

Accepted Manuscript

Analytical methods

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PII: S0308-8146(13)01635-X

DOI: <http://dx.doi.org/10.1016/j.foodchem.2013.11.012>

Reference: FOCH 14962

To appear in: *Food Chemistry*

Received Date: 30 January 2013

Revised Date: 3 September 2013

Accepted Date: 1 November 2013



Please cite this article as: Fu, L-l., Chen, X., Wang, Y., Quality evaluation of farmed whiteleg shrimp, *Litopenaeus vannamei*, treated with different slaughter processing by infrared spectroscopy, *Food Chemistry* (2013), doi: <http://dx.doi.org/10.1016/j.foodchem.2013.11.012>

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**Quality evaluation of farmed whiteleg shrimp, *Litopenaeus vannamei*, treated
with different slaughter processing by infrared spectroscopy**

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Abstract: The present study was to evaluate two slaughter processing methods (ice water immersion (T-1) and individual beheaded (T-2)) on whiteleg shrimp quality farmed in freshwater culture systems using attenuated total reflection infrared (ATR-IR) technology. In addition, the corresponding physical, chemical and microbial properties of shrimp samples were also determined. No significant differences were observed in pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA) and *K* value as well as the contents of moisture, crude protein, crude fat and ash between groups of T-1 and T-2. However, significantly higher springiness and chewiness ($P < 0.05$) were observed in T-1 as compared to those of T-2. As for the result of total viable counts (TVC), significantly lower value ($P < 0.05$) was shown in T-1 than that of T-2, indicating that the quality and shelf life of whiteleg shrimp killed by ice water immersion could be improved and prolonged. Furthermore, all the samples were successfully divided into two categories regarding the two slaughter methods by principal component analysis (PCA) according to the infrared spectra. By analysis of the regression coefficients of PLS-DA, it can be supposed that the quality differences of whiteleg shrimp with different slaughter processing are largely caused by structural changes of their protein and fat. All together, our results indicated that the springiness and chewiness of whiteleg shrimp with different processing could be correctly distinguished using infrared spectroscopy.

Keywords

Quality evaluation; whiteleg shrimp; slaughter processing; infrared spectroscopy

1. Introduction

Whiteleg shrimp, *Litopenaeus vannamei*, is a popular and also valuable species for consumers in the world due to its characteristic taste quality. To date, the world's main producer of farmed whiteleg shrimp is China with production levels of approximately 1,120,000 tons in 2010. However, the majority of shrimp is consumed in the United States, the European Union and Japan. As the increase of the shrimp production and consumption, the interest of maintaining the quality properties has been a rising concern for the shrimp industry. In addition, the shrimp industry is also concerned with the ethical and welfare issues highlighted by the overall animal production sector. According to the Scientific Panel on Animal health and welfare of the European Food safety authority, an opinion related to the animal welfare during killing procedures in farms has been issued in 2004. Hence, the need for corresponding slaughter methods and procedures is increasing to improve quality and welfare of shrimp.

Applying the principles of ethics and animal welfare to poikilothermic aquatic animals involves supplying the things necessary for sustaining life, optimising health and minimising visible discomfort (e.g. pain, stress and fear) (Håstein, Scarfe & Lund, 2005). Thus the handling methods of aquatic animals such as killing are very important and essential which contribute to quality defects and welfare. Various methods have been used to slaughter fish and there is no doubt that many of them can

be considered totally unsatisfactory from an animal welfare point of view (Ashley, 2007; Bovenkerk & Meijboom, 2012; Robb, Wotton, McKinstry, Sørensen & Kestin, 2000). However, Parisi, Mecatti, Lupi, Scappini & Poli (2002) found that ice water is the preferred method for killing edible-portion-sized sea bass (*Dicentrarchus labrax*) (of about 250 grams to 300 grams), for which individual killing is not feasible. The related information about the effect of these slaughter methods on farmed whiteleg shrimp quality is still limited. Indeed, using this method to kill marine species is not advised because the osmotic shock caused by melting ice diluting the water's salinity adversely affects their welfare. However, whiteleg shrimp can grow and survive in freshwater (Araneda, Pérez & Gasca-Leyva, 2008) and thus related culture systems have been established in rural areas in China with equal production yield as compared to that of from marine culture.

