A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Application of 915 MHz Microwave Heating for Inactivation of Foodborne Pathogen

식중독균 제어를 위한 915 MHz 마이크로파 가열의 활용

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석사학위논문

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이 논문을 석사학위 논문으로 제출함

2018 년 8 월

서울대학교 대학원 농생명공학부

김 우 주

김우주의 석사 학위논문을 인준함

2018 년 8 월

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ABSTRACT

Microwave heating is a form of dielectric heating, generating thermal energy inside food material. In comparison to the conventional heating, as microwave heating shows rapid heat generation and improved heat distribution, microwave can heat food product more effectively with less quality deterioration. There are several factors influencing microwave heating pattern including food composition, packaging material, microwave frequency, and microwave power. Among various factors, research concerning dielectric properties and packaging material has been limited. Thus, in this study, I studied the effect of dielectric properties of food product and plastic packaging material on 915 MHz microwave heating. Also, in order to improve 915 MHZ microwave heating efficiency, I investigated which essential oil can be utilized to induce synergic effect in inactivation of foodborne pathogen. First, I studied the effect of sugar contents on the dielectric properties of hot-chili sauce, and further heating rate when exposed to 915 MHz microwave heating. Sugar

contents did not affect the heating rate of hot-chili sauce when subjected to conventional heating (P>0.05), while it is important factor when treated with 915 MHz microwave heating (P<0.05). Under 915 MHz microwave energy, hot-chili sauce with lower sugar contents showed faster heating rate due to higher dielectric constant and dielectric loss factor. This trend linked to inactivation of *Escherichia coli* O157:H7, *Salmonella*. Typhimurium, and *Listeria monocytogenes*. When hot-chili sauce with higher dielectric properties were applied to 915 MHz microwave heating, it took shortest time to eliminate foodborne pathogen under detection limit. Also, I investigated the effect of plastic packaging material on the heating pattern of 915 MHz microwave heating. I utilized PE, PP, Nylon and PEI. PEI had highest value of dielectric constant and dielectric loss factor in this study. No significant difference in heating rate was observed among PE, PP and Nylon (P>0.05), but PEI showed slowest heating rate. Also, this trend linked to inactivation efficiency. PE, PP and Nylon showed better inactivation of foodborne pathogen compared to PEI. As microwave heating is thermal treatment, it can cause quality deterioration. In addition, in mass production, non-uniform heating can be one of problem in food processing, though 915 MHz had improved uniform heating distribution in comparison to 2450 MHz microwave heating and conventional heating. Thus, I researched the method to improve inactivation efficiency of 915 MHz microwave heating. When 3 mM of essential oil including carvacrol, eugenol, carvone, and citral was added to hot-chili sauce, no significant difference in dielectric properties and heating rate was observed. However, carvacrol showed most effective inactivation efficiency among various essential oil. I could observe that individual carvacrol treatment and combination of microwave and carvacrol treatment showed higher value of PI than other essential oils (P<0.05), indicating that these treatment causes most severe damage to cell membrane. This is because carvacrol induce destruction of cell membrane potential as indicated by the results of $DiBAC_4(3)$. Carvacrol treatment showed highest value. Thus, I can conclude that dielectric properties and packaging material

is important factor for 915 MHZ microwave heating and 915 MHz microwave heating can be improved with addition of natural antimicrobial compound.

Keywords: **915 MHz microwave heating, food-borne pathogens, packaging material, dielectric properties, essential oil**

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Inactivation of foodborne pathogens influenced by dielectric properties, relevant to sugar contents, in chili sauce by 915 MHz microwaves

I.1. INTRODUCTION

Sauce products are widely consumed as they are essential for food moisture, flavor, and visual effect. In the Korean food industry, the sales value of various sauces accounts for a considerable part of their profit, which is approximately 1.37 billon U.S dollars (Statista, 2017). Also, sales for chili sauce in the U.S rose by approximately 8.1 percent in 2011 (Statista, 2016). However, in spite of advances in food science and technology for ensuring food safety, foodborne illnesses continue to occur worldwide. Unfortunately, sauces are not an exception, and several cases of sauce-related foodborne diseases have been reported. Salsa had been implicated in 70 foodborne illness outbreaks, and 2,280 illness cases occurred between 1990 and 2006 (Franco and Simonne, 2009). Also, The U.S Centers for Disease Control and Prevention (CDC) reported 15 foodborne outbreaks related to sauces between 2010 and 2015 (CDC, 2017a)

In general, acidic or acidified food products have been regarded as biologically safe since many bacteria cannot grow under acidic conditions (below pH 4.6, which is the minimum hydrogen ion concentration supporting growth of *Clostridium botulinum*) (van Schothorst et al., 2009). However, this does not mean that an acidic environment fully inactivates foodborne pathogens. This is because *Escherichia coli* O157:H7 can survive at pH 4

(Glass et al., 1992). Also, a chili sauce-related foodborne outbreak caused by *Salmonella enteritica* occurred in 2013 and 2015 (CDC, 2017a), even though chili sauce is very acidic, implying that an acidic condition below pH 4.6 cannot ensure biologically safety. Moreover, jalapeño and serrano peppers, which are primary ingredients of chili sauces, were involved in a multi-state foodborne outbreak associated with *Salmonella* spp. (CDC, 2008) . Additionally, it was reported that fresh produce can contain several foodborne pathogens such as *Listeria monocytogenes* (Harris et al., 2003). As hot chili sauce can be prepared from fresh produce it can harbor foodborne pathogens.

Salmonella enterica serovar Typhimurium is the main cause of non-typhoidal salmonellosis, involving diarrhea, abdominal cramping, and fever within 72 h of infection (Baird-Parker, 1990). Also, *E. coli* O157:H7 causes severe illnesses which include bloody diarrhea and hemolytic uremic syndrome (Besser et al., 1999). *L. monocytogenes* causes a severe syndrome called Listeriosis involving neonatal death, septicemia, abortion and meningitis, and has a high mortality rate of about 24 % (CDC, 2017b). Thus, appropriate methods need to be developed to inactivate these foodborne pathogens for the biological safety of hot chili sauce.

For pasteurization, conventional heating is typically utilized by the food industry. Heating occurs through conduction and convection when food is subjected to conventional heating. In this process, the outer part of the food can be over-heated while the interior (cold spot) can be underheated; thus conventional heating results in non-uniform heat distribution and takes more time for proper pasteurization than other methods such as ohmic heating and RF heating. Consequently, pasteurization by means of conventional heating is not effective for inactivating foodborne pathogens and can result in deterioration of food quality.

For these reasons, it is necessary to develop useful alternatives to conventional heating, and microwave heating has been considered as an alternative in several studies. Microwave heating quickly generates volumetric heat inside food as microwaves can penetrate the interior of food, implying that food can processed with better efficiency. Since it can process food products much faster, it can maintain desirable food quality attributes such as nutrition or flavor in comparison to conventional heating treatment ((Vadivambal and Jayas, 2010; Zhu et al., 2007)). Since microwave heating is a form of dielectric heating and dielectric properties can influence the heating rate, dielectric properties of food products should be considered.

Dielectric properties can be affected by several factors such as salt content, moisture content, frequency of applied electromagnetic waves, and temperature. Among these factors, water is the main factor influencing heating rate of food exposed to microwave energy. Thus, sugar content was selected as a factor for dielectric properties to investigate the effect of microwave heating on chili sauce pasteurization, since the sugar content of chili sauce varies from 10% to 38% in the market and affects water activity (a_w)of chili sauce as sugar binds with water molecules.

There have been several studies investigating the efficiency of microwave heating for inactivation of foodborne pathogens depending on the water activity of food products (Song and Kang, 2016b). However, it has not been reported how dielectric properties of food products affect the pasteurization of food under microwave energy. Thus, in this study, I compared the efficiency of microwave heating and conventional heating and investigated how dielectric properties of food affect the inactivation of foodborne pathogens in chili sauce.

I.2. MATERIALS AND METHODS

I.2.1. Bacterial strains and culture preparation

Strains of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S*. Typhimurium (ATCC 19585, ATCC 19115, DT 104), and *L. monocytogenes* (ATCC 15313, ATCC 19111, ATCC 19115) were obtained from the bacterial cell culture collection of Seoul National University (Seoul, Republic of Korea) for study. Stock cultures were stored frozen at -80°C in 0.7 ml of Tryptic Soy Broth (TSB; Difco, BD, Sparks, MD) and 0.3 ml of sterile 50% (V/V) glycerol. Cultures for this study were streaked onto Tryptic Soy Agar (TSA; Difco, BD), incubated at 37°C for 24h h and stored at 4°C. A single colony of each strain was transferred to 5 ml of TSB and incubated at 37°C for 24 h. Then, 1 ml of the overnight culture of each strain was spread onto TSA, followed by 37°C incubation for 24 h. Five ml of sterile 0.2% peptone water (PW) was added to each plate to harvest the bacterial cells, and the cell suspensions were collected by scrubbing the agar surfaces with sterile cotton swabs (3M pipette swab, 3M Korea Ltd) to remove cells. Cell pellets were obtained by centrifugation of the cell suspension at 4000 g for 20 min at 4°C and washing three times with 0.2% peptone water (PW; Bacto, Sparks, MD) The final cell pellets were resuspended in PW and the population of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* was 10^8 – 10^9 CFU/ml

I.2.2. Sample preparation and inoculation

Experiments were performed using commercially processed hot chili sauce. Shelf-stable chili sauce of 10% sugar content was purchased at a local grocery store (Seoul, Republic of Korea) for this study and stored at room temperature (22 ± 1 °C). Sugar content was adjusted by addition of sucrose. Twenty-five g of chili sauce sample were aseptically placed in sterile 100 ml Pyrex beakers. For inoculation, one ml of bacterial cell culture was inoculated into each sample and thoroughly mixed for 1 min with a sterile spoon to ensure even distribution of bacterial cells. The populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* recovered from inoculated samples were 10^7 – 10^8 CFU/ml

I.2.3. Microwave heating treatment and Conventional heating treatment

Microwave treatment was performed in a previously described apparatus (Sung and Kang, 2014) for 915 MHz treatment. A beaker containing 25 g of chili sauce sample was located at the center of the turntable, and subjected to microwave heating. For inactivation of pathogens and quality evaluation, samples were treated at 3.0 kW for the 915 MHz treatment.

For conventional heating, a constant-temperature water bath (BW-10G; Jeio Tech, Seoul, South Korea) was utilized in this study. The conventional heating chamber was made of stainless steel (2 x 15 x 6 cm) of 0.2 cm thickness. Temperature of the water bath was fixed at 100°C. Samples were prepared as above and treated for up to 6 min.

I.2.4. Temperature measurement

For temperature measurement of microwave heating and conventional heating, the geometric center temperature of a non-inoculated sample adjusted to 10 %, 25%, and 40% sugar content in a beaker was measured with a fiber optic sensor (FOT-L; FISO Technologies INC, Quebec, Canada) connected to a temperature signal conditioner. This optic sensor measures real-time temperature in samples during microwave heating and conventional heating and was recorded at 1 s intervals. The rate of temperature increase was calculated by dividing the difference in temperature between the beginning and the end by the treatment time

I.2.5. Dielectric properties measurement

Dielectric properties of chili sauce of varying sugar content were measured using a network analyzer coupled with a dielectric probe kit (85070E, Agilent Technologies). Calibration was conducted using three standards: air, a standard shorting block (85070-60003, Agilent Technologies), and distilled water at 25°C. During measurement, room temperature was maintained at 25°C

I.2.6. Bacterial cell enumeration

After conventional heating treatment and 915 MHz microwave heating treatment, twenty-five g of sample was immediately mixed with 25 ml of 4.0°C 0.2% sterile peptone water (PW) to cool the sample and arrest thermal inactivation. Then, the mixture was poured into a stomacher bag containing 200 ml of PW and homogenized for 2 min in a stomacher (EASY MIS, AES Chemunex, Rennes, France). After homogenization, one ml aliquots of samples were 10-fold serially diluted in 9 ml of 0.2% sterile peptone water and then 0.1 ml of diluent was spread-plated onto Sorbitol MacConkey agar (SMAC; Difco), Xylose Lysine Deoxycholate agar (XLD; Difco), and Oxford Agar Base with antimicrobial supplement (OAB; MB cell) for enumeration of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively. In order to enumerate sub-lethally injured cells of *E. coli* O157:H7, the same volume of diluents were spread-plated onto phenol red agar base with 1% sorbitol (SPRAB; Difco) (Rocelle et al., 1995). For enumeration of injured cells of *S.* Typhimurium and *L. monocytogenes*, the overlay (OV) method was used (Lee and Kang, 2001). TSA was utilized as a nonselective agar to resuscitate injured bacteria. One hundred μl of selected dilutions were spread-plated onto TSA medium, incubated for 2 h at 37°C, then overlaid with 10 ml selective medium such as XLD or OAB for *S.* Typhimurium or *L. monocytogenes*, respectively. After solidification of the medium, overlaid plates were further incubated for 22 h at 37°C.

