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Chemical and structural characteristics of frankfurters during in vitro gastric digestion as influenced by cooking method and severity



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ABSTRACT

Cooking processes influence structural properties of foods, and may lead to differences in digestive behavior of food products. In this study, the effects of cooking method, cooking severity and digestion time on moisture, pH, texture (hardness), microstructure and effective diffusivity of frankfurters were investigated. Boiled (3, 6, 9, 12 and 15 mins) and fried (3, 6 and 9 mins) beef frankfurters were cut into cubes and digested in simulated oral and gastric conditions for up to 240 mins. Cooking method, cooking severity lowered the hardness values of the samples (p < 0.05). SEM images showed that raw and cooked samples were different from each other before and after 240 min of simulated gastric digestion. The estimated effective diffusivity of water into the boiled and fried samples were lower than that of the raw frankfurter. These results can help provide recommendations on cooking protocols of meat products that result in specific digestive outcomes.

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1. Introduction

The structural and physicochemical characteristics of foods play an important role during digestion due to enzymatic reactions and physical breakdown in the gastrointestinal system (Borreani et al., 2017; Zhang and Vardhanabhuti, 2014). Structural changes in foods that occur during processing and cooking may lead to substantial modifications in digestive properties and disintegration rates of food products (Kong et al., 2013). These differences in food structure and breakdown may influence the nutritional and functional performance of foods during digestion. By understanding food digestion processes, food functional properties can be optimized to result in specific digestive outcomes for consumers (Drechsler and Ferrua, 2016; Mat et al., 2016; Norton et al., 2014). For example, during cooking of meat, proteins are denatured, which results in shrinkage and hardening of meat tissue. In addition, hightemperature cooking causes formation of intermolecular crosslinks and aggregates. Both protein denaturation as well as crosslinking and aggregation can make proteins less susceptibility to enzymatic proteolysis and amino acid release and bioavailability

* Corresponding author. E-mail address: bozvural@karatekin.edu.tr (E.B. Ozvural). during digestion (Kaur et al., 2014). This information suggests that investigation of the specific influence of cooking process on meat digestion is warranted.

Frankfurter-type sausages are one of the most popular and widely consumed meat products around the world (Kang et al., 2016; Sousa et al., 2016; Tobin et al., 2012). Since frankfurters usually contain up to 30% fat and salt concentration ranging from 2% and higher (Sousa et al., 2016; Tobin et al., 2012), many studies have been performed in order to develop healthier and lower-cost alternatives to these products (Choi et al., 2014, 2010; Jiménez-Colmenero et al., 2010; Kang et al., 2014; López-López et al., 2009; Panagiotopoulou et al., 2016; Tobin et al., 2012). However, very few studies have been done regarding the digestion properties of frankfurters, despite their widespread consumption. There is limited information in the literature regarding textural changes and diffusion characteristics of water in these kind of sausages during gastric digestion. Previous studies in sweet potatoes have shown that the effective diffusivity of acid and moisture into food during gastric digestion is influenced by the microstructural changes that occurred during cooking, and varied in foods cooked with different methods and of varying severity (Mennah-Govela and Bornhorst, 2016a, 2016b). As such, the aims of this study were to examine the textural and microstructural changes of boiled and fried frankfurters with different cooking severity and to estimate the



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effective diffusivity of water into these products during simulated gastric digestion. This information can be used by food producers and consumers to optimize the processing and cooking of meat products to control food breakdown during digestion and provide consumers with specific digestive benefits.

2. Materials & methods

2.1. Raw material

Beef frankfurters were purchased from a local supermarket (Davis, CA, USA) and stored at 4 °C. For all experiments, the same brand of frankfurters (Ball Park) was utilized. The frankfurter composition, as indicated on the package for 1 frankfurter (53 g) was: 15 g total fat (6 g saturated fat, 1 g trans fat), 4 g total carbohydrate, 6 g protein, 30 mg cholesterol, 510 mg sodium, and 230 mg potassium.

