



Effect of Different Tumbling Marination Methods and Time on the Water Status and Protein Properties of Prepared Pork Chops

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ABSTRACT: The combined effect of tumbling marination methods (vacuum continuous tumbling marination, CT; vacuum intermittent tumbling marination, IT) and effective tumbling time (4, 6, 8, and 10 h) on the water status and protein properties of prepared pork chops was investigated. Results showed that regardless of tumbling time, CT method significantly decreased the muscle fiber diameter (MD) and significantly increased the total moisture content, product yield, salt soluble proteins (SSP) solubility, immobilized water component ($p < 0.05$) compared with IT method. With the effective tumbling time increased from 4 h to 10 h, the fat content and the MD were significantly decreased ($p < 0.05$), whereas the SSP solubility of prepared pork chops increased firstly and then decreased. Besides, an interactive effect between CT method and effective tumbling time was also observed for the chemical composition and proportion of immobilized water ($p < 0.05$). These results demonstrated that CT method of 8 h was the most beneficial for improving the muscle structure and water distribution status, increasing the water-binding capacity and accelerating the marinade efficiency of pork chops; and thus, it should be chosen as the most optimal treatment method for the processing production of prepared pork chops. (**Key Words:** Continuous, Intermittent, Tumbling Time, Water Status, Protein Property, Prepared Pork Chop)

INTRODUCTION

Today, 70% to 80% of the meat products in the world are processed to some extent, and there are varieties of prepared meat products in the market (Hullberg et al., 2005). When focusing on the worldwide meat industry, pork products dominate the market in most of the eastern countries, especially in China, which is closely associated with the traditional eating habits. The production of pork meats such as prepared pork chops is developing to cater to the growing demand by consumers. The prepared pork chop is characterized by its convenient, higher value-added and versatile qualities (Gurikar et al., 2014).

In the present study, prepared pork chops were cut from pork loins with approximately the same weight and size (100 g and 10 cm×5 cm×2 cm). For this type of prepared pork chops, the commercial marinade solutions including water, salt (NaCl), phosphates and other additives are added to the meat (Murphy and Zerby, 2004). It is well known that, salt not only aids the solubility of meat proteins but also promotes the swelling of myofibrils. As a natural flavor enhancer, salt also increases the flavor intensity of meat or meat products through the marination process (Gillette, 1985). Whereas, the fundamental purpose of applying phosphates is to increase the water-binding capacity (WBC) and stabilize meat emulsions of processed meat products.

There are various methods (tumbling, blade tenderization, massaging, etc) used to accelerate marinade solution penetration into the meat products. The marination process is mostly complemented with tumbling technology (Pietrasik and Shand, 2003). This combination facilitates the marinade's even distribution, disrupting muscle structure, promoting the extraction and solubilisation of

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Submitted Dec. 3, 2014; Revised Jan. 12, 2015; Accepted Feb. 3, 2015

myofibrillar protein complex, and consequently changing the protein properties and increasing the WBC of meat products (Cassidy et al., 1978; Krause et al., 1978; Motycka and Bechtel, 1983; Plimpton et al., 1991; Alvarado and McKee, 2007).

There are many differences in the technical parameters of tumbling marination (continuous versus intermittent tumbling, tumbling marination time, drum speed and degree of loading of drum, etc) of prepared pork chops. Any changes in these parameters would affect the quality characteristics and consumer satisfaction of the final products. However, there is little information available on the effect of critical technical parameters on prepared pork chops. Hence, the objective of this study was to investigate the effect of the two most critical factors of tumbling marination methods (continuous vs intermittent tumbling) and actual tumbling time (4, 6, 8, and 10 h) on the water status and protein properties of prepared pork chops, thus obtaining the optimal technological parameters to produce high-quality prepared pork chops.

MATERIALS AND METHODS

Meat samples and experimental design

Fresh whole pork loins (*Longissimus dorsi*) were obtained randomly from a local slaughterhouse at 48 h post-mortem. The average weight of the slaughtered crossbred barrow pigs ([Yorkshire×Landrace]×Duroc) was approximately 95 kg. The pork loins were chosen as they are a relatively homogenous muscle with the normal pH value 5.57 ± 0.03 , total moisture content $73.47\pm 0.35\%$, crude protein content $22.11\pm 0.11\%$ and fat content (ether extractable) $3.09\pm 0.26\%$. After the removal of all external fat, fascia and separable connective tissue, the pork loins were packed in low density polyethylene bags temporarily and stored at 2°C for subsequent experiments.