I.2.7. Color and water activity measurement

To measure the effect of conventional heating and microwave heating on the color of chili sauce of varying sugar content, a Minolta colorimeter (CR400; Minolta Co., Osaka, Japan) was used to measure the color changes of heat-treated samples. The value of L*, a*, and b* were utilized to quantify color attributes. L*, a*, and b* indicate color lightness, redness, and yellowness of sample, respectively. For measurement of the influence of sugar content on water activity of chili sauce, an Aqualab model 4TE a_w

meter (METER Group, Pullman, WA) was used to measure the water activity of treated samples.

I.2.8. Statistical analysis

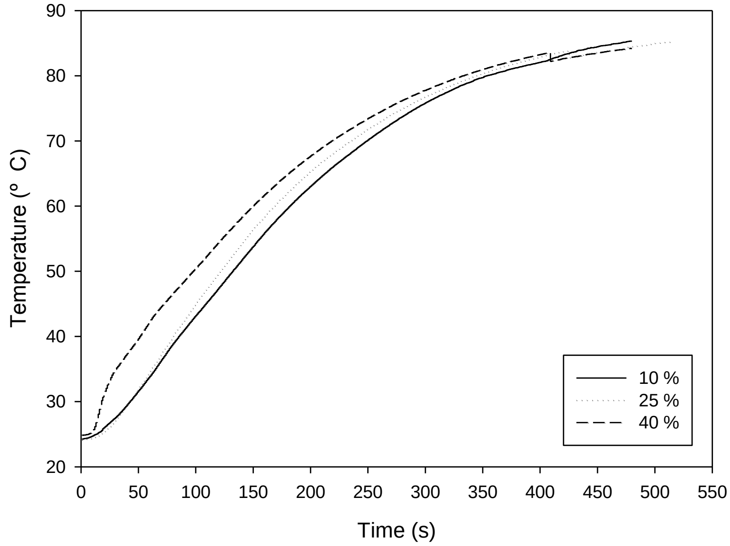
All experiments were repeated at least three times with independently prepared samples. Data were analyzed by analysis of variance (ANOVA) and t-test (LSD) using the Statistical Analysis System (SAS Institute, Cary, NC, USA). A p value of <0.05 was used to indicate significant difference.

I.3 RESULTS

I.3.1. Influence of sugar content on conventional heating and 915 MHz microwave heating treatment.

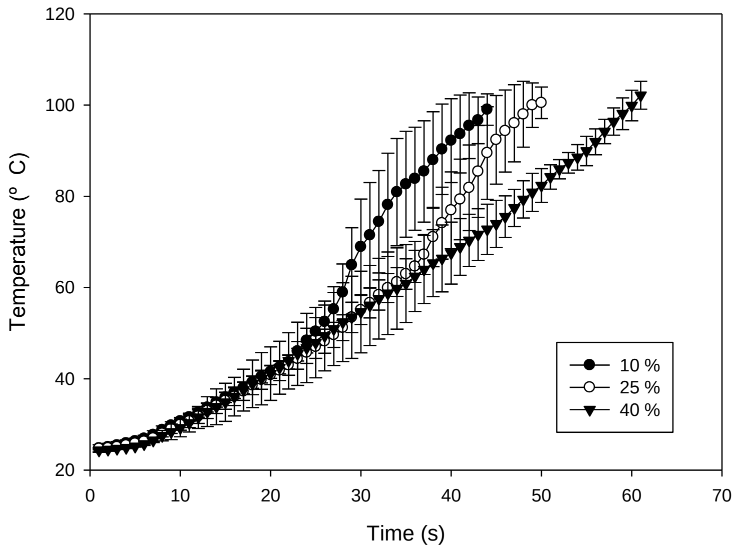
The average temperatures of chili sauce of varying sugar content (10% to 40%) during conventional heating and 915 MHz microwave heating at 3.0kW are shown in Figure I.1. Among different sugar levels, there was no significant difference in the rate of temperature increase when treated with conventional heating ($P > 0.05$). On the other hand, the rate of increase was dependent on sugar content when exposed to microwave energy. Following 915 MHz microwave heating treatment at 1.5kW, the temperature of 10%, 25%, and 40% sugar content chili sauce increased from ca. 20°C to 100°C after 44 s, 50s, and 61s, respectively. Also, it took 28 s, 34 s, and 48 s, respectively, to reach 100°C when chili sauce of 10%, 25%, 40% sugar content was treated with 915 MHz microwave heating at 3.0 kW. Lower sugar content combined with a higher power level of microwave heating accelerated the rate of temperature increase.

(a)



90
80
70
60
50
40
30
20
Temperature (° C)
0
50
100
150
200
250
300
350
400
450
500
550
Time (s)
10 %
25 %
40 %

(b)



120
100
80
60
40
20
Temperature (° C)
0
10
20
30
40
50
60
70
Time (s)
10 %
25 %
40 %

(c)

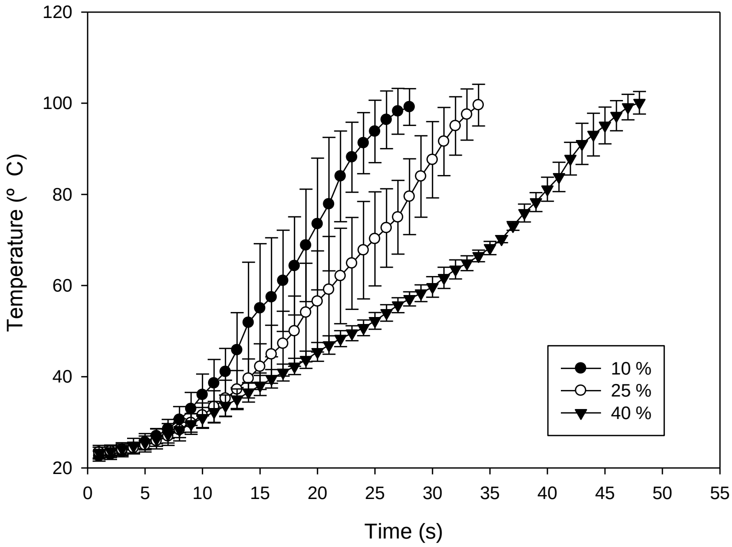
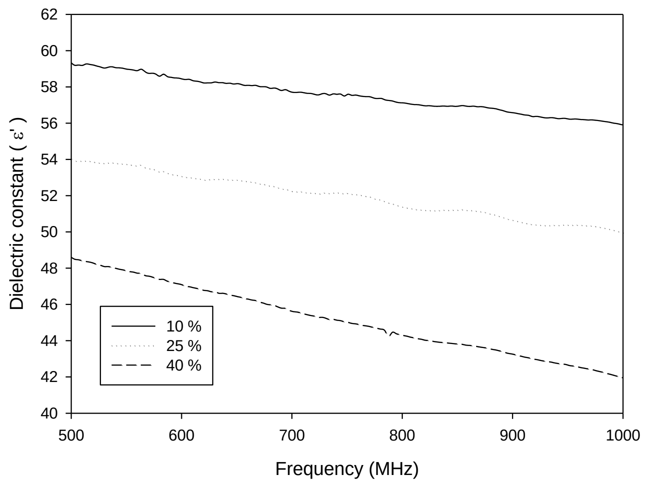


Fig. I.1. Temperature curves of 10%, 25%, and 40 % sugar content chili sauce after (a) conventional heating, (b) 915 MHz microwave heating at 1.5 kW, and (c) 915 MHz microwave heating at 3.0

I.3.2. Influence of sugar content on dielectric properties of chili sauce.

In The dielectric properties of chili sauce at each of the three sugar levels were analyzed from 500 MHz to 1 GHz as shown in Fig. I.2. Both dielectric constant and dielectric loss factor of chili sauce significantly increased with decreasing sugar content. At 915 MHz, the dielectric constant of chili sauce of 10%, 25%, and 40% sugar content were 56.4, 50.4, and 43.1, respectively. Also, the dielectric loss factor of chili sauce of these sugar contents were 99.5, 58.0, and 31.7.

(a)



(b)

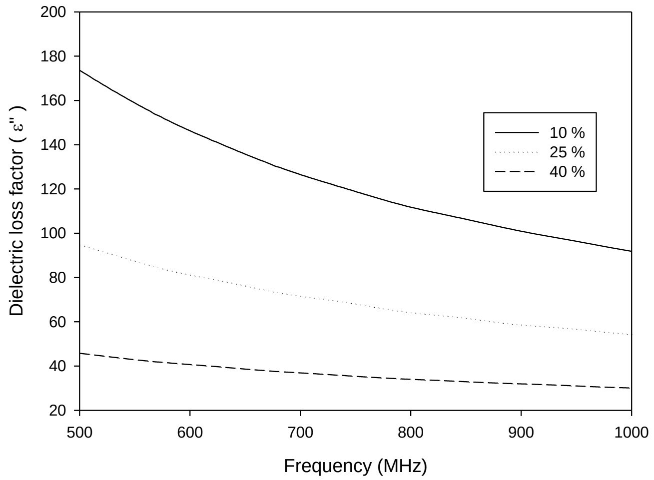


Fig. I.2. Dielectric properties from 500 MHz to 1 GHz of chili sauce of varying sugar content: (a) Dielectric constant, (b) Dielectric loss factor

I.3.3. Relationship between the rate of temperature increase, dielectric loss factor, and sugar content of chili sauce.

The results of the rate of temperature increase, dielectric loss factor, and sugar content of chili sauce were analyzed for the relationships among them and shown in Fig. I.3. My results revealed that the dielectric loss factor was inversely proportional to sugar content as shown in Fig. I.3. Also, the rate of temperature increase highly relied on the sugar content of chili sauce, since the rate of temperature increase fell as sugar content of chili sauce increased.

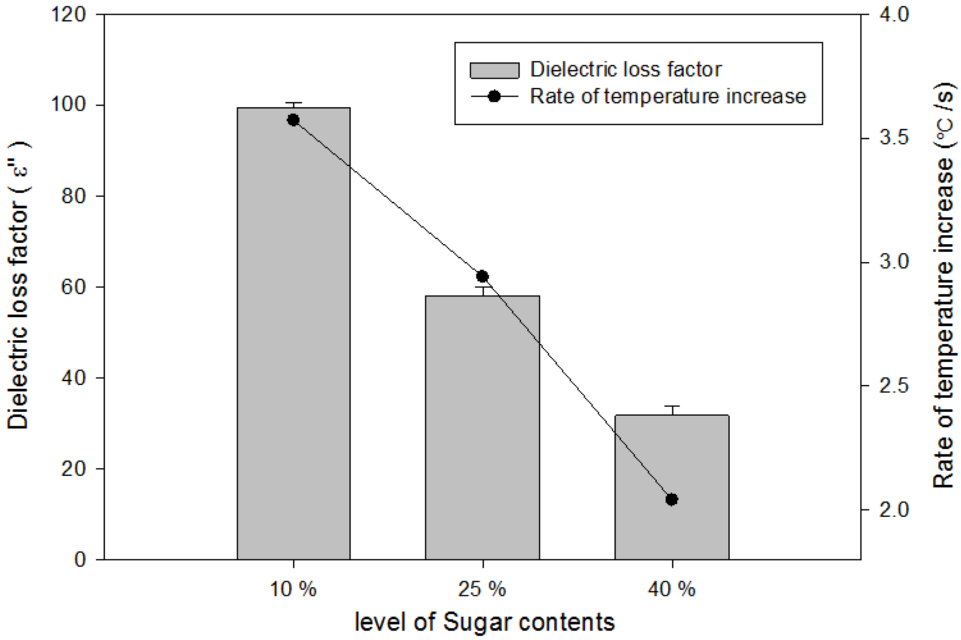


Fig. I.3. Relationship between dielectric loss factor of chili sauce of varying sugar content and rate of temperature increase during microwave heating at 3.0 kW.■, rate of temperature increase; ●, dielectric loss factor

I.3.4. Inactivation of foodborne pathogens in chili sauce by conventional heating and microwave heating.

As Fig. I.1 shows that there was no significant difference in the rate of temperature increase when samples were subjected to conventional heating, I conducted an inactivation study only using chili sauce of 10% sugar content in conventional heating. The reduction of foodborne pathogens increased as treatment time increased as shown in Table. I.1. When treated with conventional heating for 5 min, all pathogens were reduced to under the detection limit (1 Log CFU/ml) whereas sub-lethally injured cells were not detected after 6 min of treatment. All three pathogens showed a similar reduction tendency. Microwave heating showed much higher inactivation efficiency. Table. I.1 shows that when samples of 10%, 25%, and 40% sugar content were treated with 915 MHz microwave energy of 3.0 kW, all pathogens on selective media were inactivated to under the detection limit after 20 s, 25 s, and 40 s, respectively. Additionally, the result reveals that sub-lethally injured cells also follow a similar trend, as they likewise were not detected after 20 s, 25 s, and 40 s.

