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REGULAR ARTICLE

Effects of thermal processing on nutritional characteristics and non-volatile flavor components from *Tricholoma lobayense*

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ABSTRACT

In order to explore a better method to process the fruiting body of *Tricholoma lobayense*. Through the determination of proximate compositions, total soluble protein, sugar content, amino acids composition and 5′-nucleotides content, the effects of thermal processing on the nutritional compositions and non-volatile flavor components of the fruiting body of *Tricholoma lobayense* were evaluated. Our study showed that the level of the proximate compositions, total soluble protein and sugar content in *Tricholoma lobayense*, reduced except the total phenolics during cooking. Amino acids composition analysis illustrated that the boiling raised total free amino acids content, but microwaving indicated an opposite effect. Boiling and microwaving could considerably raise the total 5′-nucleotides content. Microwaving reduced the EUC (Equivalent umami concentration) while boiling raised EUC. Both boiling and microwaving significantly raised the bio-accessibility of soluble sugar and protein but boiling was almost doubling that of control. All results suggested that boiling method could effectively preserve the nutritional characteristics of *Tricholoma lobayense*, and make *Tricholoma lobayense* more delicious and easier to be digested compared with microwaving.

Keywords: Bio-accessibility; Non-volatile flavor components; Nutritional characteristics; Thermal processing; *Tricholoma lobayense*

INTRODUCTION

Edible fungus, which have long been cultivated and studied, are widely distributed in Asia and are famous for their abundant nutritive and medicinal value. In general, mushroom contains a lot of carbohydrates and protein, and a little of fat (Kalač, 2009). Proteins from mushroom contain entire essential amino-acids which could only be provided via foods instead of through demic synthesis pathways. Edible fungus contains lower content of total fat and higher content of PUFA ranged from seventy-two to eighty-five percent (Kalač, 2013). Moreover, there is high consumption demand of mushroom, due to the strong flavor and taste. The representative flavor of edible fungus comes from volatile and non-volatile compound (Phat et al., 2016). Non-volatile ingredients include polyols, soluble saccharides, 5'-nucleotides as well as free amino-acids which are pivotal to the taste of mushrooms (Tian et al., 2016). 5'-nucleotides and monosodium glutamate (MSG) commonly induce or enhance umami, which acts as the No.5 gustation in addition to 4 fundamental gustation including saline taste, bitterness taste, sweet taste as well as sour taste. Equivalent umami concentrations (EUC) of mushrooms, which are the MSG concentration amounting to the intensity of umami originated from mixed 5'-nucleotides and MSG, were calculated by Mau (2005), according to non-volatile ingredient levels.

Edible fungus is commonly dried for preservation or applied in the production of pickles, condiments, puree or canned food. The nutritional properties of mushrooms can be altered by the processing, which influences mushrooms' chemical composition. Consumers consider that process with high temperature could have deleterious effects on food's chemical composition. Manzi et al. (2004) have examined proximate composition and some nutritional components, such as chitin, β-glucan, dietary fiber and total phenols in untreated and cooked products.

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Meanwhile, the flavor compounds of mushrooms changed due to various chemical reactions during processes. The changes in taste components have been examined by many studies. It was revealed by Li et al. (2011) that non-volatile compositions in mushroom soup could be influenced by different cooking methods due to many reactions (the Maillard reaction). Chiang et al. (2006) revealed that non-volatile component content had been markedly decreased in canned mushroom. Previous results suggested that some thermal processing could affect nutritional characteristics and the taste of mushrooms. However, no reports have illustrated the change of EUC during cooking.

The bio-accessibility of nutrients has a very important position in researches on bromatology and nutriology. The ingredient of a nutriment after digestion, which could be utilized by organisms, is significant. The bio-accessibility of nutrients is influenced by many factors: The chemical state of the nutrient, potential interactions with other food components, the releasing condition, co-existing suppressive factors or co-factors as well as generated substances with slow metabolizing velocity. Nevertheless, the bio-accessibility may be improved by food processing, for example, polishing, fermenting as well as heating which is probably due to disrupted cellular wall, dissociation of matrix and nutrient complex, or higher reactivity resulted from structural change (Parada and Aguilera, 2007). Carbohydrates and proteins are the main macromolecular nutrients of mushrooms. Therefore it is necessary to study the effect of cooking methods on the bio-accessibility of carbohydrates and proteins.

