International Food Research Journal 25(4): 1384-1390 (August 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of microwave heating on soluble proteins of porcine muscle

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Article history

Received: 11 April 2017
Received in revised form: 29 May 2017
Accepted: 7 June 2017

Keywords

Heating rate
Porcine muscle
Protein
Microwave
Gel electrophoresis

Abstract

Pork, like other meat products, is normally cooked prior to consumption. These methods of cooking produce different meat flavors which are influenced by water-soluble components that contribute to taste, such as the soluble proteins (sarcoplasmic and myofibrillar proteins) of porcine muscle. In this work, the effect of different cooking methods (boiling and microwaving) on porcine muscle is studied by modifying a microwave oven with a fiber optic temperature probe for online temperature effect on sarcoplasmic protein (SP) and myofibrillar protein (MP) extracted from pork samples and semi-quantitative determination (SDS-PAGE) of these soluble proteins. The heating rate behavior of both cooking methods was studied. The results show that heating rate of microwave heating at power 100 W and 300 W shared semblance with that of WBH. However, at microwave powers of 500 W, 700 W and 900 W had a linear heating rate which ignited responses from some proteins, such as creatine kinase and glyceraldehyde-3-phosphate dehydrogenase, actinin, TN–T, and tropomyosin. These changes indicated that these proteins were thermosensitve and comprised of a structure which was ignited by the heating rate of the cooking methods.

Introduction

Meat and its products are considered to be good reservoirs of protein, minerals, vitamins (B), as well as other bioactive compounds (Baldwin, 2012; Lombradi-Boccia *et al*., 2005). Pork (porcine muscle), which happens to be the most widely consumed meat worldwide, like other meat products, is normally cooked prior to consumption. Raw meat is cooked to achieve a tastier and hygienic product to improve its consumer acceptability and product shelf life (Broncano *et al*., 2014; Tornberg, 2005). The increase in consumer demand for high quality food products has led to a growth in the use of new cooking technologies (Schellekens, 1996). Microwave ovens for meal preparation has become a preferred choice in today's modern fast-paced life for its convenience, reduced cooking time, safe handling, economic consideration among several other reasons (Zhang *et al*., 2006; Salazar-González *et al*., 2012) . For instance, its nutrient retention ability. A great deal of early research works were devoted to studying nutrient retention in vegetables (Cross *et al*., 1982), spaghetti (Cocci *et al.,* 2008) and meat (Maranesi *et al*., 2005) cooked in a microwave oven. They showed that nutrient retention was greater with electronic heating than with conventional cooking. Cocci *et al* (2008), for instance concluded that cooking spaghetti with the microwave resulted in a better color retention, higher gel degree and a more compact gluten network in the spaghetti outer layer which, in turn, resulted in lower cooking loss than the traditional cooking method. The retention of nutrient was attributed to less water-to-product ratio and consequent reduced leaching in foods cooked with microwave energy, and as a consequence increased retention of nutrients.

The effect of microwave heating on dietary protein has also been the subject of much research but not much data and literature is available on the effect of microwave heating on meat proteins. Roberts and Lawrie (1974) for instance, researched on the effects of conventional and microwave heating on relative percentage of crude myofibrillar protein (MP) in bovine muscle. They found that with conventional heating, after heating at 65°C for 20min or more, most of the sarcoplasmic protein (SP) were completely denatured, the only stained band still prominent

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was probably myoglobin but SP was still visible at 75°C for a shorter time. With the microwave heating however, myoglobin survived higher temperatures when the exposure time was in seconds. The shortfall, however, was that they were not able to measure the temperature of the microwaved samples in real-time and so nominal temperatures were used instead of actual temperatures.