Table. I.1. Comparison of Pathogen population between surviving cells and cells including heat-injured cells in 10% chili sauce following conventional heating

Time (min)	***E. coli* O157:H7**		***S.* Typhimurium**		***L. monocytogenes***	
	SAMC	**SPRAB**	**XLD**	**TSA-XLD**	**OAB**	**TSA-OAB**
	Population (log CFU/cm^2)					
0	8.00 ± 0.52^{aA}	8.11 ± 0.51^{aA}	8.03 ± 0.39^{aA}	8.52 ± 0.16^{aA}	7.64 ± 0.22^{aA}	7.91 ± 0.22^{aA}
1	6.78 ± 1.22^{abA}	7.12 ± 0.20^{bA}	6.84 ± 0.88^{aA}	7.79 ± 0.32^{abA}	6.93 ± 0.11^{aA}	6.76 ± 0.68^{abA}
2	5.73 ± 0.80^{bA}	6.08 ± 0.07^{cA}	5.21 ± 1.34^{bA}	6.48 ± 0.68^{bA}	5.88 ± 0.10^{bA}	6.02 ± 0.16^{bA}
3	2.80 ± 0.98^{cA}	4.26 ± 0.27^{dB}	2.88 ± 0.92^{cA}	4.57 ± 1.34^{cA}	4.66 ± 0.68^{cA}	4.36 ± 1.46^{cA}
4	2.83 ± 0.98^{cA}	3.02 ± 0.69^{eA}	2.10 ± 0.69^{cA}	3.17 ± 0.87^{dA}	2.33 ± 0.90^{dA}	2.73 ± 0.99^{dA}
5	ND	2.38 ± 0.27^{f}	ND	2.30 ± 0.72^{d}	ND	1.87 ± 0.55d
6	ND	ND	ND	ND	ND	ND

a Means ± standard deviation from three replications. Values followed by the same letters within the row per foodborne pathogen are not significantly different (*P* > 0.05) Also, Values followed by the same letters within the column per foodborne pathogen are not significantly different (*P* > 0.05)

Table. I.2. Comparison of Pathogen population between surviving cells and cells including heat-injured cells in chili containing various sugar contents sauce following 915 MHz microwave heating at 3.0 kW.

Time (s)		***E. coli* O157:H7**		***S.* Typhimurium**		***L. monocytogenes***	
		SAMC	SPRAB	XLD	TSA-XLD	OAB	TSA-OAB
		Population (log CFU/cm^2)					
10%	**0**	7.43 ± 0.14a	7.83 ± 0.28a	7.73 ± 0.27a	8.22 ± 0.13b	7.25 ± 0.11a	7.65 ± 0.13b
	10	5.30 ± 0.40a	7.51 ± 0.36b	6.29 ± 0.13a	7.53 ± 0.37b	6.60 ± 0.42a	6.75 ± 0.48a
	15	3.31 ± 0.77a	3.35 ± 0.99a	2.89 ± 0.86a	4.43 ± 1.35a	3.28 ± 0.70a	3.81 ± 0.66a
	20	ND	ND	ND	ND	ND	ND
25%	**10**	5.80 ± 0.34a	6.73 ± 1.20a	7.18 ± 0.52a	7.59 ± 0.20a	6.74 ± 0.48a	6.84 ± 0.06a
	15	4.61 ± 0.90a	6.23 ± 0.55a	4.64 ± 0.88a	6.70 ± 0.52b	5.50 ± 1.41a	5.18 ± 0.82a
	20	2.19 ± 0.77a	2.43 ± 0.42a	2.40 ± 1.17a	3.18 ± 1.37a	3.48 ± 1.06a	3.53 ± 0.88a
	25	ND	ND	ND	ND	ND	ND

40%	**10**	6.03 ± 0.18a	7.53 ± 0.64b	7.44 ± 0.85a	7.50 ± 0.36a	6.92 ± 0.16a	7.10 ± 0.16a
	15	5.02 ± 0.47a	6.39 ± 0.19b	6.07 ± 0.08a	6.44 ± 0.10b	6.48 ± 0.23a	6.86 ± 0.11b
	20	5.22 ± 0.38a	6.24 ± 0.14b	5.81 ± 0.28a	6.35 ± 0.52a	6.15 ± 0.12a	6.28 ± 0.25a
	25	4.32 ± 1.47a	5.47 ± 1.42a	4.13 ± 1.91a	5.49 ± 0.94a	5.45 ± 0.13a	5.45 ± 0.89a
	30	2.39 ± 0.98a	2.71 ± 1.34a	2.60 ± 0.38a	2.88 ± 1.30a	4.11 ± 0.17a	4.44 ± 0 02b
	40	ND	ND	ND	ND	ND	ND

a Means ± standard deviation from three replications. Values followed by the same letters within the row per foodborne pathogen are not significantly different (*P* > 0.05)

I.3.5. Measurement of chili sauce quality after conventional heating and microwave heating.

Table. I.3 shows the color and water activity changes of chili sauce samples of varying sugar content after conventional heating and microwave heating treatment at 3.0 kW for the treatment times needed to reduce populations of foodborne pathogens to below the detection limit. Overall, there was no significant change in color after heating treatment, except for samples of 25% and 40% sugar content exposed to 915 MHz microwave heating at 3.0 kW. a* and b* values were reduced by 0.54 and 0.61, respectively, when chili sauce of 25% sugar content was treated with 915 MHz microwave heating at 3.0 kW, while these values for 40% sugar content chili sauce were reduced by 0.49 and 1.18, respectively. Water activity was reduced in all heating treatments. However, no significant difference was observed after conventional heating and microwave heating of chili sauce of 10% sugar content. On the other hand, sugar content also influenced the change of water activity. The degree of change was 0.008 a_w when sugar content was 10%, but this change in chili sauce of 40% sugar content were 0.031 a_w when exposed to microwave energy at 3.0 kW .

Table. I.3. Color and water activity change following 915 MHz microwave heating at 3.0 kW.

Sugar contents	Time (s)	Color[a]			Water activity[a]
		L*	a*	b*	
10%	0	29.50 ± 0.23[ab]	8.52 ± 0.34[a]	10.33 ± 0.33[ab]	0.919 ± 0.003[a]
	10	28.59 ± 0.42[abcd]	8.77 ± 1.33[a]	10.89 ± 1.16[a]	0.911 ± 0.001[ab]
	480	29.48 ± 1.72[abc]	8.74 ± 0.37[a]	9.92 ± 0.94[b]	0.913 ± 0.004[ab]
25%	10	28.02 ± 0.73[cde]	4.43 ± 0.15[c]	6.89 ± 0.06[cd]	0.894 ± 0.003[c]
	15	26.63 ± 0.88[e]	3.89 ± 0.25[c]	6.28 ± 0.25[d]	0.879 ± 0.003[d]
40%	20	27.95 ± 0.16[de]	2.02 ± 0.27[d]	5.02 ± 0.04[f]	0.863 ± 0.0.6[e]
	25	27.49 ± 0.13[de]	1.53 ± 0.07[d]	3.84 ± 0.06[g]	0.832 ± 0.001[g]

a Means ± standard deviation from three replications. Values followed by the same letters within the column are not significantly different (P > 0.05)

b Color parameters are L*(Lightness), a*(Redness), b*(Yellowness)

I.4. Discussion

Microwave heating treatment is widely used in the food industry and in household appliances. In the USA, most industrial microwave systems operate at 915 MHz, since it penetrates more deeply than 2450 MHz microwave systems (Ramaswamy and Tang, 2008a). In this study, I analyzed the heating rate profile after treatment with conventional heating and 915 MHz microwave heating at 1.5 kW and 3.0 kW. I observed the temperature increase of chili sauce of 10~40% sugar content. In the case of conventional heating, there was no significant difference in heating rate among chili sauces of differing sugar content. As there was no significant difference in heating rate when treated with conventional heating, we conducted further study with only 10% sugar content chili sauce in the case of conventional heating treatment. However, chili sauce exposed to 915 MHz microwave energy showed a different tendency. Chili sauce of 10% sugar content exhibited the highest rate of temperature increase, whereas the heating rate was slowest for chili sauce of 40% sugar content. Water activity is one major factor influencing microwave heating rate. The study conducted by Song and Kang showed that peanut butter of differing water activity revealed different heating rates, and the sample of highest water activity had the highest rate of temperature increase (Song and Kang, 2016b). My results also showed positive correlation with water activity and rate of temperature increase.

Microwaves are a form of dielectric heating, and thus dielectric properties should be taken into consideration in order to explain the different tendencies in heating rate. Dielectric properties can be described by dielectric constant and dielectric loss factor. Dielectric constant describes the ability of a material to store, transmit, and reflect electromagnetic energy when it is exposed to microwave energy (Sosa-Morales et al., 2010). Whereas, dielectric loss factor describes the dissipation of electric energy, which indicates the amount of heat generated by microwave energy (Datta and Davidson, 2000; Ramaswamy and Tang, 2008a). My results showed that adding more sugar slightly decreased the dielectric constant and sharply reduced the dielectric loss factor, and this had direct correlation with water activity. The study conducted by Roth et al,, also showed that soil of higher moisture content had a higher dielectric constant (Roth et al., 1992). This is due to the relation between water activity and dielectric properties of food materials. The water molecule has a high dipolar property, and it aligns its molecular orientation following the direction of microwave radiation. Microwave frequencies are very high, thus the rapid oscillation and realignment of water molecules causes friction inside the food matrix, resulting in volumetric heating. Therefore, water activity or moisture content has an important impact on dielectric properties of foods and is one of the main factors influencing microwave heating (Tanaka and Sato, 2007). Therefore, understanding dielectric properties of food relevant to water content and water activity is important for characterizing microwave heating effectiveness.

My results showed that there was a direct correlation between the rate of temperature increase and dielectric loss factor. The effect of dielectric properties on the rate of temperature increase can be explained by the equation $P = 2\pi f V \varepsilon_0 \varepsilon''$, where P is the electrical power which is transferred to the food matrix in the form of heat, V is the electrical field strength, ε_0 is the dielectric constant of a vacuum (8.85 x 10-12 F/m), and ε'' is the dielectric loss factor of the sample (Marra et al., 2007). I can calculate how much heat is generated inside of a food matrix based on this equation. As the microwave power was fixed at 3.0 kW and 1.5 kW, it is easily assumed that electric field strength was also fixed. Also, I conducted my study at 915 MHz. Thus, I consider only dielectric loss factor as determining the rate of temperature increase. My results showed agreement with the above equations. The higher the loss factor the sample possessed due to higher water activity, the faster the rate of temperature increase.

Also, I investigated the inactivation efficiency of foodborne pathogens. My results revealed higher inactivation efficiency of 915 MHz microwave heating treatment at 3.0 kW compared to conventional heating. The population of *E. coli* O157:H7 dropped to below the detection limit after 5 min treatment when treated with conventional heating, whereas it took only 20 s when exposed to 915 MHz at 3.0 kW. *S.* Typhimurium and *L. monocytogenes* also showed similar inactivation trends. This means that a more rapid temperature increase induces better inactivation of foodborne pathogens. Additionally, sugar content of chili sauce also affected foodborne pathogen inactivation. Our results revealed that chili sauce of 10% sugar content had higher inactivation efficiency than chili sauce of 25% and 40% sugar content. This is because the rate of temperature increase for chili sauce of 10% sugar content was higher than that of chili sauce of 25% or 40% sugar content due to a higher dielectric loss factor.

I also investigated populations of sub-lethally injured cells after heating treatment. After certain treatments, sub-lethally injured bacterial cells can remain inside a food matrix and under suitable conditions resuscitate and produce colonies even on selective agar (Bozoglu et al., 2004). Thus, it is an important issue to the food industry, as well as a serious food safety concern. Our results showed overall that populations of injured cells were slightly higher than non-injured cells on selective agar, though no significant difference was observed. However, 915 MHz microwave heating at 3.0 kW reduced levels of injured cells as effectively as populations on selective agar, as it took the same time to inactivate both groups to under the detection limit. Meanwhile, conventional heating showed less effectiveness, since it took 6 min to reduce all injured bacterial cells under the detection limit. Our results suggest that microwave heating can be a very useful tool for inactivating bacterial cells to below the detection limit without generating a significant population of injured cells that can be a food safety concern.

Also, I investigated quality changes after conventional heating treatment and 915 MHz heating treatment at 3.0 kW. I focused our investigation on color values (L*, a*, b*) and water activity after treatment, since they can change after heating treatment. Heating treatment inevitably results in quality loss of food product due to destruction of heat sensitive food components. Chemat and Khan (2011) reported that conventional heating can cause quality deterioration in food ingredients (Chemat and Khan, 2011). However, Song and Kang (2016)reported that peanut butter treated with 915 MHz microwave heating treatment showed no quality change (Song and Kang, 2016a). Our results showed that when comparing quality change, chili sauce of 10% sugar content after conventional heating or 915 Microwave heating at 3.0 kW showed no significant differences in color attributes, but water activity slightly decreased. When chili sauce had a lower sugar content, there was also less quality loss. These results indicate that microwave heating did not induce worse quality changes in comparison to conventional heating and chili sauce of high dielectric constant and dielectric loss factor contents could show better quality after microwave heating treatment.

