Effect of the levels of transglutaminase in frankfurters: a physical–chemical and Raman spectroscopy study

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\textbf{ABSTRACT}

Microbial transglutaminase (MTGase) is a kind of enzyme preparation which is used in the processing of emulsified meat products for improving the quality. In this paper, the effect of MTGase content on water-holding capacity, texture and protein structure was studied by using Raman spectroscopy. The results showed that emulsion stability and cooking yield were significantly (P < 0.05) improved with increased MTGase content. But when the contents were 0.67% and 1%, the emulsion stability and cooking yield had no significant difference (P > 0.05). The hardness, springiness, cohesiveness and chewiness also were significantly (P < 0.05) improved with increased MTGase content. The result of Raman spectroscopy found that the wave peak of amide I shifted from 1658 to 1660, 1661 and 1661 cm\(^{-1}\) when added various amounts of MTGase, \(\beta\)-sheet and \(\beta\)-turn contents were significantly (P < 0.05) increased with decreased \(\alpha\)-helix content, and random coil was not a significant difference (P > 0.05); the wave peak of 3100–3500 cm\(^{-1}\) shifted from 3226 to 3228, 3229 and 3229 cm\(^{-1}\) that indicated OH stretching motions were stronger. Overall, when added appropriately, MTGase to frankfurters could change the protein conformation, improve the texture and water-holding capacity.

1. Introduction

Frankfurters are a type of frequently consumed meat products, which have enjoyed widely consumer acceptance in certain sections of the global population (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, & Jiménez-Colmenero, 2010). But traditional frankfurters have high salt level (Desmond, 2006). Excessive dietary salt intake can cause cardiovascular disease and other diseases (Askin & Kilic, 2009; Yağcı & Seker, 2016). Emulsion formation and stability of emulsified meats are markedly affected by salt content. One of salt’s main functions in emulsified meats is the solubilization of the myofibrillar proteins as it is these proteins that determine the binding and textural characteristics of the product (Pietrasik & Li-Chang, 2002).

Thus, reduction of salt in frankfurters has therefore become an important research field (Kang et al., 2014; Ma et al., 2012).

The microbial transglutaminase (MTGase; proteineglutamyl g-glutamyltransferase, EC 2.3.2.13) is an efficient and promising alternative, which can catalyze the formation of \(\varepsilon\)-(g-glutamyl) lysyl cross-links between glutamine and lysine residues. It has been used to catalyze the intra- and intermolecular transverse cross-linking of meat proteins to enhance their interactions and functionality (Chanarat & Benjakul, 2013; Han, Zhang, Fei, Xu, & Zhou, 2009; Trespalacios & Pla, 2007). MTGase has been used to improve textural properties and water-holding capacity of meat protein gel, which is one of the most important functional properties of proteins in low-salt meat systems

\textbf{KEYWORDS}

Water holding capacity; Raman spectroscopy; pork meat; secondary structure; water structure

\textbf{PALABRAS CLAVE}

Capacidad de retención de agua; espectroscopia Raman; carne de cerdo; estructura secundaria; estructura del agua

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Raman spectroscopy is a direct and noninvasive technique, which can provide information on peptide backbone conformations such as secondary and water structure of meat proteins (Li-Chan, Nakai, & Hirostuka, 1994). Due to weak background scattering from water, it is directly applicable to aqueous systems (Li-Chan, 1996; Liu, Zhao, Xie, & Xiong, 2011). This method has been used to study the structural changes of meat systems (Herrero, Cambero, Ordóñez, Hoz, & Carmona, 2008; Schmidt, Scheier, & Hopkins, 2013; Wang et al., 2013). However, as far as our knowledge, little papers reported that Raman spectroscopy was used to examine the structural changes of meat protein with various amounts of MTGase. Therefore, the objective of the present study was to determine protein structural and physical–chemical differences on low-salt frankfurters with various amounts of MTGase, and thereby to obtain an appropriate concentration of MTGase in frankfurters.

2. Materials and methods

2.1. Raw materials and ingredients

Pork leg lean meat (after 24–48 h of slaughtering, pH 5.75, 72.05% moisture, 20.10% protein, 7.34% fat) and back fat (8.50% moisture, 1.75% protein and 90.12% fat, AOAC 2000) were purchased four times in 2 days from a local meat market (Xinxiang, China). All subcutaneous and intramuscular fat and visible connective tissues were removed from the lean meat. The lean meat and back fat were passed through a grinder (JR-120, Shandong, China) fitted with a plate having 6-mm diameter holes. The ground meat (500 g each) was packaged in nylon/polyethylene bags and stored at −20°C until use within 4 weeks. MTGase (1% MTGase, 99% maltodextrin) was purchased from Dongsheng food science and technology Co. Ltd, China. The raw materials and other ingredients are shown in Table 1.

