 3-2), there were more spherical shaped particles distributed on the porous fibrils, which indicated a higher CoQ10 amount in the food-grade iBG. Later, this was confirmed by determining the CoQ10 content according to the protocol described in Section 3.2.8. The CoQ10 loading in the food-grade iBG was found to be around 3.3% (w/w), which was almost twice as that of the non-food grade iBG. This difference was due to the different PGX processing conditions employed by the ingredient supplier.

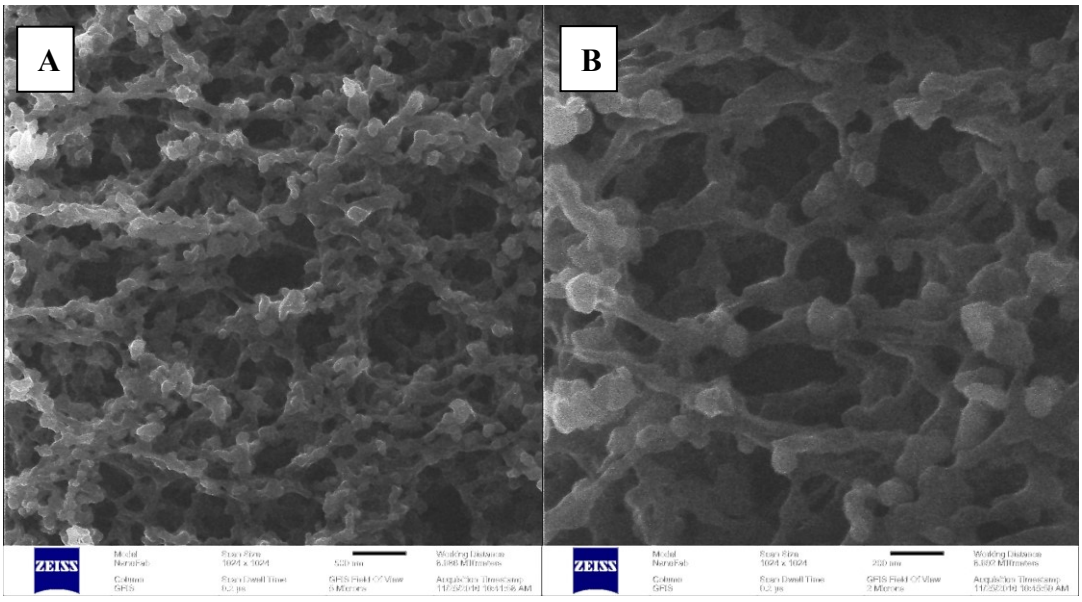


Figure 4-5. Helium ion microscopy images of food-grade iBG. Scale bars represent (A) 500 nm and (B) 200 nm.

The color of these 0.2% BG and 0.2% iBG beverages were measured to see if there is any difference in appearance. Total color difference and yellowness index were calculated and presented in Fig. 4-6, which showed the similarity of the two beverages, indicating that the orange colorant dominated the yellow color induced by iBG.

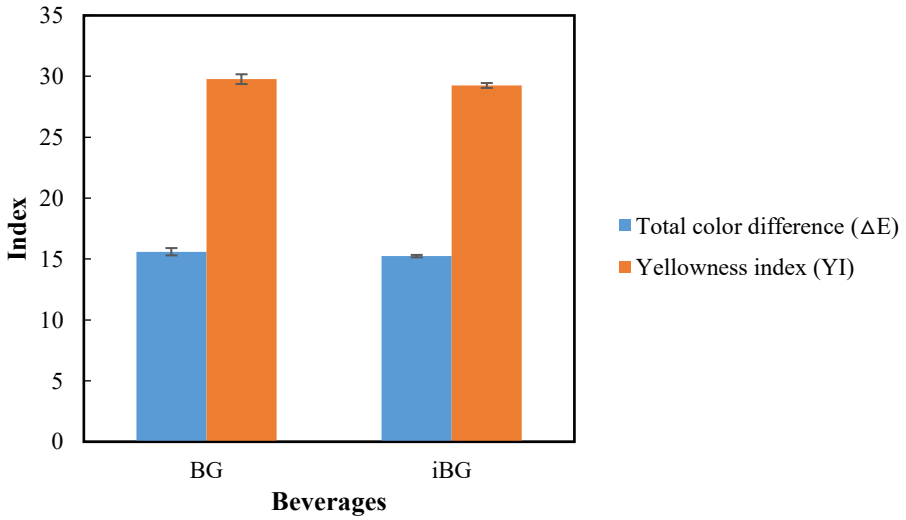


Figure 4-6. Total color difference (ΔE) and yellowness index (YI) of beverage samples prepared with 0.2% (w/v) BG and iBG ($p>0.05$).

4.3.3 Sensory evaluation

4.3.3.1 Trained panel

The beverage product had complex interactions between its various attributes evaluated (orange flavor, sweetness, sourness, aftertaste, metallic taste, bitterness and viscosity). It was anticipated to quantify and describe such interactions using the trained panel; however, upon data

analysis, it became apparent that 7 sessions of training were not sufficient and no solid conclusions could be drawn due to significant panelist effect. The results obtained, problems and recommendation for future research are presented in Appendix B. Regardless, this semi-trained panel indicated that they liked the 0.2% BG product served at 4 ^{o}C the best, and therefore these levels were adopted for the consumer panel.

4.3.3.2 Triangle test

Triangle test was performed to determine if consumers could distinguish between BG and iBG beverages. The orange colorant used was physically ground annatto seeds, and its dispersibility in water was not uniform due to impurities, therefore red light was used to avoid any interference due to the beverage appearance. A total of 6 out of the 30 assessors correctly identified the odd sample in the test. Therefore, it could be concluded with 99% certainty (i.e. $\beta = 0.01$) that no more than 5.5% of the population are able to detect a difference between the beverages prepared with 0.2% iBG and 0.2% BG. The addition of this small amount (3.3%, w/w) of CoQ10 would not affect the beverage flavor. Overall, no difference in the sensory attributes, including taste and appearance could be detected between the orange-flavored functional beverages prepared with iBG and BG.

4.3.3.3 Consumer panel

The beverage formulated with 0.2% iBG and served at 4 ºC was selected for the consumer panel, based on the feedback given by the semi-trained panel. They found that the sourness and aftertaste were less intense at the cold temperature. In addition, the viscosity of 0.2% iBG beverage at 4 ºC contributed to a smooth mouthfeel, while the 0.3% iBG beverage was described as slimy. For the consumer panel, Vitaminwater®, which is one of the popular functional beverages in the market, was taken as the reference. Also, a beverage without iBG was provided to determine whether consumers accepted the addition of iBG or not.

IPM was applied in the consumer panel to generate a beverage profile, an ideal product profile and overall acceptance of beverages. A total of 102 participants were recruited, and their demographic information is provided in Table 4-4. However, 11 participants were removed prior to data analysis, because they indicated that they have never consumed functional beverages before. The attribute intensities of beverages and ideal product evaluated by the consumer panel (n=91) are shown in Table 4-5. Overall, all three beverages were weak in bitterness, metallic taste and thickness, but moderate in orange flavor, sweetness, sourness and aftertaste. Vitaminwater® was similar to the ideal product in terms of all the attributes other than metallic taste, which explained its popularity. As expected, the 0.2% iBG beverage was thicker than that without iBG, due to the presence of soluble dietary fiber. There was no significant difference between 0% iBG and 0.2% iBG beverages, in terms of orange flavor, sweetness, aftertaste, sourness, bitterness and metallic taste.

