REDUCTION OF RED BLOOD SPOTS IN COOKED MARINATED CHICKEN BREAST MEAT BY COMBINATION OF MICROWAVE HEATING AND STEAMING

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การลดจุดเลือดแดงในเนื้ออกไก่นวดด้วยการให้ความร้อนแบบผสมของไมโครเวฟและการนึ่งไอน้ำ

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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โดยทั่วไปจุดเลือดแดงถูกพบบริเวณด้านในของเนื้ออกไก่นวดหลังการหั่นตามขวาง เนื่องจากเลือดตกค้างอยู่ในหลอดเลือด ความคงตัวของฮีโมโกลบินที่เป็นผลจากโซเดียมคลอไรด์ กลูโคส และโซเดียมไตรโพลีฟอสเฟตถูกตรวจสอบถึงความสัมพันธ์ต่อการเกิดจุดเลือดแดง โซเดียมคลอไรด์ถูกดูดซึมเข้าสู่กึ่งกลางของเนื้ออกไก่หลังการนวดในระบบสุญญากาศที่ 12 ชั่วโมง ขณะที่โซเดียมไตรโพลีฟอสเฟตและกลูโคสไม่ถูกดูดซึม โซเดียมคลอไรด์ 1.5 โมลาร์ ทำให้เกิดการสูญเสียความคงตัวของโครงสร้างฮีโมโกลบิน ส่งผลต่อการลดลงของอุณหภูมิการสูญเสียสภาพธรรมชาติจาก 69.4°ซ เป็น 65.8°ซ โซเดียมไตรโพลีฟอสเฟตที่พีเอช 9 ลดอุณหภูมิการสูญเสียสภาพธรรมชาติของฮีโมโกลบินลงที่ 61.4°ซ ส่วนผสมอาหารมีผลเพียงเล็กน้อยต่อการเกิดจุดเลือดแดง เนื่องจากข้อจำกัดของการดูดซึมเข้าสู่เนื้ออกไก่ จุดเลือดแดงถูกกำจัดได้อย่างสมบูรณ์ด้วยการให้ความร้อนที่อุณหภูมิใจกลาง 85°ซ ซึ่งสัมพันธ์กับการเสียสภาพธรรมชาติของฮีโมโกลบิน

คุณสมบัติทางไดอิเล็กทริครวมถึงค่าคงตัวของไดอิเล็กทริค (ε') และค่าการสูญเสียไดอิเล็กทริค (ε'') มีผลอย่างมากต่อการให้ความร้อนด้วยไมโครเวฟ การเติมโซเดียมคลอไรด์ลดค่า ε' และเพิ่มค่า ε'' ของเนื้ออกไก่นวดทั้งสองความถี่ที่ 915 และ 2,450 เมกะเฮิร์ต ค่าความลึกของการแทรกผ่านลดลงในเนื้ออกไก่นวดโซเดียมคลอไรด์ ทำให้เกิดความไม่สม่ำเสมอด้วยการให้ความร้อนของไมโครเวฟ ค่า ε'' เพิ่มขึ้นตามอุณหภูมิ ขณะที่ค่า ε' ลดลงที่อุณหภูมิตัวอย่าง >40°ซ เนื่องจากการเปลี่ยนแปลงโครงสร้างของเนื้อ

การให้ความร้อนด้วยไมโครเวฟกับเนื้ออกไก่นวดเป็นเวลา 7 นาที ร่วมกับการนึ่งไอน้ำสามารถกำจัดจุดเลือดแดงได้อย่างสมบูรณ์เมื่ออุณหภูมิใจกลางถึง 82°ซ โครงสร้างทุติยภูมิของเลือดที่ค้างอยู่ในหลอดเลือดถูกวิเคราะห์ด้วยเทคนิคฟูเรียร์ทรานสฟอร์มอินฟาเรดสเปกโทรสโกปีจากแหล่งแสงซินโครตรอน ปริมาณของโครงสร้างแอลฟา-ฮีลิกซ์ลดลงเมื่อเนื้ออกไก่นวดถูกทำสุกด้วยการให้ความร้อนของไมโครเวฟเป็นเวลา 7 นาที ตามด้วยการนึ่งไอน้ำถึงอุณหภูมิใจกลาง 82°ซ ขณะที่การนึ่งไอน้ำเพียงอย่างเดียวแสดงถึงปริมาณของโครงสร้างแอลฟา-ฮีลิกซ์ที่สูงกว่า บ่งบอกถึงการเสียสภาพธรรมชาติของโปรตีนซึ่งเกิดมากขึ้นด้วยการให้ความร้อนแบบผสม เวลาที่ใช้ในการทำสุกของกระบวนการให้ความร้อนแบบผสมลดลง 28-48% ค่าการสูญเสียจากการทำสุก ค่าพีเอช ค่าความอุ้มน้ำ และเนื้อสัมผัสเทียบเคียงได้ระหว่างเนื้อที่ทำสุกด้วยการให้ความร้อนแบบผสมและ

การนึ่งไอน้ำเพียงอย่างเดียว การให้ความร้อนแบบผสมด้วยไมโครเวฟและการนึ่งไอน้ำทำให้เกิดประสิทธิภาพของการให้ความร้อน สามารถลดการเกิดจุดเลือดแดงได้

สาขาวิชาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2562 ลายมือชื่ออาจารย์ที่ปรึกษา_____

MATTHANEE JANTARANIKORN : REDUCTION OF RED BLOOD SPOTS IN COOKED MARINATED CHICKEN BREAST MEAT BY COMBINATION OF MICROWAVE HEATING AND STEAMING. THESIS ADVISOR : ASSOC. PROF. JIRAWAT YONGSAWADIGUL, Ph.D., 109 PP.

RED BLOOD SPOT/MARINATED CHICKEN BREAST/MICROWAVE HEATING/STEAMING/HEMOGLOBIN/THERMAL DENATURATION/DIELECTRIC PROPERTIES

Red blood spots (RBSs) are normally found in the internal area of cooked marinated chicken breast after transverse cutting due to the blood remaining in the vessels. The effect of sodium chloride (NaCl), glucose and sodium tripolyphosphate (STPP) on hemoglobin (Hb) stability were investigated in relation to RBS formation. NaCl was absorbed into the center of chicken breast after vacuum tumbling for 12 hr while STPP and glucose were not absorbed. NaCl at 1.5 M destabilized the Hb structure, resulting in a decrease of the denaturation temperature (T_d) from 69.4 to 65.8°C. STPP at pH 9 decreased T_d of Hb to 61.4°C. The marinated ingredients appeared to have a minimal effect on RBS formation due to their limited absorption into the chicken breast meat. RBSs could be completely eliminated by heating to a core temperature of 85 °C which is likely related to denaturation of Hb.

Dielectric properties including the dielectric constant (ε') and the dielectric loss factor (ε'') greatly govern the heating profile of microwave heating (MW). Addition of NaCl in marinade decreased ε' values and increased ε'' values of marinated chicken breast at both frequencies of 915 and 2,450 MHz. Penetration depth (d_p) also

decreased in chicken breast marinated with NaCl, which could lead to non-uniform heating by MW. The ε'' values increased with temperature, while the ε' values decreased at sample temperature > 40°C due to changes of the meat structure.

MW heating of marinated chicken breast for 7 min combined with steaming completely eliminated the RBSs when the core temperature reached 82°C. The secondary structure of blood which remained in the vessel was analyzed by Synchrotron-based Fourier Transform Infrared Spectroscopy (SR-FTIR). The α-helical content decreased when marinated chicken breast was cooked by MW heating for 7 min followed by steaming to the core temperature of 82°C while steaming alone showed high α-helical content. This indicated that protein denaturation occurred to a greater extent in the combined heating regimes. Cooking time of the combined heating process was also reduced by 28-48%. Cooking loss, pH, water holding capacity and texture were comparable between meat cooked by the combined heating and that cooked by steaming alone ($P>0.05$). The combined MW heating and steaming appeared to be an effective heating regime that could reduce the incidence of RBS.

School of Food Technology Student's Signature _____

Academic Year 2019 Advisor's Signature _____

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LIST OF ABBREVIATIONS

°C	=	Degree Celsius
cm	=	Centimeter
cm^{-1}	=	Reciprocal centimeter
cm^2	=	Square centimeter
DNS	=	Dinitrosalicylic acid
d_p	=	Penetration depth
ε'	=	Dielectric constant
ε"	=	Dielectric loss factor
EM	=	Electromagnetics
EU	=	European Union
FTIR	=	Fourier Transform Infrared spectroscopy
g	=	Gram
hr	=	Hour
Hb	=	Hemoglobin
Hz	=	Hertz
ΔH	=	Enthalpy
H&E	=	Hematoxylin and eosin
ICP-OES	=	Inductively coupled plasma - optical emission spectroscopy
IR	=	Infrared radiation
J/g	=	Joules/gram

LIST OF ABBREVIATIONS (Continued)

kDa	=	Kilodalton
kg	=	Kilogram
kPa	=	Kilopascal
kW	=	Kilowatt
kWh	=	Kilowatt hour
L	=	Liter
M	=	Molar
mA	=	Milliampere
mg	=	Milligram
MHz	=	Megahertz
MicroDSC	=	Micro-Differential scanning calorimeter
min	=	Minute
mL	=	Milliliter
mm	=	Millimeter
mM	=	Millimolar
mW	=	Milliwatt
MW	=	Microwave
µg	=	Microgram
µL	=	Microliter
µm	=	Micrometer
OCT	=	Optimal cutting temperature

LIST OF ABBREVIATIONS (Continued)

N	=	Newton
nm	=	Nanometer
NM	=	Non-marinated
RBC	=	Red blood cell
RBSs	=	Red blood spots
rpm	=	Revolutions per minute
s	=	Second
SDS-PAGE	=	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SR-FTIR	=	Synchrotron radiation based Fourier Transform Infrared spectroscopy
STPP	=	Sodium tripolyphophate
S80	=	Steaming at core temperature 80°C
S82	=	Steaming at core temperature 82°C
S85	=	Steaming at core temperature 85°C
T_d	=	Thermal denaturation
V	=	Volt
W	=	Watt
WHC	=	Water holding capacity
w/w	=	Weight by weight

LIST OF ABBREVIATIONS (Continued)

W6S80	=	Microwave pre-heating for 6 min and steaming at core temperature 80°C
W6S82	=	Microwave pre-heating for 6 min and steaming at core temperature 82°C
W6S85	=	Microwave pre-heating for 6 min and steaming at core temperature 85°C
W7S80	=	Microwave pre-heating for 7 min and steaming at core temperature 80°C
W7S82	=	Microwave pre-heating for 7 min and steaming at core temperature 82°C
W7S85	=	Microwave pre-heating for 7 min and steaming at core temperature 85°C
x g	=	Relative centrifuge force

CHAPTER I

INTRODUCTION

1.1 Introduction

Chicken breast meat gains more popularity for health conscious consumers due to its relatively low fat content. Chicken meat production worldwide is increased from 95.5 million metric tons in 2018 to 98.4 million metric tons in 2019 (USDA Worldwide Agricultural Service, 2019). In Thailand, chicken meat production is estimated to be 3.3 million MT in 2019 (USDA Foreign Agricultural Service, 2019). The main export countries are Japan and EU with 49% and 40% of total production, respectively. Cooked chicken products are exported about 68% of total production in 2019 (USDA Foreign Agricultural Service, 2019). Typical defect of cooked chicken product is red blood spots (RBSs), which is found when cooked breast meat is transversely cut. Chicken breast muscle contains many blood vessels called pectoral vessels for delivery blood to whole breast muscle. Blood remaining in these vessels after slaughtering process results in RBS formation in cooked chicken breast (Farner, King, and Parkes, 1972; Ritchison, 2008). Hemoglobin (Hb) is a main pigment responsible for red color in native state, while heat-induced denaturation of Hb is brown (Elmasry, Barbin, Sun, and Allen, 2012; Suman, Nair, Joseph, and Hunt, 2016). One of the causes of RBS incidence is incomplete thermal denaturation of Hb in blood remaining in vessels. Heat-induced denaturation of Hb depends on several factors, including pH, redox state and ingredients in marination (Ahn and Maurer, 1990;

Suman et al, 2016; Jeong, 2017). Influence of marination ingredients on denaturation of Hb has not been well characterized in the cooked chicken model. Changes in denaturation behavior of Hb as affected by these ingredients would greatly affect RBS formation.

Marination is one of important steps for cooked chicken meat production. Typical ingredients used in marination include sodium chloride (NaCl), sodium tripolyphosphate (STPP) and glucose, which are commonly applied in the vacuum tumbler (Kirmaci and Singh, 2012). NaCl is used to improve flavor and tenderize meat by increasing solubility of salt soluble proteins and improving water holding capacity (WHC) of the meat (Kirmaci, 2009). STPP can increase WHC and cooked yield of the chicken breast meat (Puolanne, Ruusunen, and Vainionp, 2001; Xue et al., 2016). Glucose is added to enhance sweet taste and brown color through maillard reaction. Nevertheless, each ingredient has different ability to diffuse into chicken breast meat depending on its molecular size, water solubility, concentration and others. Xiong and Kupski (1999) reported that different phosphates showed different penetration rate into chicken fillets. The authors found that tripolyphosphate and pyrophosphate with smaller molecular size could diffuse faster, and subsequently, showed higher concentration in the meat than did hexametaphosphate. Furthermore, Kay (2001) explained that chicken breast tumbled with only NaCl had a distinct layer of about 50 μm depth from the surface of the meat, while phosphate showed a more typical diffusion layer of about 2,000 μm. These penetrated ingredients could affect thermal denaturation (T_d) behavior of Hb, thus, changes in T_d of Hb as affected by these ingredients should be investigated.

Nowadays, manufacturers of cooked chicken product attempt to minimize RBS problem by increasing heating temperature or heating time to assure complete denaturation of blood residues. But this approach results in significant loss of yield and time. Consequently, alternative approach should be sought.

Dielectric heating known as microwave (MW) and radio frequency (RF) heating can generate heat internally by dipole rotation of polar molecules like water. When chicken breast meats are placed in an alternating electromagnetic field, positive and negative charges of polar molecules migrate to different ends and continuously rotate with alternating electromagnetic field. This process induces molecular friction and generates heat within the chicken breast meat. In addition, another mechanism known as ionic conduction takes place. Ions in the chicken breast meat can disassociate during applied electrical field and migrate in direction that varies with alternating electrical fields. It causes molecular friction by oscillation of ions in the meat (Awuah, Ramaswamy, and Tang, 2015). MW and RF heating can reduce cooking time as compared with conventional methods (steaming, roasting, grilling or frying, and etc.), which heat is transferred from heating source to chicken breast by means of convection or radiation and conduction (McKenna, Lyng, Brunton, and Shirsat, 2006; Farag, Lyng, Morgan, and Cronin, 2011; Kirmaci and Singh, 2012; Rincon, Singh, and Stelzleni, 2015). Frequencies used for commercial MW heating are 915 and 2,450 MHz, while those of RF heating ranges 1-300 MHz (Piyasena, Dussault, Koutchma, Ramaswamy, and Awuah, 2003). MW heating has advantage over RF heating as it provides faster heating rate and can be applied to food containing high moisture and high salt content better than did RF. Since low frequencies of RF could increase dielectric loss factor greater than MW heating, resulting in nonuniform

heat distribution and thermal runaway (McKenna et al., 2006; Kirmaci and Singh, 2012; Awuah et al., 2015).

Qualities of MW cooked meat were comparable with those cooked by conventional methods (Yarmand and Homayouni, 2014; Ergönül, 2017; Li, Tang, Yan, and Li, 2019). MW cooked chicken breast meat had lower redness than conventionally cooked sample (Ergönül, 2017). This is because heme protein in meat were totally denatured under MW heating (Zhang, Lyng, and Brunton, 2004; Pathare and Roskilly, 2016). Therefore, MW heating could have a potential to completely denature blood residues in vessels without significant overheating.

Dielectric properties of foods are factors governing heating rate (Kirmaci and Singh, 2012). This term includes dielectric constant (ε') that is related to the capacity for electrical energy storage in the material (Zhuang, Nelson, Trabelsi, and Savage, 2007). Dielectric loss factor (ε") is associated with the electrical energy dissipation in the material (Lyng, Zhang, and Brunton, 2005) and converts that energy into heat. Another important factor affecting MW heating is penetration depth. The penetration depth (d_p) is defined as the distance that electromagnetics (EM) power reduces to 1/e (1/2.72) or 36.9% of surface value (Llave, Terada, Fukuoka, and Sakai, 2014). The d_p is related to frequency and dielectric properties (Awuah et al., 2015). Both ε' and ε" values vary with frequency, moisture content and ingredients present in meat (Zhuang et al., 2007). Lyng et al., (2005) reported that beef muscle mixed with salt or STPP resulted in an increase of ε" and ε' values. These results illustrated that ingredients could directly affect ε" and ε' values. Thus, dielectric properties of chicken breast tumbled with different ingredient marination should be investigated.

This study was aim at developing a cooking method to minimize RBSs in marinated chicken breast. Dielectric properties, namely ε' and ε" were evaluated as functions of marinated ingredients. Furthermore, the effect of marinated ingredients and their concentrations on heat denaturation of chicken Hb and optimal temperature for reduction of RBS formation in cooked marinated chicken breast were determined.