2.2. Preparation of frankfurter

Three different frankfurters were prepared with four replications at different occasions. The preparation of frankfurter was as described by Kang et al. (2014) with slight modification. Pork meat was thawed at 4°C overnight prior to use. The thawed meat was processed using a beating machine (MC-7, Shangdong, China) according to the following processing cycle: dry-weighed. The heat is processed in smokehouse (T1900 El619, Germany) according to the following processing cycle: drying for 15 min at 50°C and 60% relative humidity (RH), and steam cooking for 25 min at 80°C and 100% RH (the internal temperature of frankfurters at 72°C for 3 min), then showered for 15 min and drying for 10 min at 30°C and 60% RH and finally removed from the smokehouse and chilled at 2°C overnight.

2.3. Emulsion stability

Emulsion stability using the method of Fernández-Martin, López-lópez, Cofrads and Jiménez-Colmenero (2009) with slight modification is as follows: 25 g meat batter was placed in a 50-mL centrifuge tube and centrifuged at 500g for 15 min at 3°C to eliminate any air bubbles. Then each tube was heated in 80°C water bath for 20 min, immediately removed, uncapped and the left inverted on paper tissues to release any exudate at room temperature for 50 min. The water-released component (WR, % of the initial sample weight) was determined from the dry matter content of the total fluid release (TR) after heating at 105°C for 16 h. The TR was expressed as % of the initial sample weight. The fat-released component (FR, % of the initial sample weight) ignored any minor protein or salt components and was taken as the difference between TR and WR. Four determinations (four tubes) were made on each formulation per batch.

2.4. Cooking yield

After cooling at 2°C overnight, the frankfurters were weighed and the percentage weight yield was calculated using the following formula:

\[
\text{Cooking yield} = \frac{\text{weight of frankfurter after cooking}}{\text{weight of frankfurter before cooking}} \times 100
\]

2.5. Texture profile analysis

After cooling at 2°C overnight, the frankfurters were left at room temperature for 2 h. The texture profile analysis (TPA) attributes of the frankfurters were determined using a texture analyzer (TA-XT. plus, Stable Micro system Ltd., Surrey, UK) fitted with a cylindrical probe (P/50, 50 mm stainless cylinder). The conditions were as follows: pretest speed 2.0 mm/s, test speed 2.0 mm/s, post-test speed 5.0 mm/s, strain 50%, time 5.0 s and trigger force 5 g for TPA measurement. The frankfurters were cut in two to obtain a 20-mm depth and 20-mm diameter strips. Attributes were calculated as follows: hardness (Hd), the peak force (N) required for first compression; cohesiveness (Ch), the ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp), distance

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| Sample (g) | T1 | T2 | T3 | T4 |
|-----------|----|----|----|----|
| Pork meat | 1500 | 1500 | 1500 | 1500 |
| Back fat | 480 | 480 | 480 | 480 |
| Ice water | 408 | 408 | 408 | 408 |
| Sodium tripolyphosphate | 7.2 | 7.2 | 7.2 | 7.2 |
| Salt | 24 | 24 | 24 | 24 |
| MTGase | 0 | 8 | 16 | 24 |
| Spices | 15.6 | 15.6 | 15.6 | 15.6 |

T1: Without MTGase; T2: 0.33% MTGase; T3: 0.67% MTGase; T4: 1% MTGase. T1: Sin MTGase; T2: 0.33% de MTGase; T3: 0.67% de MTGase; T4: 1% de MTGase.
Cooking yield (%) of frankfurters with various amounts of MTGase.

2.6. Raman spectroscopic

Raman experiments were determined using a modified procedure of Shao, Zou, Xu, Wu and Zhou (2011). Spectra were smoothed, baselines corrected and normalized against the phenylalanine band at 1003 cm$^{-1}$ (Herrero, 2008; Li-Chan et al., 1994) using Labspec version 3.01c (Horiba/Jobin Yvon, Long-jumeau, France). The secondary structures of the proteins were determined as percentages of α-helix, β-sheet, β-turn and random coil or unordered conformations (Alix, Pedanou, & Berjot, 1988). With this aim, the water spectrum was subtracted from the spectra by following the same criteria as that described previously (Alix et al., 1988; Herrero, Carmona, Pintado, Jiménez-Colmenero, & Ruiz-Capillas, 2011).