2.2. Cooking of frankfurters

Two groups of frankfurters were prepared using different cooking methods. In the first group, frankfurters were boiled in distilled water for 3, 6, 9, 12 or 15 min and strained from the water after cooking. In the second group, frankfurters were deep-fried for 3, 6 or 9 min. After 9 min of frying, frankfurters were burned, so this was the longest frying time tested. Raw (uncooked) sample was used in both of the groups for comparison. Crust formation occurred, particularly in fried frankfurters. This crust layer was removed from the sausages to obtain homogeneous products. After cooking, frankfurters were cut into cubes (approximately 0.007 m \times 0.007 m x 0.007 m) in order to standardize the samples.

2.3. In vitro digestion of frankfurters

2.3.1. Simulated saliva and gastric fluid formulations

Simulated saliva was formulated by dissolving the following in deionized water: 1 g/L of mucin (Type II, Sigma-Aldrich, catalog number M2378, St Louis, MO, U.S.A.), 1.8 g/L of α -amylase (from Bacillus subtilis, MP Biomedicals, catalog number 100447, activity of 160,000 BAU/g, Solon, OH, USA), 0.117 g/L of NaCl, 0.149 g/L of KCl, and 2.10 g/L of NaHCO₃. The solution was adjusted to pH 7 using 0.01 N NaOH. Simulated gastric juice was formulated by dissolving the following in deionized water: 1.5 g/L of mucin, 8.78 g/L of NaCl, and 1.0 g/L of pepsin (from porcine gastric mucosa, MP Biomedicals, Solon, OH, USA, measured activity of 242 U/mg). The pH of the simulated gastric juice was adjusted to 1.8 using 1 N HCl. Both solutions were prepared without enzymes and stored at 4 °C, and the enzymes (α -amylase or pepsin) were added into the solutions immediately before digestion experiments (Mennah-Govela and Bornhorst, 2016a).

2.3.2. Oral and gastric digestion conditions

Frankfurter cubes (approx. 20 g) were placed in a 250 mL beaker. For simulated oral digestion, previously prepared saliva solution (0.2 mL saliva/g sample) was added to the samples and agitated gently for 30 s. After mixing, 100 mL of gastric juice (preheated to 37 °C) was added into each beaker, the beakers were covered, and placed in a shaking water bath (37 °C, 100 rpm) for up to 240 min.

2.4. Moisture, pH and texture values of the frankfurters

Samples (cubes) were analyzed for moisture and pH after digestion times of 0 (no digestion), 30, 60, 90, 120, 180, and 240 min. Moisture of the samples was calculated by oven drying method according to AOAC (1990). For pH analysis, one cube from

each sample was mixed in 12.5 mL of deionized water and homogenized at 10000 rpm for 30 s (IKA T18 Ultra Turrax, Wilmington, NC, U.S.A.). The pH of the homogenized samples were read using a pH-meter (HANNA instruments, Woonsocket, RI, U.S.A). Both moisture and pH values were measured in quadruplicate in four separate digestion experiments.

Hardness values were determined before (0 min) and after (240 min) simulated gastric digestion using a TA.HD Plus Texture Analyzer (Texture Technologies Corp., Hamilton, MA, U.S.A.). For texture analysis, one cube was compressed by a 0.045 m diameter cylinder probe with a test speed of 0.001 m/s to 0.035 m (50% strain) (Mennah-Govela and Bornhorst, 2016b). During each digestion experiment, eight cubes were analyzed for each treatment.

2.5. SEM analysis

Structural changes in the boiled and fried frankfurters were viewed by scanning electron microscopy (QUANTA 400F Field Emission SEM) before and after 240 min gastric digestion. The samples were coated by gold under vacuum and the images were observed using $100 \times$ magnification at 10 and 20 kV voltage.

