The improvement effect and mechanism of citrus fiber on the water-binding ability of low-fat frankfurters

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Abstract Water-binding ability is a major quality attribute of low-fat meat products. This study aimed to evaluate the effects of citrus fiber on the water-binding ability of low-fat frankfurters. Low-fat (10% fat) frankfurters with different contents (0, 1, 2, and 3%) of citrus fiber were prepared. Their cooking loss, water distribution, microstructure, and protein structure were assessed and compared to those of normal-fat (20% fat) frankfurters. Results demonstrated that citrus fiber could significantly reduce the cooking loss of low-fat frankfurters from 12.69 to 4.71%. Adding citrus fiber led to a faster relaxation time in low-fat frankfurters, and a significant increase in the proportion of immobilized water. Frankfurters with citrus fiber exhibited a more compact, continuous protein network structure. Furthermore, the intensity changes in the Raman bands near 760 and 2930 cm^{-1} showed that citrus fiber increased hydrophobic interactions around hydrocarbon chains and tryptophan residues in myofibrillar proteins. In conclusion, citrus fiber effectively improved the water-binding ability of low-fat frankfurters by increasing the hydrophobic

interactions of myofibrillar proteins and changing the frankfurter microstructure.

Keywords Low-fat frankfurters · Water-binding ability · Citrus fiber · Microstructure · Raman spectroscopy

Introduction

Frankfurters are one of the most popular meat products in the world, but the fat content of conventional frankfurters can be as high as 30% (Choi et al. 2009). Health organizations worldwide have proposed that animal fat intake is related to obesity, hypertension, and cardiovascular disease (Choi et al. 2010). Consequently, low-fat meat products are becoming more popular. Consistent with this trend, the meat industry has focused on reducing the fat in traditional frankfurters.

Fat reduction significantly affects the water-binding ability, texture properties, and mouth feel of meat products (Cengiz and Gokoglu 2007; Muguerza et al. 2002). Water-binding ability, regarded as one of the most important functional properties of meat products, is directly related to the cooking yield, juiciness, and flavor of the products (Weiss et al. 2010). Thus, several additives, including citrus fiber, have been proposed as fat substitutes for their ability to bind water in low-fat meat products (McAfee et al. 2010; Tobin et al. 2013).

Extracted from citrus fruits, citrus fiber is well known for its functional and nutritional properties, and has been demonstrated to reduce the risk of cancer, coronary heart diseases, and obesity (Hallfrisch et al. 1995). Citrus fiber has also been applied in meat products not only for its beneficial effects on health but also for its ability to improve the water-binding ability of the products

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(Yalinkiliç et al. 2012). However, the mechanism by which citrus fiber improves the water-binding ability of low-fat frankfurters remains unclear.

Therefore, the principle objective of this study was to evaluate the effects of citrus fiber on the proximate composition, cooking loss, textural properties, and water distribution of low-fat frankfurters. This study also attempts to explore the mechanism of how citrus fiber improves the water-binding ability in low-fat frankfurters by analyzing the microstructure and changes in protein structure.

Materials and methods

Materials

Lean pork and back fat were thawed at 4 °C for 24 h and reground through a 3-mm plate. Appropriate amounts of pork and fat calculated for five groups of frankfurter batters were weighed and stored at 4 °C for subsequent use on the day of frankfurter preparation. Citrus fiber (AQ Plus Citrus-F HO083) was obtained from Herbafood Ingredients GmbH (Werder, Germany). All other chemicals used in this work were from commercial sources and of analytical grade.

Preparation of frankfurters

Five groups of batters were prepared (as shown in Table 1) by chopping lean pork, fat, and other ingredients in a cutter following the procedure of Choi et al. (2014). The groups consisted of (1) normal-fat control (NFC), (2) low-fat control (LFC), (3) 1% citrus fiber-added group (CF-1), (4) 2% citrus fiber-added group (CF-2), and (5) 3% citrus fiber-added group (CF-3). Batters were vacuum stuffed into approximately 20-mm-diameter natural casings (sheep intestines) and hand-linked to form links approximately 15 cm long. The meat batters were then heated to 75 ± 2 °C for 30 min in a water bath (XMTD-6000,

Changfeng Corporation, Beijing, China) and then cooled in water (21 °C). The frankfurters were placed in polyethylene bags, vacuum packaged, and maintained below 10 °C during preparation.