2.7. Statistical analysis

The experiment was repeated four times. The data were analyzed using the one-way ANOVA program. The difference between means was considered significant at $P < 0.05$. Significant differences between means were identified by the least significant difference procedure using the statistical software package SPSS v.18.0 for windows.

3. Results and discussion

3.1. Emulsion stability

Emulsion stability is an important parameter that is critical to the stability of raw meat batter. MTGase level had significant effects ($P < 0.05$) on the stability of raw meat emulsions (Table 2). Emulsion stability of the raw meat batters is increasing with improved MTGase content from 0% to 0.67%. When the MTGase content was 1% (T4), the emulsion stability had not increased significantly ($P > 0.05$). The samples of T3 and T4 had the lowest TR, WR and FR, and T1 decreased largely. Different TR suggested the differences in water- and fat-holding capacity of gel network. MTGase and heat treatment could induce denaturation of protein structure and the subsequent ordered aggregation of protein forming a well-structured gel on protein interactions and having a good capacity to hold water (Martínez et al., 2014; Wang et al., 2013). Martínez et al. (2014) reported that gels obtained by adding 6 g/kg MTGase showed higher value of water-holding capacity than gels cooked directly. Wu, He, and Wang (2016) found that the treatment with MTGase, the emulsifying properties of chicken myofibrillar protein isolate/kidney bean protein isolate mixtures increased significantly ($P < 0.05$) and the emulsifying properties of preheated samples exceeded the non-preheated samples which incubated 240 min.

3.2. Cooking yield

The MTGase content had significant effects ($P < 0.05$) on cooking yield of frankfurters (Figure 1). The treatments of T3 and T4 had the highest cooking yield, and T1 had significantly ($P < 0.05$) lower than other treatments. When improved the enzyme concentration, the number of inter- and intra-chain peptide cross-links increased and formed good gel structure and enhanced the water- and fat-binding capacity of meat batters (Ahmmed, Nasu, & Muguruma, 2009). The cooking yield of T3 and T4 was not significantly different ($P > 0.05$), the reason is the higher the enzyme concentration, the lower the protein–water interaction, and water-holding capacity was decreased (Gaspar & Góes-Favoni, 2015). In agreement with our findings, Canto et al. (2014) reported that only added MTGase increased cooking yield of low-sodium-restructured caiman steaks. Pietrasik and Li-Chang (2002) found that MTGase addition reduced cooking loss of gels.

3.3. TPA

TPA parameters were affected by MTGase content which is presented in Table 3. Increasing MTGase levels significantly increased ($P < 0.05$) the hardness, springiness, adhesiveness and chewiness of frankfurters. The value of hardness was improved from 54.23 to 59.09 N. It was in agreement with Herrero et al. (2008), who reported that the hardness, springiness, adhesiveness and gumminess of pork meat gel

![Figure 1. Rendimiento del cocinado (%) de las salchichas Frankfurt con diferentes cantidades de MTGase.](image-url)

**Table 2. Emulsion stability of raw batters with various amounts of MTGase.**

| Sample | TR (%) | WR (%) | FR (%) |
|--------|--------|--------|--------|
| T1     | 13.33 ± 0.32$^a$ | 8.13 ± 0.16$^c$ | 5.22 ± 0.13$^c$ |
| T2     | 11.23 ± 0.28$^a$ | 6.82 ± 0.15$^c$ | 4.46 ± 0.15$^c$ |
| T3     | 9.55 ± 0.27$^a$ | 5.80 ± 0.18$^c$ | 3.79 ± 0.17$^c$ |
| T4     | 9.06 ± 0.31$^a$ | 5.55 ± 0.16$^c$ | 3.53 ± 0.15$^c$ |

$^a$Different superscripts in the same column indicate significant differences ($P < 0.05$).

$^b$Without MTGase; T2: 0.33% MTGase; T3: 0.67% MTGase; T4: 1% MTGase; TR: total fluid release; WR: water-released component; FR: fat-released component.

Each value represents the mean ± SD, n = 4.

$^c$Los diferentes superíndices en la misma columna indican diferencias significativas ($P < 0.05$).

T1: Sin MTGase; T2: 0.33% de MTGase; T3: 0.67% de MTGase; T4: 1% de MTGase. Cada valor representa el promedio±SD, n=4.
were significantly improved ($P < 0.05$) when MTGase is added. The proteins were aggregated, and protein interactions were enhanced after adding MTGase, then forming a well gel network (Gaspar & Góes-Favoni, 2015). Meanwhile, the deamidation promoted by the action of MTGase can increase the proteins' solubility (Renzetti, Bello, & Arendt, 2008). A higher level of salt-soluble protein concentrations enhanced gel network and protein matrix formation (Somboonpanyakula, Barbut, Jantawata, & Chinpraphasta, 2007) and increased the number of sites in the polypeptide chains capable of interacting during heating, allowing it to form a stable, elastic and rigid protein gel matrix (Verma, Sharma, & Banerjee, 2010).

