International Food Research Journal 28(4): 726 - 736 (August 2021)

Journal homepage: *http://www.ifrj.upm.edu.my*

Physicochemical properties and microstructure of mung bean starch noodles fortified with sea bass (*Lateolabrax japonicus*) actomyosin

[1]Mi, G., [2]Wang, T., [2]Li, J. R., [2]Li, X. P. and [1]*Xie, J.

[1]*College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China*
[2]*College of Food Science and Project Engineering, National R＆D Branch Centre of Surimi and Surimi Products Processing, College of Food Science and Engineering, Bohai University, Jinzhou 121013, China*

Article history

Received: 15 September 2020
Received in revised form:
16 December 2020
Accepted:
22 January 2021

Keywords

fish actomyosin,
gel network,
mung bean starch,
steaming noodles,
phase separation

Abstract

It is important to develop protein-fortified starch noodles that are both high in nutrition value and good quality. Fish protein is an ideal additive to starch noodles; but, relevant research on it is rather scarce. Noodles comprising mung bean starch and fish actomyosin at different mixing ratios (10:0, 9:1, 8:2, 7:3, 6:4, and 5:5) were prepared. The cooking quality, extension property, texture profile, moisture distribution, and microstructure of the resulting noodles were then investigated. With an increase in protein levels, the transparency of noodles significantly decreased from 15.07 to 8.21, while the whiteness and springiness significantly increased from 78.13 to 88.55, and from 0.81 to 0.96, respectively. Moreover, a higher protein mixing ratio resulted in noodles with low firmness, higher tensile strength, and decreased water solubility. Water distribution analysis indicated that the addition of protein significantly increased the amount of water that was trapped in the protein network. Using microstructure analysis, phase separation of protein and starch were observed in all noodles. Lastly, a sensory evaluation was performed, and noodles prepared with higher protein addition amount were determined to have better quality. An increase in protein content led to a sticky mouthfeel when teasing. Taken together, 6:4 was thought to be the most suitable mixing ratio for making actomyosin-mung bean starch blended noodles, and can be well applied in noodle production.

Introduction

Consuming starch noodles is one of the most distinguished and established cultures around Asia. There are many varieties of starch noodles, and they are known differently in different countries such as *fensi* in China, *dang myun* in Korea, *harusame* in Japan, *wún-sên* in Thailand, and *bihon* in the Philippines (Tam *et al.,* 2004; Tan *et al.,* 2009). Mung bean starch is considered as the best raw material for noodle making, and the need for it has gradually increased worldwide. Mung bean starch has the highest proportion of amylose-amylopectin of all legume starches; this makes it being preferred over other starches, and imparts desirable attributes such as transparent appearance, fine thread-like structure, high tensile strength, and low cooking loss to noodles (Kaur *et al.,* 2015; Thapnak *et al.,* 2019). Mung bean starch also contains high amount of "mung bean resistant starch", which is widely used as raw material in the modern food industry for the development of new functional foods (Zhang *et al.,* 2019). However, the production of mung bean starch is limited, and it is more expensive as compared to other starches. This makes it difficult to meet the market needs and bridge the gap. Therefore, it is worthwhile to look for substitutes of mung bean to partly reduce the production costs.

Hydrocolloids are the most commonly used and frequently studied food additives. Most hydrocolloids have excellent water-holding capacity and strong gelling abilities at room temperature (around 25°C). They are useful in enhancing the texture and rheology of starch noodles (Lee *et al.,* 2002; Funami *et al.,* 2005; Silva *et al.,* 2013). In addition, chitosan, glycerol monostearate, and other modified starches are also among the good choices in noodle making (Kaur *et al.,* 2005; Saito *et al.,* 2019). However, it has been reported that the use of these compounds has led consumers to perceive such products as "artificial foods", thus rendering them less desirable (Marti and Pagani, 2013). Therefore, the use of natural proteins as additives is a more suitable alternative, which explains the use of egg-white powder as an additive instead in commercial noodles.

The addition of protein to starch noodles has two other benefits. Firstly, studies have shown that

*Corresponding author.
Email: jxie@shou.edu.cn

DOI:
https://doi.org/10.47836/ifrj.28.4.09

the starch in mung bean is more slowly-digestible starch (SDS) variant than that present in other legumes (Sandhu and Lim, 2008). Moreover, the extent of hydrolysis of retrograded starch noodles was lower than the fresh ones (Hoover and Zhou, 2003; Wang *et al.,* 2015). However, starch is a polysaccharide which rapidly breaks down into large amounts of glucose upon consumption. Therefore, it is unsuitable for individuals with diabetes. The addition of protein can effectively reduce the relative levels of starch. Moreover, some studies (Desai *et al.,* 2019) have shown that protein addition or increasing the content of resistant starch (RS) in foods could effectively delay the release of glucose. Secondly, from the nutritional point of view, starch noodles are inferior owing to the lack of protein, micronutrients, vitamins, and other nutrients; therefore, fortification with protein can make up for this deficit.

The most common proteins used as additives in starch noodles are soybean, egg-white, and whey protein. Studies show that protein levels have a significant effect on the quality of starch noodles (Menon *et al.,* 2016; Phongthai *et al.,* 2017; Rachman *et al.,* 2019). To the best of our knowledge, there are only a few studies that have explored the effect of fish protein on the quality of mung bean starch noodles. The preparation process of traditional starch noodles is as follows: starch dough is extruded using an extruder or dropped by gravity using a stainless-steel cylinder with several perforations at its bottom. Next, the raw starch noodles are directly dropped into hot water (90 - 100°C), and cooked. One of the drawbacks of this process is that the cooking loss of starch noodles is high; consequently, a part of noodles dissolves in water during cooking. Steaming is another method to prepare noodles. The processing and consumption of steamed noodles is a tradition with a long history in Asia (Fu, 2008). There are several foods made from steamed starch in China such as (1) *shuijin pi*, a steamed preparation made from wheat starch or sweet potato starch, transparent, and used as dumpling wrapping, and (2) *liang pi*, another type of steamed food made using wheat starch, and used for making cold dishes. However, there are few studies that discuss the steaming of mung bean starch noodles.

