

**Utilization of a By-Product from Goji Berry Beverage as a Value-Added
Ingredient in Chinese Steamed Bread**

by

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ABSTRACT

UTILIZATION OF A BY-PRODUCT FROM GOJI BERRY BEVERAGE AS A VALUE-ADDED INGREDIENT IN CHINESE STEAMED BREAD

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Chinese steamed bread (CSB) is an Asian staple food made by steaming fermented wheat dough. Goji berry (*Lycium barbarum*) is considered an excellent source of several bioactive components which have been associated with several health benefits. During processing of beverages from goji berries, a significant amount of solid material is produced as a waste. This study was designed to evaluate the nutritional quality of goji berry by-product (GBP) and the feasibility of fortifying CSB with the GBP. Incorporating the GBP in the formula had significant adverse effect on the rheological properties of wheat flour to different extents. On the other hand, increasing the GBP concentration in the formula increased the free phenolics and consequently the antioxidant capacities of the CSB. Sensory evaluation of the composite breads indicated acceptability comparable to that of the control wheat bread. These findings suggest the possibility of utilizing GBP as a value added ingredient to produce functional CSB.

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CHAPTER 1: INTRODUCTION

In China, Chinese steamed bread (CSB), which is made by steaming fermented wheat dough, has been a major staple food for thousands of years. CSB can be roughly classified into two styles: northern-style and southern-style CSB. They represent approximately 40% of wheat consumption in China (Kim *et al.*, 2009). Moreover, over the last twenty years, CSB has gradually become popular in western countries due to food culture intercommunication among different countries and cultures.

In fact, the worldwide market for functional ingredients and foods has experienced significant growth in the past two decades and is due to increasing consumer awareness and promotion of healthy eating and life style. A commonly accepted definition for “functional foods” is foods or natural ingredients that provides addition physiological benefits beyond basic nutrient (Wang *et al.*, 2014), such as vegetables and fruits. Goji berry represents one of simplest forms of functional ingredients which can be added to existing foods. Goji berry is the fruit of *lycium barbarum*, which belongs to *solanaceous* family. Goji berry is considered an excellent source of macronutrient, including carbohydrate (46%), protein (13%), fat (1.5%), and dietary fiber (16%). It also contains significant concentrations of micronutrients such as riboflavin, thiamine, nicotinic acid, and minerals (Cu, Mn, Mg, and Se) (Li, 2001). In addition to the macro- and micro-nutrients present, several bioactive components such as carotenoids, and phenolics have been identified and associated with the health benefits of goji berries (Gan *et al.*, 2003). Bioactive

phytochemicals, such as polyphenols and carotenoids are known to have antioxidant activity in protecting body's cells against oxidative damage and reducing the risk of developing certain types of cancers (Potterat, 2010).

To date, there are relatively few studies regarding the development of goji-related novel products. For example, it was shown that betaine, β -carotene, and phenolic compounds from goji berry increased the antioxidant capacities when added to a liquor product (Song & Xu, 2013). Goji berry improved the probiotic levels and consumer acceptance when added to yogurt (Rotar *et al.*, 2015). Whole-fruit, rehydrated powder was incorporated into muffins and cookies, that resulted in improving consumer acceptance in sensory evaluation study (Pop *et al.*, 2013). Studies (Amaya-Cruz *et al.*, 2015) have demonstrated that berry by-products could be a good source of dietary fibers and other associated bioactive compounds such as polyphenolic compounds (Saura-Calixto, 2010; Pérez-Jiménez *et al.*, 2013).

During processing of beverages from goji berries, a significant amount of solid material is produced as waste (10 kg residue per 90 kg of goji berry beverage), which is typically discarded. Taking into consideration that food wastes could seriously have negative effect on the urban environment and human health (Lan *et al.*, 2012), actions have to be taken to utilize these waste into more valuable products that could be used as value-added ingredients in the functional food industry. According to previous utilization of by-products from juice production, the by-product from goji berry beverage could have a potential as a functional ingredient.

In 1998, the Food and Agriculture Organization (FAO) recommended an increased intake of low glycemic index (GI) foods, which particularly emphasized on diabetics and subjects with impaired glucose tolerance (FAO, 1998). Since then consumers are becoming more aware of the relationship between diet and disease. Fiber fortified cereal products are good examples of low GI foods since the high concentration of dietary fiber would help in slowing sugar release (American Heart Association, 2003; Buttriss, 2006). Therefore, the food industry is focusing on producing functional foods based on various ingredients.

The basic ingredients of northern-style CSB are wheat flour, yeast and water. Over the past decades, many studies investigated the fortification of CSB with various functional ingredients on a laboratory scale. For example, CSB (southern-style) fortified with barley flour (15%) increased the content of beta-glucan (Lin *et al.*, 2012). Similarly, quinoa flour was added into the northern-style CSB formula, which resulted in a mold-free shelf-life of CSB increased at 15, 30 and 60% replacement levels of quinoa flour (Wang *et al.*, 2015). Furthermore, barley hull and flaxseed hull extracts were added to CSB formula and showed greater enhancement in phytochemicals. Phytochemicals from natural sources such as fruits and vegetables have been reported to have human health benefits (Liu, 2004). Therefore, incorporation of ingredients rich in phytochemicals could be a good approach to increase nutritional value and shelf-life of CSB.

The aim of this study was to investigate the nutritional quality of a by-product from goji berry beverage and the visibility of its utilization as functional ingredient in the formulation of CSB. It was hypothesized that the addition of goji berry by-product (GBP) might affect the quality, nutritional value, shelf-life, and consumer acceptance of

northernstyle CSB. Therefore, the GBP was added to soft wheat flour at different replacement levels to produce high quality CSB with high nutritional value. Specifically, the following objectives were studied:

- 1) To investigate the nutritional value of the GBP.
- 2) To investigate the maximum replacement level of the GBP that could be incorporated into the wheat flour for the production of CSB without significant effect on the functional properties of the dough or the quality of the final product.
- 3) To investigate the nutritional value of the CSB made from the composite wheat flour.
- 4) To investigate the sensory acceptability of the CSB fortified with the GBP at different replacement levels.

This study may help better understand the effect of GBP on wheat flour products. Moreover, it would stimulate further interest in using GBP as a source of dietary fibers, phenolic compounds and antioxidants.

CHAPTER 2: LITERATURE REVIEW

2.1. Chinese steamed bread

In Asia, Chinese steamed bread (CSB) is a staple food. It has been recorded that CSB originated and was eaten in Western Han Dynasty, approximately 2,000 years ago (Su, 2005). In the major wheat-growing provinces of China, individuals may consume CSB at every meal and usually eat fresh CSB. The basic ingredients used in making CSB are wheat flour (soft or hard wheat), yeast, and water. However, recipes for CSB vary with respect to the diversity in culture and geography (Zhu, 2014).

Those formulas can roughly be classified into two styles: northern and southern styles, according to ingredient and technology (Figure 2.1) (Zhu, 2014). The southern style CSB is sweet and soft by using soft wheat flour, yeast, water, shortening, and sugar. The northern CSB style is a simpler formula, which consists of only wheat flour (either soft or hard), water, and yeast, and has a dense, chewy texture. In this study, the northern style CSB is used.



Figure 2.1 Southern-style CSB (left) and northern-style CSB (right) (Shutterstock, 2016).

During processing of CSB (Figure 2.2), all the ingredients are mixed in the first step. Then, the well-mixed dough is kneaded by hand or by using a mixer, followed by fermentation in a proofing cabinet for few minutes. After the first fermentation, the dough is kneaded again and put back into the proofing cabinet for few more minutes. Then, the dough is shaped and placed in a steamer for further proofing before steaming. However, processing procedures are different among countries, or even within the same country (Huang *et al.*, 1993). Mixing and remixing time, proofing temperature and time, and steaming time can affect the quality of CSB. The preparation method used in the present research is based on a Chinese standard (SB/T 10139-1993), which is mention above (Zhu, 2014).

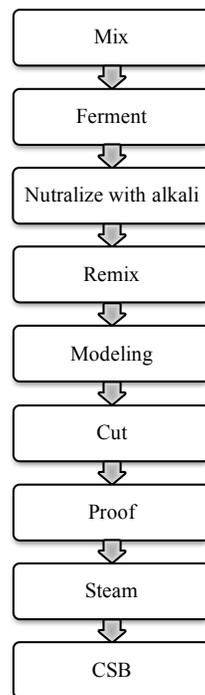


Figure 2.2 Representative processes in the production of northern-style CSB (Zhu *et al.*, 2016)

The sourdough starter could also be used for fermentation of CSB instead of yeast, which is often used at home and in small-scale production (Huang & Miskelly, 1991; Kim *et al.*, 2009). One cultural tradition associated with producing sourdough starters is to collect the starter from the previously fermented dough. Then the sourdough starter is mixed with approximately 90% of the flour and proper water amount for fermentation. Arendt, (2007) reported that the lactic acid bacteria in the sourdough formula have positive effects on the texture and staling of CSB. After full fermentation, the dough becomes sour and requires neutralization with an alkaline substance to eliminate the sour odor and taste (Zhu, 2014). However, if the dough is over-neutralized, the color of the CSB will be yellow or tawny with a harsh alkaline flavor because of peptides that hydrolyzed from proteins (Huang & Miskelly, 1991).

2.2. CSB quality

Similar to baked bread, loaf specific volume, the ratio of bread volume to bread weight, is often used to evaluate the quality of CSB. The common methods of measuring volume are by the rapeseed displacement method (AACCI, 2011) or by using a Volscan Profiler, which is a volume measuring instrument. Moreover, the texture assessments are considered main objective measurements of CSB quality (Huang *et al.*, 1996). The common textural parameters of CSB crumb is firmness. Texture profile analyses (TPA) could be obtained by compressing the crumb using a Texture Analyzer and recording the force. Also, the external appearance of CSB can be an important factor for consumer acceptance. A smooth, blister-free external surface is an important quality criterion for CSB

(Huang *et al.*, 1996). However, the diversity in color is not necessarily a negative factor for consumer acceptance in the development of new products (Zhu *et al.*, 2008).

Besides objective measurements, many researchers have used sensory tests to assess CSB quality (Huang *et al.*, 1996). The sensory evaluation system focuses on both exterior and interior quality attributes of CSB, such as whiteness of skin, brightness, smoothness, crumb structure, stickiness and odor (Zhu, 2014). Huang *et al.* (1996) reported that the results of sensory analysis of CSB are correlated with the results from objective testing methods. However, various evaluation criteria have been established and applied by researchers over the years. Because of the inconsistency in the evaluation criteria, the CSB quality results are difficult to compare among different studies (Zhu, 2014). In this study, both textural profile analysis and sensory evaluation were performed to evaluate the textural properties and consumer acceptance of CSB. A texture analyzer was used to analyze the textural profiles of the CSBs. While, sensory panelists evaluated the appearance, flavor, and texture of CSB to determine the consumer acceptability for the developed breads.