Apart from welfare, consumers are gradually looking for quality seals and trust marks on shrimp and related products, and expect manufacturers and retailers to provide shrimp of high quality. All of these factors have underlined the need for fast and reliable technique such as infrared spectroscopy (IR) to evaluate the shrimp quality. IR as one of the -fast testing techniques not only allows the characterization of natural and synthetic products, but also contains both physical and chemical information of determined samples. Thus, IR is currently more and more involved in the quality evaluation of food such as meat, dairy, fish, beverage, cereal, fruit, vegetable and so on (Chen, Wu, He & Liu 2009; Karoui, Downey & Blecker, 2010; Wu, He, Nie, Cao & Bao, 2010). Compared with traditional potassium bromide

tableting method, attenuated total reflection infrared (ATR-IR) technology is a complete non-destructive analysis, that is sample cannot be damaged because of no treatment required during testing process (Schmidtke, Smith, Müller & Holzapfel, 2012). Therefore, this method is more effective and convenient, especially being suitable for the samples that cannot be easily prepared by general sample preparation method.

The present study was undertaken to evaluate the comparison of two slaughter processing methods (ice water immersion and individual beheaded) on whiteleg shrimp quality farmed in freshwater culture systems using ATR-IR technology. In addition, the corresponding physical, chemical and microbial properties of shrimp samples were also determined using traditional methods.

2. Materials and methods

2.1. Shrimp and sample collection

Healthy farmed whiteleg shrimp (*Litopenaeus vannamei*) were obtained from the Shrimp Hatchery of Xiaoshan, Hangzhou, China and acclimatized in a storage tank (1 m³ of water), supplied with freshwater and fed the commercial diet (Tiannong Bio-nutrition Technology Co., Hangzhou, China) three times daily for two weeks. At the end of 14-day culture, shrimp were starved for 24 h before processing. Then 180 shrimp with similar sizes (13.5 ± 0.8 g) were processed individually and removed from the storage tank with an aquarium net to place randomly into six 50 L glass tank at initial density of 30 shrimp per tank. Shrimp were divided randomly into two treatments with three replicates to avoid cross-contamination of treatments. The

shrimp slaughter methods were carried out with ice water immersion and individual beheaded in treatment 1 (T-1) and treatment 2 (T-2), respectively.

For the ice water immersion treatment, a reservoir of ice and freshwater (w/v = 1:2) was maintained in a plastic container ($40 \times 60 \times 30 \text{ cm}^3$). The slurry was mixed until water temperature was about 0°C . Following each group, the container was emptied, and rinsed with freshwater ready for the next ice water immersion treatment. As for the individual beheaded treatment, shrimp were washed with freshwater and then processed in as uniform a manner as possible using techniques commonly employed in the local shrimp industry. Death was assumed when shrimp were immobile and showed no response when touched with a glass rod. Shrimp were also left in the treatment for one additional minute to be certain that death was achieved, and then immediately transferred to further analyses.

2.2. Chemical analysis

The proximate composition including crude protein, crude fat, ash (muffle furnace, oven incineration at 550°C for 24 h) and moisture (oven drying at 105°C for 24 h) of diets and shrimp muscle was determined using standard methodology of AOAC (1990). Crude protein was determined using the Kjeltec Analyzer Unit (2300, Sweden) after acid digestion and crude fat was measured using the Soxtec Auto Extraction Unit (2050, Sweden). Gross energy of diets was determined with an adiabatic bomb calorimeter (PARR 1281, USA).