2.6. Diffusion model for estimation of effective diffusivity

Fick's second law was used to estimate the effective diffusivity (D_{eff}) of water into the frankfurters. As the frankfurters were cut into 0.007 m \times 0.007 m x 0.007 m symmetrical cubes, the equation was modified according to Neumann's rule for infinite slab as shown in Eq. (1) (Markowski et al., 2009; Mennah-Govela and Bornhorst, 2016a).

$$\frac{C-C_e}{C_0-C_e} = \frac{8^3}{\pi^6} \left[\sum_{n=1}^{\infty} \frac{1}{(2n+1)^2} exp\left(-(2n+1)^2 \frac{D_{eff,\pi^2,t}}{L^2} \right) \right]^3$$
(1)

Where: C is the concentration of water (g H₂O/g dry matter (DM)), C₀ is the initial concentration (g H₂O/g DM), C_e is the equilibrium concentration (g H₂O/g DM), L is the length of frankfurter cube (m) and t is the digestion time (s). Although the length of the cube varied slightly after cooking, these differences were neglected and L was taken as 0.007 m for the calculations for all cooking treatments. The following assumptions were utilized in developing the model: the system is isothermal (37 °C), the size and shape of the cubes was constant during digestion, the gastric medium was homogenous and isotropic, effective diffusivity was independent of time, mass transfer was occurred by only diffusion. For the mathematical modelling, nonlinear regression function based on ordinary least squares was used to fit the model in MATLAB R2009b.

2.7. Statistical analysis

Statistical evaluation of all the data was performed using a oneway ANOVA in Minitab 16 software. When main effects were significant, Tukey's test was used to determine the differences between individual mean values. Significance was assessed at p < 0.05.

3. Results and discussion

3.1. Moisture and pH changes during in vitro gastric digestion

Moisture and pH values of the frankfurters during simulated gastric digestion are given in Tables 1 and 2, respectively. For both boiled and fried frankfurters, moisture content was significantly

Table 1

Moisture contents (% wet basis) of boiled and fried frankfurter	rs during 240 min of in vitro gastric digestion.	. Values represent averages $(n = 4) \pm$ standard deviation.
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Treatment	Digestion time ((minute)					
	0	30	60	90	120	180	240
R	49.23 ^{aB}	58.04 ^{aA}	58.59 ^{abA}	57.83 ^{bA}	58.05 ^{abA}	57.46 ^{bA}	57.90 ^{abA}
	±0.17	±0.51	±1.07	±0.80	±0.67	±0.27	±0.58
B3	48.57 ^{abb}	59.02 ^{4A}	59.38 ^{abA}	59.57 ^{bA}	59.42 ^{abA}	58.64 ^{abA}	57.68 ^{abA}
	±0.33	±1.97	±2.02	±0.89	±0.48	±0.73	±0.59
B6	47.75 ^{abcB}	57.93 ^{aA}	57.40 ^{abA}	57.54 ^{bA}	58.95 ^{abA}	57.48 ^{bA}	57.49 ^{abA}
	±0.14	±1.29	±1.15	±0.79	±1.79	±0.61	±1.41
B9	47.63 ^{abcB}	56.30 ^{aA}	58.29 ^{abA}	59.01 ^{bA}	56.72 ^{bA}	57.88 ^{bA}	57.30 ^{abA}
	±0.62	±0.42	±2.36	±0.68	±2.74	±1.89	±1.01
B12	47.65 ^{abcB}	57.92 ^{aA}	57.68 ^{abA}	57.64 ^{bA}	57.57 ^{abA}	57.49 ^{bA}	57.22 ^{abA}
	±0.45	±1.97	±1.56	±0.47	±2.07	±1.39	±1.58
B15	46.42 ^{bcB}	56.91 ^{aA}	56.72 ^{bA}	57.35 ^{bA}	57.69 ^{abA}	57.68 ^{bA}	57.40 ^{abA}
	±0.46	±0.59	±0.68	±0.88	±0.95	±1.87	±1.33
F3	45.96 ^{cB}	57.85 ^{aA}	58.33 ^{abA}	57.90 ^{bA}	57.51 ^{abA}	57.96 ^{abA}	58.05 ^{bA}
	±0.86	±1.27	±0.43	±1.27	±0.54	±0.63	±1.06
F6	41.62 ^{dB}	58.84 ^{aA}	58.47 ^{abA}	59.38 ^{bA}	58.93 ^{abA}	58.77 ^{abA}	60.07 ^{abA}
	±1.15	±1.21	±1.32	±0.86	±1.64	±0.19	±1.21
F9	31.14 ^{eC}	58.11 ^{aB}	60.43 ^{aAB}	61.97 ^{aAB}	61.38 ^{aAB}	60.93 ^{aAB}	62.53 ^{aA}
	±2.11	±2.27	±1.20	±1.54	±2.13	±1.94	±1.76

R: Raw frankfurter.