The composition of the marinade solution, optimized in preliminary investigations, was designed to give the following concentration of ingredients, percentage by raw meat weight: sodium chloride (NaCl) 1.50%, sodium pyrophosphate (TSPP) 0.14%, sodium tripolyphosphate (STPP) 0.08%, sodium hexametphosphate (SHMP) 0.08% and white pepper powder 0.30%. NaCl and polyphosphates were analytical grade and were purchased from Xuzhou Tianjia Chemical Plant Co. Limited (Xuzhou, China). The white pepper powder was purchased from Kunshan Spices Co. Limited (Kunshan, China). When testing, the pork loins were cut into chop samples of approximately the same weight and size (100 g and 10 cm×5 cm×2 cm). The length of pork chops was 10 cm and parallel to the muscle fiber direction. The ratio of meat weight to marinade weight was 100:35 for all treatments.

This study applied a 2×4 factorial experimental design. Eight experimental treatments were formulated to contain two tumbling marination methods (vacuum continuous tumbling marination, CT; vacuum intermittent tumbling marination, IT) and four effective tumbling times (4, 6, 8, and 10 h). The intermittent schedule was 20 min on and 10 min off for the total treatment time of 6, 9, 12, and 15 h with the effective tumbling time equal to 4, 6, 8, and 10 h, respectively. The cut pork chops and marinade solution were placed in a vacuum tumbler (ESK-125, Kakona GmbH Company, Kempten, Germany) with tumbling conditions of 11 revolutions per minute, vacuum at 90% and temperature at 2°C for all treatments. Eight treatments were named as CT-4 h, CT-6 h, CT-8 h, CT-10 h, IT-4 h, IT-6 h, IT-8 h, and IT-10 h, respectively. Each treatment consisted of three replicates of four samples. After the marination experiments, all samples were dabbed with tissue paper to absorb surface water for the further analysis.

Chemical composition analysis

The chemical composition of chop samples at the end of every tumbling experiment was determined. The total moisture content was determined by the drying method as recommended by the Association of Official Analytical Chemists (AOAC, 2000). Fat content was determined by using an ether solvent extraction system. Crude protein content was determined by the Kjeldahl method with an automatic nitrogen analyzer (Kjeltec 2300 Analyzer Unit, Foss Tecator A B, Höganäs, Sweden).

Product yield

The weights of pork chops before marinating (W_1/g) and after marinating (W_2/g) were recorded individually. The following formula was used to calculate the product yield as described by Barbut et al. (2005) with some modifications: Product yield (%) = $W_2/W_1 \times 100$.

Salt soluble proteins extraction and solubility determination

Salt soluble proteins (SSP) solubility was determined with a modification of the procedures of Frye et al. (1986). Small sponges (2 cm×2 cm×2 cm) were added into tumbler together with the chop samples and marinade at the start of marination experiments. Then the sponge was removed immediately after experiments and pressed by a 50 mL volumetric syringe to get 4 g slurry. After vacuum treatment for 15 min, the slurry was added in 200 mL of 3% NaCl solution. After storing at 4°C for 24 h, the slurry solution was centrifuged for 15 min at 10,400×g at 4°C (Beckman Allegra 64R, Beckman-Coulter Company, Fullerton, CA, USA). The total protein content in the supernatant was determined by the Coomassie brilliant blue reagent

(Nanjing Jiancheng Bioengineering Institute, Nanjing, China) from the absorbance at 595 nm.

