IMPACTS OF CONTINUOUS FLOW HIGH PRESSURE PROCESSING ON THE TEXTURE OF TOFU SUPPLEMENTED WITH VARIOUS RICE COMPONENTS

by

ENQI ZHANG

(Under the Direction of William L. Kerr)

ABSTRACT

Combining soybean with rice-based ingredients can improve the nutrition value and introduce a new flavor to tofu. Soybeans are partially replaced by various rice components (white/brown rice, rice bran, and isolated rice protein powder) to make tofu. Continuous flow high pressure throttling (CFHPT) is introduced to better breakdown and distribute the rice substitutions. Pure soy tofu was used as a control. Texture profile analysis, color, water holding capacity, and yield were measured to determine the quality change. Results show that all rice components tend to decrease the hardness and chewiness of tofu. 2.5% of rice bran decreases the hardness to 1/4 of the control. After CFHPT treatment, up to 7.5% of rice bran can be added without significantly change the texture.

INDEX WORDS: Continuous High Pressure, Rice Components, Soymilk, Tofu, TPA

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ENQI ZHANG

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Major Professor: Willam L. Kerr
Committee: Fanbin Kong
George Cavender

Electronic Version Approved:

Ron Walcott
Interim Dean of the Graduate School
The University of Georgia
August 2020

DEDICATION

I want to dedicate this thesis to my parents, who have supported me throughout the process, both financially and mentally.

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First, I would like to thank all my committee members, Dr. William Kerr, Dr. George Cavender and Dr. Fanbin Kong. Without their guidance and patience, I would not be able to finish the project. I would also like to acknowledge the help of Carl Ruiz in completing this project. Thank you Carl for guarding me using the equipment. I also need to thank my lab mates Taryn Kormanik and Kay Hyun Joo as well as Karen Simmons, Dr. Harrison, Dr. Adhikari, and all other people in my department. Thanks them for helping me catching up and dealing with things other than studying. Lastly, I would like to thank all my friends and family who have encouraged me and inspired me, making this process more manageable. This endeavor would truly not have been possible without you.

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Chapter 1

INTRODUCTION

Tofu is a popular traditional food in East Asia and has seen increasing consumption in the western world. It is often touted as a great plant protein source for vegetarians, vegans, and those looking for more sustainable and healthy diets. The tofu-making process used today is essentially the same as the process developed in Asia thousands of years ago. In order to meet the needs of better flavor and more balanced nutrition, improving tofu quality through innovative ingredients and processing is needed. Adding rice and its derivatives into tofu should increase the content of essential nutrients, such as fiber, which tofu lacks, but rice contains at high levels. Moreover, even though soy protein has been proven to be an excellent protein resource, the nutritional quality is lower than animal-sourced proteins, owing to the fact that it is slightly deficient in the essential amino acid methionine. By including methionine rich food ingredients such as isolated rice protein powder, we should be able to create a new product with more complete amino acid composition.

While promising, the added rice ingredients, including white rice, brown rice, rice bran, and isolated rice protein may inhibit soy protein coagulation and/or change the texture of tofu significantly. One possible technology to diminish negative influences is continuous flow high pressure throttling (CFHPT). CFHPT has been shown to improve the texture and sensory of several protein-rich liquid foods. It does so by reducing particle size and causing changes to protein structure. And these two properties are directly related to the ability to manufacture high-quality tofu from soy milk. In the current study, we created tofu by fortification of soy milk with four levels (2.5%, 5%, 7.5%, and 10%) of various rice ingredients. The texture and physical characteristics (color, yield, water holding capacity) of rice incorporated tofu were measured and compared with those made with CFHPT treated soy-rice mixture in order to compare the impacts of CFHPT.

Specific objectives:

1.To determine how different types (white rice, brown rice, rice bran, rice protein) and levels (2.5%, 5%, 7.5%, 10%) of rice components change the characteristics of rice-soy tofu, specifically the texture (hardness, cohesiveness, springiness, chewiness) and physical properties (color, water holding capacity, yield) of the samples.

2.To determine whether CFHPT can offset the influence of rice components on the texture and physical properties of rice-ingredient fortified tofu.

Chapter 2

LITERATURE REVIEWS

2.1 Tofu history and markets

Tofu originated in Asia during ancient times and has been increasing in popularity in the western world. In 2018, the global tofu market size was approximately 2.31 billion dollars and it is expected to expand at a continuous annual growth rate (CAGR) of 5.2% between 2019 and 2025. While the Asian Pacific region held the largest portion of the global market share(56.3%) in 2018, North America is estimated to register the fastest CAGR (6.1%) from 2019 to 2025 (Grand View Research, 2019).

Tofu contains an abundance of protein, typically over 50%, based on a dry basis. Tofu also has a low-fat content, which is about 2% ~5% (Smith, Watanabe, & Nash, 1960). It has long been one of the major sources of protein in Asia and is a common protein source for vegans and vegetarians. Rising preference for vegan diet in developed countries, including the U.S. and Germany, account for not much of the increasing tofu market. In addition, roughly 65% of the global population is lactose intolerant (Bayless, Brown, & Paige, 2017), and this is anticipated to drive the demand for lactose- and dairy-free alternative protein sources such as tofu among non-vegans. Moreover, the increasing number of consumers aimed at reducing animal slaughter is expected to be another key driving factor (Grand View Research,2019).

2.2 Soybean protein

Consumption of soybeans has been linked to beneficial physiological effects on the human body, such as lowering cholesterol, anti-cancer activity, reducing obesity, and preventing diabetes (Friedman & Brandon, 2001). When pH is near 8.5, soy protein has the highest solubility, and the isoelectric pH of soybean protein is around 4.5. These proteins are mainly globulin, which makes up 90% of the total soy protein and comprises two major fractionsβ-glycinin (11S) and β-conglycinin (7S). The 11S glycinin consists of 2 polypeptide components, the acidic and the basic chains of 38 kDa and 20 kDa, respectively (Staswick, Hermodson, & Nielsen, 1984).

Soy protein denaturation is essential for tofu structure formation. At acid condition (pH 1.5 to 2.0), the structure of glycinin unfolds, likely due to electrostatic repulsions (Thanh & Shibasaki, 1977). In addition, the thermal denaturation of soy proteins is also a pre-requisite for tofu-gel formation. Studies have shown that the denaturation temperature of glycinin is about 92°C and is 71°C forβ-conglycinin (Liu, Chang, & Li, 2004). Further, both fractions are necessary, as the elimination of the 11S subunit has been shown to result in an insufficient ability to form a gel resulting in incomplete coagulation of the soybean curd (Poysa V, Woodrow, & Yu, 2006).

2.3 Rice components

Rice has been cultivated worldwide since antiquity. Like most cereal grains, carbohydrates make up the main component of both white and brown rice, with only a small amount of those carbohydrates are from fiber, the majority of the rest existing as starch.

Besides the carbohydrate fraction, another important component of rice is protein. Rice protein has been recognized as an excellent protein resource among cereal grains. It contains higher levels of the essential amino acid methionine than most other non-animal proteins. Rice protein also has high digestibility, which is 96.7% (Qiang te al., 2014). Compared with wheat protein, rice protein has a much lower risk of allergies and sensitivities.

Despite the benefits mentioned above, rice protein has some shortcomings. The first is low solubility-rice protein contains 75% to 80% of alkaline soluble glutenin, and these glutenin fragments form large molecules through disulfide bonds, cross-linking, and cohesion. Soluble albumins are also present in the protein fraction, but only account for 2% ~ 5% of the total protein (Samson Agboola, Darren, & Dominic, 2005). There are some methods to improve solubility, as researchers have found that between pH 4 and 7, glutenin protein solubility increases slowly, and when close to pH 9, the protein solubility rapidly increases. In contrast, heating will have a negative impact on rice protein solubility, with thermally denatured rice protein showing very low solubility, and sometimes even solidifying into insoluble complexes (Wang et al., 2008).

Rice bran is a major by-product of rice milling, and retains nearly all of the rice protein, and has a protein content around 20%. This protein is similar to rice endosperm protein, which contains alkali-soluble glutelin (60%~80%), albumin (4% ~ 9%), salt-soluble globulin (10% ~ 11 %), and alcohol-soluble glutenin (3%) (Shih et al., 1999). In addition to protein, rice bran is also a rich source of vitamins, minerals, essential fatty acids, dietary fiber, and other sterols (Gul, Yousuf, Singh, Singh, & Wani, 2015).

Milled white rice, brown rice, rice bran, and isolated rice protein all contain ingredients that could have beneficial nutritional and functional properties. Unfortunately, due to the low solubility of some fractions, in particular, rice protein, fiber, and starch, avoiding sedimentation and maintaining a homogeneous distribution of rice ingredients during tofu processing may pose a challenge.