2.3. Rheological properties of dough and their roles in CSB quality

2.3.1. Type of wheat flour used in CSB

Wheat flour is the most important ingredient used in CSB formula. Basically, there are hard and soft wheat flours. Hard wheat flour is high in gluten content, which is preferred in bread making, while soft wheat flour is used for pastries (Ragae *et al.*, 2012). For CSB quality, it is generally agreed that protein content and gluten strength are the most important factors (He *et al.*, 2003). The quality of wheat flour is altered profoundly by various factors,

such as grain cultivar and growing conditions (Hou *et al.*, 1991). However, there are conflicting results on the effects of wheat flour protein content and gluten strength on CSB quality (Lukow *et al.*, 1990; Chen, 1988; Zhang *et al.*, 1993; Zhu *et al.*, 2001; He *et al.*, 2003). The differences in methodology of these reports may explain the conflicts in the results (He *et al.*, 2003).

Hou *et al.* (1991) reported that some soft wheat flours were suitable for making CSB with desired quality. Wen *et al.* (1996) reported that CSB made of soft wheat flour has better specific volume, softer texture and higher carbohydrate digestibility and resistant starch compared to CSB from hard wheat. In our study, soft wheat flour was used for the preparation of CSB.

2.3.2. Protein

Gluten protein is the main structure-forming component in wheat flour, responsible for the formation of cohesive elastic dough that can be produced into acceptable fluffy yummy products. Gluten consists of glutenin and gliadin. Glutenin subunits consist of two unequal groups, the predominant low-molecular-weight species and the high molecular weight subunits. The latter subunits of glutenin are particularly important in determining gluten and dough viscoelasticity (Shewry *et al.*, 2002). The protein in wheat flour contributes to the textural and nutritional quality of CSB (Huang *et al.*, 1996). Protein content is an important factor to determine the suitability of the wheat flour for bread making process. Protein content could be determined by different procedures such Kjeldahl and Dumas (combustion) methods.

Gluten strength is a major factor that determines the baking quality of wheat flour. Several studies indicate that moderate levels of protein and gluten strength are suitable for making northern-style CSB (Huang *et al.*, 1996). Moreover, different conditions in processing require gluten of various strength. For example, for manual processing, weak-to-medium gluten can be suitable, whereas medium-to-strong gluten is desirable for mechanized methods of CSB making (He *et al.*, 2003).

Gluten strength and functionality could be evaluated using different instruments such as Farinograph, Gluten Peak Tester (GPT) and baking process. Farinograph is a standard method that determines quality of the flour based on several parameters such as water absorption, dough development time, mixing tolerance index and other dough parameters within approximately 20 min using a low-mass flour sample (Shahzadi *et al.*, 2005).

The Gluten Peak Tester (GPT) is a useful tool for evaluating differences in gluten aggregation properties (Melnik *et al.*, 2012). The GPT produces rapid shear to mix appropriate amounts of flour and water (or CaCl₂) in a mixing cylinder. The instrument records gluten quality parameters such as maximum torque and aggregation time over a test period of 10 min. The key features of the GPT are high sensitivity, short time of analysis (<10 min) and low sample mass (<10 g), which make this method comprehensive (Chandi & Seetharaman, 2012). In this study, we used the Farinograph test and the GPT to assess the quality and functionality of the flour used to make CSB.

2.3.3. Starch

Wheat flour contains approximately 63% to 72% starch (Hoseney, 1994). Amylose and amylopectin are the main components of starch account for 25% and 75%, respectively (Bemiller & Huber, 1996). Starches play very important roles in determining the characteristics of flours from cereal grains. Starch characteristics such as pasting behavior, gelatinization, retrogradation, and allied properties determine final product quality (Hagenimana & Ding, 2005).

The Rapid Visco Analyzer (RVA) is an important tool used to determine the pasting properties of starch, such as peak viscosity, final viscosity, breakdown viscosity setback viscosity, pasting temperature, and peak time. In the RVA test, starch is rapidly hydrated with water upon heating. The heating temperature is held for a predetermined amount of time when starch granules swell. Then pasting properties of starch are observed and measured. The temperature at which the viscosity starts to increase is referred to as the pasting temperature. The viscosity increases during swelling to reach the peak viscosity. Thus, the peak viscosity indirectly indicates the final product quality because the swollen and collapsed granules are correlated with the texture of cooked starch (Wani *et al.*, 2012). After reaching the peak viscosity, a breakdown in viscosity is measured due to the disintegration of granules. As cooling begins, the viscosity rises for a second time as a result of the retrogradation of starch. This viscosity is termed setback viscosity, which indicates the texture of final product (Wani *et al.*, 2012). Finally, the viscosity stabilizes at the final viscosity, and the RVA test ends.

2.4. Additional ingredients of CSB

CSB represents about 40% of wheat consumption in China (Kim *et al.*, 2009). Due to food culture intercommunication among different countries, CSB becomes more popular in some western countries. Yu *et al.* (2012) reported the daily consumption of Chinese steamed food (such as buns and Dim sum) of Canadian-born Chinese adults in Edmonton (Alberta, Canada) was 34.40 g per day.

However, the glycemic index (GI) of CSB, which reveals the extent to which CSB can increase the blood glucose in human, is relatively high (88.1) (Yang *et al.*, 2006). Diet GI is positively linked with some pathological conditions such as obesity, heart disease, and diabetes (Yang *et al.*, 2006). The rise in health food market demands CSB with novel and optimal nutritional quality (Zhu *et al.*, 2008). Moreover, The shelf-life of fresh CSB is usually less than three days because of its high moisture content and water activity (Laohasongkram *et al.*, 2011). Therefore, there is great potential to expand and adequately satisfy consumer needs regarding CSB.

Recently, several optimizations in the formulation and processing of CSB have been studied to improve nutritional value and extend the shelf-life (Huang & Miskelly, 1991; Zhu, 2014). The impacts of different value-added ingredients on the nutritional value of CSB are summarized based on our understanding of the literature (Table 2.1).

Table 2.1 Effect of additional ingredients on the nutritional value and qualities of CSB.

CSB type	Ingredient	Source of	Reference
Southern	Barley flour (10, 20 and 30%)	Dietary fiber and β -glucan	Lin <i>et al.</i> , 2012
Southern	Wheat bran (10, 20 and 30%)	Dietary fiber and phenolic compounds	Wu <i>et al.</i> , 2012
Northern	Barley hull and flaxseed hull extract	Phytochemicals and antioxidant capacity	Hao & Beta, 2012
Northern	Tartary buckwheat-sprout (4, 8 and 12%)	Bioactive compounds particularly flavonoids	Xu <i>et al.</i> , 2014
Northern	Quinoa flour (15, 30, 45, 60, 75, and 90%)	Phytochemicals particularly polyphenols and protein	Wang <i>et al.</i> , 2015

2.4.1. Sourdough starter

Sourdough starter can also be used in the fermentation of CSB. During fermentation, sourdough starter can produce carbon dioxide and flavor components, as a result of dough structure and unique flavor. Once fermentation starts, the sourdough starter starts to consume the sugar in dough and produce the carbon dioxide, ethanol and flavor components. The rate of gas production is dependent on the temperature of dough, for which the optimum temperature is 40°C.

Apart from the production of carbon dioxide and flavor compounds, sourdough has been proved to produce components such as glucan, fructans and gluco- and fructo-oligosaccharides which demonstrate potential health benefits (Poutanen *et al.*, 2009).

2.4.2. Dietary fiber and resistant starch

Dietary fiber is the indigestible part of edible plants. The term “dietary fiber” is used in nutrition to describe components with certain chemical and physical properties (BeMiller & Huber, 2008). Dietary fiber can be divided into soluble and insoluble fibers. The benefits of consuming fiber include the production of healthful compounds during the fermentation of soluble fiber, and insoluble fiber's ability to add bulk to stool, soften stool, and reduce the transit time of stool through the intestinal tract (Cummings, 1973).

The study of the incorporation of dietary fiber into CSB has become popular over the past decade. Barley is an ancient cereal grain crop that is widely used in baby food and breakfast cereals to provide both insoluble and soluble dietary fiber (Baik & Ullrich, 2008). The advantages of barley are improvements in gastrointestinal health, weight management, and glucose tolerance, as well as a reduction in cardiovascular diseases, colorectal cancer, and blood cholesterol and glucose levels (Bourdon *et al.*, 1999). With 15% replacement level of pearl barley flour, the content of β -glucan increases (Lin *et al.*, 2012). Moreover, Cao (2010) incorporated soluble and insoluble fiber from barley grains (3%) into northern-style CSB, which enhanced the textural and sensory quality of CSB.

Wheat bran rich in dietary fiber was used in the preparation of CSB (Wu *et al.*, 2012). The addition of wheat bran increased the elasticity and sensory acceptability of southern-style CSB. In addition to dietary fiber, the phenolic compounds in wheat bran contribute to the nutritional profile of the resulting product, which plays a functional role in human health (Zhu, 2014). When incorporating more dietary ingredients, the sensory properties of CSB should also be considered.

Furthermore, resistant starch (RS) represents a fraction of dietary starch, which is indigestible by human enzymes (Sajilata *et al.*, 2006). There are five types (type I, II, III, IV and V) of resistant starches. Type I starch is physically trapped starch that is heat-stable. Type II starch is high-amylose starch and digested very slowly by enzymes. Type III starch represents retrograded amylose that formed during storage. Type IV starch is modified starch which can be physically, chemically or enzymatically modified. Finally, type V starch is amylose-lipid complex starch (Ai, 2013). The small intestine cannot digest RS, but RS can be used by probiotics in the colon (Guan, 2007). Moreover, RS barely affects the quality or sensory properties of CSB, which means that RS improves the nutritional value of CSB beyond its original values (Fu *et al.*, 2010). Wen *et al.* (1996) reported that processing conditions such as temperature, water content, starch damage and amylase activity affect the amount of RS (Wen *et al.*, 1996).

2.4.3. Phenolic

Phytochemicals are natural bioactive compounds produced by fruits, vegetables, grains, and other plant sources, that provide protection from external stress and pathogenic attack (Liu, 2004). Beneficial bioactivities have been investigated in recent studies and mounting evidence suggests that phytochemicals have health benefits (Liu, 2004; Lako *et al.*, 2007). Therefore, the phytochemical compounds provide biological functions in healthy modified food. The enhancement of CSB with phytochemicals has drawn attention.

Barley hull and flaxseed hull are regarded as cost-effective agricultural materials enriched in phytochemicals. The CSB enriched in phytochemicals from barley hull extract, and flaxseed hull extract was analyzed by high-performance liquid chromatography

coupled with mass spectrometry (Hao & Beta, 2012). Buckwheat that is rich in bioactive compounds grows mainly in northern China. Buckwheat can be used in bread and biscuits as a high-nutritional-value ingredient (Lin *et al.*, 2012). Tartary buckwheat (*Fagopyrum tataricum*), which is in the same genus with typical buckwheat, can be considered a dietary source of phytochemicals especially flavonoids. Tartary buckwheat sprouts were incorporated into CSB to improve its total phenolic, flavonoids, and gamma-aminobutyric acid content and in vitro antioxidant activity (Xu *et al.*, 2014).

In addition to buckwheat, quinoa is a unique nutrient and functional component that has become significantly popular over the past decade. Polyphenols in quinoa flour improved antioxidant capacity when incorporated into baked products (Alvarez-Jubete *et al.*, 2010). When using quinoa to modify CSB for the first time, the characteristics of the product, such as color and shelf life, were altered which might be related to the phytochemicals in quinoa (Wang *et al.*, 2015).

