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Relationship of changes in quality attributes and protein solubility of ground beef under pasteurization conditions

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ABSTRACT

Cook loss and shrinkage are important parameters for evaluating quality attributes of processed meats with the most important determinant being the thermal stability of proteins in the muscle tissue. In this study, we found that these attributes correlated with a loss of soluble sarcoplasmic proteins (SSP) and soluble myofibrillar proteins (SMP) in ground beef patties during pasteurization (65–90 °C, 1–60 min). Cook loss in beef patties was linearly correlated with the area shrinkage ($R^2 = 0.95$), and with the loss of SSP ($R^2 = 0.87$), SMP ($R^2 = 0.85$), and total soluble proteins (TSP; $R^2 = 0.89$). About 60–80% of SSP and SMP were rapidly lost in the first 2–5 min of heating, then decreased at lower rate until reaching an equilibrium value of approximately 22% of SSP and 20% of SMP of their initial values. Cook loss (29–35%) and area shrinkage (19–28%) also occurred rapidly in the first 2–5 min of heating. The concentration of TSP in exudate decreased, but the net weight of TSP and percentage of TSP (2–10%) lost in the exudate increased with heating time while the sum of TSP in exudate and meat decreased.

1. Introduction

Thermal processing is the most commonly used and effective method to inactivate microorganisms, particularly pathogenic microorganisms, such as *E. coli O157:H7*, *Salmonella* spp. and norovirus, and spoilage bacteria, such as *Hafnia alvei* in beef products. In addition, thermal processing provides desirable texture and flavors mainly through protein denaturation, the Maillard reaction, and lipid oxidation. Careful control of thermal processing condition, in particular the processing time and temperature, is important to ensure that cooked beef is safe and of high quality.

The effects of thermal processing on the quality of ground beef and beef muscle tissues have been extensively studied, with most of the research focusing on how the quality attributes were affected by heating temperature, heating rate and heating time. These attributes mainly include cook loss (Goñi & Salvadori, 2010; Oillic, Lemoine, Gros, & Kondjoyan, 2011; Palka & Daun, 1999; Pan & Paul, 2001), color (Brewer & Novakofski, 1999; García-Segovia, Andrés-Bello, & Martínez-Monzó, 2007; Okayama, Fujii, & Yamanoue, 1991), microstructure (Palka, 1999; Palka & Daun, 1999), tenderness, hardness or toughness (Christensen et al., 2013; García-Segovia et al., 2007; Martens, Stabursvik, & Martens, 1982; Palka & Daun, 1999). Quality attributes, such as cook loss, color, and texture of beef products, are closely tied to the chemical and physical characteristics of the muscle proteins.

Meat proteins are comprised of myofibrillar proteins (mainly myosin and actin), sarcoplasmic proteins, and connective tissue proteins (mainly collagen and elastin). In 100 g of meat proteins, there is about 50–55 g of myofibrillar proteins, 30–35 g of sarcoplasmic proteins, and 10–15 g of connective tissue proteins (Tornberg, 2005). It is widely accepted that heat induces protein denaturation, causes shrinkages of myofibers and collagen fibers as well as gelation of sarcoplasmic proteins and soluble myofibrillar proteins, which in turn affects quality attributes such as texture and juiciness of cooked meat (Tornberg, 2005). Quality attributes of beef which is correlated with protein denaturation can be predicted to some extent by differential scanning calorimetry (Bertola, Bevilacqua, & Zaritzky, 1994; Ishiwatari, Fukuoka, & Sakai, 2013; Martens et al., 1982; Wagner & Añon, 1985). However, there is a lack of systematic studies on the quantitative relationship between changes in quality properties of meat and changes of proteins during thermal processing. Although sarcoplasmic proteins and

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myofibrillar proteins accounted for about 85–90% of total proteins in meat, there were very few studies on how these two types of proteins affect meat quality during thermal processes (Bowker & Zhuang, 2013; Li, Wang, Xu, Gao, & Zhou, 2013; Marcos, Joseph, & Mullen, 2010). Also much of the work with meat proteins during thermal processing is conducted in model system rather than in actual meat systems which are more difficult to study due to non-uniform heating leading to non-uniform protein denaturation (Ishiwatari et al., 2013).