Although microwave-cooked meat have been consumed in the last decade (Yarmand and Homayouni, 2009; Guzmán-Guillén *et al.*, 2011), very few research focused on the effect of microwave heating on the soluble protein denaturation of pork applying real time temperature measurement while microwaving. This work focusses on the effect of two everyday cooking practices; microwave heating (MH) and water bath heating (WBH) on soluble protein denaturation of pork (porcine muscle). A fiber optic temperature probe was connected to the microwave oven for online temperature measurement. The effect of microwave heating rate at different microwave power (100 W, 300 W, 500 W, 700 W and 900 W) on sarcoplasmic proteins (SP) and myofibrillar proteins (MP) denaturation and their contents in pork were detected via SDS-PAGE and BSA semi-quantitative method. To the best of our knowledge, there has not been any report on the effect of the heating rates of these two methods on pork protein.

Materials and Methods

Sample preparation

Pork longissimus dorsi muscles were obtained at 36h postmortem from the supermarket (Chengdu, China). This was cut into cubes of 2 cm x 2 cm x 2 cm after all visible fat and connective tissues were removed. These were divided into groups A and B. Group A was heated with electric-heated thermostatic controlled water bath (Kangsheng-SYC, China) at varying temperatures; 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C and 97°C. A temperature probe was inserted in the center of each muscle to monitor the internal temperature. The meat samples were immediately removed when the core temperature reached the target temperature as previously described (Laycock *et al*., 2003).

A microwave oven (Midea EG823MF7-NRH3, output power 600 W to 900 W with a capacity of 22~25L) modified with multi-channel sensor collector and an attached fiber-optic temperature probe inserted in the sample vessel for one channel online temperature measurement. The second group was heated using the modified microwave oven at different temperatures; 25°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C and 97°C. This aspect (Groups A and B) was to establish the effect of temperature on the protein contents.

Pork longissimus dorsi muscles were again cut weighing about 6.200 ± 0.300 g and again divided into groups C and D. Group C was heated with electric-heated thermostatic water bath at different times; 1 min, 2 min, 3 min, 5 min, 7 min, 10 min, 15 min, 20 min and 25 min. Group D was heated with the modified microwave oven for 10 s, 15 s, 20 s, 25s, 30 s, 40 s, 50 s, 60 s, and 120 s. This was done to establish the heating rate as well as the heating time-temperature relationship of the cooking methods.

Protein extraction

The process of the extraction of SP and MP was done using the Diaz (Díaz *et al*., 1997) and Hughes' (Hughes *et al*., 2002) methods. Five milliliter (5ml) 0.03 $molL^{-1}$ PBS (pH 7.4) was added to 0.5 g of the heat treated pork and homogenized for about 2 min with tissue grinders. The homogenate was then centrifuged at 10000 × g for 20 min at 4°C and the supernatant was collected as sarcoplasmic proteins. Subsequently 8M urea solution and 1% mercaptoethanol buffer was added to the residue and mixed for about 2 min and finally centrifuged again at 10,000 × g for 20 min at 4°C. The supernatant was collected as myofibrillar proteins.

The solubility of the proteins in the aqueous solution depend on the number of water molecules around the surface of the protein molecule, which is mainly determined by the degree to which the hydrophilic group of the peripheral molecules of the protein molecule forms a hydration film with water and the charge of the protein molecule. By adding PBS to protein solution, the affinity of protein to water molecules causes protein molecules around the hydration layer to weakened or even disappear. Body inclusion proteins which include myofibrils are dissolvable in urea and so the use of 8M urea solution in the extraction of myofibrillar proteins.

Gel electrophoresis and protein identification

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to characterize the sarcoplasmic and myofibrillar protein profiles of the various groups of treated samples. The extracted protein samples, BSA solution (as an internal standard) and glycerin buffer in the ratio of 1:1:1 were mixed. A total of 15 µl of each sample was loaded on 4-12% Bis-Tris Criterion precast gels. The gels were run in 900 ml running buffer at 80V for approximately 2 h, until the bromophenol blue dye front just disappeared. After electrophoresis, the

proteins were stained with Coomassie Brilliant Blue R250 for 3h and de-stained until the bands were clear.

Semi-quantitative determination of proteins

Images of the gels were taken using a Canon camera after de-staining and scanned in the Gel-Pro analyzer.