Tricholoma lobayense Heim, a kind of valuable precious edible fungus, is commercially cultivated for the health food market. It is not only abundant in nutrients but also palatable. Many important bioactive compounds in *Tricholoma lobayense* Heim exhibited immunomodulation and antitumor activity (Liu et al., 1996). Our previous study of *in vitro* antioxidant activities revealed that TLH-3 among the *T.lobayense* polysaccharides had the strongest antioxidant activity (Wang et al., 2012). Related experts pointed out that *T. lobayense* Heim could become a popular commodity domestically in annuals to come. Until now, it is ascertained by our document research that systematic studies on nutritional characteristics and taste components of *T.lobayense*are extremely limited.

It was aimed to compare and examine the variety of nutritional characteristics and non-volatile taste ingredients from *Tricholoma lobayense* Heim in different cooking methods. The study focused on total phenols, total soluble sugars, total soluble proteins, 5'-nucleotides as well as free amino-acids, even change of bio-accessibility of total soluble sugars and total soluble proteins *in vitro* gastrointestinal digestion.

MATERIALS AND METHODS

Sampling

Fresh fruiting bodies of *Tricholoma lobayense* (FBTL) were purchased from Hainan Qingqing Agriculture Co., Ltd. in Hainan province, P.R. China; they were cut into small pieces before cooking procedure. The FBTL were divided into three portions, processed with different cooking treatments.

Cooking

Boiling: Mushroom samples (100 g) were soaked in 1000 mL distilled water. The soaked mixture was cooked to tender for 15 min using induction cooker (DL-D100, Donlim, China) at 400W (Thermal efficiency:86%). Then the surface water was removed.

Microwaving: Mushroom samples (100 g) were placed in a glass beaker containing 1000 mL distilled water, and cooked to tender for 10 min in a microwave oven (P70D20AP-TD(w0), Galanze, China) at 900 Watts (Thermal efficiency:58%). Then the surface water was removed.

FBTL treated with different ways were freeze-dried. Three dried samples were randomly grinded to obtain coarse powder (8 openings/cm).

Proximate composition determination

The proximate compositions of the three different samples, including crude ash, crude fat, crude protein, were determined using the AOAC (2012). The nitrogen factor used for crude protein calculation was 4.38 (Liu et al., 2014). All results were showed as g 100 g^{-1} dry weight (DW).

Total soluble protein and sugar determination

Total soluble protein and sugar were extracted and analyzed as Sun et al. with some modifications (Sun et al., 2011). Samples (100 mg) were polished, homogenized after adding 5 mL PBS (50 mM, pH 7.8), then were centrifuged at 10,000 rpm at 4ºC for 15 min. Total soluble protein content in the supernatant was analyzed based on a calibration curve of bovine serum albumin. Results were showed as g/100 gDW.

Samples (100 mg) were polished and added with 5 mL distilled water in 80ºC for 2.5 h, then centrifuged at 10,000 rpm at 4ºC for 20 min. Total soluble sugar content in the supernatant was analyzed based on a calibration curve of glucose. Results were showed as g 100 g^{-1}DW.

Total phenolic content determination

For total phenolic extraction according to Jiang et al. with some modifications (Jiang et al., 2015), dried samples (0.5 g) was homogenised with 5 mL 80% ethanol solution for 5 min. After 2 h in the dark, the slurry was centrifuged at 10,000 rpm at 4ºC for 15 min. Afterwards, total phenolic in the supernatant was determined according to the method of Li et al. with some modifications (Li et al., 2014). In brief, total phenolic content was assessed by blending 200 µL of deionised water, 50 µL of the diluted extracts, and 50 µL of Folin–Ciocalteu reagent. Six minutes later, 500 µL of 7.5% Na_2CO_3 was added, then the distilled water was added to make the mixture volume to 1.3 mL and allowed to stand at room temperature for 60 min. At 765 nm the mixture absorbance was read using a Microplate reader (Spectra MAX-M2e, Molecular Devices, USA). The calibration curve was constructed using gallic acid (ranging from 0 to 100 µg mL^{-1}, $R^2 = 0.9968$). The results were showed as mg of gallic acid equivalents (GAE) 100 g^{-1}DW.