In the present work, actomyosin from fish was mixed with mung bean starch at different ratios, and then the blend doughs were made into noodles using the steaming method. The quality change and mechanism of noodle formation were the main research objectives.

Materials and methods

Materials

Fresh sea bass (*Lateolabrax japonicus*) was purchased from a local aquatic product market. The white muscle on the back was cut and collected (yield was about 30%, w/w), and then rinsed twice using cold deionised water. After that, they were stored in a -80°C freezer until further usage. Mung bean starch was kindly provided by Fu Qiao Starch Co., Ltd. (Hebei, China).

Preparation of actomyosin paste

Actomyosin was prepared following the method of Donald and Lanier (1994) with some modifications. Sea bass muscle (50 g) was minced for 2 min using a meat chopper in 500 mL extraction solution (0.6 M KCl, 4°C), then homogenised for 4 min at 10,000 rpm using a homogeniser (FJ300-S, Shanghai, China). The beaker containing the sample was placed on ice, blended for 20 s, followed by 20 s rest interval to avoid overheating during extraction. The extract was centrifuged at 5,000 *g* for 30 min at 4°C. Three volumes of chilled deionised water were added to precipitate the actomyosin. Actomyosin was collected by centrifuging at 5,000 *g* for 20 min at 4°C, and the pellet was dissolved by stirring for 30 min at 4°C in an equal volume of chilled 1.2 M KCl at pH 7.0. Undissolved material was removed from the preparation by centrifugation at 5,000 *g* for 20 min at 4°C. Then, the actomyosin was collected by precipitating with three volumes of chilled deionised water and centrifugation at 5,000 *g* for 20 min at 4°C. The content of protein was measured as 60.54 ± 0.41 mg/mL (approximately 6%) according to the Biuret method (Layene, 1957).

Preparation of raw actomyosin-starch dough and steamed noodles

Starch dough was prepared following the method of Wang *et al.* (2018) with some modifications combined with the traditional handmade procedure from China. Thirty-gram dry starch was added with 70 g deionised water, and stirred for 2 min with a rod, then 200 g boiling water was slowly poured while stirring. Later, 300 g dry starch was added and mixed with a blender (ZG-LZ906, Chigo, Zhejiang, China) at 100 rpm for 5 min to form a starch dough. After that, raw actomyosin-starch dough of five different mixing ratios (9:1, 8:2, 7:3, 6:4, and 5:5) were prepared separately by adding actomyosin to starch dough (w/w) and mixed with the blender at 100 rpm for 10 min.

All the dough were extruded in a flat-bottomed steel plate separately using an electric noodle extruder (HX-DMJ-01, Junxifu, Shanxi, China) to form raw noodles with a diameter of about 3 mm, and then they were put into a steamer and steamed for 15 min. Later, the cooked noodle were removed into cold water for 10 min, and drained for 5 min. The noodles of different mixing ratios from 9:1 to 5:5 were marked sequentially as R_1 ~ R_5. The starch noodles without adding actomyosin served as the control, and marked as R_0. All samples were stored in refrigerator at 4°C for 4 h before testing.

Cooking quality

The swelling index (SI) of steamed noodles was determined following the method of Rachman *et al.* (2019). One-hundred-gram dough was made into steamed noodles, weighed (recorded as W_c, g), then dried at 105°C until a constant weight (recorded as W_d, g). The SI was calculated using Eq. 1:

$$SI = \frac{Wc - Wd}{Wd} \quad \text{(Eq. 1)}$$

The water solubility (WS) was determined by the following procedure: 20 strands of steamed noodles (approximately 100 g) were weighed (W_m), put in cold water, and refrigerated at 4°C. Every 4 h, the noodles were stirred gently for 1 min, taken out, drained for 15 min, and weighed (W_m). The WS was calculated using Eq. 2:

$$WS = \frac{Wm - Wn}{Wm} \quad \text{(Eq. 2)}$$

The transparency was determined following the method of Li *et al.* (2010). Thin sheet of noodle of 4 mm wide and 1 mm thick was extruded using the extruder equipped with a narrow mouth mould, and steamed as described earlier. A single strand of noodle was cut into 4 cm long and put into 1 cm cuvette, and then the transmittance was detected using a spectrophotometer (UV-2550, Shimadzu, Japan) at 690 nm.

The whiteness (W) is measured using a tristimulus colour analyser (CR-400, Konica Minolta, Japan). The $L*$ (brightness), $a*$ (redness), and $b*$ (yellowness) were recorded to calculate the W using Eq. 3:

$$W = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2} \quad \text{(Eq. 3)}$$

Extension and textural profile analysis (TPA)

A texture analyser (TA-XT plus, TA, Godalming, UK) with a 5 kg force transducer was used. The extension was determined following the method of Chen *et al.* (2002); one strand of noodle was wrapped with tape at both ends to prevent breaking from the edges, and then fixed using a clamp probe (CA/SPR, TA, UK). The test mode was tension, and the test speed was 0.1 mm/s. The extension modulus (E) and the relative extension (r_e) were calculated using Eq. 4 and Eq. 5:

$$E = (F/\Delta L)(L/A) \quad \text{(Eq. 4)}$$

$$r_e = \Delta L/L \quad \text{(Eq. 5)}$$

where, F = extension force, and A = cross-sectional area of the starch noodle, ΔL = increased length, and L = original length of starch noodle.

Five strands of noodles were cut into 4 cm long, placed on the test platform parallelly, and a cylindrical probe (model: P50) was used to perform the TPA with the following setting: pre-test speed, 1 mm/s; post-test speed, 5 mm/s; target mode, 75% strain; trigger mode, auto with a 5.0 g force; test speed, 0.5 mm/s; and interval time between the two simulated chewing, 5 s.

Moisture distribution testing

One gram of noodles was accurately weighed and placed into a flat-bottom glass tube. Later, the tube was placed into the spectrometer (Niumag, Shanghai, China) to carry out proton relaxation studies. The resonance frequency was 23 MHz, and Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences were employed to measure the spin-spin relaxation time (T_2). The main parameters of the pulse were: frequency offset, 911.6; receiver gain 1, 20; receiver gain 2, 3; 90° pulse, 14.5 µs; 180° pulse, 29 µs; dwell time, 3500 ms; recycle time, 150 µs; echo count, 5,000; scan repetitions, 32; and intensity of signal acquisition, 150016. The software T_invfit which integrated a step-by-step iterative algorithm was used to calculate the T_2 value.