Moreover, some plant extracts can be incorporated into CSB. However, the extracts may induce inhibition and interaction, affecting the fermentation of dough. Green tea extract has long been a very popular functional ingredient. Although green tea extract provides significant antioxidant activity, α -amylase activity might be inhibited and gluten functionality might be affected, causing adverse effects on dough development and final loaf volume (Ananingsih *et al.*, 2013). Nevertheless, Ananingsih *et al.* (2013) also found that fungal α -amylase could suppress these adverse effects and allow for the fortification of green tea extract into CSB. Likewise, Zhu *et al.* (2016) incorporated black

tea extract into northern-style CSB, which increased the antioxidant activity and slightly affected the textural properties.

2.5. Goji berry

Goji berry (*Lycium barbarum*) belongs to the *Solanaceous* family. The fruit is usually harvested from June to October and consumed dry. Approximately 70 species of goji plant are distributed in temperate and sub-temperate regions around the world, such as North and South America, South Africa, East Asia and Australia. Goji berry is one of the most important medicinal foods in East Asia.

Goji berry contains considerable fruits polysaccharides or *L. barbarum* polysaccharides (LBP), which also represent the most important component in goji berry quantitatively (Potterat, 2010). LBP contains a relatively high-quality mixture of branched and characterized polysaccharides and proteoglycans. Approximately 90-95% of the mass is the glycosidic part which consists of arabinose, glucose, galactose, mannose, rhamnose, xylose, and galacturonic acid (Potterat, 2010).

2.5.1. Phytochemical profile of goji berry

Goji berry is a rich source of various phytochemicals that can give several protective health benefits by protecting proteins, DNA, and lipids from free radical damage (Lako *et al.*, 2007). Certain phytochemicals in goji berry have been identified by high-performance liquid chromatography (HPLC) (Forino *et al.*, 2015; Shen *et al.*, 2015; Zhang *et al.*, 2016). Carotenoids are important compounds in goji berry which contribute to human health because of their antioxidant activity. Carotenoids can become radicals after reacting with free radicals. Among common dietary carotenoids, astaxanthin, zeaxanthin, and lutein

are excellent lipid-soluble antioxidants. At the appropriate concentration, carotenoids can exhibit antioxidant capacity under oxidative stress (Liu, 2004). In addition, flavonoids are another important compound in goji berry. Flavonoids reveal potent antioxidant capacity and are also related to reducing risk of major chronic diseases (Liu, 2010).

2.5.2. Antioxidant activity of goji berry

Goji berry has a long tradition as a food and medicinal plant in China and other Asian countries for eyes, liver and kidney (Amagase *et al.*, 2011). The studies of antioxidant activities of goji berry have received attention over the past decades. Several reports demonstrated high antioxidant activity of goji berry (Potterat, 2010; Amagase *et al.*, 2011; Ulbricht *et al.*, 2015). The identified phytochemicals are the significant contributor for the goji phytocomplex and antioxidant activity in goji berry (Donno *et al.*, 2014). Moreover, a few clinical reports show that goji berry has an effect on aging, endurance, metabolism, glucose control in diabetics and glaucoma, anti-tumor activity, and some other biological activities (Donno *et al.*, 2014). An *in vivo* study showed that 14 to 30 days consumption of goji berry standardized juice enhanced the function of the nervous system, the cardiovascular system, joints, and muscles without any observed side effects (Hsu *et al.*, 2012).

2.5.3. Applications of goji berry

Due to its potential human health benefits, goji berry has become popular in the healthy food market, particularly in Europe and North America, over the past decades. The consumption of goji berry is as safe as that of other fruits in the market. Rare information exists about the toxicity of goji berry as I evidenced in the scientific literature or traditional

Asian herbal medicine textbooks. Thus, import of dried fruit is rarely limited. However, Adams *et al.* (2006) analyzed eight samples of berries from China and Thailand for traces of atropine by using HPLC-MS methods. In all analyzed samples, the maximum concentration of atropine was found to be 19 ppb (w/w), which is far below toxic. Thus, goji berry can be considered a safe and healthy supplement (Adams *et al.*, 2006).

In this case, goji berry could serve as a functional ingredient in a variety of food applications. For example, it is shown that goji berry could enhance antioxidant capacity by endogenous factors when applied in a standardized beverage (Amagase *et al.*, 2009). Betaine, β -carotene, and phenolic compounds from goji berry increased their antioxidant capacities in steeping liquor (Song & Xu, 2013). Goji berry improved the probiotic levels and sensory acceptance of consumers when added to yogurt (Rotar *et al.*, 2015). It is worth noting that goji berry could be a suitable ingredient in pastry products. For example, whole-fruit rehydrated goji powder incorporated in muffins and spritz cookies resulted in improving consumer acceptance in the sensory analysis (Pop *et al.*, 2013).

Moreover, an investigation of northern-style CSB fortified with goji berry extract was reported (Chen & Song, 2004). However, there were some flaws in the reports. First of all, the methods to determine the quality of CSB were based only on sensory evaluations, which could be better if objective measurements, such as specific volume and textural properties, were applied. Moreover, the impact of goji berry on the nutritional value of CSB was not determined. The last but not the least, the rheology properties of the dough were not determined in the study. Therefore, there is still a gap in the incorporation of goji berry into CSB.

2.5.4. Applications of by-products from beverage production

The beverage processing industries produce large amounts of materials (about 20–60% of raw material) as waste (Amaya-curz *et al.*, 2015). Taking into consideration that food wastes could seriously have negative effects on the urban environment and human health (Lan *et al.*, 2012), actions have to be taken to utilize these waste into more valuable products because of its abundant organic compounds (Ezejiofor *et al.*, 2014). For example, grape pomace from the wine and juice production has been used for the production of enzymes (Botella *et al.*, 2007). According to the previous utilization of other by-products from juice production, the by-product from goji berry beverage could have a potential as a value-add ingredient to improve the nutritional value of food products.

To our knowledge, there is no study on the utilization of goji berry by-product as functional ingredient in cereal products. Therefore, the main goal of the present study was to investigate the possibility of utilizing GBP as a value added ingredient to produce functional CSB.

CHAPTER 3:
IMPACT OF THE GOJI BY-PRODUCT ON THE RHEOLOGICAL
PROPERTIES OF WHEAT FLOUR

3.1. Abstract

Goji berry is a good source of dietary fiber and phenolic antioxidants, which has various health benefits. The goji berry by-product (GBP) is derived from goji berry beverage production. The aim of this study was to investigate the nutritional quality of the GBP and the effect of different replacement levels of GBP on rheological properties of soft wheat flour. The chemical composition of the GBP indicated that it is a rich source of protein, lipid, fiber and free phenolic contents compared to soft wheat flour. The GBP was dehydrated, ground and added to soft wheat flour at replacement levels of 5, 10, 15 and 20%. The rheological properties of the wheat flour were affected by the GBP to different extents, among which the 20% GBP replacement exhibited the most significant changes. At 20% GBP replacement level, the significant reduction in Farinograph water absorption (53.6 to 50.0%), and maximum torque (27.67 to 21.00 BE) was observed, while significant increase in dough development time (1.27 to 3.40 min) and peak maximum time (108 to 157 s) were observed. RVA data indicated the reduction in peak viscosity (2480 to 1243 cP), breakdown viscosity (1020 to 521 cP) and final viscosity (2756 to 1268 cP) at 20% replacement level. The results suggest that the GBP affected the rheological properties and pasting characterizes of soft wheat flour at replacement levels of 10, 15, and 20%, among which 20% GBP affected most.

3.2. Introduction

In recent years, goji berry (*L. barbarum*) has been praised as a health food product in western countries because of its various nutrients and bioactivities. Goji berries are considered an excellent source of macronutrient, including carbohydrate (46%), protein (13%), fat (1.5%), and dietary fiber (16%). They also contain significant concentration of micronutrients such as riboflavin, thiamine, nicotinic acid, and minerals (Cu, Mn, Mg, and Se) (Li, 2001). In addition to the macro- and micro-nutrients present, several bioactive components such as carotenoids, and phenolics have been identified and associated with the health benefits of Goji berries (Gan *et al.*, 2003).

Studies (Amaya-Cruz *et al.*, 2015) have demonstrated that berry by-products could be a good source of dietary fibers and other associated bioactive compounds such as polyphenols. During processing of beverages from berries such as goji, a significant amount of solid material is produced as waste. Taking into consideration that food wastes could have serious negative effects on the urban environment and human health, actions have to be taken to utilize these waste into value-added products that could be used in the food industry.

Goji berry has also been incorporated into some food products such as wine, yogurt and cookies (Amagase & Nance, 2009; Song & Xu, 2013; Rotar *et al.*, 2014). In this study, we used a by-product (GBP) that was obtained from the production of goji berry juice. During processing of beverages from goji, a significant amount of solid material is produced as waste (10 kg residue per 90 kg of goji beverage) which is typically discarded.

Taking into consideration the high nutritional value of the GBP and that food wastes could seriously have negative effect on the urban environment and human health (Lan *et al.*, 2012), actions have to be taken to utilize these wastes into more valuable products that could be used as value-added ingredients in the functional food industry. Chen and Song (2004) and Pop *et al.* (2013) reported that incorporation of goji berry in refined flour products resulted in negative impact on quality of the final products. Taking into consideration the high dietary fiber concentration in the GBP we expect significant effects on the rheological properties of the wheat flour. Therefore, the main goal of the present study was to investigate the effect of different replacement levels of GBP on the rheological properties of soft wheat flour since the rheology of wheat flour plays a significant role in making appropriate dough for a given end product. The findings from this study would help better understand the effect of GBP on wheat flour functionality.

3.3. Materials

Soft wheat flour was kindly provided by P&H milling group (Cambridge, Ontario, Canada). Goji berry by-product was kindly provided by Beveragist Company (Ancaster, Ontario, Canada).

3.4. Methods

3.4.1. Preparation of goji berry by-product powder and composite wheat flours

Goji berry by-product was dehydrated in a TSM 14 Insulated Food Dehydrator at 70°C for ten hours. The dried samples were then ground using two types of equipment, a Retsch mill (Centrifugal Mill Type ZM1) and a Quadro Comil (Model 197, Quadro

Engineering Inc. Waterloo, ON), to pass through 0.85 mm screen. The motor speed of the Quadro Comil was set to 100%, and a “2A040G03122329” screen was used.

Wheat composite flours were prepared for four different replacement levels (5%, 10%, 15%, and 20%) of goji berry by-product powder. The prepared goji berry by-product powder was measured and evenly added to pre-calculated contents of flour. All samples were mixed to create uniform flour samples for later experiments.

3.4.2. Proximate analysis

Ash and fat contents in the soft wheat flour and dry goji by-product samples were analyzed according to Methods 08-01.01 and 30-25.01, respectively, of the Approved Methods of the American Association of Cereal Chemists (AACCI, 2011). Moisture content was measured using a halogen moisture analyzer (Ohaus, Switzerland). Protein content was determined by the Dumas method (FP-528 Leco Instrument Ltd. Mississauga, ON, Canada).