Analysis for free amino acids

The free amino acid contents of the samples were determined using the methods of Melo-Ruiz et al. (2015). Dried samples (0.5 g) were extracted with 50 mL of 0.1 M HCl. The sample was stood at room temperature for 45 min and then centrifuged at 10,000 rpm for 15 min. The collected supernatant liquid was filtered with a MCE syringe filter and the resulting liquid was determined by an amino acids analyzer (S-433D, SYKAM, Germany).

Analysis for 5′-nucleotide

The nucleotides were extracted using a modified method of Wang et al. (2016). Dried samples(500 mg) were grinded fully, and extracted with 10 mL of distilled water. The suspension was placed in a water bath (100ºC) for 1 min, stirred for 15 min until cooling to room temperature and then centrifuged at 10,000 rpm for 20 min. The precipitation was extracted for two times with 10 mL of distilled water. All supernatant was evaporated and re-dissolved in a final volume of 10 mL with distilled water, then filtrated with a 0.22-µm MCE syringe filter before HPLC.

5'-Nucleotides were analyzed as described by Tsai et al. (2009). The assay was performed on a Zorbax Eclipse XDB C18 column (4.6×150,5 µm, Agilent), controlled at 30ºC temperature. The $H_2O/CH_3OH/CH_3COOH/C_{16}H_{37}NO$ (tetrabutylammonium hydroxide) (894.5/100/5/0.5, v/v/v/v) was used as the mobile phase, and the flow rate was 0.7 mL/min. All samples were detected by UV at 254 nm with 10 µL injection volume. All 5'-nucleotides were identified and quantified by constructing the calibration curve, using the authentic 5'-nucleotide (Aladdin Reagent (Shanghai) Co., Ltd, Shanghai, China).

Equivalent umami concentration (EUC)

The EUC value (mg MSG/g) reflects the concentration of MSG, which is equivalent to the umami intensity given by a mixture of MSG and 5'-nucleotides. The EUC value is calculated by the following equation (Yamaguchi et al., 1971):

$$Y = \sum a_i b_i + 1.218\left(\sum a_i b_i\right)\left(\sum a_j b_j\right)$$

Y: The EUC of the mixture (mg MSG/100 g);
a_i: The concentration (mg/100 g) of each umami amino acid [aspartic acid (Asp) or glutamic acid (Glu)];
a_j: The concentration (mg/100 g) of each umami 5'-nucleotide [5'-inosine monophosphate (5'-IMP), 5'-guanosine monophosphate (5'-GMP), 5'-xanthosine monophosphate(5'-XMP) or 5'-adenosine monophosphate (5'-AMP)];
b_i: The relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1 and Asp, 0.077);
b_j: The RUC for each umami 5'-nucleotide to 5'-IMP (5'-IMP, 1; 5'-GMP, 2.3; 5'-XMP, 0.61 and 5'-AMP, 0.18); and 1.218 is a synergistic constant based on the concentration (mg/100 g) used.

In vitro gastrointestinal digestion

The *in vitro* gastrointestinal digestion could be divided into two successive phases: Gastric and intestinal digestion, as previously reported by Rodríguez-Roque et al. with some modifications (Rodríguez-Roque et al., 2013).

Gastric digestion: Dried samples (1.00 g) were ground with 100 mL of distilled water. Then the pH was adjusted immediately to 2.00 by addition of HCl (12 M). 15,720 Units of pepsin were added and the mixture was incubated at 37ºC and 90 rpm for 2 hours in an air bath shaker (HQ45, China). After the 2-hour incubation, aliquots of 30 mL were collected from each vessel and centrifuged using a SIGMA 3K15 centrifuge at 10,000 rpm at 4ºC for 15 min. The supernatant was stored at -20ºC for further analysis.