2.4 Nutrition value of soy tofu and rice substituted tofu

The calculated nutrition for traditionally made soy tofu and rice-substituted tofu are shown in table 2. Pure soy tofu is a high protein low-fat food, and according to USDA, 100g of firm tofu contains about 17.3g of protein and 8.7g of lipids. WHO recommends that adults consume 0.8g/kg of protein per kilogram weight every day, and thus consuming 300g of firm tofu would meet the protein need of a 65kg adult. As previously mentioned, tofu protein also has a reasonably good amino acid balance, albeit slightly deficient in methionine, one of the indispensable amino acids that must be included in the diet. Non-vegans rarely have difficulty meeting the recommended amount (about 676 mg for a 65 kg adult), as animal proteins such as meat and milk are rich in methionines. Vegans or vegetarians are vulnerable to methionine deficiency, as even the 300g of firm tofu that would provide all of the protein a person needs, would only provide 633 mg methionine, which is close to the recommended amount but still over 5% deficient.

Further, despite being an excellent source of protein, tofu has very low fiber and carbohydrate content. The American heart association suggests eating 25-30g of fiber per day. According to table 2, adding 40% rice bran would increase the fiber content from 2.3g/100g to 7.6g/100g, and adding rice milk made from either white or brown rice would allow the creation of food with a better balance of carbohydrate, protein, and lipids.

2.5 Tofu coagulant

Various acids and salts are commonly used in tofu making. For salt coagulant, calcium sulfate (gypsum) is the most widely used in China for several reasons. In addition to being inexpensive, the use of this coagulant also increases the calcium content of tofu. In fact, many tofu manufacturers choose to use this coagulant in order to be able to market their tofu as a good source of dietary calcium (Prabhakaran, Perera, & Valiyaveettil, 2006). Another common type of salt coagulant is chloride salts, either magnesium chloride (the principal component of Japanese Nigari) or calcium chloride (called Lushui in China). Both chloride-based coagulants are highly soluble in water and affect soy protein in the same way. They have no detectable taste themselves, except at levels so high as to be impractical for tofu production.

The study of salt coagulants shows that with the increase of concentration, the strength of tofu gel increases, the network structure becomes coarse, and the water holding capacity is reduced. When the concentration of coagulant exceeds a certain value, the gravitational and repulsive forces between soybean protein are broken, and the coagulated material loses the necessary honeycomb network structure. Therefore, for each salt coagulant, there is a critical concentration value for making tofu. In general, these coagulants work by decreasing soymilk's pH, and coagulants with more exceptional ability to decrease pH often have smaller critical concentrations (Kao, Su, & Lee, 2003). Furthermore, due to reaction kinetics, as temperature increases, the critical concentration of a given coagulant also decreases (Obatolu, 2008).

In addition to the traditional salt coagulants, manufacturers may utilize organic acids as coagulants. One of the most popular organic acid coagulants is Glucono-delta-lactone (GDL), which is commonly used for soft tofu. Unlike traditional coagulants, GDL will give tofu a slightly sour taste. When the pH of tofu gel is higher than the isoelectric point of soybean protein (pH 4.5-5.0), the binding strength between soybean protein will be enhanced with GDL concentration. However, the network structure and water holding capacity are not affected. Compared with the salt coagulant, the water holding capacity of GDL coagulated tofu is less influenced by mixing temperature (Liu & Chang, 2004). Higher tofu gel strength and water holding capacity require sufficiently higher curdling temperature and longer settling time. However, the long settling time can have a negative impact on tofu's water holding capacity.

The current tofu factory usually blends different coagulants to achieve desired textural properties and processing parameters. In this study, GDL, nigari, and gypsum were blended in a radio of 1:1:1 to make firm texture tofu.

2.6 Tofu quality

The yield, moisture content, textural characteristics, and color of tofu are the most important indices of tofu quality. Those properties are essential to tofu acceptability (Cai and Chang 1997), and in this study, yield, water holding capacity (WHC), color, and texture were tested to evaluate the quality change.

Most tofu manufactures prefer a high yield for obvious economic reasons. For traditional soy tofu, this high yield is achieved through trapping more water in the tofu gel matrix. Thus higher yield usually accompanies higher moisture content and a softer texture. Texture preference among consumers is varied, and there are no definitive scientific conclusions on the relationship between region and texture preference, but “common knowledge” is that North Americans tend to prefer harder textures, as the resultant meat-like texture and stronger water holding ability during frying make firm tofu better fit in American cuisine. Silken tofu is widely used in soups and desserts, and is considered to be more popular in East Asia.

Besides texture, WHC is another important index to evaluate tofu’s performance during cooking. WHC can imitate the tofu water loss during cooking. A higher WHC value allows for less water loss during cooking and better retention of volume and shape. Finally, color is also an important measure of quality, with a light yellow color typically considered to be a desirable tofu characteristic (Abd Karim, Sulebele, Azhar, & Ping, 1999; Hou & Chang, 2004).

2.7 Factors affecting quality

Many factors, such as soybean cultivar, bean to water ratio, storage condition, soymilk heating rate and time, stirring speeds, coagulation time and temperature, pressing time and weight can influence tofu quality (Rekha & Vijayalakshmi, 2013; Kong & Chang, 2013). Bean to water ratio and soymilk heating processing determine the protein concentration of the resultant soymilk. The higher the milk protein concentration is, the greater the strength of tofu gel, and the smaller the water loss rate will be. However, when the milk protein concentration is too high (> 7%), tofu gel strength ceases to increase with the increase of protein concentration (Lee & Rha, 1978). Soy protein composition also plays an essential role in the tofu texture (Guo & Ono, 2005). This composition can be influenced by a variety of factors, including cultivar differences, growing conditions, and storage time/conditions. Analysis of soymilk with different 11S/7S ratios has shown that 11S-rich soy milk has a higher content of protein particles, and results in higher strength in the corresponding tofu (Schaefer & Love, 1992). Further, small molecular species, like phytate, are also considered to have an important buffering effect on soy milk coagulation and tofu texture, with a higher amount of phytate in soy milk corresponding to a higher optimal coagulant concentration and softer tofu (Guo & Ono, 2005). In addition, due to reaction kinetics, adding coagulant at a lower temperature is often beneficial not only to the control of the operating conditions but also in obtaining a homogeneous tofu gel (Liu & Chang, 2004).

2.8 High pressure processing

2.8.1 High pressure treating type and equipment

Any food processing with pressure over 100 MPa can be regarded as high-pressure processing, and these processes can be broken down into two types: high hydrostatic pressure (HHP) processing and continuous high pressure processing (CHPP). HHP is a batch process that allows for the treatment of a wide variety of solid and liquid foods across a wide range of pressures (typically from 100MPa to 800MPa). The product is held at this pressure for a specified holding time, which may vary from several minutes to a few hours. Different from HHP, CHPP is a continuous system which pumps liquid or semisolid foods through a system directly, exposing them to more brief periods of high pressure, but also shear effects at the outlet. Continuous flow high pressure throttling (CFHPT) is one type of CHPP, and was first developed at the University of Georgia, Athens, Georgia, U.S.A. (Thiebaud, Dumay, Picart, Guiraud, & Cheftel, 2003). Compared to HHP, it has a lower pressure range (100MPa~350MPa) and treatment time (based on flow rate) of mere seconds. During CFHPT, fluid product is pumped through a system that pressurizes them above 100 MPa by one or more piston pumps. The fluid then passes through a pressure relief device (typically a valve) where the fluid is subjected to shear, cavitation, and friction effects (Cavender, 2011). In this study, CFHPT was used to treat rice substituted soymilk prior to tofu production.

2.8.2 Application of high pressure processing on safety improvement

High pressure processing (HPP) has been approved to increase the shelf life of foods by destroying microbes and inactivating enzymes. In the United States and Europe, there are a variety of ultra-high pressure products available on the market, with manufacturers using HPP to replace heat treatment. HPP improves product safety while reducing the undesirable impacts of heat treatment on quality. Cold-pressed juices and milk are the most common examples, and the effect is profound- for example, treating orange juice with HPP can extend the shelf life up to 12 weeks (Bull et al., 2004; Goodner; Braddock, Parish, & Sims, 1999). HPP is also widely used in meat, poultry and seafood, and is often used as a post-packaging step to help control some food-borne pathogens (particularly Listeria monocytogenes). When combined with thermal treatment, even sterilization of food with limited nutrition and sensory loss can be achieved, though this has yet to be successfully commercialized (Ohshima, Ushio, & Koizumi, 1993). Overall, high pressure treatment has a less detrimental effect on flavor and nutritional quality, while elongating shelf life. (Peck, 2004; Sivanandan, Toledo, & Singh, 2008).

Commercially, HPP, which aims to increase the safety of food, relies more on high hydrostatic processing. In these processes, the microorganism disinfection and enzyme inactivation usually require treatment pressures between 400MPa and 700MPa, and long treating time (minutes to hours). As a result of this, and availability of production-scale equipment CFHPT is not currently used for anti-microbial applications, due to its narrow pressure range (100~350MPa) and short treating time (seconds).