In conclusion, this study demonstrated that microwave heating can not only reduce foodborne pathogens effectively but also does not induce quality loss as seen in conventional heating. In light of our results, microwave processing can be considered an alternative to conventional heating. Moreover, I investigated the effect of dielectric constants on microwave heating trends. As sugar content affects water activity, chili sauce of lower sugar content had a higher dielectric constant and dielectric loss factor resulting in a higher rate of temperature increase. Thus, chili sauce containing a small amount of sugar facilitates better inactivation of foodborne pathogens. The results of this study can be used for understating the relationships among sugar content, dielectric properties, and microwave heating treatment for pasteurization of chili sauce and for further practical application by the food industry

Chapter II.

Effect of plastic packaging on inactivation of foodborne pathogens in hot chili sauce by 915 MHz microwave heating

II.1. INTRODUCTION

Post-packaging pasteurization is one solution for ensuring the safety of chili sauce. This is because a considerable number of chili sauce products are marketed in packaged form and post-packaging pasteurization already has been used as an effective method for reducing foodborne pathogens in ready-to-eat food products. Also, post-packaging thermal sterilization can reduce the possibility of cross-contamination, which is a serious issue in the food industry (Jung et al., 2014; Muriana et al., 2002). For this purpose, microwave heating can be utilized to inactivate microorganisms and ensure the safety of packaged food, since microwave heating is volumetric and microwave energy which penetrates the food packaging material generates heat inside foods. Also, microwave heating is a form of dielectric heating thus its heating pattern depends on various factors including dielectric properties of the food product as well as packaging material.

There are a number of materials which can be used for packaging chili sauce. Among them, plastic materials such as polyethylene (PE), polypropylene (PP), and polycaprolactam (Nylon) are candidates for packaging chili sauce. This is because they are economical, possess high mechanical performance and they can act as a physical barrier to not only external oxygen and various gases but also to retain internal volatile substances such as aromatic compounds (Siracusa et al., 2008). Furthermore, these packaging materials have been extensively used by the food industry due to their high heat resistance. Thus, they are suitable for pasteurization of food products by microwave heating treatment (Ozen and Floros, 2001).

Polyetherimide (PEI) can also be used for microwave pasteurization. Several papers have reported the usage of PEI film in Radio-Frequency (RF) heating. Liu et al. used PEI containers in their investigation of microbial validation by RF heating (Liu et al., 2018). Also, Yu et al. researched temperature distribution of samples inside PEI blocks under RF energy (Yu et al., 2016). Additionally, the possibility of using RF heating to process heterogeneous foods inside PEI containers was also investigated (Wang et al., 2012). These research studies indicate that PEI has high heat resistance, and thus can be used as packaging material and applied for post-pasteurization processing.

There have been some papers studying the influence of different food packaging materials on the inactivation of foodborne pathogens under 27.12 MHz RF heating treatment (Jeong, 2017). However, to date, no study has reported on the effect of food packaging materials on the inactivation of foodborne pathogens under 915 MHz microwave energy. Thus, in this study, I investigated the efficiency of heating rate and inactivation of foodborne pathogens in chili sauce packaged by various food packaging materials such as PE, PP, Nylon and PEI film treated with 915 MHz microwave heating.

II.2. MATERIALS AND METHODS

II.2.1. Bacterial strains and culture preparation

Bacterial strains and culture preparation was conducted according to the method described in chapter I.2.1.

II.2.2. Sample preparation and inoculation

Shelf-stable chili sauce was purchased at a local grocery store (Seoul, Republic of Korea) for this study and stored at room temperature (22 ± 1 °C); Twenty-five g chili sauce samples were aseptically placed in sterile plastic material (PE, PP, Nylon and PEI). The size of the sample bags was 10 × 15 cm and thickness was 0.7 mm. For inoculation, 300 μl of bacterial cell culture was inoculated into each sample and thoroughly mixed for 1 min with a sterile spoon to ensure even distribution of bacterial cells. The populations of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* recovered from inoculated samples were 10^7 – 10^8 CFU/ml. The packaged samples were subjected to microwave heating immediately after inoculation.

II.2.3. Microwave heating treatment

Microwave treatment was performed in a previously described apparatus (Sung and Kang, 2014) designed for 915 MHz treatment. A plastic material containing a 25 g sample of chili sauce was placed at the center of the turntable and subjected to microwave heating. For inactivation of pathogens and quality evaluation, samples were subjected to 915 MHz microwave heating at 1.5 kW.

II.2.4. Temperature measurement

Temperature measurement was conducted according to the method described in chapter I.2.4.

II.2.5. Dielectric properties measurement

Dielectric properties measurement of various plastic material was conducted according to the method described in chapter I.2.5.

II.2.6. Bacterial cell enumeration

Bacterial cell enumeration was conducted according to the method described in chapter I.2.6.

II.2.7. Color and water activity measurement

Color and water activity measurement was conducted according to the method described in chapter I.2.7.

II.2.8. Volatile flavor compound quantification

Capsaicinoid contents including capsaicin and dihydrocapsaicin were measured by the following procedure adapted from a previous study: Extraction and separation (Hartley et al., 2013). For capsaicinoid extraction, 3 g of chili sauce was added to a mixture of 5 ml phosphate-buffered saline (PBS; pH 7.4) and 5 ml methyl isobutyl ketone (Sigma Chemical Co., St. Louis, MO) in a 15 ml conical centrifuge tube. The mixture was gently inverted and shaken at 250 rpm for 10 min. After centrifugation at 1922 rcf for 20 min, the upper organic layer was transferred to a 5 ml Eppendorf tube. Then, the solvent was evaporated using multipurpose centrifugation connected to a freeze drier at 2000 rpm for 2 hr. Two ml of acetonitrile (Sigma Chemical Co., St. Louis, MO) was added to the remaining dried residue in order to re-extract capsaicinoid, and the extracted solution was used for further analysis. For capsaicinoid separation, a high-performance liquid chromatography apparatus (HPLC; Waters 2695; Waters) equipped with an autosampler and a photodiode array detector (Waters 996; Waters) was utilized. The wavelength was set at 280 nm, and a reversed-phase C18 column (5-mm particle size, 4.6- mm diameter, 250-mm length; Young Jin Biochrom Co. Ltd., Gyeonggi, South Korea) was used with these conditions of the mobile phase: methanol and triple distilled water (70:30 [v/v]) at a flow rate of 1 ml/min. A standard calibration curve was obtained by using pure capsaicin (Sigma Chemical Co., St. Louis, MO) and pure dihydrocapsaicin (Sigma Chemical Co.) prepared in acetonitrile.

II.2.9. Statistical analysis

All experiments were repeated at least three times with independently prepared samples. Data were analyzed by analysis of variance (ANOVA) and t-test (LSD) using the Statistical Analysis System (SAS Institute, Cary, NC, USA). A p value of <0.05 was used to indicate significant difference.

II.3 RESULTS

II.3.1. Temperature curve of chili sauce inside various plastic material bags during 915 MHz microwave heating at 1.5 kW

Fig. II.1 shows the temperature increase of chili sauce when treated with 915 MHz at 1.5 kW. My results revealed that there was no significant difference between PE, PP and Nylon. It took 51 s for chili sauce inside PE, PP and Nylon films to reach 85 °C, while it took 77 s when PEI film was used as packaging material. My results revealed that the rate of temperature increase was slowest when chili sauce inside PEI bags was exposed to 915 MHz microwave energy.

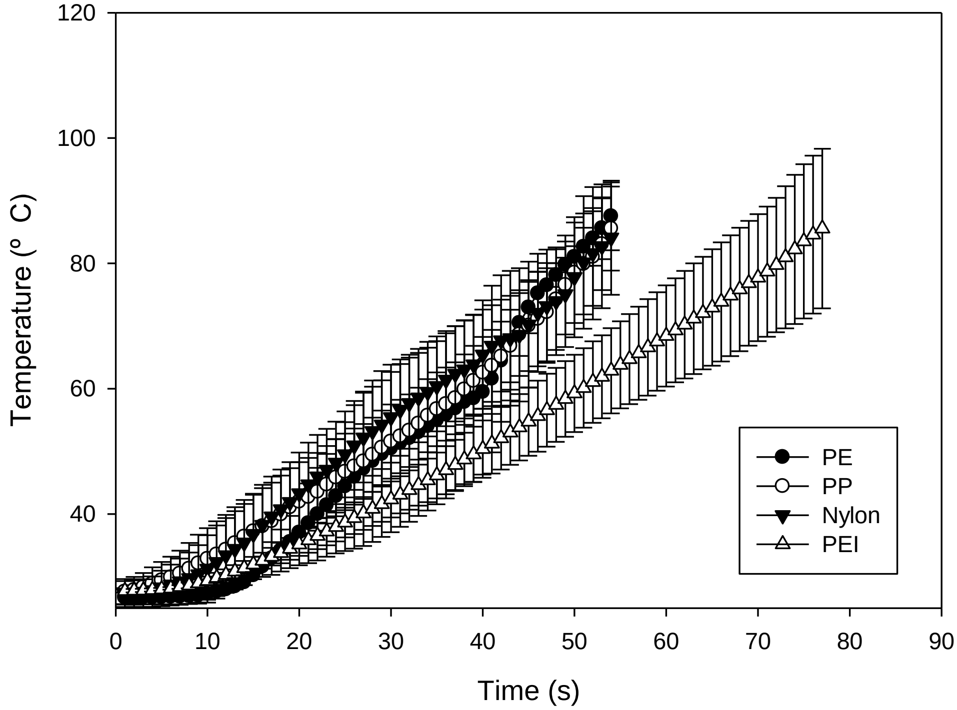
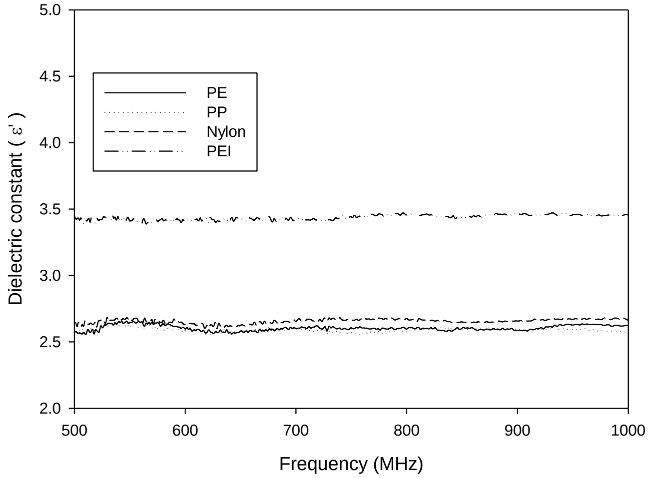


Fig. II.1. Comparison of heating rate among various plastic packaging material when exposed to 915 MHz microwave heating at 1.5 kW

II.3.2. Dielectric properties of PE, PP, Nylon and PEI

Fig. II.2 shows the dielectric constant of various plastic films. PEI had the highest dielectric constant for 915 MHz: 3.461. Also, the dielectric constants of PE, PP and Nylon were 2.593, 2.587 and 2.671, respectively. However, no significant difference in dielectric constant among PE, PP and Nylon was observed (P>0.05). Also, Fig. II.2 reveals the dielectric loss factors of PE, PP, Nylon and PEI films. It followed a similar trend to that of the dielectric constants. The dielectric loss factor of PEI at 915 MHz was 0.210, but the dielectric loss factors of PE, PP and Nylon were 0.090, 0.108. 0.089, respectively, and there was no significant difference between PE, PP and Nylon (P>0.05). Thus, our results show that PEI had the highest dielectric constant and dielectric loss factor at 915 MHz microwave energy.