Table 4-4. Demographic information of the consumer panel (n=102).

Question	Category	Frequency
Gender	Male	59%
	Female	41%
Age	18-39	94%
	40-59	6%
Frequency of consuming a functional drink		
	Once or more per day	5%
	Once per week	20%
	Once per month	25%
	Several times per year	39%
	Never	11%
Ingredient(s) that is looked for, when consuming functional beverages (select all that apply)	Dietary fiber	22%
	Vitamin	81%
	Low calories or zero calorie	40%
	Protein	21%
	Caffeinated	22%
	Minerals	40%
Sweetener(s) consumed/purchased in the past 6 months (select all that apply)	White granulated sugar	75%
	High fructose corn syrup	23%
	Aspartame	23%
	Sucralose	17%
	Saccharin	10%
	Acesulfame K	1%
	Stevia extract	12%
Consistency preferred in a functional beverage		
	Watery	56%
	Medium	24%
	Thick, smoothie-type	20%
Flavour(s) preferred in functional beverages (select all that apply)	Orange	74%
	Berry	34%
	Pineapple	37%
	Apple	36%
	Peach	47%
	Cherry	23%

Table 4-5. Attributes* of beverages and ideal product evaluated by the consumer panel (n=91).

	0% iBG	0.2% iBG	Vitaminwater®	Ideal product
Orange flavor	4.0±1.5a	4.2±1.6a	5.6±1.5b	6.3±1.3b
Sweetness	4.0±1.6a	4.2±1.5a	5.2±1.5b	4.6±1.3ab
Aftertaste	3.9±1.8	4.0±1.8	4.4±1.9	3.2±1.9
Sourness	4.2±2.0b	4.2±1.9b	3.3±1.9a	3.8±1.4ab
Bitterness	2.1±1.5	2.0±1.4	1.8±1.2	1.7±1.3
Metallic taste	2.2±1.6b	2.4±1.7b	2.2±1.7b	1.4±1.1a
Thickness	1.9±1.3a	2.5±1.6b	2.4±1.5b	3.5±1.8b

[a,b] Values followed by different letters in the same row are significantly different ($p<0.05$).

* Attributes evaluated on a nine-point scale with "Weak" scored as 1 and "Strong" scored as 9.

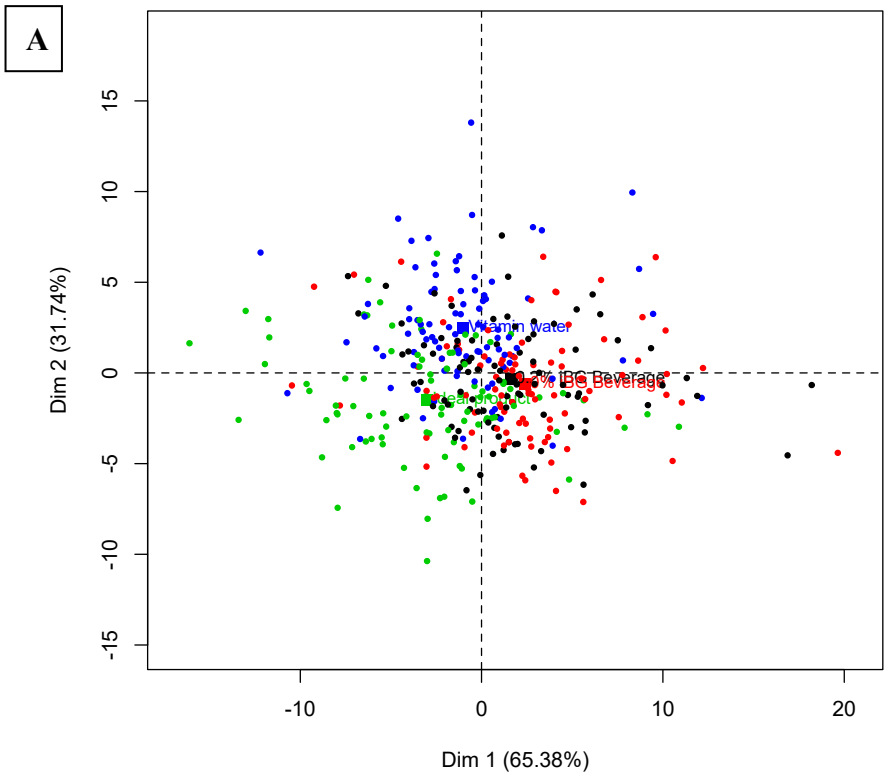
The addition of beta-glucan had no effect on the perception of aftertaste, which is in agreement with Mielby et al. (2016), who incorporated 0.5-1% (w/v) oat beta-glucan into a fruit beverage. However, Lyly et al. (2007) found that the addition of 1% (w/w) oat beta-glucan increased the intensity of oat flavor, but decreased the intensities of sourness, fruit aroma and total flavour of their apple-pear beverage. In addition, Mielby et al. (2016) also stated that increased concentration of beta-glucan resulted in decreased sourness, but increased the sweetness of their fruit beverages. The different results of these studies would be due to the different purity and concentrations of beta-glucan used in each study.

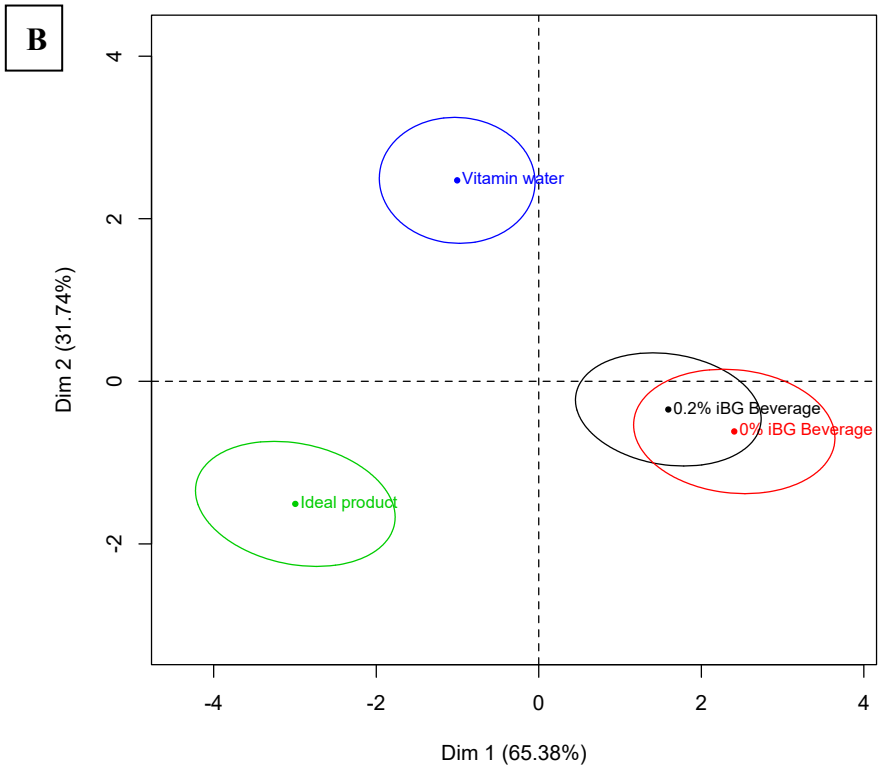
The mean values of attributes provided by the 91 participants were analyzed using PCA to examine the product space (i.e., the distance between a pair of samples). Two significant principal components accounted for 97.12% of the total variation with 65.38% for the first dimension and 31.74% for the second dimension. The PCA factor loading of each attribute is

presented in Table 4-6. All the variance of the seven attributes was explained with three significant principal components, indicating that some attributes were highly associated. Evaluation of each participant showed that except for a few outliers, most participants evaluated each sample quite consistently (Fig. 4-7A). The product space is presented in Fig. 4-7B, showing that the ideal product had a higher factor loading on the negative half of the first dimension, while 0.2% iBG and 0% iBG beverages were located close to each other on the positive half of the first dimension (Table 4-7). Vitaminwater® was characterized in the positive half of the second principle component (Table 4-7). By combining the attribute information on the variable factor map (Fig. 4-7C), it was found that Vitaminwater® had higher factor loading for "sweet", while the attribute for the ideal product was "thick". Additionally, "bitter", "sour" and to a smaller extent "metallic taste" were attributes that characterized both 0.2% iBG and 0% iBG beverages. The interpretation from PCA was consistent with the findings in Table 4-5.