Intestinal digestion: Dialysis bag was held with 25 mL water–$NaHCO_3$ (0.5 N) mixture to make the gastric digest pH 7.5. To simulate intestinal digestion, the dialysis bag (containing the water-$NaHCO_3$ mixture) was completely immersed in that digest until reaching pH 5.0. Later, 5 mL of pancreatin (4 g/L) - bile (25 g/L) mixture was added, and the incubation lasted for 2 h at 37ºC and 90 rpm. The dialysis bag was removed and washed with distilled water, then the dialysate was studied. Therefore, two fractions were got after intestinal digestion: Duodenal and dialysed fractions. Aliquots were collected after each digestive phase and immediately cooled to room temperature. Afterwards, aliquots were frozen (-20ºC) until the analyses of total soluble protein and total soluble sugar.

Bio-accessibility was considered as the concentration of bioactive compounds released from the food matrix by *in vitro* gastrointestinal digestion. Bio-accessibility was calculated using the following formula (Rodríguez-Roque et al., 2013):

$$\text{Bio}-\text{accessibility}(\%)=\left(\frac{\text{C dialysed}}{\text{C non}-\text{digested}}\right)*100$$

where $C_{dialysed}$ and $C_{non\text{-}digested}$ referred to the nutrient content concentration (mg/100 mL) in dialysed fraction and non-digested samples (as initially determined from samples), respectively.

Statistical analysis

All experiments were performed three times. The results were expressed as mean ± standard deviation (SD). The analysis of variance was executed using one-way analysis of variance (ANOVA). The differences between the means of samples were analyzed by Duncan's test at a significance level of 0.05.

RESULTS AND DISCUSSION

Effects of thermal processing on chemical components

The proximate compositions of the samples are listed in Fig. 1A. Crude protein content of untreated samples was 17.34 g 100 g^{-1}DW, and the crude protein content in boiling FBTL was 17.28 g 100 g^{-1}DW. No significant differences were observed between control and boiling treatment. However, microwaving treatment caused a reduction in crude protein to 90.25% and its effect was significantly higher than that of boiling. Previous studies revealed that these reductions might be related to the protein denaturation at high temperature (Ahmed and Ali, 2013). It has been reported that there is much protein and a little lipid in the mushrooms. In our results, lipid content of control was 11.92 g 100 g^{-1}DW. During boiling and microwaving processes, the total lipid content decreased by 20.22% and 54.36%, respectively. Both processes showed a decrease in ash contents of FBTL, whereas microwaving displayed higher effect.

The total soluble sugar and protein contents of untreated and processed FBTL were listed in Fig. 1B. In untreated samples, the total sugar and protein were 30.32 and 2.84 g 100 g^{-1}DW, respectively. Their contents could be reduced by the methods of boiling and microwaving. The lowest contents of total soluble sugar and protein were displayed in microwaving treatment group, which are 25.41 and 0.95 g 100 g^{-1}DW, respectively. The effects of both heat treatments on total soluble sugar and protein had obviously differences. The two heat treatment methods have little

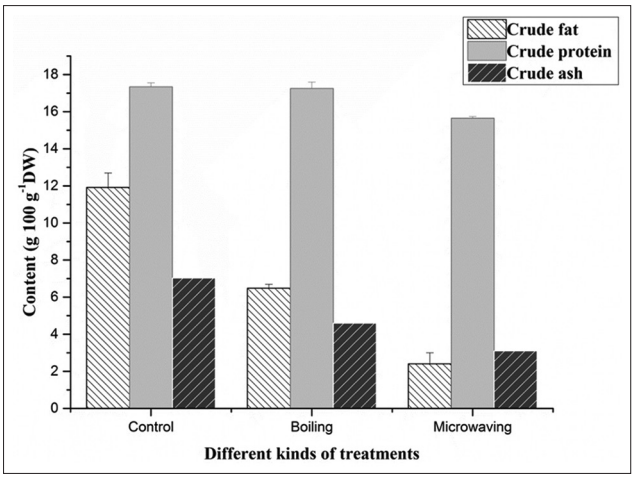

Fig 1A. The proximate composition of untreated and processed FBTL on dry weight basis. Results represent the means of three experiments (p <0.05).