Proximate composition

Compositional properties of the frankfurters were determined using Association of Official Analytical Chemists methods (AOAC 2000). The moisture contents of the samples were determined by drying each sample in an oven at 103 ± 2 °C until a constant weight was obtained. Crude protein content was calculated from the nitrogen content, as determined using Kjeldahl's method ($6.25 \times N$). Fat content was determined using a Soxhlet solvent extraction system. Ash content was determined by drying ashes in a furnace oven at 550 °C.

Cooking loss

Before and after being heated to 75 ± 2 °C for 30 min in the water bath, the weight of the frankfurters was measured to determine cooking loss, which was calculated as follows (Choi et al. 2014):

$$\text{Cooking loss (g/100 g)} = [(\text{weight of raw meat batter (g)} - \text{weight of cooked meat batter (g)}) / \text{weight of raw meat batter (g)}] \times 100.$$

Texture profile analysis

The texture profile analysis (TPA) followed the method of Shan et al. (2015). TPA was performed in triplicate on each sample at room temperature with a TMS-Pro Texture Analyzer (Food Technology Corp., Sterling, VA, USA). The 2.0×1.0 cm (diameter $\times$ length) samples were cut from the central portion of each frankfurter. A probe with a diameter of 50 mm was used. Samples were axially compressed (crosshead speed 12 mm/min) twice to 50% of

Table 1 Frankfurter formulations with varying concentrations of added fat and citrus fiber (units: g/100 g)

Treatments	Ingredients						
	Lean pork	Back fat	Citrus fiber	Ice	Salt	Sugar	NaNO ₂ , polyphosphates
NFC	50	20	0	21	6	2.5	0.5
LFC	50	10	0	31	6	2.5	0.5
CF-1	50	10	1	30	6	2.5	0.5
CF-2	50	10	2	29	6	2.5	0.5
CF-3	50	10	3	28	6	2.5	0.5

NFC normal-fat control with 20% pork back fat and without citrus fiber, *LFC* low-fat control with 10% pork back fat and without citrus fiber, *CF-1* treatment with 10% pork back fat and 1% citrus fiber, *CF-2* treatment with 10% pork back fat and 2% citrus fiber, *CF-3* treatment with 10% pork back fat and 3% citrus fiber

their original height between flat plates, and values for hardness (N), springiness (mm), cohesiveness, adhesiveness (N), and chewiness (N × mm) were recorded.

Nuclear magnetic resonance spin–spin relaxation time (T_2) measurements

Spin–spin relaxation time measurements were performed using an NMR-PQ001 (Niumag Corporation, Shanghai, China) at a measurement temperature of 32 °C. The raw sausage samples (2 g) were placed in glass tubes (15 mm in diameter) and then inserted into the nuclear magnetic resonance (NMR) probe. Carr–Purcell–Meiboom–Gill sequences were employed to measure T_2 according to the method of Villacis et al. (2008). Typical pulse parameters were as follows: τ -value (time between 90° and 180° pulses) of 200 μ s. Data from 6000 echoes were acquired as 32 scan repetitions. The repeat time between subsequent scans was 6 s. The multi-exponential decay curve was obtained from NMR relaxation processing, and multi-exponential decay analysis was performed using the Multi Exp Inv Analysis program (Niumag Corporation, Shanghai, China).

Scanning electron microscopy

Samples for scanning electron microscopy (SEM) were prepared and analyzed according to the procedure described by Haga and Ohashi (1984). Sausage samples ($3 \times 3 \times 3$ mm³) were fixed for 48 h at 4 °C in 0.1 M phosphate buffer (pH 7.0) containing 2.5% glutaraldehyde. The fixed samples were washed using 0.1 M phosphate buffer (pH 7.0) and postfixed for 5 h in 0.1 M phosphate buffer containing 1% osmium tetroxide (pH 7.0). The postfixed samples were then washed three times with 0.1 M phosphate buffer (pH 7.0) for 10 min, followed by dehydration in incremental concentrations of ethanol (30, 50, 70, 75, 80, 90, 95%, and three times with 100%, respectively) for 15 min per solution, and finally dipped into isoamyl acetate for 10 min. The dehydrated samples were then dried using supercritical carbon dioxide. The dried samples were gold-coated (approximately 10-nm-thick layer) using a high-vacuum ion sputter. Samples were observed using a scanning electron microscope (Quanta 200, FEI, Hillsboro, OR, USA) with an accelerating voltage of 15 kV and a magnification of 1000 $\times$.