Histological observation

Histological sections were made according to the procedures of Li et al. (2008) with some modifications. A sample of 5 mm×5 mm×10 mm was cut out parallel to the muscle fiber direction, from the middle part of each raw pork chop after marination treatment. The cut sample was frozen in liquid nitrogen and then stored at -80°C . The sample was sectioned into 10 μm slices with a cryostat microtome (CM1900, Leica, Nussloch, Germany) at -24°C . The sections were placed on glass slides, contrast-stained with hematoxylin and eosin, dehydrated by using a series of gradually increasing concentrations of ethanol (70% to 100%) and sealed with Canada balsam. Sealed samples were visually examined using a light microscope (Olympus BX41, Olympus Optical Co. Ltd., Tokyo, Japan) with a 10×objective for cross sections and images were captured using an Olympus DP12 CCD digital camera (Olympus Optical Co. Ltd., Tokyo, Japan). Measurement points were randomly selected from each photograph and muscle fiber diameter (MD, μm) was determined by the Image-Pro Plus 6.0 software (Media Cybernetics, Bethesda, Silver Spring, MD, USA).

Nuclear magnetic resonance transverse relaxation (T_2) measurement

Nuclear magnetic resonance (NMR) relaxation measurements were performed on a Niumag Pulsed NMR analyzer (PQ001, Niumag Corporation, Shanghai, China) using a modification of the procedures of Straadt et al. (2007). The NMR instrument was equipped with a 15 mm variable temperature probe. Approximately 3 g of raw meat sample from the center of each pork chop was individually wrapped in parafilm membrane, placed in a cylindrical glass tube and inserted in the NMR probe. The analyzer was operated at a resonance frequency of 22.4 MHz at 32°C . Transverse relaxation times (T_2) were measured using the Carr-Purcell-Meiboom-Gill sequence. The T_2 measurements were performed with a τ -value (time between 90° pulse and 180° pulse) of 150 μs . Data from 4,096 echoes were acquired as 16 scan repetitions. The obtained T_2 data were analyzed using a multi-exponential model under the program Multi Exp Inv Analysis (Niumag Corporation, Shanghai, China).

Statistical analysis

All data were analyzed by analysis of variance using the general linear model procedure of the SAS statistical package (Statistics Analysis System 8.1, SAS Inc., Cary, NC, USA) for significant differences among treatment means based on tumbling marination methods, tumbling

time and their interactions. If significant differences ($p<0.05$) were found in factors, the Duncan's new multiple range test was used to rank the means.

RESULTS

Chemical composition

The total moisture content (%) of prepared pork chops significantly increased ($p<0.05$) for CT method compared with that of IT method regardless of tumbling time, as shown in Table 1. However, the crude protein content (%) and fat content (%) significantly decreased ($p<0.05$) with the effective tumbling time increased from 4 h to 10 h regardless of tumbling methods, and the lowest values for both methods were obtained at 10 h. Additionally, there were interactive effects on the chemical composition between tumbling methods and effective tumbling time ($p<0.05$).

Product yield and salt soluble proteins solubility

The product yield (%) and SSP solubility (mg/mL) of prepared pork chops significantly increased ($p<0.05$) for CT method compared with those of IT method regardless of tumbling time, as shown in Table 1 and 2. Whereas the SSP solubility increased firstly and then decreased with the effective tumbling time increasing from 4 h to 10 h, and the SSP solubility of 8 h was significantly higher ($p<0.05$) than the other groups in terms of tumbling time (Table 2).

Muscle fiber determination

The MD of prepared pork chops from the CT method was lower than that of those from the IT method for the same effective tumbling time, as shown in Figure 1. For the same tumbling method, the MD had a trend towards decreasing with the increasing of effective tumbling time. After 4 h, single muscle fibers and muscle bundles could be distinguish, with the muscle microstructure of integrated tissue in clear outline. The muscle fiber of 6 h began to display slight extrusion deformation with smooth swelling. The muscle fibers after 8 h showed much more severe distortions and individual dissolved broken phenomena (as shown by the arrow), the effect of CT method was greater than IT method. The muscle fiber of 10 h displayed the severest distortions and dissolved broken phenomena along with the smallest diameter and the biggest gap (as shown by the arrow, especially for CT method).