A known amount of BSA was added to each gel to serve as an internal standard. Peak areas were determined and protein concentrations (expressed as BSA protein equivalents) were calculated as follows:

$$\mu g \text{ target protein/ mg pork muscle} = \frac{peak\ area\ protein\ band}{peak\ area\ BSA\ band} \times mBSA \quad (1)$$

In the sarcoplasmic protein, mBSA was 4ug BSA/mg pork whiles the myofibrillar crude protein, mBSA was 3.2 ug BSA/mg pork.

Temperature measurement

The microwave oven (Midea, China) used was modified with a fiber-optic temperature probe for the real-time temperature measurement of the samples.

Statistical analysis

SP and MP protein levels for the various groups of processed samples were compared using analysis of variance (ANOVA) and Student's t-test (Microsoft Excel software), with $P<0.05$ being considered as statistically significant. The results are as shown in Tables 1 and 2.

Results and Discussion

The effect of WBH and MH on sarcoplasmic protein denaturation

Sarcoplasmic proteins account for about 35% of the total muscle protein (Dalmış and Soyer, 2008; Marcos *et al*., 2010) and are mainly composed of enzymes associated with energy-producing metabolism and are closely related to protein solubility and meat quality traits particularly meat color (Ladrat *et al.*, 2003; Kim *et al*., 2005; Choi *et al*., 2008; Marcos *et al*., 2010).

Processing the sarcoplasmic proteins at room temperature, eight main bands were displayed in the gel electrophoresis images. These were glycogen phosphorylase (96.9 kDa), glucose-6-phosphate isomerase (62.9 kDa), enolase B muscle isoform (46.8 kDa), creatine kinase (43 kDa), fructose-bisphosphate aldolase A (39.2 kDa), glyceraldehyde-3-phosphate dehydrogenase (35.7 kDa), carbonic anhydrase (29.4 kDa) and myoglobin (16.9 kDa) in decreasing molecular sizes. Denaturation of SP began after 40°C in both methods. With rise in temperature (>70°C),

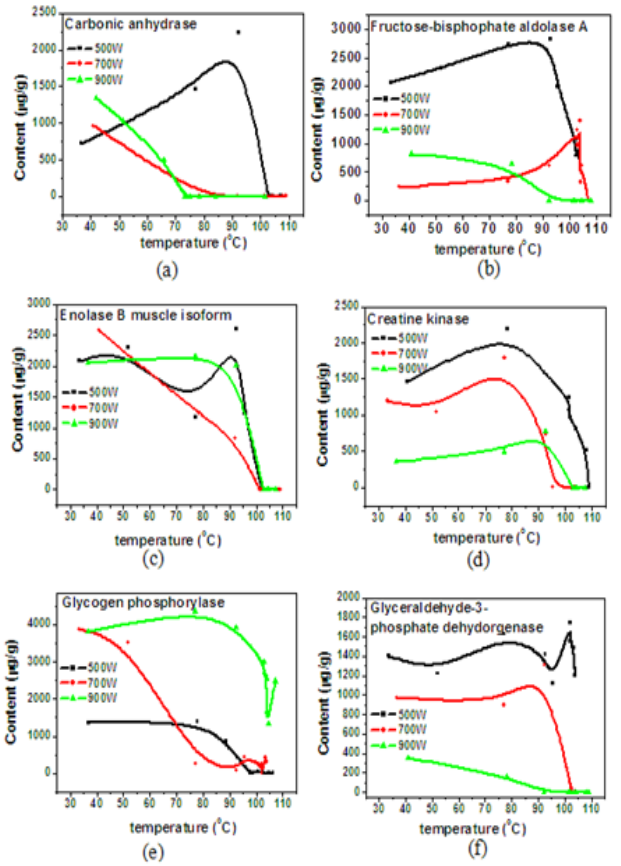

Figure 1(a-f). Effect of microwave average power on the contents of carbonic anhydrase (a), fructose-bisphosphate aldolase A (b), enolase B muscle isoform (c), creatine kinase (d), glycogen phosphorylase (e) and glyceraldehyde -3- phosphate dehydrogenase (f)