B3, B6, B9, B12 and B15: Frankfurters boiled for 3, 6, 9, 12 or 15 min, respectively.

F3, F6, F9: Frankfurters fried for 3, 6 or 9 min, respectively.

 a^{-e} Means within each column followed by different superscript letters are significantly different (p < 0.05).

 $^{A-C}$ Means within each line followed by different superscript letters are significantly different (p < 0.05).

Table 2

pH values of boiled and fried frankfurters during 240 min of in vitro gastric digestion. Values represent averages (n = 4) ± standard deviation.

Treatment	Digestion time (minute)						
	0	30	60	90	120	180	240
R	5.71 ^{dA}	4.78 ^{dB}	4.79 ^{bB}	4.55 ^{eC}	4.32 ^{fD}	4.28 ^{eE}	4.17 ^{deF}
	±0.01	±0.02	±0.01	±0.03	±0.01	±0.01	±0.01
B3	5.73 ^{cdA}	5.02 ^{bB}	4.83 ^{aC}	4.85 ^{aC}	4.46 ^{eD}	4.34 ^{dE}	4.32 ^{bE}
	±0.01	±0.02	±0.01	±0.01	±0.03	±0.01	±0.01
B6	5.74 ^{bcA}	5.11 ^{aB}	4.71 ^{cD}	4.81 ^{bC}	4.47 ^{deF}	4.51 ^{aE}	4.32 ^{bG}
	±0.01	±0.01	± 0.00	±0.01	± 0.00	±0.02	±0.01
B9	5.73 ^{cdA}	5.13 ^{aB}	4.83 ^{aC}	4.62 ^{dD}	4.50 ^{cE}	4.41 ^{cF}	4.27 ^{cG}
	±0.02	±0.01	±0.01	±0.02	±0.01	±0.01	±0.01
B12	5.77 ^{bA}	4.99 ^{bB}	4.82 ^{abC}	4.72 ^{cD}	4.64 ^{aE}	4.47 ^{bF}	4.41 ^{aG}
	±0.01	±0.01	±0.01	±0.01	±0.01	±0.01	±0.02
B15	5.77 ^{bA}	5.00 ^{bB}	4.69 ^{cC}	4.60 ^{dD}	4.60 ^{bD}	4.47 ^{bE}	4.25 ^{cF}
	±0.01	± 0.00	±0.01	±0.02	±0.01	±0.01	±0.01
F3	5.88 ^{aA}	4.77 ^{dC}	4.80 ^{bB}	4.69 ^{cD}	4.47 ^{cdeE}	4.28 ^{eF}	4.13 ^{efG}
	±0.01	±0.01	±0.01	±0.01	±0.01	±0.02	±0.01
F6	5.85 ^{aA}	4.87 ^{cB}	4.70 ^{cC}	4.45 ^{fE}	4.50 ^{cdD}	4.29 ^{eF}	4.19 ^{dG}
	±0.01	±0.03	±0.01	±0.01	±0.02	±0.01	±0.01
F9	5.77 ^{bA}	4.70 ^{eB}	4.51 ^{dC}	4.34 ^{gD}	4.31 ^{fD}	4.30 ^{eD}	4.12 ^{fE}
	±0.02	±0.01	±0.03	±0.01	±0.01	±0.02	± 0.05

R: Raw frankfurter.

B3, B6, B9, B12 and B15: Frankfurters boiled for 3, 6, 9, 12 or 15 min, respectively. F3, F6, F9: Frankfurters fried for 3, 6 or 9 min, respectively.

 $^{\rm a-g}$ Means within each column followed by different superscript letters are significantly different (p < 0.05).