Microstructure

Thin sheet noodles were made following the method mentioned in "Cooking quality". Briefly, 1 cm long noodle strand was cut and stuck to the sample holder using a glue. Later, the samples were frozen and sliced into 15 µm sheets using a frozen slicer (CM1850, Leica, Germany), and then put on the slide and stained with 1/5 concentration of Lugol solution (I_2 = 0.33%, KI = 0.67%, w/v) for 1 min, and Fast Green (0.1%, w/v) for 1 min. Following this, the samples were rinsed with ironless water for 1 min, covered with glass, and observed under a 100× optical microscope (80i, Nikon, Japan).

Sensory evaluation

The steamed noodles were evaluated by a group of seven people, and given a score based on the seven sensory attributes: aroma (smell), surface quality (vision), softness (touch), stickiness (touch), chewiness (mouthfeel), and smoothness (mouthfeel). Each attribute was given three levels: good, fair, and poor with a score of 3, 2, and 1. Next, 50 g noodles of different mixing ratios were coded and offered randomly in batch to the panellists. After each evaluation, they were instructed to rinse their mouth with warm water twice. All tests were performed under the same lighting conditions.

Statistical analysis

All tests were performed in triplicate unless specified, and the mean scores were taken as the result. The data were evaluated by an analysis of variance (ANOVA), and a comparison of means was carried out with Duncan's test. Differences were considered significant at $p < 0.05$. Statistical computation and analysis were conducted using SPSS (18.0).

Results and discussion

Effect of actomyosin on the cooking qualities of starch noodles

Cooking qualities of the steamed noodles were analysed by measuring WS, SI, transparency, and whiteness,. The results are listed in Table 1. All dough variants made using different mixing ratios (R_1 to R_5) could produce steamed noodles with acceptable qualities including firmness, colour, and flexibility. Therefore, the preparation of steamed noodles by adding actomyosin to mung bean starch was proven to be successful. Previous studies have shown feasibility to improve the quality of cooked starch noodles by the addition of egg or soy protein (Limroongreungrat and Huang, 2007).

Transparency is an important parameter in evaluating noodles made of pure starch. It varies greatly between preparations owing to the intrinsic properties of different starches. Generally, it is considered that the transparency of noodles made from starch paste obtained from potatoes is better than those obtained from beans and corn (Singh *et al.,* 2003; Mishra and Rai, 2006; Jinzhe, 2011). There is not yet adequate knowledge regarding the effect of fish protein on the transparency and whiteness of starch noodles. Based on our results, the addition of protein significantly reduced the transparency of noodles but increased the whiteness as compared to the control. In R^5, the transparency decreased to a minimum of 8.2%, while the whiteness increased to a maximum of 88.55. This phenomenon was most likely related to the gel-like network structure of noodles. Previous studies have shown that the crosslinking degree of starch molecules is negatively correlated with transparency (Yamamori, 2009). We speculated that in the sample prepared using a low protein mixing ratio (R^1), the gel network was mainly formed by starch, while the protein molecules were scattered within the network; thus, the transparency levels were similar to that observed in the control. As the level of protein increased, a protein network gradually formed and dominated. Consequently, the noodles gradually lost their transparency, and turned whiter. We have confirmed the accuracy of our speculation in subsequent microstructural experiments.

When the ratios R^1 to R^5 were used, the SI

Table 1. Cooking properties of steamed noodles.

Sample	SI (g/g, water/dry noodle)	WS (g/100 g)	Whiteness	Transparency (%)
R_0	3.17 ± 0.07^a	7.09 ± 0.07^b	74.09 ± 0.67^f	15.79 ± 0.37^a
R_1	2.93 ± 0.04^b	8.58 ± 0.03^a	78.13 ± 1.06^e	15.07 ± 0.42^a
R_2	2.77 ± 0.11^c	7.12 ± 0.05^b	82.09 ± 0.61^d	13.13 ± 0.39^b
R_3	2.54 ± 0.07^d	7.09 ± 0.05^b	85.33 ± 0.37^c	11.09 ± 0.15^c
R_4	2.43 ± 0.04^d	6.59 ± 0.09^c	87.46 ± 0.59^b	10.59 ± 0.23^d
R_5	2.30 ± 0.05^e	5.51 ± 0.04^d	88.55 ± 0.33^a	8.21 ± 0.38^e

R_1 - R_5 = steamed noodles prepared form actomyosin and mung bean starch with mixing ratio from 9:1 to 5:5; and R_0 = steamed noodles prepared from mung bean starch (control). Values are mean ± standard deviation of triplicate measurements (n = 3). SI = swelling index; WS = water solubility. Different letters in each column indicated significant differences ($p < 0.05$).

of cooked noodles gradually decreased from 3.17 to 2.30. The reduction in SI was probably due to the formation of protein network in the noodles, thus resulting in water being trapped within the network and limiting the supply of available water for the swelling of starch. A similar tendency was observed by Desai *et al.* (2019) when they investigated the SI effect on fish powder to pasta, and found that the SI significantly decreased from 2.29 to 1.95 as the level of fish powder increased. On the other hand, the SI of the control was 3.17 ± 0.07. Chen *et al.* (2002) reported that the SI of mung bean starch noodles made with boiling method ranged from 3.5 - 6.5 g/g. It was obvious that the SI of our steamed noodles was significantly lower than that value. Cooking with boiling water resulted in the water molecules coming in direct contact with the noodles and enabling their absorption. Therefore, it appeared that steaming was a useful technique to limit water absorption and reduce noodle swelling. This view was also supported by the results reported by Luo *et al.* (2015).

Water solubility is an important parameter to be considered during noodle preparation. The ideal batch of noodles should stay firm and not breakdown even after it is soaked in water for prolonged periods. Our results showed that samples with a high starch ratio had a higher WS value, and as the level of protein increased, the water solubility decreased. We attributed this to the fact that actomyosin was likely having a coating effect on starch, thereby reducing its contact with water.