Raman spectroscopy analysis

Raman spectroscopy experiments were conducted with a Jobin-Yvon Labram HR800 spectrometer (HORIBA Jobin-Yvon, Longjumeau, France) according to the method of Xu et al. (2011). A microscope equipped with a 50 $\times$ lens was used to focus the excitation laser beam (514.5 nm

excitation line of a Spectra-Physics Ar-laser; Spectra-Physics, Santa Clara, CA, USA) on the sample and to collect the Raman signal in the backscattered direction. The laser power at the sample surface was controlled to be approximately 100 mW. The laser spot diameter reaching the sample was approximately 1 μ m. During the measurement, the gel sample was spread on a glass slide, and the spectra were recorded in the range of 500–3100 cm^{−1}. Each spectrum was obtained under the following conditions: 3 scans, 60 s exposure time, 2 cm^{−1} resolution, and a sampling speed of 120 cm^{−1}/min, with data collected every 1 cm^{−1}. Spectra were smoothed, baseline leveled, and normalized against the phenylalanine band at 1003 cm^{−1} using Labspec version 3.01c (HORIBA Jobin-Yvon). The Phe v-ring band located near 1003 cm^{−1} was used as the internal standard to normalize the spectra, as it has been reported to be insensitive to the microenvironment (Li-Chan 1996). The intensity values of Raman bands from various atomic groups were determined after spectral normalization.

Statistical analysis

Statistical analysis of the results was performed using Statistical Analysis System version 8.12 (SAS Institute Inc., Cary, NC, USA). Analysis of variance was employed to determine the significance of main effects. Significant differences ($p < 0.05$) between means were identified using Duncan's multiple range test.

Results and discussion

Proximate composition

The proximate compositions of the frankfurters formulated with different amounts of fat and citrus fiber are shown in Table 2. The moisture content increased significantly as the amount of back fat added decreased from 20% to 10% ($p < 0.05$). The moisture content of the LFC was higher than that of the low-fat frankfurters containing citrus fiber ($p < 0.05$) because the fat in the low-fat samples was replaced by water and citrus fiber (Table 1). Similar trends in moisture content have been reported by Cengiz and Gokoglu (2007) when citrus fiber and soy protein concentrate were added to frankfurters with different fat levels.

The fat content was significantly lower in the frankfurters formulated with reduced-fat and citrus fiber compared with the NFC ($p < 0.05$). The overall fat contents of the frankfurters were close to the target levels of 20 and 10%. Among the different samples, those containing 3% citrus fiber had the lowest fat content. Similar results have been reported by Vural et al. (2004), who found that the fat

Table 2 Proximate composition of frankfurters with varying concentrations of added fat and citrus fiber (units: g/100 g)

Treatments	Traits			
	Moisture	Fat	Protein	Ash
NFC	61.33 ± 0.68 ^a	23.60 ± 0.82 ^c	12.34 ± 0.26 ^a	2.07 ± 0.04 ^a
LFC	69.89 ± 0.41 ^c	12.98 ± 0.61 ^b	13.16 ± 0.47 ^a	2.40 ± 0.09 ^b
CF-1	68.72 ± 0.29 ^d	12.11 ± 0.40 ^{ab}	13.08 ± 0.19 ^a	2.50 ± 0.02 ^b
CF-2	66.86 ± 0.32 ^c	11.42 ± 0.23 ^a	13.08 ± 0.02 ^a	2.68 ± 0.04 ^c
CF-3	65.80 ± 0.35 ^b	10.97 ± 0.43 ^a	12.39 ± 0.54 ^a	2.87 ± 0.01 ^d

All values are mean ± SD

^{a–c} Means within a column with different letters are significantly different ($p < 0.05$)

content was lower in frankfurters with added sugar beet fiber.

The protein content of the low-fat frankfurter samples was not significantly different from that of the NFC samples ($p > 0.05$). Choi et al. (2010) reported similar results; i.e., that replacing pork back fat with different grape seed oil levels and 2% rice bran fiber in the reduced-fat meat emulsion systems did not significantly affect the protein content. The addition of citrus fiber significantly increased the ash content of frankfurter samples ($p < 0.05$) because the citrus fiber contains higher minerals.

Cooking loss and texture profile analysis

The cooking losses of the samples are shown in Table 3. Decreasing the fat levels from 20 to 10% significantly increased cooking loss from 4.94 to 12.96% ($p < 0.05$), and the cooking loss decreased from 12.69 to 4.71% with the addition of 3% citrus fiber to low-fat frankfurters ($p < 0.05$). The cooking loss was the lowest in the low-fat frankfurters with 3% citrus fiber, but there was no significant difference between the CF-2, CF-3, and NFC samples ($p > 0.05$).