3.4.3. Color measurement of soft wheat and composite flours

The color of each flour sample was determined using a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Sensing, Inc., New Jersey, USA) equipped with a SpectraMagic NX CM-S100. The spectral attributes were based on the CIE system: lightness (L^*), greenness/redness (a^*), and blueness/yellowness (b^*). The CIE whiteness index of each flour sample was calculated by the following equation (Zhu *et al.*, 2016):

$$\text{Whiteness Index} = \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

3.4.4. Rheological properties of soft wheat and composite flours

A Brabender Farinograph-E (GmbH & Co. KG, Duisburg, Germany) equipped with a 10 g bowl was used to measure water absorption, dough development time, stability time, and mixing tolerance index (BU) according to the AACC approved method 54-21-02 (AACCI, 2011). Gluten strength and quality of soft wheat and composite flour were assessed using a GPT (Brabender GmbH and Co. KG, Duisburg, Germany). CaCl₂ solution (9.7 g, 0.5 M) was weighed in the GPT stainless steel mixing cylinder. A flour sample (8.3 g) was added to the solvent, and the test was initiated at 1,900 rpm and run for 10 min. The test temperature was held constant at 34°C using a Brabender water bath connected to the GPT. The torque (Brabender equivalents, BE) and peak maximum time (PMT) were calculated using GPT software (version 1, Brabender GmbH and Co., Duisburg, Germany).

3.4.5. Pasting properties of soft wheat and composite flours

The pasting properties of the soft wheat composite flours were determined on a Rapid Visco Analyzer (RVA-4) (Newport Scientific Pty, Ltd, Warriewood, Australia) according to the method of Ragae and Abdel-Aal (2006). A 3.5 g flour sample (14% moisture basis) and approximately 25±0.1 mL distilled water (corrected to compensate for 14% moisture basis) were transferred to an RVA canister. The slurry was heated to 50°C and stirred at 160 rpm for 10 seconds for thorough dispersion. The test temperature was held at 50°C for the first minute and rapidly ramped from 50 to 95°C over the next 7.3 min. Then, the temperature was held at 95°C for 5 min and finally decreased to 50°C over the

next 7.7 min. All viscosities and other data were recorded and calculated from the pasting curve using Thermocline software (TCW version 3, Newport Scientific Pty. Ltd., Warriewood, Australia). The pasting temperature (the temperature at which the viscosity increased by approximately 25 centipoises (cP) over a 20 sec period), peak time (the time at which the peak viscosity occurred), holding strength or trough viscosity (the trough at the minimum hot paste viscosity), final viscosity (the paste viscosity recorded when the paste temperature was 50°C after the cooling stage at the end of the test), breakdown viscosity (peak viscosity holding strength or trough viscosity) and setback viscosity (final viscosity holding strength) were determined.

3.4.6. Statistical analysis

Each test was in performed as an independent replicate. Statistical analysis was carried out by one-way ANOVA using SPSS software. Significance differences were calculated by Tukey's HSD. *P* values < 0.05 were considered significant.

3.5. Results and discussion

3.5.1. Chemical composition

The chemical composition of the GBP and soft wheat flour are presented in Table 3.1. As expected, the GBP had higher protein (136 g/kg), ash (32.7 g/kg) and lipids contents (103.2 g/kg) compared to the soft wheat flour. Based on the results, GBP could be considered an ideal source of dietary fiber and minerals. The composite flour samples could obtain proximate intermediate composition according to the ratios of GBP and soft wheat flour. Thus, the replacement of wheat flour with the GBP could be utilized to improve the nutritional value of different cereal in refined flour products.

Table 3.1 Nutritional quality of goji berry by-product and soft wheat flour (% dry basis).

Component	Content (% dry basis)	
	GBP	Soft wheat flour
Protein	13.6±0.056 _a	7.47±0.05 _b
Ash	3.27±0.07 _a	0.59±0.03 _b
Fat	10.32±0.05 _a	0.97±0.03 _b
Moisture	11.30±0.23 _a	14.26±0.11 _a
Soluble dietary fiber	7.14±0.01	1.5*
Insoluble dietary fiber	46.86±2.5	2.1*
Total dietary fiber	54.00±2.5	3.6*
Free phenolic (µg/g)	1307±37	-

Values with the same letter in the same row are not significantly different ($p < 0.05$). GBP: goji berry by-product from goji berry beverage.

*Values obtained from Ragaee *et al.* (2006).

3.5.2. Color of soft wheat and composite flours

Soft wheat flour and GBP presented significant differences in L^* , a^* and b^* values, as shown in Table 3.2. As expected soft wheat flour (L^* value was 91.44) was significantly lighter than that of GBP (L^* value was 36.28). The a^* value of soft wheat flour (0.35), which indicates redness, was significantly lower than that of GBP (14.20). The b^* value of GBP (27.10) was significantly higher than that of soft wheat (6.10), suggesting that the GBP was a darker shade of yellowness. With increasing the replacement level of GBP incorporated into the soft wheat flour, the color characteristics tended to be darker, redder and yellower. Therefore, we expected differences in the colors of the composite CSBs

which could have impact on the sensory evaluation of the final products.

Table 3.2 Color of soft wheat and composite flours, and goji berry by-product.

Control and composite flours	L^*	a^*	b^*	Whiteness index
GBP0 (control)	91.44±0.01 _a	0.35±0.00 _a	6.10±0.02 _a	10.51±0.01 _a
GBP	36.28±0.01 _f	14.20±0.01 _f	27.10±0.02 _f	70.68±0.01 _f
GBP5	86.93±0.01 _b	1.24±0.01 _b	9.39±0.01 _b	16.14±0.01 _b
GBP10	83.86±0.01 _c	1.88±0.01 _c	11.59±0.01 _c	19.96±0.01 _c
GBP15	80.57±0.01 _d	2.57±0.02 _d	13.51±0.01 _d	23.81±0.01 _d
GBP20	77.24±0.01 _e	3.17±0.02 _e	15.65±0.03 _e	27.80±0.01 _e

Values with the same letter in the same column are not significantly different ($p < 0.05$). Three coordinates of CIELAB, namely L^* , a^* and b^* , describe the lightness, position between red/magenta and green, as well as position between yellow and blue, respectively. GBP 0: soft wheat flour without GBP; GBP 5-20: soft wheat flour with 5-20% GBP.

3.5.3. Rheology of soft wheat and composite flours

Farinograph results revealed significant differences between the control (0% replacement level) and the composite flour-water systems (Table 3.3). When soft wheat flour was replaced by 5, 10, 15, and 20% GBP, flour water absorption decreased by 0.4, 3.7, 4.7, and 6.7%, respectively. In addition, the flours with 10, 15 and 20% GBP demonstrated higher dough developing time (2.10, 2.81, and 3.70 min, respectively) than that of the control (1.13 min). Similar results were obtained by Ragae and Abdel-Aal (2006) when whole meal flours from different cereals were incorporated in the wheat flour.

Also, Hamed *et al.*, (2014) incorporated barley flour rich in β -glucan into refined wheat bread and reported similar results.

Table 3.3 Farinograph results of soft wheat and composite flours.

Control and composite flours	Farinograph parameters			
	Water	Development	Mixing	Consistency
	absorption		Tolerance index	
	(%)	time (min)	(FU)	(FU)
GBP0 (control)	53.6±0.2 _c	1.13±0.11 _{ab}	82.0±10.6 _c	499.3±2.6 _a
GBP5	53.4±0.2 _c	1.00±0.10 _a	128±9.1 _d	507.0±2.1 _c
GBP10	51.6±0.7 _b	2.10±0.59 _{bc}	79.5±10.7 _{bc}	508.0±4.2 _c
GBP15	51.1±0.3 _b	2.81±0.12 _c	75±9.2 _b	502.0±2.0 _{bc}
GBP20	50.0±0.3 _a	3.70±0.21 _d	62±8.9 _a	500.5±2.3 _{ab}

Values with the same letter in the same column are not significantly different ($p < 0.05$). FU: Farinograph unit. GBP: goji berry by-product. GBP 0: soft wheat flour without GBP; GBP 5-20: soft wheat flour with 5-20% GBP.

GPT analysis is a tool to explain the functionality of gluten in the wheat flour-water system. Maximum torque (MT) and peak maximum time (PMT) are two major parameters, which refer to the torque of developed gluten and aggregation time (gluten network formation), respectively. The GPT results revealed lower MT and longer PMT response for 5, 10, and 15% GBP composite flour-water systems compared to those of control wheat (Table 3.4). No significant differences in the torque values were observed between GBP15 and GBP20 flour-water systems while significant differences between these two samples

were observed in their PMT. The GPT results were in agreement with the results of Farinograph. Brunnbauer *et al.* (2014) reported that positive correlation between PMT and Farinograph dough development time. The long PMT indicates a delaying in developing gluten network (Hamed *et al.*, 2014). Moreover, low MT due to the addition of GBP reflected the weakening in dough strength. However, the increase of PMT and decrease of MT cannot be explained simply by adding GBP. The interaction between flour and GBP could be complex and dynamic, possibly changing dough rheological properties.

Table 3.4 Gluten Peak Tester (GPT) results of soft wheat and composite flours.

Control and composite flours	Maximum torque (BE)	Peak maximum time (s)	Lift off time (s)
GBP0	27.67±0.71 _c	108±0.7 _a	82±2.1 _a
GBP5	24.50±0.71 _b	131±1.4 _{ab}	107±1.4 _b
GBP10	22.50±0.71 _{ab}	143±0.7 _b	118±2.1 _{bc}
GBP15	21.50±0.00 _{ab}	138±2.1 _b	110±1.4 _b
GBP20	21.00±1.15 _a	107±1.4 _b	129±7.1 _c

Values with the same letter in the same row are not significantly different ($p < 0.05$). BE: Brabender equivalents. GBP: goji berry by-product powder. GBP 0: soft wheat flour without GBP; GBP 5-20: soft wheat flour with 5-20% GBP.

3.5.4. Pasting properties of soft wheat and composite flours

Pasting properties of starch can explain the behavior of starch during processing, and they are regarded as the assessment of the suitability of starch as a functional ingredient in food products (Wani *et al.*, 2012). In RVA test, the samples start pasting during the

starch granules gelatinizing and swelling after or simultaneously absorbing water. The viscosities of starch, which is the most important pasting property of granular starch dispersion (Wani *et al.*, 2012) were determined.

The RVA result (Table 3.5) indicated that the addition of GBP significantly affected the pasting behavior of composite wheat flour-water systems. As the GBP replacement level increased in the formula, significant reductions in all RVA parameters were observed except for the pasting temperature, which was increased as the percentage of the GBP increased in the formula. For instance, at 20% GBP replacement level, peak time was shifted to 8.93 min compared to that of the control (9.25 min). Also at 20% replacement level of GBP, we observed significant reductions (approximately 50%) in peak-, breakdown-, setback- and final viscosities, suggesting the weaker water-holding capacity of starch and resistance to swell under heating compared to the control flour. The dilution of starch concentration by the GBP could contribute to the low viscosity values of composite flours (Ragae & Abdel-Aal, 2006). Also, some interaction could occur between phenolic compounds present in GBP and starch granules in the wheat flour which could affect its pasting properties (Guzar *et al.*, 2012).

Table 3.5 Rapid Visco Analyzer (RVA) pasting properties of soft wheat and composite flours.