Values of pH were determined with a digital pH meter (PHS-3C digital precision pH meter, Leici, Shanghai, China). The Kjeltec Analyzer Unit (2300, Foss Tecator AB,

Sweden) was also used to detect total volatile basic nitrogen (TVB-N) amount of shrimp muscle. The unit of TVB-N amount was shown as mg N/100 g. The thiobarbutiric acid (TBA) analysis distillation method was performed as described by Tarladgis, Watts, Younathan & Dugan (1960) using a UV-Vis spectrophotometer (Schimadzu UV-160A, Japan). The TBA results were expressed as mg/kg shrimp flesh. As for *K* value, it was determined by high-performance liquid chromatography (HPLC, Agilent 1100 series, California, US), with a Thermo Hypersil BDS C₁₈ (4.6 × 250 mm, 5 μm) and defined as follows equation: $K (\%) = ([\text{Ino}] + [\text{Hx}]) / ([\text{ATP}] + [\text{ADP}] + [\text{AMP}] + [\text{IMP}] + [\text{Ino}] + [\text{Hx}]) \times 100$ (Ino, inosine; Hx, hypoxanthine; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine monophosphate).

2.3. Physical texture analysis

For the physical texture analysis of shrimp muscle, a texture analyzer (LFRA 4500, TA6502421, US) was used based on instrumental method of Texture Profile Analysis (TPA) (Cadun, Schubring & Cakli, 2009). Smooth abdominal muscle (the front 2 sections of abdominal) was selected for the texture profile analysis at room temperature (25 °C). A double compression cycle test was performed at up to 50% compression of the original sample height with a cylindrical probe of 5.0 mm diameter. The cross-head moved at a constant speed of 1 mm s⁻¹. In the present study, hardness, springiness and chewiness were determined as texture parameters of shrimp muscle.

2.4. Microbial analysis

As described by the method of Harrigan & McCance (1976), total viable counts (TVC) were determined using plate count agar (Merck, Shanghai, China). The shrimp muscle sample (10 g) was collected aseptically and placed in stomacher bag containing 90 ml of saline water (0.85%). After mixing for 2 min in a Stomacher blender (M400, Seward, UK), further serial dilution was done using the same diluent. Thereafter, 0.1 mL of appropriate dilution was used for microbiological analysis by spread plate method. The sample plates were then incubated at 30 °C for 48 h. The result was expressed as log N , where N was the number of colony-forming units (cfu) per gram of material (cfu/g).

2.5. IR collection

Shrimp samples treated with different slaughter processing methods were prepared for the IR analysis. The IR spectra (400-4000 cm^{-1}) were determined using U-4100 spectrophotometer (Bruker, Germany) with Golden Gate Diamond ATR sampling accessory. All samples were scanned in fifteen times and the results were averaged using OMNIC software (Version 5.2, Bruker). The collection of all samples was completed in an airtight collection box. In this study, 24 shrimp samples were used which 12 shrimps for each slaughter method.

2.6. Statistical analysis

A completely random design was used in the present study. Data are presented as the mean values with standard deviations (SD) when and where necessary. Data were analysed statistically by the analysis of variance test (ANOVA). $P < 0.05$ was taken to indicate statistical significance in all cases.

3. Results

3.1. Chemical, physical and microbial analysis

Proximate composition of the farmed whiteleg shrimp treated with different slaughter processing was shown in Table 1. No significant differences were observed in contents of moisture, crude protein, crude fat and ash between groups of T-1 and T-2. Table 2 presented the effect of two different processing methods on other chemical parameters of shrimp muscle. Similarly, there were no significant differences in pH, TVB-N, TBA and *K* value between groups of T-1 and T-2 although relatively higher values were found in shrimp treated with individual beheaded (7.17, 7.53 mg N/100g, 0.085 mg/kg and 3.25%, respectively) as compared to those of the shrimp treated with ice water immersion (7.14, 6.05 mg N/100g, 0.065 mg/kg and 2.62 %, respectively).

