Agr. Nat. Resour. 54 (2020) 39–47

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Effect of thermosonication or microwave heating for post pasteurization on chemical, physical, and sensory characteristics of prototype sausage

Pawinee Inmanee[a,†], Wannasawat Ratphitagsanti[a,†], Pitiya Kamonpatana[b,†] Tantawan Pirak[a,*]

[a] *Department of Product Development, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.*

[b] *Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.*

Article Info

Article history:
Received 17 May 2018
Revised 13 December 2018
Accepted 26 February 2019
Available online 28 February 2020

Keywords:
Microwave heating,
Quality,
Sausage,
Shelf life,
Thermosonication

Abstract

Thermosonication or microwave techniques were applied to sausage samples as post pasteurization methods. The pasteurization was performed either in an ultrasonic bath at 80°C for 20 min, 30 min and 40 min or in a microwave at power levels of 400 W, 600 W and 800 W for 15 s or 30 s and compared to conventional pasteurization (75°C for 15 min) and the control (without pasteurization). The results showed that thermosonication and microwaves effectively inhibited microbial growth during storage. However, these techniques significantly ($p < 0.05$) affected the hardness, springiness, cohesiveness and chewiness of the treated sausages. There were no significant ($p > 0.05$) differences in the sensory characteristics of the treated samples from those of the conventional pasteurization samples. The optimal thermosonication conditions were 80°C for 20 min and maintained the quality and extended the shelf life, while microwave heating at a power level of 400 W for 30 s produced similar inhibition results. Hence, thermosonication or microwave heating have potential for application in sausage production to extend the shelf life of sausage products and to maintain overall quality with greater energy efficiency.

Introduction

Sausage is a perishable food that is easily spoiled when stored at high temperature as the product can become unsafe due to microbial growth and the formation of toxins (Berrang et al., 2002). The sources of contamination of meat and meat products are not restricted to the processing line in slaughter and meat plants, but also involve the prevention of microbial adhesion and the physical removal and destruction of microorganisms on the meat during the production steps (Sofos, 2008; Sofos and Geornaras, 2010). Nowadays, consumers prefer to select high quality and safe foods which has driven the food industry and academic research to develop new preservation methods with minimized impact on product quality (Knorr, 1993).

Pasteurization, a thermal processing technology, has been used for microbial inactivation to ensure safety and extended the shelf life of foods as traditional thermal pasteurization effectively retards the growth of microorganism (Piyasena et al., 2003). However, it leads to nutrition loss and the development of undesirable changes in the color, texture and flavor of the processed meat products (Selby et al., 2006; Cichoski et al., 2015). Therefore, in the post-pasteurization step, some technologies have been proposed to minimize the losses arising from conventional methods, including high pressure processing, pulsed electric fields, UV light and ohmic heating (Zell et al., 2010; Chen et al., 2012). Thermosonication and microwave heating have been proposed to reduce the processing time and temperature, while maintaining product quality and reducing energy consumption.

† Equal contribution.
* Corresponding author.
E-mail address: fagitwk@ku.ac.th, tantawan.k@hotmail.com (T. Pirak)

https://doi.org/10.34044/j.anres.2020.54.1.06

Power ultrasound in combination with moderate heat or thermosonication has been proposed as one an alternative process for preserving food products, as this treatment is more energy efficient and is able to kill microorganisms effectively (Ordonez et al., 1987; Feng, 2011). Various studies have reported on the application of this treatment in foods such as lettuce, rice porridge, beef slurry and cheese slurry (Seymour et al., 2002; Piyasena et al., 2003; Alves et al., 2018). The induction of heat and cavitation from the growth and explosion of bubbles in a liquid is a response to microbial inactivation, where the cavitation causes disruption of the cell membrane and the production of free radicals by both temperature and pressure changes (Atchley and Crum, 1998; Abdullah and Chin, 2014). Piyasena et al. (2003) revealed that ultrasound with high power and low frequency provided a large amount of cavitation. Moreover, power ultrasound (32–40 kHz) helped to increase the antimicrobial activity of chlorine against *Salmonella typhimurium* on lettuce (Seymour et al., 2002). The application of thermosonication was also reported in reducing *Bacillus cereus* spores in rice porridge, beef slurry and cheese slurry by 7-folds, 6-folds and 4-folds, respectively (Evelyn and Silva, 2015). Various researchers have implied that thermosonication has a positive effect on the destruction of *Escherichia coli*, *Penicillium digitatum*, *Salmonella* sp. and *Listeria monocytogenes* without changing the product quality (Sams and Feria, 1991; Manas et al., 2000; Lopez-Malo et al., 2001; Lee et al., 2009; Mansour and Oh, 2015). Moreover, some studies have reported effective inhibition in meat and meat products such as beef and pork (McDonnell et al., 2014), Italian salami (Alves et al., 2018) and sausage (Cichoski et al., 2015).

Pasteurization of ready-to-eat meals using a microwave technique has been recognized for many years as a means of enhancing the product shelf life (Burfoot et al., 1988; Cross et al., 2009). Microwave pasteurization is able to destroy microorganisms at temperatures lower than that of conventional pasteurization and furthermore, this method uses high heating rates that result in a significant reduction in the cooking time (Salazar-Gonzalez et al., 2012; Chandrasekaran et al., 2013). Microwave heating processing at 80–85°C for a few minutes is considered sufficient, with a margin of safety, to inactivate vegetative pathogenic microorganisms such as *Salmonella* and *Campylobacter* (Chandrasekaran et al., 2013). Song and Kang (2016) found that microwave heating inactivated pathogenic bacteria (*Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella typhymurium*) in peanut butter. The effects of various combinations of microwave oven and frequency were studied in the pasteurization of ready-to-eat spaghetti bolognaise meals in retail packaging and to extend the product shelf life (Burfoot et al., 1988; Ohlsson, 1991). Ryynanen and Ohlsson (1996) studied the importance of chemical and physical modifications in four components of chilled ready-to-eat meals during microwave heating. They reported that the placement and geometry of the food component in the packages had a significant role in providing microwave heating uniformity in multicomponent food systems. Until now, microwave techniques have been used widely as an effective cooking method for preserving food nutrients (Burfoot et al., 1988; Tang et al., 2018). However, the application of this treatment as a post pasteurization method is still lacking.