Table 4-6. Loadings of 7 attributes on three significant principal components (n=91).

Attribute	**Dim.1**	**Dim.2**	**Dim.3**
Orange flavor	-0.99	0.08	-0.08
Sweetness	-0.69	0.73	0.01
Aftertaste	0.46	0.88	0.08
Sourness	0.68	-0.73	0.10
Bitterness	0.99	-0.08	-0.12
Metallic taste	0.85	0.46	0.25
Thickness	-0.85	-0.42	0.32


A
Dim 2 (31.74%)
Dim 1 (65.38%)
Vitamin water
0.2% iBG Beverage
0% iBG Beverage
Ideal product


B
Dim 2 (31.74%)
Dim 1 (65.38%)
Vitamin water
0.2% iBG Beverage
0% iBG Beverage
Ideal product

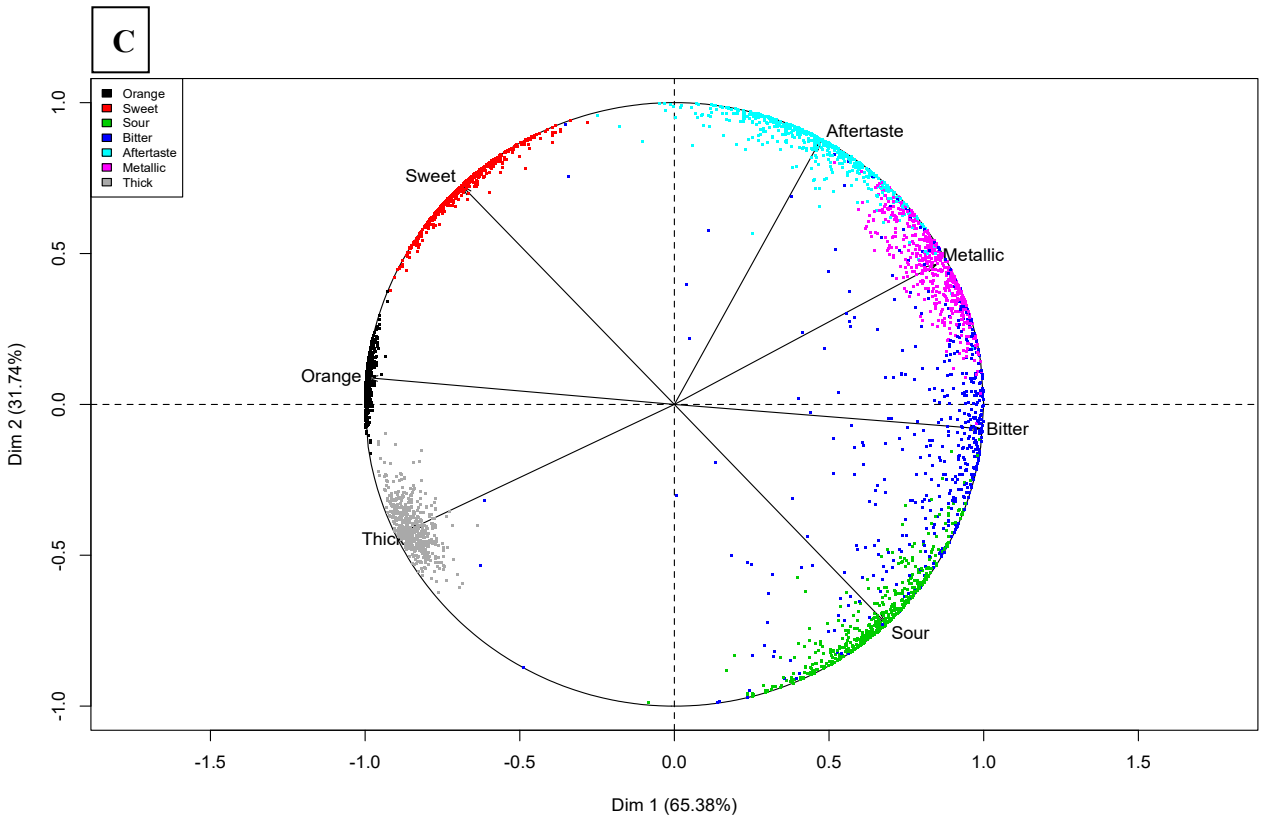


Figure 4-7. Principal component analysis (PCA) of the data of ideal product (green), Vitaminwater® (blue), 0% iBG beverage (red) and 0.2% iBG beverage (black), in terms of (A) individual description with individual scores (•) and means (□); (B) confidence ellipses of mean points; and (C) variable factor map.

Table 4-7. Loadings of 4 products on three significant principal components (n=91).

	Dim.1	**Dim.2**	**Dim.3**
0% iBG beverage	2.39	-0.63	-0.56
0.2% iBG beverage	1.60	-0.35	0.70
Vitaminwater®	-0.99	2.48	-0.08
Ideal product	-3.00	-1.51	-0.05

The perception of bitterness, aftertaste and metallic taste induced by stevia extract would have a negative effect on the sensory satisfaction of the targeted beverages, but a health claim was reported to have a higher potential added value on the overall acceptance of a product with negative attributes (Tuorila and Cardello, 2002; Sabbe et al., 2009). Therefore, the effect of health information (Table 4-3) on the overall acceptance was evaluated. There was no significant difference between the pre-information (5.1±1.6) and the post-information (5.3±1.6) acceptance of the beverage without iBG. But a positive effect of the provided health information on the overall acceptance was found for the 0.2% iBG beverage with the pre-information acceptance of 5.2±1.5 and the post-information acceptance of 5.9±1.4.

By evaluating the overall acceptability difference (Fig. 4-8), over half of the participants (52%) liked the 0.2% iBG beverage more after providing health information, while 33% did not change their mind, probably because they were not interested in the ingredients in these beverages. Among these 30 participants who did not change their mind, 83% of them selected "vitamin" as their ingredient of interest in a functional beverage, while only 4 participants chose "dietary fiber". In addition, there was almost 2.5 times as many participants who liked the 0% iBG beverage less after reading the provided health information, compared with the 0.2% iBG beverage. Health information for these two beverages were provided side by side in the current study, and thus a potential comparison between beverages could exist, such that the beverage with more ingredients providing health benefits would reduce the acceptance of the beverage with fewer healthy ingredients for participants who responded favorably to the information.