2.8.3 Application of high pressure processing on sensory improvement

Beside longer shelf life, high pressure treatment also influences the sensory properties of food. Treating fish under 200 MPa leads to a firmer texture and more vivid color, increasing the sensory value of fish (Matser, Stegeman, Kals, & Bartels, 2000). The observed sensory changes may be associated with protein denaturation, as high pressure processing has been reported to result in the formation of a new type of protein network in cod (Angsupanich & Ledward, 1998). Though it has a low pressure range and short treating time, CFHPT has also been used to improve the sensory properties of liquid food. The throttling valve in the CFHPT system reduces the size of suspended particles and thereby creating more stable emulsions (Sidhu, 2013), and Laneuville, Paquin, and Turgeon (2000) used continuous high pressure processing to produce novel complexes of whey protein and xanthan gum that showed potential for use as fat substitutes. They noted that the continuous high pressure treatment prevented the formation of fibrous complexes, making the products unsuitable for use as a fat substitute. While treating liquid food with high pressure leads to a smoother and more stable liquid, it also influences the sensory properties of foods made from the liquid. For example, treating milk has been proven to change the cheese made from the milk, with Tunick et al. (2000) examining the ultrastructural differences in cheese made from non-homogenized milk and that made from CHPP milk. They found that continuous high pressure treatment reduces the fat globule size, and rearranges the pattern of electron-dense regions surrounding fat globules. Treatment at higher pressures led to changes in the nanostructure. The full-fat cheese made from the highest pressure resulted in the best dispersion, with similar trends of structural rearrangement being seen after six weeks of storage (Tunick et al., 2000).

In general, continuous flow high pressure throttling systems can reduce particle size, improve particle dispersion, and denature the protein in ways different from thermal processing. As processing pressure increases, there is typically an associated reduction of particle size, improved distribution of components, and more significant protein denaturation. In the current work, CFHPT will be used to reduce the particle size and better distribute substituted rice ingredients in soymilk, with a pressure of 300 MPa being applied to maximize the influence the protein denaturation, changing the tofu texture.

Table 1. Nutrition Value of Rice Components

	Protein (g/100g)	Methionine (mg/100g)	Carbohydrate (exclusive fiber) (g/100g)	Fiber (g/100g)	Lipids (g/100g)
White rice	6.6	155	79.3	0	0.6
Brown rice	7.7	163	71.8	2.6	2.6
Rice bran	15.0	328	8.0	51.0	21.4
Rice protein	80	2300	6.7	<6.7	0

Table 2. Calculated Nutrition Value for Rice Substituted Tofu*

	Substitution	Protein (g/100g)	Methionine	Carbohydrate	Fiber (g/100g)	Lipids (g/100g)	Reference
Pure Soy Tofu	NA	17.3	211.0	1.6	2.3	8.7	USDA,2019
White Rice Substituted Tofu	10%	16.7	208.2	5.5	2.2	8.3	USDA,2019; Nutrition Label
	20%	16.2	205.4	9.4	2.1	7.9	
	30%	15.7	202.6	13.3	2.0	7.5	
	40%	15.1	199.8	17.1	1.8	7.1	
Brown Rice Substituted Tofu	10%	16.7	208.2	5.5	2.2	8.3	USDA,2019; Nutrition Label
	20%	15.1	187.4	4.9	2.0	7.5	
	30%	14.9	199.0	18.7	2.1	7.1	
	40%	12.5	162.1	10.6	1.6	6.0	
Rice Bran Substituted Tofu	10%	17.2	216.9	2.6	3.6	9.3	USDA,2019
	20%	17.0	222.7	3.6	5.0	9.9	
	30%	16.9	228.6	4.7	6.3	10.6	
	40%	16.8	234.4	5.7	7.6	11.2	
Rice Protein Substituted Tofu	10%	20.4	315.5	2.0	2.4	8.3	USDA,2019 Nutribiotic, 2020
	20%	23.5	419.9	2.4	2.4	7.8	
	30%	26.7	524.4	2.9	2.5	7.4	
	40%	29.8	628.8	3.3	2.5	7.0	

* Assuming all added rice components are kept in tofu gel.

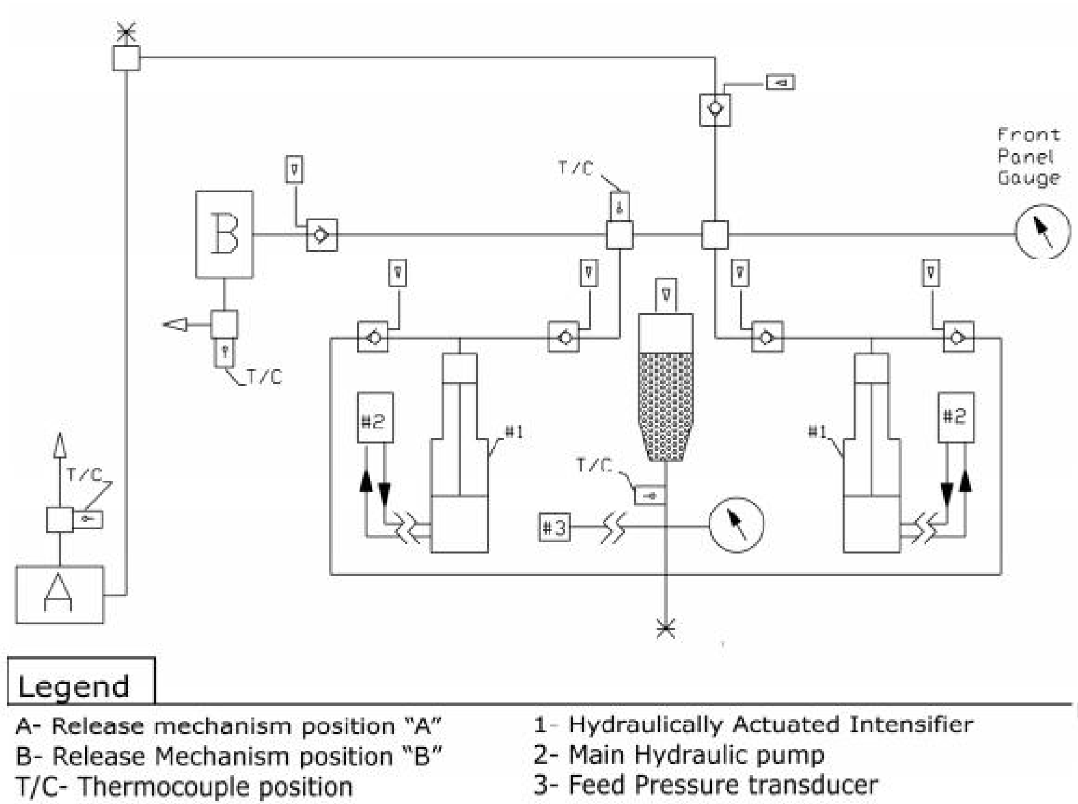

Figure 1. Schematic of High-Pressure System. Adapted from Cavender (2011)

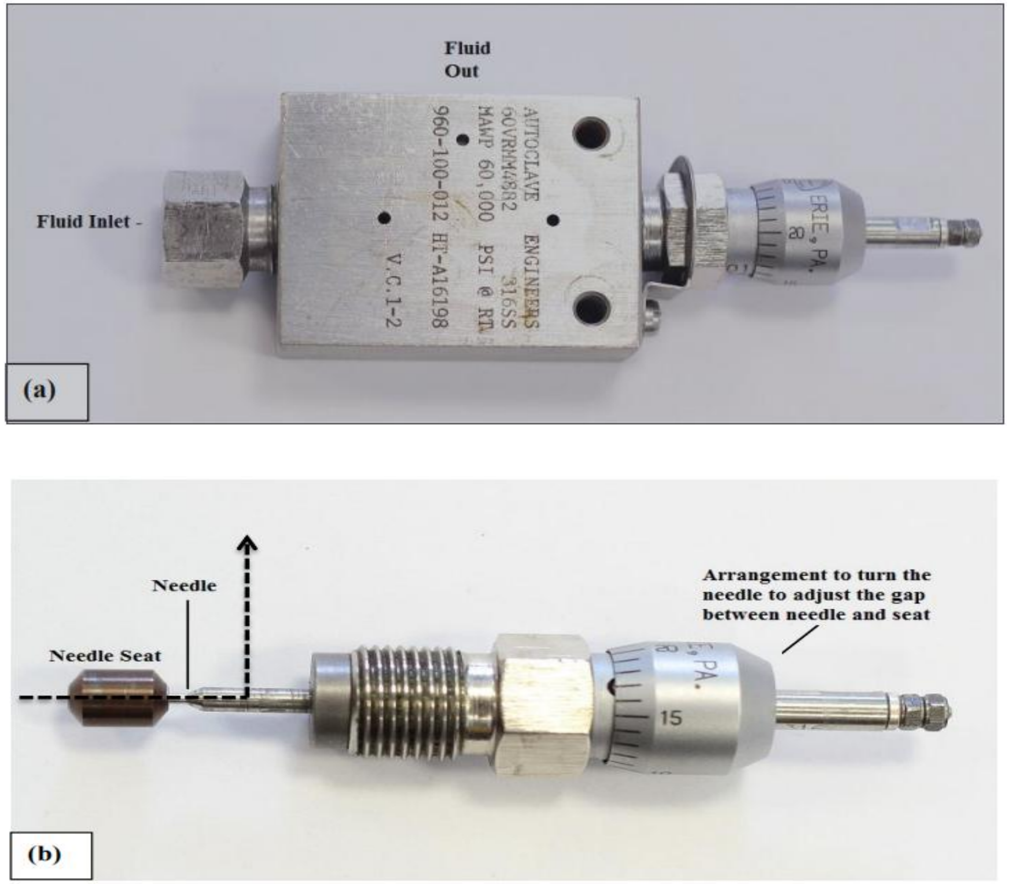

Figure 2. Throttling valve (Autoclave Engineers, Erie, PA) with – (a) the needle assembly fitted in; (b) the needle and its seat shown separately. The dotted line represents the direction and path of fluid flow. Adapted from Sidhu (2007)