Effect of two different slaughter methods on shrimp muscle texture properties such as hardness, springiness and chewiness was given in Table 3. No significant difference was found in shrimp hardness between T-1 and T-2. However, shrimp killed by ice water immersion showed significantly higher springiness ($P < 0.05$) as compared to that of T-2 shrimp killed by individual beheaded. In addition, there was also significant difference ($P < 0.05$) in chewiness between groups of T-1 and T-2 and higher value was obtained in T-1 (184.33 ± 12.09) as compared to that of T-2 (140.09 ± 13.18). According to the result of TVC illustrated in Fig. 1, significantly lower value ($P < 0.05$) was shown in T-1 shrimp (2.15 ± 0.28 log cfu/g) than that of T-2 shrimp (4.08 ± 0.52 log cfu/g), which increased by 89.72%.

3.2. IR evaluation

The whole IR spectra of total 24 samples were shown in Fig. 2A. The spectra containing useful information were determined as being the region between 623 and 4000 cm^{-1} , and a total number of 3500 variables were obtained. On the spectra, the distinct characteristic absorption peaks were appeared: i) at 3400 cm^{-1} attributed to the stretching vibration of O-H; ii) at 2350 cm^{-1} , which was probably the characteristic absorption peak of the water vapour in the air or CO_2 ; iii) at 1650 cm^{-1} that was the stretching absorption peak of C=O of proteins; iv) at 600 cm^{-1} defined as the absorption peak of nucleic acid. In addition, principal component analysis (PCA) method was employed for cluster analysis of all the samples, which was shown in Fig. 2B. All the samples were successfully divided into two categories, which clustered as shrimp treated by ice water immersion stunning processing and individual beheaded slaughter method. Furthermore, 16 samples were randomly selected as a training sample set from all the samples. Then, a recognition model was built to predict the remaining 8 samples by the commonly used partial least square-discriminant analysis (PLS-DA) model. The total identification percentage reached 100%. Therefore, it was indicated that the quality of whiteleg shrimp with different slaughter methods could be correctly distinguished using IR spectroscopy technology.

Besides, the regression coefficients of PLS-DA (Fig. 2C) were analysed to mainly investigate where the different spectral information on the quality of whiteleg shrimp with different slaughter methods located. It was observed that the absolute value is relatively larger around 3070 cm^{-1} , 1750 cm^{-1} , 1650 cm^{-1} , 1230 cm^{-1} , 1060

cm⁻¹ and 500-600 cm⁻¹.

4. Discussion

As clearly proved in fish, good welfare means good production. The end point of fish, i.e. the pre-killing procedures management and slaughter methods, if carried out without care to avoid severe stress conditions to the fish, can heavily influence the expression of quality and the subsequent changes during storage of the final product (Poli, Parisi, Scappini & Zampacavallo, 2005). This is an ethic aspect that also has clear reflexes on shrimp quality. In the present study, two different slaughter processing methods were carried out to maintain the best original quality of whiteleg shrimp and the results showed that shrimp killed by ice water immersion presented better springiness and chewiness as compared to those of shrimp killed by individual beheaded. Similar results were obtained in bream, *Nematolosa erebi*, using the ice-slurry as killing method and it suggested that this method should be accepted as a standard humane method and considered similarly for other warm-water species (Blessing, Marshall & Balcombe, 2010). However, unsatisfactory results were observed in the previous studies of Van De Vis *et al.* (2003) and Morzel, Sohler & Van De Vis (2003), who concluded that the killing method of ice water immersion was as unacceptable technique due to the prolonged period of distress before death. The contrast results could be explained by important differences in the species, size and physiology of the aquatic animals used. As outlined above, prior evaluations of ice-water killing methods have focused on fish species that were both large-bodied and from cold-climate regions, whereas the studies shown beneficial effectiveness

used relative small bodied shrimp and fish cultured in tropical and sub-tropical ponds.