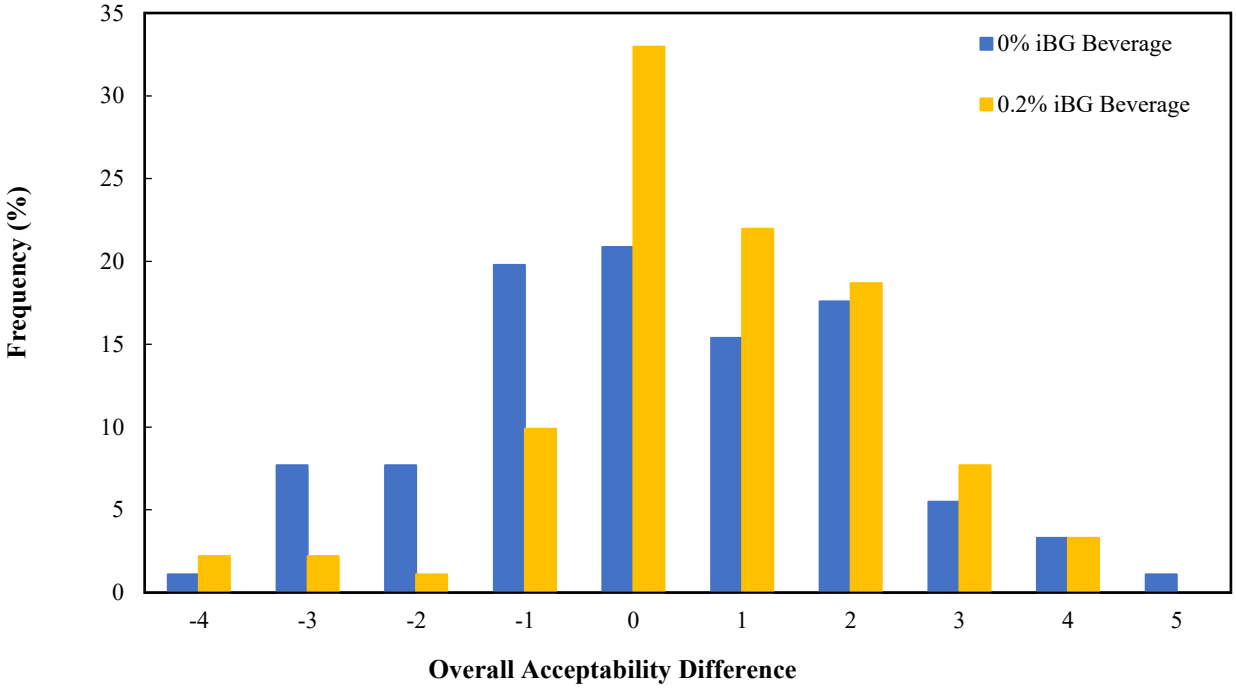


Figure 4-8. Frequency distribution of the overall acceptability difference for consumer panel scores (on a 9-point hedonic scale) for beverages with 0.2% (w/v) iBG and 0% iBG. The difference was calculated as "post-information acceptance" minus "pre-information acceptance". Positive values=like more; zero=no change; negative values=like less.

In a study of fruit juices with different concentrations of açaí, overall acceptance was highly influenced by participant gender, age, personal characteristics (i.e., food neophobia) and education background (Sabbe et al., 2009). Women and elder people were reported as the group who cared the most about the health-promoting ingredients, especially fiber and antioxidants, and a higher overall acceptance was generally associated with this group when rating a functional product with provided ingredient information (Ares et al., 2009). Also, health and

nutrition claims were only positively perceived by specific target consumers who are interested in the ingredient, understand the benefits, and expect the product with such claims to help them in reaching their overall health goals (Buul and Brouns, 2015). Thus, in the present study, a target group comprised of 47 participants who scored higher on post-information acceptance (overall acceptance difference = 1, 2, 3, 4) was selected to determine how they evaluated the target beverages, which would help with beverage formulation improvement. In this target group, there were 30 women, which could explain the reason for positive values of the overall acceptance difference. However, a total of 40 out of the 47 participants were 18-29 years of age, which was not in agreement with the previous findings. The main reason would be the location of recruitment. All participants were recruited at the Agriculture/Forestry Centre, University of Alberta, so most were highly educated university students. Evaluation of the influence of demographic factors on product acceptance cannot be done with this convenience sample of university students. However, as this group of educated individuals responded positively to health messages, they may be a target market for these functional beverage products and their insight on the product attributes is valuable. Furthermore, the format of the health information presentation was also found to have an effect on the overall product acceptance in a previous study, stating that disease-related health benefits with a healthy image or health positioning history could result in a preference of a functional product (Kleef et al., 2005).

To improve the beverage formulation, attribute intensity of beverages and the ideal product evaluated by the target group of 47 participants was assessed further (Table 4-8). The product

space among samples (Appendix C) was similar to that generated with the data of 91 participants (Fig. 4-7). In contrast to the evaluation of the whole group of 91 consumers (Table 4-5), the target group of 47 consumers found the 0.2% iBG beverage to be similar to their ideal product in terms of metallic taste (Table 4-8). Even though the thickness of the ideal product was not significantly different from that of the 0.2% iBG beverage, the radar chart (Fig. 4-9) indicated

Table 4-8. Attributes* of beverages and ideal product evaluated by the target consumer group (n=47)

	0% iBG	**0.2% iBG**	**Vitaminwater®**	**Ideal product**
Orange flavor	4.0±1.6a	3.9±1.6a	5.7±1.5b	6.4±1.2b
Sweetness	3.7±1.5a	4.1±1.7ab	5.0±1.5c	4.8±1.3bc
Aftertaste	3.8±1.5	4.3±1.9	4.4±2.1	3.3±1.9
Sourness	4.4±2.1b	4.3±2.1b	3.4±1.8a	3.8±1.3ab
Bitterness	1.6±1.6	2.3±1.5	1.9±1.2	1.8±1.3
Metallic taste	2.4±1.7	2.7±1.8	2.4±1.8	1.5±1.0
Thickness	1.8±1.1a	2.6±1.6b	2.4±1.5ab	3.6±1.7b

[a,b] Values followed by different letters in the same row are significantly different ($p<0.05$).

* Attributes evaluated on a nine-point scale with "Weak" scored as 1 and "Strong" scored as 9.

that there was still opportunity to incorporate a higher concentration of iBG, because from a health perspective, a higher concentration of beta-glucan is desired. When it is indicated that dietary fiber is added to a beverage, the consumers would expect a more viscous mouthfeel to confirm its presence; therefore, they sought a thicker beverage in this study, although around 55% of the 91 participants stated they prefer the consistency of water for functional beverages.

Additionally, off–flavors introduced by the stevia extract, including bitterness, metallic taste and aftertaste were expected to be as low as possible. As well, a more intense orange flavor was expected, a more intense orange flavor was expected, which could be improved by adding orange essential oil or orange peel oil.

Figure 4-9. Sensory profiles of the beverage samples and ideal product evaluated by a consumer panel. Means of 47 evaluations. * indicates statistically significant difference at $p<0.05$.

4.4 Conclusions

The formulation of an orange-flavored functional beverage containing PGX-processed BG or iBG and sweetened with stevia extract was successfully developed. The addition of citric acid had no effect on the viscosity of beverages prepared with either iBG or BG. There was no

detectable difference between iBG and BG beverages, indicating that the presence of 3.3% (w/w) CoQ10 did not introduce any effect on the sensory attributes of the functional beverages. Also, the addition of 0.2% iBG had no effect on any of the sensory attributes evaluated other than thickness when compared with the 0% iBG beverage. Providing health information regarding the ingredients yielded a positive overall acceptance for the 0.2% iBG beverage. The formulation can be further improved with the addition of a higher iBG concentration and orange essential oil in the future to satisfy the sensory expectations of the target group of consumers. Overall, the potential use of both PGX-processed BG and iBG as nutraceutical ingredients in functional beverages was demonstrated in this study.