Texture analysis

The TPA test, also known as twice-bite test, consists of a probe which simulates human teeth, and compresses the food twice. The result shows two force peaks over a function of time. The bite characteristics of food including firmness and springiness can be quantitatively evaluated by the calculation of peak height and area. The TPA test results are shown in Table 2. The brittleness data of noodles are not shown because there were no obvious fracture peaks in the left shoulder for the first main compression peak of all samples, thus indicating that there was no breaking occurred during the compression process. Thus, the texture of all noodles was satisfactory although different mixing ratios were used.

The firmness of noodles from R_1 to R_5 significantly decreased from 8,403.3 to 3,314.6 g, thus indicating that the addition of actomyosin conferred more softness to the noodles than that observed in the control. Similar results were reported by Menon *et al.* (2016) who investigated the effects of whey protein concentrates on noodles made using sweet potato starch, and found that the firmness decreased from 206 to 190 N as the protein mixing ratio increased from 10 to 30%. Chen *et al.* (2002) and Fan *et al.* (2017) also reported a similar tendency. This reason could likely be attributed to the higher water capacity of the protein network in samples like R_5, with a higher mixing ratio.

Springiness is a textural attribute related to the rapidity and degree of recovery from a deforming force (Monaco *et al.,* 2008). Both springiness and cohesiveness showed an initial trend of decline followed by a rise. R_3 showed the lowest values of 0.76 and 0.70 for springiness and cohesiveness, respectively. This could be due to the weak protein and starch networks at this ratio. The springiness of noodles prepared using R_5 was higher than that obtained using R_1, and even that of the control. This can be explained by the fact that the gel elasticity of fish protein is generally higher than that of starch.

Table 2. Texture profile analysis and extension results of steamed noodles.

Sample	Texture attribute					Extension	
	Firmness (g)	Springiness	Cohesiveness	Resilience	Adhesiveness (g.sec)	E (g/mm^2)	r_e
R_0	8,907.8 ± 141.4^a	0.89 ± 0.04^b	0.87 ± 0.02^a	0.64 ± 0.03^a	-2.0 ± 0.7^a	1.43 ± 1.5^a	1.7 ± 0.2^a
R_1	8,403.3 ± 197.8^b	0.81 ± 0.01^c	0.81 ± 0.03^b	0.63 ± 0.02^a	-5.0 ± 0.9^b	1.25 ± 1.3^a	2.1 ± 0.2^b
R_2	7,335.2 ± 144.3^c	0.87 ± 0.04^b	0.75 ± 0.02^c	0.51 ± 0.04^b	-7.0 ± 1.0^c	1.07 ± 1.1^a	2.9 ± 0.3^c
R_3	5,276.2 ± 201.4^d	0.76 ± 0.02^d	0.70 ± 0.02^d	0.45 ± 0.03^b	-10.0 ± 1.1^d	8.9 ± 0.5^b	3.5 ± 0.3^d
R_4	4,430.3 ± 159.9^e	0.85 ± 0.02^b	0.83 ± 0.04^{ab}	0.47 ± 0.03^b	-18.4 ± 1.5^e	7.2 ± 0.6^c	4.0 ± 0.1^e
R_5	3,314.6 ± 97.9^f	0.96 ± 0.02^a	0.85 ± 0.06^{ab}	0.36 ± 0.02^c	-26.3 ± 2.2^f	3.6 ± 0.8^d	4.3 ± 0.2^f

R_1 - R_5 = steamed noodles prepared form actomyosin and mung bean starch with mixing ratio from 9:1 to 5:5; and R_0 = steamed noodles prepared from mung bean starch (control). Values are mean ± standard deviation of triplicate measurements (n = 3). Different lowercase superscripts in each column indicate significant differences ($p < 0.05$).

Resilience represents spring-back ability and the ratio of elastic energy released by the sample during the first compression cycle to the energy consumption of the probe during compression. Results showed that the resilience value of the control was the highest. The addition of proteins resulted in an obvious decline in resilience values from 0.64 to 0.36. Joshi *et al.* (2014) researched the resilience of lentil starch-lentil protein composite pastes, and obtained similar results.

All samples analysed in the present work had a certain value of adhesiveness. Results showed an obvious increasing tendency although this increase was not statistically significant ($p > 0.05$). Higher values indicated that a high ratio of protein addition might have caused an adhesion feeling on teeth during noodle consumption.

Extension modulus (E) represents the stretch firmness of a substrate, while relative extension (r_e) is a measure of the extent of stretchability of s substrate. Table 2 shows that E exhibited a significantly declining tendency, while r_e exhibited the opposite. This indicates that noodles with a high ratio of protein were firm but easy to break, while noodles with lower protein ratios (R_4 and R_5) could pull up to at least four times their original length although their tensile strengths were relatively poor. These findings can be attributed to the protein network density of the noodles.

Moisture distribution analysis

The moisture distribution in the starch-actomyosin noodles was analysed to further explore the mechanism of the influence of actomyosin on their cooking quality and texture. The continuous distribution of the spin-spin relaxation time of the noodles prepared using different mixing ratios is shown in Figure 1. Four typical peaks were observed in all samples except in the control (T_{24} was not observed). These peaks were T_{21}, T_{22}, T_{23}, and T_{24}, and their corresponding areas were A_{21}, A_{22}, A_{23}, and A_{24}, respectively.

T_{21}, T_{22}, T_{23}, and T_{24} appeared at around 1, 10, 100, and 400 ms. This result is similar to those of previous studies involving a system of starch and fish-protein blends (Bertram *et al.,* 2002; Sun *et al.,* 2014). Generally, T_{21} represents the water linked to the polar groups on the surface of proteins, starch by hydrogen bonds, and proton that is located on the inherent structure of molecules. T_{22} refers to the water that is linked to the amide group of proteins and hydroxyl group of starch through weak hydrogen bonds. Since bond energies are relatively low, T_{22} is usually known as semi-bound water. T_{23} represents water retained by the network structure of protein or starch, while T_{24} represents water that can move freely outside the structure of the noodle network.