To serve consumer needs for high quality and safe food, the current research aims were: 1) to investigate the possibility of using thermosonication or microwave heating as post pasteurization techniques on vacuum packaged sausages; and 2) to determine the effect of thermosonication and microwave heating on the texture, color, and sensory characteristics of treated vacuum-packaged sausage. These technologies were expected to maintain the quality and extend the shelf life.

Materials and Methods

Preparation of samples, packaging and storage

Commercial pork sausage was obtained from a local industry in Thailand. Raw sausage emulsion batter without adding antimicrobial preservatives was prepared in a commercialized scaled production line, stuffed into edible collagen casing with a diameter of 22 mm using an automatic stuffer system and linked in intervals of 10 cm. The sausages were cooked in a smokehouse until their internal temperature was 72°C and then they were cooled down with cold water until reaching 20°C. The sausages were vacuum-packed with five sausages per pack, stored in a refrigerator at 4°C and sent to the laboratory within 2 hr. Normally, the shelf life of this product is expected to be around 30 d. The chemical, physical, microbiological and sensory characteristics of all samples were analyzed on the first day of arrival and then they were treated using thermosonication or microwave procedures under various conditions. All experiments were performed in duplicate.

Thermosonication treatment

The thermosonication treatment was performed using different periods (10 min, 20 min, 30 min and 40 min) using an ultrasonic bath (VGT-1860QTD; GT Sonic; Guangdong; China) at a frequency of 40 kHz. A completely randomized design was used to investigate the effect of the period of thermosonication on the chemical, physical and microbiological properties and the consumer preference level of sausage samples. The temperature inside the reactor (80°C) and samples were monitored using a K-type thermocouple (FLIR Commercial Systems, Inc.; New York, NY; USA). The vacuum-packaged sausage samples were submerged in an ultrasonic bath under different conditions. Sausage samples were removed from the ultrasonic bath and cooled by immersing in ice water for 10 min before storage at 4°C until analysis. All treatments were performed in duplicate.

Microwave heating treatment

The microwave treatment of the sausages used a household microwave oven (EMM2007X; Electrolux; Bangkok, Thailand) at 2,450 MHz. A 3 × 2 factorial randomized complete block design was used with two factors (power level and time) applied to the sausages. The three power levels were 400 W, 600 W or 800 W for

periods of 15 s or 30 s. The vacuum packaged sausage samples were placed in the microwave chamber and treated under the specified conditions. Then, samples were removed, cooled by immersing in ice water for 10 min and stored at 4°C prior for analysis. All treatments were performed in duplicate.

Conventional thermal treatment

The vacuum-packaged sausage samples were submerged into hot water on a hotplate (OMI Induction Cooker IC1; OMI; Guangdong, China) and the temperature of the water was maintained at 80°C. The internal temperature was monitored using a thermocouple (FLIR Commercial Systems, Inc.; Nashua, NH; USA). Once the internal temperature reached 75°C for 15 min, the samples were removed from the water bath and cooled by immersing in ice water.

All treatments were subjected to analysis for chemical, physical, microbial and sensorial characteristics. The methods applied for these analyses are described below.

Proximate composition

Proximate composition assessment of the sausage samples was performed. The moisture content was based on Method 950.46 (Association of Official Analytical Chemists, 2000) and the weight loss after drying for 12 hr at 105°C in an oven (model FD115; Binder Inc.; New York, NY; USA). The fat content was determined using solvent extraction system (petroleum ether; Fisher Chemical, Thermo Fisher Scientific (Thailand) Co Ltd; Bangkok, Thailand) system and a Soxhlet apparatus according to Method 960.39 (Association of Official Analytical Chemists, 2000). The protein content was determined using an automatic Kjeldahl nitrogen analyzer (model KjelFlex K360; Buchi; Flawil, Switzerland). Ash was determined based on Method 920.153 (Association of Official Analytical Chemists, 2000). All measurements were performed in triplicate.

pH

10 grams of sausages were homogenized (model AT716170; Tefal; Tournus, France) with 100 mL of distilled water and the pH value of each sausage sample was measured using a pH meter (model Docu-pH +; Sartorius; Göttingen, Germany). The pH values were measured in triplicate for all samples.

Thiobarbituric acid value

The 2-thiobarbituric acid (TBA) value was determined using a spectrophotometer (Biomate 3S; Thermoscientific; Gloucester, UK) according to the method of Tarladgis et al. (1960) with slight modifications. Ten grams of sausages were blended with 97.5 mL distilled water and then 2.5 mL 4 M HCL were added. Following mixing to ensure homogeneity, the solution was placed in the distillation apparatus and then 50 mL of distillate were collected. A 5 mL distillate of sample and 5 mL of thiobabituric acid (0.02 M 2-thiobarbituric acid in 90% acetic acid) were mixed homogeneously (Vortex-Genie 2; Scientific Industries, Inc; Bangkok, Thailand) and incubated in boiling water for 35 min before cooling to room temperature (under tap water for 10 min). The absorbance of the solution was measured at 538 nm. The optical density was multiplied by the constant 7.8 to give the TBA value. TBA values were expressed as milligrams of malondialdehyde per kilogram of sample. The TBA test was performed on all samples from each batch in triplicate.

Color

The internal color of the sausage samples was determined based on the CIE L*, a* and b* system using a spectrophotometer (CR-3500d; Minolta; Tokyo, Japan). The color was measured using reflectance and expressed in terms of L* (lightness), a* (redness) and b* (yellowness) values. The spectrophotometer was standardized using a black-and-white plate. The illuminant used was D65, with a standard observer angle of 10°. Sausages were cut into slices (diameter 2.2 cm, length 2 cm) and the data were collected at five different points on the surface and inside each sample. Each sample was measured five times and the experiment was performed in triplicate.

Texture profile analysis

The texture profile analysis (TPA) was applied to the products based on a method described by Barbut (2006). A texture analyzer (TA-XT-plus; Stable Micro Systems; Godalming, UK) with a load cell of 2 kg was used to measure the textural properties of the sausages. For each treatment, the sausages were cut (diameter 2.2 cm, length 2 cm) and compressed twice to 50% of their original height using the texture profile analysis program. Attributes were collected and expressed in term of hardness, springiness, cohesiveness and chewiness. Five samples were randomly measured and the experiment was performed in duplicate.