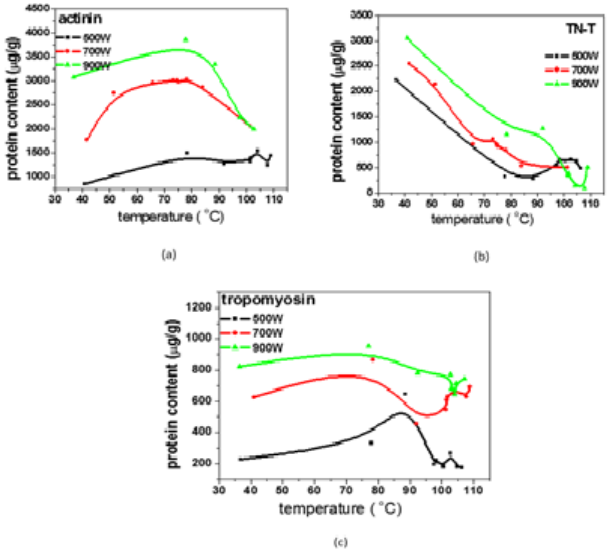

Figure 2. Effect of microwave average power on actinin (a), TN-T (b) and Tropomyosin (c)

all SP protein bands became weak or disappeared in both methods. There was no evident change for sarcoplasmic proteins above 70°C as a result of their complete denaturation. These results are consistent with earlier research works (Ryu *et al*., 2005; Choi *et*

Table 1. Sarcoplasmic protein contents at different temperatures for WBH and MH

	Glycogen phosphorylase (µg/mg)			Glucose-6-phosphate isomerase (µg/mg)			BSA (µg/mg)		
Temp(°C)	WBH	MH	P(t-test)	WBH	MH	P(t-test)	WBH	MH	P(t-test)
25	9.27E-04	9.27E-04	0.95	1.59E-03	1.59E-03	0.92	0.002	0.002	
40	1.46E-03	1.56E-03		1.82E-03	1.92E-03		0.002	0.002	
50	5.40E-04	5.50E-04		1.72E-03	1.74E-03		0.002	0.002	
60	4.53E-04	4.73E-04		1.66E-03	1.86E-03		0.002	0.002	
70	7.56E-05	7.63E-05		2.36E-04	2.46E-04		0.002	0.002	
80	5.16E-05	5.26E-05		1.40E-04	1.50E-04		0.002	0.002	
90	2.29E-05	2.32E-05		1.03E-04	1.05E-04		0.002	0.002	
97	2.29E-05	2.32E-05		0.0001	0.00011		0.002	0.002	
	fructose-bisphosphate aldolase A (µg/mg)			glyceraldehyde-3-phosphate dehydrogenase (µg/mg)			carbonic anhydrase (µg/mg)		
Temp(°C)	WBH	MH	P(t-test)	WBH	MH	P(t-test)	WBH	MH	P(t-test)
25	2.47E-03	2.47E-03	1.00	6.52E-04	6.52E-04	1.00	5.28E-04	5.28E-04	1.00
40	2.58E-03	2.58E-03		1.01E-03	1.01E-03		6.99E-04	6.99E-04	
50	2.53E-03	2.55E-03		8.39E-04	8.39E-04		5.10E-04	5.10E-04	
60	2.10E-03	2.10E-03		8.67E-04	8.67E-04		1.94E-04	1.94E-04	
70	2.55E-04	2.66E-04		5.51E-05	5.51E-05		3.43E-05	3.43E-05	
80	8.13E-05	8.13E-05		2.99E-05	2.99E-05		4.44E-05	4.44E-05	
90	6.78E-05	6.78E-05		1.23E-05	1.23E-05		2.82E-05	2.82E-05	
97	6.78E-05	6.78E-05		1.23E-05	1.23E-05		2.82E-05	2.82E-05	
	Enolase B muscle isoform (µg/mg)			Creatine kinase (µg/mg)			myoglobin (µg/mg)		
Temp(°C)	WBH	MH	P(t-test)	WBH	MH	P(t-test)	WBH	MH	P(t-test)
25	1.56E-03	1.56E-03	0.90	7.76E-04	7.76E-04	0.99	7.29E-05	7.29E-05	1.00
40	1.60E-03	1.89E-03		9.03E-04	9.08E-04		7.17E-05	7.17E-05	
50	1.52E-03	1.54E-03		7.82E-04	7.85E-04		5.68E-05	5.68E-05	
60	1.55E-03	1.57E-03		2.51E-04	2.54E-04		4.96E-05	4.96E-05	
70	2.36E-04	2.96E-04		2.54E-04	2.55E-04		2.62E-05	2.62E-05	
80	1.40E-04	1.40E-04		9.93E-05	9.93E-05		4.03E-05	4.03E-05	
90	1.03E-04	1.03E-04		4.39E-05	4.39E-05		1.99E-05	1.99E-05	
97	0.0001	0.000103		4.39E-05	4.39E-05		1.99E-05	1.99E-05	