Chapter 3

METHODS

3.1 Impact of Continuous High Pressure Processing on the Texture of Tofu Supplemented with White and Brown Rice

Four groups of tofu, soy-white rice milk tofu with/without CFHPT and soy-brown rice milk tofu with/without CFHPT, were prepared using the process summarized in Figure 3. Soy-white rice milk tofu was abbreviated as WR/WRC tofu. Soy-brown rice milk tofu was abbreviated as BR/ BRC tofu. Each group of tofu contained 5 different levels of white/brown rice milk (0%, 2.5%, 5%, 7.5% and 10% by weight). All samples were prepared in triplicate.

3.1.1 Soy Milk And Rice Milk Preparation

US grown organic soybeans (Gain Place foods, Inc., NE, USA), which had been stored at room temperature from the time of purchase until the time of use, were used to prepare rice milk for all trials. For each replication, samples (150g) of soybeans were soaked in 900 ml of water for 12 hours at 4 °C and then ground using a blender (Vita-Max Professional series 500, OH, USA). Soy solids (okara) was removed by straining the slurry through four layers of grade 90 cheesecloth.

To prepare ricemilk, either medium grain white rice (Nishiki Medium Grain Rice Specially Selected, CA, USA) or medium grain brown rice (Nishiki Premium Brown Rice, CA, USA) were used. Rice was added to water in a 1:6 rice: water ratio by weight) and placed into an automatic rice cooker (SANYO Electric Co. ECJ-D100S 10-Cup Micro-Computerized Rice Cooker, JP) for 30 minutes using the "porridge" function, before grinding into a paste using the blender mentioned above.

To create plant milk blends for the experimental samples, ricemilk was mixed with soy milk at either 2.5%, 5%, 7.5%, and 10% (w/w), and mixed in the blender at speed 10 for 60 seconds prior to further use.

3.1.2 Continuous Flow High Pressure Throttling Treatment

For treated samples, either pure soymilk or the soy-rice milk mixture were loaded into the feeding tank of a CFHPT system (Model nG7900, Stansted Fluid Power Ltd., U.K.), fitted with a metered throttling valve (Autoclave engineers, Model 60VRMM4882, Erie, PA), and processed at 300 MPa (4.4×104 Psi) and a flow rate of 1 L/Min. The inlet temperature was kept at 22 ± 2 °C. After processing, the treated milk was rapidly cooled to room temperature (22 ± 2 °C) by immersing their containers into a water-ice bath immediately following treatment. Cooled samples were then transferred to refrigerated storage for later use.

3.1.3 Tofu Preparation

Tofu was prepared from the prepared soymilk and soy/ricemilk blends, using the method developed by Kong (2008) with modifications. For non-CFHPT treated groups, the soymilk or the soy-rice milk mixture (For ease of reading, the term “milk” will be used to refer to the products) were heated to 95 °C and held at that temperature for 3 minutes. After heating, milk was allowed to cool to 80°C, before the introduction of a coagulant suspension at a rate of 6% of the soymilk by volume. The coagulant suspension was made by dissolving 10g of coagulant mixture (Nigari (Magnesium chloride, Ohsawa, CA, USA), Gypsum (Calcium Sulfate Dihydrate, LD Carlson Co., OH, USA) and Glucono-delta-Lactone (Jungbunzlauer, Switzerland) in a ratio of 1:1:1) in 100ml of water. Milk was allowed to rest for 30 minutes to allow the formation of curds, which were then broken into small cubes and transferred to cheesecloth-lined perforated molds. The molds were capped, and pressure (1.17 kPa) was applied to the caps for 12 hours to express the whey and form a cohesive block. Samples were then de-molded and stored under refrigeration for later tests.

3.1.4 Yield

Yield was defined as the ratio of the weight of final tofu and the weight of the soymilk or soy-rice milk mixture and was calculated using equation 1. Measurements were made in triplicate, and all data are reported as mean ± SD.

$$Yield = \frac{W_p}{W_s + W_a} \times 100\% \quad (1)$$

W_p = the wight of tofu (g)

W_s = the weight of soy milk (g)

W_a = the weight of rice additives (g)

3.1.5 Texture Profile Analysis (TPA)

Tofu samples were allowed to equilibrate to room temperature prior to measurement. From each block of tofu, four cylindrical samples (1.5 cm dia×1.5 cm height) were excised using a stainless steel cylindrical cutter. During testing, samples were compressed to 50% of their original height twice, using a texture analyzer fitted with a 35 kg load cell (TA.XT2i, Stable Micro Systems, Godalming, Surrey, United Kingdom) and 40 mm cylindrical probe (Model TA-94) according to the method of (Rekha & Vijayalakshmi, 2013), with modification. The pre-test speed was 2 mm/s, test and post-test speed were 5 mm/s, and the trigger force was 20 g. Hardness, cohesiveness, springiness and chewiness were calculated from the texture profile analysis curve as described by Bourne (1978), and all results were reported as mean ± SD.

3.1.6 Color

A colorimeter (Model CR-410, Konica Minolta Sensing Americas, Inc., Ramsey, NJ) was used to measure the surface color using a CIE L*a*b* system. Prior to use, the colorimeter was calibrated with a white D65 standard ($Y = 94.7$, $x = 0.3156$ and $y = 0.3319$) according to manufacturer instructions. For each sample, three spots were chosen on the surface of a given block, and each spot was measured twice, with all data reported as mean ± SD.

3.1.7 Water Holding Capacity

Water Holding Capacity (WHC) measurements were performed using the method described by Li et al. (2014). Measurement was performed immediately after removing the tofu from the refrigerator. For each measurement, a cylindrical sample (1.5cm in diameter and 1cm in length) was excised and placed into a 50 ml conical centrifuge tube. Samples were centrifuged at 1600 x g for 15 minutes using a benchtop centrifuge (Sorvall RC6 PLUS, F21S-8 * 50; Thermo Fisher Scientific, Waltham, Massachusetts, USA) with a fixed angle rotor. Immediately after centrifugation, expressed water was decanted from the tube and weighed. Total WHC was calculated using equation 2, and all measurements were performed in triplicate, with results expressed as mean ± SD.

$$Water\ Holding\ Capacity = \frac{m_o - m_w}{m_o} \times 100\% \qquad (2)$$

$m_o = the\ weight\ of\ tofu\ before\ centrifigation$

$m_w = the\ weight\ of\ water\ removed\ through\ centrifigation$

3.1.8 Statistic Analysis

The statistical significance of the observed differences between the experimental results was determined by one-way ANOVA and post-hoc testing by Fisher's Least Significant Difference (LSD) method. Calculations were performed using statistical software (JMP version 14.1.0, RStudio, Inc. Boston, MA, USA) with differences considered significant if alpha ≤ 0.05. A quantile range screening method was used to determine the outlier.

3.2 Impact of Continuous High Pressure Processing on the Texture of Tofu Supplemented with Rice Bran and Rice Protein Powder

Four groups of tofu, rice bran powder added tofu with/without CFHPT and rice protein powder added tofu with/without CFHPT, were prepared using the processing in Figure 4.

Soy-rice bran milk tofu was abbreviated as RB/RBC tofu. Soy-rice protein milk tofu was abbreviated as RP/RPC tofu. Each group of tofu contained 5 different levels of rice bran/rice protein powder (0%, 2.5%, 5%, 7.5% and 10% by weight). All samples were prepared in triplicate.

3.2.1 Soymilk Preparation

Soymilk was prepared through the method mentioned in section 3.1.1.

Rice bran (Rice Plus stabilized rice bran, USA) or rice protein powder (Nutribiotic raw rice protein powder, CA, USA) was added into soymilk directly. The powder was blended with soymilk in Vita Max blender at the speed level 10 for 60s. Rice bran was vacuum packaged and stored under -20 °C to avoid oxidation.

3.2.2 Continuous Flow High Pressure Throttling

Soymilk or soy-rice mixture was treated at the same conditions as in the section 3.1.2.

3.2.3Tofu Preparation and Characterization

Tofu preparation and characterization (yield, texture, color) were conducted as described above.

3.2.4 Statistic Analysis

The statistical significance was measured through the method described in 3.1.8.