This study was conducted to provide a better understanding of the relationship between the changes in quality attributes (including cook loss and shrinkage) and solubility of proteins (including myofibrillar and sarcoplasmic proteins) in ground beef under a wide range of pasteurization temperatures at various heating times. The information will help optimize thermal processing conditions to better control the quality of meat products.

2. Materials and methods

2.1. Sample preparation and thermal treatments

Ground beef products were purchased from local retailers, and contained 17.7–17.9 g protein, 18.2–20.4 g fat, 61.4–61.9 g moisture per 100 g meat. About 11.5 g ground beef was sealed into a cylindrical aluminum test cell (internal thickness: 5.0 mm; diameter: 50.0 mm) designed for fast heat transfer (Kong, Tang, Rasco, Crapo, & Smiley, 2007). The cell was heated in a water bath (HAAKE DC30, or Isotemp 5150 H7, Thermo Fisher Scientific, Waltham, MA, USA) at 65, 70, 75, 80, 85, or 90 °C for a specified period of time (1–60 min) (Table 1). The heating times and temperatures were selected to obtain equivalent lethality for inactivation of *Listeria monocytogen* and norovirus (Buckow, Isbarn, Knorr, Heinz, & Lehmacher, 2008; Gaze, Boyd, & Shaw, 2006; Gibson & Schwab, 2011; Ovissipour, Rasco, Tang, & Sablani, 2013). Immediately after the thermal treatment, the cell was cooled in a mixture of ice and water ($n = 4$). The come-up time, defined as the time required for the sample core temperature to reach 0.5 °C below the targeted temperature, was determined based upon the method of Ovissipour et al. (2013).

2.2. Cook loss and area shrinkage

After a thermal treatment, the liquid exudate from the meat was collected for protein analysis. Cook loss, expressed in percentage, was calculated as the ratio of the difference of weight between raw and cooked beef patty to the weight of raw beef.

A computer vision system described in detail by Pandit, Tang, Liu, and Mikhaylenko (2007) was applied to capture images of ground beef samples immediately before and after thermal treatment (Canon EOS-60D Digital camera; Canon USA Inc., Melville, NY, USA). The surface area of beef patty was determined using ImageJ software (Version 1.47a, National Institute of Health, USA). Area shrinkage was calculated as the percentage decrease in surface area following the thermal process.

Table 1
Heating temperatures and times for ground beef.

Temperature	Heating time (min:sec)							
65 °C	1	3	4	5	10	20	40	60
70 °C	2	3	4	5	7	12	20	40
75 °C	2	2:30	3	4	5	7	10	20
80 °C	1	1:30	2	2:30	3	5	12	18
85 °C	1	1:30	2	2:30	3	5	7	12
90 °C	1	1:20	1:40	2	3	4	8	12

2.3. Soluble sarcoplasmic proteins and total soluble proteins

Soluble sarcoplasmic proteins (SSP) and total soluble proteins (TSP) content in raw and thermally treated ground beef samples were analyzed based upon the method of Joo, Kauffman, Kim, and Park (1999). To determine SSP, 1 g of beef sample was mixed into 10 ml of 0.025 mol/L potassium phosphate buffer (pH 7.2, stored at 4 °C) using a homogenizer (F6/10, Fluko Equipment Shanghai Co., Ltd, Shanghai, China) at medium speed (15,000 rpm) for 15 s. The homogenized sample was transferred to a shaking incubator (IS-RDD3, Incushaker, Crystal Technology & Industries Inc, USA) set at 4 °C and 250 rpm overnight; then the homogenate was centrifuged (1500 g, CT14RD, Tabletop refrigerated centrifuge, Tianmei Biochemical Instruments Ltd, Shanghai, China) for 20 min at 4 °C. The supernatant was collected and analyzed for protein content using the Biuret method (Gornall, Bardawill, & David, 1949).