Table 3 shows the peak areas (A_{21} to A_{24}) which represent water content. It is apparent A_{21}

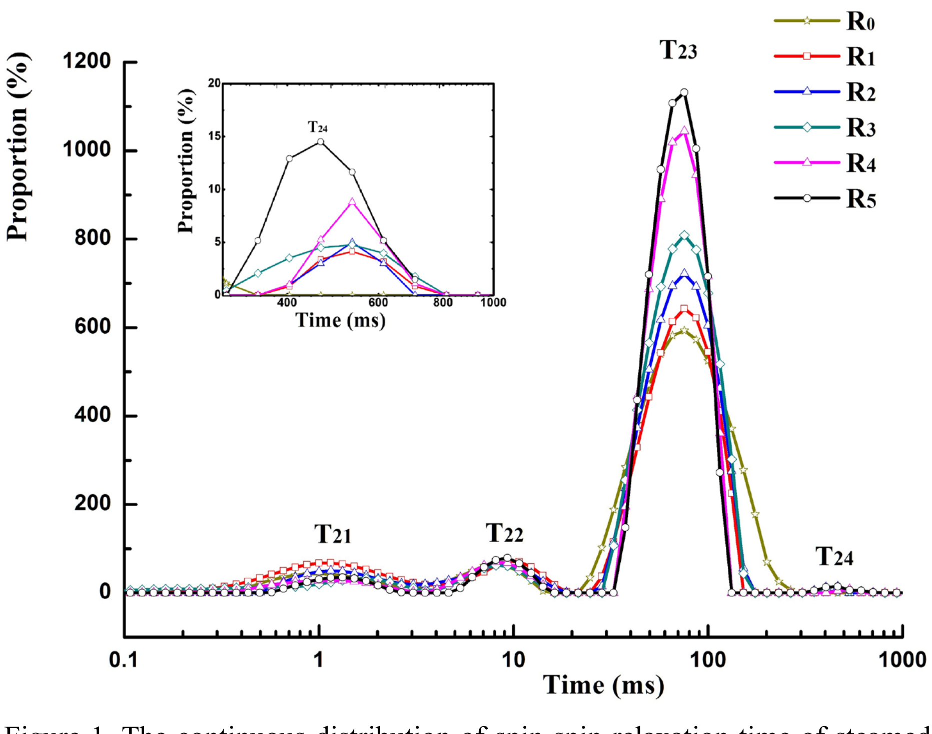

Figure 1. The continuous distribution of spin-spin relaxation time of steamed noodles. R_1 - R_5 = steamed noodles prepared form actomyosin and mung bean starch with mixing ratio from 9:1 to 5:5; and R_0 = steamed noodles prepared from mung bean starch (control). The insert is the T_{24} at 20× magnification.

showed a tendency of decline as compared to the control. Generally, starches and proteins have different water-binding capacities. When heated, starch granules gelatinise and bind more water molecules on its surface than protein (Bushuk and Winkler, 1957). In the present work, as the protein mixing ratio increased, the relative amount of added starch decreased which resulted in a significant decrease in A_{21}. With regard to A_{22}, there were no significant differences between groups; therefore, we speculated that it might be due to the fact that the number of water-binding sites of mung bean starch and fish actomyosin were similar at a given weight. However, this hypothesis needs to be further confirmed in subsequent studies.

Table 3. Integral results of the four peaks in the water distribution.

Sample	A_{21}	A_{22}	A_{23}	A_{24}
R_0	444.89	419.18	5,649.76	0
R_1	477.04	453.35	4,741.53	8.05
R_2	446.53	429.80	5,369.46	8.11
R_3	410.34	454.00	5,937.81	14.05
R_4	299.17	422.11	6,301.64	14.86
R_5	238.06	479.85	6,492.65	32.18

R_1 - R_5 = steamed noodles prepared form actomyosin and mung bean starch with mixing ratio from 9:1 to 5:5; and R_0 = steamed noodles prepared from mung bean starch (control).

With an increase in actomyosin levels, the A_{23} of the noodles exhibited an interesting tendency where it first decreased and then increased from 4,741.53 to 6,492.65. Both protein and starch bind to water molecules through a gel network (Xu *et al.,* 2020). The reasons for this phenomenon are as follows: In the control, amylose leached from the starch granules during heating, which was followed by the formation of a double helix that was intertwined during cooling. This process resulted in a continuous phase with a three-dimensional network structure with some water trapped in the network (Ozel *et al.,* 2017; Matignon and Tecante, 2017). With regard to R_1, protein addition was likely to break the continuity of the starch network; however, at the instance when the protein network was not formed, water could escape from the matrix. With an increase in the proportion of actomyosin, the starch network weakened, and the protein network formed gradually with increasing levels of water being trapped within this network. This speculation has been confirmed experimentally using subsequent microstructural observations. Furthermore, the tendency of A_{23} provides a reasonable explanation for SI as earlier discussed.

Although A_{24} constituted a very small proportion of the whole water distribution, we found an interesting phenomenon upon zooming in. A_{24} exhibited a tendency similar to that of A_{23}. For the control, no T_{24} was observed. We hypothesised that this had something to do with the fact that the pure starch noodles had a very smooth surface. As the ratio increased from R_1 to R_5, the noodle surface appeared sticky, most likely because it combined with some water that could flow freely.

Microstructure of noodles

Microstructure photographs of R_1 - R_5 and the control, R_0, are shown in Figure 2. These findings provided a further objective approach to understand the mechanism of how actomyosin affected the quality of steamed noodles. Actomyosin was dyed green using Fast Green, and the starch was dyed light purple (amylose) or dark purple (amylopectin) using iodine owing to the difference in binding abilities of the two starch components.

For the control, the light purple substances were found to be evenly distributed in the dark purple spots, thus indicating that most of the amylose leached from the starch granules. These findings also suggested that the starchy dough gelatinised completely when steamed. When protein was added to the starch noodles, we observed an interesting phase-separation process between actomyosin and the mung bean starch. In R_1, it was obvious that starch formed continuous phase, while protein constituted dispersed phase. In R_2, the amount of dispersed protein increased significantly but had not formed a network. In R_3, a bi-continuous phase of starch and protein was formed, and an interpenetrating network of actomyosin and mung bean starch was observed. In R_4, the continuity of the protein network increased gradually, and the starch network broke down. In R_5, the protein network appeared to be dominant with the starch dispersed within it. With the gradual formation of protein network, the water retention ability of noodles was enhanced gradually. Our results corresponded to those from the previous water-distribution and texture-analysis studies.