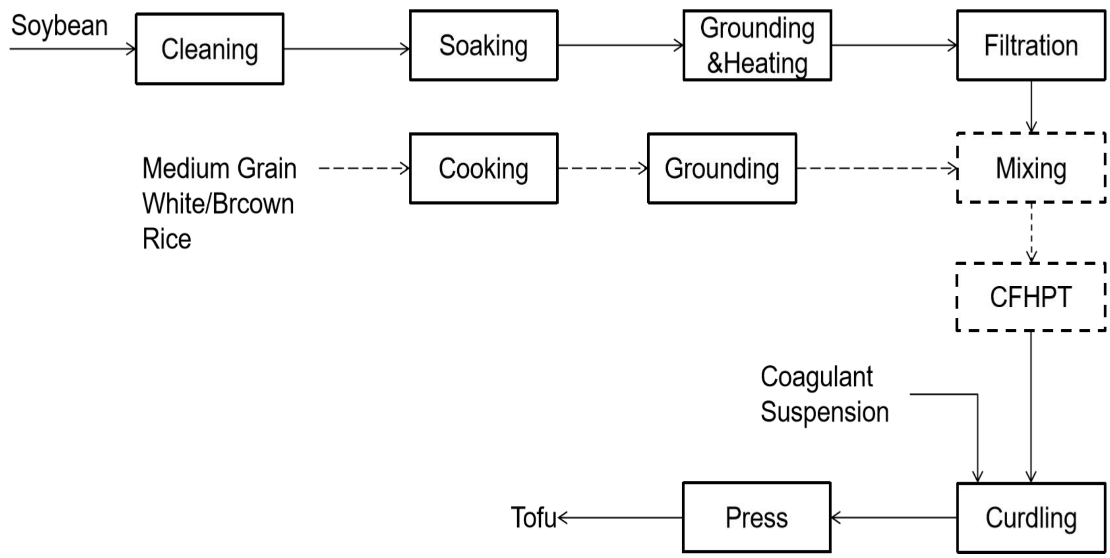

Figure 3. Flow Chart for White Rice and Brown Rice Incorporated Tofu

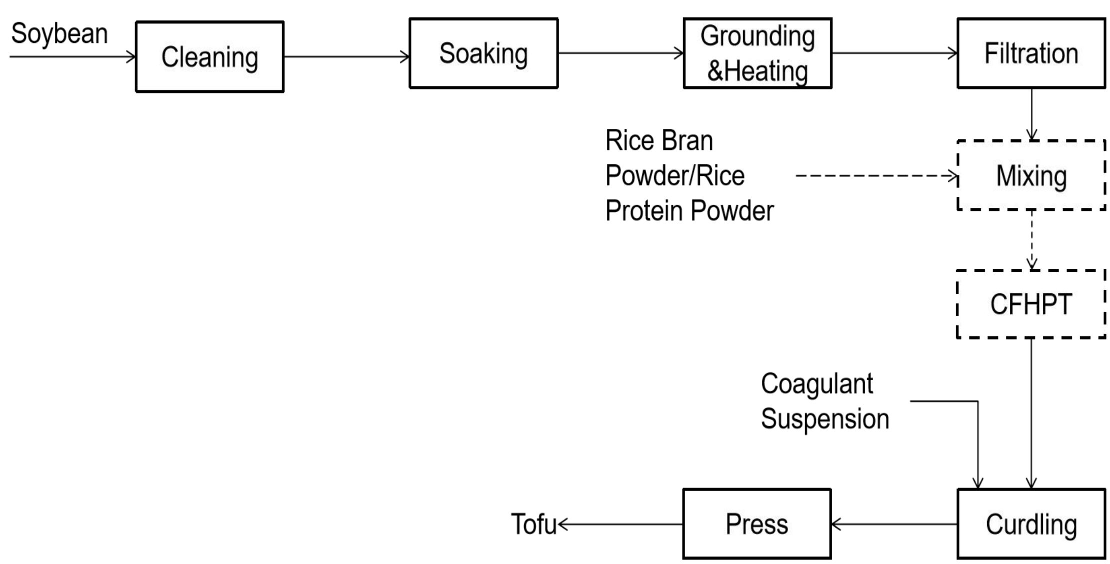

Figure 4. Flow Chart for Rice Bran and Rice Protein Incorporated Tofu

Chapter 4

RESULTS

4.1 Impact of Continuous High Pressure Processing on the Texture of Tofu supplemented with White and Brown Rice

4.1.1 TPA

The results of the TPA are presented in Table 3. For WR tofu samples, the addition of white rice milk had no significant influence on cohesiveness, but at higher levels, it significantly ($p<0.05$) decreased the hardness, chewiness, and springiness of the tofu. In particular, when the white rice level was higher than 5%, both hardness and chewiness decreased rapidly. At the 10% level, both hardness and chewiness were less than 50% of the non-supplemented samples.

Similarly, adding brown rice decreased hardness, chewiness, and springiness. Of note is the curious case of the 5% brown rice samples, which were fundamentally lower than all other levels. Initially, it was thought that this figure might be an outliner, but no after testing via quantile range screening (tail quantile=0.25, Q=1.5), this was not the case. In general, the relationship between the brown rice level and the textural properties was not linear, suggesting that brown rice influences the formation of tofu gel.

Continuous flow high-pressure treatments had no significant influence on WR tofu's hardness (Figure 4.). Instead of diminishing the influence of white rice milk created, the high-pressure treatment led to a more erratic result.

For BR tofu groups, the hardness of CFHPT treated tofu had a less erratic result, with the 5% level of brown rice substitution showing no significant decrease in hardness.

4.1.2 Yield and Water Holding Capacity

Results for yield and water holding capacity are presented in Table 4. There were no significant differences in the water holding capacity among all WR/BR tofu samples, while the yield of the WR/BR tofu samples were significantly ($P < 0.05$) lower than those of the 100% soy tofu sample(0% WR/BR). Water holding capacity, which is indicative of the ability of tofu gel to trap water in its matrix, showed no statistical differences among any of the samples.

4.1.3 Color

The mean ± SD values for L*, a*, and b* color coordinates of tofu samples are given in Table 5. The original tofu (0%) sample had a creamy white color with a hint of yellow. Brown rice substitute alone significantly ($P < 0.05$) affects the color, and the differences were mainly due to a decrease in the L * (lightness) value. There were no significant differences in either a* (green/red) or b* (blue/yellow) values among all tofu samples without CFHPT treatment.

WRC and BRC tofu had color much more close to the original tofu sample, and overall, CFHPT treatment significantly changed the a* and b* value of the white rice tofu group.

However, the change would likely be difficult to be detected with the untrained eye. CFHPT treated tofu samples also showed smaller standard deviations, indicating a more uniform color among the various batches. The significant increase in lightness seen in the treated brown rice tofu might increase consumer acceptability, as a light yellow color has long been considered a desirable tofu characteristic (Abd Karim et al., 1999; Hou & Chang, 2004).

4.2 Impacts of Continuous High Pressure Processing on the Texture of Tofu Supplemented with Rice Bran and Rice Protein Powder

4.2.1 TPA

Textural properties of RB and RP tofu were given in Table 6. RB tofu showed erratic results for hardness, chewiness, cohesiveness, and springiness. Some tofu samples in 2.5% RB and 5% RB groups were so paste-like that they did not recover after the first compression, preventing a second compression from measuring anything meaningful. The erratic results and paste-like samples suggest that the addition of rice bran severely interferes with the formation of the desired soy protein structure, making it impossible to produce tofu with stable textural quality from those milk.

CFHPT treatment diminished the unfavorable effects of rice bran addition. While samples made from untreated 2.5% supplemented soymilk were so plastic that they could not be properly measured, samples made from CFHPT treated milks showed no significant differences in hardness and springiness between the non-supplemented samples and those with supplementation up to 7.5%. However, the benefits of CFHPT were not seen in the 10% rice bran samples, as those tofus started to be paste-like again.

Rice protein substitution significantly decreased the hardness of tofu, while significantly increasing the cohesiveness. The hardness decreased to approximately 67% of the pure soy tofu, and the cohesiveness increased by 50%. These two changes suggest that the product will have a softer texture and a better resistance to cohesiveness. Different rice protein levels had no significant impacts on textural properties. Thus possibly less than 2.5% of rice protein could be trapped in tofu gel, with the extra rice protein being lost to the whey instead of becoming part of the tofu.

4.2.2 Yield and Water Holding Capacity

The results for yield and WHC are presented in Table 7. There was no significant difference in yield among tofu with different rice bran levels, and the overall mean yield of RB tofu was around 31%. Treatment with CFHPT significantly reduced the yield of RBC tofu, and with both 10% of rice bran substitution, the decrease in yield got as high as 30%. Despite the changes due to CFHPT, no significant difference was found for water holding capacity among all tofu samples.

4.2.3 Color

As shown in table 8, the addition of either rice bran or rice protein powder significantly decreased the L* (lightness) of tofu samples. Moreover, RP tofu showed significant increases in a*(redness) and b*(yellowness). The increase was unexpected, as rice protein powder naturally has a dark reddish-brown color, which translated into similar shifts in the tofu. Unsurprisingly, CFHPT treatment did not influence the color of both RB and RP tofu.