 $^{A-G}$ Means within each line followed by different superscript letters are significantly different (p < 0.05).

influenced by cooking method, cooking severity and digestion time (p < 0.05). After cooking, moisture content of boiled samples were not significantly (p > 0.05) influenced by longer boiling time. However, with longer frying time, the initial moisture content significantly decreased (p < 0.05) from 45.96%, wet basis after 3 min frying to 31.14%, wet basis after 9 min frying. After 30 min of simulated gastric digestion, all samples, regardless of cooking treatment, had similar moisture content values (p > 0.05). This indicates that the samples with lower initial moisture content (e.g. severely fried) had a greater rate of initial moisture absorption during the first 30 mins of digestion. A similar trend was observed

by (Dalmau et al., 2017) when they examined the simulated gastric digestion of apples; those samples that had a lower initial moisture content (e.g. dried and frozen) had greater increases in moisture content compared to raw apple samples during the first 30-60 min of simulated gastric digestion. In boiled and fried frankfurters, moisture content increased after 30 mins, but did not show significantly different values between 60 and 240 min of digestion (p < 0.05), except severely fried treatment (F9).

Cooking severity and digestion time significantly influenced the pH values of the samples during simulated digestion of both boiled and fried frankfurters (p < 0.05). Unlike moisture changes, there was a pronounced decrease in pH values after 30 min gastric digestion, with a continued decrease during the rest of the 240 min gastric digestion period for both boiled and fried frankfurters (p < 0.05). The pH values observed in this study are in a similar range to what has been observed in vivo (using pigs as a model for an adult human) after consumption of another high protein, high lipid food product (almonds). In this study, they observed decreases from an initial pH of ~6.5 to a pH of 4.5-5.2 in the proximal stomach region after 300 min of gastric digestion of raw and roasted almonds (Bornhorst et al., 2013). In both this study, as well as previous works on high protein and high lipid foods (Bornhorst et al., 2013), the pH during gastric digestion was higher than may be traditionally described (\sim 1.5–3). This may be the result of the higher buffering capacity of these products (e.g. frankfurters, almonds) due to their protein and lipid content. However, the relationship between food buffering capacity and pH change during food gastric digestion is an area that requires future investigation.

Water and acid transfer into food during digestion have recently been examined in different food products in order to help understand changes structural breakdown and nutrient uptake in different food products. These studies have focused on the effects of different cooking methods and cooking severity which may be commonly used either in household cooking or in the food industry. Cooking method (boiling, frying, microwave heating, roasting etc.) and cooking severity (the time of cooking process) affects the structure of foods, and these changes in the structure may influence the transfer, bioaccessibility and bioavailability of nutrients (Kaur et al., 2014; Simonetti et al., 2016; Zeng et al., 2016) Mennah-Govela and Bornhorst (2016a) examined acid and moisture uptake in steamed and boiled cubes of orange-fleshed sweet potato. They found that digestion time significantly influenced moisture and total acidity, but cooking severity only affected moisture uptake (p > 0.0001). Chen et al. (2011) loaded non-roasted and roasted peanuts into a vessel containing gastric juice. They found that pH of the gastric juice (including peanuts) showed an initially rapid increase probably due to immediate consumption of acid and quick digestion action in the digestion process, but afterwards the value tended to ascend slowly. Although the pH change was just the opposite compared to our study, the pH behavior may be similar to the results of the current study, as this previous work recorded the pH of gastric solution with samples, but in the current study, the pH of the samples during simulated digestion was measured.

3.2. Texture values

Hardness was measured to determine the textural characteristics of the frankfurters before and after simulated gastric digestion (Table 3). Cooking method and severity significantly affected the hardness values (p < 0.05). In boiled frankfurters, cooking severity reduced the hardness of the frankfurters from 7.0 to 6.5 N (average of all cooking times) compared to a decrease from 10.1 to 6.7 N for raw frankfurters. In fried products, cooking severity caused the structure to soften, because the initial hardness of F9 was significantly lower than the hardness of F3 (p < 0.05) probably due to the higher fat content of F9. However, the hardness after 240 min of gastric digestion was only significantly different (p < 0.05) between the value of the cooked sample with the value after digestion in raw and boiled for 6 min treatments. For fried frankfurters, there were no significant differences between the hardness values of 0 and 240 mins of simulated gastric digestion (p > 0.05). Interestingly, there was a trend in the percent change of the mean hardness value

Table 3

Texture values (hardness and compression work) of boiled and fried frankfurters before (0 min) and after 240 min in vitro gastric digestion. Values represent averages $(n = 8) \pm$ standard deviation.