1.2 Research objectives

Objectives of this research were:

1.2.1 To minimize red blood spots in cooked marinated chicken breast meat by combination of MW heating and steaming.

1.2.2 To investigate the effect of marinated ingredients on dielectric properties of chicken breast

1.2.3. To evaluate the effect of ingredients used in commercial marination on thermal denaturation of chicken Hb.

1.2.4. To determine optimal heating regime for red blood spot reduction.

1.3 Research hypotheses

Combination of MW heating and steaming can be used to minimize red blood spots on cooked chicken breast. Heating rate of MW heating is governed by dielectric properties, which are, in turn, varied by marinated ingredients applied. RBS is caused by incomplete thermal denaturation of Hb retained in blood vessels. Types of ingredients in marination process greatly affect denaturation pattern of Hb, leading to varied degree of red spot formation. Optimal cooking temperature can be reduced RBS formation and maintain reasonable yield.

1.4 Scope of the study

Concentration of marinated ingredients, namely NaCl, sodium tripolyphosphate (STPP) and glucose that penetrated into chicken breast after tumbling at storage time of 0, 2, 6, 12, 16 and 20 hr was determined. Thermal denaturation of Hb from chicken blood as affected by marinating ingredients was also investigated. Optimal cooking temperature for red blood spot reduction was determined.

Chicken breast meat samples were vacuum tumbled with different marinated ingredients, namely 5% NaCl, 3% glucose, 2% STPP and mixed ingredients (5% NaCl, 3% glucose, 2% STPP) as commercial formula. Dielectric properties (ε' and ε") of marinated chicken breast samples were elucidated at frequency of 915 and 2,450 MHz. Dielectric properties of marinated samples were examined at various temperatures of 4, 20, 40, 60 and 85°C. Penetration depth (d_p) was also calculated.

Non-marinated and marinated chicken breast meat samples (5% NaCl, 3% glucose, 2% STPP) were heated until the core temperature reaches 85°C using MW heating coupling with steaming. Degree of red blood spots were analyzed by visual inspection and IR spectroscopy. Cooking loss, moisture content, water holding capacity, shear force, pH value and color of cooked meat were evaluated.

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CHAPTER II

LITERATURE REVIEWS

2. 1 Red blood spots in chicken breast

A big problem of cooked chicken meat products is the red color defects in meat which they are not acceptable to consumers, leading to rejection. It has been reported that commercial cooked chicken products were complained and rejected by the consumers to 11% of random products in supermarkets due to extensive and severe internal red color (Smith and Northcutt, 2003). Red blood spots (RBSs) are which sporadically occurred in cooked chicken breast production. They are caused by blood remaining in vessels of internal chicken breast muscles (Ahmed, 2003). RBSs are observed after transverse cutting of cooked chicken breast meat. Factors affecting RBS formation have been studied, including the slaughter process, bleeding time and stunning methods (Veerkamp, 1988; Gregory and Wilkins, 1989; Contreras and Beraquet, 2001; Ahmed, 2003; Ali, Lawson, Tauson, Jensen, and Chwalibog, 2007). The optimal condition of slaughter process and electrical stunning results in a complete bleeding, leading to a reduction of the RBSs. However, the variation of each factors are limited. The slaughter process is recommended by Halal regulations (The Central Islamic Council, 2016) that the trachea, carotid artery and jugular vein of the live chicken are cut at one time with sharp knife. Likewise, bleeding time is recommended at least 90 s. The prolonged bleeding time had no effects on the amount of blood removing (Kuenzel and Walther, 1978). Bleeding could remove a little blood

from the chicken breast muscle because the blood pressure rapidly drops after cutting the neck, hence there is no enough driving force to empty blood in vessels of breast muscle (Alvarado, Richards, O'Keefe, and Wang, 2007). Minimum stunning current at 100 mA per bird and frequency <200 Hz is required for EU regulation (The Council of the European Union, 2009). Ahmed (2003) reported that the electrical stunning at 45 mA and frequency of 50 Hz could minimize the RBSs. This could not be commercially practiced due to animal welfare regulations. In addition, stunning is prohibited by Halal regulation (The Central Islamic Council, 2016). This could result in an incomplete bleeding, leading to blood remaining in vessels. Kotula and Helbacka (1965) found that blood remaining in chicken breast muscle was 4.93% after electrical stunning. It is likely a cause of RBS when heating process is insufficient.

2.2 Hemoglobin (Hb)

Hemoglobin (Hb) is a main pigment, responding for red color of blood (Undeland, Kristinsson, and Hultin, 2004). The structure of globular proteins is the interaction of hydrophilic outer layer and hydrophobic inner core. Hydrophobic interactions have an important role to maintain the conformation of globular protein (Garrett, Stairs, and Annett, 1988). Hb is the tetrameric protein with four subunits and each subunit contains a globular protein which has heme group at its core (Dafre and Reischl, 2006). Heme group is ferrous iron (Fe^{2+}) that dictates Hb derivatives with attachment of ligand to the sixth ligand binding, and shows the typical color (Abbasi and Lutfullah, 2002). Oxyhemoglobin (oxy-Hb) is formed by oxygen-bind Fe^{2+}, resulting in cherry-red, while no oxygen combination changes to form of deoxyhemoglobin (deoxy-Hb) and purplish-red. The oxidation of Fe^{2+} to Fe^{3+} is a

form of methemoglobin (Met-Hb) which results in the brown color (Hammad, Meihu, Guofeng, and Lichao, 2017).

Thermal denaturation of Hb leads to conformational change, precipitation and aggregation (Ajloo, Moosavi-Movahedi, Sadeghi, and Gharibi, 2002). Previous study reported that type of ingredient had an effect on the stability of Hb. Ahn and Maurer (1990) investigated the effects of NaCl, sodium tripolyphosphate (STPP) and dextrose on heat denaturation of purified Hb from turkey blood. NaCl (2.5%) destabilized structure of turkey Hb and subsequently decreased its thermal denaturation as compared with purified Hb. This was because exposure of heme group could react with chloride ion (Ledward, 1971). STPP (0.5%) did not significantly affect the thermal denaturation of Hb, while 1% dextrose increased thermal denaturation of turkey Hb because the hydroxyl groups in dextrose molecules and water formed hydrogen bond. This leads to an increase of surface tension of water and free energy of unfolded protein (Alfonso et al., 2007). Likewise, sucrose and glucose could increase the thermal denaturation of protein which increased with increasing of concentration. Sucrose had a stronger effect on protein stability than glucose (Garrett et al., 1988; Oshima and Kinoshita, 2013). This is because sucrose has a larger amount of hydroxyl groups than glucose (Oshima and Kinoshita, 2013). It can be seen that Hb stability varies with ingredients used in chicken marination.

2.3 Measurement of protein denaturation

Protein denaturation can be measured using various methods, namely circular dichroism spectroscopy (CD), isothermal titration microcalorimetry (ITC), infrared spectroscopy (IR), differential scanning calorimetry (DSC), Raman spectroscopy, and

nuclear magnetic resonance (NMR) (Lepock, 2005). These methods have a different principle, for example DSC measures thermodynamic transition, particularly transition temperature (T_m) which is denaturation temperature of protein (Deshpande, 2008). Changes related to endothermic and exothermic process can also be monitored (Ariaeenejada et al., 2013). DSC was used to determine T_m of protein, such as Hb in human blood and odorant binding proteins from pig nasal mucosa (Lepock, 2005). In case of multiple domains are presented in a protein, they lead to more complex denaturation profile with multiple transitions using DSC. Deshpande (2008) reported that T_m of fresh chicken breast meat had two peaks at 58ºC and 77ºC and sarcoplasmic protein had three peak transitions at 27ºC, 52ºC and 75ºC. Additionally, Fourier Transform Infrared spectroscopy (FT-IR) is used to measure protein denaturation in secondary structure with a vibrational spectroscopic technique. FT-IR spectroscopy has a great advantage over conventional methods since it is on-line monitoring, non-destructive, rapid technique and multiple analysis without any additional sample preparation (Boyaci, 2015; Yang and Ying, 2011). IR spectral data of protein are interpreted in term of the vibrations of chemical bonds in molecules which can absorb or emit infrared light and changes vibration state (Kong and Yu, 2007). Nine characteristic IR absorption bands in the protein repeat unit compose of amide A, B and I to VII. Both amide I and II bands are the most predominant vibrational bands of the protein backbone. Amide I and II band have an approximate frequency of 1700 to 1600 cm^{-1} and 1480 to 1575 cm^{-1}, respectively which amide I is approximately 80% of the peptide linkages of C=O stretch vibrations and amide II is 40 to 60% of in-plane NH bending and 18 to 40% of CN stretching vibration (Kong and Yu, 2007). The spectral profile of the amide I band is used to quantify the secondary structure of

proteins, namely α-helix, β-sheet, β-turn, and random coil, while amide II vibration usually cannot be detected because of a small change in polarizability (Carey, 1996). The relative percentage of the secondary structure may influence protein quality (Yu, McKinnon, Soita, Christensen, and Christensen, 2005). However, the overlap of secondary structural components is common in the amide I region. The curve fitting procedure is commonly applied to quantitate each secondary structure (Dong, Huang, and Caughey, 1990). Other amide vibrational bands have a few practical use in the protein conformational studies because they are very complex and related to the details of force field and the nature of side chains and hydrogen bonding. FT-IR spectroscopy has been applied to analyze protein denaturation in meat products for comparing the cooking ability. Calabro and Magazu (2012) reported that protein denaturation of bovine breast meat cooked by microwave heating at power of 800W for 95 s and conventional convective heating was analyzed using FT-IR spectroscopy. The results indicated that the samples cooked at 800W for 95 s exhibited higher intensity of β-turns observed at 1665-1690 cm^{-1} than that conventional convective heating. This implied that protein denaturation occurred to a greater extent in the microwave heating. Yan, Wang, He, and Zhou (2004) also investigated heat-induced unfolding and aggregation of bovine Hb using FT-IR spectroscopy. The Hb solution heated <60ºC showed a predominant α-helix band at 1651 cm^{-1}. When the Hb solution were heated >60ºC, FT-IR spectra showed two new bands at 1681 cm^{-1} and 1618 cm^{-1} which defined to hydrogen-bond extended intermolecular ß-sheet structures formed upon aggregation. When FT-IR spectrometer is combined with synchrotron light source and microscopy, it is called synchrotron radiation-based FTIR microspectroscopy (SR-FTIR). The synchrotron light is usually 100-1000 times

brighter than conventional globar source (Pascolo et al., 2014), thus it is capable of exploring microstructures of biological samples with high signal to noise ratio at ultraspatial resolutions as fine as 3-10 μm (Yu, 2006). Kirschner, Ofstad, Skarpeid, Host, and Kohler (2004) report that protein secondary structure of bovine *longissimus dorsi* myofibre proteins was analyzed using SR-FTIR and found that major changes in response to heat treatment were in the amide I region, which exhibited two well defined peaks at 1628 and 1655 cm^{-1}. The band at 1655 cm^{-1} assigned to α-helical structure decreased with temperature, while the band at 1628 cm^{-1}, which was assigned to aggregated β-sheet, increased. SR-FTIR spectroscopy could be applied to determine in situ protein denaturation in cooked meat products.

2.4 Absorption of marinated ingredients

Marinated ingredients have a different absorption ability into meat after vacuum tumbling. Xiong and Kupski (1999) revealed that phosphates, namely pyrophosphate (PP), tripolyphosphate (TPP), and hexametaphosphate (HMP) greatly enhanced the marinade absorption due to the increase of protein solubility and swelling of muscle fibers. The PP and TPP had a faster diffusion than HMP, and concentration of both PP and TPP was found to be lower at the outer layer of the meat. This indicated that molecular size of ingredient has an effect on the absorption into meat as well as meat temperature during tumbling. Palang (2004) reported that the marinade penetration of meat marinated at 4ºC and 10ºC was analyzed by fluorescing trivalent europium ion (Eu^{+3}) under microscope. $EuCl.6H_2O$ (0.003 M) was mixed in marination with 6% NaCl, 2.1% sodium tripolyphosphate. The results showed that meat temperature during vacuum tumbling significantly affected marinade

penetration. The chicken breast meat marinated at 10ºC and 4ºC showed diffusion layer depth of 4.7 and 3.4 mm, respectively. Higher meat temperatures (10ºC) during vacuum tumbling could promote deeper absorption.

The absorbed sodium and phosphate in meat after tumbling can be determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Vallapragada, Inti, and Ramulu, 2011). The ICP-OES is emission spectrometric technique which detects the excited atoms emitting energy at a given wavelength as the electrons return to their ground state. The amount of element in the sample is proportional to intensity of the energy emitted at that wavelength. The ICP-OES has the advantages over other techniques in terms of detection limits and speed of analysis. Quantitation values of the elements in ICP-OES can be verified in parts per million and even parts per billion. Chicken breast and thigh meat were quantified for Mg, Ca, Fe and Zn content in parts per million by ICP-OES (Jeon et al., 2010). In addition, Vallapragada et al., (2011) applied ICP-OES to estimate calcium and phosphorus in Eliphos tablets which were found in the small amount at 0.01 mM with 98.5% accuracy. Therefore, ICP-OES is a selective technique and capable detecting small amount of elements contained the meat products.

2.5 Microwave (MW) heating

Microwave (MW) heating relies on internal heat generation of the sample due to polar molecules, like water. When meat products are placed in an alternating electromagnetic field, then, positive and negative charges in polar molecules migrate to different ends and lead to polarization. If polar molecules continuously rotate with alternating EM field as called dipole rotation. This process induces molecular friction

and generates heat within meat products (Awuah, Ramaswamy, and Tang, 2015). Additionally, ions in the meat products can disassociate during applied electrical field and migrate in direction that varies with alternating electrical fields. It causes molecular friction by oscillation of ions in the meat products. This mechanism is known as ionic conduction (Hebbar and Rastogi, 2012). In industry, the frequency range of MW heating is 915 and 2,450 MHz (Piyasena, Dussault, Koutchma, Ramaswamy, and Awuah, 2003; Kirmaci and Singh, 2012). In MW heating, dissipation of electromagnetics power to the food material is induced by special oscillator tubes known as magnetrons which emit the microwaves. The MW energy is transferred by the waveguide into a metal chamber or a cavity which waves bounce around off the metal walls of cavity and subsequently dissipates to meat products from many directions (Marra, Zhang, and Lyng, 2009).

The advantage of MW heating is short processing time, leading to more nutrient retention, and less processing cost and energy usage (McKenna, Lyng, Brunton, and Shirsat, 2006; Yarmand and Homayouni, 2011; Awuah et al., 2015; Rincon, Singh, and Stelzleni, 2015). Yarmand and Homayouni (2011) reported that cooking time of chicken breast cooked by MW was reduced, resulting in higher retention of thiamin of 98%, while thiamin retention of roasted chicken breast was 77%. Moreover, Jouquand et al., (2015) reported that MW cooked beef burgundy (4.6 kWh) had lower energy consumption than convection oven cooking (6.5 kWh) due to 56% shorter cooking time. Moreover, qualities (pH, moisture content, juiciness, water holding capacity, tenderness and flavor) of meat cooked by MW were comparable with those cooked conventionally (Yarmand and Homayouni, 2009; Nikmaram, Yarmand, Emamjomeh, and Darehabi, 2011; Ergonul, 2017). However, MW cooked

meat showed a decrease in a* (redness). Ergonul (2017) reported that MW- and conventionally-cooked chicken breast showed comparable L* (lightness) value, whereas less a* and b* (yellowness) values were observed for MW cooked chicken breast. Protein pigment undergoes faster oxidation and denaturation during MW cooking, leading to formation of ferrihemochrome with brown color (Suman, Nair, Joseph, and Hunt, 2016).

Cooking loss is an important factor for chicken meat processing. Yarmand and Homayouni (2009) reported that MW (12000W) cooked lamb and goat meat to reached internal temperature of 70ºC, showed higher cooking loss than those cooked by a convection oven. High power and short time of MW heating have an effect on protein denaturation, breaking down texture matrix and protein destruction. This would eventually lead to an increase of cooking loss. However, Ergonul (2017) observed that cooking loss of MW, 720W for 15 min, cooked chicken breast was comparable to that cooked in a convection oven. The cooking loss of MW heating might be depended on the power and cooking time of MW heating.

MW heating alone poses problem on non-uniform heating in whole meat. Goksoy, James, and James (1999) reported that MW heated whole chicken carcass at 2,450 MHz, 500W for 5 min, resulted in a different temperature about 15-45ºC between breast and leg. Similarly, Jeong et al., (2007) demonstrated that ground pork patties with salt cooked by MW at 2,450 MHz had a differential temperature around 13ºC between edge and central area of sample. The combined heating process of MW heating and conventional method has been reported to improve uniform heating, process efficiency and costs (Geedipalli, Datta, and Rakesh, 2008; Hebber and Rastogi, 2012; Datta and Rakesh, 2013). Geedipalli et al., (2008) reported that the

combined microwave-jet impingement heating increased a heating uniformity to 22-30% over microwave-only heating. The jet impingement is a special form of hot air heating in which jets of hot air at high velocities impinge on the product for faster heat transfer (Wahlby, Skjoldebrand, and Junker, 2000). The jet impingement dominates over MW heating near the surface, with MW heating being more significant in the interior. This helped the product achieving the uniform heating. Steaming is a common heating process applied in chicken meat industry. Moist heat using steam generates the temperature of 100ºC (Datta and Rakesh, 2013). This is quite a long processing time to reach the desired core temperature as heat dissipated from the surfaces into internal part (Li, Tang, Yan, and Li, 2019). It typically requires processing time of 30-50 min to reach core temperatures of 80-82ºC (Pathare and Roskilly, 2016). Therefore, combination of MW heating and steaming could be explored to reduce RBS incidence.