To determine TSP, 1 g beef was dispersed into 20 ml of 1.1 mol/L potassium iodide in 0.1 mol/L phosphate buffer (pH 7.2, stored at 4 °C) and treated as described above for SSP.

The soluble myofibrillar protein (SMP) content was defined as the difference between TSP and SSP since a negligible amount of soluble connective proteins would be extracted under the test conditions.

To determine the TSP in ground beef exudates, the exudate from each beef patty was collected, diluted to 10 ml with 1.1 mol/L potassium iodide in 0.1 mol/L phosphate buffer and then incubated overnight, centrifuged and analyzed for TSP following the procedure described above.

3. Results and discussion

3.1. Cook loss

As shown in Fig. 1, in general, the cook loss increased with heating time or with increasing temperature. Most of the cook loss occurred within the first 2–5 min. After 5 min of thermal treatments, cook losses for ground beef patties were: 16% (65 °C), 22% (70 °C), 28% (75 °C), 29% (80 °C), and 32% (85 °C), respectively. At 90 °C, the cook loss of ground beef reached 30% after only 3 min. The cook loss of beef patties appeared to reach an equilibrium value after extended thermal treatment, and this value increased with heating temperature. For example, the cook loss of 65 °C treated beef patties was about 29% after 60 min, while the cook loss of 85–90 °C treated beef patties was about 35% after 12 min. Variation in denaturation rate accounted for the difference in maximum cook loss of beef patties at different temperatures. Denaturation temperatures for sarcoplasmic proteins are normally between 40 and 65 °C, but could be as high as 90 °C, while denaturation temperature for myosin is 56–58 °C, for actin is 73–80 °C, and for collagen is 65–67 °C (Tornberg, 2005; Wagner & Añon, 1985). Although gelation due to denaturation of soluble proteins helps retain water, protein denaturation in meat generally leads to cook loss. A relatively low cook loss for ground beef after a prolonged heat treatment at 65 °C was due to a lower extent of protein denaturation compared to that at a higher temperature. Similar trends in cook loss were observed for salmon muscle (Kong, Tang, Rasco, & Crapo, 2007) and blue mussel (Ovissipour et al., 2013).

Kinetic functions including zero-, first-, and second-order reactions were applied to model the changes in cook loss with heating time, but no single kinetic function could fit the data well. The changes in cook loss with time at a specific temperature exhibited two phases: a rapid increase stage followed by a slow increase one until reaching a constant value, and the time required to pass the first phase was much less at a higher temperature.

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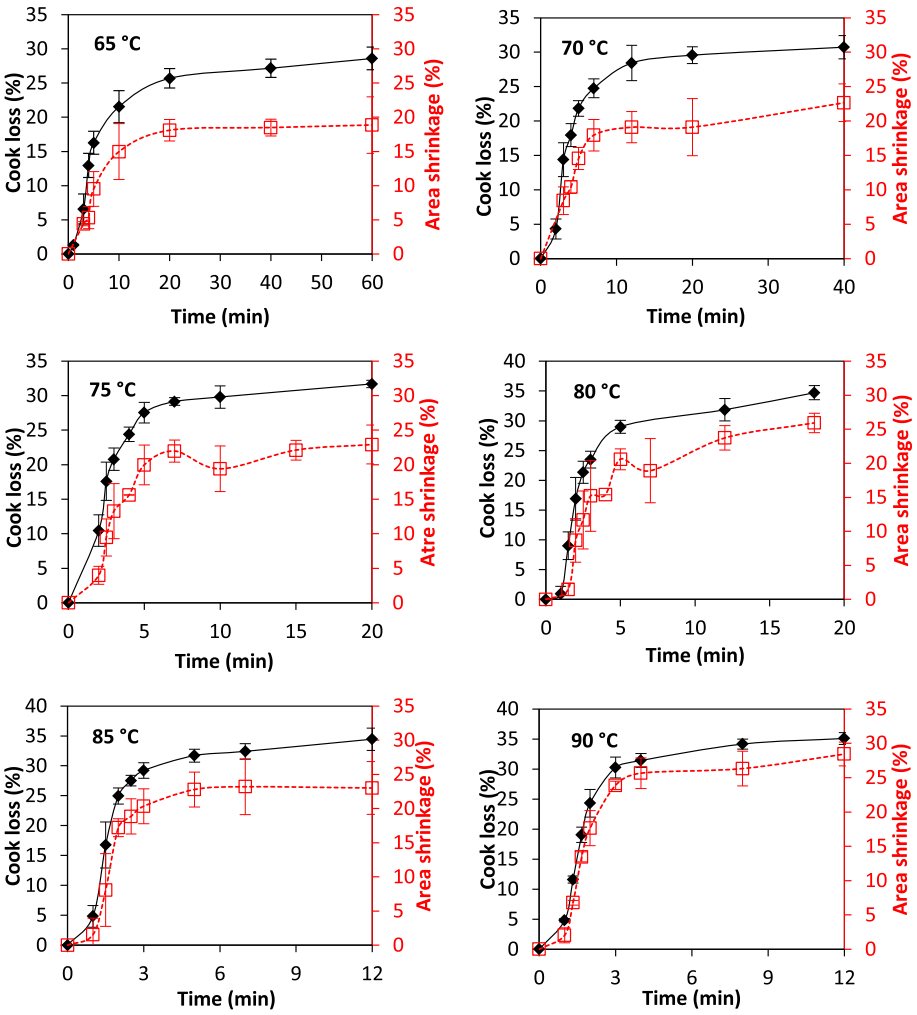