MH: Microwave heating at 100W power; WBH: Water bath heating

al., 2008; Huang *et al*., 2011;) and showed that severe denaturation of SP in meat was related to processing and cooking treatments. The SDS-PAGE results of SP profiles showed that slightly more denaturation occurred in the WB treated samples, especially after 70°C. In both methods, solubility decreased as the temperature increased.

The effect of WBH and MH on myofibrillar protein denaturation

Myofibrillar proteins are not only important in muscles for their role in contraction (Pearson and Young, 1989) but also for their importance in the functional properties of meat products such as water-holding, emulsifying capacity, binding ability and gelation (Asghar *et al.*, 1985).

When myofibrillar proteins were processed at room temperature, there were eight main bands displayed in the gel electrophoresis. These were myosin (220 kDa), α-actinin (100 kDa), desmin (53 kDa), actin (42 kDa), TN-T (37 kDa), tropomyosin (36 kDa), TN-I (23 kDa) and TN-C (18 kDa). Myofibrillar proteins were observed to have a better thermo-stability than SP. The bands from 36 kDa to 42 kDa became darkened with an increase in temperature which resulted in the generation of more actin, TN-T and tropomyosin with more contents observed in the microwaved samples.

Quantification of porcine protein by SDS-PAGE

To ascertain the accuracy of the BSA quantitative method, standard curves of two purified proteins (carbonic anhydrase and myoglobin) were generated. Six mixtures each of known amounts (between 0.25 and 4 µg) of BSA was loaded together with the proteins to serve as an internal standard. There was a good linearity between the exact amounts of loaded proteins and the amounts calculated as BSA-equivalents when the standard graphs of myoglobin and carbonic anhydrase were plotted. Regression lines passed through the origin (intercepts did not differ significantly from zero, $P < 0.05$). With the equations for carbonic anhydrase and myoglobin plots being (2) and (3) respectively;

$$M_{ca} = 0.08 + 2.136C_{BSA} \quad (2)$$

$$M_{my} = 0.0147 + 0.794C_{BSA} \quad (3)$$

It was also observed that different proteins bind to different amounts of Coomassie brilliant blue R.50 and this observation corresponds to the findings of Tal *et al* (1985).

Effect of WBH and MH on sarcoplasmic protein content

Using the BSA semi-quantitative method and the Gel-pro analyzer software to analyze the gel images, the protein content at the respective temperatures