(a)



(b)

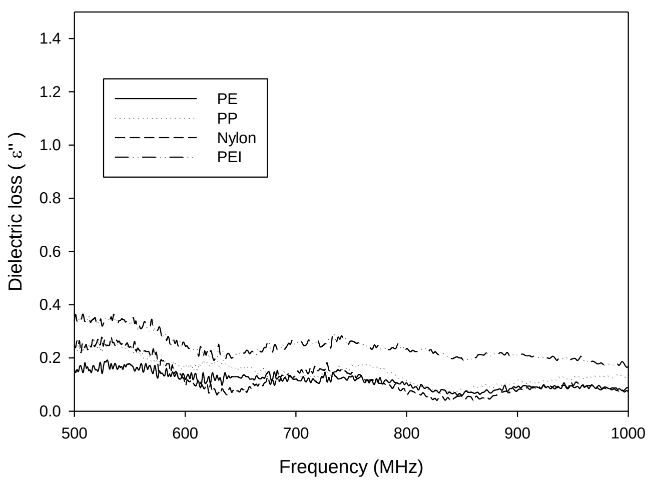
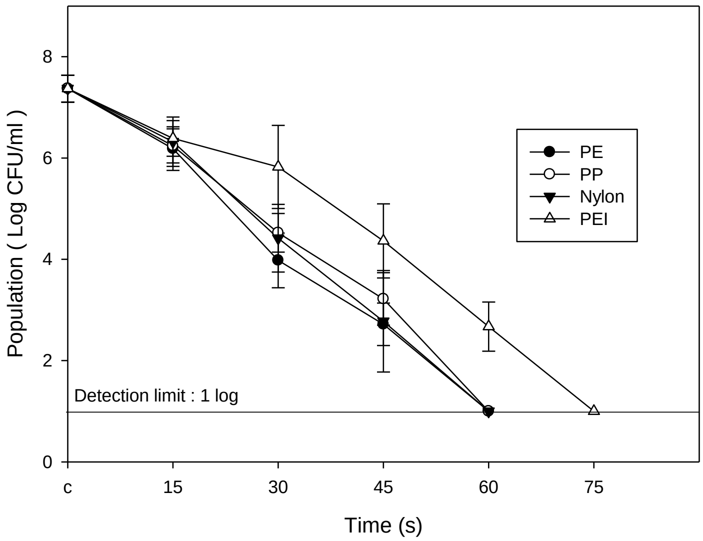


Fig. II.2. Dielectric constant and dielectric loss factor of various plastic material

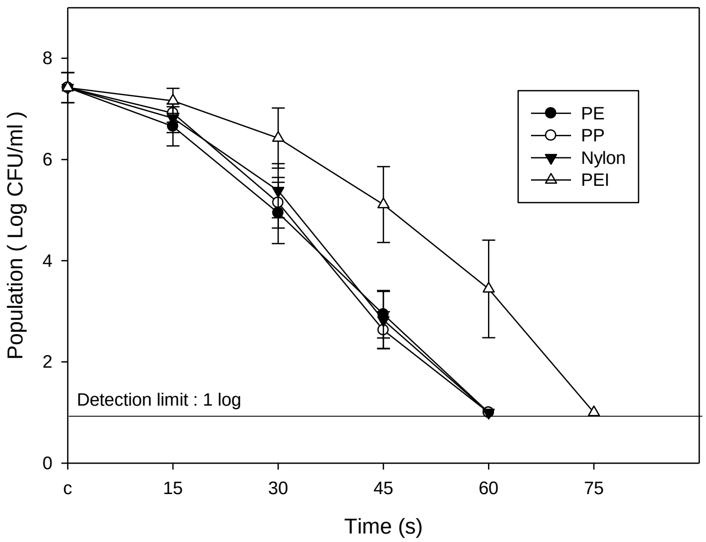
II.3.3. Inactivation of foodborne pathogens using 915 MHz microwave heating.

Fig. II.3 shows the population of foodborne pathogens on selective media when treated with microwave heating. The rate of temperature increase was related to inactivation efficiency of foodborne pathogens in chili sauce. When chili sauce surrounded by plastic material was treated with microwave energy at 1.5 kW, the time required to inactivate *E. coli* O157:H7 to under the detection limit was 60 s for PE, PP and Nylon but 75 s for PEI film. The same trend was shown for *L. monocytogenes* and *S*. Typhimurium. Fig. II.4 reveals that the time required to inactivate foodborne pathogens to under the detection limit was still 60 s on resuscitative media. Also, the populations of foodborne pathogens on resuscitative media were slightly higher in the case of PEI compared to population on selective media, though it took the same time to reduce populations to under the detection limit. These results reveal that PEI had lower efficiency than other plastic films when exposed to 915 MHz microwave energy.

(a)


PE
PP
Nylon
PEI
Detection limit : 1 log
Population (Log CFU/ml)
Time (s)
0
2
4
6
8
c
15
30
45
60
75

(b)


PE
PP
Nylon
PEI
Detection limit : 1 log
Population (Log CFU/ml)
Time (s)
0
2
4
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8
c
15
30
45
60
75

(c)

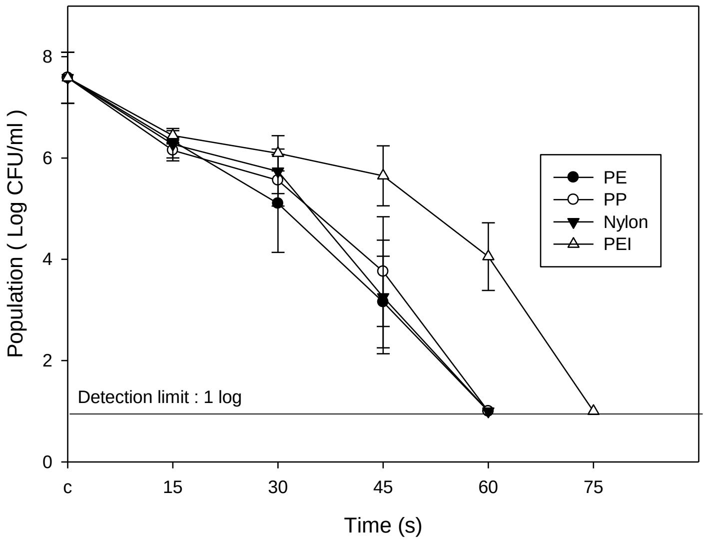
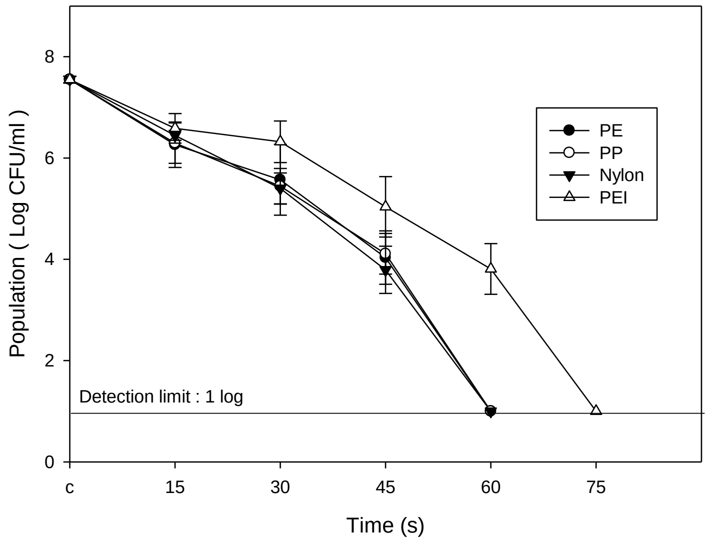


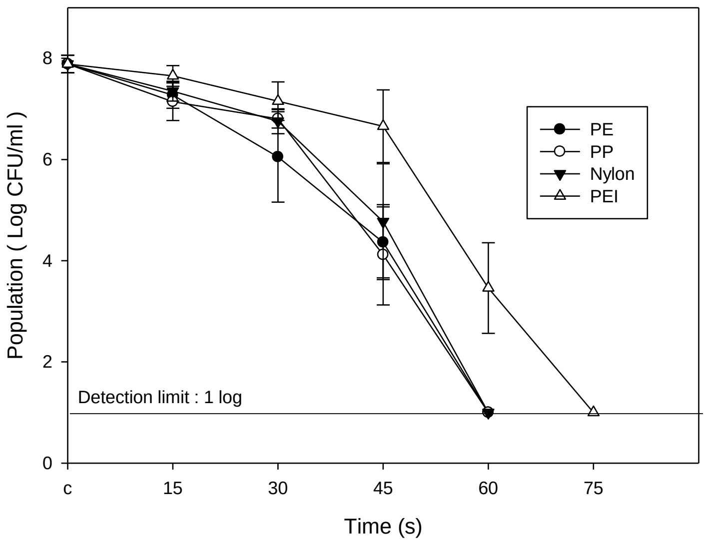
Fig. II.3. Population of foodborne pathogen in selective media when exposed to 915 MHz microwave heating at 1.5 kW : (a) *E. coli* O157:H7 (b) *S.* Typhimurium, (c) *L. monocytogenes*

(a)



Population (Log CFU/ml)
Time (s)
PE
PP
Nylon
PEI
Detection limit : 1 log
0
2
4
6
8
c
15
30
45
60
75

(b)



Population (Log CFU/ml)
Time (s)
PE
PP
Nylon
PEI
Detection limit : 1 log
0
2
4
6
8
c
15
30
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60
75

(c)

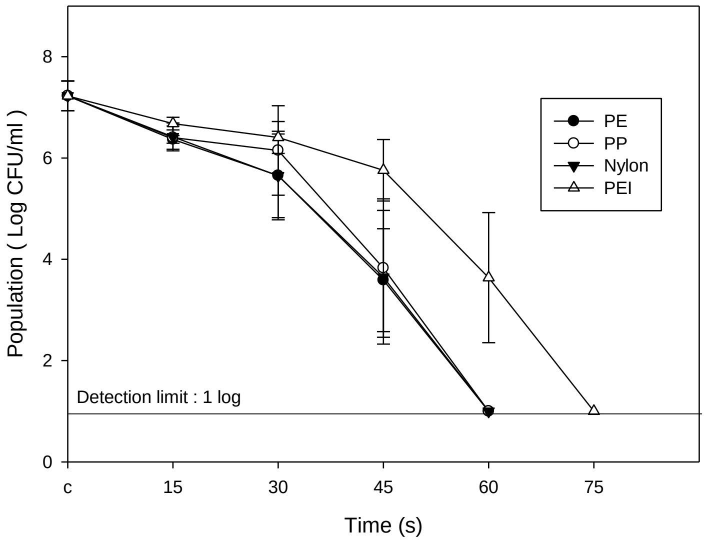


Fig. II.4. Population of foodborne pathogen in resuscitative media when exposed to 915 MHz microwave heating at 1.5 kW : (a) *E. coli* O157:H7 (b) *S.* Typhimurium, (c) *L. monocytogenes*

II.3.4. Chili sauce quality after 915 MHz microwave heating treatment

Table. II.1 show color and water activity changes after 915 MHz microwave heating treatment at 1.5 kW for the time intervals needed to reduce foodborne pathogen populations to under the detection limit. When chili sauce surrounded by plastic material was treated with 915 MHz microwave heating system at 1.5 kW, water activity was not significantly changed. However, a slight color change was observed. When chili sauce surrounded by PE was subjected to 1.5 kW power, b* value was slightly reduced. In the case of Nylon and PEI, the value of a* and b* decreased when exposed to 1.5 kW power. Also, I quantified the amount of volatile flavor compounds after 915 microwave heating in order to investigate the flavor changes. I then estimated the amount of capsaicin and dihydrocapsaicin in Fig. II.5. When chili sauce was exposed to microwave heating at 1.5 kW, the amount of capsaicin was 61.9, 69.4, 69.5, 71.1, and 65.7 ppm for the control, PE, PP, Nylon and PEI, respectively, but no significant difference was observed. Also, the amount of dihydrocapsaicin was not significantly different, except for PEI which had 26.6 ppm compared to the control which had 22.0 ppm. These results indicate that none of the packaging materials induced any significant quality deterioration when subjected to 915 MHz microwave heating.

Table. II.1 Color value and water activity of treated chili sauce packaged with various plastic material subjected 915 MHz microwave heating at 1.5 kW .

Sugar contents	Time (s)	Color[a]			Water activity[a]
		L*	a*	b*	
Control	**0**	**29.84 ± 1.47a**	**9.70 ± 0.65a**	**12.04 ± 0.81a**	**0.917 ± 0.006a**
PE	**60**	**29.41 ± 1.12a**	**9.45 ± 1.27ab**	**10.91 ± 1.80ab**	**0.914 ± 0.005a**
PP	**60**	**30.14 ± 0.74a**	**8.21 ± 0.41b**	**9.21 ± 0.74b**	**0.913 ± 0.003a**
Nylon	**60**	**29.64 ± 0.45a**	**8.62 ± 0.76ab**	**10.25 ± 1.28ab**	**0.914 ± 0.002a**
PEI	**75**	**31.02 ± 0.35a**	**9.17 ± 0.55ab**	**10.46 ± 0.71ab**	**0.914 ± 0.001a**

a Means ± standard deviation from three replications. Values followed by the same letters within the column are not significantly different (P > 0.05)

b Color parameters are L*(Lightness), a*(Redness), b*(Yellowness)

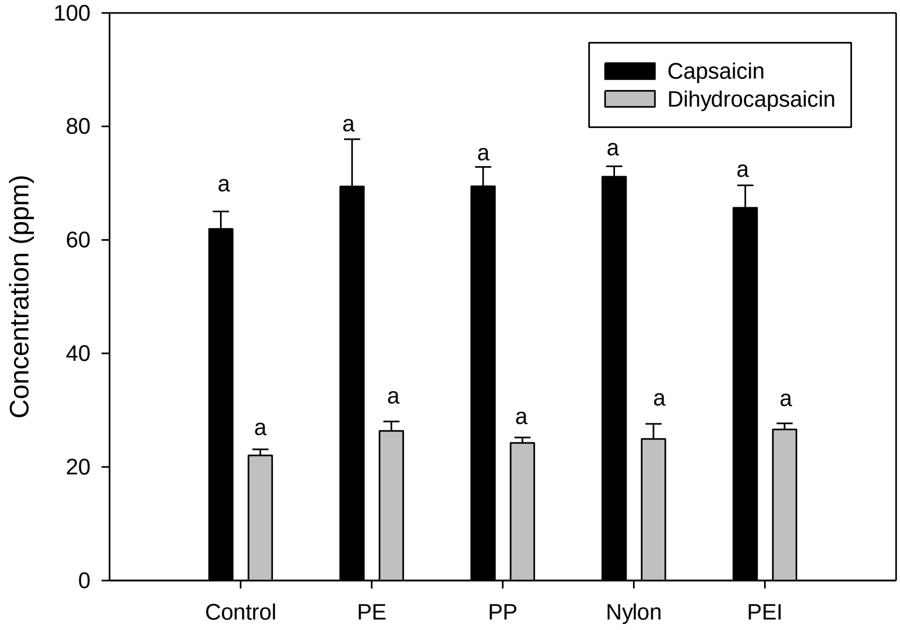


Fig. II.5. The amount of volatile flavor compounds of chili sauce packaged in various plastic film bags after 915 MHz microwave heating at 1.5 kW

II.4. Discussion

Cross-contamination is a serious issue in the food industry. In the food processing line, there are many chances for foodborne pathogens to adhere and contaminate food products. For example, workers' hands or abiotic surfaces can reintroduce foodborne pathogens into pasteurized product (DORSA et al., 1993). Thus, post-packaging pasteurization is required to reduce the risk of cross-contamination. For packaged food, conventional heating treatment utilizing hot water or steam can be applied. However, these methods transfer heat through convection or conduction which takes more time and results in a cold spot due to non-uniform heat distribution (Ramaswamy and Tang, 2008b). Microwave heating can be a good alternative for conventional heating treatment, since microwave heating is volumetric and thus generates thermal energy inside food, penetrating the food packaging material. Thus, food can be processed with better efficiency as described in Chapter I.