Cooking loss occurs through the release of fat and moisture, and was related to the binding ability between meat protein, fat, and moisture (Choi et al. 2009). In this study, the addition of citrus fiber improved the water-

binding ability of low-fat frankfurters. The citrus fiber was mostly composed of pectin, cellulose, and hemicellulose. The cellulose has a certain swelling capacity in water, so it can absorb more moisture. The hemicellulose has a good water-binding ability because of its branched, generally amorphous, and non-crystalline structure (Wen et al. 1998). Therefore, the higher water-binding ability of the CF-1, CF-2, and CF-3 samples absorption of water by citrus fiber water into their structure and expanding. Similar results were observed for frankfurters made with peach dietary fiber (Grigelmo-Miguel et al. 1999). This was reinforced by Kim et al. (2013), who reported that cooking loss was lower in chicken patties with added dietary fiber extracted from brewer's spent grain than that of those without fiber. Some of the earlier studies have also reported that the addition of dietary fiber from soy fiber, rice bran fiber, and oat fiber reduced cooking loss in meat products (Choi et al. 2008; Cofrades et al. 2000; Hughes et al. 1997).

Fat reduction resulted in significant decrease in the hardness and chewiness of frankfurters ($p < 0.05$), but no significant changes in cohesiveness, adhesiveness, and springiness were observed ($p > 0.05$). The hardness and chewiness decreased as the citrus fiber levels changed from 3 to 0% in the low-fat frankfurters ($p < 0.05$) (Table 3). The softer structure and lower hardness values of low-fat frankfurters may be attributed to the replacement of fat with water and incorporation of citrus fiber in low-fat

Table 3 Effects of various concentrations of added fat and citrus fiber on the cooking loss and textural attributes of frankfurters

Treatments	Traits					
	Cooking loss (%)	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N × mm)	Adhesiveness (N)
NFC	4.94 ± 0.93 ^a	20.25 ± 1.25 ^b	0.76 ± 0.01 ^a	0.66 ± 0.02 ^b	10.01 ± 0.29 ^b	1.57 ± 0.48 ^a
LFC	12.69 ± 1.20 ^c	18.71 ± 0.41 ^a	0.73 ± 0.02 ^a	0.66 ± 0.01 ^b	9.09 ± 0.22 ^a	1.58 ± 0.35 ^a
CF-1	8.56 ± 1.27 ^b	22.88 ± 0.71 ^b	0.73 ± 0.04 ^a	0.65 ± 0.01 ^b	10.73 ± 0.52 ^b	2.35 ± 0.41 ^b
CF-2	5.03 ± 0.88 ^a	29.90 ± 2.13 ^c	0.75 ± 0.01 ^a	0.66 ± 0.01 ^b	14.92 ± 1.00 ^c	1.94 ± 0.39 ^{ab}
CF-3	4.71 ± 0.62 ^a	36.91 ± 3.30 ^d	0.71 ± 0.04 ^a	0.59 ± 0.02 ^a	15.43 ± 1.82 ^{cd}	2.25 ± 0.25 ^{ab}

All values are mean ± SD

^{a–d} Means within a column with different letters are significantly different ($p < 0.05$)

frankfurters. There was no significant difference in the springiness of all samples ($p > 0.05$). The addition of citrus fiber was associated with increased adhesiveness and chewiness. These findings agreed with those reported by Salcedo-Sandoval et al. (2013), who observed that reducing the fat content by replacing pork back fat with konjac gel increased hardness, adhesiveness, and chewiness. Crehan et al. (2000) reported that reducing fat from 30 to 5% significantly decreased the hardness, cohesiveness, gumminess, and chewiness of frankfurters. Many studies have also reported that the addition of citrus fiber increased the hardness of various meat products (Backers and Noli 1997; Fernández-López et al. 2004).

Low-field nuclear magnetic resonance proton relaxation

The low-field NMR technique is useful for measuring properties such as T_2 relaxation times, which are sensitive to molecular motion, and an estimate can be made for the mobility and structural properties of different fractions of water molecules that are partially immobilized by the protein in the gel system (Han M et al. 2014). Table 4 shows the distribution of T_2 relaxation times in various samples. The NMR decay curve was fitted to three components: T_{21} , T_{22} , T_{23} . It has been suggested that the T_{21} , T_{22} , and T_{23} components, reflect water closely associated with macromolecules (bound water), water trapped within the myofibrils (immobilized water), and water outside the myofibrillar lattice (free water), respectively in meat (Han et al. 2014).