Starch is a typical polysaccharide. Proteins and polysaccharides are macromolecules that are abundant in food. Generally, protein-polysaccharide mixtures are thermodynamically incompatible at

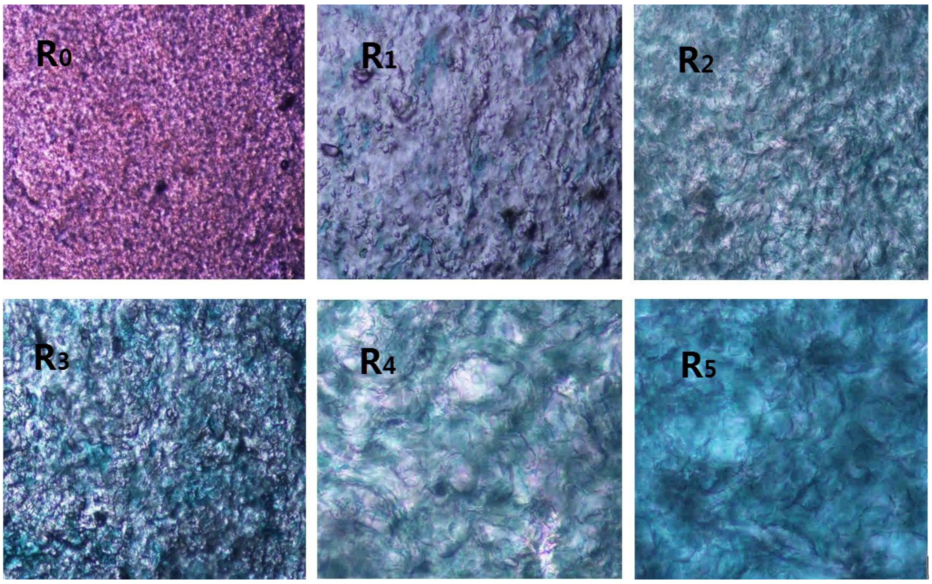

Figure 2. Microstructure of noodles. R_1 - R_5 = steamed noodles prepared form actomyosin and mung bean starch with mixing ratio from 9:1 to 5:5; and R_0 = steamed noodles prepared from mung bean starch (control). Starch was dyed purple, and protein was dyed green.

neutral pH, which causes phase separation and the formation of a two-phase system (Le *et al.,* 2017). In the case of mung bean starch and actomyosin, a gel network was formed independently when heating. This resulted in a common state in which one phase was continuous, while the other was dispersed. When the mixing ratio is suitable, a bi-continuous phase will be formed. Schorsch *et al.* (1999) reported a similar phase separation of micellar casein and locust bean gum blends in which they stated that different mixing ratios led to different continuous appearances. Fan *et al.* (2017) reported that when the mixing ratio of fish myofibrillar protein and cassava starch was 0.5, an interpenetrating network was formed.

Sensory evaluation

During extrusion, it was found that the raw noodles made from a higher mixed ratio (*e.g.,* 9:1) appeared straighter than those made using low ratios. As the ratio of actomyosin in the mixture increased, the curling of the raw noodles increased, thus rendering them stickier. Visualisation of all steamed noodles are shown in Figure 3.

All five groups of noodles with added actomyosin as well as the control were evaluated for smell, appearance, touch, and mouthfeel using sensory evaluation (Table 4). For the sake of comparison, a total score was given at the end. The single score of the control was better as compared to

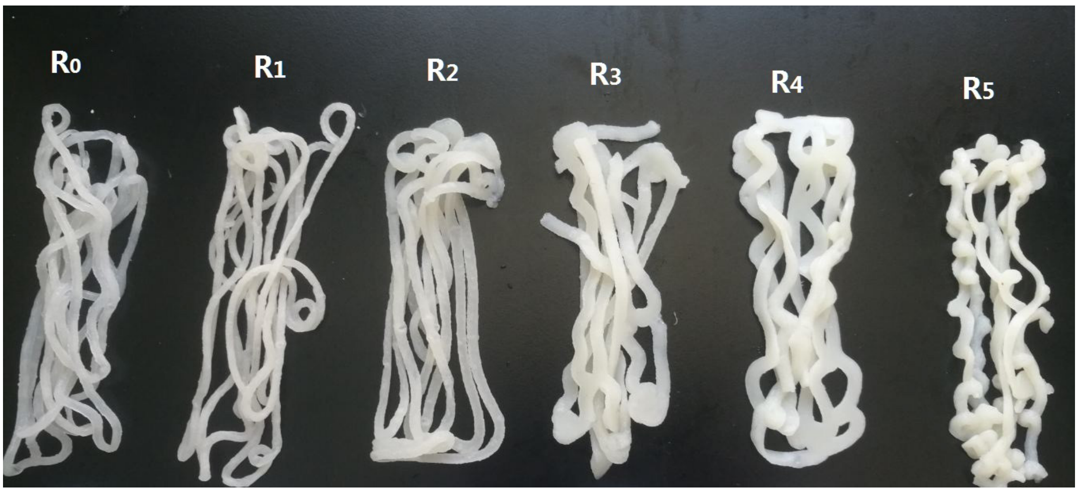

Figure 3. Visualisation of steamed noodles. R_1 - R_5 = steamed noodles prepared form actomyosin and mung bean starch with mixing ratio from 9:1 to 5:5, and R_0 = steamed noodles prepared from mung bean starch (control).

Table 4. Sensory scores of steamed noodles.