The microwave heating pattern depends on the dielectric properties of the heated materials. In general, dielectric properties of materials can be described via two terms : permeability and permittivity (Marra et al., 2009). Permittivity is term related to the electric field, whereas permeability pertains to the magnetic field of electromagnetic wave energy (Minowa et al., 2008). In microwave heating, the dielectric constant (ε') and dielectric loss factor (ε''), which are the real and imaginary parts of permittivity, are important to generating thermal energy inside dielectric materials. (Chandrasekaran et al., 2013; Marra et al., 2009). Thus, an understanding of dielectric properties is essential to predict the microwave heating pattern.

My results showed that the dielectric constant of PEI was higher than that of PE, PP or Nylon, but no significant difference among them was observed. The dielectric loss factor showed similar trends. PEI had the highest dielectric loss factor. In previous studies, Jeong reported that the dielectric constant of PEI was higher than that of PE, PP or Nylon but the dielectric constant of Nylon was not significantly different from that of PEI (Jeong, 2017). The results for dielectric loss factor showed different trends. The order of decreasing dielectric loss factor in the previous study was Nylon, PEI, PE and PP. No significant difference between PE and PP was observed (Jeong, 2017). This might be due to the difference in frequency. Several factors such as electromagnetic wave frequency, water content, ionic compounds and temperature can influence the dielectric constant and dielectric loss factor (Chandrasekaran et al., 2013).

The heating rate of chili sauce inside various packaging materials showed different tendencies compared to the dielectric loss factor or dielectric constant. The rate of temperature increase of chili sauce with PEI was slowest with 915 MHz microwave heating at 1.5 kW. This is in contrast to a previous study of RF heating. In the previous study, Jeong reported that when red or black pepper was treated, the order of decreasing heating rate was PEI, PE/PP and Nylon under a RF heating system (Jeong, 2017).

These differences in heating rates can be explained by reduction of microwave power passing through packaging material. In other words, microwave energy can be attenuated as it passes through dielectric materials. This reduction of microwave energy can be explained by Lambert's Law :

$$P(x) = P0e^{-2\beta x}.$$

where P_0 is the initial power of microwave energy, P(x) is power dissipated in food sample and x is the site of temperature measurement, constant $\beta$is attenuation constant (m^{-1}). Using this equation, I can calculate how much microwave energy was attenuated as it passed through packaging material. In this equation constant β is very important in calculation of heating rate. β can be explained by following equation.

$$\beta = \frac{2\pi f}{c}\sqrt{\frac{k'\sqrt{1+(tan\,\delta)2}-1}{2}}$$

Where f is frequency of radiation , k' is dielectric constant and (tan δ) 2 is loss tangent which is (Dielectric constant/Dielectric loss factor). Higher β means lower power dissipated in food sample. The dielectric constant of PE, PP, Nylon and PEI was 2.59, 2.59, 2.67 and 3.46, respectively. Also, the dielectric loss factor of PE, PP, Nylon and PEI was 0.09, 0.11, 0.09 and 0.21, respectively, thus β is highest in PEI, indicating that the $P(x)$will be lowest.

Our results of the correlation between dielectric loss factor and heating rate coincide with those of a previous study. In that study, when the packaging material had a high loss factor, it indicated a decreased heating rate of treated samples such as red or black pepper (Jeong, 2017). Another study reported that in electroheating, dielectric loss factor of packaging material significantly influenced heating rate. When packaging material has a higher dielectric loss factor it is more difficult for electromagnetic wave energy to permeate the packaging material (Metaxas, 1996).

In thermal treatment heating rate is very important for effective inactivation of foodborne pathogens. In general, a higher heating rate leads to better inactivation efficiency against foodborne pathogens. Song et al. reported that peanut butter with higher water activity showed faster foodborne pathogen inactivation due to faster heating rate (Song and Kang, 2016b). Also, under higher microwave power, better pasteurization was shown due to faster heating rate (Song and Kang, 2016a). In other treatments similar trends were shown. When milk of different fat content was treated with ohmic heating, lower fat content milk showed better inactivation due to higher heating rate (Kim and Kang, 2015). In this study, when chili sauce packaged with PE, PP or Nylon film bags was treated with 915 MHz microwave energy at 1.5 kW, higher inactivation efficiency was shown. This can be explained by a faster heating rate.

Also, I investigated the population of sub-lethally injured cells after microwave heating. In the current study, there was no significant difference between selective and non-selective media in the time needed to reduce cell populations to under the detection limit, indicating that microwave heating can inactivate microorganisms without generating significant levels of injured bacterial cells .

Processed foods must be of very high quality to compete in the marketplace. No significant difference in water activity was observed in our study. Although significant differences in b* or a* were observed in some cases following microwave heating, there was no change in L*. Chili peppers are the main component of chili sauce, thus I investigated changes in capsaicin and dihydrocapsaicin content after heating treatment, but no significant change was observed. Other researchers reported that microwave heating can retain food product quality due to its high heating rate. Sung et al. reported that no significant difference in lycopene content was observed after microwave heating treatment of salsa. Also, in a previous study, microwave heating did not induce significant lipid oxidation in peanut butter (Song and Kang, 2016a). Based on our results, microwave heating treatment can inactivate foodborne pathogens without inducing critical quality change of chili sauce when packaged in various plastic films.

In conclusion, microwave heating is highly efficacious for inactivating *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* while maintaining chili sauce quality. Also, the treatment time required to reduce foodborne pathogens to under the detection limit differed depending on the plastic packaging material. PE, PP and Nylon are applicable, but PEI is not recommended for microwave heating. This is in contrast to RF heating, where PEI was revealed to be the most effective packaging material for maintaining spice safety and quality. These results are fundamental to understanding the difference between RF and microwave heating systems and further application of 915 MHz microwave energy according to the type of packaging material. With further investigation of the influence of various plastic materials on the heating rate in chili sauce, 915 MHz microwave heating can be a promising technology for post-packaging commercial sterilization.

Chapter III.

Verification of synergic effect of 915 MHz microwave heating and various Essential Oil for inactivation of foodborne pathogen in hot-chili sauce

I.1. INTRODUCTION

Department of Economic and Social Affairs (DESA) in United Nations (UN) reported that the population of world might reach around 10.0 billion in 2050 (DeSA, 2013). This implies that the demand of stable and bulk production of food product might be increased. It was also reported that the amount of absolute production of food would increase though production growth rate would decrease (Alexandratos and Bruinsma, 2012). For the secure and stable bulk distribution, several issues should be dealt. One of key factors to be managed is to maintain the quality and the safe of food product during distribution into market. From this perspective, food additive can provide good solutions for that problem.

Food additive is the substance which has the various purpose such as improving flavor properties, enhancing physical appearance including color and texture, maintaining its nutritional quality and keeping the food safety against microorganism or fungi. Thus, in the food industry, a wide range of food additive have been used for those purpose. Food additive can be classified into two group : Synthetic food additive and natural food additive (Carocho et al., 2014). However, customers are more reluctant to purchase the food product including synthetic food additive. Thus, many users have tendency utilize natural food additive which is obtained from natural sources.

Among them, essential oil can be a good candidate as the food preservatives. Essential oils are aromatic compound which is hydrophobic and volatile. Essential oils are produced by aromatic or medicinal plant as mostly secondary metabolites (Pandey et al., 2017). As many of these compounds are considered generally recognized as safe (GRAS) and antioxidant property and broad range of antibacterial or antifungal activity, it had been wieldy used in food industry (Moleyar and Narasimham, 1992; Pandey et al., 2017; Vardar-Ünlü et al., 2003). There are many kinds of essential oil. Among them, in this study, I utilize carvacrol (CL), carvone (CN), eugenol (EU) and citral (CI) as food preservative as they has been recognized as safe food additive by European commission (Di Pasqua et al., 2006).

Hot-Chili sauce is condiment which is mainly made of chili pepper, but it includes tomato as primary ingredient and sometimes other substances including vinegar, garlic and sugar are also contained. It has been reported that several foodborne outbreak related to chili sauce in Unites States occurred (CDC, 2017a). Thus, essential oil can be also one of ingredient in order to keep the microbial safety of hot-chili sauce. However, in general, resistance of foodborne pathogen rises in food product due to effect of food matrix. Thus, higher concentration of essential oil is required to reduce the population of microorganism effectively. However, since most of essential oil has strong unique flavor, it has negative influence on the product acceptance if high amount of essential oil is applies to food product. To overcome this limitation, many researchers have reported the method to improve inactivation efficiency, combining other treatment such as ultrasound (Millan-Sango et al., 2015), high pressure (Palhano et al., 2004) and pulsed electric field (Arroyo et al., 2010).

Thermal treatment can be utilized so as to enhance antimicrobial activity of essential oil. There are many possible thermal treatments which can be applied to hot-chili sauce. Among them, 915 MHz microwave heating can be a good candidate, which is applicable to hot-chili sauce with various essential oil, alternatively to conventional heating. Microwave heating is a volumetric, thus generate thermal energy inside food matrix very quickly. Also, compared to 2450 MHz microwave heating, 915 MHz microwave has deeper penetration depth thanks to longer wavelength resulting in better heating uniformity. To the best of our knowledge, no study regarding the comparing combination efficiency of 915 MHz microwave heating system and various essential oil including carvacrol, eugenol, carvone and citral has been reported.

Thus, in the present study, I compared the inactivation efficiency on *E. coli* O157:H7, *Salmonella* Typhimurium and *L. monocytogenes*, when these chemicals were combined with 915 MHz microwave heating system. First, I compared and identified the synergic effect of combination treatment in hot-chili sauce. Second, I investigated foodborne pathogen inactivation mechanism using PI uptake test and $DiBAC_3(4)$ test. Finally, quality properties including color, water activity and principal flavor compound contents after combination treatments were compared.

III.2. MATERIALS AND METHODS

III.2.1. Bacterial strains and culture preparation

Bacterial strains and culture preparation was conducted according to the method described in chapter I.2.1.

III.2.2. Sample preparation and inoculation

Sample preparation and was conducted according to the method described in chapter I.2.2.

III.2.3. Essential oil, Microwave heating and combination treatment

Inoculated hot-chili sauce was treated with essential oil, Microwave heating (M), or combination treatment (essential oil + M). For various essential treatment, extreme pure carvacrol (CL, 98%), eugenol (EU), carvone (CN) and citral (CI) were purchased from Sigma-Aldrich (St. Louis, MO). For essential oil stock solution, each essential oil was mixed with 99.5% ethanol and used within 1 week after stock preparation (Kim and Kang, 2017b). Final concentration of essential oil component was adjusted to 3 mM in each sample. I determined the final concentration based on the preliminary experiment. For 915 MHz microwave heating treatment, was conducted according to the method described in chapter I.2.3. Treatment time was 40 s for hot-chili sauce and treatment time was also determined by preliminary study.

III.2.4. Temperature measurement

Temperature measurement was conducted according to the method described in chapter I.2.4.

III.2.5. Dielectric properties measurement

Dielectric properties measurement was conducted according to the method described in chapter I.2.5.

III.2.6. Bacterial cell enumeration

Bacterial cell enumeration was conducted according to the method described in chapter I.2.6.