Control and composite flours	Peak Time (min)	Peak viscosity (cP)	Breakdown Viscosity (cP)	Setback Viscosity (cP)	Final Viscosity (cP)	Pasting Temperature (°C)
GBP0	9.25±0.03 _a	2480±1 _a	1020±7 _a	1296±4 _a	2756±15 _a	85.48±0.1 _a
GBP5	9.10±0.04 _{ab}	1853±3 _b	785±8 _b	934±11 _b	1997±42 _b	85.85±0.1 _a
GBP10	9.06±0.01 _{ab}	1582±4 _c	663±16 _c	761±37 _c	1680±69 _c	86.18±0.5 _{ab}
GBP15	9.07±0.00 _{bc}	1435±0 _d	598±11 _d	676±8 _d	1513±20 _d	86.95±0.3 _b
GBP20	8.93±0.08 _c	1243±1 _e	521±5 _e	546±0 _e	1268±8 _e	87.15±0.1 _b

For each pasting parameter, values with the same letter in the same column are not significantly different ($p < 0.05$). GBP: goji berry by-product. GBP 0: soft wheat flour without GBP; GBP 5-20: soft wheat flour with 5-20% GBP.

3.6. Conclusion

The chemical composition of GBP indicated the high nutritional value of the goji by-product. Goji by-product had high concentrations of insoluble dietary fiber (IDF, 46.9%), soluble dietary fiber (SDF, 7.1%), lipid content (10.3%), free phenolic (1307 µg/g) and ash (3.3%), while had very reasonable concentrations of protein (13.6%). Therefore, the by-product from goji beverage production could be considered a valuable and economic ingredient for the functional food industry. The addition of GBP greatly affected the rheological properties of the soft wheat flour to different extents depending on the replacement level. The GBP had darker color compared to wheat flour. Further research is underway to better understand the effect of different replacement levels of GBP on the quality of the CSB.

CHAPTER 4:
EFFECT OF GOJI BERRY BY-PRODUCT ON QUALITY OF CHINESE
STEAMED BREAD

4.1. Abstract

Goji berry (*lycium barbarum*) is considered an excellent source of macronutrient, including carbohydrate, protein, fat, and dietary fiber. It also contains significant concentrations of micronutrients such as riboflavin, thiamine, nicotinic acid, and minerals. In addition to the macro- and micro-nutrients present, several bioactive components such as carotenoids, and phenolics have been identified and associated with the health benefits of goji berries. During processing of beverages from berries such as goji, a significant amount of solid material is produced as waste. Studies have demonstrated that berries by-products could be a good source of dietary fibers and other associated bioactive compounds such as polyphenols. Chinese steamed bread (CSB) is a staple food in China but its nutritional value needs to be improved. GBP was incorporated in the CSB formula at different levels (0, 5, 10, 15 and 20%). As the replacement level of the GBP increased in the formula, the quality of the CSB was significantly affected. However, the mold-free shelf-life was extended at the 15 and 20% replacement levels of GBP. The nutritional quality of CSB was improved when GBP was incorporated at 10, 15 and 20% replacement levels. For instance, we observed the significant increase in the free phenolic contents and the antioxidant capacities of the composite breads compared to those of the control. Sensory evaluation indicated good overall acceptance of CSB at all replacement levels of GBP. The results indicated that GBP could be utilized as a functional ingredient that would

enhance the phytochemical content and shelf-life of CSB.

4.2. Introduction

Chinese steamed bread (CSB), also known as “mantou,” is a popular staple food in China. CSB represents approximately 40% of wheat consumption in China (Kim *et al.*, 2009). CSB is made of wheat flour and cooked by steaming after fermentation. Steaming makes CSB soft, moist and uniform with a thin and smooth skin and a healthy product with a low salt content (Huang *et al.*, 1996).

CSB production faces challenges such as rapid staling behavior and short shelf-life due to a high moisture content and water activity (Wu *et al.*, 2012). These limitations hinder further development of CSB on a commercial scale. Over the past decade, various additives for increasing the nutritional value and quality of CSB have been studied. Wang *et al.* (2015) fortified CSB with quinoa flour. The incorporation of high-fiber and polyphenol-rich ingredients or additives into CSB could be considered a practical method for promoting the nutritional quality of CSB, as indicated by previous studies (Zhu *et al.*, 2015). Wu *et al.* (2012) incorporated wheat bran as a rich source of dietary fiber in the preparation of southern-style CSB to produce CSB with improved nutritional quality and sensory characteristics.

Goji berry by-product (GBP) is an inexpensive by-product that is collected after the processing of goji berry juice. Studies (Amaya-Cruz *et al.*, 2015) have demonstrated that berry by-products could be a good source of dietary fiber and other associated bioactive compounds such as polyphenol compounds (Pérez-Jiménez *et al.*, 2013; Saura-Calixto, 2010).

To our knowledge, the feasibility of incorporating GBP into CSB has not been reported yet. Therefore, the main goal of the present study was to investigate the maximum replacement level of GBP that could be incorporated in wheat flour for the production of CSB without significant effect on the quality of the final product and also to evaluate the nutritional quality of the CSB and its organoleptic acceptability.

4.3. Materials

Soft wheat flour was kindly provided by P&H Milling Group (Cambridge, Ontario, Canada). Goji berry raw by-product was kindly provided by Beveragist Co. (Ancaster, Ontario, Canada). Commercial dry yeast (Fleischmann's Traditional Active Dry Yeast) was purchased from the local Market.

4.4. Methods

4.4.1. Preparation of CSB

Water absorption of soft wheat flour was determined using the Farinograph (Brabender Farinograph-E) as described in Chapter 3. Chinese steamed bread was prepared according to a protocol described by Wang *et al.* (2015), with slight modifications. The water absorption values obtained by the Farinograph were modified (increased by 10% for GBP0 and GBP5; 15% for GBP10; and 18% for GBP15 and GBP20) to produce good quality CSB. Soft wheat and composite wheat flours (100 g) were mixed with yeast/water slurry. As indicated in Table 4.1, the slurries were prepared at five different ratios to produce 0-20% replacement levels of goji berry by-product samples. The well-mixed dough was then kneaded in a KitchenAid stand mixer (Mississauga, ON, Canada) for 3 min and fermented in a proofing cabinet (30°C, relative humidity of 85%) for 35 min.

Following first fermentation, the dough was kneaded by hand for another 3 min and placed back in the proofing cabinet for 25 min. The dough was then rounded by hand and placed in a steamer for another 15 min of proofing in the proofing cabinet before steaming for 20 min (Figure 4.1).

Table 4.1 Chinese steamed bread formulation based on 100 g flour.

CSB	Ingredient (g)			
	Soft wheat flour	GBP	Water	Yeast
GBP0	100	0	59.18	1.0
GBP5	95	5	59.18	1.0
GBP10	90	10	61.87	1.0
GBP15	85	15	63.50	1.0
GBP20	80	20	63.50	1.0

GBP: goji berry by-product. GBP 0: CSB without GBP; GBP 5-20: CSB with 5-20% GBP.

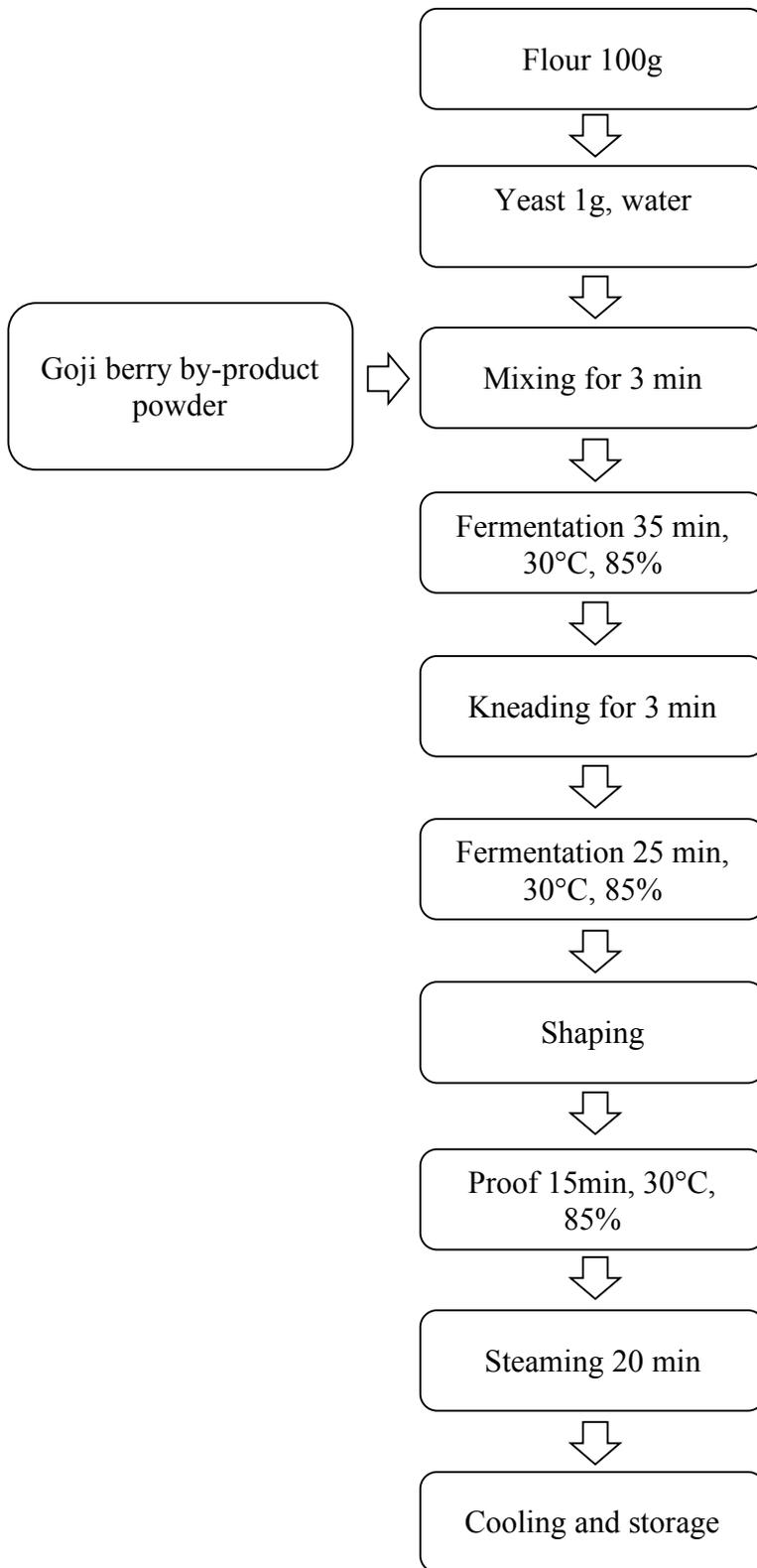


Figure 4.1 CSB making process.

4.4.2. Specific volume of CSB

After steaming the bread, each Chinese steamed bread was allowed to cool to room temperature for approximately one hour before quality measurement. The volume of the bread was determined by the rapeseed displacement method in cubic centimeters (cm³), as described by the AACC Intl. approved method 10-05.01 (AACCI 2011). The weight of each CSB was measured in grams using an analytical balance. The specific volume was calculated in units of cm³/g by dividing the volume of each CSB by its weight.