Chapter 5: Conclusions and recommendations

5.1 Summary of key findings

Beta-glucan obtained from oat and barley is a linear polysaccharide with a large molecular weight, indicating its great potential for use as a carrier of other bioactive components in particle formation as delivery systems. However, only a limited number of studies have evaluated this aspect. In this thesis research, the physicochemical properties of the oat beta-glucan powder (BG) and CoQ10 impregnated BG powder (iBG) produced by the pressurized gas-expanded liquid (PGX) technology were studied to characterize such ingredients and explore their potential for product applications. BG powder was white fibrils with a porous structure (half pore width = 27.9 nm) and high molecular weight (>> 361,000 Da). The purity of BG was 83.6% (w/w) with impurities including 7.4% (w/w) moisture, 1.5% (w/w) protein, 2.9% (w/w) ash and 4.6% (w/w) other carbohydrate components. The viscosity of BG solution increased as the concentration increased from 0.15% to 0.3% (w/v), while exhibiting Newtonian behavior with a constant viscosity through the shear rate range of 1.29 s^{-1} to 129 s^{-1}. Also, a decrease in viscosity was observed at the shear rate of 50 s^{-1} when temperature was increased from 0 ºC to 80 ºC. Furthermore, it was shown that impregnation of BG particles with CoQ10 using the PGX process did not interrupt the porous structure or affect the viscosity of BG. Compared with BG, iBG was a yellowish powder, due to the presence of 1.4% (w/w) CoQ10 in its amorphous form. Both BG (7.7 μm) and iBG (6.1 μm) particles were micrometer-sized particles, while CoQ10 in iBG had a

spherical shape and a particle size between 35 nm and 160 nm with the average diameter of 92 nm.

Another major goal of this thesis research was to utilize iBG as a functional ingredient in a beverage product. An orange-flavored functional beverage formulated with the PGX-processed BG or iBG, and sweetened with stevia extract was developed. The addition of citric acid had no effect on the viscosity of beverages prepared with either iBG or BG. The sensory quality of the beverages was assessed by three different panels. A semi-trained panel (n=12) recommended the beverage formulated with 0.2% iBG served at 4 ºC. The overall difference between beverages prepared with 0.2% iBG and BG was determined by a triangle test (n=30), and there was 99% certainty (i.e. $\beta = 0.01$) that no more than 5.5% of the population are able to detect a difference between these two beverages. Ideal profile method was applied to evaluate the consumer acceptance (n=91) of the 0.2% iBG beverage served at 4 ºC. The addition of 0.2% iBG had no effect on all the sensory attributes evaluated (i.e., orange flavor, sourness, sweetness, bitterness, metallic taste and aftertaste) other than thickness, when compared with the beverage without iBG. The effect of health information on the overall acceptance of beverages was also evaluated. After providing the health information for stevia extract for the beverage without iBG and that for stevia extract plus beta-glucan and CoQ10 for the 0.2% iBG beverage, around 52% of the participants liked the 0.2% iBG beverage more (n=47), while 15% of participants liked the product less. However, for the beverage without iBG, 41% of participants liked it more, while 37% liked it less after providing health information. Principle component analysis demonstrated the

distinction between iBG beverage and the ideal beverage. Based on a comparison with the ideal product, improvements can be made on the formulation to make the iBG beverage closer to the ideal product of consumers, including an increase in orange flavor, sweetness and thickness.

Overall, this thesis presents fundamental characterization of iBG and BG ingredients produced using the PGX process, which should be useful for optimizing the impregnation conditions and developing novel combinations for the delivery of different bioactives. In addition, the prototype functional beverage developed exhibited the potential of iBG as a functional ingredient for incorporation into beverages, and may inspire new applications in food products or natural health products.

5.2 Recommendations for future work

Based on the findings, it would be worthwhile to investigate the following aspects in future research:

- More analysis, including the proximate composition of iBG, molecular weight of BG, the effect of boiling on iBG, especially CoQ10 should be done in the future.
- The bioavailability of CoQ10 in the iBG should be evaluated to determine if the impregnation of CoQ10 onto the BG powder helps increase its bioavailability.
- The stability of the beverage containing 0.2% iBG, specifically the stability of the bioactives over the shelf life of the beverage should be evaluated.
- The addition of beta-glucan was anticipated to have a masking effect on the off-flavor

introduced by stevia extract. Due to the insufficient training of the panelists, it was not possible to assess this effect fully. Therefore, the potential masking effect of beta-glucan on the off-flavor of stevia can be evaluated in the future.

- For the trained panel, more training time is needed to obtain well-trained panelists and avoid the panelist effect on the results. Despite the efforts to add more training time in this study, it was not possible. Alternatively, other methods requiring shorter time, like free-choice profiling method could be used to do quantitative descriptive analysis.
- Recruitment location for the consumer panel could be varied to reflect the product acceptance of the public. Alternatively, the location could be very specific, like a gym or physical fitness facility, because the target population of functional beverages is commonly those who care about their dietary and life style.

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Appendix A: Questionnaires

A.1 Trained panel screening questionnaire

Questionnaire for Screening

Date: 2016/9/21 Participant:_____

1. **Color identification test**
   Take a look at the 7 samples. Please rank them from light to dark, and write down the code on the following scale:

Color	Code
Light ⇓ Dark	

2. **Odor identification test**
   Open the lid, and smell the 3 samples in the bottles labelled with 3-digit code, and identify the sensory attributes for them. Please match the code to the following odors. Please refresh between each sample by smelling the provided coffee beans.

Odor	Orange	Vanilla	Floral
Code			

Date: 2016/9/21 Participant:_____

3. Taste identification test

Taste the 4 samples in the cups with 3-digit code (**726**, **480**, **201**, **069**), and identify the sensory attributes for them. Please write down the code to the 4 basic tastes.
Please cleanse your palate between each sample by taking a sip of water.

Taste	Sweetness	Sourness	Bitterness	Metallic Taste
Code				

4. Texture test

Taste the 3 samples in the cups with 3-digit code (**825**, **653**, **163**). Please describe the attribute of the sample that you received, and rank them from low to high intensity.
Please cleanse your palate between each sample by taking a sip of water.

Descriptors you may need:
juicy, viscous, clean, crunchy, crispy, hard, chewy, tough, grainy, runny, creamy, brothy

Intensity	Code	Descriptor
Low ⇩ **High**		

Thanks for your participation. We will contact you shortly via email!
☺

A.2 Trained panel evaluation questionnaire

Date: 2016/10/07 Participant #: _____

Beverage sensory evaluation form for trained panel

You will be evaluating **8** samples (**4 at 4°C** and **4 at 25°C**) with a break of **5-10** min in between each set of 4 samples.

Please rinse your mouth with water before starting and between samples. Unsalted crackers are also provided for refreshing your palate.