Table 2. Myofibrillar protein contents at different temperatures for WBH and MH

	BSA (µg/mg)			TN-C (µg/mg)			TN-1(µg/mg)		
Temp(°C)	WBH	MH		WBH	MH	P(t-test)	WBH	MH	P(t-test)
25	0.002	0.002		0.00188	1.88E-03	0.99	0.00158	1.58E-03	0.98
40	0.002	0.002		0.00146	1.48E-03		0.00154	1.54E-03	
50	0.002	0.002		0.00211	2.13E-03		0.00168	1.68E-03	
60	0.002	0.002		0.00225	2.27E-03		0.00178	1.78E-03	
70	0.002	0.002		0.00242	2.43E-03		0.00253	2.55E-03	
80	0.002	0.002		4.80E-04	4.82E-04		0.00203	2.04E-03	
90	0.002	0.002		1.42E-04	1.43E-04		0.00133	1.33E-03	
97	0.002	0.002		1.42E-04	0.00014		0.00133	0.00133	
	Actin (µg/mg)			Desmin (µg/mg)			a-actinin (µg/mg)		
Temp(°C)	WBH	MH	P(t-test)	WBH	MH	P(t-test)	WBH	MH	P(t-test)
25	4.08E-04	4.08E-04	0.98	3.81E-04	3.81E-04	1.00	9.94E-05	9.94E-05	1.00
40	4.26E-04	4.36E-04		3.96E-04	3.96E-04		9.95E-05	9.95E-05	
50	4.30E-04	4.28E-04		3.58E-04	3.58E-04		6.53E-05	6.53E-05	
60	4.91E-04	4.91E-04		4.51E-04	4.51E-04		1.06E-04	1.06E-04	
70	6.00E-04	6.00E-04		5.70E-04	0.00057		1.61E-04	1.61E-04	
80	3.24E-04	3.24E-04		2.24E-04	2.24E-04		8.19E-05	8.19E-05	
90	3.88E-04	0.00039		3.28E-04	3.28E-04		3.29E-05	3.29E-05	
97	3.88E-04	0.00039		3.18E-04	0.00032		0	0.00	
	Tropomyosin (µg/mg)			TN-T (µg/mg)			Myosin (µg/mg)		
Temp(°C)	WBH	MH	P(t-test)	WBH	MH	P(t-test)	WBH	MH	P(t-test)
25	2.03E-04	2.03E-04	1.00	2.83E-04	2.83E-04	1.00	8.94E-05	8.94E-05	1.00
40	2.10E-04	2.10E-04		2.71E-04	2.71E-04		8.95E-05	8.95E-05	
50	1.55E-04	1.55E-04		8.91E-05	8.91E-05		5.53E-05	5.53E-05	
60	4.37E-04	4.37E-04		2.59E-04	2.59E-04		8.56E-05	8.56E-05	
70	4.69E-04	4.69E-04		3.09E-04	3.09E-04		8.56E-05	8.56E-05	
80	2.17E-04	2.17E-04		1.00E-04	1.00E-04		9.56E-05	9.56E-05	
90	7.57E-05	7.57E-05		8.51E-05	8.51E-05		7.86E-05	7.86E-05	
97	2.01E-05	2.01E-05		8.51E-05	8.51E-05		8.86E-05	8.86E-05	

MH: Microwave heating at 100W power; WBH: Water bath heating

were calculated and a graph of protein content (µg/g) against temperature (°C) for the various SPs were plotted. The contents of SPs such as carbonic anhydrase, creatine kinase, enolase B muscle isoform, fructose-bisphosphate aldolase A, glyceraldehyde-3-phosphate dehydrogenase and glycogen phosphorylase neared zero when the temperature was close to 100°C. This was attributable to the proteins changing from solubility at relatively low temperatures to insolubility at relatively high temperatures.

The temperature increased linearly with time at microwave average power of 500 W, 700 W and 900 W except for the difference in their heating rates. It was discovered that the average power also affected the content distribution of different soluble proteins of sarcoplasmic proteins ($p<0.05$) (Figure 1). Additionally, the content of creatine kinase and glyceraldehyde-3-phosphate dehydrogenase increased with an increase in microwave power when the temperature was lower than 80°C ($P<0.05$), probably as a result of the proteins comprising of a structure that was internally provided with an ignitionability of heating rate. For the WB treatment, the various SP contents decreased steadily and neared zero as the temperature increased (Table 1).