4.4.3. Firmness of CSB

The textural properties of CSB crumbs were analyzed using a texture analyzer (TA.XT2. Plus, Texture Technologies. Corp. Scarsdale, NY, USA) according to the AACC Intl. Approved Method 74-09.01 (AACCI 2011). The bread loaves (25 mm) were sliced manually from the middle of each CSB using a knife. The test was conducted with the trigger force of 5 g and a plunger speed of 5mm/sec. The firmness of the crumbs was recorded and calculated using Texture Exponent 32 software (Texture Technologies. Corp. Scarsdale, NY, USA).

4.4.4. Color measurement of CSB

The color of each CSB was determined using a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Sensing, Inc., New Jersey, USA) equipped with a SpectraMagic NX CM-S100. The spectral attributes were based on the CIE system: lightness (L^*), greenness/redness (a^*), and blueness/yellowness (b^*). The CIE whiteness index of each CSB was calculated by the following equation (Zhu *et al.*, 2016):

$$\text{Whiteness Index} = \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

4.4.5. Microbiological shelf-life of CSB

Microbiological shelf-life of CSB was determined according to a protocol described by Zhu *et al.* (2016), with slight modifications. Each bread, which was allowed to cool for one hour after steaming, was sliced (20 mm in thickness) and exposed to the air for 5 min on each side. Then, each slice was packaged into a plastic bag, in which small slits were made. All bags were placed under comparable aerobic conditions. All samples were stored at room temperature (approximately 20°C) and examined for mold growth at the same time. The visual appearance and water activity of each sample were recorded for three days. Mold growth was quantified as the number of aerial mycelia on the front and back of each slice. The shelf life of samples was determined by the appearance of the first mold colony. Water activity was determined using a water activity analyzer (Aqua Lab 4TE, Decagon Devices, USA).

4.4.6. Sensory evaluation

Fifteen sensory panelists were invited to evaluate the appearance, texture, flavor and overall liking of soft wheat and composite CSBs. This evaluation was performed using a 9-point hedonic scale. The panelists were then asked to check the aftertaste of CSB by using 5-point category scale. Finally, the panelists were asked to indicate their opinion regarding aftertaste (pleasant, neither pleasant nor unpleasant or unpleasant).

4.4.7. Free phenolic content (FPC)

For each replacement level, each CSB loaf was sliced and ground. The ground particles were dehydrated in an oven at 65°C overnight. Prepared samples were analyzed for free phenolics using the procedure described by Ragaee *et al.* (2012). 500 mg of each sample was placed in a 50-mL white round-bottom tube. Five milliliters of 80% methanol were added to the tube with filling nitrogen, and the samples were then placed on a shaker for 30 minutes at 350 rpm. The samples were then centrifuged at 16800 x g for 5 min. The supernatant was gently decanted into a new cone centrifuge tube, and the remaining sediment solids were extracted again by adding 80% methanol. The supernatant was collected into the same cone tube. The supernatant was evaporated to 5 mL under nitrogen and diluted to 10 mL with distilled water. Then, 10 mL of 1/1 ethyl ether/ ethyl acetate was added, and the sample was shaken for 2 min, followed by centrifugation at 16800 x g for 5 min. The extraction was performed in triplicate, with all residues collected and filtered through glass wool and anhydrous sodium sulfate. The solvent was then evaporated under nitrogen, and the subsequent sample was reconstituted with 1 mL of 50.0% methanol. The free phenolic content in the extraction was determined by using Folin-Ciocalteu's reagent (Ragaee *et al.*, 2012). Absorbance was measured at 725 nm by a spectrophotometer. We standardized the phenolic level by using gallic acid. The results are expressed as micrograms of gallic acid per gram of sample.

4.4.8. Ferric-reducing antioxidant power (FRAP) assay

The FRAP activity of CSB was determined according to previously reported procedures (Chen *et al.*, 2015). Briefly, 10 µL CSB phenolic extract was mixed with 300

μL of ferric-TPTZ reagent, a mixture of 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ at a ratio of 10:1:1 (v/v/v). The reaction mixtures were then allowed to react at room temperature for 2 h before the absorbance was recorded at 593 nm on a UV/Vis microplate reader. FRAP value was expressed as μmol of L-ascorbic acid equivalent (ACE) per gram sample ($\mu\text{mol AAE/g}$).

4.4.9. Radical scavenging activity (DPPH) assay

DPPH assay followed the method as previously reported (Chen *et al.*, 2015). Before the measurement, a stock solution (3.5 mM) of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was prepared daily by dissolving 34.5 mg of DPPH in 25 mL of methanol, while a working solution (350 μM) was also prepared by adding 2.5 mL of 3.5 mM stock solution into 22.5 mL methanol. Both Trolox standard solution and working solution were prepared by dissolving 25 mg of Trolox in 5 mL of methanol and diluted into a concentration range between 62.5 to 1000 μM . A volume of 25 μL of diluted CSB extract was mixed with 200 μL of the DPPH working solution. For CSB extract sample, 2 times dilution was required to obtain the absorbance within the linear standard range. The entire sample, blank and standard wells were incubated at room temperature in the dark for 6 hours after gently swirl. The absorbance was read at 517 nm. The results are expressed as $\mu\text{mol/L}$ Trolox equivalent (TE)/g sample.

4.4.10. Statistical analysis

Each test was performed as an independent replicate. Statistical analysis was carried out by one-way ANOVA using SPSS software. Significance differences were calculated

by Tukey's HSD. *P* values < 0.05 were considered significant.

4.5. Results and discussion

4.5.1. Specific volume of CSB

As shown in Table 4.2, as the percentage of GBP increased in the formula, the specific volume (SV) of the CSB was decreased. At 20% replacement level, we observed 22% reduction in the bread SV, compared with that of the control CSB. This could be due to the dilution of gluten content and disruption of the gluten network in the dough by the GBP. Moreover, fibers and polyphenols could interact with gluten (Wang *et al.*, 2015). These results are consistent with others (Lin *et al.*, 2012; Ananingsih *et al.*, 2013; Wang *et al.*, 2015; Zhu *et al.*, 2016). Lin *et al.* (2012) reported the reduction in the SV of CSB fortified with barley flour (SV decreased from 3.95 to 2.43 mL/g by replacing 30% wheat flour with barley flour).

Table 4.2 Quality of CSB made from soft wheat and composite flours.

CSB	Specific volume (mL/g)	Firmness (g)	Water activity (a_w)	Moisture content (%)
GBP0	3.4±0.0 _a	1669±79 _c	0.968±0.01 _{ab}	39.89±0.9 _a
GBP5	3.3±0.1 _a	1550±86 _{bc}	0.966±0.01 _a	40.30±0.2 _a
GBP10	3.2±0.1 _a	1368±104 _{ab}	0.976±0.01 _b	39.58±0.4 _a
GBP15	3.2±0.1 _a	1353±25 _a	0.970±0.03 _{ab}	41.33±1.1 _a
GBP20	2.6±0.1 _b	1414±91 _{ab}	0.966±0.01 _a	41.79±0.0 _a

Values with the same letter in the same column are not significantly different ($p < 0.05$). GBP: goji berry by-product. GBP 0: CSB without GBP; GBP 5-20: CSB with 5-20% GBP.

4.5.2. Firmness of CSB

The addition of GBP decreased the firmness of CSB at all replacement levels (Figure 4.2). CSB fortified with 15% GBP exhibited the lowest crumb firmness (1352 g) among all breads. The low firmness is mainly due to the large cell size presenting a heterogeneous crumb structure with a worse and more asymmetrical pore distribution. This could be due to the high contraction of fiber in GBP. Fiber could affect the gluten formation and functionality (Adams, 2015). Similarly, increasing the replacement levels of quinoa flour, which is rich in fiber and polyphenols, increased the hardness and chewiness of CSB, and decreased the cohesiveness texture of the product (Wang *et al.*, 2015).

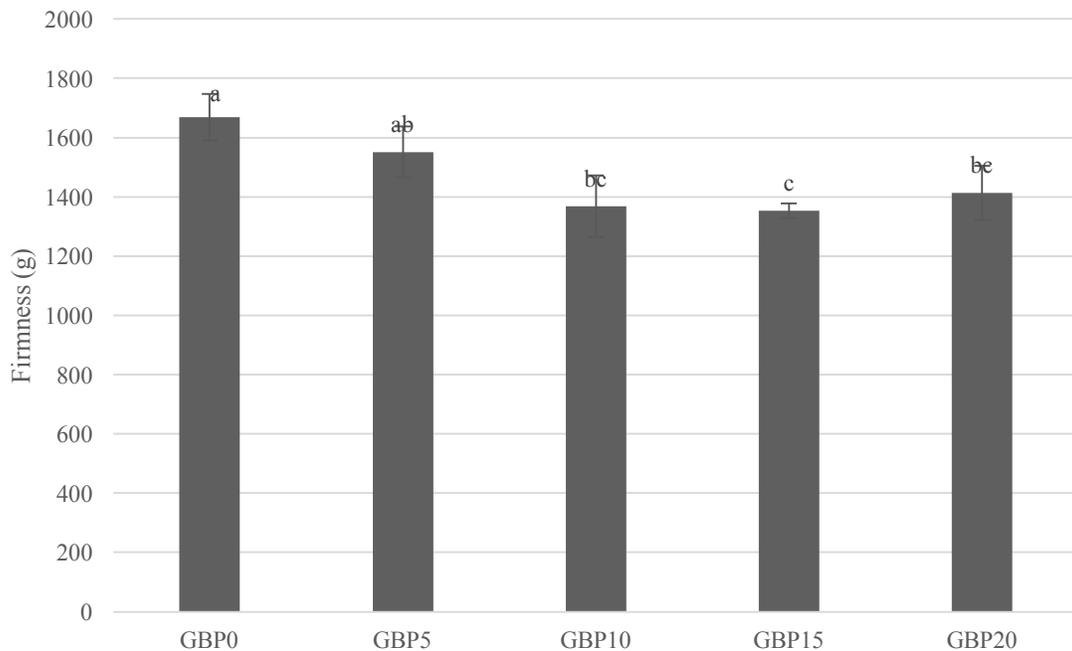


Figure 4.2 Crumb firmness of CSB made from soft wheat and composite flours.

4.5.3. CSB Color

The color parameters of CSB crumbs are presented in Table 4.3. Increment the replacement level of GBP decreased the L^* index and increased the a^* and b^* indices of CSB. The composite CSBs with added GBP were redder than CSB made from the control soft wheat flour. GBP20 had the highest a^* and b^* indices but the lowest L^* (12.89, 25.86 and 33.94, respectively). The changes were partially attributed to the impact of GBP on the color of flour samples as discussed in Chapter 3. However, the difference in color is not necessarily a negative factor for consumer acceptance in the development of new products (Zhu *et al.*, 2008).

Table 4.3 Color of CSB made from soft wheat and composite flours.

CSB	L^*	a^*	b^*	Whiteness index
GBP0	74.11±0.64 _a	0.61 ±0.24 _a	15.83±0.53 _a	30.36±0.48 _a
GBP5	55.03±1.04 _b	9.67±0.11 _b	32.53±0.58 _b	56.34±0.52 _b
GBP10	43.11±0.46 _c	11.42±0.34 _c	27.95±1.23 _b	64.42±0.39 _c
GBP15	37.96±1.10 _d	12.64±0.17 _d	28.03±0.60 _b	69.25±0.74 _d
GBP20	33.94±1.37 _e	12.89±0.33 _d	25.86±1.05 _c	72.12±0.98 _e

Three coordinates of CIE, namely L^* , a^* and b^* , describe the lightness, position between red/magenta and green, as well as position between yellow and blue, respectively. Values with the same letter in the same column are not significantly different ($p < 0.05$). GBP: goji berry by-product powder. GBP 0: CSB without GBP; GBP 5-20: CSB with 5-20% GBP.