Please evaluate each sample for **SWEETNESS, AFTERTASTE, METALLIC TASTE, BITTERNESS, VISCOSITY, ORANGE FLAVOR** and **SOURNESS**

Sample: 440

ORANGE FLAVOR

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

SWEETNESS

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

AFTERTASTE

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

SOURNESS

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

BITTERNESS

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

METALLIC TASTE

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

VISCOSITY

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

Date: 2016/10/07 Participant #: _____

Sample: 421

ORANGE FLAVOR

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

SWEETNESS

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

AFTERTASTE

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

SOURNESS

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Weak Strong

BITTERNESS

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

METALLIC TASTE

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

VISCOSITY

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

Date: 2016/10/07 Participant #: _____

Sample: 784

ORANGE FLAVOR

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

SWEETNESS

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Weak Strong

AFTERTASTE

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Weak Strong

SOURNESS

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Weak Strong

BITTERNESS

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Weak Strong

METALLIC TASTE

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Weak Strong

VISCOSITY

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Weak Strong

Date: 2016/10/07 Participant #: _____

Sample: 151

ORANGE FLAVOR

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

SWEETNESS

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Weak Strong

AFTERTASTE

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SOURNESS

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METALLIC TASTE

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Weak Strong

VISCOSITY

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Weak Strong

Now, please take a break of 5-10min.

Date: 2016/10/07 Participant #: _____

Sample: 729

ORANGE FLAVOR

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

SWEETNESS

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Weak Strong

AFTERTASTE

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METALLIC TASTE

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Weak Strong

VISCOSITY

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Weak Strong

Date: 2016/10/07 Participant #: _____

Sample: 426

ORANGE FLAVOR

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Weak Strong

SWEETNESS

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Weak Strong

AFTERTASTE

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Date: 2016/10/07 Participant #: _____

Sample: 670

ORANGE FLAVOR

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Weak Strong

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Weak Strong

Date: 2016/10/07 Participant #: _____

Sample: 632

ORANGE FLAVOR

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SWEETNESS

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Weak Strong

BITTERNESS

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

METALLIC TASTE

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

VISCOSITY

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Weak Strong

**Among these 8 samples, please let us know which one you prefer. Please write down the sample code here:_____

----Here is the end of the evaluation. Thanks for your interest, efforts and time. Really appreciate it!----

8

A.3 Triangle test questionnaire

Date:_____ Participant #:_____

Triangle Test for orange-flavored functional beverage

Demographic Information
Please indicate your gender: ❒Male ❒ Female
Please indicate the age group that you belong to: ❒18-29 years ❒50-59 years ❒30-39 years ❒60-69 years ❒40-49 years ❒70 years plus

Triangle test
Instructions Taste samples from left to right. Two samples are alike; one is different. Circle the sample that differs from the others. If you are not sure, record your best guess; you may note under remarks that you were guessing. **485 146 324** Remarks:_____ _____

Thank you very much and have a nice day☺.

A.4 Consumer panel questionnaire

Date:_____ **Participant #:**_____

Consumer Evaluation of Orange-flavored Functional Beverage

Demographic Information

1. Please indicate your gender:
   ❒Male ❒ Female

2. Please indicate the age group that you belong to:
   ❒18-29 years ❒50-59 years
   ❒30-39 years ❒60-69 years
   ❒40-49 years ❒70 years plus

3. A "functional beverage" is a drink formulated with health benefiting ingredients, for example, vitamin water. On average, how often do you consume functional drink?
   ❒Once or more per day
   ❒Once per week
   ❒Once per month
   ❒Several times per year
   ❒Never

4. When you consume functional beverage, which ingredient(s) do you look for? Select all that apply.
   ❒Dietary fiber
   ❒Vitamin
   ❒Low calories or zero calorie
   ❒Protein
   ❒Caffeinated
   ❒Minerals
   ❒Other:_____

5. What type of sweeteners did you consume, and/or purchase in the past 6 months? Select all that apply.
   ❒White granulated sugar
   ❒High fructose corn syrup
   ❒Aspartame (Equal, NutraSweet)
   ❒Sucralose (Splenda)
   ❒Saccharin (Sugar twin, Sweet'N Low)
   ❒Acesulfame Potassium (Sunett, Sweet One)
   ❒Stevia extract (Steviol glycodies)

Date:_____ **Participant #:**_____

6. Which consistency do you prefer in a functional beverage?
   - ❒ Watery
   - ❒ Medium
   - ❒ Thick, smoothie-type

7. Do you have a preference for any of the following flavours in functional beverages? Select all that apply.
   - ❒ Orange
   - ❒ Berry
   - ❒ Pineapple
   - ❒ Apple
   - ❒ Peach
   - ❒ Cherry
   - ❒ Others:_____

Date:_____ **Participant #:**_____

Consumer Evaluation of Orange-flavored Functional Beverage

Sensory questionnaire

You have 2 sets of samples to evaluate: **SET #1** and **SET #2**. Please start with the **first** set.

SET #1

In this set, you have **three** coded samples of beverages in front of you. Evaluate the samples in the order shown below.

Please cleanse your palate before you begin with a sip of water.

Date:_____ **Participant #:**_____

Sample:_____

How is the **orange flavor** of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **sweet** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **sour** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **bitter** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How is the **aftertaste** of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How is the **metallic taste** of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **thick** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

Overall, what is your **opinion** of this beverage?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like nor dislike | Like slightly | Like moderately | Like very much | Like extremely

Date:_____ **Participant #:**_____

Please cleanse your palate with water and unsalted crackers before you evaluating the next sample.

Sample: _____

How is the **orange flavor** of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **sweet** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **sour** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **bitter** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How is the **aftertaste** of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How is the **metallic taste** of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **thick** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

Overall, what is your **opinion** of this beverage?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like nor dislike | Like slightly | Like moderately | Like very much | Like extremely

Date:_____ **Participant #:**_____

Please cleanse your palate with water and unsalted crackers before you evaluating the next sample.

Sample:_____

How is the **orange flavor** of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **sweet** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **sour** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **bitter** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How is the **aftertaste** of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How is the **metallic taste** of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

How **thick** is this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

Overall, what is your **opinion** of this beverage?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like nor dislike | Like slightly | Like moderately | Like very much | Like extremely

Date:_____ **Participant #:**_____

If you can prepare this orange-flavored beverage by yourself, then

Your Ideal Sample:

What is your ideal or preferred level of **orange flavor** for the beverage?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

What is your ideal or preferred level of **sweetness** for the beverage?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

What is your ideal or preferred level of **sourness** for the beverage?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

What is your ideal or preferred level of **bitterness** for the beverage?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

What is your ideal or preferred level of **aftertaste** for the beverage?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

What is your ideal or preferred level of **metallic taste** for the beverage?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

What is your ideal or preferred level of **thickness/mouthfeel** for the beverage?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

None Slight Moderate Very Extremely

Please notify one of our research investigators when you finish the first set. They will provide you the second set of samples.

Date:_____ **Participant #:**_____

Consumer Evaluation of Orange-flavored Functional Beverage

SET #2

You have **two** samples of beverages in front of you. Information about the ingredients and their health benefits are provided below. Please read the information and evaluate the samples for overall acceptance.

Sample 946	**Sample 457**
Stevia Extract (Rebaudioside A): • non-sugar sweetener • natural sweetener • zero calories Beta-glucan: • soluble dietary fiber • 0.75g per serving beta-glucan helps reduce/lower cholesterol, a risk factor for heart disease • health claim approved by Health Canada Coenzyme Q10: • vitamin-like antioxidant • help maintain cardiovascular health	Stevia Extract (Rebaudioside A): • non-sugar sweetener • natural sweetener • zero calories

Date:_____ **Participant #:**_____

Please cleanse your palate with water and unsalted crackers before you start.