Sensory attribute	Score					
	R_0	R_1	R_2	R_3	R_4	R_5
Aroma (smell)	1.00	1.14	1.86	2.71	2.86	2.86
Surface smoothness (vision)	3.00	1.14	1.57	2.14	3.00	2.86
Softness (touch)	1.71	1.57	2.00	2.51	2.86	3.00
Stickiness (touch)	3.00	3.00	2.86	1.86	2.00	1.56
Chewiness (mouthfeel)	2.43	2.86	2.00	2.14	2.71	2.51
Smoothness (mouthfeel)	2.71	1.14	1.71	2.71	2.86	2.86
Total score	13.85	10.85	12	14.07	16.29	15.65

R_1 - R_5 = steamed noodles prepared form actomyosin and mung bean starch with mixing ratio from 9:1 to 5:5; and R_0 = steamed noodles prepared from mung bean starch (control).

those of R_1 to R_5; however, the drawback was regarding the lack of fish protein aroma. R_4 had the highest score, thus indicating that the 6:4 mixing ratio was optimal for noodle making. Softness was one of the characteristic properties of noodles considered in the present work. Pure starch noodles tend to easily harden due to retrogradation. It was found that starch noodles tended to get softer in texture with the addition of protein. This finding highlighted the potential of fish protein in improving noodle quality. Limroongreungrat and Huang (2007) investigated the effect of soy protein in noodles containing sweet potato starch, and found similar results. The firmness of noodles decreased from 1.0 to 0.4 N when the protein content increased from 15 to 45%, respectively.

TPA results indicated that all samples containing actomyosin exhibited a certain adhesiveness. This made us worry that the taste would be compromised. During the actual sensory evaluation, it was found that adhesiveness had little effect on mouthfeel except in the case of R_5, which had a sticky feel, thus lowering the score.

Conclusions

The present work established the possibility of producing steamed noodles using fish actomyosin and mung bean starch. When the mixing ratio of actomyosin and starch was 6:4, the quality of the steamed noodles was proven to be optimal. With an increase in the added protein, noodle whiteness increased from 78.13 to 88.55, springiness increased from 0.81 to 0.85, and firmness decreased from 8403.3 to 3314.6. Furthermore, the addition of fish protein improved the surface smoothness and tensile properties as well as the resistance of noodles to water solubility. High protein content (as in sample R_5) made the noodles sticky. Additionally, the noodles appeared curly, irrespective of whether they were raw or cooked, which led to a lower sensory score. Results from the water-distribution study showed four peaks of T_2 spin-spin relaxation time for all steamed noodles (T_{24} was not observed in R_0), and their areas varied significantly; A_{21} showed a tendency of decline; A_{22} showed no significant differences between groups; A_{23} first decreased, and then increased from 4,741.53 to 6,492.65; and A_{24} had a tendency similar to that of A23, although it took a relatively small proportion of the water distribution. Microstructure photographs revealed that there was an obvious phase separation of actomyosin and mung bean starch in the prepared noodles. In R_1, starch was in the continuous phase, while protein was in the dispersed phase; in R_3, a bi-continuous phase structure was observed; in R_5, actomyosin was in the continuous phase, while mung bean starch was in the dispersed phase. Combined with the application in actual production, the ideal noodles should have both good processing quality and mouthfeel characters. Based on the results obtained, the noodles made from actomyosin and mung bean starch at a mixing ratio of 6:4 met the demand, and were concluded to be the best. However, actomyosin-mung bean starch noodles formulated in the present work still require further investigation on certain aspects such as their digestive properties, rheological characteristics, and interaction of actomyosin and starch.

Acknowledgement

The present work was financially supported by the National Natural Science Foundation of China (31972107 and U20A2067), and Liaoning Revitalisation Talents Program (XLYC 1907040). The authors acknowledge Profs. Shumin Yi and Zhipeng Yu for their help with the latest version of the manuscript.

References

Bertram, H. C., Donstrup, S., Karlsson, A. and Andersen, H. J. 2002. Continuous distribution analysis of T_2 relaxation in meat—an approach in the determination of water-holding capacity. Meat Science 60(3): 279-285.

Bushuk, W. and Winkler, C. A. 1957. Sorption of water vapor on wheat flour, starch, and gluten. Cereal Chemistry 34(2): 73-86.

Chen, Z., Sagis, L. M., Legger, A., Linssen, J. P., Schols, H. A. and Voragen, A. G. 2002. Evaluation of starch noodles made from three typical Chinese sweet-potato starches. Journal of Food Science 67(9): 3342-3347.

Desai, A. S., Brennan, M. A. and Brennan, C. S. 2019. Influence of semolina replacement with salmon (*Oncorhynchus tschawytscha*) powder on the physicochemical attributes of fresh pasta. International Journal of Food Science and Technology 54(5): 1497-1505.

Donald, G. A. and Lanier, T. C. 1994. Actomyosin stabilization to freeze-thaw and heat denaturation by lactate salts. Journal of Food Science 59(1): 101-105.

Fan, M., Hu, T., Zhao, S., Xiong, S., Xie, J. and Huang, Q. 2017. Gel characteristics and microstructure of fish myofibrillar protein-cassava starch composites. Food Chemistry 218: 221-230.

Fu, B. X. 2008. Asian noodles: history, classification, raw materials, and processing. Food Research International 41(9): 888-902.

Funami, T., Kataoka, Y., Omoto, T., Goto, Y., Asai, I. and Nishinari, K. 2005. Effects of non-ionic polysaccharides on the gelatinization and retrogradation behavior of wheat starch. Food Hydrocolloids 19(1): 1-13.

Hoover, R. and Zhou, Y. 2003. *In vitro* and *in vivo* hydrolysis of legume starches by α-amylase and resistant starch formation in legumes—a review. Carbohydrate Polymers 54(4): 401-417.

Jinzhe, H. 2011. Physicochemical properties of 10 plant starch pastes. Journal of the Chinese Cereals and Oils Association 26(4): 37-41.

Joshi, M., Aldred, P., Panozzo, J., Kasapis, S. and Adhikari, B. 2014. Rheological and microstructural characteristics of lentil starch-lentil protein composite pastes and gels. Food Hydrocolloids 35(3): 226-237.

Kaur, A., Shevkani, K., Singh, N., Sharma, P. and Kaur, S. 2015. Effect of guar gum and xanthan gum on pasting and noodle-making properties of potato, corn and mung bean starches. Journal of Food Science and Technology 52(12): 8113-8121.