The obtained MD (μm) results were summarized in Table 2. The MD of prepared pork chops significantly decreased ($p<0.05$) for CT method in comparison to that of IT method regardless of tumbling time. Whereas the MD decreased with the increasing of effective tumbling time from 4 h to 10 h, and the MD of 10 h was significantly lower ($p<0.05$) than the other treatments. Additionally, there

Table 1. Effect of different tumbling marination methods and time on the chemical composition and product yield of prepared pork chops

Items	Protein (%)	Moisture (%)	Fat (%)	Product yield (%)
CT-4h	20.48±0.14 ^a	75.45±0.08 ^c	2.18±0.11 ^a	103.26±1.05
CT-6h	20.32±0.21 ^a	75.46±0.05 ^c	1.98±0.09 ^b	103.83±0.72
CT-8h	19.95±0.18 ^b	76.49±0.12 ^b	1.67±0.09 ^{cd}	103.79±1.17
CT-10h	19.25±0.15 ^d	76.83±0.05 ^a	1.17±0.06 ^e	104.73±1.72
IT-4h	20.44±0.08 ^a	73.91±0.03 ^d	2.18±0.16 ^a	101.23±1.00
IT-6h	19.96±0.14 ^b	73.48±0.19 ^e	1.74±0.17 ^c	100.95±1.56
IT-8h	19.65±0.22 ^c	75.32±0.11 ^c	1.54±0.05 ^d	101.74±0.72
IT-10h	19.05±0.12 ^d	72.98±0.14 ^f	1.29±0.02 ^e	101.86±1.98
Methods				
CT	20.25±0.24	76.06±0.64 ^a	1.75±0.40	103.91±1.18 ^a
IT	19.77±0.54	73.92±0.91 ^b	1.69±0.35	101.44±1.26 ^b
Time				
4 h	20.46±0.11 ^a	74.68±0.85	2.18±0.07 ^a	102.24±1.44
6 h	20.14±0.24 ^b	74.47±1.09	1.86±0.17 ^b	102.39±1.92
8 h	19.80±0.22 ^c	75.90±0.65	1.61±0.09 ^c	102.76±1.42
10 h	19.65±0.67 ^c	74.91±2.11	1.23±0.08 ^d	103.29±2.28
Two-way analysis of variance				
Methods	0.073	<0.0001	0.113	0.001
Time	0.006	0.264	<0.0001	0.530
Interaction	<0.0001	<0.0001	0.014	0.893

CT, vacuum continuous tumbling marination; IT, vacuum intermittent tumbling marination.

^{a-e} Means with different letters in the same column (within each main effect or interaction) are significantly different ($p < 0.05$). Values are reported as means±standard deviation of three replicates.

Table 2. Effect of different tumbling marination methods and time on the SSP solubility, muscle fiber diameter and the T₂ peak area ratio of prepared pork chops

Items	SSP solubility (mg/mL)	MD (μm)	PI (%)	PII (%)	PIII (%)
CT-4h	0.98±0.01	78.08±0.95	0.044±0.001 ^a	0.949±0.001 ^c	0.007±0.001 ^c
CT-6h	1.05±0.02	74.19±0.96	0.034±0.001 ^{bc}	0.949±0.002 ^c	0.017±0.002 ^{ab}
CT-8h	1.16±0.02	68.87±1.32	0.032±0.002 ^{cd}	0.964±0.002 ^a	0.005±0.001 ^c
CT-10h	0.89±0.01	59.52±0.62	0.035±0.003 ^b	0.959±0.003 ^a	0.006±0.003 ^c
IT-4h	0.88±0.03	82.89±0.98	0.030±0.002 ^d	0.950±0.002 ^c	0.019±0.003 ^{ab}
IT-6h	1.04±0.07	77.09±1.25	0.031±0.003 ^{cd}	0.949±0.004 ^d	0.020±0.005 ^a
IT-8h	1.06±0.06	73.72±0.92	0.029±0.001 ^d	0.951±0.002 ^{bc}	0.022±0.004 ^a
IT-10h	0.85±0.01	62.38±0.78	0.031±0.001 ^{cd}	0.955±0.004 ^b	0.015±0.002 ^b
Methods					
CT	1.02±0.10 ^a	70.17±7.29 ^b	0.036±0.005 ^a	0.955±0.007 ^a	0.009±0.005 ^b
IT	0.96±0.10 ^b	74.02±7.86 ^a	0.030±0.001 ^b	0.951±0.003 ^b	0.019±0.004 ^a
Time					
4 h	0.93±0.06 ^c	80.49±2.76 ^a	0.037±0.007 ^a	0.950±0.001 ^b	0.014±0.007 ^{ab}
6 h	1.04±0.05 ^b	75.64±1.87 ^b	0.032±0.002 ^{ab}	0.949±0.003 ^b	0.019±0.004 ^a
8 h	1.11±0.07 ^a	71.29±2.72 ^c	0.030±0.002 ^b	0.957±0.007 ^a	0.013±0.009 ^{ab}
10 h	0.87±0.03 ^c	60.95±1.68 ^d	0.033±0.003 ^{ab}	0.956±0.004 ^a	0.010±0.005 ^b
Two-way analysis of variance					
Methods	0.001	<0.0001	<0.0001	0.001	<0.0001
Time	<0.0001	<0.0001	0.001	0.006	0.001
Interaction	0.114	0.092	0.001	0.005	0.008