Sample 946

Overall, what is your opinion of this beverage?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please cleanse your palate with water and unsalted crackers before you evaluating the next sample.

Sample 457

Overall, what is your opinion of this beverage?

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Additional Comments:

_____

_____

_____

_____

_____

Thanks for your participation. Your responses are very precious for us.

Appendix B: Trained panel

B.1 Objective

The objective of this study was to determine the effect of beta-glucan (BG) concentration (0.15%, 0.2%, 0.3% w/v) and storage temperature (25 ^{o}C or 4 ^{o}C) on the sensory characteristics (sweetness, sourness, aftertaste, mouthfeel, orange flavor, bitterness and metallic taste) of stevia-sweetened orange-flavored beverage.

B.2 Methods

B.2.1 Screening procedure

An online registration form was firstly sent to potential participants to collect their contact information, demographic information, availability, and experience with functional beverages and non-sugar sweeteners. Then participants were invited to a screening session which involved 4 sensory tests: 1) color vision test; 2) taste identification test; 3) odor identification test; and 4) texture test. In the color vision test, participants were asked to rank 7 orange solutions in terms of color intensity. The result helped to determine their color vision and judgement. In the taste identification test, participants were asked to identify the taste of four solutions (sweet, metallic taste, bitter and sour). This part was designed to determine their sensitivity to substances, which may be present in small concentrations in the products. In odor identification test, participants were asked to identify the odor of three materials. This part could help detect anosmia or possible lack of sensitivity. In texture test, participants were asked to rank three different

solutions in terms of mouthfeel. This part was to determine their ability to discriminate the intensity of a texture property. Participants who passed the screening session with more than 80% correct answers were invited to participate in the following training sessions.

B.2.2 Training

A total of 12 panelists (11 females, aged from 18 to 29 years) were selected for training. In total, 7 training sessions were held to help participants: 1) familiarize with basic principles of sensory evaluation, test procedures and scaling methods; 2) improve their ability to recognize and identify seven important sensory attributes of the test beverages, including sweetness, sourness, aftertaste, bitterness, aftertaste, metallic taste and orange flavor; and 3) improve sensitivity and memory to make consistent sensory judgments while evaluating samples. Each session focused on one of the attributes. Participants were instructed on how to evaluate the attributes individually and rate the intensity for each attribute. For each attribute, two extremely intense samples were provided to help participants have a general idea of the scaling on this attribute.

B.2.3 Evaluation

After training, participants were asked to attend three evaluation sessions. In each session, 8 beverages at two different temperatures (4 samples at 25 ^{o}C and 4 samples at 4 ^{o}C) were evaluated on their sweetness, sourness, aftertaste, bitterness, aftertaste, metallic taste and orange

flavor using the scale adopted during the training sessions. At each temperature, four beverages containing beta-glucan at the concentrations of 0%, 0.15%, 0.2% and 0.3% (w/v) were provided. Participants started with the 4 samples at 25 ^{o}C, then after a 15 min rest, they were asked to evaluate the other 4 samples at 4 ^{o}C.

B.3 Results

The performance of the trained panel was shown in Fig. B-1, with product p-values provided. Generally, a well-trained panel should have no colored box shown under the column of "Participant", indicating no significant participant effect. However, in this study, the p values under that column were much smaller than 0.05, which indicated that participants evaluated the samples differently from each other. Removing certain outlier participants did not improve the overall panel performance. Therefore, in this study, participants did not all agree with the scale they developed and they required further training, but unfortunately, it was not possible to extend the training time due to unavailability of the participants.

However, a two-way ANOVA was still applied to determine the effect of temperature and BG concentration and their interaction on the sensory attributes of the orange-flavored functional beverages. The model applied in ANOVA was:

Beverage=Concentration*Temperature + Random effect (Participant).

	Product	Participant	Replicate	Product:Participant	Product:Replicate	Participant:Replicate	median
Viscosity	1.908e-23	0.0007979	0.2399	9.036e-06	0.9084	0.03301	0.01691
Orange.Flavor	0.01553	3.361e-10	0.3787	0.0005866	0.0966	0.3148	0.05606
Sweetness	0.2355	2.195e-10	0.119	8.404e-10	0.04837	0.5391	0.08369
Aftertaste	0.3214	4.195e-11	0.3337	1.086e-08	0.9995	0.1475	0.2344
Bitterness	0.7307	6.342e-06	0.6262	0.1028	0.4329	0.7329	0.5295
Sourness	0.841	4.361e-06	0.9352	0.005573	0.4469	0.9345	0.644
Metallic.taste	0.8904	7.793e-11	0.4682	0.9016	0.9908	0.9533	0.896

Figure B-1. Panel performance sorted by product p-value.

The results of the ANOVA were presented in Table B-1. BG concentration, serving temperature and their interaction had significant effects on orange flavor and viscosity of beverages (Table B-2), while sweetness (Table B-3) and sourness (Table B-4) were significantly affected by only concentration or temperature, respectively. It was found that beverages served at 4 ºC had a higher sourness intensity. The sweetness intensity increased by the addition of BG, but was not affected by the increased concentration of BG. The beverage prepared with 0.3% BG and served at 4 ºC had the strongest orange flavor. Furthermore, viscosity detected by participants reflected the same trend as instrumentally measured values: viscosity increased as temperature decreased and as BG concentration increased. Considering the significant panelist effect on the results obtained from the trained panel, no solid conclusion could be drawn; but this semi-trained panel provided some direction for the product selection (0.2% BG product served at 4 ºC) for the subsequent consumer panel.

Table B-1. ANOVA P-values.

	Concentration	**Temperature**	**Interaction**
Orange flavor	0.0001*	0.0011*	0.0017*
Sweetness	<0.0001*	0.8686	-
Aftertaste	0.0559	0.7393	-
Sourness	0.4321	<0.0001*	-
Bitterness	0.2094	0.8366	-
Metallic taste	0.9738	0.6375	-
Viscosity	<0.0001*	<0.0001*	<0.0001*

* indicates significant effect at $p<0.05$.

Table B-2. The intensity* of orange flavor and viscosity of beverages prepared with four concentrations of BG and served at 25 ºC and 4 ºC.

	BG concentration	Orange flavor	Viscosity
T=25ºC	0%	7.7±1.1a	1.4±0.9a
	0.15%	8.5±0.7bc	4.9±0.7b
	0.2%	7.9±1.2ab	7.5±1.4c
	0.3%	8.1±0.8ab	10.9±1.2d
T=4ºC	0%	8.1±0.8ab	1.1±0.4a
	0.15%	8.3±1.2ab	4.4±0.6b
	0.2%	8.3±0.9ab	7.6±1.0c
	0.3%	8.9±1.0c	13.5±0.5e

[a-e] Values followed by different letters in the same column are significantly different at $p<0.05$.

* Intensity evaluated on a 15-point scale with "Weak" scored as 1 and "Strong" scored as 15.

Table B-3. The sweetness intensity* of beverages prepared with four concentrations of BG.

BG concentration	Sweetness
0%	7.3±1.3a
0.15%	7.9±1.1b
0.2%	7.9±1.0b
0.3%	8.2±1.2b

[a,b] Values followed by different letters are significantly different at $p<0.05$.

* Intensity evaluated on a 15-point scale with "Weak" scored as 1 and "Strong" scored as 15.

Table B-4. The sourness intensity* of beverages served at 25 ºC and 4 ºC.

Temperature(ºC)	Sourness
25	8.4±1.4a
4	9.2±1.4b

[a,b] Values followed by different letters are significantly different at $p<0.05$.