### 3.4. Raman spectroscopy analysis

The Raman spectroscopy of frankfurters with different MTGase concentrations on the 1550–1800 and 3100–3500 cm$^{-1}$ region and quantitative analysis of the selected peaks are shown in Figures 2 and 3 and Table 4, respectively. The Raman bands were assigned according to the previous literature (Chen & Han, 2011; Li-Chan et al., 1994). The changes of frequency and intensity in the Raman bands were mainly indicative of changes in the secondary structure and water structure of the pork meat proteins.

#### 3.4.1. Changes of secondary structures

The Raman spectra of meat products in 1600–1700 cm$^{-1}$ region showed that the most prominent band has been assigned to the amide I vibrational mode, which centered near 1665 cm$^{-1}$ region (Herrero et al., 2011; Krimm & Bandekar, 1986). This Raman band could provide secondary structural information about protein (Alix et al., 1988; Herrero, 2008; Tu, 1982), which involves mainly C=O stretching and to lesser degrees C–N stretching, C=O–C=N bending and N–H in-plane bending of peptide groups. Raman spectra (Figure 2) showed a slight shift from 1658 cm$^{-1}$ to higher frequencies of the maximum intensity of this band (1660, 1661 and 1661 cm$^{-1}$) after MTGase was added to frankfurters, indicating a reduction in the $\alpha$-helical structure.

![Figure 2](image2.png)

**Figure 2.** Raman spectra of frankfurters with various amounts of MTGase in the region 1550–1800 cm$^{-1}$. T1: Without MTGase; T2: 0.33% MTGase; T3: 0.67% MTGase; T4: 1% MTGase.

### Table 4. Percentages of protein secondary structures $\alpha$-helix, $\beta$-sheet, $\beta$-turns, unordered of frankfurters with various amounts of MTGase.

| Sample | $\alpha$-Helices (%) | $\beta$-Sheets (%) | $\beta$-Turn (%) | Random coil (%) |
|--------|----------------------|-------------------|-----------------|-----------------|
| T1     | 53.52 ± 3.15$^a$     | 20.02 ± 1.26$^a$  | 15.09 ± 0.63$^a$| 10.31 ± 0.33$^a$|
| T2     | 49.39 ± 1.96$^b$     | 23.93 ± 1.38$^b$  | 16.11 ± 0.57$^b$| 10.66 ± 0.26$^b$|
| T3     | 47.01 ± 2.08$^c$     | 25.75 ± 1.42$^c$  | 16.50 ± 0.55$^c$| 10.78 ± 0.31$^c$|
| T4     | 45.82 ± 1.86$^d$     | 26.66 ± 1.41$^d$  | 16.66 ± 0.69$^d$| 10.85 ± 0.29$^d$|

**Notes:**
- $^a$ Different parameter superscripts in the same column indicate significant differences ($P < 0.05$).
- T1: Without MTGase; T2: 0.33% MTGase; T3: 0.67% MTGase; T4: 1% MTGase.
- Each value represents the mean ± SD, n = 4.
- $^b$, $^c$, $^d$, $^e$: Los diferentes parámetros en los superíndices de la misma columna indican diferencias significativas ($P < 0.05$).

The Raman spectra is sensitive to changes in the hydrogen-bonding scheme involving the peptide linkages, the amide I band consists of overlapping band components falling in the 1650–1660, 1665–1680, 1680 and 1660–1665 cm$^{-1}$ ranges, which are attributable to $\alpha$-helices, $\beta$-sheets, $\beta$-turn and random coil structures, respectively (Li-Chan, 1996; Ngarize,

### Table 3. Texture profile analysis of frankfurters with various amounts of MTGase.

| Sample | Hardness (N) | Springiness | Adhesiveness (mm) | Chewiness (N × mm) |
|--------|--------------|-------------|-------------------|--------------------|
| T1     | 54.23 ± 0.81$^d$ | 0.859 ± 0.008$^d$ | 0.681 ± 0.004$^d$ | 30.92 ± 0.72$^d$ |
| T2     | 56.86 ± 0.88$^c$ | 0.869 ± 0.007$^c$ | 0.691 ± 0.005$^c$ | 33.63 ± 0.89$^c$ |
| T3     | 57.82 ± 0.75$^b$ | 0.883 ± 0.006$^b$ | 0.708 ± 0.003$^b$ | 35.19 ± 0.91$^b$ |
| T4     | 59.09 ± 0.91$^a$ | 0.903 ± 0.005$^a$ | 0.712 ± 0.006$^a$ | 36.93 ± 0.75$^a$ |