It can therefore be proved that both the size difference and the difference in climate are important in understanding these different results.

Microbial deterioration is one of the most common causes of fish and shrimp spoilage. Results from growth of microorganisms, evaluated with TVC, introduced through the meat handling chain and presents as the changes of nutritional quality, appearance, odor and flavor. Based on the present results, significant reduction of TVC was found in shrimp samples killed by ice water immersion. Proliferation of microorganisms requires appropriate high temperatures, while at lower temperatures such as close to 0 °C in ice water container, their activity is reduced, thereby decreasing the TVC values. Apart from an ethic reason, it could be benefit to the quality and shelf life of whiteleg shrimp. In fact, even if at the end point of shrimp life some quality traits are already fixed, some other indexes of quality will be still developing during the post-mortem muscle turning into meat and changeable according to external stress and conditions. Indeed, shrimp killing represents a highly stressful moment and can cause significant impact on flesh quality. From this point, it is suggested that ice water immersion is a suitable processing method for whiteleg shrimp with cold tolerance thresholds higher than the temperature of an ice-water.

This work was the first time to use IR for quality distinguishing of shrimp with different slaughter processing methods. The results indicated that the differences of shrimp quality potentially caused by stress with different killing methods could be effectively distinguished using first three components of PCA, whilst the

identification result reached 100% using the recognition model of PLS-DA. By analysis of the regression coefficients of PLS-DA, the main differences of shrimp muscle using different processing methods can be revealed in the region of the larger absolute value. Through band assignments of the IR spectrum (Akkas, Severcan, Yilmaz & Severcan, 2007; Cakmak, Togan & Severcan, 2006; Dogan, Siyakus & Severcan, 2007), the larger absolute values of 3070 cm^{-1} , 1750 cm^{-1} , 1650 cm^{-1} , 1230 cm^{-1} , 1060 cm^{-1} and $500\text{-}600\text{ cm}^{-1}$ are mainly considered as the absorption peak of N-H stretching of proteins, carbonyl C=O stretch of lipids, C=O stretch of proteins, PO_2^- symmetric stretch, C-O stretching of polysaccharides and nucleic acid, respectively.

Since it is known that physicochemical processes as well as protein and fat compositional or structural changes play an important role in *post mortem* texture development (Bhavan & Geraldine, 2001; Chomnawang, Nantachai, Yongsawatdigul, Thawornchinsombut & Tungkawachara, 2007; Kjaersgård & Jessen, 2003), functional disturbances caused by pre-slaughter stress may affect the hardness, springiness or chewiness. Therefore, our results showed that the quality differences of whiteleg shrimp with different slaughter processing methods are largely caused by structural changes of their protein and fat (Fig. 2C), other than the composition changes (table 1), which may ultimately contribute to the differences of springiness and chewiness (table 3).

Acknowledgements

This study was supported by the National Natural Science Foundation of China (31072221 and 31101921), Zhejiang Provincial Natural Science Foundation of China (R3110345), Zhejiang Provincial Science and Technology Foundation of China (2012C24024) and Agricultural Scientific Program of Hangzhou Science & Technology Bureau (20120232B58).

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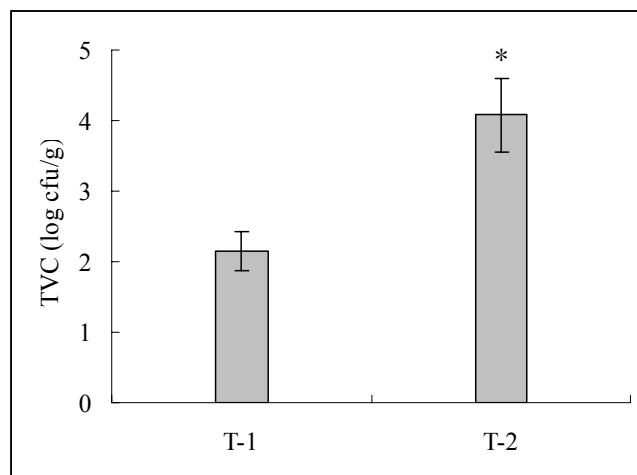
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FIGURE CAPTIONS