2.6 Dielectric properties

Factors affecting heating rate of MW cooking are dielectric properties, penetration depth, the thickness and the geometry of meat (Vadivambal and Jayas, 2010). Dielectric properties are defined as relative permittivity (ε). The permittivity of the material relative to free space or vacuum as $\varepsilon = \varepsilon' - j\varepsilon''$ where ε' is the dielectric constant, ε'' is the dielectric loss factor and j is $\sqrt{-1}$. The dielectric constant (ε') is related to the capacity for electrical energy storage in the material. It also affects the amount of electromagnetic energy reflected or transmitted into the product (Zhuang, Nelson, Trabelsi, and Savage, 2007). The dielectric loss factor (ε'') is associated with the electrical energy dissipation in the material and conversion energy to heat (Lyng,

Zhang, and Brunton, 2005). Dielectric properties governed heating rate of MW heating as shown in equation (1) (Nelson, 1996).

$$\frac{\Delta T}{\Delta t} = \frac{2\pi f \varepsilon_0 \varepsilon_r'' \tan \delta \, E^2}{C_p \rho} \quad (1)$$

Where ΔT is temperature increase (°C) in t sec, f is the frequency (Hz), ε_0 is dielectric constant of vacuum, ε''_r is relative dielectric loss factor, E is electric field intensity (V/m), C_p is specific heat (J/kg.°C) and ρ is density (kg/m^3)

Penetration depth (d_p) is defined as the distance that electromagnetic power dissipates into the food material and reduces to 1/e (1/2.72) or 36.9% of surface value (Llave, Terada, Fukuoka, and Sakai, 2014). The d_p values of meat batters were lower at MW frequencies as compared to radio frequencies (Zhang, Lyng, and Brunton, 2004). Zhang et al., (2004) reported that d_p of pork meat batters (1.2% salt) ranged between 1.8-2.5 cm at 915 MHz and 1.2-1.4 cm at 2,450 MHz, and Lyng et al., (2005) who stated d_p of chicken breast meat at 2,450 MHz was 1.7 cm. The consequence is non-uniformity in meat product during MW heating. The knowledge of d_p is useful to design appropriate thickness of food material to ensure a relatively uniform heating by MW. The d_p is inversely proportional to frequency and dielectric properties as shown in equation (2) (Buffler, 1993);

$$d_p = \frac{c}{2\pi f \sqrt{2\varepsilon_r' \left[\sqrt{1 + (\varepsilon_r''/\varepsilon_r')^2} - 1\right]}} = \frac{c}{2\pi f \sqrt{2\varepsilon_r' \left[\sqrt{1 + \tan^2 \delta} - 1\right]}} \quad (2)$$

where c is the speed of the propagation of waves in a vacuum (3 x 10^8 m/s), f is the frequency (Hz), and d_p is the penetration depth (m)

Dielectric properties of the foods are affected by frequency, moisture content and ingredients. At MW frequencies, the ε' and ε'' values of lean meat (chicken breast, turkey breast, lamb leg and beef forequarter trimmings) decreased with increasing frequency (Lyng et al., 2005; Zhuang et al., 2007). The polar and ion in food materials that tend to realign itself with the changes in the direction of electric field at low frequency. On the other hand, when frequency increases, it leads to rapid change of polarity, thereby, the polar and ion in food materials cannot follow with the change of electrical field (Icier and Baysal, 2004; Rahman, Kasapis, Guizani, and Al-Amri, 2003). Dielectric properties increase with moisture content due to the effect on the dominant dipole rotation mechanism of water (Farag, Lyng, Morgan, and Cronin, 2011). Lyng et al., (2005) reported that the similar moisture contents of lean meats were similar magnitude of ε' and ε'' values at frequency of 2,450 MHz, while low moisture content of the pork fat showed lower ε' and ε'' values than lean meats.

In general, the ε'' values of biological tissues in MW frequency region are influenced by combined effects of polarization (dipole loss component, ε''_D) and ionic conductivity (ionic loss component, ε''_σ) (Icier and Baysal, 2004). The ε'' values greatly affected by ionic conductivity at low frequency and high temperature, while polarization has a strong effect at high frequency (Tanaka, Mallikarjunan, Kim, and Hung, 2000). Therefore, polarization is an important mechanism with dielectric properties at MW frequencies. Ingredients used in meat marination have an effect on dielectric properties. Moreover, the ionic compounds in meat product can dissociate in water, resulting in an increase of ε'' values (Lyng et al., 2005). The addition of salt

contributes to an increase in conductive charge. Ingredients, namely pork fat, potato starch, wheat gluten, water, nitrite, phosphate, or NaCl were blended with beef muscle. The batter with 1.5% NaCl showed the highest of ε'' values, while the batter containing pork fat had the lowest ε'' values.

Furthermore, the change of meat temperature affects the dielectric properties. The chicken breast samples with marinated solution of 0.5 and 1% NaCl were heated to reach internal temperature from 3 to 75ºC, the values of ε' were decreased with increasing temperature (Tanaka et al., 2000). Zhang et al., (2004) demonstrated that ε' of pork meat batter (2.3% salt in final product) significantly decreased at temperature >45ºC at frequencies of 896 and 915 MHz. A decrease of ε' might be caused by the effect of temperature on protein denaturation. The myofibrillar proteins of whole chicken breast was transversely shrunk at 35-40ºC, resulting in changes of muscle structure and water loss (Tornberg, 2005). Water molecule plays a significant role on absorption of microwave energy. Therefore, release of water at higher temperatures leads to a decrease of ε' values. This was because most free water was held in meat structure at temperature <40ºC, thus water content had influence on ε' values (Ngadi, Satyanarayan, Vijaya, and Kazemi, 2015). At sample temperature >60ºC, the meat juice held by capillary forces was expelled, resulting in a decrease of ε' values (Bircan and Barringer, 2002). While the ε'' value increased with temperature. The ε'' value significantly increased at low frequencies, and gradually increased at high frequencies (Tanaka et al., 2000). An increase in temperature results in viscosity reduction and increased ion mobility, leading to high differential of heat dissipation in meat sample during MW heating at lower frequency (Hebbar and Rastogi, 2012). The synergy of temperature and ε'' values lead to thermal runaway affecting uniform heat distribution

during MW heating (Wang, Wig, Tang, and Hallberg, 2003). The moist foods with salt, loss factors generally increase with increasing temperatures at lower MW frequencies, which often results in a phenomenon commonly referred to as “thermal runway” (Metaxas and Meredith, 1983). Thermal runaway is explained as accumulation of heat due to continuous energy input in some area of sample, which is associated with increased current flow and power dissipation (Kirmaci, 2009). That is, a preferentially heated part of the food in an electromagnetic field accelerates its heating, often causing non-uniform heating. This problem can be improved by using a turntable, optimal power and a mode stirrer in the MW oven (Datta and Rakesh, 2013). Heddleson, Doores, and Anantheswaran (1994) reported that MW heating with oven of lower power of 450W was less effective in destroying pathogenic bacteria as compared to heating with ovens of higher wattage of 700W. Moreover, stirring sample immediately after heating was effective in reducing numbers of viable *Salmonella spp.* because large temperature gradients within foods was eliminated (Heddleson, and Doores, 1994). Therefore, the optimal design of MW heating would improve uniformity of heat distribution in MW heating.

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CHAPTER III

EFFECT OF MARINATING INGREDIENTS ON THE DENATURATION OF HEMOGLOBIN AS RELATED TO RED BLOOD SPOT FORMATION IN COOKED CHICKEN BREAST MEAT

3.1 Abstract

The objectives of this study were to investigate the effects of salt (NaCl), sodium tripolyphosphate (STPP) and glucose on the denaturation of hemoglobin (Hb) and the formation of red blood spots (RBSs) in cooked marinated chicken breast. After vacuum tumbling, STPP and glucose were not absorbed into the center of chicken breast after 20 hr, while Na^+ was absorbed after 12 hr. The denaturation temperature (T_d) of chicken Hb decreased to 65.8ºC in the presence of 1.5 M NaCl, while that of the control was 69.4°C. STPP at pH 9 decreased the T_d of Hb to 61.4°C. The alkaline pH induced by STPP destabilized the Hb structure .RBSs were observed at 100% incidence at core temperatures of 50 and 70°C for 1 min, and 3.3% incidence was found at 80°C for 1 min. When chicken breast was heated to 85ºC, RBSs did not take place. The ingredients used during marination appeared to have a minimal effect on RBS formation due to their limited absorption into the chicken breast meat. The cooking temperature is a major factor governing RBSs, as it directly affects the denaturation of Hb.

Key words: Red blood spot, Chicken breast meat, Hemoglobin, Thermal denaturation

3.2 Introduction

Cooked chicken breast meat is an important food product with significant nutritional value. It has been popular with health-conscious customers due to its lower fat content (USDA Foreign Agricultural Service, 2019). A red blood spot (RBS) is described as a red spot observed inside chicken breast after transverse cutting and is considered a defect of cooked chicken meat products. It is not acceptable to most consumers, as they often deem that the product is undercooked and not safe for consumption (Suman, Nair, Joseph, and Hunt, 2016; Bae, Cho, Hong, and Jeong, 2018). RBSs have sporadically occurred in cooked chicken breast production (Smith and Northcutt, 2003), which has caused significant economic loss in the processed chicken meat industry (Holownia, Chinnan, and Reynolds, 2004).

Red spots could be related to the blood remaining in blood vessels after slaughtering. Factors affecting RBS formation have been studied, including the slaughter process, bleeding time and stunning methods (Veerkamp, 1988; Gregory and Wilkins, 1989; Contreras and Beraquet, 2001; Ahmed, 2003; Ali, Lawson, Tauson, Jensen, and Chwalibog, 2007). Electrical stunning at 45 mA has been reported to minimize RBSs (Ahmed, 2003). This was because when low current levels are used during stunning, cardiac pumping activity is slowly reduced, resulting in continued bleeding after slaughtering (Gregory and Wilkins, 1989). However, this method cannot be commercially practiced due to animal welfare regulations. A minimum stunning current of 100 mA per bird and a frequency <200 Hz is required by EU regulations (The Council of the European Union, 2009). A high current level during

stunning could induce sudden cardiac arrest, leading to more blood remaining in the vessels after death (Gregory and Wilkins, 1989). In addition, stunning is prohibited by Halal regulations (The Central Islamic Council, 2016). When complete bleeding is not achieved, blood is likely to remain in vessels, causing RBS when the heating process is insufficient.

Marination is an important step in which ingredients, such as NaCl, sodium tripolyphosphate (STPP) and glucose, are commonly applied during vacuum tumbling (Khiari, Omana, Pietrasik, and Betti, 2013). These ingredients are used to tenderize the meat, increase the water holding capacity and enhance the flavor (Kijowski and Mast, 1988; Dhanda, Pegg, and Shand, 2003). Nevertheless, each ingredient has a different ability to diffuse into chicken breast meat, depending on its molecular size, water solubility, concentration and other factors. Li, Kerr, Toledo, and Carpenter (2000) reported that different phosphates showed different penetration rates in chicken breast. Tripolyphosphate and pyrophosphate, with smaller molecular sizes, diffused faster, and subsequently, they presented higher concentrations than did hexametaphosphate (Xiong and Kupski, 1999). Thus far, the diffusibility of NaCl and glucose into chicken breast has not been well established. The ingredients used during marination could also affect the thermal denaturation of hemoglobin (Hb), which would, in turn, impact the color of blood residues in vessels upon cooking. The native form of Hb is responsible for its red color, while the heat-induced denaturation of Hb results in a brown color, which is an indicator of fully cooked chicken meat (Suman et al., 2016). Jeong (2017) reported that 2% NaCl decreased the stability of the heme pigment, resulting in lower thermal denaturation temperatures. The thermal

denaturation of Hb affected by common ingredients in marination should be investigated to gain insight into their roles in RBS formation.

The objectives of this study were to investigate the effects of common ingredients used during chicken meat marination, namely, NaCl, STPP and glucose, and their concentrations on RBS formation and the thermal denaturation of chicken Hb .Additionally, the optimal cooking temperature for RBS reduction was determined.

3.3 Materials and methods

3.3.1 Ingredients and chemicals

NaCl was purchased from Pimai Salt Co., Ltd. (Nakhon Ratchasima, Thailand). Glucose powder was obtained from Kornthai Co., Ltd. (Ratchaburi, Thailand). STPP was purchased from Aditya Birla Chemicals Ltd. (Samutprakan, Thailand). Nitric acid (HNO_3) and hydrochloric acid (HCl) were ordered from Carlo Erba Reagents S.A.S. Inc. (Val-de-Reuil, France). Ethanol was purchased from Merck KGaA Inc. (Darmstadt, Germany). Dinitrosalicylic (DNS) acid, sodium citrate and Tris-maleate were purchased from Sigma-Aldrich Co. (St Louis, MO, USA).

3.3.2 Preparation of marinated chicken breast

Boneless, skinless chicken breast (*Pectoralis major*) (Ross male, 48±2 days old, 3.0±0.2 kg body weight) with sizes of 250-280 g per piece and maximum thicknesses of 4-5 cm were obtained from a chicken processing plant (Charoen Pokphand Foods PCL., Nakhon Ratchasima, Thailand). The samples were refrigerated at 4°C for about 15 hr after slaughtering. In each treatment, 5.0-5.5 kg of chicken breast meat was vacuum-tumbled at 80 kPa for 65 min at a temperature less than 8°C using a vacuum tumbler (DVTS-50, Davison's Butcher Supply, Los Angeles, CA,

USA). Three different solutions, including 5% NaCl, 3% glucose powder and 2% sodium tripolyphosphate (STPP), were separately applied at a ratio of 16.5% (w/w) of the chicken breast samples. The percentage of marinade uptake was calculated as follows:

$$\text{Marinated uptake (\%)} = \left[\frac{\text{Weight after marination} - \text{Weight before marination}}{\text{Weight before marination}}\right] \text{x } 100$$

After marination, the samples were stored at 4°C for 20 hr and randomly taken at various time intervals of 0, 2, 6, 12, 16, and 20 hr. The breast meat was dissected, and the center of the thickest part was cut to the size of 2 x 2 x 2 cm (Figure 3.1). The concentration of each ingredient at the center of the chicken breast was determined.

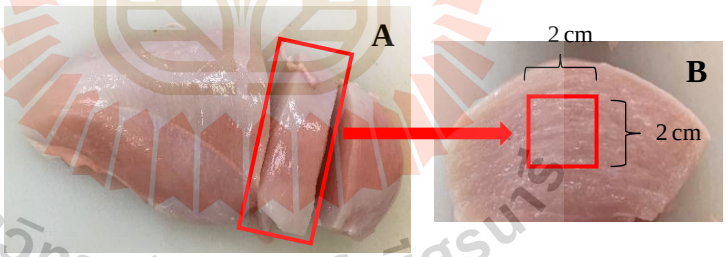


Figure 3.1 Cuts of (A) marinated chicken breast meat and (B) the area of meat used for analyses.

3.3.3 Determination of sodium and phosphorus

Minced samples (300 mg) were digested with 5 mL of 65% HNO_3 and 1 mL of conc. HCl. Digestion was conducted using a microwave digestion oven (Multiwave 3000, Anton Paar, Ashland, VA, USA) at 1200W, 30 bar, and 200°C for

15 min, as described by Bou, Guardiola, Padro, Pelfortb, and Codonya (2004). The volumes of the digested samples were adjusted to 50 mL with deionized water. Sodium and phosphorus were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 8000, Perkin-Elmer, Waltham, USA). The instrument was operated under a radio frequency power of 1300W, a plasma flow of 15 L/min, a nebulizer gas flow of 0.55 L/min, an auxiliary gas flow of 0.2 L/min and a pump rate of 1.5 mL/min. Sodium and phosphorus were determined at wavelengths of 589.592 nm and 213.617 nm using the axial mode of the plasma viewing position, respectively. ICP standards of sodium and phosphorus (SCP Science, Québec, Canada) were used.

3.3.4 Determination of reducing sugar

Reducing sugar was determined according to Jayasena et al., (2014). A minced sample (1 g) was mixed with 5 mL of hot 80% ethanol (50°C). The extracted samples were centrifuged at 2,000 x g (Sorvall Legend MACH 1.6R, Thermo Electron LED GmbH, Lengensellbold, Germany) for 10 min at 4°C. The extraction was repeated twice. The supernatants were filtered through a Whatman filter paper no.1 and evaporated using N_2 gas (99.999%). Dried residues were dissolved in 2 mL distilled water and centrifuged at 10,000 x g for 10 min. The reducing sugar content was measured by a dinitrosalicylic (DNS) acid method. Each sample was mixed with 1 mL of DNS solution and heated in a 90°C water bath for 5 min. The mixtures were cooled, and the absorbance was measured at 550 nm using a spectrophotometer (Jenway, Bibby Scientific Ltd., Staffordshire, UK). The amount of reducing sugar was calculated using a series of glucose standards and expressed as mg/100g meat sample.