A statistically significant effect on T_2 was found with the different levels of fat and citrus fiber ($p < 0.05$). No significant effects were found on the positions and proportions of T_{21} with different amounts of fat added ($p > 0.05$). The reduction of fat led to a somewhat slower relaxation time ($p < 0.05$), as T_{22} increased from 62.36 for NFC to 71.44 ms for LFC, and T_{23} increased from 546.23

for NFC to 642.81 ms for LFC. Yasui et al. (1979) found that a faster T_2 indicated that the outer hydration layers were more effectively immobilized. A longer T_2 of water usually means higher mobility. However, adding citrus fiber led to a faster relaxation time ($p < 0.05$), as T_{22} decreased from 71.44 to 46.75 ms, and T_{23} decreased from 642.81 to 394.42 ms. The decrease was in line with increasing water-binding ability. P_{21} , P_{22} , and P_{23} were also significantly affected by the addition of citrus fiber ($p < 0.05$). Compared with the LFC, adding citrus fiber caused a significant increase in P_{21} and P_{22} , and a significant decrease in P_{23} ($p < 0.05$). This result suggested that the addition of citrus fiber could have beneficial effects in converting free water to bound and immobilized water.

The analysis revealed pronounced effects of citrus fiber on the distribution of the T_2 relaxation time, indicating that the addition of citrus fiber caused migration of water from the loosely bound water fraction to the more tightly bound water fraction, and improved the binding ability between water and meat tissue, which was generally considered to be a reason for the reduction in cooking loss.

Scanning electron microscopy

Cooked frankfurters with different fat and citrus fiber contents were selected for SEM observation, as shown in Fig. 1. Observing these microstructural changes helped to explain the textural properties and water-binding abilities of the samples.

The samples exhibited a fibrous, porous, three-dimensional network structure with cross-linked strands. Fat reduction could have caused the formation of a coarse network with capillaries where water could escape the structures (NFC and LFC in Fig. 1). Generally, gels with a fine network of protein matrix can effectively bind water and fat, resulting in a smoother texture, while gels with a coarse texture were brittle and had poor binding ability

Table 4 Effects of various concentrations of added fat and citrus fiber on the relaxation time and proportion of water in frankfurters

Treatments	Traits					
	T_{21} (ms)	T_{22} (ms)	T_{23} (ms)	P_{21} (%)	P_{22} (%)	P_{23} (%)
NFC	1.53 ± 0.27^a	62.36 ± 2.97^c	546.23 ± 19.21^c	0.5 ± 0.2^{ab}	95.17 ± 0.23^b	4.37 ± 0.06^c
LFC	1.36 ± 0.06^a	71.44 ± 2.90^d	642.81 ± 23.64^e	0.43 ± 0.21^{ab}	94.03 ± 0.42^a	5.53 ± 0.15^c
CF-1	1.30 ± 0.23^a	65.92 ± 3.24^c	592.55 ± 18.29^d	0.37 ± 0.15^a	94.77 ± 0.23^b	5.00 ± 0^d
CF-2	1.44 ± 0.47^a	55.91 ± 2.21^b	503.52 ± 26.67^b	0.7 ± 0.2^b	95.77 ± 0.25^c	3.57 ± 0.06^a
CF-3	1.70 ± 0.23^a	46.75 ± 1.31^a	394.42 ± 20.08^a	1.1 ± 0.1^c	95.1 ± 0.1^b	3.77 ± 0^b

All values are mean $\pm$ SD

T_{21} relaxation time of bound water, T_{22} relaxation time of immobilized water, T_{23} relaxation time of free water, P_{21} proportion of bound water, P_{22} proportion of immobilized water, P_{23} proportion of free water

^{a-c} Means within a column with different letters are significantly different ($p < 0.05$)

(Yang et al. 2015). Figure 1 (CF-1, CF-2, and CF-3) shows the effect of adding citrus fiber on the microstructure of low-fat frankfurters. The addition of citrus fiber converted the network structure to a compact, continuous structure with a high density of strands, which may have led to higher water-binding ability. These findings agreed with those reported by Sun et al. (2011), who observed that the presence of flaxseed gum transformed the protein emulsion into a highly interactive matrix system that was more conducive to water immobilization.