Cooking loss and water holding capacity

Cooking loss was determined by weighing the sausages before and after cooking and calculating the percentage weight loss. The water holding capacity (WHC) was determined in the sausages before and after the thermosonication or microwave heating treatments using the centrifugation method (Honikel and Hamm, 1994). Ten grams of sausages were placed in a centrifuge tube and centrifuged (Rotina 380R; Hettich centrifuge; Buckinghamshire, UK) at 10,000 revolutions per minute and 4°C for 30 min. After centrifugation, the free water in the centrifuge tubes was removed and weighed. Each sample was measured five times and the experiment was performed in duplicate. The WHC was calculated using Equation 1:

$$WHC\ (\%) = \frac{(W_s - W_w)}{W_s} \times 100 \quad (1)$$

where, *Ws* is the weight of the initial sample and *Ww* is the weight of water loss with all weights measured in grams.

Microbial analysis

10 grams of sausage were randomly selected and homogenized in a laboratory blender (Stomacher 400 circulator; Seward; West Sussex, UK) using sterile peptone water for 1 min. To determine the number of aerobic mesophiles, inoculation was performed in standard plate count agar followed by incubation for 48 h at 37 ± 1°C. Yeast and mold were inoculated on potato dextrose agar and incubated at 25 ± 1°C for 5–7 d. The results were expressed as colony forming units (CFU) per gram of sausage. The microbial analysis of all samples was performed on day 1 and day 30.

Sensory evaluation

Sensory evaluation was performed using a 9-point hedonic scale test with 50 untrained panelists. Sausages were reheated using a steamer for 5 min until their internal temperature reached 70°C. Samples were labeled using 3-digit random numbers, cooked and then served within 2 min in random order to the panelists who were in individual booths. Panelists were instructed to cleanse their palates with water between samples. The untrained panelists evaluated each sample using the 9-point hedonic scale with a score of 1 being "dislike extremely" and a score of 9 being "like extremely".

Statistical analysis

All experiments were performed in duplicate. Data were expressed as the mean ± SD. Data were then analyzed using one-way analysis of variance and significant differences among means were determined using Duncan New Multiple Range Test in the SPSS program (Version 12; SPSS Inc.; Chicago, IL, USA). Significant differences were tested at $p < 0.05$.

Results and Discussion

Quality of prototype sausages

The qualities of prototype sausage are reported in Table 1. The prototype sausage had a moisture content of 59.72%, a protein content of 12.98% and a fat content of 23.10%. The hardness, springiness, cohesiveness and chewiness values of the samples were 54.07 N, 0.95 cm, 0.77 N/cm and 39.52 N/cm, respectively. The lightness (L*), redness (a*) and yellowness (b*) values of the samples were 68.58, 8.76 and 20.30, respectively. The microbial loads of the prototype sausage (total variable count, yeast and mold, *Pseudomonas* spp., *Escherichia coli*, *Clostridium perfringens*, *Salmonella* spp. and *Staphylococcus aureus*) were not over the limitations for sausage specified in TIS 2300-2549 by the Thai Industrial Standards Institute (2007). The sensory scores of the prototype sausage samples revealed that consumers liked the sausage moderately with an overall liking score of 7.5.

Chemical characteristics of sausages

The chemical properties of the sausage samples treated using the thermosonication or microwave technologies are presented in Table 2 and Table 3, respectively. Untreated sausage and conventional treated sausage were used as the control. Treating sausage with thermosonication resulted in no significant change in the moisture content (62.24–62.93%). For the samples, the protein content was 12.88–13.16%, the fat content was 20.67–21.25% and the ash content was 2.39– 2.50%. The proximate compositions of sausage treated with microwave were also studied, resulting in a moisture content of 60.52–62.53%, a protein content of 12.88–13.16%, a fat content of 20.16–21.25% and an ash content of 2.39–2.62%. These results indicated that post pasteurization using thermosonication or microwave techniques did not affect the major composition of sausages ($p > 0.05$). The proximate compositions of sausage with and without post pasteurization were not significantly different, indicating there was a negligible effect from the post pasteurization treatment. These results were in agreement with Cross et al. (2009) regarding the minimal nutritional effects of microwaves on protein, lipid, and minerals.

Table 1 Characteristics of prototype sausage

Chemical characteristic	Mean ± SD
Moisture content (%)	59.72 ± 0.05
Fat (%)	23.10 ± 0.30
Protein (%)	12.98 ± 0.35
Water holding capacity (%)	99.23 ± 0.25
pH [ns]	6.32 ± 0.06
TBA (mg malonaldehyde/kg)	0.30 ± 0.01
Physical characteristic	
L*	68.58 ± 0.25
a*	8.76 ± 0.09
b*	19.21 ± 0.22
Hardness	54.07 ± 0.80
Springiness	0.95 ± 0.13
Cohesiveness	0.77 ± 0.29
Chewiness	39.52 ± 0.13
Microbiological characteristic	
TPC (CFU/g)	< 10 est.
Yeast & mold (CFU/g)	< 10 est.
Pseudomonas spp. (CFU/g)	< 10 est.
Escherichia coli (MPN/g)	< 3.0
Clostridium perfringens (per 0.01g)	Not Detected
Salmonella spp. (per 25 g)	Not Detected
Staphylococcus aureus (per 0.1g)	Not Detected
Sensorial characteristic	
Appearance	7.6 ± 0.78
Color	7.5 ± 0.81
Odor	7.3 ± 1.17
Flavor	7.2 ± 1.12
Texture	7.4 ± 0.98
Taste	7.4 ± 1.36
Overall liking	7.5 ± 0.79

TBA = thiobarbituric acid; TPC = total plate count; CFU = colony forming units; MPN = most probable number

Lipid oxidation was evaluated and expressed in terms of the TBA value as shown in Table 2 and Table 3. The TBA values of the thermosonication samples were not significantly different from those of the control and the conventional pasteurized samples. The TBA values after treatment using the thermal method for 1 d were 0.28–0.34 mg malondialdehyde/kg sausage The TBA values of sausages treated with microwaves at 400 W or 600 W for 15 s or 30 s did not differ significantly from the control and the conventional thermal pasteurization samples. Microwaving resulted in a significant increase in the TBA values in sausages treated at 800W, with the TBA values at 800 W for 15 s or 30 s being 0.20 mg malondialdehyde/kg and 0.22 mg malondialdehyde/kg, respectively. The TBA value will be reduced income of using vacuum packing (below 1 mg malonaldehyde/kg) (Martinez et al., 2006; Rubio et al., 2008), which indicate little development in the thermosonication and microwave process. Selby et al. (2006) reported that in-package pasteurization enhanced the lipid oxidation of bologna samples.