Fig. 1. Changes in cook loss (—◆—) and area shrinkage (----□----) of ground beef patties with heating time at different temperatures.

3.2. Area shrinkage

Area shrinkages of ground beef patties showed similar trend as the cook losses during thermal treatments (Fig. 1). The area shrinkage increased rapidly within the first few minutes, and then slowly increased until reaching a constant value (about 19% for 65 °C; 23% for 70–75 °C; 26–28% for 80–90 °C). In addition, the area shrinkage had a strong linear relationship (R^2 = 0.95) with the cook loss of ground beef patties (Fig. 2). Although nonlinear correlation between cook loss and area shrinkage was found for salmon fillets cooked at 100–131 °C (Kong, Tang, Rasco, Crapo, & Smiley, 2007), a positive linear relationship (R^2 = 0.74–0.99 at different temperature) was found for blue mussel heated over the same temperature range (65–90 °C) with the use of similar sample cells for heat treatments (Ovissipour et al., 2013). Protein denaturation was the underlying mechanism accounting for the area shrinkage of beef patties, as observed for cook loss.

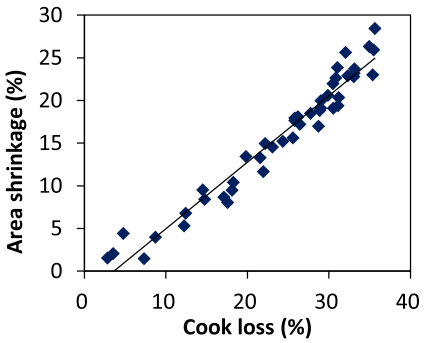

Fig. 2. Plot of cook loss versus area shrinkage during heating, R^2 = 0.951.

3.3. Soluble proteins

3.3.1. Soluble proteins in beef patties

During heating, both SSP and SMP in beef patties were lost rapidly (Fig. 3). For SSP, about 67–73% was lost at 80–90 °C, and 48–56% lost at 70–75 °C within 2 min. Similarly, 63–78% of SMP was lost at 80–90 °C, and 41–57% lost at 70–75 °C within 2 min. A higher temperature resulted in a faster decrease of SSP or SMP which reached an equilibrium value (about 22% of SSP and 20% of SMP left) in a shorter time; however, these equilibrium values were very close for heat treatments at different temperatures. What is more, the percentage changes of SSP and SMP in beef patties with time at the same temperature were very similar. Although denaturation temperatures for sarcoplasmic proteins and myofibrillar proteins vary, these differences in denaturation