Table 3. Impacts of CFHPT on the Textural Properties of Tofu Made with Different Rice Milk

		Hardness			Chewiness			Cohesiveness			Springiness		
WHITE RICE	0%	960	±	79a	461	±	50a	0.47	±	0.02a	0.99	±	0.02a
	2.5	943	±	317a	425	±	164ab	0.46	±	0.03a	0.97	±	0.04b
	5%	917	±	329a	408	±	145b	0.45	±	0.02a	0.99	±	0.15abc
	7.5	614	±	135ab	268	±	76b	0.45	±	0.03a	0.94	±	0.05bc
	10%	476	±	141b	200	±	64b	0.46	±	0.03a	0.91	±	0.05c
BROWN RICE	0%	960	±	79ab	461	±	50a	0.47	±	0.02a	0.99	±	0.02a
	2.5	1035	±	208a	483	±	29ab	0.48	±	0.04a	0.96	±	0.05ab
	5%	376	±	68c	130	±	19c	0.42	±	0.03b	0.83	±	0.05c
	7.5	804	±	189ab	357	±	95bc	0.48	±	0.03a	0.92	±	0.07ab
	10%	604	±	118bc	258	±	62bc	0.47	±	0.03ab	0.91	±	0.06b
CFHPT WHITE RICE	0%	896	±	107a	280	±	48a	0.32	±	0.03a	0.96	±	0.02ab
	2.5	1233	±	257b	556	±	171b	0.47	±	0.06b	0.93	±	0.06b
	5%	719	±	166a	201	±	63a	0.29	±	0.06ac	0.95	±	0.03ab
	7.5	458	±	77c	97	±	41c	0.21	±	0.03c	0.83	±	0.09c
	10%	708	±	96a	216	±	49a	0.31	±	0.05a	0.98	±	0.02a
CFHPT BROWN RICE	0%	896	±	107a	280	±	48a	0.32	±	0.03a	0.96	±	0.02a
	2.5	733	±	135ab	292	±	51a	0.42	±	0.02b	0.96	±	0.04a
	5%	847	±	85a	363	±	61b	0.45	±	0.11b	0.96	±	0.06a
	7.5	590	±	64bc	227	±	23a	0.41	±	0.02b	0.95	±	0.02a
	10%	454	±	103c	107	±	27c	0.30	±	0.04a	0.80	±	0.08b

Different letters indicate significant difference within one tofu sample and one textural property. ($p < 0.05$)

Table 4. Yield and Water Holding Capacity for White Rice Tofu and Brown Rice Tofu Before and After CFHPT Treatment

		Yield (%)			Water holding capacity (%)		
WHITE RICE	0%	32.88	±	0.27a	95.58	±	1.21a
	2.5%	27.37	±	2.11b	95.36	±	1.05a
	5%	27.03	±	1.36b	94.61	±	1.18a
	7.5%	25.89	±	1.95b	96.51	±	1.08a
	10%	27.95	±	2.31b	95.76	±	1.77a
BROWN RICE	0%	32.88	±	0.27a	95.58	±	1.21a
	2.5%	24.29	±	2.68b	96.71	±	0.64a
	5%	29.58	±	3.30b	92.88	±	3.63a
	7.5%	25.37	±	0.76ab	96.36	±	0.70a
	10%	24.91	±	1.16b	95.85	±	1.08a
CFHPT WHITE RICE	0%	32.30	±	1.27a	96.88	±	0.95a
	2.5%	29.81	±	1.48a	95.76	±	2.00a
	5%	29.31	±	1.36a	96.47	±	2.12a
	7.5%	29.29	±	1.33a	96.48	±	2.32a
	10%	30.35	±	0.57a	96.98	±	1.61a
CFHPT BROWN RICE	0%	32.30	±	1.27ab	96.88	±	0.95a
	2.5%	29.45	±	1.79a	96.54	±	1.81a
	5%	34.20	±	0.78b	97.11	±	1.83a
	7.5%	34.19	±	1.03b	97.01	±	1.94a
	10%	30.03	±	1.14a	95.58	±	2.01a

Different letters indicate significant difference within one tofu sample and one property. (p<0.05)

Table 5. Color For White Rice Tofu and Brown Rice Tofu Before and After CFHPT Treatment

		L*	a*	b*
WHITE RICE	0%	86.12 ± 0.19a	-0.71 ± 0.29a	11.01 ± 0.60a
	2.5%	85.75 ± 0.31a	-0.48 ± 0.27a	11.00 ± 0.43a
	5%	85.21 ± 0.21a	-0.74 ± 0.07a	10.62 ± 0.28a
	7.5%	86.08 ± 0.28a	-0.29 ± 0.10a	10.44 ± 0.07a
	10%	85.82 ± 0.78a	-0.51 ± 0.20a	10.44 ± 0.42a
BROWN RICE	0%	86.12 ± 0.19a	-0.71 ± 0.29a	11.01 ± 0.60a
	2.5%	84.33 ± 0.20b	-0.34 ± 0.19a	10.44 ± 1.34a
	5%	84.48 ± 0.46ab	-0.52 ± 0.28a	10.43 ± 0.96a
	7.5%	84.02 ± 0.81b	-0.41 ± 0.32a	9.83 ± 0.73a
	10%	84.27 ± 1.03b	-0.25 ± 0.19a	10.52 ± 0.61a
CFHPT WHITE RICE	0%	84.10 ± 0.29a	-0.41 ± 0.12a	12.35 ± 0.30a
	2.5%	84.76 ± 0.77a	-0.02 ± 0.17ab	11.82 ± 0.58b
	5%	84.53 ± 0.17a	0.12 ± 0.14b	10.61 ± 0.39b
	7.5%	84.41 ± 0.63a	0.12 ± 0.03b	11.00 ± 0.30b
	10%	85.31 ± 0.45a	-0.19 ± 0.05ab	10.26 ± 0.09b
CFHPT BROWN RICE	0%	84.10 ± 0.29a	-0.41 ± 0.12a	12.35 ± 0.30a
	2.5%	84.02 ± 0.23a	-0.14 ± 0.23a	11.49 ± 1.23a
	5%	83.52 ± 0.50a	-0.34 ± 0.09a	11.55 ± 0.83a
	7.5%	83.91 ± 0.29a	-0.21 ± 0.53a	11.20 ± 0.68a
	10%	83.32 ± 0.88a	-0.59 ± 0.31a	11.41 ± 0.69a

Different letters indicate significant difference within one tofu sample and one property. ($p<0.05$)

Table 6. Impacts of CFHPT on The Textural Properties of Tofu Made with Rice Bran and Rice Protein

		hardness	chewiness	cohesiveness	springiness
RICE BRAN	0%	960 ± 79a	461 ± 50a	0.47 ± 0.02a	0.99 ± 0.02a
	*2.5%	268 ±	49 ±	0.35 ±	0.52 ±
	*5%	224 ±	98 ±	0.52 ±	0.86 ±
	7.5%	381 ± 73b	212 ± 51b	0.60 ± 0.02b	0.93 ± 0.06a
	10%	607 ± 129b	348 ± 70b	0.62 ± 0.04b	0.93 ± 0.05a
RICE PROTEIN	0%	960 ± 79a	461 ± 51a	0.47 ± 0.02a	0.99 ± 0.02a
	2.5%	698 ± 172b	462 ± 132a	0.68 ± 0.11b	0.97 ± 0.02a
	5%	648 ± 130b	461 ± 91a	0.73 ± 0.05b	0.98 ± 0.02a
	7.5%	649 ± 120b	482 ± 105a	0.76 ± 0.05b	0.97 ± 0.01a
	10%	627 ± 68b	429 ± 72a	0.70 ± 0.05b	0.98 ± 0.01a
CFHPT RICE BRAN	0%	896 ± 107a	280 ± 48a	0.32 ± 0.03a	0.96 ± 0.02a
	2.5%	949 ± 156a	405 ± 71b	0.44 ± 0.01b	0.97 ± 0.01a
	5%	823 ± 98a	306 ± 46ab	0.39 ± 0.02b	0.94 ± 0.04a
	7.5%	818 ± 82a	351 ± 34ab	0.45 ± 0.01b	0.96 ± 0.02a
	10%	295 ± 51b	55 ± 36c	0.30 ± 0.09a	0.58 ± 0.15b
CFHPT RICE PROTEIN	0%	896 ± 107a	280 ± 48a	0.32 ± 0.03a	0.96 ± 0.02a
	2.5%	792 ± 129ab	294 ± 44ab	0.39 ± 0.02b	0.94 ± 0.03a
	5%	595 ± 88ab	193 ± 50ac	0.38 ± 0.03b	0.84 ± 0.05b
	7.5%	1000 ± 179a	387 ± 81b	0.39 ± 0.02b	0.96 ± 0.02a
	10%	584 ± 102b	163 ± 53c	0.29 ± 0.06a	0.94 ± 0.05a

* only one batch was measured due to the paste-like consistency of the products

Different letters indicate significant difference within one tofu sample and one textural property. ($p<0.05$)