Kaur, L., Singh, J. and Singh, N. 2005. Effect of glycerol monostearate on the physico-chemical, thermal, rheological and noodle making properties of corn and potato starches. Food Hydrocolloids 19(5): 839-849.

Layene, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. Methods in Enzymology 10(73): 447-454.

Le, X. T., Rioux, L. and Turgeon, S. L. 2017. Formation and functional properties of protein-polysaccharide electrostatic hydrogels in comparison to protein or polysaccharide hydrogels. Advances in Colloid and Interface Science 239(1): 127-135.

Lee, M. H., Baek, M., Cha, D., Park, H. J. and Lim, S. T. 2002. Freeze-thaw stabilization of sweet potato starch gel by polysaccharide gums. Food Hydrocolloids 16(4): 345-352.

Li, G. L., Gang, L., Jie, Z. and Hua, G. 2010. Study on the transparency of corn starch Chinese dumpling wrappers. Science and Technology of Food Industry 31(9): 89-90.

Limroongreungrat, K. and Huang, Y. W. 2007. Pasta products made from sweet potato fortified with soy protein. LWT – Food Science and Technology 40(2): 200-206.

Luo, L., Guo, X. and Zhu, K. 2015. Effect of steaming on the quality characteristics of frozen cooked noodles. LWT – Food Science and Technology 62(2): 1134-1140.

Marti, A. and Pagani, M. A. 2013. What can play the role of gluten in gluten free pasta. Trends in Food Science and Technology 31(1): 63-71.

Matignon, A. and Tecante, A. 2017. Starch retrogradation: from starch components to cereal products. Food Hydrocolloids 68: 43-52.

Menon, R., Padmaja, G., Jyothi, A. N., Asha, V. and Sajeev, M. S. 2016. Gluten-free starch noodles from sweet potato with reduced starch digestibility and enhanced protein content. Journal of Food Science and Technology 53(9): 3532-3542.

Mishra, S. and Rai, T. 2006. Morphology and functional properties of corn, potato and tapioca starches. Food Hydrocolloids 20(5): 557-566.

Monaco, R. D., Cavella, S. and Masi, P. 2008. Predicting sensory cohesiveness, hardness and springiness of solid foods from instrumental measurements. Journal of Texture Studies 39(2): 129-149.

Ozel, B., Dag, D., Kilercioglu, M., Sumnu, S. G. and Oztop, M. H. 2017. NMR relaxometry as a tool to understand the effect of microwave heating on starch-water interactions and gelatinization behavior. LWT – Food Science and Technology 83: 10-17.

Phongthai, S., Damico, S., Schoenlechner, R., Homthawornchoo, W. and Rawdkuen, S. 2017. Effects of protein enrichment on the properties of rice flour based gluten-free pasta. LWT – Food Science and Technology 80: 378-385.

Rachman, A., Brennan, M. A., Morton, J. D. and Brennan, C. S. 2019. Effect of egg white protein and soy protein fortification on physicochemical characteristics of banana pasta. Journal of Food Processing and Preservation 43(9): 1-7.

Saito, H., Tamura, M. and Ogawa, Y. 2019. Starch digestibility of various Japanese commercial noodles made from different starch sources. Food Chemistry 283(15): 390-396.

Sandhu, K. S. and Lim, S. T. 2008. Digestibility of legume starches as influenced by their physical and structural properties. Carbohydrate Polymers 71(2): 245-252.

Schorsch, C., Clark, A. H., Jones, M. G. and Norton, I. T. 1999. Behaviour of milk protein/polysaccharide systems in high sucrose. Colloids and Surfaces B - Biointerfaces 12(3): 317-329.

Silva, E., Birkenhake, M., Scholten, E., Sagis, L. M. and Der Linden, E. V. 2013. Controlling rheology and structure of sweet potato starch noodles with high broccoli powder content by hydrocolloids. Food Hydrocolloids 30(1): 42-52.

Singh, N., Singh, J., Kaur, L., Sodhi, N. S. and Gill, B. S. 2003. Morphological, thermal and rheological properties of starches from different botanical sources. Food Chemistry 81(2): 219-231.

Sun, F., Huang, Q., Hu, T., Xiong, S. and Zhao, S. 2014. Effects and mechanism of modified starches on the gel properties of myofibrillar protein from grass carp. International Journal of Biological Macromolecules 64(2): 17-24.

Tam, L. M., Corke, H., Tan, W. T., Li, J. and Collado, L. S. 2004. Production of *bihon*-type noodles from maize starch differing in amylose content. Cereal Chemistry 81(4): 475-480.

Tan, H., Li, Z. and Tan, B. 2009. Starch noodles: history, classification, materials, processing, structure, nutrition, quality evaluating and improving. Food Research International 42(42): 551-576.

Thapnak, C., Rungsardthong, V., Vatanyoopaisarn, S., Vimolchalao, C. and Thumthanaruk, B. 2019. Effect of barrel temperatures and starch type on some properties of extruded glass noodles. IOP Conference Series Earth and Environmental Science 346: article ID 012050.

Wang, S., Li, C., Copeland, L., Niu, Q. and Wang, S. 2015. Starch retrogradation: a comprehensive review. Comprehensive Reviews in Food Science and Food Safety 14(5): 568-585.

Wang, Y., Ye, F., Liu, J., Zhou, Y., Lei, L. and Zhao, G. 2018. Rheological nature and dropping performance of sweet potato starch dough as influenced by the binder pastes. Food Hydrocolloids 85(12): 39-50.

Xu, X., Liu, H. P., Duan, S. M., Liu, X. H. and Tu, J. Q. 2020. A novel pumpkin seeds protein-pea starch edible film: mechanical, moisture distribution, surface hydrophobicity, UV-barrier properties and potential application. Materials Research Express 6(12): 1-26.

Yamamori, M. 2009. Amylose content and starch properties generated by five variant *Wx* alleles for granule-bound starch synthase in common wheat (*Triticum aestivum* L.). Euphytica 165(3): 607-614.

Zhang, H., Meng, Y., Liu, X., Guan, X. and Li, S. 2019. Effect of extruded mung bean flour on dough rheology and quality of Chinese noodles. Cereal Chemistry 96(5): 1-11.