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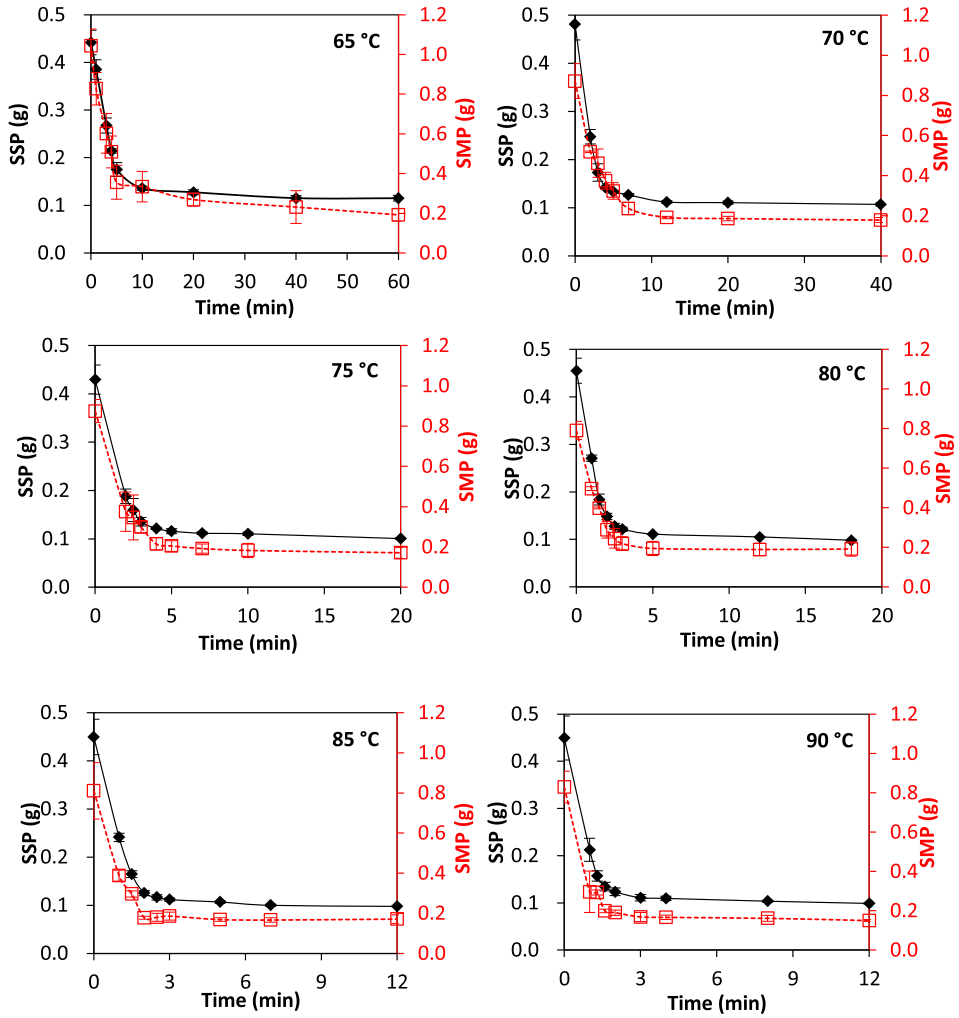

Fig. 3. Changes in soluble sarcoplasmic proteins (SSP, ——◆——) and soluble myofibrillar proteins (SMP, ----□----) in beef patties with heating time at different temperatures.

temperature were not apparent in the data for soluble sacroplasmic and myofibrillar proteins in the ground meat system studied here.

SSP was linearly related to SMP in beef patties ($R^2 = 0.85$). Linear relationship was also found between cook loss and loss of SSP ($R^2 = 0.87$), loss of SMP ($R^2 = 0.85$) or loss of TSP ($R^2 = 0.89$). To the best of our knowledge, no similar quantitative relationship between cook loss and loss of protein solubility during thermal processing have been reported, although some studies have shown a correlation between these two parameters. For example, the study of Joo et al. (1999) showed somewhat linear relationship between soluble sarcoplasmic proteins and drip loss ($R = -0.72$) for 60 pork loins varying in quality, but the effects of heating time or temperature on this relationship was not investigated. The study of Li et al. (2013) indicated that the solubility of myofibrillar proteins and sarcoplasmic proteins in duck breast muscle was affected by final cook temperatures, but the data presented were insufficient to quantitatively show the relationship between cook temperature (or time) and protein solubility.