Raman spectroscopic analysis

The Raman spectra of the different frankfurter samples in the 500–3100 cm^{-1} region are shown in Fig. 2. The intensity changes in the Raman bands (Table 5) were mainly indicative of changes in the secondary structure and variations in the local environments of pork myofibrillar

proteins. The normalized intensity of the Raman band near 760 cm^{-1} was assigned to the stretching vibration of the tryptophan residues. The intensity of the 760 cm^{-1} band decreased significantly when the fat level decreased from 20 to 10% ($p < 0.05$), but increased from 0.75 to 0.85 with the addition of citrus fiber, which indicated that more tryptophan residues were exposed in the aqueous environment, and that the hydrophobic interactions were strengthened (Zhang et al. 2015). Hydrophobic interactions played an important role in maintaining the water-binding ability and textural properties of proteins.

The intensity of the doublet bands located near 830 (I830) and 850 cm^{-1} (I850) were assigned to vibrations of the para-substituted benzene ring of tyrosine residues. The tyrosyl doublet ratio (I850/I830) has been proposed to determine whether the tyrosine residue was exposed or buried in the solvent (Li-Chan et al. 1994). When the doublet bands ratio ranged from 0.90 to 2.5, or the intensity

Fig. 1 SEM of frankfurters with different fat levels and citrus fiber concentrations ($\times 1.0$ K). *MF* muscle fiber; *FG* fat globule

Fig. 2 Raman spectra in the 500–3100 cm^{-1} region from the different frankfurter samples analyzed

Table 5 Normalized intensities of the 760 cm^{-1} (tryptophan) band, 850/830 cm^{-1} (tyrosine) doublet band and 2395 cm^{-1} (C–H stretching vibrations of aliphatic residues) band of the frankfurters with varying concentrations of added fat and citrus fiber

Treatments	Traits		
	I760/I1003	I850/I830	I2930/I1003
NFC	0.81 $\pm$ 0.02 ^b	1.13 $\pm$ 0.05 ^b	1.81 $\pm$ 0.02 ^b
LFC	0.75 $\pm$ 0.02 ^a	1.04 $\pm$ 0.01 ^a	1.96 $\pm$ 0.04 ^c
CF-1	0.74 $\pm$ 0.01 ^a	1.10 $\pm$ 0.01 ^{ab}	1.76 $\pm$ 0.02 ^b
CF-2	0.77 $\pm$ 0.02 ^{ab}	1.06 $\pm$ 0.01 ^{ab}	1.42 $\pm$ 0.06 ^a
CF-3	0.85 $\pm$ 0.01 ^c	1.04 $\pm$ 0.03 ^a	1.79 $\pm$ 0.05 ^b

All values are mean $\pm$ SD

^{a–c} Means within a column with different letters are significantly different ($p < 0.05$)

of the 850 cm^{-1} band was higher than the I830, tyrosine residues were exposed to the aqueous or polar environment and acted as simultaneous acceptors and donors of moderate to weak hydrogen bonds (Kang et al. 2014). By contrast, when the ratio ranged from 0.7 to 1.0, or the I850 was less than the I830, this could be interpreted as indicator of an increase in tyrosine residues buried in a hydrophobic environment and acting as hydrogen donors (Li-Chan 1996; Xu et al. 2011). Results showed that all tyrosine doublet ratio of the frankfurters ranged from 1.04 to 1.13, indicating that the tyrosine residues of proteins were exposed to a polar environment, and acted as simultaneous acceptors and donors of moderate to weak hydrogen bonds. Compared with the NFC, the doublet bands ratio in the LFC were significantly decreased ($p < 0.05$), indicating that more tyrosine residues were buried in a hydrophobic environment and acting as hydrogen donors. There was no difference in the doublet bands ratio among low-fat frankfurters ($p > 0.05$), suggesting that there was no change in the exposure of tyrosine to the solvent with the addition of citrus fiber.

In the range between 1600–1800 cm^{-1} , the exact location of the most prominent band depends on the secondary structure of the polypeptide chain. In general, the amide I band consisted of overlapped band components falling in the 1650–1658, 1665–1680, and 1660–1665 cm^{-1} ranges, which were attributed to α -helices, β -sheets and random coil structures, respectively (Frushour and Koenig 1975). The amide I band of samples appeared between 1655–1658 cm^{-1} suggested that the secondary structure of the samples was mainly α -helices. There were no significant differences between any of the control or citrus fiber-added samples ($p > 0.05$).