3.3.5 Isolation of Hb from chicken blood

Chicken blood was collected from 48±2 days old of broiler chickens at the processing line of a commercial chicken slaughterhouse (Charoen Pokphand Foods PCL.). Blood samples were immediately mixed with sodium citrate to attain a final concentration of 3.8% to prevent clotting. Isolation of Hb from the blood samples was performed according to Lueangsakulthai et al., (2017). Whole blood was centrifuged at 2,500 x g (Thermo Electron LED GmbH) for 15 min at 4°C to remove plasma and leukocytes. The red blood cells were collected, 5 volumes of deionized water were added, and the sample was mixed vigorously for 5 min. The hemolysate was centrifuged at 4,500 x g for 40 min at 4°C. The middle layer containing hemoglobin (Hb) was collected and lyophilized (Lyovac GT2-S, GEA Lyophil GmbH, Huerth, Germany). The dried samples were then kept at 5°C for further analyses. The purity of Hb was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with a 4% stacking gel and 12.5% running gel according to Laemmli (1970).

3.3.6 Thermal denaturation of Hb

To the lyophilized Hb was added 50 mM Tris-maleate buffer (pH 7) containing varied concentrations of NaCl (500, 1000 and 1500 mM, pH 7), glucose (150, 350 and 600 mM, pH 7) and STPP (10, 55 and 100 mM) at pH 7 and pH 9. All samples contained 20 mg/mL protein .The effect of the combined ingredients, namely, 1 M NaCl, 350 mM glucose and 55 mM STPP at pH 7 and pH 9, was also investigated. The samples were analyzed by microdifferential scanning calorimetry (Micro DSC7 evo, Setaram Instrumentation, Caluire, France). Samples (250 µL) were placed in a stainless-steel crucible. Tris-maleate buffe (pH 7) was used as a reference. The

samples were heated from 20 to 90ºC at a heating rate of 1ºC/min. The denaturation temperatures and enthalpy values were determined in triplicate.

3.3.7 Effect of the heating temperature on RBS

Marinated chicken breast samples were prepared as previously described with a combination of 3 common ingredients (5% NaCl, 3% glucose powder and 2% STPP). Chicken breast without marination was used as a control. Samples were packed in a vacuum bag (1 piece/bag) and heated in a water bath until the core temperature reached 50, 70, 80 and 85ºC with a holding time of 1 min and until the core temperature reached 85ºC with a holding time of 5 min. The core temperature was measured using a thermocouple type K (54 II, 80PT-5A, Fluke Corp., Moorpark, CA, USA). Twenty samples were prepared at each studied core temperature. Three different lots of chicken were prepared. All heated samples were transversely cut. RBSs were visually inspected which the color of blood spot was evaluated by color chart. Moreover, the cooking loss at each core temperature was determined and calculated as follows:

$$\text{Cooking loss (\%)} = \frac{(\text{Weight before cooking} - \text{Weight after cooking}) \times 100}{\text{Weight before cooking}}$$

3.3.8 Microscopy

Changes of the blood in the cooked marinated chicken breast samples were investigated using a microscopic technique. Samples were cut at blood spot areas to an approximate size of 1.5 x 1.5 cm^2. The cut samples were placed on specimen chucks and then embedded using OCT® (Bio-optica, Milano, Italy). A cryostat microtome (AST500, AMOS Scientific, Clayton South Victoria, Australia) was used to cut the samples to 8-µm thick. Each sample was attached to glass slide and stained

with hematoxylin and eosin (H&E) following the standard protocols (Cardiff, Miller, and Munn, 2014). Blood residues remaining in the vessels were observed under a light microscope with a 40X objective and a 10X eye-piece lens (SMZ-U, Nikon Corp., Tokyo, Japan).

3.3.9 Statistical analysis

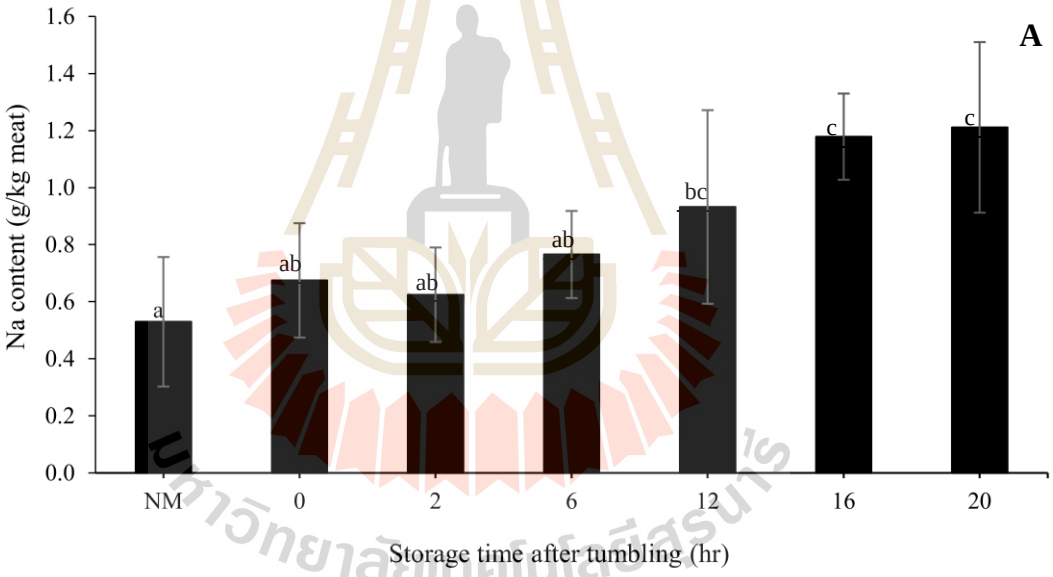
Three independent lots of chicken breast samples were prepared. Mean values ± standard deviations were reported. An analysis of variance (ANOVA) was conducted to determine the effect of the ingredients on the diffusibility and the thermal denaturation of Hb using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). A comparison of the means was performed using Duncan's test with $P<0.05$.

3.4 Results and discussion

3.4.1 Diffusibility of ingredients

The sodium content at the center of the thickest part of the marinated chicken breast increased with the storage time until 12 hr and remained stable afterward ($P<0.05$, Figure 3.2A). In contrast, the phosphate and glucose contents did not change throughout the storage time ($P>0.05$, Figure 3.2B, C). The penetration of phosphate and glucose into the chicken breast meat appeared to be lower than that of Na^+. The structure of chicken breast muscle is disrupted during vacuum tumbling, as it undergoes repeated squeezing and relaxing, allowing the marinade ingredients to penetrate into the meat (Lee, Youm, Owens, and Meullenet, 2011; Gao et al., 2015). The percentage of marinade uptake ranged from 6 to 10%. Storage after tumbling allowed more solubilization of myosin and simultaneous changes in the microstructure of the meat matrix (Andersen, Andersen, and Bertram, 2007; Chupaj, Malila, Petracci,

Benjakul, and Visessanguan, 2016). This resulted in more sodium penetration into the center of chicken meat. However, larger-molecular weight (MW) compounds like phosphate (~370 g/mol) and glucose (~180 g/mol) showed limited diffusion (Xiong and Kupski, 1999; Graiver, Pinotti, Califano, and Zaritzky, 2006). Our studies indicated that NaCl is the main ingredient that can diffuse to the center of chicken breast, and it could thus have a greater effect on the denaturation of myoglobin and Hb, if any, in blood vessels than could phosphate and glucose, which are rarely absorbed into the center of the breast muscle.



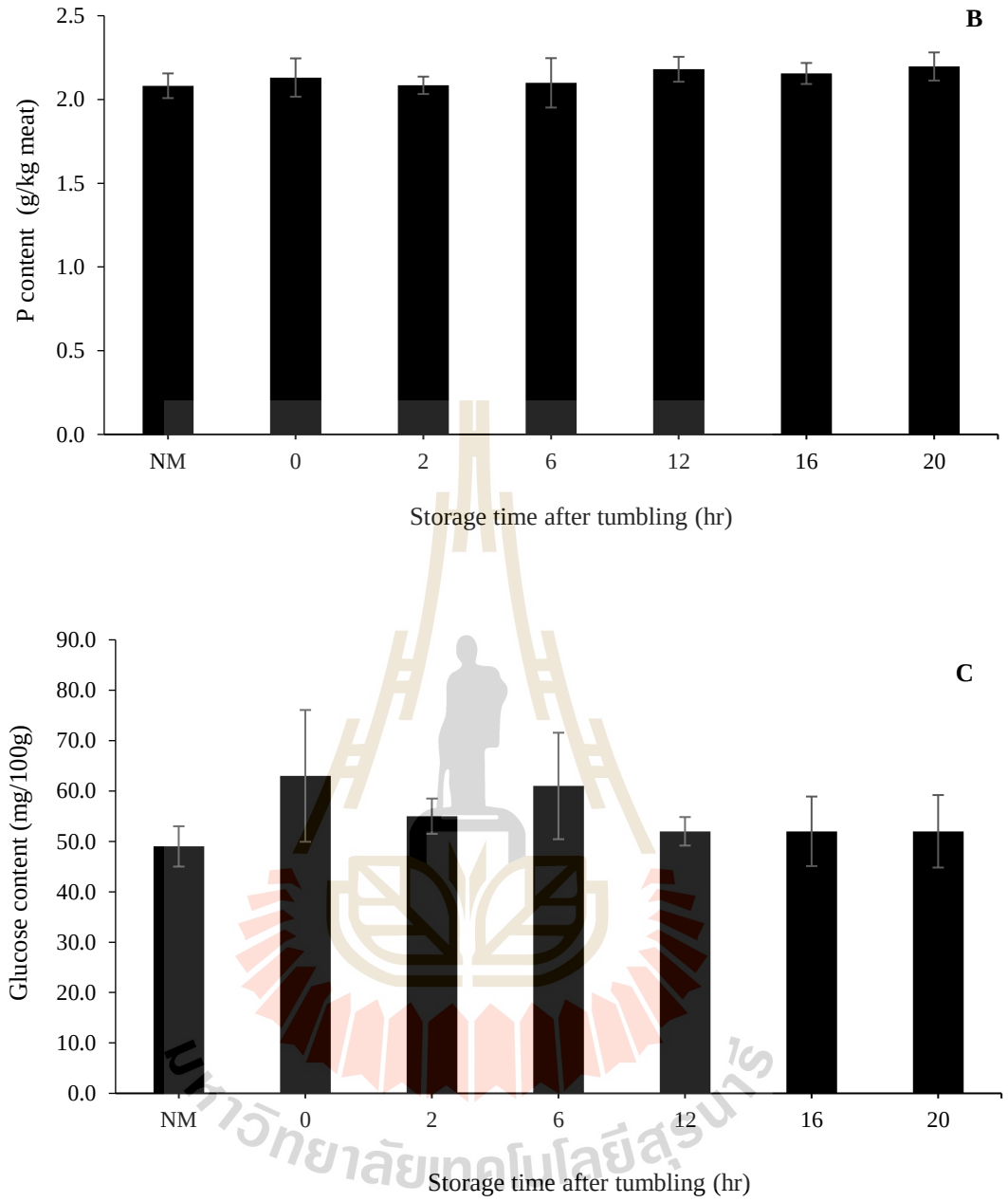


Figure 3.2 Changes of ingredient contents at the center of marinated chicken breast meat at various storage times after vacuum tumbling: (A) sodium, (B) phosphorus, and (C) glucose contents. Different letters indicate significant differences ($P<0.05$). [NM denotes non-marinated samples].

3.4.2 Effects of ingredients on thermal denaturation of chicken Hb

Isolated chicken Hb had a molecular weight (MW) of about 15.6 kDa with a purity of 93% (Figure 3.3). Hb is a tetrameric protein (MW ~ 64.5 kDa) with 4 subunits (Shi et al., 2015). The MW of the monomer of poultry Hb ranged from 12.5 to 16.3 kDa (Ahn and Maurer, 1989; Kranen et al., 1999; Abbasi and Lutfullah, 2002). The T_d of Hb in the presence of NaCl decreased from 67 to 65.8ºC as the NaCl concentration increased from 0 to 1.5 M ($P<0.05$, Table 3.1), indicating a destabilizing effect of NaCl. NaCl decreases interactions between aqueous solvents and Hb through preferential hydration, disrupting hydrophobic interactions and unfolding of the Hb structure (Mao, Sheng, and Pan, 2007). Ahn and Maurer (1989) also reported that 2.5% NaCl decreased the T_d of turkey Hb to 68ºC. In addition, Hb mixed with a low concentration of STPP (10 mM) at pH 7 showed a higher thermal stability up to 72.5ºC (Table 3.1). Phosphate ions increase the solvent surface tension and decrease the solubility of nonpolar molecules, leading to an increase in hydrophobic interactions and higher thermal stability (Lindman et al., 2006; Moller et al., 2012). However, the typical pH of cooked chicken marinated with STPP is around pH 9, which is slightly higher than that of the studied model. At pH 9, the structure of Hb appeared to be further destabilized as the concentration of phosphate increased ($P<0.05$, Table 3.1). This also corresponded with a decrease in enthalpy values (ΔH, Table 3.1). At pH 9, muscle proteins become negatively charged, which promotes intramolecular repulsions and protein unfolding, resulting in a lower T_d and ΔH. The T_d values of Hb with glucose added increased with the glucose concentration concomitantly with an increase in ΔH ($P<0.05$, Table 3.1). Glucose is known to decrease the hydrated radius of protein and enhance hydrogen bonds with water,

resulting in an increase in thermal stability (Alfonso et al., 2007; Oshima and Kinoshita, 2013). Hb with combined ingredients added at either pH 7 or pH 9 showed less thermal stability compared to the control ($P<0.05$, Table 3.1). The destabilizing effect of mixed ingredients indicated that Hb could undergo denaturation at lower temperatures. In the meat system, NaCl is the only main ingredient that penetrates into the chicken muscle, with an estimated NaCl content of 3.1 g/kg meat, which is equivalent to about 66 mM NaCl based on an 80% moisture content. This concentration was far lower than the minimum NaCl concentration tested in the Hb model. Therefore, it is reasonable to presume that denaturation of Hb at the center of the meat is likely to occur at temperatures greater than 69ºC, which is the T_d of the control Hb. In contrast, Hb at the meat surface exposed to marinade at pH 9 would undergo denaturation at the lower temperature of 65.8ºC (Table 3.1).

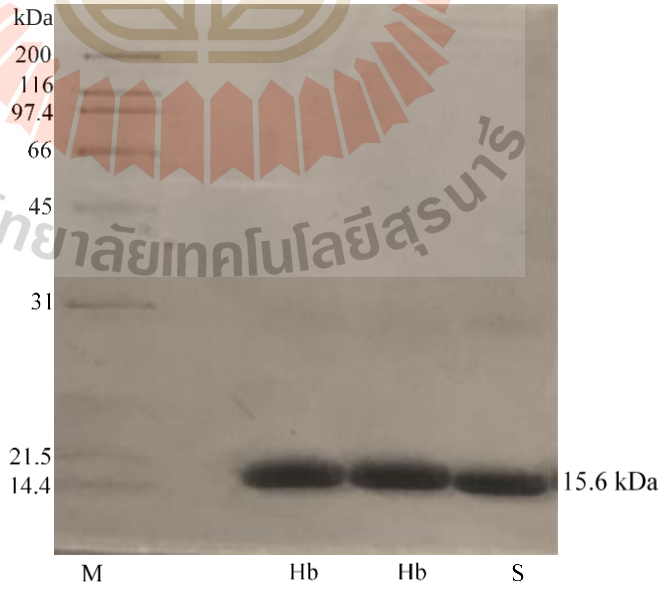


Figure 3.3 SDS-PAGE pattern of chicken hemoglobin (Hb) on a 12.5% polyacrylamide gel. (M) Marker, and (S) standard bovine Hb.

Table 3.1 Effect of the marinade ingredients on the thermal denaturation (T_d) and enthalpy (ΔH) of chicken Hb.

Ingredients	**Concentration (mM)**	**T_d (ºC)**	**ΔH, Enthalpy of denaturation, (J/g)**
Control	-	69.4±0.35^{e}	15.21±0.32^{h}
NaCl	500	67.0±0.25^{cd}	11.51±0.17^{de}
	1000	66.3±0.51^{bc}	9.78±0.10^{c}
	1500	65.8±0.74^{b}	7.82±0.18^{a}
STPP (pH~7)	10	72.5±0.31^{g}	16.57±0.24^{k}
	55	69.4±0.14^{e}	12.09±0.21^{fg}
	100	69.5±0.39^{e}	11.88±0.53^{ef}
STPP (pH~9)	10	66.1±0.12^{bc}	12.50±0.40^{g}
	55	61.9±0.77^{a}	11.21±0.20^{d}
	100	61.4±1.09^{a}	8.53±0.60^{b}
Glucose	150	69.6±0.38^{e}	15.82±0.12^{i}
	350	70.0±0.28^{ef}	16.08±0.15^{i}
	600	70.6±0.69^{f}	18.07±0.04^{l}
Hb (pH~7) in mixed ingredients	1000 (NaCl) 55 (STPP) 350 (Glucose)	67.9±0.38^{d}	10.21±0.19^{c}
Hb (pH~9) in mixed ingredients	1000 (NaCl) 55 (STPP) 350 (Glucose)	65.8±0.63^{b}	10.00±0.15^{c}

Different letters in the same column indicate significant differences ($P<0.05$).