There were no significant changes in the pH values in all treatments. The pH values of thermosonication samples were 6.31–6.35. The pH values (6.30–6.39) of the microwave-treated samples were not significantly different compared with the control and conventional samples. The microwave samples had slightly increased pH values compared with the conventional treatment (Roldan et al., 2014). Therefore, the thermal treatment did not affect the pH values of sausage during post pasteurization under different conditions.

Physical characteristics of sausage

The internal color of the sausage samples treated using thermosonication or microwaves are presented in Table 2 and Table 3, respectively. Thermosonication, within the range of the experimental conditions of the current study, caused a slight decrease in the a* values with increased thermosonication time. The L* and b* values of the sausage samples were not affected by the thermosonication treatments. This was in agreement with Cichoski et al. (2015) who reported no significant differences in the L* and b* values for the inner and outer parts of sausages treated with ultrasound. Within the range of the experimental conditions of the current study, the L*, a* and b*values of the sausages treated with microwaves were not significantly different for the different power levels and contact times of microwave treatment. These results agreed with Evrendilek and Balasubramaniam (2011) who reported that the color of the sausage samples was not significantly ($p > 0.05$) influenced by the different pressures involved in non thermal processing and high-pressure processing.

Table 2 Effect of contact time in thermosonicator on physicochemical, chemical and microbiological characteristic in sausages for control (without thermosonication or heat treatment); conventional (75°C for 15 min) and thermosonication (80°C for 10, 20, 30 and 40 min).

Sample parameter	Control	Conventional	10 min	20 min	30 min	40 min
Physicochemical characteristic						
Hardness	$54.07^{a} \pm 1.43$	$53.11^{a} \pm 2.15$	$53.17^{a} \pm 4.37$	$49.80^{b} \pm 4.29$	$43.02^{c} \pm 4.46$	$46.08^{c} \pm 4.52$
Springiness^{NS}	0.94 ± 0.01	0.95 ± 0.01	0.95 ± 0.02	0.95 ± 0.01	0.95 ± 0.02	0.95 ± 0.01
Cohesiveness	$0.69^{c} \pm 0.05$	$0.76^{a} \pm 0.02$	$0.72^{b} \pm 0.04$	$0.75^{ab} \pm 0.01$	$0.75^{ab} \pm 0.01$	$0.77^{a} \pm 0.01$
Chewiness	$47.09^{ab} \pm 4.40$	$48.67^{ab} \pm 1.81$	$49.95^{a} \pm 3.00$	$49.80^{a} \pm 2.96$	$44.72^{b} \pm 2.56$	$40.85^{c} \pm 2.91$
L*^{NS}	68.58 ± 0.25	69.35 ± 0.34	69.64 ± 0.43	69.43 ± 0.26	68.62 ± 0.52	69.30 ± 0.47
a*	$9.27^{a} \pm 0.10$	$9.12^{b} \pm 0.12$	$9.21^{ab} \pm 0.09$	$9.10^{b} \pm 0.13$	$9.28^{a} \pm 0.15$	$9.17^{b} \pm 0.22$
b*^{NS}	20.30 ± 0.18	20.22 ± 0.15	20.04 ± 0.22	20.06 ± 0.26	20.26 ± 0.24	20.47 ± 0.09
WHC (%)^{NS}	96.63 ± 0.21	96.43 ± 0.25	96.60 ± 0.17	96.60 ± 0.26	96.40 ± 0.87	96.77 ± 0.21
Cooking loss (%)	$1.96^{a} \pm 0.07$	$1.87^{a} \pm 0.07$	$1.14^{ab} \pm 0.12$	$1.14^{ab} \pm 0.02$	$1.35^{b} \pm 0.15$	$0.87^{bc} \pm 0.07$
Chemical characteristic						
TBA (mg malonaldehyde/kg)	$0.28^{ab} \pm 0.05$	$0.29^{ab} \pm 0.02$	$0.34^{a} \pm 0.02$	$0.30^{b} \pm 0.02$	$0.28^{ab} \pm 0.03$	$0.30^{b} \pm 0.02$
pH^{ns}	6.35 ± 0.00	6.32 ± 0.01	6.34 ± 0.00	6.31 ± 0.01	6.35 ± 0.00	6.34 ± 0.03
Proximate composition						
Moisture content (%)	62.93 ± 0.53	62.60 ± 0.11	62.84 ± 0.12	62.26 ± 0.40	62.24 ± 0.07	62.76 ± 0.17
Fat content (%)	20.67 ± 0.03	21.25 ± 0.01	20.16 ± 0.02	20.98 ± 0.02	20.87 ± 0.02	21.07 ± 0.01
Protein (%)	13.03 ± 0.23	12.88 ± 0.27	13.07 ± 0.29	13.16 ± 0.25	12.92 ± 0.25	12.96 ± 0.52
Ash (%)	2.50 ± 0.03	2.39 ± 0.09	2.42 ± 0.00	2.46 ± 0.01	2.48 ± 0.01	2.40 ± 0.01
Microbiological characteristic						
TPC (CFU/g)						
Day1	< 10 est.	< 10 est.	< 10 est.	< 10 est.	< 10 est.	< 10 est.
Day30	2.0×10^{5}	1.5×10^{2}	1.0×10^{2}	5.0×10^{1}	1.0×10^{1}	< 10 est.
Yeast & mold (CFU/g)						
Day1	< 10 est.	< 10 est.	< 10 est.	< 10 est.	< 10 est.	< 10 est.
Day30	< 10 est.	< 10 est.	< 10 est.	< 10 est.	< 10 est.	< 10 est.

WHC = water holding capacity; TBA = thiobarbituric acid; TPC = total plate count; CFU = colony forming units; est. = estimate

means ± SD within the same raw with different lowercase superscript letters are significantly different ($p < 0.05$).

^{NS} = not significantly different ($p > 0.05$)

Table 3 Effect of microwave heating on physicochemical and microbiological characteristics of vacuum-packaged sausages.