Treatment	Digestion time (min)		% change of mean hardness	
	0	240	values between 0 and 240 min	
	Hardness (N)	Hardness (N)		
R	10.06 ^{aA}	6.71 ^{bcB}	33.30	
B3	±0.94 8.26 ^{bcA}	±1.06 7.23 ^{abA}	12.47	
BG	±1.24 7.14 ^{cdeA}	±0.82 6.14 ^{bcB}	14.01	
	±0.65	±0.72		
B9	6.92 ^{cdex} +0.91	6.81 ^{5CA} +0.52	1.59	
B12	6.52 ^{deA}	6.51 ^{bcA}	0.15	
B15	±0.79 6.01 ^{eA}	±0.43 5.70 ^{cA}	5.16	
F3	±0.51 9.30 ^{abA}	±1.21 8.74 ^{aA}	6.02	
70	± 1.00	±1.26		
F6	7.96 ^{bcarr} ±0.93	7.33 ^{ab/r} ±0.91	7.91	
F9	7.20 ^{cdeA}	5.95 ^{bcA}	17.36	
	± 1.69	± 1.20		

R: Raw frankfurter.

B3, B6, B9, B12 and B15: Frankfurters boiled for 3, 6, 9, 12 or 15 min, respectively. F3, F6, F9: Frankfurters fried for 3, 6 or 9 min, respectively.

 $^{\rm a-e}$ Means within each column followed by different superscript letters are significantly different (p < 0.05).

^{A-C} Means within each line followed by different superscript letters are significantly different (p < 0.05).

from not digested samples to those digested for 240 min with respect to cooking severity. With the exception of samples that were boiled for 15 min, with increasing boiling time (e.g. severity), the percent change in hardness decreased, from 12.5% change for samples boiled for 3 min to 0.15% change for samples boiled for 12 min (Table 3). The opposite trend was observed in the fried samples: the frankfurters that had a greater frying time showed a greater decrease in hardness after simulated gastric digestion. The frankfurters that were fried for 3 min had a 6% decrease in hardness compared to a 17.4% change in hardness for the frankfurters that were fried for 9 min. These trends may be the result of structural changes that occur in the samples during cooking. The hardness change in boiled frankfurters is similar to what was previously observed in boiled sweet potatoes that were boiled for 6 (mild) or 20 (severe) minutes. In this study, they observed that the samples that were boiled for a shorter time had a greater change in hardness during 240 min of in vitro gastric digestion compared to those that were boiled for a longer time (Mennah-Govela and Bornhorst, 2016a).

Previously published works have also examined texture changes in food during simulated gastric digestion. In a study regarding the digestive behavior of commercial cheeses with different textures, it was shown that cheese disintegration was influenced by cheese texture and composition. A soft cheese with lower initial hardness and cohesiveness exhibited fast digestibility due to attenuation of matrix forming forces by pepsin hydrolysis while a hard cheese was disintegrated slowly (Fang et al., 2016). Mennah-Govela and Bornhorst (2016b) cooked sweet potatoes using different cooking methods (boiling, steaming, microwave seaming and frving) and examined the mass transport and textural characteristics of the products after 240 min of in vitro gastric digestion. It was indicated that moisture uptake and hardness were significantly influenced by cooking method and digestion time. Kong and Singh (2009) examined characteristics of raw and roasted almonds in simulated gastric environment and suggested that hardness values of raw and roasted almonds were approximately similar before soaking into simulated gastric juice, but after soaking both of the hardness values decreased and hardness of the roasted almond was lower than the raw sample, likely due to cellular and structural disruption. These are similar to the results of the current study, as there were differences between the texture changes of boiled and fried frankfurters, which varied with cooking severity. The raw and boiled (6 min) frankfurters showed significant changes in hardness during digestion, which may have been due to structural breakdown and the added moisture content into the matrix. However, the other boiled treatments and the fried frankfurters did not show significant decreases in hardness after 240 min. This may indicate that the soft meat structure of the fried frankfurters did not significantly change during digestion. However, these results may have been influenced by the removal of the crust after cooking, which may increase hardness and act as a barrier for extensive moisture and acid uptake by samples.