3.4.3 RBS formation and cooking loss

RBS formation was noticeable in all samples heated to core temperatures of 50 and 70ºC for 1 min (Table 3.2). The incidence of RBS decreased to 1.7% and 3.3% when the core temperature increased to 80ºC in non-marinated and marinated samples, respectively. Marinated samples appeared to have a higher

incidence of RBSs compared to the non-marinated counterparts. Water uptake was greater in the marinated samples, resulting in a higher moisture content and energy of heat conduction (Alvarado and Mckee, 2007). Although the T_d of purified chicken Hb in the model system was around 69ºC, RBSs were still observed, even after heating to 80ºC. No evidence of RBSs was found at 85ºC with a holding time of either 1 or 5 min in both non-marinated and marinated samples (Table 3.2). Thus, heating to a core temperature of 85ºC could overcome the RBS problem.

Cooking loss is one of the important factors that affects the yield of cooked chicken products. The cooking loss of marinated chicken breast was lower than that of the non-marinated counterparts. NaCl and/or phosphate induce unfolding of myofibrillar proteins, which subsequently increases the space between filaments to retain more water (Smith and Young, 2007; Khan et al., 2016). Cooking loss tended to increase with the cooking temperature ($P<0.05$), but core temperatures of 80 and 85ºC produced comparable cooking losses ($P>0.05$, Figure 3.4). These results were in agreement with Bae et al., (2018), who reported that the cooking yields of chicken breast products containing 1-2% NaCl were similar at 80 and 85ºC ($P>0.05$). Previous studies reported that the cooking loss in marinated meat ranged from 24 to 30% (Barbantia and Pasquini, 2005; Pérez-Juan, Kondjoyan, Picouet, and Realini, 2012; Chupaj et al., 2016; Gamage, Mutucumarana, and Andrew, 2017). Our results demonstrated that heating chicken breast until the core temperature reaches 85ºC for 1 min could completely eliminate RBS formation without a significant loss in yield.

3.4.4 Microscopic analyses

The microstructure of the blood retained in the blood vessels within *Pectoris major* was investigated. Red blood cells (RBCs) of raw chicken breast

were observed as having a round shape and being distributed within the blood vessel (Figure 3.5A), while coagulation took place in samples cooked to a core temperature of 50ºC (Figure 3.5B). When the core temperature reached 85ºC, RBCs appeared to be brown, and extensive aggregation was clearly visualized (Figure 3.5C). Heat-induced denaturation of globin leads to unfolding and the formation of ferrihemochrome, which is responsible for a brown color (Suman et al., 2016). Some RBCs existed in native form with red color at 50ºC. When thermal denaturation completely occurred at 85ºC, brown-colored RBCs were observed, corresponding to the disappearance of RBSs. Microscopic data confirmed that RBSs are caused by the incomplete denaturation of the blood remaining in the vessels. Thus, sufficient heating leading to the complete thermal denaturation of Hb can eliminate RBS incidence.

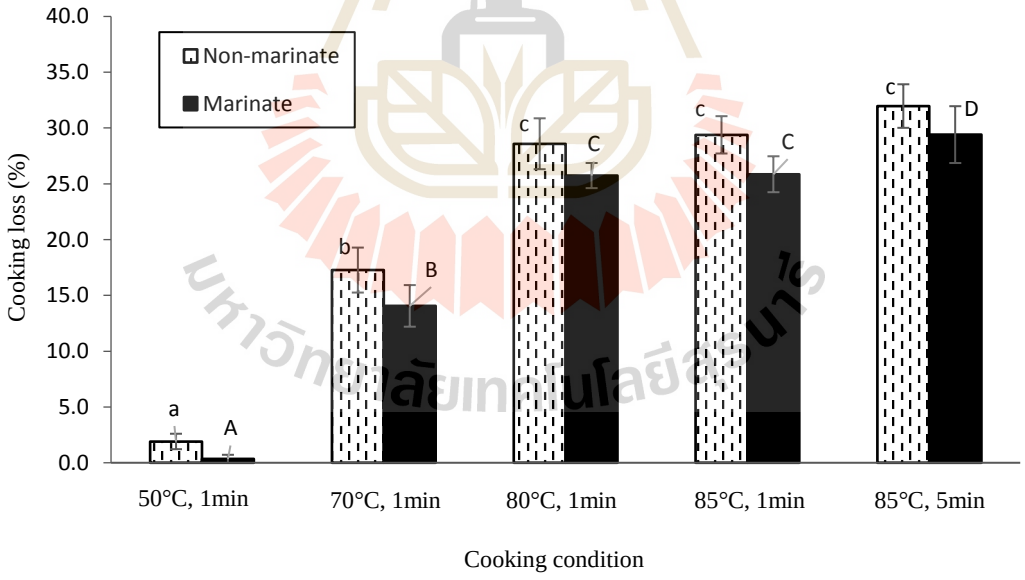
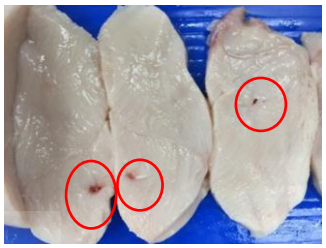
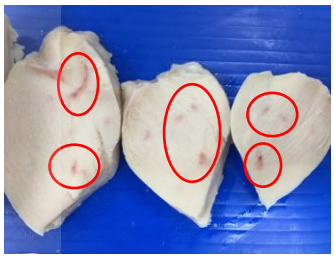


Figure 3.4 Effect of the core temperature on the cooking loss of marinated and non-marinated chicken breast samples. Different lowercase and uppercase letters indicate significant differences within non-marinated and marinated samples, respectively ($P<0.05$)

Table 3.2 Red blood spot (RBS) formation at varied core temperatures (n=60)

Core temperature, holding time (min)	**Type**	**RBS formation (%)**	**Representative images**
50°C, 1 min	Non-marinated	100	
	Marinated	100	
70°C, 1 min	Non-marinated	100	
	Marinated	100	
80°C, 1 min	Non-marinated	1.7	
	Marinated	3.3	
85°C, 1 min	Non-marinated	0	
	Marinated	0	
85°C, 5 min	Non-marinated	0	
	Marinated	0	

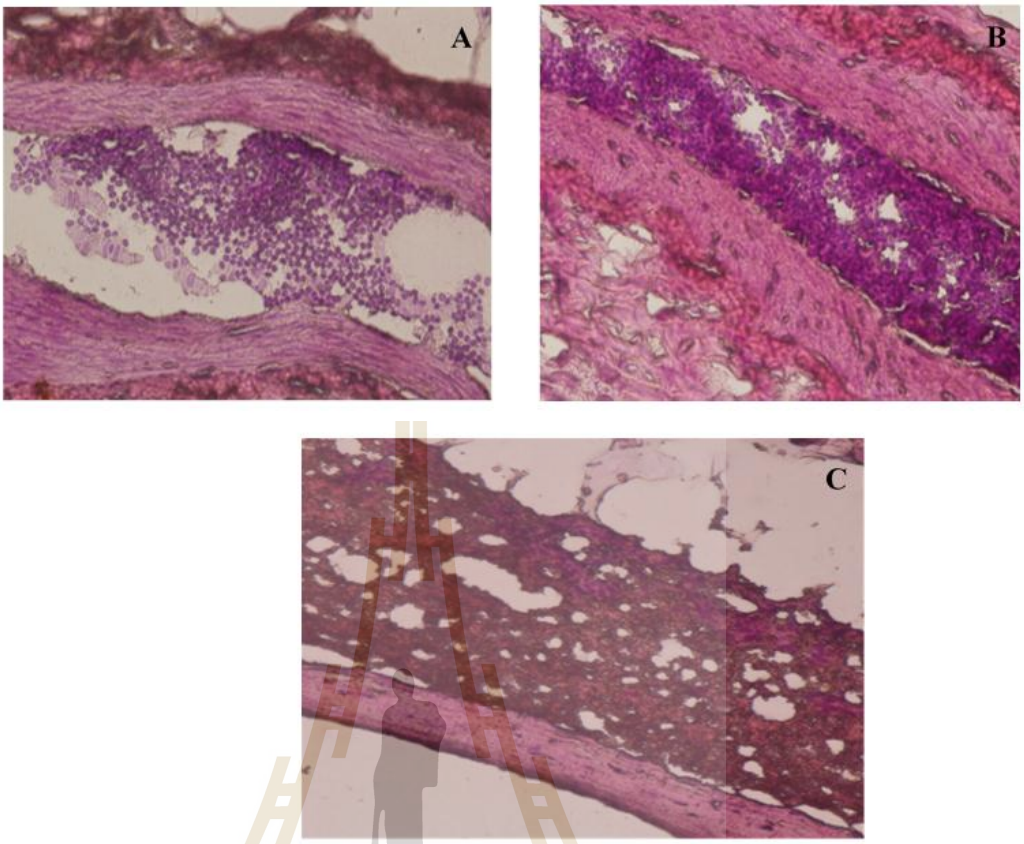


Figure 3.5 Red blood cells of blood remaining in the vessels of marinated chicken breast meat cooked under various conditions: (A) raw meat, (B) meat cooked to a core temperature of 50ºC for 1 min, and (C) meat cooked to a core temperature of 85ºC for 5 min.

3.5 Conclusions

Among the marinade ingredients studied, NaCl showed the greatest absorption after tumbling. NaCl alone and STPP at alkaline pH destabilized the Hb structure, resulting in lower T_d values. Glucose alone increased the thermal stability of Hb. However, the combined ingredients destabilized the Hb structure, resulting in a lower T_d. RBS was mainly caused by the incomplete denaturation of Hb in the blood vessels.

Marinated chicken breast should be cooked to reach a core temperature of 85ºC for at least 1 min to completely eliminate RBSs.

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CHAPTER IV

DIELECTRIC PROPERTIES OF MARINATED CHICKEN BREAST MEAT

4.1 Abstract

The objective of this study was to elucidate the effect of sodium chloride (NaCl), sodium tripolyphosphate (STPP) and glucose in marinated chicken breast meat on dielectric properties at microwave (MW) frequencies of 915 and 2,450 MHz at various internal temperatures. Dielectric constant (ε') values were lowest in chicken breast tumbled with marinade composing of 5% NaCl, 3% glucose and 2% STPP at both frequencies, while its dielectric loss factor (ε'') values were highest. The ε' values were reduced at temperatures above 40°C. Chicken breast tumbled with NaCl and mixed ingredients showed the highest increase of ε'' values with temperature at 915 MHz, while a lesser extent was observed at 2,450 MHz. Penetration depth (d_p) values of marinated chicken breast were 1.5-3.9 cm at 915 MHz and 1.1-1.7 cm at 2,450 MHz. NaCl resulted in a decrease in d_p values, which could lead to a non-uniform heating by MW.

Key words: Microwave heating, Dielectric constant, Dielectric loss factor, Penetration depth, Marinated chicken breast

4.2 Introduction

Chicken breast meat is one of significant protein sources. A red blood spot (RBS) is one of common defects found in cooked chicken breast products (Smith and Northcutt, 2003). This is caused by incomplete denaturation of blood remained in vessels (Friesen and Marcy, 2000). Conventional cooking methods applied in chicken meat processing typically transfer heat from heating medium to meat by convection or radiation and conduction (Rincon, Singh, and Stelzleni, 2015). Limited heat transfer results in undercooked meat. Dielectric heating, particularly microwave (MW) heating, exhibits different heat transfer. Heat is generated internally by dipole rotation and ionic conductive (Awuah, Ramaswamy, and Tang, 2015). This could reduce RBS which is normally found at internal chicken breast meat.

MW heating applies in industry at frequency of 300 MHz to 3 GHz (Piyasena, Dussault, Koutchma, Ramaswamy, and Awuah, 2003; Kirmaci and Singh, 2012). MW frequencies of 915 and 2,450 MHz are allocated for use in Industrial, Scientific, and Medical (ISM) heating applications according to international agreement (Wang, Wig, Tang, and Hallberg, 2003). The efficiency of MW heating is governed by the dielectric properties of the foods (Samuel and Trabelsi, 2012). Dielectric properties include dielectric constant (ε') and dielectric loss factor (ε''). The ε' is related to the capacity for electrical energy storage in the material (Zhuang, Nelson, Trabelsi, and Savage, 2007), while ε'' is associated with the electrical energy dissipation in the material and conversion to heat (Awuah et al., 2015). Changes of dielectric properties of food materials are varied with various factors, including, ingredients, temperature, frequency and moisture content (Lemos, Nunes, and Viana, 1999). To achieve high efficiency of MW heating, dielectric properties of food must be elucidated.

Ingredients absorbed into meat have the effect on dielectric properties. Lyng, Zhang, and Brunton (2005) reported that beef muscle blends with salt had highest ε'' values and ε'' values decreased when blended with starch. Ingredients commonly used in marinade of chicken breast meat included NaCl, glucose and sodium tripolyphosphate (STPP) (Khiari, Omana, Pietrasik, and Betti, 2013). NaCl is used to improve flavor and tenderize meat. Glucose provides sweet taste and brown color. STPP is added to increase water holding capacity and cook yield (Dhanda, Pegg, and Shand, 2003). These ingredients would affect dielectric properties of chicken breast to varied extent. Moreover, dielectric properties changes with temperature. Zhuang et al., (2007) reported that ε' of chicken breast meat decreased as the temperature increased. Tanaka, Mallikarjunan, Kim, and Hung (2000) described that ε'' in salted meat increased with temperature. Therefore, it is critical to understand changes of dielectric properties of chicken breast meat as affected by these factors in order to effectively design microwave heating process.

The objective of this study was to determine dielectric properties at MW frequencies (915 and 2,450 MHz) of chicken breast meat marinated with different ingredients at various temperatures.

4.3 Materials and methods

4.3.1 Ingredients and chemicals

NaCl was purchased from Pimai Salt Co., Ltd. (Nakhon Ratchasima, Thailand). Glucose powder were obtained from Kornthai Co., Ltd. (Ratchaburi, Thailand). STPP were purchased from Aditya Birla Chemicals Ltd. (Samutprakan, Thailand). Nitric acid (HNO_3) and hydrochloric acid (HCl) were ordered from Carlo

Erba Reagents S.A.S. Inc. (Val-de-Reuil, France). Ethanol was purchased from Merck KGaA Inc. (Darmstadt, Germany). Dinitrosalicylic (DNS) acid, sodium citrate and Tris-maleate were purchased from Sigma-Aldrich Co. (St Louis, MO, USA).

4.3.2 Preparation of marinated chicken breast meat

Boneless skinless chicken breast (*Pectoralis major*) (Ross male, 48±2 days old, live body weight of 3.0±0.2 kg) were obtained from a commercial chicken processing plant (Charoen Pokphand Foods PCL., Nakhon Ratchasima, Thailand) with size of 250-280 g per piece and maximum thickness of 4-5 cm. Samples were refrigerated at 4°C for about 24 hr after slaughtering. Four marinade solutions were 5% NaCl, 3% glucose, 2% sodium tripolyphosphate (STPP) and mixed ingredients of 5% NaCl, 3% glucose and 2% STPP, representing a commercial formula. Chicken breast samples (5-6 kg) were added 16.5% (w/w) marinade and tumbled at temperature less than 8°C for 65 min by a vacuum tumbler (DVTS-50, Davison's Butcher Supply, Los Angeles, CA, USA). Consequently, a 35-mm diameter cork borer was placed at the maximum thickness of marinated chicken to obtain a cut sample with height of 30 mm. The cut sample was trimmed using a scalpel to provide smooth surface and put into the stainless steel (SUS304) sample holder. Dielectric properties of marinated chicken breast samples were determined after 20 hr of tumbling. Five samples were determined in each treatment. Two independent lots of chicken were used for vacuum tumbling.

4.3.3 Temperature control

Dielectric properties of each sample were measured at 4, 20, 40, 60 and 85°C. The filled sample holder with a closing lid was placed in a refrigerated circulating bath (CBN 8-30, Heto-Holten, Allerød, Denmark) at 4 or 20°C to reach equilibrium for 2 min before measurement. For higher temperatures, the filled sample holder was heated

in a 90°C water bath until internal temperature reached the set temperatures. Subsequently, the sample holder was immediately placed to an oil bath set at the set temperature. Dielectric properties were measured when sample was equilibrated for 2 min. The lid had an opening designed to insert a dielectric probe (Figure 4.1). The K-type thermocouple probe connected to a handheld digital thermometer (54 II, 80PT-5A, Fluke, Moorpark, CA, USA) was inserted into the opening on the lid for monitoring internal temperature.

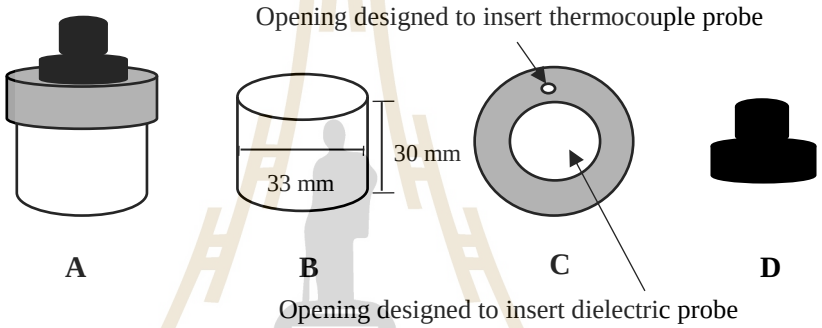

Figure 4.1 Illustration of (A) sample holder, (B) stainless steel cylinder, (C) lid, (D) covered lid during heating.