Collagen characteristics such as solubility may be an important factor when considering the texture of beef or bull muscles processed at high temperature (such as 100–200 °C) for extended time, and various studies have been reported on the changes in collagen characteristics during thermal treatments (Christensen et al., 2011; Kong, Tang, Lin, & Rasco, 2008; Lepetit, 2008; Palka, 1999). Connective tissue proteins, accounting for only 10–15% of total proteins, play a less important role in the quality of ground beef compared to beef steaks with relatively intact muscles, and collagen solubility was not an important factor for quality properties of ground beef treated at pasteurization temperatures. In addition, current methods used to analyze soluble collagen require extraction of soluble collagen at 75–90 °C for about 120 min and the effects of this preparation method on the sample would mask the impact of the lower temperature pasteurization processes (60–90 °C, 1–60 min) evaluated here.

3.3.2. *Total soluble proteins in beef patty exudate*

Total soluble proteins in exudate increased rapidly during the first few minutes of heating, and then slowly reached a constant value which was about 25–28 mg for all heat treated samples except for those at 65 °C (maximum TSP loss was about 32 mg) (Fig. 4). In addition, the cook loss of beef patties and loss of TSP in exudates were highly linear correlated ($R^2 = 0.96$) at 70–90 °C, but the relationship between cook loss and TPS lost in ground beef heated at 65 °C was offset from that for samples treated at 70–90 °C (Fig. 5). Compared to beef patties heated at 70 °C or higher temperatures, the amount of TSP in exudates from beef patties treated at 65 °C were higher when the cook loss were similar (Fig. 5). This implies a higher concentration of TSP in exudates for 65 °C treated samples due to lower extent of protein denaturation and the

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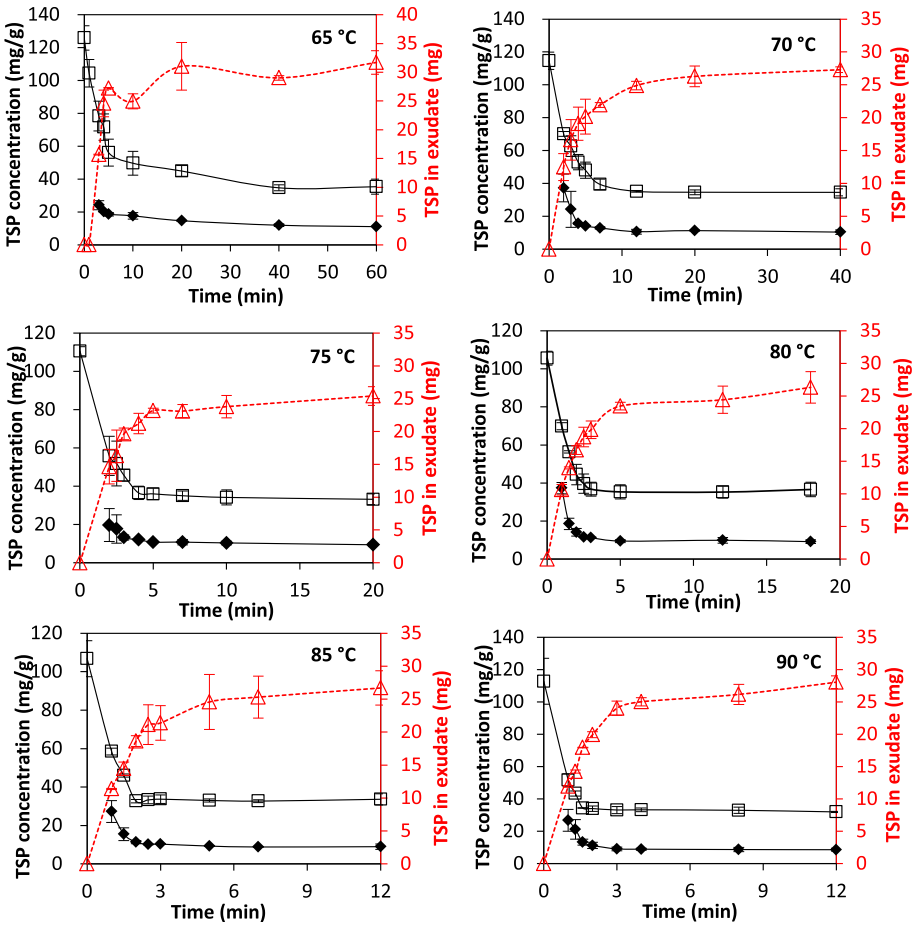