Sample parameter			Microwave heating level		
			400 W	600 W	800 W
Physical characteristic					
Hardness (N)		15 s	$40.10^{aNS} \pm 1.35$	$38.61^{aA} \pm 0.34$	$35.93^{bAB} \pm 1.71$
		30 s	$39.22^{aNS} \pm 0.22$	$34.37^{bB} \pm 0.14$	$34.30^{bB} \pm 0.76$
Springiness ^{NS}		15 s	0.92 ± 0.02	0.92 ± 0.02	0.92 ± 0.02
		30 s	0.94 ± 0.02	0.92 ± 0.01	0.92 ± 0.01
Cohesiveness ^{NS}		15 s	0.78 ± 0.01	0.76 ± 0.02	0.79 ± 0.01
		30 s	0.77 ± 0.02	0.78 ± 0.01	0.78 ± 0.01
Chewiness		15 s	$28.94^{aNS} \pm 1.18$	$27.24^{bA} \pm 0.78$	$25.27^{cNS} \pm 1.22$
		30 s	$28.15^{abNS} \pm 0.88$	$24.70^{bB} \pm 0.25$	$24.79^{bNS} \pm 0.08$
L* ^{NS}		15 s	69.44 ± 0.10	69.40 ± 0.19	69.78 ± 0.09
		30 s	69.44 ± 0.16	69.83 ± 0.06	69.37 ± 0.19
a* ^{NS}		15 s	8.94 ± 0.06	9.24 ± 0.10	9.13 ± 0.20
		30 s	8.95 ± 0.34	9.10 ± 0.19	9.30 ± 0.12
b* ^{NS}		15 s	20.39 ± 0.38	20.22 ± 0.24	20.38 ± 0.19
		30 s	20.53 ± 0.52	20.49 ± 0.04	20.48 ± 0.25
WHC (%)		15 s	$97.37^{aNS} \pm 0.15$	$95.73^{cNS} \pm 0.15$	$96.57^{bNS} \pm 0.61$
		30 s	$97.17^{aNS} \pm 0.64$	$96.47^{bNS} \pm 0.21$	$96.80^{bNS} \pm 0.26$
Cooking loss (%)		15 s	$1.14^{aNS} \pm 0.12$	$0.87^{bNS} \pm 0.07$	$0.89^{bNS} \pm 0.02$
		30 s	$1.14^{aNS} \pm 0.02$	$0.89^{bNS} \pm 0.23$	$0.90^{bNS} \pm 0.01$
Chemical characteristics					
Moisture content (%) ^{NS}		15 s	61.28 ± 0.29	60.52 ± 0.29	61.46 ± 0.30
		30 s	60.75 ± 0.37	61.47 ± 0.05	61.23 ± 0.06
Protein (%) ^{NS}		15 s	13.03 ± 0.23	13.07 ± 0.29	12.92 ± 0.25
		30 s	12.88 ± 0.27	13.16 ± 0.25	12.96 ± 0.52
Fat (%) ^{NS}		15 s	20.67 ± 0.03	20.16 ± 0.02	20.87 ± 0.02
		30 s	21.25 ± 0.01	20.98 ± 0.02	21.07 ± 0.01
Ash (%) ^{NS}		15 s	2.54 ± 0.02	2.62 ± 0.03	2.51 ± 0.06
		30 s	2.58 ± 0.02	2.55 ± 0.03	2.51 ± 0.02
pH ^{NS}		15 s	6.35 ± 0.00	6.36 ± 0.00	6.34 ± 0.00
		30 s	6.34 ± 0.00	6.34 ± 0.01	6.30 ± 0.00
TBA (mg malonaldehyde/kg)		15 s	$0.30^{aA} \pm 0.03$	$0.30^{aNS} \pm 0.02$	$0.20^{bNS} \pm 0.01$
		30 s	$0.28^{abB} \pm 0.01$	$0.31^{aNS} \pm 0.03$	$0.22^{bNS} \pm 0.01$
Microbiological characteristics					
TPC (CFU/g)	day 1	15 s	< 10 est.	< 10 est.	< 10 est.
		30 s	< 10 est.	< 10 est.	< 10 est.
	day 30	15 s	2.0×10^3	1.1×10^3	1.3×10^2
		30 s	6.1×10^2	3.5×10^2	1.1×10^2
Yeast & mold	day 1	15 s	< 10 est.	< 10 est.	< 10 est.
		30 s	< 10 est.	< 10 est.	< 10 est.
	day 30	15 s	< 10 est.	< 10 est.	< 10 est.
		30 s	< 10 est.	< 10 est.	< 10 est.

WHC = water holding capacity; TBA = thiobarbituric acid; TPC = total plate count; CFU = colony forming units.

$^{A-B}$ = means ± SD within the same column with different uppercase superscript letters within each parameter are significantly different ($p \leq 0.05$).

$^{a-c}$ = means ± SD within the same row with different lowercase superscript letters within each parameter are significantly different ($p \leq 0.05$).

^{NS} = not significantly different ($p > 0.05$).

The hardness, cohesiveness, springiness and chewiness values of the thermosonication samples are presented in Table 2. There was an effect due to the thermosonication time as the hardness of the sausage changed after 10 min of thermosonication ($p \leq 0.05$), while the a reduction in the cohesiveness was detected after treating for 30 min. Moreover, the cohesiveness of the treated samples was maintained until the thermosonication time reached 40 min ($p \leq 0.05$). All values were lower than the values for untreated sausage ($p \leq 0.05$) revealing that thermosonication with a contact time over 20 min directly degraded the texture quality of the sausage. However, thermosonication for 10 min and 20 min did not affect the texture of the treated sausages compared with the control sample and the conventional pasteurization sample. Dickens et al. (1991) reported that ultrasound (40 kHz, 15 min) did not affect the texture of cooked broiler breast meat. With microwaving, the hardness and chewiness values significantly decreased when the power level and contact time of microwaves increased. The cohesiveness value significantly increased compared to the control and conventional pasteurization. No

significant changes in springiness values were detected for any of the treatments conducted in the current study.

The effect of the thermosonication process on the WHC and cooking loss results are shown in Table 2. There was no significant difference in the WHC (96.40–96.77%). Cooking loss is caused by water loss during the thermal processing of meat products (Sheard et al., 1998; Scheeder et al., 2001). A change in the cooking loss resulted after treating with thermosonication for 30 min and 40 min ($p \leq 0.05$). These results might have occurred because sonication reduced the number of air bubbles in the sausage matrix and hence more water was able to be trapped inside the structure (Siró et al., 2009).