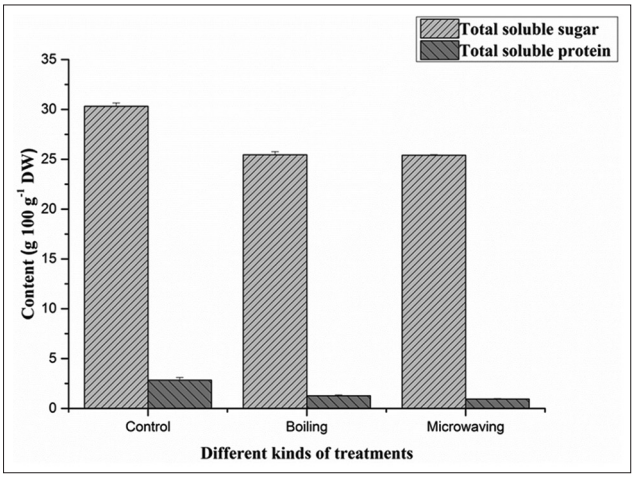

Fig 1B. Total soluble sugar and protein contents of untreated and processed FBTL on dry weight basis. Results represent the means of three experiments (p <0.05).

impact on the total soluble sugar content. However, the effect of microwaving on total soluble protein was significantly higher than that of boiling. Our study were in good agreement with the results Sun et al. (2011), which suggested that heat treatment could significantly reduced the total sugars and proteins contents in *Agaricus blazei* Murril. It could be explained that some soluble sugars and proteins in FBTL were diffused from tissue to peripheral water.

Phenolic content contributes to the antioxidant activity in *Tricholoma lobayense*. Therefore, it is necessary to investigate the change in total phenolic content. Fig. 2 showed the effect of different cooking methods on the total phenolic content of FBTL. In our work, the initial level of the total phenolic in dried FBTL was 98.79 mg GAE 100 g^{-1} DW. After cooking procedures, the total phenolic content

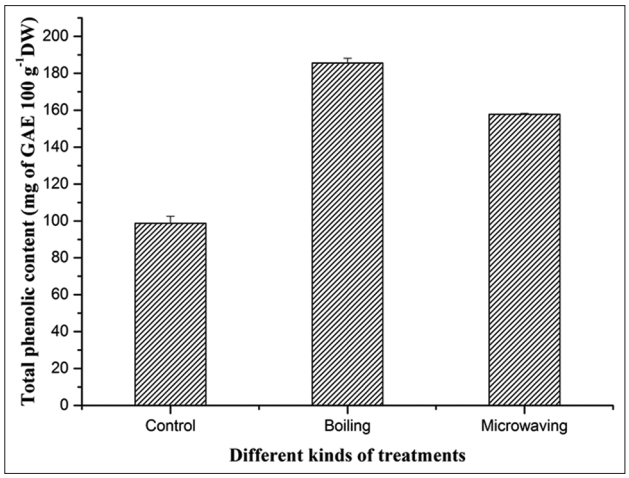

Fig 2. Total phenolic content of untreated and processed FBTL on dry weight basis. Results represent the means of three experiments (p <0.05).

was significantly (p <0.05) raised to 185.61 mg GAE 100 g^{-1} DW for boiling and 157.84 mg GAE 100 g^{-1} DW for microwaving compared with control. Boiling resulted in higher total phenolic content than microwaving. The result was consistent with Choi et al. (2006), who reported that heat treatment of Shiitake sample raised the total phenolic content. It was presumed that heat treatment might produce changes due to the destruction of the cell wall, thus bound polyphenolic could be released more easily (Peleg et al., 1991).

Effect of thermal processing on free amino acids

Several studies referred that mushrooms contain essential amino acids such as leucine, valine, threonine, lysine, methionine, and tryptophan. It was found that threonine and lysine were the major essential free amino acids in all edible fungus (Beluhan and Ranogajec, 2011). The content of free amino acids in samples was shown in Table 1. Thirteen free amino acids were detected in our untreated and processed FBTL. The total amount of 13 free amino acids in FBTL was 17.07 mg/g DW. In general, due to the mechanism of heat transfer and the particular tissue under treatment, the composition in nitrogenous compounds may change with heat treatment. Based on the results, the content of total free amino acids in boiling and microwaving FBTL should reduce, as a result of condensation between amino groups of amino acids with sugar in mushroom tissue, called maillard reaction (Candela et al., 1997). Table 1 shows that microwaving brought about a reduction of 25.01% in total free amino acids content, which is same as expected. But the total free amino acids content from boiling was raised to 18.68 mg/g DW. This result might be due to some free amino acids. These free amino acids includes $_{L}$-glutamine (from 0.21 to 0.27 mg/g DW), $_{L}$-valine (from 0.74 to 0.91 mg/g DW), $_{L}$-isoleucine (from 0.44 to 0.66 mg/g DW), $_{L}$-Leucine (from 0.88 to 1.62mg/g DW),