Fig. 4. Changes in the concentration of total soluble proteins (TSP) in beef patties (—□—) and exudates (—◆—) and total soluble proteins in exudates (···△···) with heating time at different temperatures.

resultant protein precipitation. Although the net weight of TSP in exudates increased with heating temperature and time, the concentration of TSP in exudates decreased, which followed similar trend as that for the change of the concentration of TSP in beef patties (Fig. 4). What is more, the concentration of TSP in a beef patty was always higher than that in its exudate, with a relatively constant difference of about 20–25 mg protein/g sample. A decrease of TSP concentration in exudates indicated that the percentage of water in the exudates increased during pasteurization, which is undoubtedly tied to the decrease of water holding capacity of protein because of heat-induced denaturation. The amounts of TSP lost in exudates were about 2–10% of the total TSP, and the percentage of TSP lost increased with cooking time while the sum of TSP in the exudate and meat decreased.

4. Conclusions

The loss of soluble proteins in beef patties or in juices was linearly correlated with the cook loss and shrinkage. Most of the loss of soluble proteins (including SSP and SMP) and the changes in quality attributes (including both cook loss and area shrinkage) occurred rapidly in the first 2–5 min of heating. The concentration

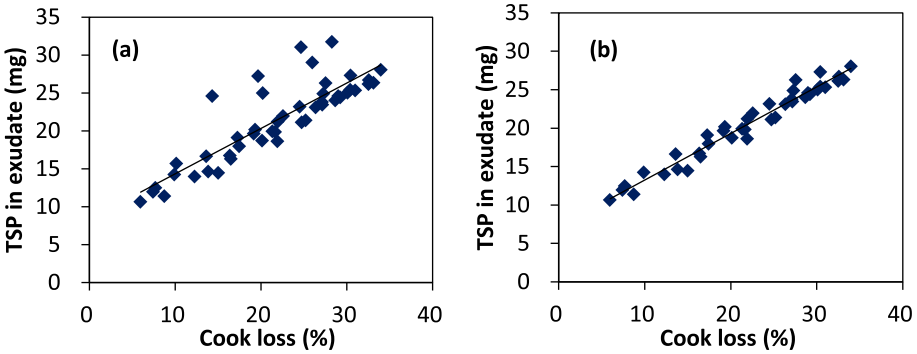

Fig. 5. Cook loss versus loss of total soluble proteins (TSP) in exudates following thermal treatments at (a) 65–90 °C, $R^2 = 0.744$, and (b) at 70–90 °C, $R^2 = 0.961$.

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of TSP in exudates decreased during pasteurization, but the net weight and percentage of TSP lost in exudate increased with time at a specific treatment temperature. No kinetic model was developed for the tested parameters because of the complex nature of the data that would have required multiple models instead of a single kinetic function to fit the data. In addition, the rapid changes of the tested attributes happened in the first 2–5 min, and the come-up time at the cold spot of ground beef patty reaching the targeted temperature in about 3.5–4 min. For samples with non-homogenous distribution of temperature like these, although it is theoretically possible to develop kinetic models, these models would be empirical and would need refinement if formulations changed.

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