**Notes:**
- $^d$ Los diferentes parámetros en los superíndices de la misma columna indican diferencias significativas ($P < 0.05$).
- T1: Without MTGase; T2: 0.33% MTGase; T3: 0.67% MTGase; T4: 1% MTGase.
- Each value represents the mean ± SD, n = 4.
Herman, Adams, & Howell, 2004). Thus, quantitative information about secondary structure of meat protein could be estimated from the amide I spectral profile (Alix et al., 1988). According to Table 4, there were significant effects ($P < 0.05$) of MTGase on the values of secondary structural percentages content in frankfurters, mainly decreased $\alpha$-helices and increased $\beta$-sheet, $\beta$-turn, which is consistent with the amide I spectral changes (Figure 2). Because of an increase in hydrogen bonding, more $\alpha$-helical structures could change into $\beta$-sheet structures during protein denaturation with MTGase addition 0.335–0.67% (Perisic, Afsetha, Ofstada, Hassani, & Kohler, 2013). When the MTGase addition was increased from 0.67% to 1%, there were no significant differences ($P > 0.05$) on the values of secondary structural percentages content. After the enzyme concentration reaches a certain point in meat proteins, it lowered the protein–water interaction, enhanced the inter- and intra-chain peptide cross-links and decreased the number of hydrogen bonding (Damodaran, 2010). Herrero et al. (2008) reported that it was a significant ($P < 0.05$) increase in the $\beta$-sheet and $\beta$-turn accompanied by a concomitant decrease in $\alpha$-helices when MTGase is added. Some researchers have reported positive correlations between the content of $\beta$-sheet and hardness, and $\beta$-sheet structure is the base of formed gel (Herrero et al., 2008; Shao et al., 2011). In the present study, an increased $\beta$-sheet content could improve emulsion stability (Table 2), cooking yield (Figure 1) and hardness (Table 3) of frankfurters.

3.4.2. Water structure

The Raman bands of 3100–3500 cm$^{-1}$ region could provide information about the changes of water structure in meat protein (Herrero et al., 2008; Maeda & Kitano, 1995). It is attributable to OH stretching vibration which has been related to intramolecular vibrations of hydrogen-bonded water. Figure 3 shows the Raman spectrum of frankfurters with various amounts of MTGase in 3100–3500 cm$^{-1}$ region. Compared with T1 (3226 cm$^{-1}$), it could be observed as light frequency upshifting of the band (3225 cm$^{-1}$) in frankfurters with various amounts of MTGase, T2, T3 and T4, were separately located at 3228, 3229 and 3229 cm$^{-1}$, respectively. The results indicated that hydrogen-bonded water was stronger (Socrates, 2001). It was in compliance with the results of emulsion stability (Table 2) and cooking yield (Figure 1).

Some authors have reported that the addition of MTGase promoted the G-L cross-links and lead to the formation of a strong and stable gel with better water-holding capacity in the gel network (Bönisch, Huss, Weitl, & Kulozik, 2007; Yokoyama, Nio, & Kikuchi, 2004). When the MTGase is added from 0.67% to 1%, the bands had little changes. Thus, excessive addition of the MTGase did not affect the OH stretching vibration of protein. The changes of water structure also affected the secondary structure of meat protein, the stronger hydrogen bonding promotes secondary structural transitions toward $\beta$-sheets (Mukherjee, Chowdhury, & Gai, 2007).

4. Conclusions

The additional difference levels of MTGase significantly affected the physical–chemical and protein structure of frankfurters. When the different levels of MTGase are added to frankfurters, the emulsion stability, cooking yield, hardness, springiness and chewiness were increased. When the MTGase content is increased to 0.67%, the emulsion stability and cooking yield increased and has little effects from 0.67% to 1%. But the hardness, springiness and chewiness of frankfurters increased with the increase in the MTGase content. In addition, the frankfurters with MTGase had a higher $\beta$-sheet and $\beta$-turn content and lower $\alpha$-helices content. Moreover, the frequency changes in OH stretching vibration band showed that the addition of MTGase to frankfurters generates stronger hydrogen bonds as water–protein interactions, which can be related to the enhancement of water binding. In conclusion, added MTGase from 0.33% to 1% could improve the quality of frankfurters and transform protein structure.

Disclosure statement

No potential conflict of interest was reported by the authors.

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