In the 2700–3100 cm^{-1} region of the Raman spectrum, aliphatic amino acids, peptides and proteins exhibited C–H stretching vibrations. The intensity of the 2930 cm^{-1} band increased with the increase in polarity of the environment around the hydrocarbon chains (Larsson and Rand 1973). With the addition of citrus fiber to the frankfurters, the intensity of the 2930 cm^{-1} band decreased significantly compared with the LFC ($p < 0.05$). Some authors have suggested that the decrease in intensity of these bands stems attributed hydrophobic interactions around the aliphatic side chains (Li-Chan et al. 1994). The Raman results suggested that the citrus fiber enhanced cross-linking of the proteins and resulted in the formation of a more compact network structure, manifested by strengthening of the hydrophobic interactions in the environments, which may have led to an increase in the water-binding ability and hardness in the frankfurters.

Adding citrus fiber can change the tertiary structure of proteins and increase hydrophobic interactions around hydrocarbon chains and tryptophan residues, but the citrus fibers have no significant effect on the secondary structure of proteins. These differences may be caused by the dispersion effect of citrus fiber on the proteins in the samples (Sánchez-González et al. 2009).

Conclusion

Citrus fiber, proposed as a fat substitute, could improve the water-binding ability of low-fat frankfurters. The results of this study showed that cooking loss in low-fat frankfurters decreased from 12.69 to 4.71% with the addition of 0 to 3% citrus fiber. With the addition of citrus fiber, T_{22} decreased from 71.44 to 46.75 ms, T_{23} decreased from 642.81 to 394.42 ms, and a more compact, continuous structure in the low-fat frankfurters was also observed. The addition of citrus fiber can increase hydrophobic interactions around the hydrocarbon chains and tryptophan residues in a meat system. In the present study, the I760/I1003 increased from 0.75 to 0.85, and the I2930/I1003 decreased from 1.96 to 1.42, probably because of the citrus fiber dispersion effect.

In conclusion, citrus fiber could have beneficial effects on water immobilization by enhancing the hydrophobic interactions of meat proteins, and lead to a significant enhancement of the water-binding ability of low-fat frankfurters.