4.5.4. Sensory evaluation

Fifteen sensory panelists tasted the CSB. The addition of GBP had little effect on texture, appearance, flavor and overall acceptances of CSB. As shown in Figure 4.3, the

composite CSBs were darker than that of the control wheat CSB due to the color of the GBP as discussed in the color section above. However, the altered color of cereal food products could be attractive to consumers (Zhu *et al.*, 2014). The surface of control wheat CSB was smoother and the crumb cells were more small and uniform than those of composite CSBs. The smoothness of CSB surface decreased in all composite CSBs, probably because GBP disrupted the gluten network. Similar results were observed in the CSB incorporated with quinoa flour (Wang *et al.*, 2015).

GBP0



GBP5



GBP10



GBP15



GBP20



Figure 4.3 Photographs of CSB made from soft wheat and composite flours.

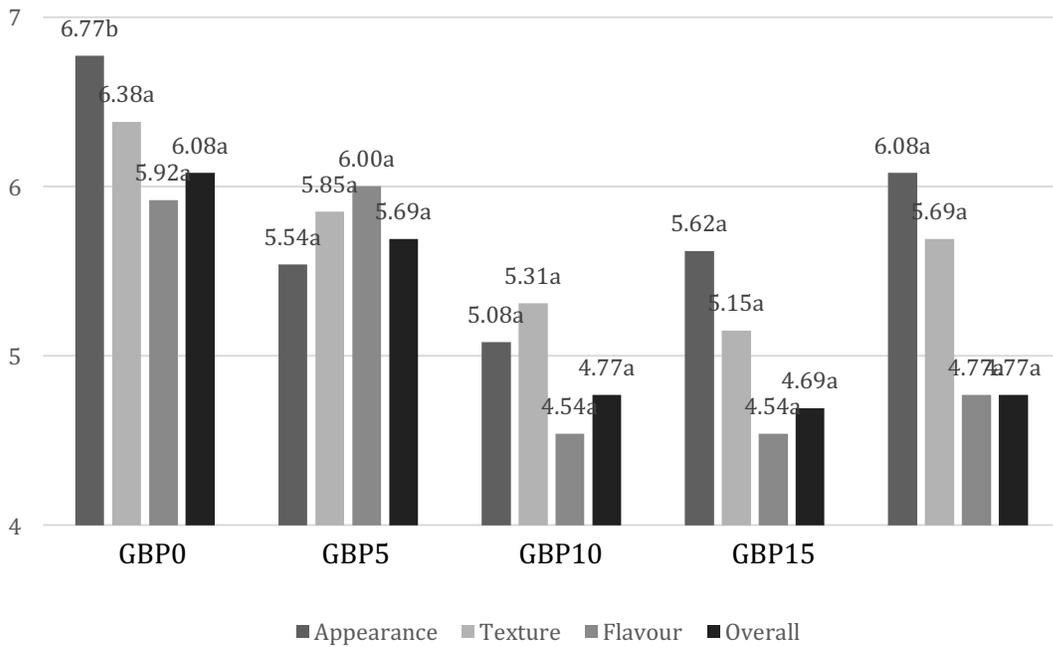
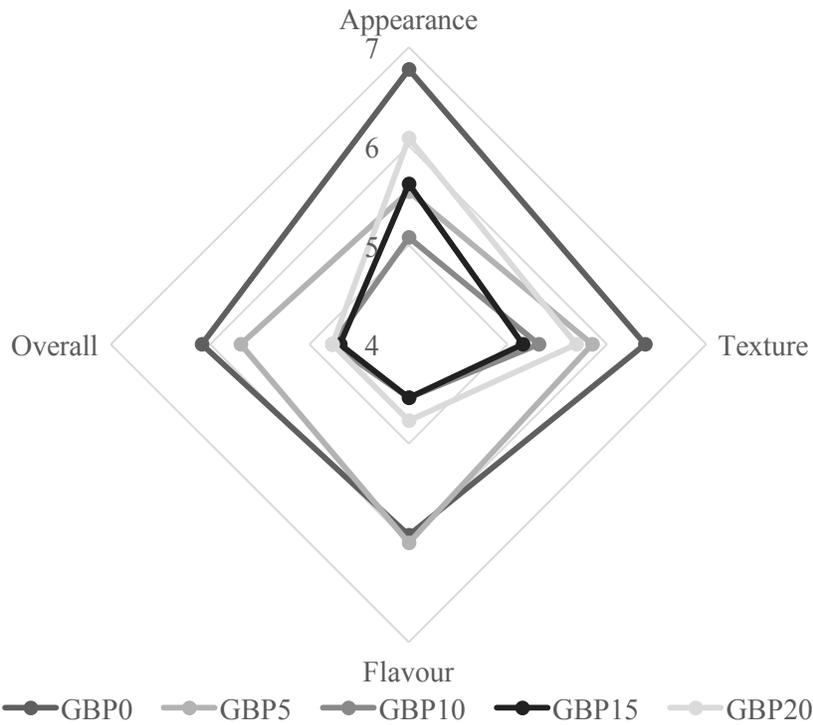
No significant differences in the scores pertaining to appearance, texture, flavor and overall acceptance were observed among all CSB including the control (Table 4.4 & Figure 4.4). At 15 and 20% replacement levels of GBP, the panelists witnessed slightly bitter aftertastes, which were mainly attributed to the presence of phenolic compounds in the GBP (Langfried, 2013). The results suggest that even though the incorporation of the GBP altered the organoleptic characteristics of CSB, the panelists liked the composite CSBs for their uniqueness as functional products. The obtained results suggested that GBP can be utilized as functional food ingredient without affecting sensory acceptance of CSB.

Table 4.4 Sensory scores for CSB made from soft wheat and composite flours.

CSB	Appearance	Texture	Flavor	Overall
GBP0	6.77±1.48 _a	6.38±1.33 _a	5.92±1.38 _a	6.08 ±1.38 _a
GBP5	5.54±1.27 _{ab}	5.85±1.46 _a	6.00±1.08 _a	5.69±1.38 _a
GBP10	5.08±1.38 _b	5.31±1.38 _a	4.54±1.05 _a	4.77±1.30 _a
GBP15	5.62±1.12 _{ab}	5.15±1.63 _a	4.54±1.50 _a	4.69±1.25 _a
GBP20	6.08±1.19 _{ab}	5.69±0.75 _a	4.77±1.69 _a	4.77±1.79 _a

A panel of 15 people used a 9-point hedonic scal: 0= disliking very much, 9=liking very much. Values with the same letter in the same column are not significantly different ($p < 0.05$). GBP: goji berry by-product. GBP 0: CSB without GBP; GBP 5-20: CSB with 5-20% GBP.

Sensory Attributes



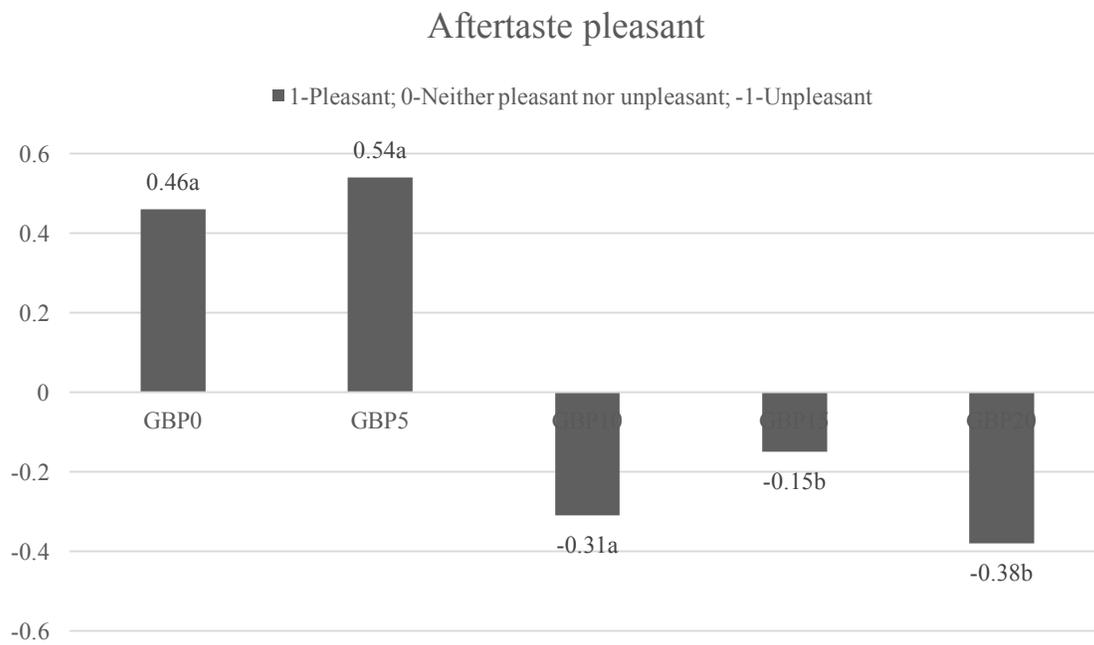
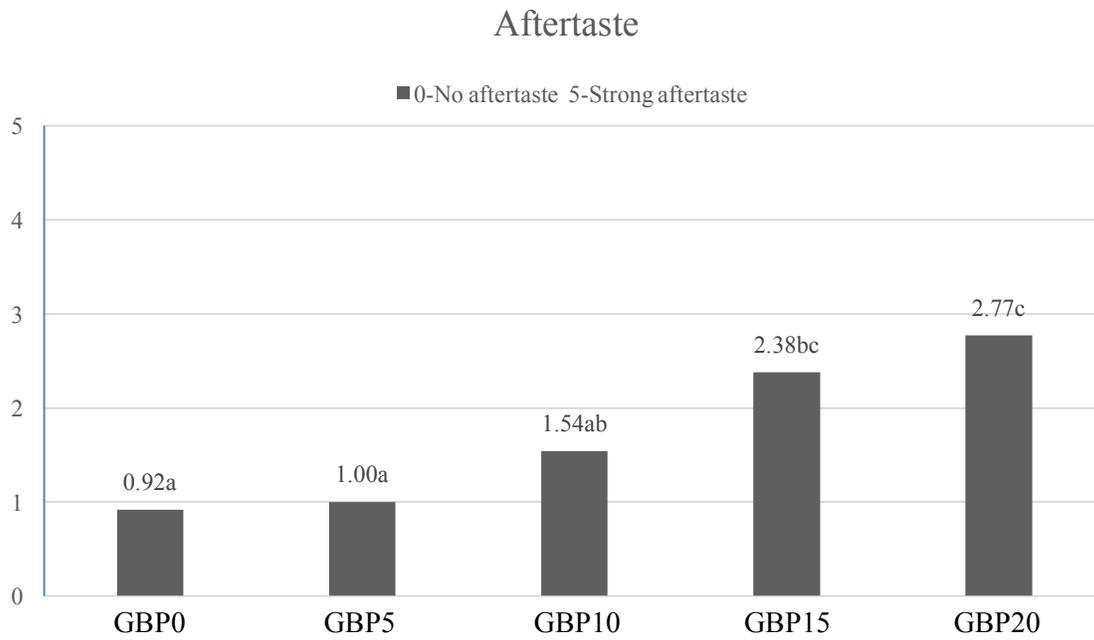


Figure 4.4 Sensory attributes of CSB made from soft wheat and composite flour.