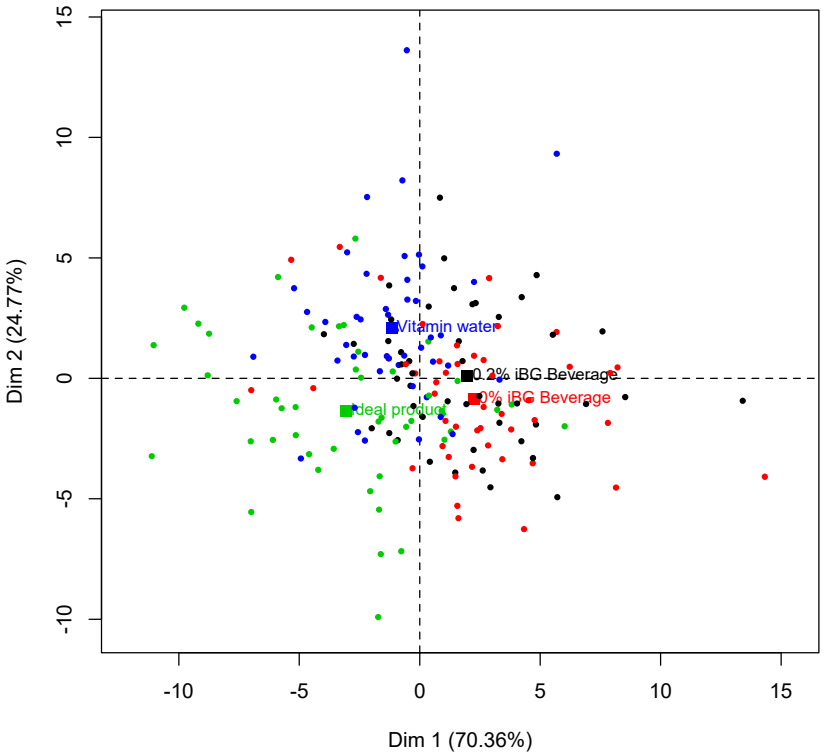
* Intensity evaluated on a 15-point scale with "Weak" scored as 1 and "Strong" scored as 15.

B.4 Recommendations

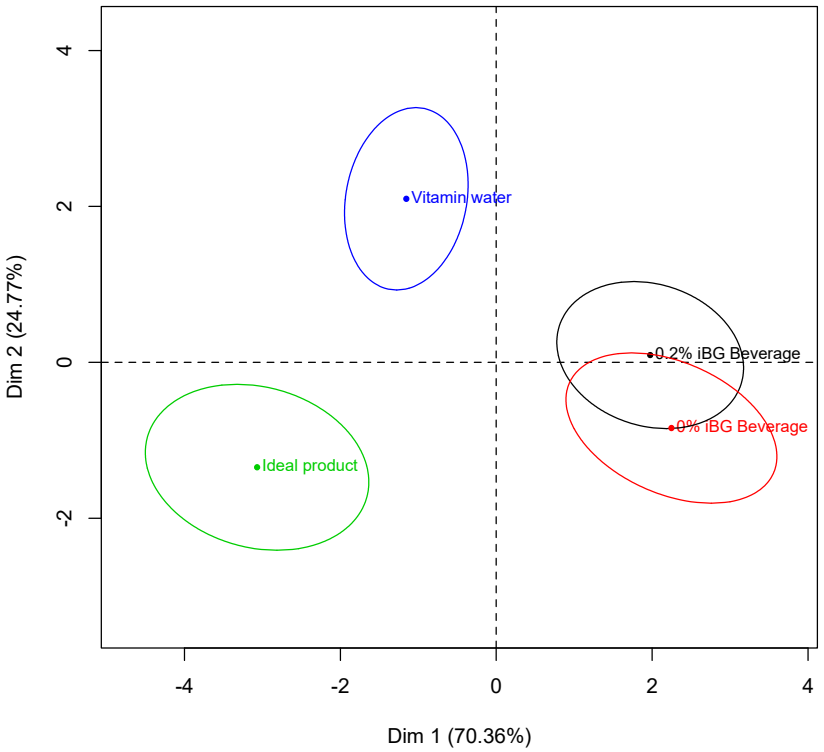
- Panelists should develop their own descriptors for beverages.
- More sessions are needed to train participants. After several sessions, the panel performance should be evaluated, and then one or more sessions could be arranged for retaining certain attributes if necessary. Until all participants reach an agreement and consistency on the use of the scale, participants are not considered to be well-trained to evaluate the test samples.
- Other than crackers and water, weak green tea could also be provided for participants to clean palate.
- During evaluation, sample order should be balanced, because carry-over from the first sample would affect the evaluation of the second sample.

Appendix C: PCA on the target group of 47 participants

A



B



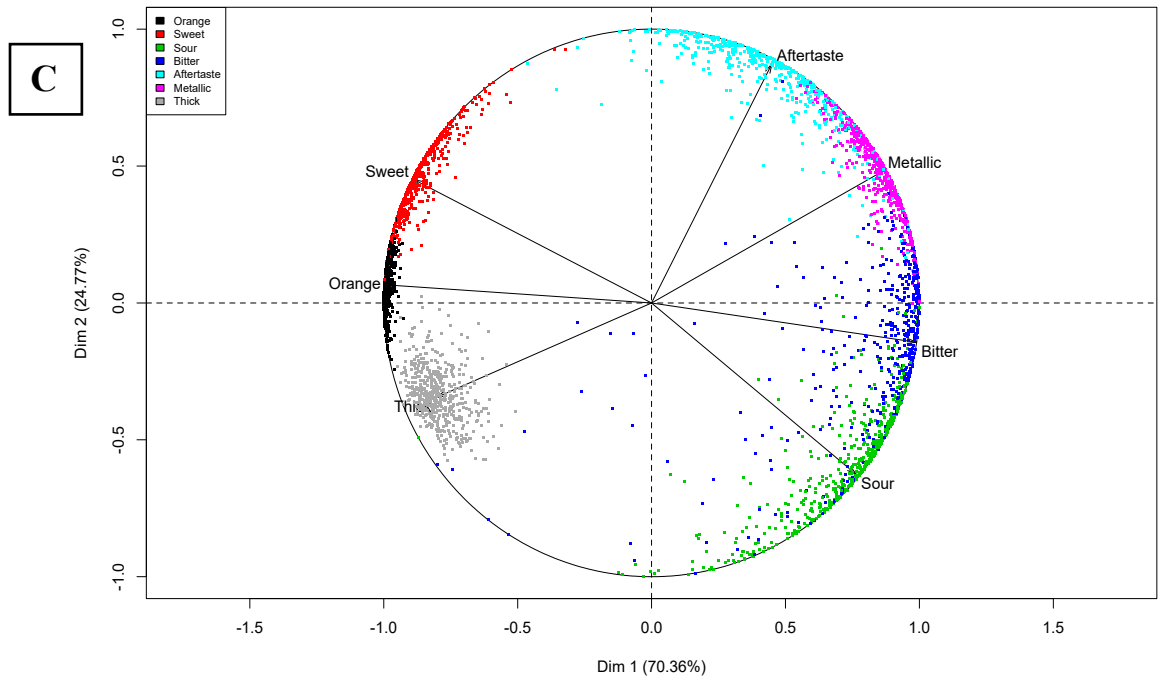


Figure C-1. Principal component analysis (PCA) on (A) individual description with individual scores (•) and means (□); (B) confidence ellipses of mean points; and (C) variable factor map.