III.2.7. Inactivation mechanism

III.2.7.1 Propidium iodide uptake test

For determination of cell membrane damage, the fluorescent dye propidium iodide (PI; Sigma-Aldrich) was utilized. This test method was adopted by Kim and Kang (2017a). I conducted our experiment using buffered-peptone water (BPW) which was treated with same condition described above. Inoculated untreated and treated BPW were centrifuged at 10,000 $\times$ g for 10 min at 4 °C. After removal of supernatant, remaining cell pellet were resuspended in 5 ml of phosphate-buffered saline (PBS; Corning, pH 7.4) to adjusted 0.2 of an optical density at 680 nm (Spectramax M2e; Molecular Device, Sunnyvale, CA) for foodborne pathogens. Their population were approximately 10^7 CFU/ml. PI was added to cell suspension and final concentration was 2.9 μM and incubated for 10 min at room temperature. After incubation, cell suspension was centrifuged at some condition described above and resuspended in 5 ml PBS, then it washed more two times. The final pellet was resuspended in 5 ml of PBS and fluorescence was measured using a spectroflurophotometer (Spectramax M2e; Molecular Device, Sunnyvale, CA) at an excitation wave length of 493 nm and an emission length of 630 nm. The PI uptake value was calculated by following equation.

PI value = (florescence value of treated cell – fluorescence value of untreated cells) / OD_{680}

III.2.7.2 DiBAC$_4$(3) uptake test

For determination of cell membrane potential, the fluorescent dye Bis (1,3-dibutylbarbituric acid) trimethine oxonal (DiBAC$_4$(3); Sigma-Aldrich) were utilized. This is also conducted by the method described by Kim and Kang (2017a). I utilized BPW which was treated with individual essential oil in the same condition. Untreated and treated with inoculated BPW was centrifuged at 10,000 × g for 10 min at 4°C. After removal of supernatants, the cell pellet were resuspended in 1 ml of PBS. DiBAC$_4$(3) was added to the cell suspension to adjusted final concentration corresponding to 2.5 μg/ml for *E. coli* O157:H7 and *S.* Typhimurium or to 0.5 μg/ml for *L. monocytogenes*, followed by incubation for 15 min at 37°C or 2 min at 25°C, respectively. After incubation, dye-added sample was washed two more times as described above in PI uptake value test. The final cell pellets were resuspended in 1 ml of PBS and fluorescence was measured with a spectrofluorophotometer at an excitation wave length of 488 nm and an emission length of 525 nm. The $DiBAC_4(3)$ uptake value was calculated by following equation.

$DiBAC_4(3)$ = florescence value of treated cell / florescence value of untreated cell

III.2.8 Color, water activity measurement and volatile flavor compound quantification

Color and water activity measurement was conducted according to the method described in chapter I.2.7. Also, I measured the contents of dihydrocapsaicin as described in chapter II.2.8

III.2.9 *Statistical analysis*

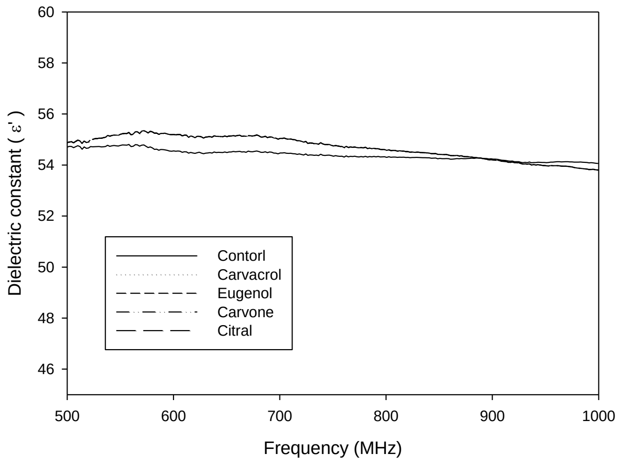
All experiments were repeated at least three times with independently prepared samples. Data were analyzed by analysis of variance (ANOVA) and t-test (LSD) using the Statistical Analysis System (SAS Institute, Cary, NC, USA). A p value of <0.05 was used to indicate significant difference.

II.3 RESULTS

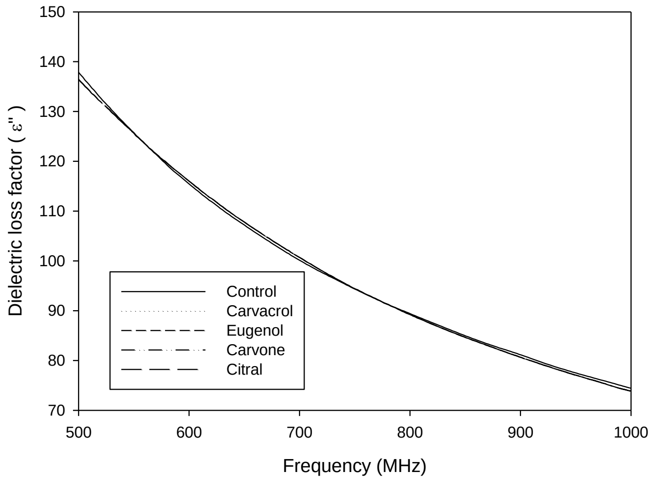
III.3.1. Dielectric properties of hot-chili sauce with or without various essential oil.

According to III.3.1, the dielectric constant of hot-chili sauce without essential oil was 54.2, and hot-chili sauce with various essential oil had 51.1, but no significant difference was observed (P>0.05) . Also, dielectric loss factor of hot-chili sauce without essential oil 80.0, whereas the value of hot-chili including various essential oil were 79.5, but still there was no significant difference compared to not including essential oil component (P>0.05).

(a)



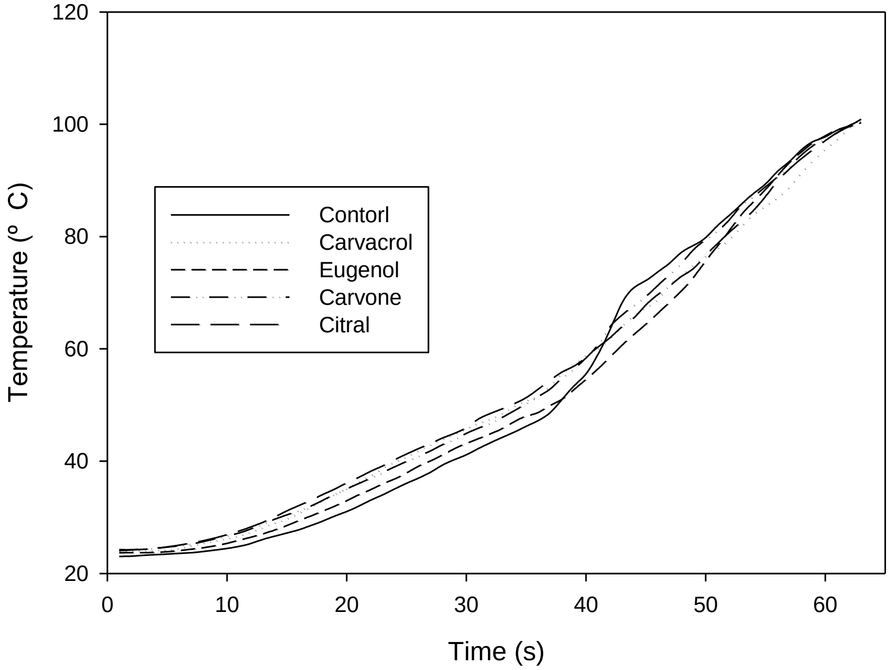
(b)



III.3.1. Dielectric constant (a) and dielectric loss factor (b) of chili sauce with or without various essential oil

III.3.2. Temperature curve of chili sauce with or without various essential oil during 915 MHz microwave heating at 1.5 kW

I investigated the influence the essential oil on the heating rate of hot-chili sauce. In III.3.2, it took 63 s to reach 100°C no matter what hot-chili sauce contains essential oil or not. No significant difference was observed among them ($P>0.05$).

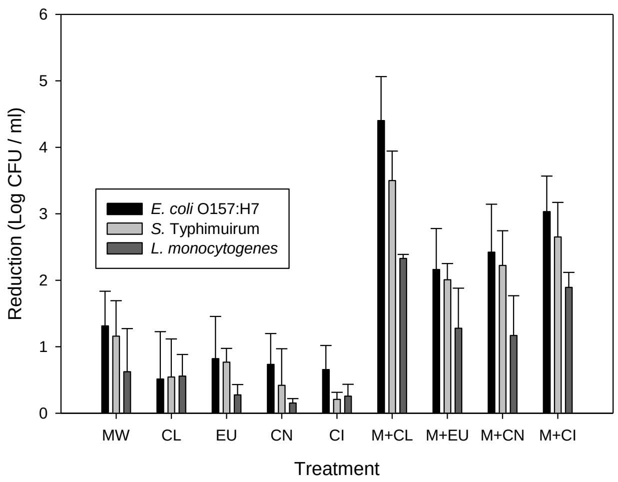


III.3.2. Measurement of heating rate of chili sauce with or without various essential oil

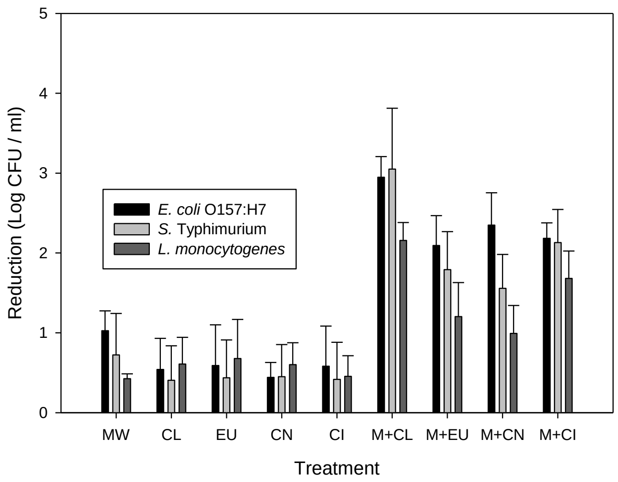
III.3.3. Inactivation of foodborne pathogen in hot-chili sauce using 915 MHz microwave, essential oil, and combination treatment.

I compared the inactivation efficiency among single microwave heating, essential oil treatment and combination treatment. According to III.3.3, in hot-chili sauce, microwave eliminated 1.3, 1.2 and 0.6 log reduction for *E. coli* O157:H7 and *S.* Typhimurium, and *L. monocytogenes* for 40 s treatment. Also, according to III.3.3, carvacrol single treatment did not result in significant difference compared to other essential oil. Moreover, all of essential oil resulted in synergic effect, but no significant difference among EU, CN, and CI though CI have tendency to have higher inactivation efficiency. However, M+CL showed most efficient inactivation effect to all pathogens. Also, sub-lethally injured bacterial cells showed similar results. Except for the population of *E. coli* O157:H7 treated with M+CL and M+CI, there was no significant difference.

(a)



(b)



III.3.3. Population of foodborne pathogen in selective media (a) and resuscitative media (b) when exposed to 915 MHz microwave heating at 1.5 kW

III.3.4. PI and DiBAC$_4$(3) uptake test

Table. III.1 shows that PI uptake value of all of pathogen was significantly larger for M+CL, followed by individual carvacrol treatment (*P*<0.05). For *E. coli* O157:H7, the PI value of M+CL and M+CI was 758.5 and 378.7 was significantly larger than M+EU (62.1) and M+CN (48.7). Similar trends were shown for *S.* Typhimurium and *L. monocytogenes*. M+CI also tended to have higher PI value, but no significant difference was observed for *L. monocytogenes* though it still had larger value. In addition, when treated with M+EU and M+CN, there was no significant difference in comparison to single essential oil treatment. Similar trends were shown in the result of DiBAC$_4$(3) according to Table. III.2. When *E. coli* O157:H7 was treated with carvacrol, the value of DiBAC$_4$(3) was 2.09, followed by 1.48 of citral treatment. The DiBAC$_4$(3) value of carvacrol was significantly larger than eugenol (0.91) and carvone (0.78) (*P*<0.05). Similar trends also observed for *S.* Typhimurium and *L. monocytogenes*, though citral had no significant difference.