SSP, salt soluble proteins; MD, muscle fiber diameter; NMR, nuclear magnetic resonance; PI, the T₂₁ peak area ratio of NMR graph; PII, the T₂₂ peak area ratio of NMR graph; PIII, the T₂₃ peak area ratio of NMR graph; CT, vacuum continuous tumbling marination; IT, vacuum intermittent tumbling marination.

^{a-f} Means with different letters in the same column (within each main effect or interaction) are significantly different ($p < 0.05$). Values are reported as means±standard deviation of three replicates.

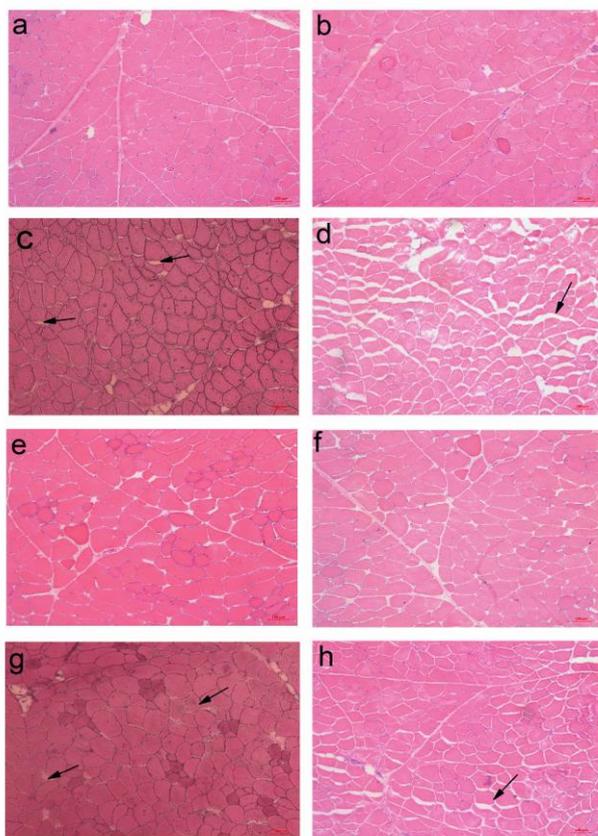


Figure 1. Light microscopy of cross sections of muscle fibers of pork chops prepared by different tumbling marination methods and time (a, vacuum continuous tumbling marination for 4 h; b, vacuum continuous tumbling marination for 6 h; c, vacuum continuous tumbling marination for 8 h; d, vacuum continuous tumbling marination for 10 h; e, vacuum intermittent tumbling marination for 6 h; f, vacuum intermittent tumbling marination for 9 h; g, vacuum intermittent tumbling marination for 12 h; h, vacuum intermittent tumbling marination for 15 h; 10× magnification, representative of n = 3).

was no interactive effect on the MD between tumbling methods and tumbling time ($p > 0.05$).

Nuclear magnetic resonance transverse relaxation (T_2) measurements

There were three water components, a minor component between 1 to 10 ms (T_{21}), a major component between 30 to 100 ms (T_{22}) and finally a much weaker component between 100 and 500 ms (T_{23}), as shown in Figure 2. The peaks of the major component of various tumbling treatments had a trend of slight shifting towards higher relaxation times with the effectively increasing tumbling time from 4 h to 10 h.