Table 1: Free amino acids contents of untreated and processed FBTL on dry weight basis, each value is expressed as mean±SD (n=3)

Free amino acids	Content (mg/g dry weight)		
	Control	Boiling	Microwaving
Asp	0.21±0.02b	0.27±0.03a	0.17±0.01b
Ser	3.62±0.03a	3.34±0.06a	2.35±0.06b
Glu	1.78±0.01a	1.73±0.09a	1.24±0.11b
Gly	1.14±0.07a	1.03±0.05a	0.67±0.13b
Ala	4.23±0.06a	3.99±0.08a	2.98±0.14b
Val	0.74±0.09b	0.91±0.08a	0.68±0.01c
Ile	0.44±0.10b	0.66±0.01a	0.40±0.06b
Leu	0.88±0.11b	1.62±0.09a	0.70±0.08b
Tyr	0.62±0.06a	0.68±0.11a	0.37±0.03b
Phe	1.00±0.05b	1.25±0.12a	0.78±0.07c
His	1.26±0.04b	1.55±0.10a	1.28±0.04b
Lys	0.72±0.06c	1.40±0.07a	1.01±0.10b
Arg	0.43±0.13a	0.25±0.14b	0.17±0.06c
Bitter	4.75±0.0.52b	6.24±0.54a	4.01±0.32c
MSG-like	1.99±0.03a	2.00±0.12a	1.41±0.12b
Sweet	4.76±0.16a	4.37±0.19a	3.02±0.33b
Tasteless	1.34±0.12b	2.08±0.18a	1.38±0.13b
Total	17.07±0.83a	18.68±1.03a	12.80±0.90b

Means with different letters within a row are significantly different (p<0.05)
aAla, L-Alanine; Arg, L-Arginine; Asp, L-Aspartic acid; Glu, L-Glutamic acid; Gly, Glycine; His, L-Histidine; Ile, L-Isoleucine; Leu, L-Leucine; Lys, L-Lysine; Phe, L-Phenylalanine; Ser, L-Serine; Tyr, L-Tyrosine; Val, L-Valine; bMSG-like: Asp+Glu; Sweet: Ala+Gly+Ser+Thr; Bitter: Arg+His+Ile+Leu+Met+Phe+Val; Tasteless: Lys+Tyr;

$_{L}$-tyrosine (from 0.62 to 0.68 mg/g DW), $_{L}$-phenylalanine (from 1.00 to 1.25 mg/g DW), L-histidine (from 1.26 to 1.55 mg/g DW) and L-lysine (from 0.72 to 1.40 mg/gDW), which were released from the proteolysis during heating (Pei et al., 2014).

According to the classification described by Mau et al. (2001), free amino acids in edible mushrooms were divided into four groups in accordance with their taste characteristics. They were monosodium glutamate-like (MSG-like) acids (aspartic and glutamic), sweet taste amino acids (alanine, glycine, serine and threonine), bitter amino acids (arginine, histidine, isoleucine, leucine, methionine, phenylalanine, and valine), and tasteless amino acids (lysine and tyrosine). It has been reported that the MSG-like and sweet components may be responsible for the natural taste of mushrooms (Beluhan et al., 2011). The sweetness from sweet components comprised mainly of high amounts of soluble sugars and polyols could probably mask the bitter taste produced by the bitter components in mushrooms. Liu et al. (2014) suggested that the taste-active MSG-like and sweet acids in common mushrooms would be the main reason of the delightful taste of mushrooms. After processing, the amount of MSG-like in microwaving FBTL (1.41 mg/g DW) was lower than that in untreated FBTL (1.99 mg/g DW), but the amount of MSG-like in boiling (2.00 mg/g DW) was almost the same with the un-processed as shown in Table 1. The results also

showed that the amount of sweet amino acids in boiling (4.37 mg/g DW) and microwaving (3.02 mg/g DW) treatment group were also lower than the control group (4.76 mg/g DW). Our results suggested that the taste of boiling FBTL was better than that of microwaving.