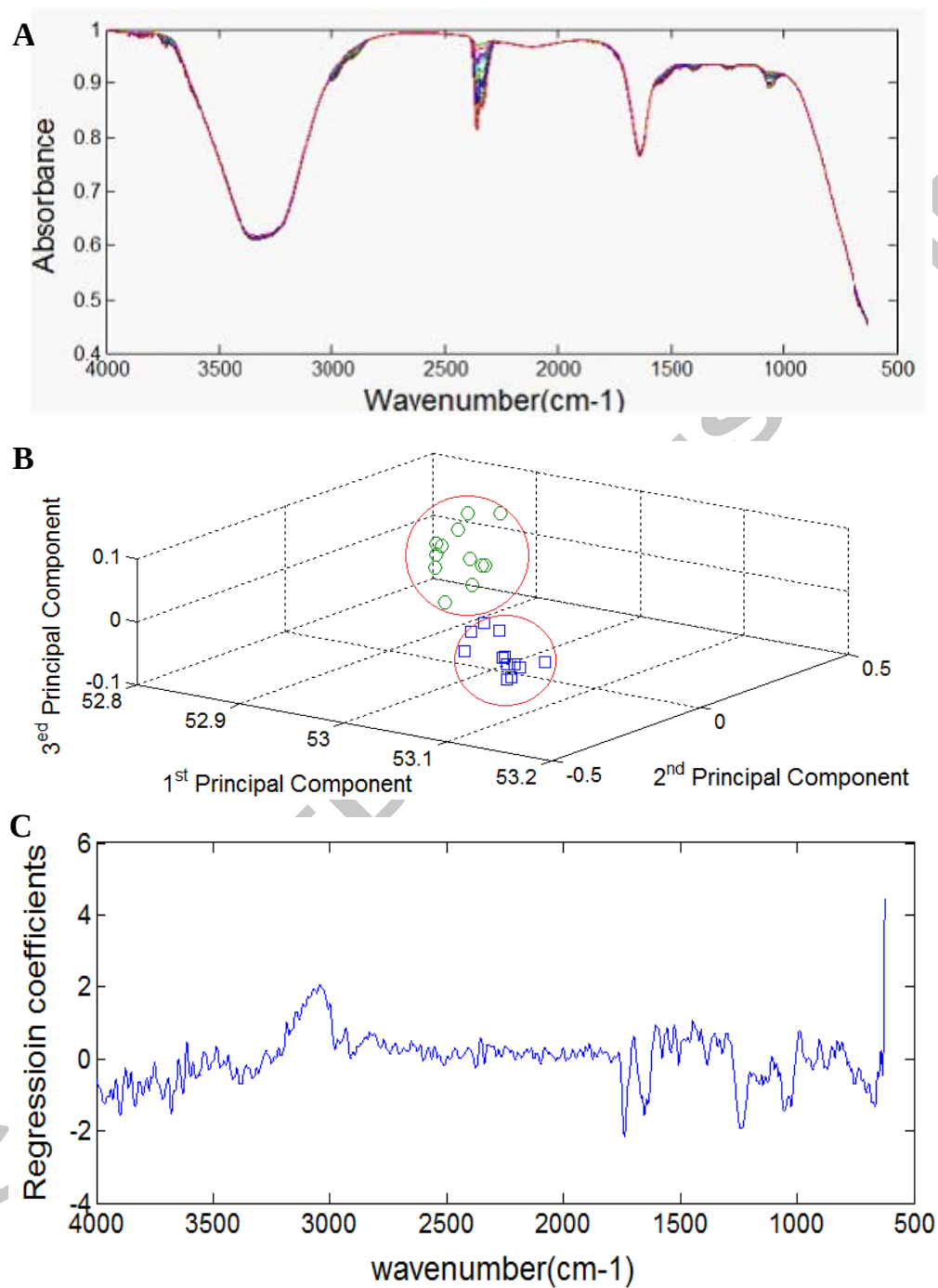
Fig.1. Total viable counts (TVC) (log cfu/g) of the farmed whiteleg shrimp, *Litopenaeus vannamei*, treated with different slaughter processing methods including both ice water immersion and individual beheaded (Treatment 1, T-1 and Treatment 2, T-2, respectively). Means with asterisk are significantly different ($P < 0.05$).