4.5.5. Water activity, moisture content and microbiological shelf-life of CSB

The water activity and moisture content of all CSBs were high (Table 4.5). These would contribute to short mold-free shelf-life of CSB (Zhu *et al.*, 2016). The addition of GBP hardly affected the moisture content or water activity of CSB.

Table 4.5 Changes in firmness (g), moisture (%) and water activity of CSB made from soft wheat and composite flours during storage.

CSB	Storage day	Firmness (g)	Moisture (%)	Water activity
GBP0	Day1	1669±79 _d	39.89±0.9 _a	0.9682±0.004 _{ab}
	Day2	1461±18 _{cd}	40.06±0.03 _{ab}	0.9604±0.001 _{ab}
GBP5	Day1	1550±86 _{cd}	40.30±0.3 _a	0.9656±0.001 _a
	Day2	1349±8 _{bc}	41.44±0.05 _a	0.9740±0.002 _{ab}
GBP10	Day1	1368±104 _b	39.58±0.4 _{ab}	0.9754±0.000 _b
	Day2	1063±121 _a	40.30±0.65 _{ab}	0.9704±0.001 _{ab}
GBP15	Day1	1353±25 _{bc}	41.33±1.1 _{ab}	0.9702±0.003 _{ab}
	Day2	1124±5 _{ab}	42.45±0.27 _b	0.9661±0.001 _a
GBP20	Day1	1414±91 _b	41.79±0.0 _{ab}	0.9655±0.001 _a
	Day2	1127±91 _{ab}	40.12±0.84 _{ab}	0.9669±0.005 _{ab}

Values with the same letter in the same column of firmness, moisture content, and water activity are not significantly different ($p < 0.05$). GBP: goji berry by-product. GBP 0: CSB without GBP; GBP 5-20: CSB with 5-20% GBP.

The shelf life of CSB is usually less than three days because of the high moisture content and water activity (Laohasongkram *et al.*, 2011). The water activity and moisture content of CSB were analyzed during the first two days of storage. Moreover, the number

of mold colonies on the surfaces of CSB slices were monitored over three days of storage at room temperature. The results (Figure 4.5) demonstrated that addition of 15 and 20% GBP in the formula delayed the mold formation in CSB, which showed no mold colonies during the first two days. A larger amount of mold colonies was observed on the control CSB on day 2. However, the water activities and moisture contents of all CSBs showed no significant changes during the storage period. Thus, the impact of GBP on the mold growth could be due to the high concentration of phenolic compounds in the GBP. The phenolic acids were reported to exhibit antimicrobial activity (Cueva *et al.*, 2010), which may explain the extension of the microbiological shelf life of CSB containing 15 and 20% GBP.

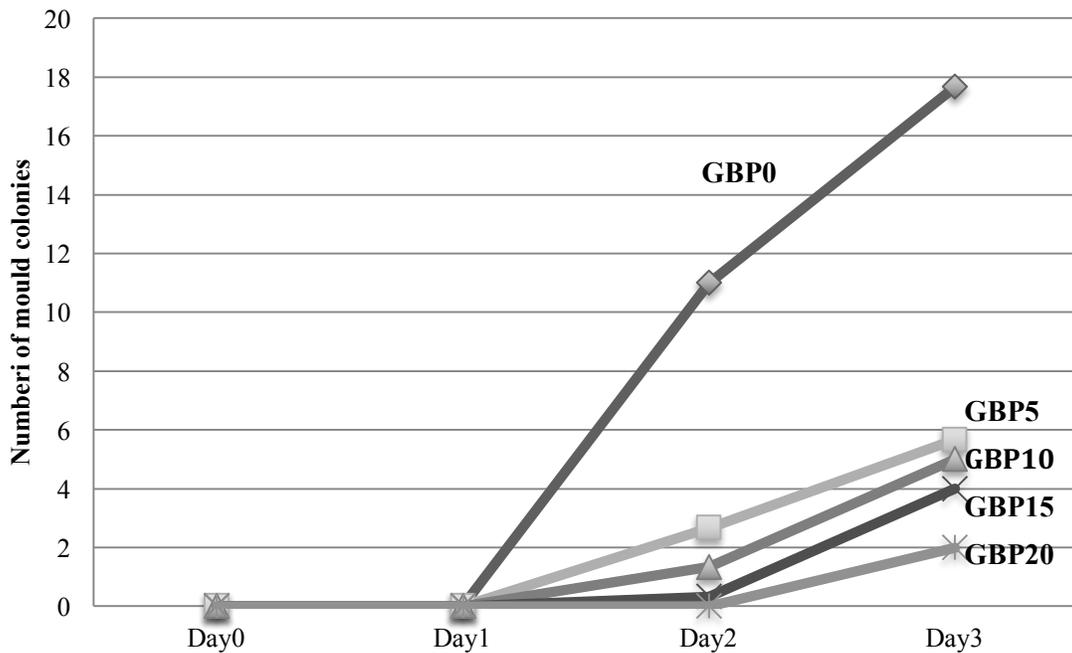


Figure 4.5 Formation of mold colonies on the CSB slices during storage.

4.5.6. Free phenolic contents (FPC), ferric-reducing antioxidant power (FRAP) assay and radical scavenging activity (DPPH) assay

Goji berry is considered a rich source of antioxidant compounds, with phytochemicals and antioxidant activity levels comparable to those in other similar fruit species (Donno *et al.*, 2014). The free phenolic content of GBP was 1,307 GAE $\mu\text{g/g}$. Donno *et al.* (2015) reported that the total phenolic content of goji berry methanolic extracts was 2683.5 GAE $\mu\text{g/g}$.

The control wheat CSB extract contains small concentration of phenolics (Table 4.6) which can be attributed to the variety of phenolics found in wheat, such as ferulic acid, flavonoids, and other phenolic acids (Hernández *et al.*, 2011). As the replacement level of GBP increased in the formula, the free phenolic contents significantly increased by 241, 377, 451, and 738%, for GBP5, GBP10, GBP15, and GBP20, respectively. Therefore, the phenolics in the composite CSBs would promote the antioxidant activity of CSB, which could play profound roles in human health (Liu, 2010).

Table 4.6 Free phenolic contents and antioxidant activities of CSB made from soft wheat and composite flours.

CSB	FPC	FRAP	DPPH
GBP0	18.81±1.15 _a	0.391±0.028 _a	59.825±0.557 _a
GBP5	64.19±0.81 _{ab}	2.258±0.214 _b	60.225±0.246 _a
GBP10	89.79±7.27 _b	3.881±0.236 _c	61.2±0.548 _b
GBP15	103.69±19.86 _b	6.296±0.091 _d	61.394±0.525 _b
GBP20	157.64±13.88 _c	8.268±0.358 _e	62.206±0.120 _b

FPC, free phenolic content (GAE $\mu\text{g/g}$ sample); DPPH assay, results are expressed as $\mu\text{mole TE/g}$ sample; FRAP, results are expressed as $\mu\text{mole L-AAC/g}$ sample; Values with the same letter in the same column are not significantly different ($p < 0.05$). GBP: goji berry by-product. GBP 0: CSB without GBP; GBP 5-20: CSB with 5-20% GBP.

In the current study, ferric ion reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to evaluate antioxidant capacity of soft wheat and composite CSBs. These two assays are commonly used for dietary antioxidants (Huang *et al.*, 2005). Two assays were both applied because currently there is no single assay that is able to characterize antioxidant capacities of foods due to the presence of multiple free radicals and oxidants in biological systems (Rahman, 2007).

In the FRAP assay, increasing the replacement level of GBP in the formula resulted in significant increase in FRAP values with the 20% composite CSB exhibited the highest value (8.268 $\mu\text{mole L-AAC/g}$). Zhang *et al.* (2016) reported a range of FRAP values between 56.3 to 92.5 $\mu\text{M TE/g}$ fresh weight for the fruits of the eight native Chinese goji genotypes. In the DPPH assay, slight increases in DPPH values were observed for the

composite CSBs, but not very profoundly compared to that of the control. One possible explanation was that the phytochemical antioxidants of the samples responded somewhat differently in each type of evaluation assays (Wang & Zhu, 2016).

Above all, the results indicated that the GBP was a good source of phenolics and antioxidants and the addition of GBP could be a practical way to enhance the free phenolic content and antioxidant properties of CSB.

4.6. Conclusion

The current study investigated the effect of different replacement levels of GBP on the specific volume, color, firmness, microbiological shelf-life, sensory attributes, free phenolic content, and antioxidant capacities of CSB. The overall quality of CSB was affected to different extents depending on the replacement level of GBP in the formula. However, at higher replacement levels (15 and 20%), we observed one extra day of mold-free shelf-life of CSB compared to the control. Sensory evaluation of the composite CSBs indicated that the addition of GBP did not significantly affect the appearance, texture, flavor or overall acceptability of CSB. The high free phenolic contents and antioxidant capacities of the composite CSBs compared with those of the control wheat CSB indicated superior nutritional values. CSB fortified with GBP could be considered a good source of dietary antioxidants with good overall consumer acceptance and extended shelf-life.

CHAPTER 5: CONCLUSION AND FUTURE OUTLOOK

Chinese steamed bread (CSB), which is made by steaming fermented wheat dough, has been a major staple food in China for thousands of years. Over the past two decades, CSB has gradually become popular in western countries. Nowadays, consumers are becoming more aware of the relationship between diet and disease. Therefore, CSB could be a good vehicle to deliver functional ingredients for the health food industry. As a result, research on CSB has been carried out to improve the quality and nutritional value of CSB by using different functional ingredients.

Goji berry (*lycium barbarum*) is considered a good source of nutrients and bioactive components, such as phenolics that associated with several health benefits. During processing of beverages from berries such as goji, a significant amount of solid material is produced as waste. Studies have demonstrated that berry by-products could be a rich source of dietary fiber and phytochemicals.

This research was designed to investigate the nutritional quality of a by-product from goji berry beverage (GBP) and its utilization as functional ingredient in the formulation of CSB. The chemical composition of GBP indicated the high nutritional value of the by-product. The addition of GBP greatly affected the rheological properties of the soft wheat flour to different extents depending on the replacement level. As expected, the quality of the CSB (specific volume, color, and firmness) was affected as the replacement level of the GBP increased in the formula. However, the mold-free shelf-life was extended

by one extra day at the 15 and 20% GBP replacement levels. The free phenolic contents and the antioxidant activity of the composite CSBs were improved when GBP was incorporated at all replacement levels. Moreover, sensory evaluation indicated good overall acceptance of CSB at all replacement levels. The results have shown that GBP has a good potential for the utilization as a value-added ingredient that can enhance the phytochemical content and shelf-life of CSB.

Future research on characterization of dietary fiber and phytochemicals is required to gain more information regarding the nutritional quality of GBP. Another future prospect could be to investigate the digestibility of starch in the composite CSB and expected the glycemic index as compared to the control wheat CSB to understand the functionality of GBP as a value-added ingredient for the functional food industry.

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