3.3. Microstructural images

Morphological changes of the frankfurters after 240 mins of in vitro digestion were observed by SEM. Different magnitudes and voltages were tested in preliminary analyses in order to obtain the most clear and appropriate images. In Fig. 1 images of only the raw and extreme treatments were given for both boiled and fried frankfurters as examples of the general trends that were observed in all samples. As seen in the images, the structure of the raw and the heat treated samples were different from each other both before and after 240 min gastric digestion. Before digestion, the structure of the raw frankfurter was rough and heterogeneous with



Raw

B15

F3

Fig. 1. SEM results of some boiled and fried frankfurters before and after simulated gastric digestion.

large holes and deep crevices (see arrows in Fig. 1). After boiling for three minutes, the deep cracks and cavities were closed and the surface became smoother. The surface of the 15 min boiled sample (severe heat treatment) was smoother and less porous than the raw frankfurter. Despite this general similarity, small holes appeared, which may have resulted from the decomposition of the structure during the longer heat treatment. Frying of frankfurters also influenced the surface microstructure. After 3 min of frying, large holes and deep cracks tended to disappear. After an increase in frying time up to 9 min, the structure was smoother, similar to the samples that were boiled for longer times. These changes in initial surface microstructure for the fried samples may also be the result of greater absorption of frying oil during cooking.

The microstructure of undigested and digested of each treatment also had visible differences. The gaps seen in the raw sausage shrank or disappeared after in vitro gastric digestion and the surface appeared less porous. Unlike the raw sample, cracks and cavities formed in the 3 min boiled samples were still present after digestion. In contrast, there were no visible difference in smoothness or roughness of the 15 min boiled samples after digestion compared to the cooked, undigested sample. Digestion also changed the structure of both fried treatments. The surface microstructure of the 3 min fried samples appeared to be smoother with fewer cracks, while the 9 min fried samples appeared more spongy and granular after 240 min of simulated gastric digestion. In a study that examined the in vitro gastric digestion of apples, raw apples had many well-arranged pores in a heterogeneous and anisotropic pattern, but after 180 min of digestion these pores disappeared, similar to the current study. The researchers suggested that this alteration was due to significant cell lysis and increases in the intercellular space between remaining cells (Dalmau et al., 2017).

3.4. Diffusion model

The effective diffusivities and equilibrium concentration of water in both boiled and fried frankfurters after different cooking times are given in Table 4. The effective diffusion coefficient of water (D_{eff}) was found to be $2.66 \times 10^{-9} \text{ m}^2/\text{s}$ for raw frankfurters. In boiled frankfurters, the effective diffusivities ranged from $5.52 \times 10^{-10} \text{ m}^2/\text{s}$ to $2.56 \times 10^{-9} \text{ m}^2/\text{s}$. For fried frankfurters, the effective diffusivities ranged from $3.38 \times 10^{-9} \text{ m}^2/\text{s}$. The D_{eff} value for raw frankfurter was higher than the values of both boiled and fried frankfurters. This may be because the structure of frankfurters became less porous, as was observed in the SEM study. These changes are likely due to the differences between cooking treatments (boiled vs. fried) and

Table 4

Effective diffusivity (D_{eff}) and equilibrium concentration (C_e) of water in boiled and fried frankfurters during 240 min of in vitro gastric digestion.

Treatment	Deff (m2/s)	C_e (g H ₂ O/g DM)	R ²
R	2.66×10^{-09}	1.39	0.96
B3	1.51×10^{-09}	1.43	0.95
B6	2.56×10^{-09}	1.38	0.97
B9	1.58×10^{-09}	1.33	0.97
B12	$5.52 imes 10^{-10}$	1.45	0.98
B15	1.88×10^{-09}	1.35	0.97
F3	1.38×10^{-09}	1.39	0.95
F6	1.64×10^{-09}	1.38	0.96
F9	8.26×10^{-10}	1.68	0.99

R: Raw frankfurter.