The obtained NMR T_2 peak area ratio (%) results are summarized in Table 2. The T_{22} peak area ratio (%) of prepared pork chops significantly increased ($p < 0.05$) for CT method compared with that of IT method in spite of

tumbling time. While the T_{22} peak area ratio increased with the increasing of effective tumbling time from 4 h to 10 h, and the highest T_{22} peak area ratio was observed at 8 h. In addition, the T_{22} peak area ratio was significantly affected by the interactive influences of tumbling methods and tumbling time ($p < 0.05$) with the highest observed in CT method of 8 h.

DISCUSSION

The compositional properties of marinated meat products can be changed by tumbling processing technology (Dolata et al., 2004). The SSP (including myosin, actin, actomyosin, etc.) are extracted from the muscle with salt (Marsh, 1977). Largely depending on salt concentration and muscle structures, the SSP solubility reflects the degree of protein denaturation and is closely correlated with meat tenderness and texture. In this study, the WBC was expressed as the product yield (Table 1). Results show that as the effective tumbling time increasing from 4 h to 8 h, tumbling marination process significantly changed the protein properties, increased SSP solubility and consequently decreased the crude protein content of prepared pork chops. Pietrasik and Shand (2004) also reported tumbling marination treatment facilitated the extraction and solubilization of myofibrillar proteins, allowing more moisture to be bound by the SSP, thus increasing the product yield and assuring better textural properties. In addition, after heating, the SSP composite formed gel network within a densely spatial grid structure, which facilitated wrapping more water and fat, or adsorbing more moisture by physical capillary force, and finally increasing the WBC (Xiong and Kupski, 1999; Pietrasik and Shand, 2004). However, as the effective tumbling time extending to 10 h from 8 h, an inverse trend of SSP solubility was observed. Frye et al. (1986) also observed that after 7 h of vacuum tumbling marination treatment of boneless ham, the total content of protein extraction started to fall, but the reason was unclear, therefore, further in-depth studies were needed to elucidate this. Meanwhile, CT method significantly increased SSP solubility and product yield compared with the IT method, this might be attributed to the continuous mechanical action being more effective in loosening muscle structure and facilitating the marinade efficiency.

The fundamental structure unit for all muscles is the muscle fiber (Lawrie and Ledward, 2006). Most previous research regarding muscle structure stated that, changes in muscle structure are strongly linked to important meat quality characteristics, such as the WBC, tenderness and textural properties, etc (Souza et al., 2011). In this study, our results of myofiber transverse section microstructure (Figure 1 and Table 2) support the findings of Ockerman

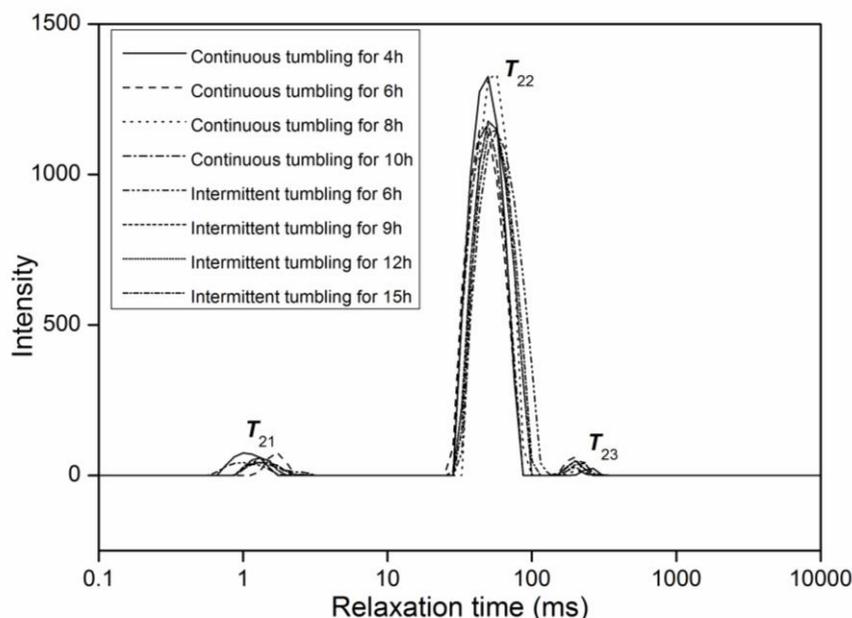


Figure 2. Effect of different tumbling marination methods and time on the T_2 relaxometry time (ms) of prepared pork chops (representative of $n = 3$).