Fig.2. Quality differentiation of whiteleg shrimp with ice water immersion and individual beheaded processing by infrared spectroscopy. (A) Original spectra of shrimp with different processing methods. (B) Clustering analysis of shrimp with different processing methods using principal component analysis (PCA). ice water immersion stunning processing (○), individual beheaded processing method (□). (C) Regression coefficients of PLS-DA for shrimp with different slaughter methods.

Fu et al. Figure 1



Fu et al. Figure 2



Fu et al. Table 1

Proximate composition (%) of the farmed whiteleg shrimp, *Litopenaeus vannamei*, treated with different slaughter processing methods including both ice water immersion and individual beheaded (T-1 and T-2, respectively). Data are presented as the mean values with standard deviations (SD). Means in the same column with asterisk are significantly different ($P < 0.05$).

Group/Parameters	Moisture	Crude protein	Crude fat	Ash
Treatment 1 (T-1)	74.32 ($\pm$ 2.41)	21.54 ($\pm$ 15.57)	1.72 ($\pm$ 0.13)	1.55 ($\pm$ 0.12)
Treatment 2 (T-2)	74.47 ($\pm$ 2.05)	20.99 ($\pm$ 1.65)	1.74 ($\pm$ 0.16)	1.57 ($\pm$ 0.12)

1 Fu et al. Table 2

2 Effect of two different slaughter processing methods (ice water immersion and

3 individual beheaded, T-1 and T-2, respectively) on pH, total volatile basic nitrogen

4 (TVB-N), thiobarbutiric acid (TBA) and K value of the farmed whiteleg shrimp,

5 *Litopenaeus vannamei*. Data are presented as the mean values with standard

6 deviations (SD). Means in the same column with asterisk are significantly different (P

7 < 0.05).

Group/Parameters	pH	TVB-N (mg N/100g)	TBA (mg/kg)	K value (%)
Treatment 1 (T-1)	7.14 $\pm$ 0.06	6.05 $\pm$ 0.58	0.065 $\pm$ 0.009	2.62 $\pm$ 0.47
Treatment 2 (T-2)	7.17 $\pm$ 0.09	7.53 $\pm$ 0.86	0.085 $\pm$ 0.012	3.25 $\pm$ 0.54

Fu et al. Table 3

Effect of two slaughter processing methods (ice water immersion and individual beheaded, T-1 and T-2, respectively) on hardness, springiness and chewiness of the farmed whiteleg shrimp, *Litopenaeus vannamei*. Data are presented as the mean values with standard deviations (SD). Means in the same column with asterisk are significantly different ($P < 0.05$).

Group/Parameters	Hardness (g)	Springiness	Chewiness
Treatment 1 (T-1)	306.15 ± 21.91	0.74 ± 0.05 *	184.33 ± 12.09 *
Treatment 2 (T-2)	292.62 ± 19.69	0.47 ± 0.06	140.09 ± 13.18

Highlights

Shrimp killed by ice water immersion presented better springiness and chewiness.

Shelf life of shrimp is prolonged after ice water immersion processing.

Quality differences of shrimp induced by pre-slaughter stress can be distinguished using IR.

Quality differences are supposed to be related to structural changes of protein and fat.