However, the WHC of the sausage samples was significantly different in the microwave process where the WHC was affected by the microwave heating level (Table 3). After the microwave process, the treated samples at 600 W and 800 W had significantly decreased WHC, while the microwave heating times (15 s and 30 s) did not affect the WHC and cooking loss of the vacuum-packaged sausages. One report indicated that microwave heating resulted in a higher moisture loss in food (Cross et al., 2009). However, the moisture loss was not restricted to the meat product during the microwave heating process but also occurred in the raw material, food composition and food additive for sausage production.

Microbial analysis of sausages

The microbiological characteristics of the sausage samples after treatment using thermosonication are shown in Table 2. The numbers of colonies of bacteria on the first day of storage were less than 10 CFU/g. In addition, the bacterial, and yeast and mold counts were inhibited by the thermosonication treatment at 80°C for 10 min, 20 min, 30 min or 40 min. The total bacterial counts decreased as the contact time of thermosonication increased. The yeast and mold levels in the all samples were less than 1 log CFU/g on all days of storage (up to day 30). Pohlman et al. (1997) applied ultrasound (frequency 40 kHz) for 30 min at 30°C in beef and evaluated the microbial growth during storage at refrigeration temperature. Compared to conventional pasteurization, the sonicated beef had a lower number of bacteria. In the current study, thermosonication at 80°C for 10 min inhibited bacterial growth. Thermosonication at 80°C for 40 min completely inhibited bacterial growth at day 30 of storage. The microbiological properties of the sausage samples treated with microwaves are shown in Table 3. Bacteria were inhibited by the microwave treatments at 400 W, 600 W or 800 W for 15 or 30 s, with the total bacterial counts decreasing as the power level of microwaving increased. The presence of yeast and mold is a common and recurrent problem during storage. Zohri et al. (2014) reported 40 samples of beef burger and sausage were contaminated with different species of yeast and mold. Variations in the density and kind of these fungi are often related to the conditions of processing and storage. The yeast and mold levels in the current samples treated with microwave heating were lower than 1 log CFU/g at the final storage date. These results suggested that thermosonication or microwave heating can be used to control yeast and mold growth during storage in sausage products.

Sensory characteristic of sausages

The results of the sensory testing of sausages treated with thermosonication are given in Fig. 1A. All sensory attributes were in the range from "like moderately" to "like very much". The appearance and color were rated high for thermosonication at 40 min and there was no significant difference with the conventional sample. There was a low liking score for the texture of the sausages following thermosonication. The odor of the control sausage without thermosonication received the lowest score. Finally, the taste and overall liking were rated high for thermosonication and conventional sausages with no significant difference detected. All sensory attributes for the microwave samples were in the range from "like moderately" to "like very much" (Fig. 1B). The samples treated with a high power

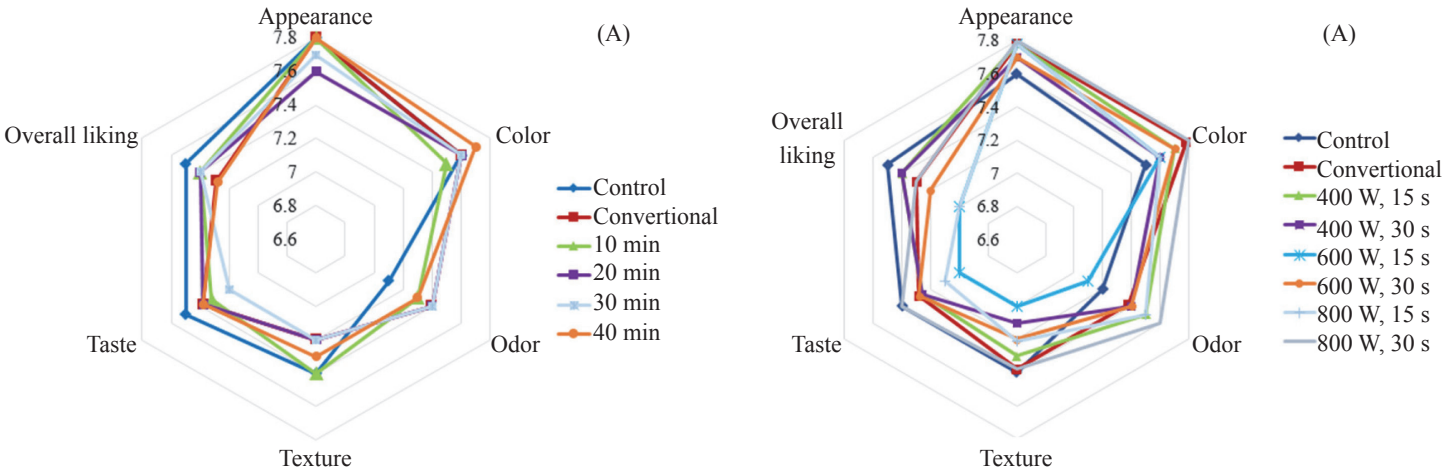

Fig. 1 Sensory scores for appearance, color, odor, texture, taste and overall liking of sausage obtained using a 9-point hedonic scale (1 = dislike extremely to 9 = like extremely): (A) thermosonication; (B) microwave heating

level of microwaves (800 W) received higher scores for appearance, color and odor than did the low power levels (400 W and 600 W); however, there were no significant differences compared with the conventional sample. All microwaved sausage samples received a low score for the texture attributes. The taste and overall liking were rated high for microwave and conventional sausages with no significant difference. The consumers gave high scores for taste and overall liking to both microwave and conventional sausages.

The results indicated that the optimum conditions for thermosonication were 80°C for 20 min. This technique effectively inhibited microbial growth during the storage period. All samples were heated to a temperature higher than 80°C which was similar to pasteurization. However, thermosonication significantly affected the hardness, springiness, cohesiveness and chewiness of the prototype sausage. The lightness of the conventional pasteurization sausage samples was significantly higher than for the thermosonicated samples. The sensory characteristics of the thermosonicated samples were not significantly different from the conventional pasteurization samples. The microwave treatment effectively inhibited microbial growth during the storage period. The yeast and mold levels in the samples treated with microwaves were less than 1 log CFU/g throughout the storage period. After heating, the temperature was measured (results not shown) and samples treated with microwaves at 800 W had a temperature higher than 80°C. However, microwave treatment affected the hardness, springiness, cohesiveness and chewiness ($p \leq 0.05$). The sensory characteristics of the microwave samples were not significantly different from the conventional pasteurization samples. In the current study, the optimal microwave conditions were microwaving at 400 W for 30 s. However, in terms of energy consumption, conventional thermal (75°C for 15 min), thermosonication (80°C for 20 min) and microwave heating (400 W for 30 s) required 1,800 kJ, 540 kJ and 12 kJ, respectively, indicating that thermosonication or using microwaves can save energy in the cost of sausage production. The shelf-life studies were performed for three pasteurization treatments (conventional, thermosonication and microwaving) and compared with the treatment without pasteurization as a control. The microbiological and physicochemical properties of sausages were monitored during a storage period of 30 days at 4°C. The shelf-life of the treated samples was extended to more than 30 days at 4°C based on the total aerobic count below the detection limit of 5 log CFU/g (Thai Industrial Standards Institute, 2007).