Effect of WBH and MH on myofibrillar proteins content

There were still some content of these proteins present even at temperatures above 97°C. For WB, the contents of the various MP was steady until after 50°C in TN-T, TN-1 and tropomyosin when there was a steady increase which started to decrease after 70°C (Table 2). At approximately 80°C the content of these proteins neared zero. For actin, the content was steady until after 60°C where it started increasing until it reached its peak at 70°C after which it started decreasing steadily.

The variations in the average power also affected the content distribution of different myofibrillar proteins such as actinin, TN-T and tropomyosin ($p<0.05$) (Figure 2). Furthermore, the content of actinin and tropomyosin increased with increasing microwave power when the temperature was below 80°C before nearing zero at higher temperatures. The shorter the heating time, the better it was for nutrient retention.

Effect of heating mechanism on temperature change

In order to study the heating mechanism of the two cooking methods, the heating rates of 500 mL distilled water under the same condition of time for the cooking methods (boiling and microwaving) were determined (Figure 3). A 20 s approximate delayed response was observed in both methods. Different temperature curves were obtained for water bath (black), microwave heating with different average powers; 100 W (red), 300 W (green), 500 W (blue), 700W (green) and 900 W (purple) respectively. The

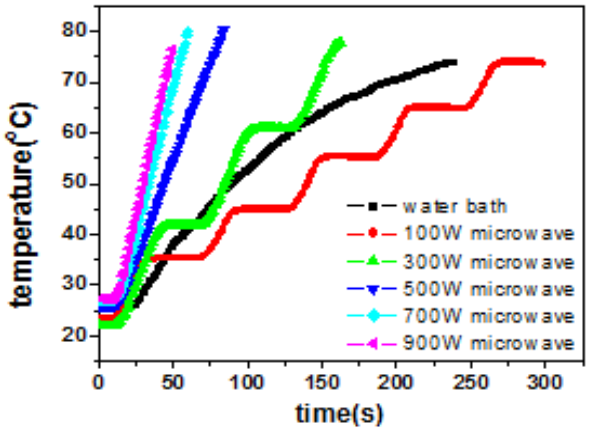

Figure 3. Temperature-time curve for WBH (black) and MH (at 100 W, 300 W, 500 W, 700 W and 900 W microwave power) of 500 ml distilled water

temperature curve for the WBH stayed between that of microwave operated at 100 W and 300 W average powers. This showed that MH at these average powers (100 W and 300 W) had similar heating rate as that of the WBH method. This similarity was also observed in their effect on the proteins content as the difference observed was statistically not significant ($P > 0.05$) as shown in Tables 1 and 2. The behavior of the 900 W, 700 W and 500W temperature curves for MH was linear with positive slopes after the 20 s delay with linear slopes of 1.19°C/s, 1.14°C/s, and 0.80°C/s respectively.

The temperature increased with time, however, a shorter time was used (approx. 2 min) in microwave than in the WB (approx. 25 min) to attain 97°C. The total time-temperature combination sustained by the proteins in the WBH, is thus greater and has a correspondingly greater denaturing effect than in MH. For both SPs and MPs, the heating rate effect was observed between 40°C and 80°C.

Conclusion

In this work, a fiber-optic temperature probe is set up in the microwave oven for detecting temperature changes of pork samples at different microwave power online. BSA semi-quantitative determination is also introduced for the proteins content analyses via SDS-PAGE. The results show that operating the microwave at 100 W and 300 W power, the heating rate is similar in behavior to that of the WBH. However, a linear response is observed in the heating rate of microwave at a power of 500 W, 700 W, and 900 W. The research also shows that from 500 W to 900 W microwave operating power and in the temperature range of 40°C to 80°C, a response to microwave heating rate is observed in some sarcoplasmic proteins (creatine kinase and glyceraldehyde -3-phosphate dehydrogenase) and myofibrillar proteins (actinin, TN –T and tropomyosin). Future work will study the denaturation mechanism of the proteins and also the effect of MH on protein digestibility and bio-availability. This will present a potential application for MH which will make meat more flavored and nutritious by applying the right cooking parameters.

Acknowledgement

We would like to appreciate the financial support, in whole or in part, by the National Natural Science Foundation of China (Nos.61071026).

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