Table. III.1. PI uptake value[b] of foodborne pathogen in hot-chili sauce subjected to microwave, essential oil, and combination treatment

Treatment[a]	***E. coli* O157:H7**	***S.* Typhimurium**	***L. monocytogenes***
Microwave	-37.4 ± 72.5a	-1.98 ± 23.7a	6.9 ± 53.6a
Carvacrol	425.7 ± 227.9d	538.2 ± 99.3b	265.3 ± 161.3b
Eugenol	46.2 ± 30.4ab	41.3 ± 16.9a	14.3 ± 29.59a
Carvone	16.8 ± 18.9a	24.8 ± 35.06a	35.4 ± 9.5a
Citral	213.1 ± 68bc	28.3 ± 34.3a	35.6 ± 24.8a
M + CL	758.5 ± 154.6e	919.5 ± 167.7c	1130.1 ± 310.5c
M + EU	62.1 ± 46.6ab	97.7 ± 70.9a	-0.33 ± 46.9a
M + CN	48.7 ± 137.9ab	103.3 ± 96.0a	14.12 ± 47.13a
M + CI	378.7 ± 75.2cd	456.7 ± 73.0b	194.0 ± 88.8a

a Means ± standard deviation from three replications. Values followed by the same letters within the row per foodborne pathogen are not significantly different ($P > 0.05$)

Table. III.2. $DiBAC_4(3)$ value[a] of foodborne pathogen in hot-chili sauce subjected to microwave, essential oil, and combination treatment

Treatment	**E. coli O157:H7**	**S. Typhimurium**	**L. monocytogenes**
Carvacrol	2.09 ± 0.36b	2.78 ± 0.33b	2.99 ± 0.15c
Eugenol	0.91 ± 0.56a	1.08 ± 0.21a	0.84 ± 0.41a
Carvone	0.78 ± 0.31a	1.10 ± 0.26a	1.16 ± 0.22ab
Citral	1.48 ± 0.12ab	1.77 ± 0.61a	1.56 ± 0.18b

a Means ± standard deviation from three replications. Values followed by the same letters within the row per foodborne pathogen are not significantly different (P > 0.05)

***III.3.5. Chili sauce quality after 915 MHz microwave heating and combination treatment*.**

I compared quality change after thermal and combination treatment. According to Table. III.3, except for M+E, no color change observed (*P*>0.05). Also, there was no significant difference between microwave treatment and combination treatment. Water activity also did not change (*P*>0.05). I investigated the volatile flavor compound in hot-chili sauce. I estimated the contents of dihydrocapsaicin among a wide range of capsaicinoid. These treatments did not resulted in change of contents of dihydrocapsaicin.

Table. III.3. Color value, water activity and volatile compound contents of treated chili sauce

Treatment	Time (s)	Color[a]			Water activity[a]	Dihydrocapsaicin[a] (ppm)
		L*	a*	b*		
Control	**0**	30.38 ± 1.44a	10.38 ± 0.77a	11.67 ± 0.28ab	0.907 ± 0.004a	22.9 ± 1.29ab
Microwave	**40**	30.20 ± 0.78ab	10.55 ± 0.98a	11.08 ± 0.98ab	0.902 ± 0.003a	23.7 ± 0.71ab
M + L	**40**	29.32 ± 0.52ab	11.01 ± 0.90a	11.38 ± 0.47ab	0.907 ± 0.004a	24.0 ± 1.36ab
M + E	**40**	28.50 ± 1.32b	11.43 ± 1.50a	12.50 ± 1.97a	0.902 ± 0.003a	22.6 ±1.20a
M + N	**40**	30.61 ± 0.48a	11.31 ± 0.70a	11.78 ± 0.72ab	0.907 ± 0.004a	25.0 ± 1.93ab
M + I	**40**	30.16 ± 0.82ab	10.42 ± 0.81a	10.73 ± 0.34b	0.902 ± 0.003a	22.9 ± 0.44ab

a Means ± standard deviation from three replications. Values followed by the same letters within the row per foodborne pathogen are not significantly different ($P > 0.05$)

III.4. Discussion

In the present study, I compared the inactivation efficiency of various combination treatments. I utilized carvacrol, eugenol, carvone and citral as food additive in hot-chili sauce, since these chemicals has classified as applicable food additive by European commission (Di Pasqua et al., 2006). However, as high concentration of essential oil can negatively influence the flavor quality, it is required to be combined with another treatment so as not only to enhance anti-microbial activity but also reduce its usage in food product. Thus, I utilized 915 MHz microwave heating system as thermal treatment for that purpose.

Dielectric properties of food can be changed depending on various factors such as temperature, water contents, ion contents, electro conductivity, frequency and physical structure of food (Chandrasekaran et al., 2013; Marra et al., 2009). In this experiment, essential oil content, 3 mM, did not significantly influence the dielectric properties of hot-chili sauce. This indicate that 3 mM of essential oil is not sufficient to change dielectric constant and dielectric loss factor and more contents might be required to affect dielectric properties in hot-chili sauce.

This trends link to temperature increase history. The addition of 3 mM essential oil did not induce change in the rate of temperature increase in hot-chili sauce. When chili sauce was exposed to 915 MHz microwave heating system at 1.5 kW, it toot 63 s to reach 100°C.

After treated with 915 MHz microwave heating for the time to induce ca. 1.0 log reduction in hot-chili sauce, I compared the inactivation efficiency of combination treatment and single treatment of various essential oil. In hot-chili sauce, CL did not reduce foodborne pathogen significantly in comparison to EU, CN and CI. However, when combined with 915 MHz microwave heating system, it eliminated highest number of population of foodborne pathogen. CI tended to show slightly higher inactivation, even though there was no significant difference between EU or CN (P>0.05).

It is well-known that chemical structure of essential oil component affects inactivation of foodborne pathogen. In general, essential oil with phenolic compound shows high inactivation efficiency (Burt, 2004). In this experiment, only EU and CN had phenolic compound in their chemical structure. However, only carvacrol showed synergic effect in hot-chili sauce. This might be due to the difference in hydrophobicity among essential oil component. Hydrophobicity is important factor influencing the inactivation effect of antimicrobial chemical, since chemical should attach to cytoplasmic membrane of bacteria so as to deliver toxicity to foodborne pathogen (Lanciotti et al., 2003; Sikkema et al., 1995; Weber and de Bont, 1996).

Referring to previous literature, I could compare hydrophobicity (log*P* ; partitioning behavior of compound in octanol and water) among CL, EU, CN, and CI. It has been known that hydrophobicity of CL was from 3.53 to 3.75 and the value of CI was from 3.37 to 3.45 which is higher than EU and CN. The hydrophobicity of EU was from 2.73 to 2.99 whereas the hydrophobicity of CN from 2.71 to 2.74 (Ben Arfa et al., 2006; Griffin et al., 1999; Takenouchi et al., 2013). Ben Arfa et al. also reported that CL showed better inactivation compared to EU due to higher hydrophobicity (Ben Arfa et al., 2006).

I investigated the inactivation mechanism of various essential oil which explain higher inactivation of CL and CI. Several researcher reported that the main inactivation mechanism of essential oil is to disrupt pH gradient, increase cell membrane permeability, and destruct bacterial cell membrane (Kim and Kang, 2017a; Lambert et al., 2001; Ultee et al., 2002). I measure membrane potential change after single treatment of essential oil BPW. Our results showed that CL induce most dramatic change in cell membrane potential followed by CI. This linked to PI uptake test. The value of PI uptake indicates the permeability and disruption of cell membrane, and I found that not only CL but also CL+M cause most severe damage to foodborne pathogen followed by CI+M, whereas EU+M and CN+M did not significant damage to cell membrane. Thus, I can conclude that carvacrol induce most severe damage to cell membrane by disrupting membrane potential seriously in compared to other essential oils. These results explain why carvacrol showed most effective to inactive foodborne pathogen in chili sauce.

I also observe the reduced inactivation efficiency in hot-chili sauce in the case of CI compared to the results of PI and $DiBAC_4(3)$. This might be because CI is less stable in low pH (Choi et al., 2010). Oxidative stress decompose the CI structure, and thus CI showed less inactivation efficiency in lower pH (Ueno et al., 2004). Somolinos et al. also reported that in neutral pH CI inactivate *E. coli* more efficiently (Somolinos et al., 2010). I suggested that these factors inhibit the inactivation activity of CI in hot-chili sauce.

I also investigated the quality change after combination treatment since quality aspect is important for customer acceptance. First, I measured the change in color after combination treatment. Except for L* value in combination of M+E, overall, no significant change was observed ($P<0.05$). Also, I observed there was no significant change in water activity. As hot-chili sauce main component is chili pepper, I measured the contents of capsaicinoid. Capsaicinoid group is main flavor volatile ingredient of the chili pepper. Among them, I investigated the dihydrocapsicin, and no significant change was observed.

In conclusion, I identified some essential oil cause disruption in cell membrane potential, leading to cell membrane destruction. Among tested essential oil, only carvacrol exhibited most effective activity to inactivation of foodborne pathogen in hot-chili sauce, though both carvacrol and citral showed synergetic bactericidal effec. Also, combination treatment did not result in significant change in quality aspect including color, water activity and volatile compound. Thus, I can conclude that it is required to determine which essential oils can be utilized for the hot-chili sauce processing, combined with 915 MHz microwave heating.

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VI. 국문초록

마이크로파 가열은 유전가열의 일종으로 식품의 내부로부터 열을 발생시키는 방식을 일컫는다. 기존의 가열방식에 비하여, 마이크로파 가열은 식품내 열에너지를 빠르게 만들어낼 뿐만 아니라 식품 내 열 에너지가 고르게 분포된 형태를 가지므로 좀 더 효과적으로 식품을 처리할 수 있다는 특징을 가진다. 다양한 요인들이 마이크로파 가열에 영향을 미치는데, 그 중 식품자체의 유전율 및 포장제의 종류에 따라 마이크로파의 가열 형태는 다르게 나타날 수 있다.

우선 마이크로파 가열은 85°C 까지 가열하는데 6 분이 소요되는 기존의 가열 방식에 비하여 10%의 당을 함유한 칠리소스를 100°C까지 25초 이내에 빠르게 가열시킬 수 있었다. 따라서, 기존 가열 방식에 비해 식품에 존재하는 식품유래 식중독 균을 효과적이고 신속하게 저감화 할 수 있음을 확인하였다. 특히, 마이크로파 가열은 기존 가열 방식에 비하여 칠리소스내 당 함량에 따라 가열형태가 다르게 나타남을 알 수 있었는데, 당의 함량이 높을수록 가열속도는 느리게 됨을 확인하였다. 이는,

소스의 유전율이 달라지기 때문인데 당의 함량이 높아질수록 식품내 수분의 양은 감속하게 되므로 이로 인해 유전상수와 유전손실인자가 감소하여 가열속도도 저하되는 것이다. 따라서 *E.coli* O157:H7, *S.* Typhimurium 및 *L. monocytogenes* 와 같은 병원성 미생물을 검출한계 이하로 저감화하는데 10% 당을 함유한 소스의 경우 가장 적은 시간이 필요하였다.

식품산업에서 플라스틱 포장재를 이용한 식품포장이 다양하게 이루어져 있다. 그 중 PE, PP, Nylon 이 가장 널리 쓰이고 있는데, 최근 고주파가열 시스템과 관련하여 PEI 도 식품포장과 관련하여 다양한 연구가 이루어지고 있다. 27.12 MHz 고주파 가열 시스템과 관련한 선행연구에 의하면, PEI 가 가장 효과적으로 쓰일 수 있음을 알 수 있다. 하지만, 915 MHz 마이크로파에서는 다소 다른 경향성이 나타났다. 유전상수 및 유전손실인자의 값이 PEI 가 가장 높게 나왔는데, 소스를 포장 후 915 MHz 마이크로파로 처리하였을 때 가장 느리게 온도가 상승함을 알 수 있었다. PE, PP 및 Nylon 사이에는 온도상승속도가 서로 유의적인 차이가 없음을 확인하였다 (P>0.05). 이는 식품유래 식중독균 저감화에도 영향을

주어, 칠리소스가 PEI 로 포장되었을 때 검출한계로 저감화하는데 가장 많은 시간이 소비되었다.

마이크로 가열의 살균효율을 증진시키 위하여 다양한 에센셜오일을 동시에 처리하여 실험을 진행하였다. 에센셜오일은 3 mM 농도의 카르바크롤, 카르본, 유게놀 및 시트랄을 이용하였다. 우선 3mM 의 에센셜오일이 가열 속도에 영향을 미치는 지 확인하였으나, 마이크로파 가열속도에는 유의적인 차이가 없었다 (P>0.05). 칠리소스에 에센셜오일과 915 MHz 마이크로파를 동시에 처리하였을 때 전체적으로 시너지 효과가 남을 확인할 수 있었다. 그 중 카르바크롤과 마이크로파를 동시에 처리하였을 때 소스의 커다란 품질변화없이 가장 효과적으로 식중독균을 저감화 할 수 있음을 확인하였다. 이러한 시너지효과를 분석하기위하여 PI uptake 실험을 진행하였고 시너지의 주된 효과는 카르바크롤에 의한 세포막 손상이 주된 원인임을 확인하였다. 또한 $DiBAC_4(3)$ 실험을 통하여 카르바크롤이 다른 에센셜오일에 비하여 세포막 전위를 가장 크게 손상시킴으로써 세포막을 파괴시키는 것을 알 수 있었다 (P<0.05).

따라서 본 연구를 통해 실제 산업에서 915 MHz 마이크로파 가열을 적용함에 있어 식품내 유전율이 중요하게 영향을 미친 다는 것과 포장재의 종류에 따라 가열형태가 달라진다는 것을 확인하였다. 이는 실제 식품산업에서 915 MHz 마이크로파 가열을 최적화함에 있어 유용한 자료가 될 수 있으며, 또한 다른 에센셜오일과 함께 동시에 처리하였을 때 마이크로파 가열의 효율이 증가함을 알 수 있다.

주요어: 915 MHz 마이크로파 가열, 식품매개 병원균, 포장재,
유전율, 에센셜오일

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