Hence, thermosonication and microwave technology both have potential in sausage production and could maintain overall quality and extend the shelf life of the sausage.

The best conditions for in-packed post pasteurization with both thermosonication and microwave heating was achieved and the shelf lives of these sausages were extended to more than 30 days at which time the conventional samples were rated as spoiled. Moreover, the sensory characteristics, chemical qualities and textural parameters of the treated samples were not changed during the post pasteurization process. Hence, post pasteurization using thermosonication or microwave heating is recommended as alternative thermal processing options for the food industry.

Conflict of Interest

The authors declare there is no conflict of interest.

Acknowledgements

The authors are grateful for financial support (Grant ID: PHD57I0028) from the Research and Researcher for Industry (RRi) PhD Scholarship awarded by the Thailand Research Fund under the Office of the Prime Minister, Royal Thai Government. The Department of Product Development, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand allowed the use of their instruments during this project.

References

Abdullah, N., Chin, N.L. 2014. Application of thermosonication treatment in processing and production of high quality and safe-to-drink fruit juices. Agric. Agric. Sci. Procedia. 2: 320–327.

Alves, L.L., da Silva, M.S., Flores, D.R.M., et al. 2018. Effect of ultrasound on the physicochemical and microbiological characteristics of Italian salami. Food Res. Int. 106: 363–373.

Association of Official Analytical Chemists. 2000. Official Methods of Analysis of AOAC International, seventeen ed. AOAC International. Gaithersburg, MD, USA.

Atchley, A.A., Crum, L.A. 1998. Acoustic cavitation and bubbles dynamics. In: Suslick, K.S. (Ed.). Ultrasounds, Its Chemical, Physical and Biological Effects. VCH Publishers. New York, NY, USA, pp. 1–64.

Barbut, S. 2006. Effects of casein, whey and milk powders on the texture and microstructure of emulsified chicken meat batters. LWT-Food Sci. Technol. 39: 660–664.

Berrang, M.E., Meinersmann, R.J., Northcutt, K., Smith, D.P. 2002. Molecular characterization of *Listeria monocytogenes* isolated from a poultry further processing facility and from fully cooked product. J. Food Prot. 65: 1574–1579.

Burfoot, D., Griffin, W.J., James, S.J. 1988. Microwave pasteurisation of prepared meals. J. Food Eng. 8: 145–156.

Chandrasekaran, S., Ramanathan, S., Basak, T. 2013. Microwave food processing-review. Food Res. Int. 52: 243–261.

Chen, J.H., Ren, Y., Seow, J., Liu, T., Bang, W.S., Yuk, H.G. 2012. Intervention technologies for ensuring microbiological safety of meat: Current and future trends. Compr. Rev. Food Sci. Food Saf. 11: 119–132.

Cichoski, A.J., Rampelotto, C., Silva, M.S., et al. 2015. Ultrasound-assisted post-packaging pasteurization of sausages. Innov. Food Sci. Emerg. Technol. 30: 132–137.

Cross, G.A., Fung, D.Y.C., Decareau, R.V. 2009. The effect of microwave on nutrition value of foods. CRC Crit. Rev. Food Sci. Nutr. 16: 355–381.

Dickens, J.A., Lyon, C.E. Wilson, R.L., 1991. Effect of ultrasonic radiation on some physical characteristics of broiler breast muscle and cooked meat. Poultry Sci. 70: 389–396.

Erkaya, T., Baslar, M., Sengul, M., Ertugay, M.F. 2015. Effect of thermosonication on physical, microbiological and sensorial characteristics of ayran during storage. Ultrason. Sonochem. 23: 406–412.

Evrendilek, G.A., Balasubramaniam, V.M., 2011. Inactivation of *Listeria monocytogenes* and *Listeria innocua* in yoghurt drink applying combination of high pressure processing and mint essential oils. Food Control. 22: 1435–1441.

Evelyn, E., Silva, F.V.M. 2015. Thermosonication versus thermal processing of skim milk and beef slurry: Modeling the inactivation kinetics of psychrotrophic *Bacillus cereus* spores. Food Res. Int. 67: 67–74.

Feng, H. 2011. The thermodynamic and kinetic aspects of power ultrasound process. In: Feng, H., Barbosa-Canovas, G.V., Weiss, J. (Eds.). Ultrasound Technologies for Food and Bioprocessing. Springer. New York, NY, USA, pp. 107–123.

Fito, P., Chiralt, A., Eugenia Martin, M. 2005. Current state of microwave applications to food processing. In: Barbosa-Canovas, G.V., Tapia, M.S., Cano, M.P. (Eds.). Novel Food Processing Technologies. CRC Press. New York, NY, USA. pp. 525–537.

Honikel, K.O., Hamm, R. 1994. Measurement of water-holding capacity and juiciness. In: Pearson, A.M., Dutson, T.R. (Eds.). Advances in Meat Research-Volume 9 Quality Attributes and Their Measurement in Meat, Poultry and Fish Product. Blackie Academic and Professional. Glasgow, UK. pp. 125–157.

Knorr, D. 1993. Effects of high-hydrostatic-pressure processes on food safety and quality. Food Techn. 46: 156–161.

Lee, H., Zhou, B., Liang, W., Feng, H., Martin, S.E. 2009. Inactivation of *Escherichia coli* cells with sonication, manosonication, thermosonication, and manothermosonication: Microbial responses and kinetics modeling. J. Food Eng. 93: 354–364.

Lopez-Malo, A., Jimenez-Fernandez, M., Palou, E. 2001. *Penicillium digitatum* spores inactivation by combining thermoultrasonication treatments and antimicrobial agents. In: Barrette, K. (Ed.). Proceedings of the IFT 2001 Annual Meeting Technical Program. Institute of Food Technologists. New Orleans, LA, USA, p. 151.