and Organisciak (1978), Pietrasik and Shand (2004) and Siró et al. (2009), which indicated that with the increasing of effective tumbling time from 4 h to 10 h, the combination of tumbling (involving meat rotating, falling and contacting with metal walls and paddles in a tumbler drum) and marinade (containing salt and polyphosphate) facilitated loosening the muscle structure, disrupting cell membranes, destroying the connection between the myofibril and collagen, promoting marinade permeation and distribution in the meat and improving the solubilization and extraction of SSP, thus reducing the MD. It is also observed that CT method was more effective in reducing MD of pork chops than IT method. It was surmised that the mechanical energy of CT method could effectively avoid the intrinsic elastic shrink of tumbled meat samples during the “rest period” in the intermittent tumbling process. Consequently, the CT method was more effective in loosening muscle structure, destroying the connection between the myofibers and the connective tissue and facilitating marinade penetration into meat uniformly. Moreover, the severest distortions and the smallest diameter of muscle fibers were obtained by CT method of 10 h.

Recently, as an advanced non-destructive technique, low field NMR relaxometry has been widely applied to obtain basic information about water mobility and distribution status; the mechanism of NMR is to probe the mobility of water protons and exchangeable protons in meat proteins (Bertram and Andersen, 2004; Straadt et al., 2007). Many studies including the determination of WBC and quality characteristics of meat or meat products were performed by the use of NMR (Brown et al., 2000; Bertram et al., 2002).

In view of previously related research results, three water components of T_{21} , T_{22} , and T_{23} (Figure 2) in raw meat of this study probably reflect the bound water, immobilized water and free water, respectively (Bertram et al., 2001; Lawrie and Ledward, 2006). Approximately 80% of the water in raw meat was immobilized water, which mainly existed in the spaces between the thick filaments of myosin and the thin filaments of actin/tropomyosin (Offer and Trinick, 1983; Straadt et al., 2007). As a result of the WBC being strongly linked to the immobilized water, so the discussion mainly focuses on the T_{22} component. NMR result from Table 2 is in agreement with observations by Dolata et al. (2004) and Cheng et al. (2011), who explained that the association of longer tumbling time and tumbling marination process facilitated disrupting the muscle fiber structure, promoting access of marinade to the intracellular myofibrillar proteins and releasing SSP into the intracellular space, and thus increasing T_{22} water component. Meanwhile, this association also improved T_{22} water mobility as shown by the T_{22} peak slightly shifting towards higher relaxation times (Figure 2). Regardless of tumbling time, the continuously mechanical effects of CT method significantly increased T_{22} peak area ratio compared with IT method, and this result reflects the increasing of the WBC and total moisture content of pork chops as noted earlier.

CONCLUSIONS

The continuous mechanical effect of CT method was more effective in decreasing the MD, increasing the total moisture content, product yield, SSP solubility and

immobilized water component of prepared pork chops regardless of tumbling time. With the effective tumbling time increased from 4 h to 10 h, the crude protein content, fat content and the MD continued to decrease, whereas the SSP solubility of prepared pork chops firstly increased and then decreased. Besides, the highest values of total moisture content, SSP solubility and immobilized water component were observed at 8 h. Thus, we believe that a 10 h tumbling time is too long and consequently not suitable for this kind of pork chops. In addition, an interactive effect between CT method and effective tumbling time was also observed for the chemical composition and proportion of immobilized water. These results demonstrated that a CT method of 8 h was the most beneficial for improving the muscle structure and water distribution status, increasing the WBC and accelerating the marinade efficiency; and thus, it should be chosen as the most optimal treatment method for the processing production of prepared pork chops.

ACKNOWLEDGMENTS

This study was funded by the National Science & Technology Pillar Program during the Twelfth Five-year Plan Period of China (2012BAD28B03), Three Agricultural Projects of Jiangsu Province of China (SX(2011)146), and the Fundamental Research Funds for the Central Universities of China (KYZ201222).

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