Effect of thermal processing on 5′-nucleotides

The amount of 5'-nucleotides in untreated and processed FBTL was shown in Table 2. The content of total 5'-nucleotides in untreated FBTL was 2.22 mg/g DW. After cooking, the total 5'-nucleotides content was raised to 3.33 and 3.19 mg/g DW by boiling and microwaving respectively. Table 2 showed that 5'-CMP (1.39 mg/g DW) occupied the high content of 5'-nucleotides, which was consistent with Tsai et al. (2009). 5'-GMP was a flavor enhancer much stronger than MSG with a meaty flavor, while the flavor could be enhanced by 5'-IMP with other 5'-nucleotides (Pei et al., 2014). Furthermore, the sweet taste could be provided by 5'-AMP for mushroom, and 5'-AMP also has the effective inhibition on bitter taste (Leksrisompong et al., 2012). Our results showed that the 5'-GMP and 5'-AMP content, consistent with the variety of total 5'-nucleotides content, raised during different cooking methods, which could assign to the degradation of deoxyribonucleic acid or ribonucleic acid in FBTL when cooking (Claudine et al., 2005). However, 5'-IMP content reduced during boiling (from 0.15 to 0.11mg/g DW) and microwaving (from 0.15 to 0.10 mg/g DW). It may be explained that 5'-IMP was susceptible of thermal sensitivity, which make 5'-IMP become ribose (Van Boekel, 2006).

Both flavors of MSG-like components and 5'-nucleotides played an important role in enhancing the umami taste of mushrooms (Yamaguchi et al.,1971). Mau (2005) reported that the mushrooms were classified into four levels according to the calculated EUC values of flavor components:>1000% (>1000 g MSG/100 g DW), 100–1000% (100–1000 g MSG/100 g DW), 10–100% (10–100 g MSG/100 g DW), <10% (<10 g MSG/100 g DW). Table 3 shows that EUC value of the FBTL (31.65 g MSG/100 g DW) was at the third level. After cooking, the EUC value of microwaving FBTL significantly ($p < 0.05$) decreased to 24.85 g MSG/100 g DW. But the EUC value of boiling FBTL was raised to 39.92 g MSG/100 g DW. The results indicated that boiling could enhance the umami taste of FBTL.

Effect of thermal processing on the bio-accessibility of soluble sugar and protein by *in vitro* gastrointestinal digestion

Effect of cooking on the bio-accessibility of soluble sugar and protein by *in vitro* gastrointestinal digestion is presented in Table 4. The results showed that cooking could significantly raise the bio-accessibility of soluble sugar and protein.

Table 2: Content of free 5'-nucleotides in untreated and processed FBTL on dry weight basis

5'-Nucleotide	Content (mg/g dry weight)		
	Control	Boiling	Microwaving
5'-AMP	0.13±0.01b	0.16±0.02a	0.14±0.01b
5'-CMP	1.39±0.01b	2.31±0.05a	2.30±0.20a
5'-GMP	0.55±0.03b	0.75±0.06a	0.65±0.05b
5'-IMP	0.15±0.01a	0.11±0.01a	0.10±0.01a
Total	2.22±0.06b	3.33±0.14a	3.19±0.27a

Means with different letters within a row are significantly different ($p<0.05$), 5'-AMP, 5'-adenosine monophosphate; 5'-CMP, 5'-cytosine monophosphate; 5'-GMP, 5'-guano-sine monophosphate; 5'-IMP, 5'-inosine monophosphate;

Table 3: The EUC of untreated and processed FBTL on dry weight basis

Samples	EUC (g MSG/100 g dry weight)
Control	31.65±1.56a
Boiling	39.92±2.25b
Microwaving	24.85±0.96c

Each value is expressed as mean±SD (n=3), means with different letters within a row are significantly different ($p<0.05$)

For the total soluble protein, after gastric digestion, small amounts of soluble protein were detected in stomach from three different samples. It was found that after intestinal digestion, the soluble protein was raised. The increase in water-soluble low molecular components level might result in the change, because of the activity of fungal proteolytic enzymes (Agosin et al., 1989). But the bio-accessibility of the soluble protein with different cooking methods (boiling and microwaving, being 21.38% and 19.74%, respectively) raised about 1-fold than the untreated samples. The increase of soluble protein bio-accessibility after cooking can be explained by heat-degradation of proteins which occurred during cooking and the removal of anti-nutrients that inhibit protein digestion, such as trypsin inhibitors and inositol phosphates (Stodolak and Anna, 2008).