Manas, P., Pagan, R., Raso, J., Sala, F.J., Condon, S. 2000. Inactivation of *Salmonella typhimurium*, and *Salmonella senftenberg* by ultrasonic waves under pressure. J. Food Prot. 63: 451–456.

Mansour, A.R., Oh, D.H. 2015. Combined effects of thermosonication and slightly acidic electrolyzed water on microbial quality and shelf life extension of fresh-cut kale during refrigeration storage. Food Microbiol. 51: 154–162.

Martínez, L., Djenane, D., Cilla, I., Beltrán, J.A., Roncalés, P. 2006. Effect of varying oxygen concentrations on the shelf-life of fresh pork sausages packaged in modified atmosphere. Food Chem. 94: 219–225.

McDonnell, C.K., Allen, P., Chardonnereau, F.S., Arimi, J.M., Lyng, J.G. 2014. The use of pulsed electric fields for accelerating the salting of pork. LWT-Food Sci. Technol. 59: 1054–1060.

Ohlsson, T. 1991. Microwave processing in the food industry. Eur. Food. Drink Rev. 7: 9–11.

Ordoñez, J.A., Aguilera, M.A., Garcia, M.L., Sanz, B. 1987. Effect of combined ultrasonic and heat treatment (thermoultrasonication) on the survival of a strain of *Staphylococcus aureus*. J. Dairy Res. 54: 61–67.

Piyasena, P., Moharep, E., Mckellar, R.C. 2003. Inactivation of microbes using ultrasound: A review. Int. J. Food Microbiol. 87: 207–216.

Pohlman, F.W., Dikeman, M.E., Zayas, J.F. 1997. The effect of low-intensity ultrasound treatment on shear properties, color stability and shelf life of vacuum packaged beef semitendinosus and biceps femoris muscles. Meat Sci. 45: 329–337.

Roldan, M., Antequera, T., Palacios, T.P., Ruiz, J. 2014. Effect of added phosphate and type of cooking method on physico-chemical and sensory features of cooked lamb loin. Meat Sci. 97: 69–75.

Rubio, B., Martínez, B., García-Cachán, M.D., Rovira, J., Jaime, I. 2008. Effect of the packaging method and the storage time on lipid oxidation and colour stability on dry fermented sausage salchichón manufactured with raw material with a high level of mono and polyunsaturated fatty acids. Meat Sci. 80: 1182–1187.

Ryynänen, S., Ohlsson, T. 1996. Microwave heating uniformity of ready meals as affected by placement, composition, and geometry. J. Food Sci. 61: 620–624.

Sala, F.J., Burgos, J., Condón, S., López, P., Raso, J. 1995. Effect of heat and ultrasound on microorganisms and enzymes. In: Gould, G.W. (Ed.), New Methods of Food Preservation. Blackie Academic and Professional. Glasgow, UK, pp. 176–204

Salazar-Gonzalez, C., San Martin-Gonzalez, M.F., Lopez-Malo, A., Sosa-Morales, M.E. 2012. Recent studies related to microwave processing of fluid foods. Food Bioproc. Tech. 5: 31–46.

Sams, A.R., Feria, R. 1991. Microbial effects of ultrasonication of broiler drumstick skin. J. Food Sci. 56: 247–248.

Scheeder, M.R.L., Casutt, M.M., Roulin, M., Escher, F., Dufey, P.A., Kreuzer, M. 2001. Fatty acid composition, cooking loss and texture of beef patties from meat of bulls fed different fats. Meat Sci. 58: 321–328.

Selby, T.L., Berzins, A., Gerrard, D.E., Corvalan, C.M., Grant, A.L., Linton, R.H. 2006. Microbial heat resistance of Listeria monocytogenes and the impact on ready-to-eat meat quality after post-pasteurization. Meat Sci. 74: 425–434.

Seymour, I.J., Burfoot, D., Smith, R.L., Cox, L.A., Lockwook, A. 2002. Ultrasound decontamination of minimally process fruits and vegetable. Int. J. Food Sci. Technol. 37: 547–557.

Sheard, P.R., Nute, G.R., Happell, A.G.C. 1998. The effect of cooking on the chemical composition of meat products with special reference to fat loss. Meat Sci. 49: 175–191.

Siró, I., Vén, Cs., Balla, Cs., Jónás, G., Zeke, I., Friedrich, L. 2009. Application of an ultrasonic assisted curing technique for improving the diffusion of sodium chloride in porcine meat. J. Food Eng. 91: 353–362.

Sofos, J.N. 2008. Challenges to meat safety in the 21st century. Meat Sci. 78: 3–13.

Sofos, J.N., Geornaras, I. 2010. Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of *Escherichia coli* O157:H7 in non intact, and *Listeria monocytogenes* in ready-to-eat, meat products. Meat Sci. 86: 2–14.

Song, W.J., Kang, D.H. 2016. Influence of water activity on inactivation of *Escherichia coli* O157:H7, *Salmonella Typhimurium* and *Listeria monocytogenes* in peanut butter by microwave heating. Food Microbiol. 60: 104–111.

Tang, J., Hong, Y.K., Inanoglu, S., Liu, F. 2018. Microwave pasteurization for ready to-eat meals. Curr. Opin. Food Sci. 133–141.

Tarladgis, B.G., Watts, B.M., Younathanm, M.T., Dugan, L. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44–52.

Thai Industrial Standards Institute. 2007. Meat Product (TISI. 2300–2549). Ministry of Industry. Bangkok, Thailand.

Walkling-Ribeiro, M., Noci, F., Rener, J. Cronin, D.A., Lyng, J.G., Morgan, D.J. 2009. The impact of thermsonication and pulse electric fields on *Staphylococcus aureus* inactivation and selected quality parameter in orange juice. Food Bioproc. Tech. 2: 422–430.

Zell, M., Lyng, J.G., Cronin, D.A., Morgan, D.J. 2010. Ohmic cooking of whole turkey meat –Effect of rapid ohmic heating on selected product parameters. Food Chem. 120: 724–729.

Zohri, A.A., Moharram, A.M., Refaie, R.R.S. 2014. Mycobiota contaminating beef burger and sausage with reference to their toxins and enzymes. J. Basic Appl. Mycol. 5: 61–73