Table 7. Yield and Water Holding Capacity for Rice Bran Tofu and Rice Protein Tofu Before and After CFHPT Treatment

		Yield (%)	Water holding capacity (%)
RICE BRAN	0%	32.88 ± 0.27a	95.58 ± 1.21a
	2.5%	30.29 ± 2.78a	95.92 ± 0.68a
	5%	30.75 ± 1.73a	95.36 ± 1.50a
	7.5%	31.05 ± 1.28a	96.17 ± 1.58a
	10%	32.84 ± 1.41a	95.38 ± 0.78a
RICE PROTEIN	0%	32.88 ± 0.27a	95.58 ± 1.21a
	2.5%	31.30 ± 1.07a	96.07 ± 0.68a
	5%	30.22 ± 1.60a	95.70 ± 0.78a
	7.5%	29.96 ± 1.09a	96.44 ± 1.31a
	10%	31.10 ± 1.81a	96.41 ± 1.16a
CFHPT RICE BRAN	0%	32.30 ± 1.27a	96.88 ± 0.95a
	2.5%	35.20 ± 2.17a	96.09 ± 2.38a
	5%	35.98 ± 1.56a	95.78 ± 2.32a
	7.5%	27.70 ± 1.24b	97.17 ± 1.82a
	10%	22.99 ± 0.24c	96.39 ± 1.77a
CFHPT RICE PROTEIN	0%	32.30 ± 1.27a	96.88 ± 0.95a
	2.5%	28.04 ± 1.43b	95.75 ± 2.47a
	5%	29.15 ± 1.06b	95.67 ± 1.97a
	7.5%	33.01 ± 0.50a	95.37 ± 2.11a
	10%	33.49 ± 0.52a	96.01 ± 1.68a

Different letters indicate significant difference within one tofu sample and one property. ($p<0.05$)

Table 8. Color for Tofu Samples Before and After CFHPT Treatment

		L*	a*	b*
RICE BRAN	0%	86.12 ± 0.19a	-0.71 ± 0.29a	11.01 ± 0.60a
	2.5%	84.43 ± 0.62b	-0.57 ± 0.30a	10.84 ± 0.34a
	5%	83.23 ± 0.26b	-1.853 ± 0.02a	10.66 ± 0.75a
	7.5%	83.21 ± 0.10b	-0.88 ± 0.52a	10.73 ± 0.58a
	10%	83.05 ± 0.41b	-0.95 ± 0.88a	11.38 ± 0.65a
RICE PROTEIN	0%	86.12 ± 0.19a	-0.71 ± 0.29a	11.01 ± 0.60a
	2.5%	80.19 ± 0.44b	0.47 ± 0.11b	12.47 ± 0.17b
	5%	79.45 ± 0.50b	0.75 ± 0.06b	12.34 ± 0.23b
	7.5%	78.38 ± 0.17c	1.30 ± 0.04c	12.44 ± 0.28b
	10%	77.31 ± 0.02d	1.16 ± 0.04c	12.79 ± 0.13b
CFHPT RICE BRAN	0%	84.10 ± 0.29a	-0.41 ± 0.12a	12.35 ± 0.30a
	2.5%	83.90 ± 0.51a	-0.27 ± 0.05a	10.22 ± 0.51b
	5%	83.52 ± 0.59a	-0.18 ± 0.10a	10.25 ± 0.16b
	7.5%	83.38 ± 0.48a	-0.14 ± 0.08a	11.30 ± 0.39ab
	10%	83.46 ± 0.70a	-0.27 ± 0.35a	10.83 ± 0.87b
CFHPT RICE PROTEIN	0%	84.10 ± 0.29a	-0.41 ± 0.12a	12.35 ± 0.30a
	2.5%	80.84 ± 0.84b	0.98 ± 0.43b	11.37 ± 0.58a
	5%	79.66 ± 0.45b	1.58 ± 0.27b	11.53 ± 1.38a
	7.5%	80.02 ± 0.12b	1.18 ± 0.32b	10.59 ± 1.57a
	10%	79.90 ± 0.78b	0.98 ± 0.07b	10.85 ± 0.18a

Different letters indicate significant difference within one tofu sample and one property.($p<0.05$)

Chapter 5

DISCUSSION

5.1 Impacts of Continuous High Pressure Processing on the Texture of Tofu Supplemented with White and Brown Rice

5.1.1 White Rice Milk Incorporated Tofu

Tofu texture is an important quality that affects consumer acceptability. The incorporation of white rice significantly decreases both the hardness and chewiness but has no influence on either the cohesiveness and springiness. As chewiness is a calculated measure (hardness × cohesiveness × springiness), the change in chewiness is essential to be expected due to the change in hardness. One possible explanation for the change in texture properties is that during the production of tofu, the protein in soy milk will denature, exposing a part of its internal structure, and then react with the tofu coagulant to form gel structure. The calcium/magnesium ion or acid species in the coagulant then reacts with the soy protein, promoting a protein matrix formation. It is likely that the large amount of gelatinized starch contained in white rice milk may either coat parts of the soy protein or act to neutralize cations, both of which would inhibit the interaction between soybean protein and coagulant, resulting in a tofu structure that is more fragile and softer.

The continuous flow high pressure throttling (CFHPT) treatments used in this study had no significant influence on the textural properties of WRC tofu, but different pressures and times might allow for significant changes. Some researchers have reported that treating firm tofu at 650MPa for 30 minutes develops a harder texture and a more compact structure. These researchers believe that high pressure promotes the denaturation of soy protein, and increases the accessibility of polar groups, which are able to form saline bridges with coagulant cations and induce aggregation (Pre´stamo & Arroyo, 1998; Cheftel, 1995). However, in this study, soymilk instead of tofu was treated with high pressure, and this change may lead to different results. Reports about softer textures and worse soy protein denaturation also can be found, particularly at lower pressures and shorter times, such as 100-200 MPa for 2-10minutes (Liu, Chien, & Kuo, 2013). And as in this research, soy milk was treated at 300MPa for only seconds- perhaps it would be possible to increase either treating pressure, hold time (probably through adding a holding tube to the system), or both.

Another significant change resulting from the addition of white rice milk is decreasing yield. White rice milk substitution decreases tofu yield by up to 22%, but no significant difference is seen between the different levels of white rice substitution. As mentioned above, white rice starch may inhibit soy protein and coagulant interaction, which could cause some of the soy protein to be lost to the whey, resulting in tofu yield decreases. This phenomenon may also explain why after CFHPT treatment, the yield of white rice tofu increases- Perhaps the high pressure breaks down the size of starch particles and allowing the coated protein regions to be more accessible.

5.1.2 Brown Rice Milk Incorporated Tofu

Brown rice substitution significantly decreases the hardness and chewiness of tofu, but the effects on hardness and chewiness are erratic. Similar to white rice, brown rice contains a lot of starch. As mentioned in section 5.1.1, starch would decrease the hardness of tofu. 5% brown rice tofu shows a sudden drop in hardness, chewiness, and springiness. The erratic textural results may be due to the uniform distribution of fiber in the brown rice tofu. The dietary fiber in brown rice, with its high affinity for water molecules, interacted with protein molecules or be trapped in a tofu gel network, thereby increasing the moisture content (Liu et al., 2016). And since the high moisture content is reported to be associated with a softer texture, this may help explain some of the mechanisms involved in the textural changes (Zhu, Wu, Saito, Tatsumi, & Yin, 2016). with the tofu samples with higher dietary fiber contents possibly having a higher moisture content and softer texture. Therefore, the lack of uniform distribution of dietary fiber would cause erratic textural results, and in future studies, perhaps increasing sample sizes might eliminate the problem.

CFHPT treated brown rice tofu samples have more stable textural results compared to non-CFHPT treated brown rice tofu. The textural properties have no significant difference when brown rice level is below 5%, but when 7.5% of brown rice is added, the hardness and chewiness start to decrease. The more stable textural results suggest that a better disperse of brown rice milk has been achieved, which is expected, given that CFHPT has been proven to reduce the particle size and improve particle dispersion in different food such as milk, soy milk, and corn starch (Liu, Wu, Chen, & Chang, 2009; Nguyen, Guillarme, Rudaz, & Veuthey, 2006). Increasing treating pressure and time might help decrease the particle size furthermore and increase the saturation point for brown rice.

Without CFHPT, the yield of the brown rice tofu significantly decreases. After CFHPT treatment, the yield initially increases but starts to drop at the 10% level of supplementation. The changes in yield may be related to some combination of functions of rice starch and fiber. As mentioned above, the native starch appears to inhibit protein aggregation and decrease the yield. Rice fiber is expected to increase the moisture content and thus increase the yield. However, the amount of starch far outweighs the fiber, so the yield increasing the effect of fiber is unseen. Further, the lack of uniform distribution of the fiber makes some samples have a larger yield and cause a larger standard deviation. CFHPT treatment breakdown starch and fiber sizes and promote even distribution. These changes are very likely to release some of the starch coated soy protein and increases the moisture content consistently. Therefore, CFHPT brown rice tofu has a higher yield at the beginning. However, once fiber levels become high enough, they would likely begin to disrupt the network of brown rice tofu, leading to a decrease of capability on trapping water (Ullah et al., 2019).