Table 4 revealed that two different cooking methods could also raise the bio-accessibility of total soluble sugar, compared with untreated FBTL (33.25%). The affection of boiling on bio-accessibility of total soluble sugar (71.25%) was stronger than that of microwaving (62.17%). Englyst and Englyst (2005) reported that there was a central position in the food matrix in the concept of carbohydrate bio-accessibility. In crude plant foods, the cell wall NSP played a structural role in maintaining the integrity of the cells. NSP produced an encapsulation effect, which limited the sugars digested and absorbed in the small intestine. Since excessive food processing destroyed the encapsulation effect, our results were consistent with Englyst and Englyst. The reason of consistency might be that the encapsulation was

Table 4: Concentration of total soluble protein and total soluble sugar by *in vitro* digestion of untreated and processed FBLT

Sample	Concentration (mg/100 ml)				Bio-accessibility (%)
	Non-digested	Gastric digestion	Intestinal digestion		
			Duodenal fraction	Dialysed fraction	
Total soluble protein					
Control	28.09±0.42a	2.70±0.04d	26.55±0.64b	3.04±0.01c	10.81
Boiling	12.50±0.24b	2.58±0.03c	30.88±1.00a	2.67±0.04c	21.38
Microwaving	8.90±0.63b	9.16±0.07b	31.53±0.69a	1.81±0.32c	19.74
Total soluble sugar					
Control	303.14±5.32a	18.08±1.17c	101.66±1.71b	100.82±7.36b	33.25
Boiling	254.55±4.33a	246.82±5.53a	221.90±7.82b	181.32±7.43c	71.25
Microwaving	252.00±2.60a	266.63±8.06a	168.76±8.08b	158.0±3.75b	62.17

Results are given as the average values±standard deviation of three independent samples, different letters in the columns represent statistically significant differences ($p<0.05$), the terms represent: Non-digested, as initially determined from sample matrix using 80% aqueous ethanol

disrupted when heated in the presence of water, so the sugar was freed from the cell and easily susceptible to digestion.

CONCLUSIONS

The present study indicated that the nutritional components including crude protein, crude fat, ash, total soluble protein and total soluble sugar reduced by the boiling and microwaving treatments, while the total phenolic was raised. Furthermore, our results showed an obvious effect of boiling and microwaving on free amino acids and 5'-nucleotides. Boiling raised the total free amino acids while microwaving reduced the total free amino acids content. Both treatments raised the 5'-nucleotides content. The EUC, which was affected by free amino acids and 5'-nucleotides, changed with different treatments. Our results suggested that boiling could raise the EUC but microwaving reduced the EUC. In addition, we have studied the effects of cooking on the bio-accessibility of soluble sugar and protein by in vitro gastrointestinal digestion. The bio-accessibility with different cooking methods was significantly higher than that in untreated FBTL measured by the total soluble protein or total soluble sugar. But the bio-accessibility of boiling was higher than that of microwaving. Our study recommends that the quality of boiling FBTL is better than the quality of microwaving FBTL in nutrition, taste and bio-accessibility.

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Authors' contributions

Li-Yuan Zhou was the project leader and performed most of the experiments. Li Wan joined in the experiment design and implementation; Ya Wang, Wen-Qiang Guo were responsible for the completion of "In vitro gastrointestinal digestion"; Zheng-Nan Cai, Wei-Wei Yang, Dan-Dan Wang were responsible for the completion of "Analysis for free amino acids" and "Analysis for 5'-nucleotide".Sajid Hussain, Wen-Juan Pan, Yan Chen were responsible for modification and processing of the manuscript.

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