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References

- AOAC (2000) Official methods of analysis, vol 41, 17th edn. Association of Official Analytical Chemists, Washington DC
- Backers T, Noli B (1997) Dietary fibers meat processing. *Int Food Mark Technol* 12:4–8
- Cengiz E, Gokoglu N (2007) Effects of fat reduction and fat replacer addition on some quality characteristics of frankfurter-type sausages. *Int J Food Sci Technol* 42:366–372
- Choi Y et al (2008) Effects of containing dietary fiber extracted from rice bran on quality characteristics of emulsion-type sausages. *Korean J Food Sci Anim* 28:14–20
- Choi Y, Choi J, Han D et al (2009) Characteristics of low-fat meat emulsion systems with pork fat replaced by vegetable oils and rice bran fiber. *Meat Sci* 82:266–271
- Choi Y, Choi J, Han D et al (2010) Optimization of replacing pork back fat with grape seed oil and rice bran fiber for reduced-fat meat emulsion systems. *Meat Sci* 84:212–218
- Choi Y, Kim H, Hwang K et al (2014) Physicochemical properties and sensory characteristics of reduced-fat frankfurters with pork back fat replaced by dietary fiber extracted from makgeolli lees. *Meat Sci* 96:892–900
- Cofrades S, Guerra M, Carballo J et al (2000) Plasma protein and soy fiber content effect on bologna sausage properties as influenced by fat level. *J Food Sci* 65:281–287
- Crehan C, Hughes E, Troy D, Buckley D (2000) Effects of fat level and maltodextrin on the functional properties of frankfurter formulated with 5, 12 and 30% fat. *Meat Sci* 55:463–469
- Fernández-López J, Fernández-Ginés J, Aleson-Carbonell L, Sendra E, Sayas-Barberá E, Pérez-Alvarez J (2004) Application of functional citrus by-products to meat products. *Trends Food Sci Technol* 15:176–185
- Frushour B, Koenig J (1975) Raman spectroscopy of proteins. In: Clark RJH, Hester RE (eds) *Advances in infrared and Raman spectroscopy*. Heyden, London, pp 35–97
- Grigelmo-Miguel N, Gorinstein S, Martín-Belloso O (1999) Characterisation of peach dietary fibre concentrate as a food ingredient. *Food Chem* 65:175–181
- Haga S, Ohashi T (1984) Heat induced gelation of a mixture of myosin B and soybean protein. *Agric Biol Chem* 48:1001–1007
- Hallfrisch J, Scholfield D, Behall K (1995) Diets containing soluble oat extracts improve glucose and insulin responses of moderately hypercholesterolemic men and women. *Am J Clin Nutr* 61:379–384
- Han M, Wang P, Xu X, Zhou G (2014) Low-field NMR study of heat-induced gelation of pork myofibrillar proteins and its relationship with microstructural characteristics. *Food Res Int* 62:1175–1182
- Hughes E, Cofrades S, Troy D (1997) Effects of fat level, oat fiber and carrageenan on frankfurters formulated with 5, 12 and 30% fat. *Meat Sci* 45:273–281
- Kang Z, Wang P, Xu X, Zhu C, Li K, Zhou G (2014) Effect of beating processing, as a means of reducing salt content in frankfurters: a physico-chemical and Raman spectroscopic study. *Meat Sci* 98:171–177
- Kim H et al (2013) Effects of dietary fiber extracts from brewer's spent grain on quality characteristics of chicken patties cooked in convective oven. *Korean J Food Sci Anim* 33:45–52
- Larsson K, Rand R (1973) Detection of changes in the environment of hydrocarbon chains by Raman spectroscopy and its application to lipid-protein systems. *Biochim Biophys Acta Lipids Lipid Metabol* 326:245–255
- Li-Chan E (1996) The applications of Raman spectroscopy in food science. *Trends Food Sci Technol* 7:361–370
- Li-Chan E, Nakai S, Hirotsuka M (1994) Raman spectroscopy as a probe of protein structure in food systems. In: Yada RY, Jackman RL, Smith JL (eds) *Protein structure-function relationships in foods*. Chapman and Hall Inc, New York, pp 163–197
- McAfee A, McSorley E, Cuskelly G et al (2010) Red meat consumption: an overview of the risks and benefits. *Meat Sci* 84:1–13
- Muguerza E, Fista G, Ansorena D et al (2002) Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Sci* 61:397–404
- Salcedo-Sandoval L, Cofrades S, Ruiz-Capillas Perez C, Solas M, Jimenez-Colmenero F (2013) Healthier oils stabilized in konjac matrix as fat replacers in n-3 PUFA enriched frankfurters. *Meat Sci* 93:757–766
- Sánchez-González I, Rodríguez-Casado A, Careche M, Carmona P (2009) Raman analysis of surimi gelation by addition of wheat dietary fibre. *Food Chem* 112:162–168
- Shan B, Li X, Pan T et al (2015) Effect of shaddock albedo addition on the properties of frankfurters. *J Food Sci Technol* 52:4572–4578
- Sun J, Li X, Xu X, Zhou G (2011) Influence of various levels of flaxseed gum addition on the water-holding capacities of heat-induced porcine myofibrillar protein. *J Food Sci* 76:C472–C478
- Tobin B, O'Sullivan M, Hamill R et al (2013) The impact of salt and fat level variation on the physicochemical properties and sensory quality of pork breakfast sausages. *Meat Sci* 93:145–152
- Villaci M, Rastogi N, Balasubramaniam V (2008) Effect of high pressure on moisture and NaCl diffusion into turkey breast. *LWT Food Sci Technol* 41:836–844
- Vural H, Javidipour I, Ozbas O (2004) Effects of interesterified vegetable oils and sugarbeet fiber on the quality of frankfurters. *Meat Sci* 67:65–72
- Weiss J, Gibis M, Schuh V et al (2010) Advances in ingredient and processing systems for meat and meat products. *Meat Sci* 86:196–213
- Wen L, Chang K, Gallaher D (1998) Isolation and characterization of hemicellulose and cellulose from sugar beet pulp. *J Food Sci* 1(1):826–829
- Xu X, Han M, Fei Y, Zhou G (2011) Raman spectroscopic study of heat-induced gelation of pork myofibrillar proteins and its relationship with textural characteristic. *Meat Sci* 87:159–164
- Yalınkılıç B, Kaban G, Kaya M (2012) The effects of different levels of orange fiber and fat on microbiological, physical, chemical and sensorial properties of sucuk. *Food Microbiol* 29:255–259
- Yang H, Han M et al (2015) High pressure processing alters water distribution enabling the production of reduced-fat and reduced-salt pork sausages. *Meat Sci* 102:69–78
- Yasui T, Ishioroshi M, Nakano H, Samejima K (1979) Changes in shear modulus, ultrastructure and spin-spin relaxation times of water associated with heat-induced gelation of myosin. *J Food Sci* 44:1201–1204
- Zhang Z, Yang Y, Tang X, Chen Y, You Y (2015) Chemical forces study of heat-induced myofibrillar protein gel as affected by partial substitution of NaCl with KCl, MgCl₂ and CaCl₂. *CYTA-J Food* 14(2):239–247