4.3.4 Dielectric properties measurement

The measurement was initiated after the sample reached the set temperature using an open-ended coaxial probe (N1501A, Agilent Technologies, Santa Clara, CA, USA) connected to a Fieldfox Keysight network analyzer (Model No. E5063A, Agilent Technologies, Santa Clara, CA, USA) as shown in Figure 4.2. All samples were measured at frequency ranging from 0.3 to 3.0 GHz. A probe was pressed firmly against the sample surface in the perpendicular direction to muscle fibers. The values of ε' and ε'' were calculated using the 85070 software package (Version

E07.01.08, Agilent Technologies, Santa Clara, CA, USA). The open-ended coaxial probe was calibrated prior to measurement using air, short-circuit block and deionized water at 25°C. Penetration depth (d_p) was calculated using the equation (Buffler, 1993);

$$d_p = \frac{c}{2\pi f \sqrt{2\varepsilon_r' \left[\sqrt{1 + (\varepsilon_r''/\varepsilon_r')^2} - 1\right]}} = \frac{c}{2\pi f \sqrt{2\varepsilon_r' \left[\sqrt{1 + \tan^2 \delta} - 1\right]}}$$

Where c is the speed of the propagation of waves in a vacuum (3 x 10^8 m /s), f is the frequency (Hz), and d_p is the penetration depth (m).

Moisture content of marinated chicken breast samples were determined according to AOAC (2010).

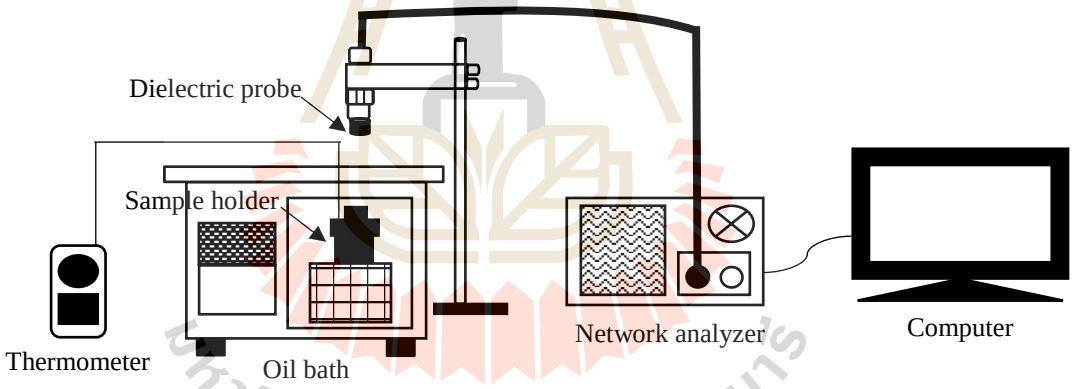

Figure 4.2 Schematic diagram of apparatus for dielectric properties measurement.

4.3.5 Determination of sodium and phosphorus

Samples from the center of marinated chicken breast was cut and minced manually. Mince (300 mg) was digested with 5 mL of 65% HNO_3 and 1 mL of 37% HCl using a microwave digestion oven (Multiwave 3000, Anton Paar, Ashland, VA, USA) at 1200W, 30 bar, and 200°C for 15 min as described by Bou, Guardiola, Padro,

Pelfortb, and Codonya (2004). The volume of digested samples was adjusted to 50 mL with deionized water. Sodium and phosphorus were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 8000, Perkin-Elmer, Waltham, Massachusetts, USA). The instrument was operated under radio frequency power of 1300W, plasma gas flow of 15 L/min, nebuliser gas flow of 0.55 L/min, auxillary gas flow of 0.2 L/min and pump rate of 1.5 mL/min. The concentration of sodium and phosphorus was determined at wavelengths of 589.592 nm and 213.617 nm with axial mode of plasma viewing position, respectively. ICP standard of sodium and phosphorus (SCP Science, Québec, Canada) was used.

4.3.6 Determination of reducing sugar

Reducing sugar was determined according to Jayasena et al., (2014). Meat at the center of marinated samples (about 1 g) was collected and mixed with 5 mL of hot 80% ethanol (50°C). Extraction was repeated twice. The extracted samples were centrifuged at 2,000 x g (Sorvall Legend MACH 1.6R, Thermo Electron LED GmbH, Lengensellbold, Germany) for 10 min at 4°C. Supernatants were filtered through Whatman filter paper no.1 and evaporated using N_2 gas (99.999%). Dried sugar was dissolved with 2 mL distilled water and centrifuged at 10,000 x g for 10 min. The reducing sugar content was measured by a dinitrosalicylic (DNS) acid method. Each sample was mixed with 1 mL of DNS solution and heated in a water bath (90°C) for 5 min. The mixtures were cooled and measured at 550 nm using a spectrophotometer (Jenway, Bibby Scientific Ltd., UK). The amount of reducing sugar in each sample was calculated using a series of glucose standard.

4.3.7 Statistical analysis

The measurement of dielectric properties and chemical analyses was carried out with at least five replicates per tumbling lot. Two lots of tumbling were prepared using 2 different lots of chicken. Analysis of variance (ANOVA) was conducted to determine the significant difference of the effects of marinade ingredients and temperature on dielectric constant and loss factor using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was accepted at $P<0.05$. Mean comparisons were carried out using Duncan's test. The mean values were expressed as mean ± standard deviation (SD).

4.4 Results and discussion

4.4.1 Effect of marinade ingredients on dielectric properties

Changes of dielectric constant (ε') and loss factor (ε'') of marinated chicken breast as a function of frequency are shown in Figure 4.3A, B. The ε' and ε'' values decreased with increasing frequency. At low frequency, polar molecules can realign themselves to the changes in the direction of electric field. When frequency continues to increase, the dipole motion can no longer keep up with the changing field (Icier and Baysal, 2004). As a result, dielectric properties decrease with frequency in this region. At 915 and 2,450 MHz, the ε' values of chicken breast tumbled with various marinade ingredients decreased in the order of STPP>NaCl>glucose>mixed ingredients (Figure 4.4A). This was mainly attributed to differences in moisture content. Chicken breast tumbled with STPP alone showed the highest moisture content (Table 4.1). An increase in electrostatic repulsive forces by phosphate create gaps between actin and myosin, leading to higher moisture retention (Nguyen-Huynh, Gál, and Buňka, 2011).

Water is a strong polar molecule that can absorb microwave energy, resulting in higher ε' values (Pongpichaiudom and Songsermpong, 2017). Chicken breast tumbled with mixed ingredients showed the lowest values of ε', corresponding to the lowest moisture content. This agreed with the result of Lopez, Schilling, Armstrong, Smith, and Corzo (2012) who reported that moisture content of chicken breast tumbled with mixture of NaCl and STPP decreased when NaCl concentration increased. Moreover, the addition of ionic ingredients in marinade decreased ε' values of chicken breast. The ε' values of mixed marinade ingredients were lower than non-marinated chicken breast despite of its higher moisture content ($P<0.05$). NaCl and STPP were absorbed in the meat and bound to free water molecules, leading to a decrease in water polarization and ε' value (Zhuang et al., 2007). This result was in agreement with the findings of Ling, Liu, Zhang, and Wang (2018) who stated that salt addition reduced free water content, leading to a decrease in ε' value as compared with non-salted products. These results indicated that the energy absorption of chicken breast greatly varies with marinade ingredients.

The ε'' values were higher in chicken breast tumbled with NaCl and mixed ingredients as compared with others at both MW frequencies ($P<0.05$, Figure 4.4B). This result was similar to the studies of Lyng et al., (2005) and Zhang, Lyng, Brunton, Morgan, and McKenna (2004). The addition of ionic ingredients tends to increase ε'' as a consequence of an increase in conductive charge carriers (Ryynanen, 1995). Sum of Na and P contents in chicken breast tumbled with mixed ingredients was 631 mg, which was higher than that of chicken breast tumbled with NaCl (496 mg) or STPP alone (369 mg) and non-marinated sample (269 mg) (Table 4.2). STPP was added only 2% in marination, thus it had little effect on Na and P content, resulting in

insignificant contribution to ε'' values. Chicken breast tumbled with glucose showed the lowest ε'' values. Glucose is an uncharged compound which could inhibit orientation polarization by the electromagnetic energy (Brinley, Truong, Coronel, Simunovic, and Sandeep, 2008). The ε'' value has a greater effect on heating rate as it governs the ability of materials to dissipate heat energy (Nelson, 2015). This implied that heating rate of MW heating at both 915 and 2,450 MHz increased with Na^+ in meat.

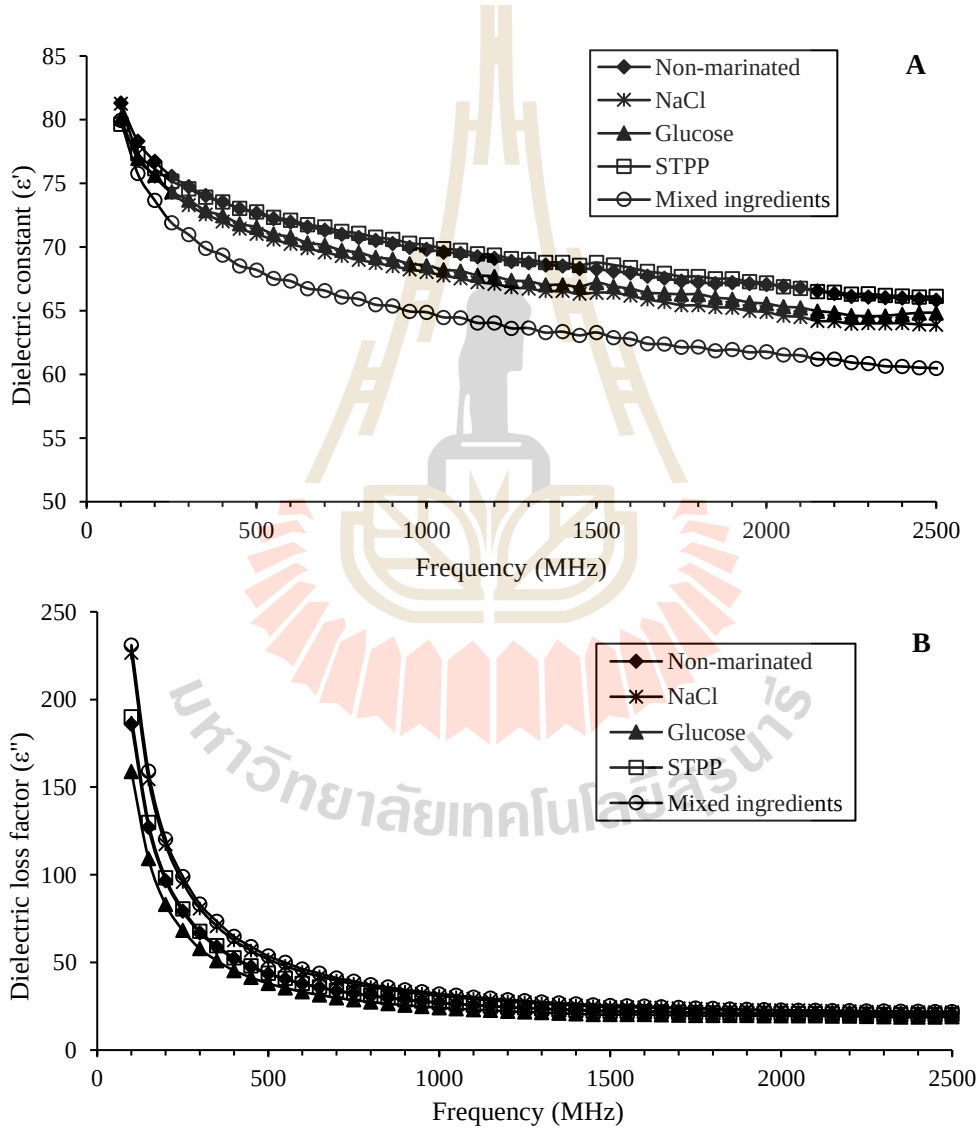

Figure 4.3 Dielectric properties of chicken breast tumbled with marinade ingredients at 20°C at 100-3,000 MHz. (A) Dielectric constant, ε' and (B) dielectric loss factor, ε''.

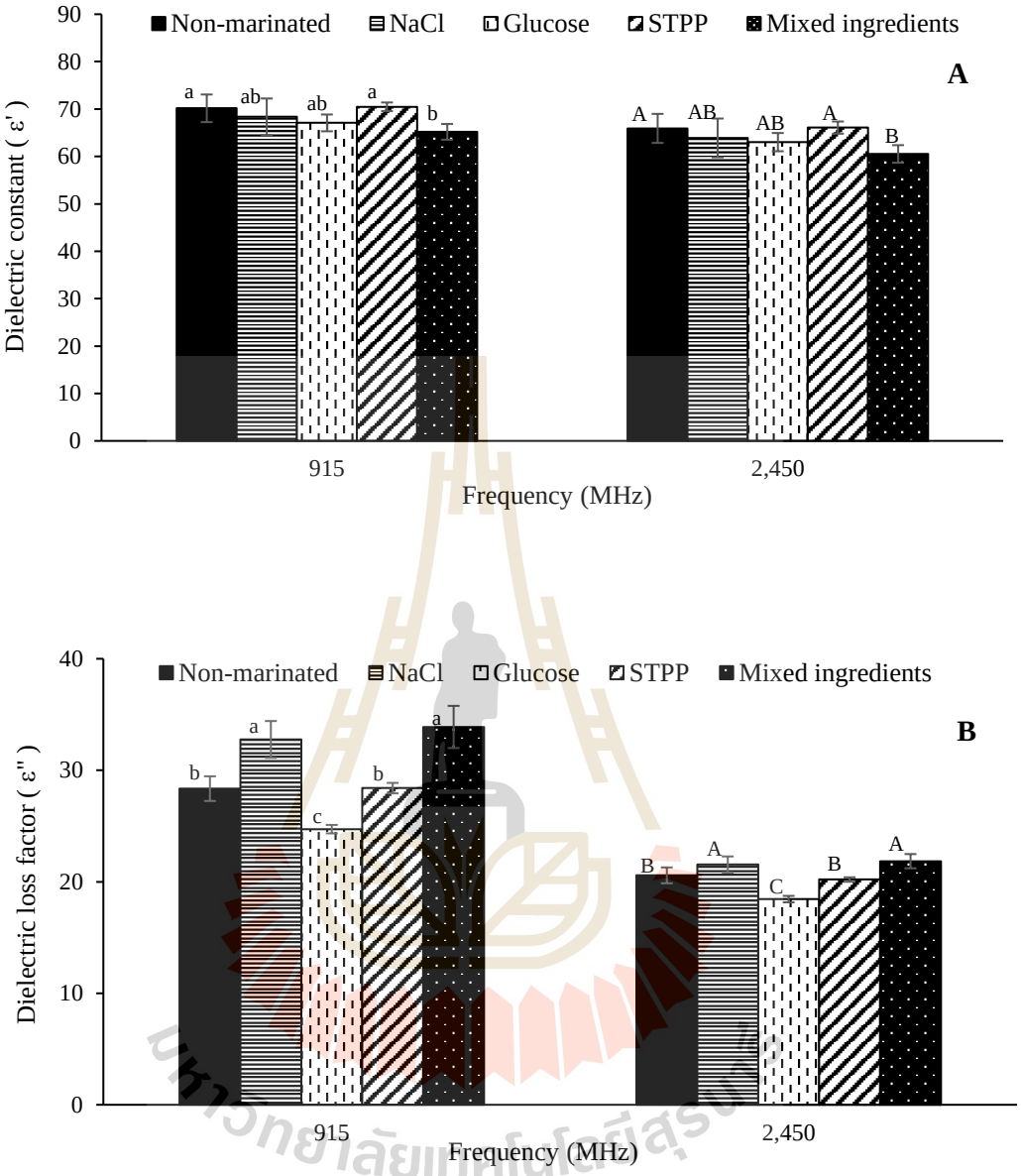

Figure 4.4 Dielectric properties of chicken breast tumbled with marinated ingredients at 20°C. (A) Dielectric constant, ε' and (B) dielectric loss factor, ε'' (mean ± standard deviation). Different letters are significantly different within same frequency ($P<0.05$).

Table 4. 1 Moisture content of chicken breast tumbled with different marinated ingredients (mean ± standard deviation).

Marinade ingredients	Moisture content (%)
Non-marinated	76.81±0.81[c]
NaCl	78.60±0.76[ab]
Glucose	78.11±0.75[ab]
STPP	79.00±0.89[a]
Mixed ingredients	77.95±0.75[b]

[a-c] means with different superscripts are significantly different (P<0.05).

Table 4.2 Contents of various ingredients in marinated chicken breast (mean ± standard deviation).

Marinade ingredients	Content (mg/100 g meat)		
	Na	P	Glucose
Non-marinated	57.39±9.40[d]	211.62±12.73[b]	46.28±3.50[b]
NaCl	305.64±42.78[b]	190.36±6.83[c]	ND
Glucose	ND	ND	101.87±7.28[a]
STPP	126.40±11.50[c]	242.42±12.44[a]	ND
Mixed ingredients	373.52±33.23[a]	257.61±7.41[a]	112.77±17.27[a]

[a-d] means with different superscripts in the same column are significantly different (P<0.05). ND means not determined.