Unsurprisingly, brown rice tofu has a darker color compared to pure soy tofu, and CFHPT treatment further decreases the lightness. The decrease in lightness in the untreated samples is mainly due to the brown color of brown rice, whereas CFHPT treatment may make more rice milk get trapped in the tofu matrix, thereby producing darker tofu. This decrease in lightness might be problematic if it reduces consumer acceptance, which is possible given that white, creamy white or light yellow color is considered as a desirable tofu characteristic (Abd Karim et al., 1999; Hou and Chang, 2004).

5.2 Impacts of Continuous High Pressure Processing on the Texture of Tofu Supplemented with Rice Bran and Rice Protein Powder

5.2.1 Rice Bran Incorporated Tofu

Rice bran substitution appears to damages the structural integrity of tofu. Every RB tofu presented a softer, paste or pudding-like texture. Some samples (2.5%, 5% BR tofu) even had extremely low chewiness and springiness that those samples were entirely damaged in the first compression and were not suitable for TPA measurement. However, when the rice bran level is larger than 7.5%, hardness, chewiness, cohesiveness, and springiness begin to increase, even when compared with the unsupplemented products. For example, cohesiveness increases from 0.47 (pure soy tofu group) to 0.62 (10% rice bran tofu). This is likely due to complex chemicals in the rice bran interacting in some manner to cause the textural change. Rice bran contains about 28.3% dietary fiber, 20.3% fat, 18% digestible carbohydrate, 12.3% protein, and some other chemicals such as minerals (Gul et al., 2015). The dietary fiber in brown rice may interact with protein molecules or get trapped in the tofu gel network, thus increasing the moisture content (Liu et al., 2016). The fiber content in 2.5% rice bran tofu corresponds to approximately 140% of that seen in the brown rice tofu. Accord to section 5.1.2, such a large amount of fiber severely damages the tofu network, resulting in tofu deformation (Ullah, I. et al., 2019). Table 6 shows that hardness, chewiness, cohesiveness, and springiness start to increase when rice bran levels are higher than 7.5%. This may be due to the additional lipids, since Escueta, Bourne, and Hood (1985) reported that 7.7% - 28.8% (adjusted percentage under the same standard) of coconut cream substitution was able to increase the hardness, springiness, cohesiveness of tofu significantly. The mechanism behind why lipids affect the texture of tofu is currently unknown, but it has been observed, and further research on the effect of lipids on tofu networks are needed to better explain this phenomenon.

CFHPT treatments significantly improve the textural properties of rice bran incorporated tofu. Without CFHPT treatment, 2.5% of rice bran is enough to prevent solid tofu from forming. After ultra-high pressure treatment, there is no noticeable difference in texture between 7.5% rice bran tofu and pure soy tofu. The change is in accordance with Préstamo and Arroyo's (2000) study. It is known that High pressure can break down particle sizes, and this phenomenon may allow it to achieve better dispersions for both rice bran and soy protein, thereby improving the tofu texture.

Smaller particle size and better distribution of the rice bran may also lead to a slight increase in the yield. However, when the rice bran level is above 7.5%, too much fiber likely became incorporated in the tofu network, causing even the CFHPT treated tofu samples to fail to maintain proper structure, and resulting in decreasing yields. Another change seen in tofu made from rice bran supplemented soymilks is a decrease in lightness. This decrease is most likely due to the brown color of rice bran, while the darkening effect of high pressure treatments might be due to the temperature increases during high pressure processing. The outlet temperature of soymilk after CFHPT can be as high as 85°C-90°C. Though soymilk is cooled with ice -water bath right after CFHPT, soymilk stays at a high temperature for a short period during which the soy protein may undergo reactions such as Maillard browning, or even pyrolysis. One possibility to reduce this effect would be the addition of an efficient heat exchanger to the outlet of the system, which could greatly shorten the time the product experiences at high temperatures.

5.2.2 Rice Protein Incorporated Tofu

Isolated rice protein powder decreases the hardness of tofu by up to 35% but substantially increases cohesiveness from 0.47 to 0.76. Despite this effect, no differences were found between the different rice protein substitution levels. These similar textural properties indicate that only a few amounts of rice protein powder can be trapped in the tofu gel; the exceeding amount of rice protein was removed with the whey. The low substituted amount may be due to the type of rice protein powder used in this research. This protein powder contains 80% rice protein, and less than 6.6% of fiber and moisture, and also contains a small amount of minerals. In brown rice, the four protein fractions of rice are glutelin (79%–83%), globulin (6%-13%), albumin, and prolamin (Cao, Wen, Li, & Gu, 2009). Albumin is the only water-soluble composition among those four proteins (Shih, 2003). Thus, rice protein powder may work similarly to fiber and decrease the hardness. The increase in cohesiveness can be explained by the creation of a better internal tofu network. 7S and 11S globulins in soy protein are essential for the tofu network (Pre' stamo et al., 2000). The globulin in the rice protein powder may have a similar function to soy globulin and help build a better tofu network.

CFHPT treatments diminish the influence of rice protein powder and make the textural properties closer to pure soy tofu. One possible explanation is that CFHPT treatments denature rice protein in beneficial ways. Guraya and James (2002) report that high pressure treatment of nonglutinous rice slurry will increase the rice protein's solubility, possibly due to the denaturation of the globulin. However, the rice slurry the Guraya study used contained significant starch, and that starch may have influenced the rice protein denaturation caused by high pressure.

Finally, the rice protein incorporated tofu has a darker brownish color, which is mainly due to the deep brown color of the rice protein itself. The unique smell of rice protein powder also carried over into the rice protein tofu, and the combination of those two properties may decrease the consumer acceptance of tofu.

Chapter 6

CONCLUSIONS

6.1 Impacts of Continuous High Pressure Processing on the Texture of Tofu Supplemented with White and Brown Rice

6.1.1 White Rice Milk Incorporated Tofu

Up to 5% of white rice can be incorporated for soybean tofu without having any significant impacts on textural properties (hardness, chewiness, cohesiveness, springiness), color, and water holding capacity. More than 5% of white rice milk substitution decreases the hardness and chewiness significantly. Continuous flow high pressure throttling has no significant improvement on the texture. In general, up to 5% of white rice milk can be substituted for soybean without significant changes in quality. In general, white rice milk is not an ideal choice for nutritional promotion due to its low saturation point and limited nutritional benefits at that level. However, it might be used as a cheap texture modifier in the production of silken tofu.

6.1.2 Brown Rice Milk Incorporated Tofu

Up to 2.5% of brown rice can be incorporated for soybean tofu without any significant impacts on textural properties (hardness, chewiness, cohesiveness, springiness), color, and water holding capacity. Tofu with more than 2.5% of brown rice milk substitution fails to maintain a stable texture. Continuous flow high pressure throttling (CFHPT) significantly improves the texture of tofu. In this research, after CFHPT, up to 5% of brown rice milk can be substituted without significant quality changes. In general, brown rice milk is a better nutritional ingredient compared to white rice milk, and longer CFHPT treatment time may increase the saturation point of brown rice milk. Future studies focus on finding better treating conditions that need to be done.

6.2 Impacts of Continuous High Pressure Processing on the Texture of Tofu Supplemented with Rice Bran and Rice Protein Powder

6.2.1 Rice Bran Incorporated Tofu

Adding rice bran can destroy the tofu network, as even supplementation at the 2.5% level can cause the failure of tofu formation. Continuous flow high pressure throttling of the supplemented milk significantly improves the texture of the resultant tofu. With CFHPT treatment, up to 7.5% of rice bran can be substituted for soybean without significant changes in texture. In short, rice bran may be a promising choice for increasing tofu's nutrition value, especially as it relates to dietary fiber content. For example, the fiber content of 7.5% rice bran tofu is 0.9%, which is three times as much as pure soy tofu. The use of rice bran also utilizes what is primarily a byproduct, providing potential increased sustainability for rice processors.

6.2.2 Rice Protein Incorporated Tofu

Rice protein substitution significantly changes the texture of tofu. The springiness of tofu increases from 0.47 to 0.70. Higher springiness indicates better internal bonding and may correspond to better customer acceptance. However, no difference was found among different substitution levels. Rice protein may not be trapped in the tofu and discarded with the whey. Continuous flow high pressure throttling slightly changes the texture of rice protein supplemented tofu, but the change is Unfavorable, and CFHPT treated tofu has a more erratic texture. The deep color and possibly unpleasant smell of rice protein carry over into the finished tofu, which may limit its utility.

6.2.3 Final Thoughts and Future studies

While this study found several important interactions and trends, fundamental questions remain, and would need to be answered before the industry would adopt either fortification or CFHPT. Among these questions are: How much of the rice ingredients stay in the tofu? Are some fractions more likely to be included, and if so, how do we maximize their positive effects? How will the changes in texture, color, smell among the various treatments and ingredients influence sensory properties and consumer acceptability?

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