B3, B6, B9, B12 and B15: Frankfurters boiled for 3, 6, 9, 12 or 15 min, respectively.

cooking severity within each treatment. Absorption of frying oil probably increased the fat content of the products, so the high fat content in the medium may have also limited the water transfer in fried sausages. There is limited information about water diffusion coefficient of foods during simulated gastric digestion in the literature. Mennah-Govela and Bornhorst (2016a) estimated effective water diffusivity for water in mild boiled and severely boiled sweet potatoes in the range of 3.52×10^{-10} m²/s and 3.81×10^{-10} m²/s, respectively. In another similar study, the D_{eff} of fried orange-fleshed sweet potato was found to be 0.2×10^{-9} m²/s which was lower than that of the boiled samples 1.0×10^{-9} m²/s (Mennah-Govela and Bornhorst, 2016b). Water diffusivity values for both of these previous studies had similar order of magnitudes to the current study.

Equilibrium water concentrations (Ce) of the all treatments were estimated. Ce for the raw frankfurters was estimated to be 1.39 g H_2O/g DM and the C_e for the boiled frankfurters was between 1.33 and 1.45 g H₂O/g DM. There were not large differences among the Ce values of boiled frankfurters, but in fried samples the most severe fried treatment (9 min fried) had the highest Ce. The values for equilibrium water concentration are similar in magnitude to previously reported results of water uptake during simulated gastric digestion. Mennah-Govela and Bornhorst (2016a) calculated Ce for mild and severely boiled sweet potatoes as 2.27 and 2.29 g H₂O/g DM, respectively. Also, Ce of boiled and fried orange-fleshed sweet potato was determined as 2.2 g H₂O/g DM and 1.9 g H₂O/g DM (Mennah-Govela and Bornhorst, 2016b). For the frankfurters, the initial porosity and water holding capacity of the samples may have influenced the Ce, but further investigations are needed to elucidate the variation of C_e between the different cooking treatments.

4. Conclusions

Frankfurters were prepared using different cooking methods (boiling and frying) and cooking severities (3–15 min cooking time) in order to compare the influence of preparation method on in vitro digestive behavior. Boiling and frying severity decreased the moisture uptake of the frankfurters. Moisture content of boiled and fried frankfurters increased up to 30 min and then became stable for the remainder of the 240 min digestion period. In contrast, the pH values of both boiled and fried frankfurters rapidly decreased up to 30 min of simulated gastric digestion, and continued to decline slowly between 60 and 240 min gastric digestion. In boiled frankfurters, cooking severity reduced the hardness of the frankfurters, but only raw and 6 min boiled samples significantly softened after gastric digestion. In fried frankfurters, although cooking severity lowered the texture values, digestion time did not affect the hardness of the treatments. These results indicate that future work is needed to fully describe the relationship between moisture uptake, pH changes, and hardness changes during digestion, as a clear trend was not observed in the current study. SEM images before and after digestion revealed that boiling and frying processes influenced the microstructure of the frankfurters. Effective diffusion coefficients for water in boiled frankfurters were in the range of $10^{-9} - 10^{-10}$. The diffusivities for water in fried frankfurters were in the range of 10^{-9} up to 6 min of heat treatment, but it decreased to the range of 10^{-10} after 9 min of frying. The current study has demonstrated that cooking method and cooking severity of frankfurters influences certain aspects of their behavior during simulated gastric digestion. These changes in water uptake, structural breakdown, and pH decrease may ultimately impact the functional properties of frankfurters, including the rate of protein hydrolysis and absorption, the release of beneficial compounds in the matrix, and the impact of the matrix on the gut microbiome. However, further studies are needed to determine the relationship between

F3, F6, F9: Frankfurters fried for 3, 6 or 9 min, respectively.

R²: Coefficient of determination. DM: Dry matter.

the properties measured in the current study with other food functional properties.

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