4.4.2 Effect of temperature on dielectric properties

Changes of ε' value were subtle at 4-40°C at both 915 and 2,450 MHz (Figure 4.5). But, it drastically decreased when temperature was >40°C at both frequencies. Zhang et al., (2004) also demonstrated that ε' of pork meat batter (2.3% salt in final product) significantly decreased at temperature >45°C at frequency of 896 and 915 MHz. A decrease of ε' might be caused by the effect of temperature on protein denaturation. Myofibrillar proteins of whole chicken breast was transversely shrunk at 35-40°C, resulting in shrinkage of muscle structure and release of water (Tornberg, 2005). Water molecule plays a significant role on absorption of microwave energy. Therefore, release of water at higher temperatures leads to a decrease of ε'. At 4-40°C, non-marinated chicken breast and samples marinated with STPP showed higher ε' values than others. This was because they contained higher amount of free water in meat. The results suggested that ε' value changed with heating temperature. High temperature decreased MW energy absorption of marinated chicken breast meat.

The ε'' values also increased with temperatures at 915 MHz (Figure 4.6A). Changes in the ε'' values with respect to temperature were less pronounced than at 2,450 MHz (Figure 4.6B). An increase in temperature resulted in viscosity reduction and increased ion mobility, leading to high differential heat dissipation in meat sample during MW heating at lower frequency (Hebbar and Rastogi, 2012). Similar trend of ε'' was reported by Tanaka et al., (2000). Samples tumbled with NaCl and mixed ingredients showed the highest increase of ε'' values at both frequencies. While chicken breast tumbled with glucose exhibited the lowest ε'' values. Generally, the ε'' values of meat sample are varied by combined effects of dipole rotation (dipole loss component, ε''_D) and conductive charge migration (ionic loss component, ε''_σ) (Icier and Baysal,

2004). At the microwave frequency, dipole rotation decreases with temperature and ionic conductivity increases due to changes of meat structure and water binding to constituents in meat (Brinley et al., 2008). Therefore, ions in marinated chicken breast increased ε'' values. The ε'' values of chicken breast tumbled with NaCl and mixed ingredients were comparable at 4-60°C. The main effect is likely due to NaCl. Temperature of marinated chicken breast had an effect on ε'' values which would greatly affect heating rate during microwave heating. These results indicated that heating rate in marinated chicken breast increased during MW heating due to an increase in ε'' values. This is quite different from conventional heating whose heating rate decreased as temperature of sample increased to medium temperature.

4.4.3 Penetration depth (d_p)

The d_p values of marinated chicken breast meat ranged between 1.5-3.9 cm at 915 MHz and 1.1-1.7 cm at 2,450 MHz. Chicken marinated with glucose showed the highest d_p (Table 4.3). Samples marinated with mixed ingredients exhibited the smallest value of d_p. This was because the d_p value was adversely proportional to that of ε'' values as seen from the above equation. As ε'' increased at higher salt concentrations, d_p decreased (Zhang, Lyng, and Brunton, 2007). When d_p values were declined, the incident electromagnetic radiation would be absorbed near the surface, leading to a large temperature differential between outer and inner meat sample. This would eventually lead to non-uniform heating, which appeared to be pronounced in chicken breast marinated with NaCl. To assure uniform heating of marinated chicken breast sample, thickness of samples should be controlled in the range of d_p values. The d_p values tended to decline with increasing temperatures from 4 to 85°C. These results agreed with the finding of Zhang et al., (2004). The incident electromagnetic radiation would be deeply absorbed at lower temperature at MW

frequencies. These results suggested that marinated chicken breast with NaCl is likely to have non-uniform heating with MW heating regime as compared to marinated chicken breast without NaCl.

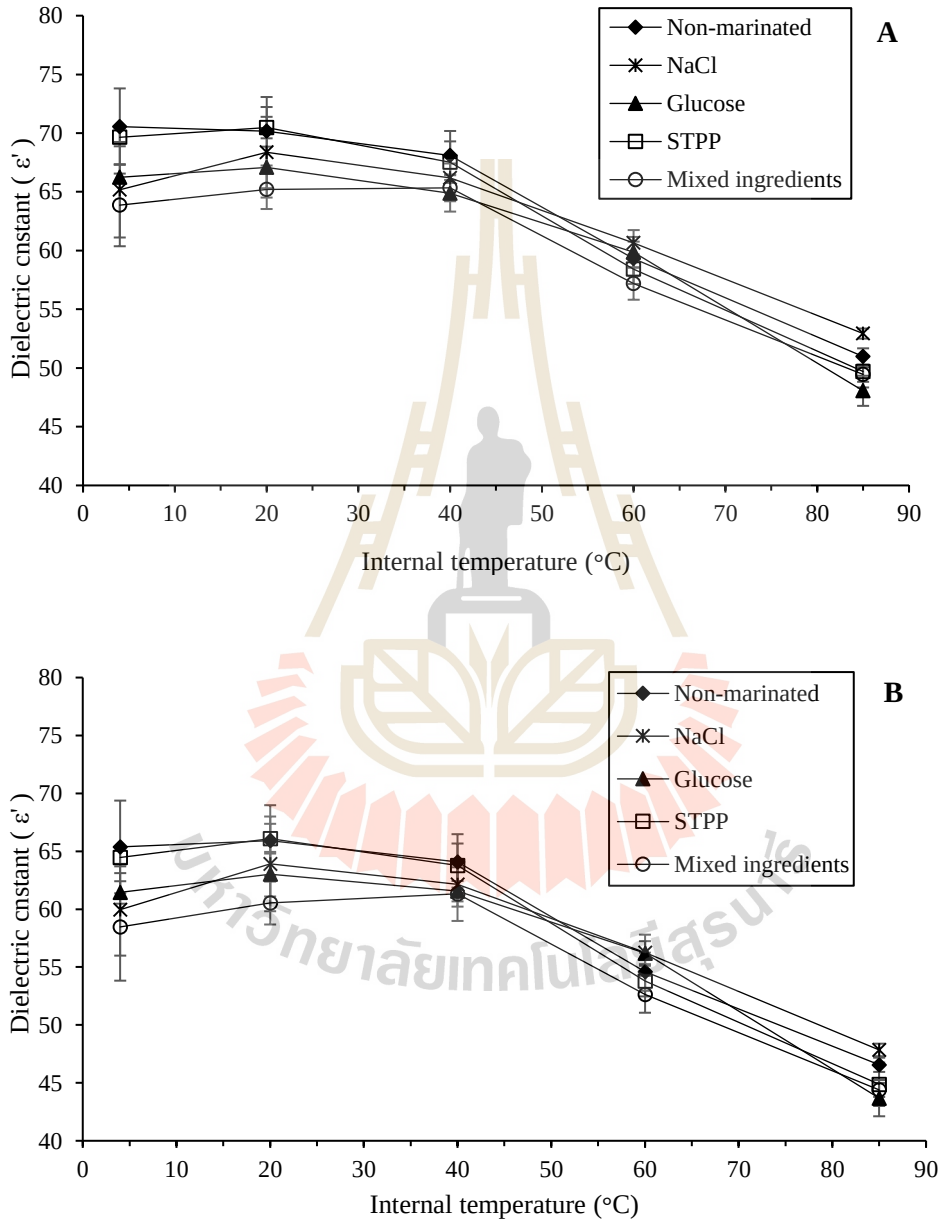

Figure 4.5 Changes of dielectric constant (ε') of marinated chicken breast at various temperatures at (A) 915 MHz and (B) 2,450 MHz. (mean ± standard deviation).

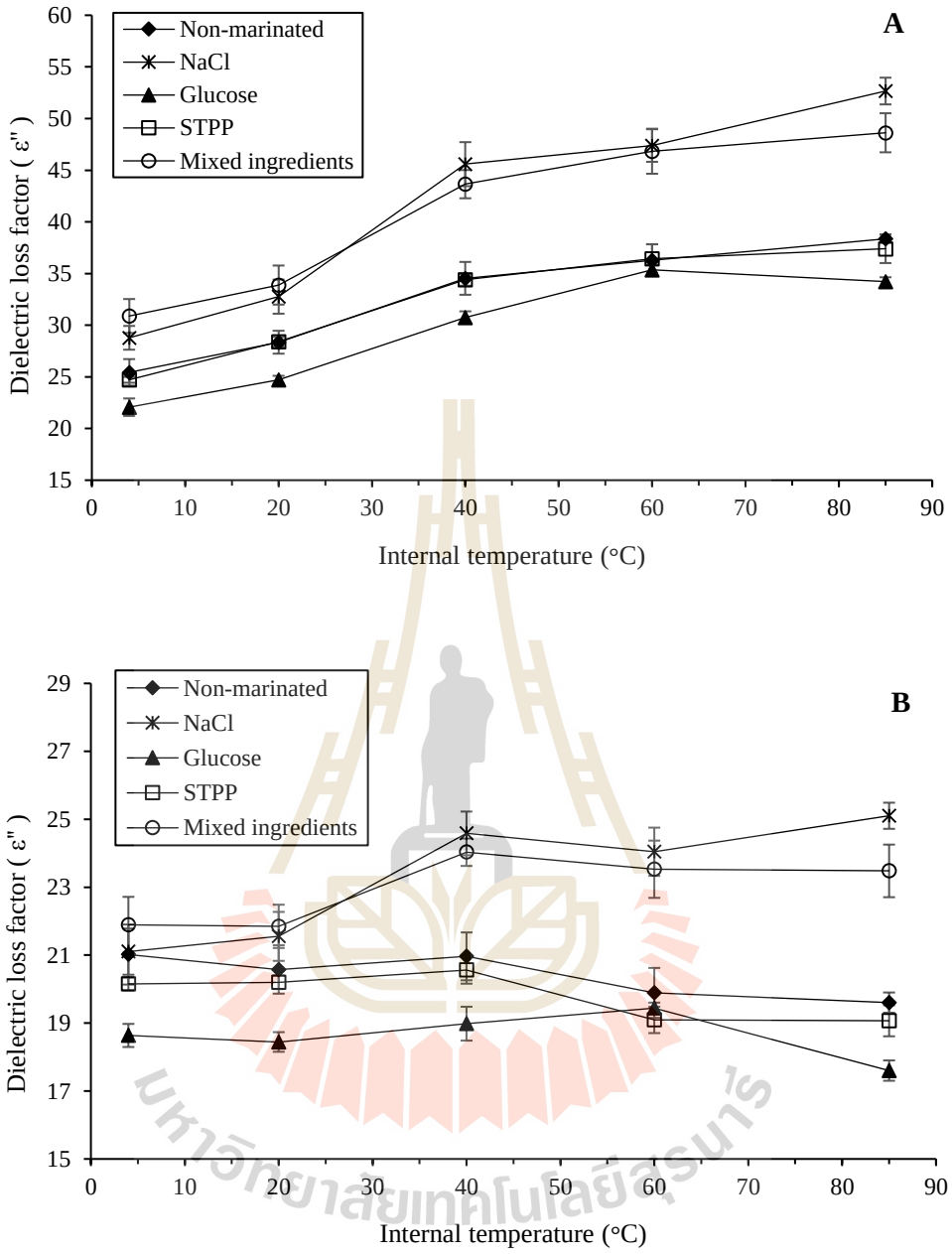

Figure 4.6 Changes of dielectric loss factor (ε'') of marinated chicken breast at various temperatures at (A) 915 MHz and (B) 2,450 MHz. (mean ± standard deviation)

Table 4.3 Calculated penetration depth (d_p) of marinated chicken breast at 2 microwave frequencies.

Sample temperature (°C)	915 MHz					2,450 MHz				
	Non-marinated	NaCl	Glucose	STPP	Mixed ingredients	Non-marinated	NaCl	Glucose	STPP	Mixed ingredients
4	3.5 ± 0.10^{Ba}	3.0 ± 0.16^{Ca}	3.9 ± 0.14^{Aa}	3.6 ± 0.05^{Ba}	2.8 ± 0.16^{Da}	1.5 ± 0.04^{Cab}	1.5 ± 0.04^{Da}	1.7 ± 0.03^{Ab}	1.6 ± 0.02^{Ba}	1.4 ± 0.04^{Ea}
20	3.2 ± 0.07^{Bb}	2.7 ± 0.16^{Cb}	3.5 ± 0.07^{Ab}	3.2 ± 0.04^{Bb}	2.6 ± 0.16^{Cb}	1.6 ± 0.03^{Ba}	1.5 ± 0.07^{Ca}	1.7 ± 0.02^{Aa}	1.6 ± 0.01^{Ba}	1.4 ± 0.06^{Ca}
40	2.6 ± 0.08^{Bc}	2.0 ± 0.09^{Cc}	2.8 ± 0.07^{Ac}	2.6 ± 0.02^{Bc}	2.0 ± 0.04^{Cc}	1.5 ± 0.03^{Bab}	1.3 ± 0.04^{Cb}	1.6 ± 0.03^{Ab}	1.5 ± 0.02^{Bb}	1.3 ± 0.01^{Cb}
60	2.3 ± 0.08^{ABd}	1.8 ± 0.04^{Cc}	2.4 ± 0.02^{Ad}	2.3 ± 0.06^{Bd}	1.8 ± 0.07^{Cd}	1.5 ± 0.05^{Bb}	1.2 ± 0.03^{Cb}	1.5 ± 0.02^{Ac}	1.5 ± 0.02^{Ab}	1.2 ± 0.03^{Cc}
85	2.1 ± 0.02^{Be}	1.5 ± 0.26^{Dd}	2.2 ± 0.01^{Ae}	2.1 ± 0.05^{ABe}	1.7 ± 0.05^{Ce}	1.4 ± 0.02^{Bc}	1.1 ± 0.02^{Dc}	1.5 ± 0.02^{Ac}	1.4 ± 0.03^{Bc}	1.1 ± 0.03^{Cd}

$^{A-E}$ means in the same row within the same frequency with different letters are significantly different ($P<0.5$).

$^{a-e}$ means in the same column with different letters are significantly different ($P<0.05$).

4.5 Conclusions

The marinated ingredients used in marination of chicken breast meat greatly affected dielectric properties, namely ε' and ε'' values. NaCl elevated the ε'' values, while glucose decreased ε'' values. Mixing of NaCl and STPP decreased ε' values. An increase of temperature resulted in the change of meat structure and the dramatic decrease of ε' values, while the ε'' values increased, especially in chicken breast marinated with NaCl. However, the addition of NaCl in marination likely led to non-uniform heating in marinated chicken breast under MW heating.

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CHAPTER V

REDUCTION OF RED BLOOD SPOTS IN COOKED MARINATED CHICKEN BREAST BY COMBINED MICROWAVE HEATING AND STEAMING

5.1 Abstract

A major defect quality of cooked marinated chicken breast products is red blood spot (RBS) which is caused by undercooked blood in the vessel. Preheating with microwave (MW) for 6-7 min followed by steaming alleviated the problem. RBS formation decreased when samples were heated to core temperature of 80°C and completely eliminated at core temperature of 82 and 85°C when MW was applied as a pre-heating step for 7 min. Based on Synchrotron-based Fourier Transform Infrared spectroscopy (SR-FTIR), α-helical content of blood cooked by the combined MW heating and steaming was lower than that of samples subjected to steaming alone ($P<0.05$). MW pre-heating decreased cooking time by 28-48% as compared to steaming alone. Heating regimes had no effect on cooking loss, pH, water holding capacity and shear force. Preheating by MW for 7 min followed by steaming to core temperature of 82°C appeared to be an effective heating regime that could reduce RBS with acceptable cooking loss.

Key words: Red blood spot, Microwave heating, Steaming, Marinated chicken breast, Blood denaturation, Synchrotron radiation Fourier Transform Infrared spectroscopy

5.2 Introduction

Red blood spots (RBSs) are problematic for cooked chicken breast products. Red spots observed after transverse cutting is due to incomplete denaturation of blood residues. The presence of RBS in the commercial cooked product is not acceptable by most consumers as red spots are deemed as undercooked meat (Smith and Northcutt, 2003; Bae, Cho, Hong, and Jeong, 2018).

Thermal processing applied in chicken meat industry includes steaming, roasting, grilling or frying. Heat is typically transferred from heating medium or heating source to chicken breast meat by convection, radiation and/or conduction (Rincon, Singh, and Stelzleni, 2015). Limited heat transfer could be a reason of incomplete denaturation of blood residues in vessels, particularly those located in the middle of breast muscle (Sturkie, 2015). Although high heating temperature and/or prolonged heating time can induce complete denaturation of blood in vessel, it causes overcooking and significant yield reduction (Pathare and Roskilly, 2016). Therefore, thermal processes that can assure denaturation of blood residues in breast muscle without significant loss of yield should be sought.

Microwave (MW) heating generates heat internally by dipole rotation and ionic conduction. This induces molecular friction and generates heat within meat products in an alternating electromagnetic field (Hebbar and Rastogi, 2012). Based on industrial, scientific, and medical (ISM) heating applications, the frequency range of MW heating

is 915 and 2,450 MHz (Piyasena, Dussault, Koutchma, Ramaswamy, and Awuah, 2003). MW heating gains more interest in food industry due to rapid heating, and low energy consumption (McKenna, Lyng, Brunton, and Shirsat, 2006; Jouquand et al., 2015). However, MW heating could lead to non-uniform heating of marinated meat due to heterogeneity and varied thickness and geometry of meat pieces (Vadivambal and Jayas, 2010). Goksoy, James, and James (1999) reported that MW heating of whole chicken carcass at 2,450 MHz, 500W for 5 min resulted in different temperatures of 15-45°C between breast and leg. In addition, Jeong et al., (2007) demonstrated that ground pork patties with salt cooked by MW at 2,450 MHz exhibited a differential temperature around 13°C between edge and the center of sample. However, combination of MW heating with conventional method has been reported to improve uniform heating, process efficiency and costs (Geedipalli, Datta, and Rakesh, 2008; Hebber and Rastogi, 2012; Datta and Rakesh, 2013). Typical commercial heating process of chicken breast relies on steaming process, which requires relatively long processing time of 30-50 min to reach core temperature at 80-82°C (Pathare and Roskilly, 2016). Combination of MW heating and steaming could be explored to evaluate the feasibility of RBS reduction.

Thermal process greatly affects structural changes of proteins, resulting in protein unfolding and aggregation (Bertram, Kohler, Bocker, Ofstad, and Andersen, 2006). This can be described by changes of secondary structure namely α-helix, β-sheet, and β-turn. Fourier Transform Infrared (FTIR) spectroscopy is normally used to elucidate secondary structure of proteins. Synchrotron radiation based-FTIR (SR-FTIR) utilizes synchrotron light source which is usually 100-1000 times brighter than a conventional globar source, allowing to selectively measure a part of interest sample area with size as small as 3 μm (Pascolo et al., 2014; Yu, 2006). The typical FTIR

measures the composition within 10 μm (Petibois, Cestelli-Guidi, Piccinini, Moenner, and Marcelli, 2010). Ellis, Santoro, Gómez, and Marco (2010) illustrated that the spectra of SR-FTIR had higher quality than those of conventional FTIR at small aperture at 3 x 3 μm^2. SR-FTIR could directly target proteins in blood vessel with the size at least 8 μm (Sturkie, 2015), leading to higher quality spectra than those obtained from the conventional FTIR. Therefore, SR-FTIR would likely be able to target the small area of interest like blood vessel.

The objectives of this study were to reduce RBS in cooked marinated chicken breast using the combined MW heating and steaming. Quality of marinated chicken breast cooked by the combined heating processes was assessed. Furthermore, the extent of denaturation of blood remaining in the vessel of cooked marinated chicken breast was elucidated by SR-FTIR.

5.3 Materials and methods

5.3.1 Marinated chicken breast preparation

Ten kg of boneless skinless chicken breast (*Pectoralis major*) were obtained from a commercial chicken processing plant (Charoen Pokphand Foods PCL., Nakhon Ratchasima, Thailand) with size of 250-280 g per piece and maximum thickness of 4-5 cm. Samples were collected immediately after slaughtering and refrigerated at 4°C for 24 hr. Chicken breast meat were vacuum tumbled (DVTS-50, Davison's Butcher Supply, Los Angeles, CA, USA) at temperature less than 8°C for 65 min. Marinated solution containing 5% NaCl (Pimai Salt Co., Ltd., Nakhon Ratchasima, Thailand), 3% glucose (Kornthai Co., Ltd., Ratchaburi, Thailand) and 2% sodium tripolyphosphate (STPP) (Aditya Birla Chemicals Ltd., Samutprakan, Thailand) was added at 16.5%

(w/w) of chicken breast sample. Three independent lots of chicken breast meat were prepared. Samples were stored in a 4°C-refrigerator for 20 hr after tumbling. The marinated samples were divided into 3 groups of different heating regimes as detailed below.

5.3.2 Cooking

Industrial MW oven (NE 1756, 18L, Panasonic Corp., Osaka, Japan) at frequency of 2,450 MHz and power output of 1.7 kW was used. Based on preliminary studies, longer pre-heating time by MW could quickly increase internal temperature but it caused burning and/or tissue explosion. Thus, MW pre-heating was set at 6 and 7 min, contributing to internal temperature around 50-70°C without quality defects. Three pieces of marinated chicken breast samples (300±20 g/piece) were put in polypropylene box, which was then placed at the middle of MW chamber and heated for 6 and 7 min. To obtain uniform heating, MW pre-heating time was paused at 2, 4, 5 and 6 min of the 6-min heating treatment and at 2, 4, 5, 6 and 7 min of the 7-min treatment. Core temperature was immediately measured at each paused time intervals using thermocouple type K (54 II, 80PT-5A, Fluke Corp., Moorpark, CA, USA). Three pieces of pre-heated samples were immediately placed in a steam oven (Model 101, Rational, Landsberg am Lech, Bavaria, Germany) set at 92°C, 100% of dew point and speed fan of level 3. Internal temperature of sample during steaming was continuously monitored using a thermocouple equipped in an oven and steaming was continued until core temperature of 80, 82 and 85°C was attained.

5.3.3 SR-FTIR Spectroscopy

Both raw and cooked samples were prepared for IR spectroscopy measurement as follows. The samples were transversely cut and embedded in OCT® (Bio-optica, Milano, Italy), and immediately frozen in liquid nitrogen and stored at -80°C prior to sectioning. Sectioning was carried out at -20°C using a cryostat microtome (AST500, AMOS Scientific, Clayton South Victoria, Australia) to obtain 8 µm thickness. The cut sample was attached on an infrared transparent, 2-mm thick Barium fluoride (BaF_2) slides and dried in a vacuum chamber for overnight. Infrared spectra were obtained with a Vortex 70 FTIR spectrometer (Bruker Optics, Ettlingen, Germany) coupled with a 36x objective microscope (Hyperion 2000, Bruker, Ettlingen, Germany) with MCT D315 detector cooled with liquid nitrogen. SR-FTIR at beamline BL4.1 of the Synchrotron Light Research Institute (SLRI) (Nakhon Ratchasima, Thailand) was used which connected with Synchrotron radiation entered the interferometer via an instrument port designed for IR emission. The measurement was performed in the mapping mode over the wavenumber range of 4000-600 cm^{-1}. The most sensitive spectral region of the secondary structure namely amide I at 1700-1600 cm^{-1}, was measured at 6 cm^{-1} resolution and 64 scans. Changes of secondary structure were acquired from a curve fitting using OPUS software (version 7.0, Bruker optics Inc, Billerica, MA, USA).

5.3.4 Qualities of cooked chicken breast

Quality of cooked marinated chicken breast samples namely cooking loss, moisture content, pH, water holding capacity (WHC), texture and color was analyzed within 24 hr after cooking.

Cooking loss was determined based on the weight after tumbling of respective samples as follows;

$$\text{Cooking loss (\%)} = \frac{(\text{Weight after tumbling} - \text{Weight after cooking})}{\text{Weight after tumbling}} \times 100$$

For pH determination, marinated chicken breast samples were homogenized with deionized water at a ratio of 1:5 (w/v) for 1 min using a homogenizer (T25 digital Ultra-Turrax, IKA Works Inc., Wilmington, NC, USA). The pH of homogenate samples was measured using a glass electrode pH meter (Mettler Toledo™ S220 SevenCompact, Schwerzenbach, Switzerland), which was calibrated against buffers pH 4.00 and pH 7.00 before use.

Moisture content of cooked samples was determined according to the Association of Official Analytical Chemists (AOAC, 2010).

WHC of cooked marinated chicken breast was determined by the centrifugal method according to Laycock, Piyasena, and Mittal (2003) with slight modifications. Minced samples (2 g) were placed on filter paper (Whatman no.1), and centrifuged (Sorvall Legend MACH 1.6R, Thermo Electron LED GmbH, Lengensellbold, Germany) at 6,000 x g for 15 min at 4°C. Samples after centrifugation were weighed and calculated based on the moisture content of the original chicken meat sample. WHC was calculated as follows;

$$Water\ loss\ (\%) = \frac{(\text{Weight before centrifuge} - \text{Weight after centrifuge})}{(\text{Sample weight before centrifuge})} \times 100$$

$$\text{WHC (\%)} = \frac{\big(\text{Moisture content (\%)} - \text{Water loss (\%)}\big)}{\text{Moisture content (\%)}} \times 100$$

Warner-Bratzler shear force was determined using a Texture Analyzer (TA.XT. Plus, Stable Micro Systems, Surrey, UK). Samples were measured using a 25-kg load cell at a crosshead speed of 10 mm/s. Cooked samples was cut to 1.9 cm wide, 2.5 cm long and 2.0 cm thick. Warner–Bratzler shear force was measured in 5 replicates for each cooking conditions.

Color of cross sectional cooked chicken breast meat was evaluated using a Hunter lab/Color Quest XE (Hunter Associates Laboratory Inc., Reston, VA., USA) and recorded the lightness (L*), redness (a*), and yellowness (b*) values. Color values were measured in 5 chicken breast strips for each treatment.

5.3.5 Statistical analysis

Three independent lots of chicken breast were tumbled. All experimental measurements otherwise stated were carried out at least 3 replicates per lot. Data were analyzed by one-way analysis of variance using SPSS (Version 23.0, SPSS Inc., Chicago, IL, USA). Mean comparisons were performed using Duncan's test to evaluate the significance of differences among mean values with $P<0.05$. Mean values ± standard deviation were reported.

5.4 Results and discussion

5.4.1 Time-temperature profiles

Temperature sharply increased during MW heating, and gradually increased during steaming (Figure 5.1). Heat generation of MW causes by dipolar rotation of polar molecules and conductive migration of dissolved ions which result in molecular friction, thus the internal temperature of marinated chicken breast is rapidly increased. In contrast, heat transfer of conventional steaming relies on convection and/or

conduction, resulting in slower heating rate (Awuah, Ramaswamy, and Tang, 2015; Li, Tang, Yan, and Li, 2019). Heating rate of MW pretreatment alone was about 7.6-8.3°C/min, while that of subsequent steaming alone was 2.4-2.7°C/min. Pérez-Juan, Kondjoyan, Picouet, and Realini (2012) reported that heating rate of cooked marinated beef by MW was 15.6-16.8°C/min. Heating rate of MW cooked marinated meat depended on the concentration of ionic ingredients added in marination as these would, in turn, increased dielectric properties (Lyng, Zhang, and Brunton, 2005). However, MW heating alone caused non-uniform heating by thermal runaway, which could eventually lead to burning. In contrast, steaming alone of marinated chicken breast required 26.2-32.1 min to reach core temperature of 80-85°C. When two processes were combined, process time reduced to 13.6-23.2 min, accounting for 28-48% process time reduction ($P<0.05$, Table 5.1). Reduction of cooking time contributes to higher nutrient retention and yield as well as energy saving (Yarmand and Homayouni, 2011; Joseph, 2017). Yarmand and Homayouni (2011) reported that MW heating shortened cooking time, resulting in thiamin retention of 98%, while thiamin retention in conventionally cooked chicken was only 77%. Moreover, Jouquand et al., (2015) reported that MW cooking of beef burgundy (4.6 kWh) had lower energy consumption than conventional cooking (6.5 kWh), which was attributed to decreased cooking time of 56%. MW cooking is more energy efficient process because of internal heat generation (Pathare and Roskilly, 2016). These results suggested that combination of MW heating and steaming reduced heating time of marinated chicken breast as compared to steaming alone.

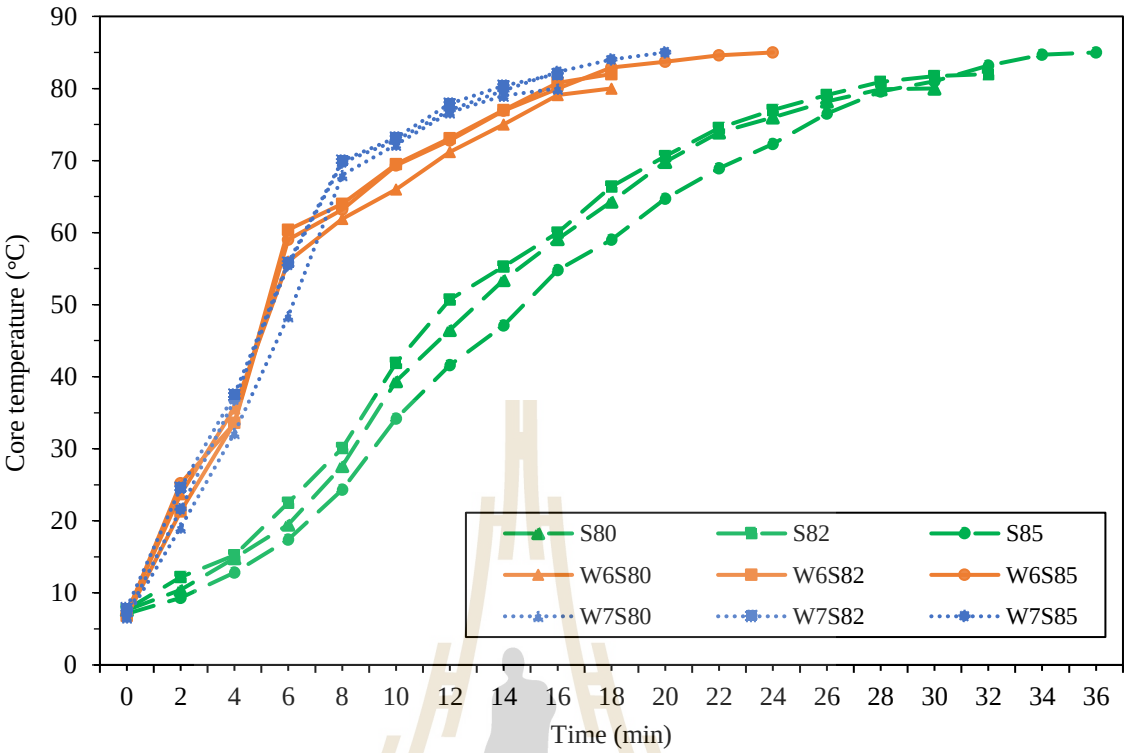

Figure 5.1 Temperature profiles of chicken breast subjected to steaming alone and combined microwave and steaming at various core temperatures. S80, S82 and S85 indicate steaming to core temperature of 80, 82 and 85°C, respectively. W6S80, W6S82 and W6S85 denote microwave preheating for 6 min, followed by steaming to core temperatures of 80, 82 and 85°C, respectively. W7S80, W7S82 and W7S85 denote microwave preheating for 7 min, followed by steaming to core temperatures of 80, 82 and 85°C, respectively.

5.4.2 Quality of cooked chicken breast meat

The combined MW heating and steaming process had no effect on cooking loss of marinated chicken breast as compared with steaming alone at the same core temperature ($P>0.05$, Table 5.1). Cooking loss appeared to increase as the final

core temperature increased. It has been reported that MW heating of meat resulted in higher cooking loss as compared to conventional methods (Yarmand and Homayouni, 2009; Dominguez, Gómez, Fonseca, and Lorenzo, 2014; Li et al., 2019). MW heating resulted in higher heating rate and greater extent of muscle destruction, and larger amount of released water upon cooking (Nikmaram, Yarmand, Emamjomeh, and Darehabi, 2011). These results indicated that the combined MW heating and steaming could increase the blood denaturation in meat without significant cooking loss.

The values of pH, WHC and shear force were comparable regardless of heating regimes applied (P>0.05, Table 5.2). The average pH values of all samples cooked both heating processes ranged between 6.30 and 6.36 (P>0.05, Table 5.2). Based on the results, the combined MW heating and steaming did not increase water expulsion in cooked meat. The shear force values correlate with meat toughness, causing by thermal denaturation of myofibrillar proteins (Pathare and Roskilly, 2016; Laakkonen, Wellintong, and Sherbon, 1972). Nikmaram et al., (2011) reported that shear force of veal muscle cooked by MW heating, roasting and braising were comparable. This probably illustrated that the extent of myofibrillar protein denaturation was similar among cooking methods. However, MW cooking at high power (700W) of yak meat resulted in lower shear values than the boiled sample, which was likely contributed from rapid thermal shrinkage of muscles (Li et al., 2019). Our study indicated that combined heating had no effect on quality of cooked meat.

However, samples cooked by combined heating showed different moisture content and color value than those cooked by steaming alone (P<0.05, Table 5.2). Moisture contents of samples cooked by longer MW heating time were lower than those of steamed samples (P<0.05). MW heating caused more moisture evaporation

from the sample (Datta and Rakesh, 2013). In addition, marinated samples cooked by the combined heating showed higher lightness (L*) and, lower redness (a*) and yellowness (b*) than steamed samples at core temperature of 82°C ($P<0.05$, Table 5.2). These results were in agreement with Ergonul (2017) who indicated that lower a* and b* values were observed in MW-cooked chicken breast as compared to samples cooked by blanching. It has been reported that a decrease in a* value of cooked meat was due to oxidation and thermal denaturation of myoglobin, leading to formation of ferrihemochrome with brown color (Suman, Nair, Joseph, and Hunt, 2016). Our results suggested that combined MW and steaming decreased redness of cooked marinated chicken breast at core temperature of 82°C.

5.4.3 RBS formation

RBS incidence of samples cooked by the combined heating was lower than that of samples cooked by steaming alone at all core temperatures studied (Table 5.1). Longer microwave pretreatment time showed greater reduction of RBS. At core temperature of 82°C, RBS was not found with MW preheating of 7 min. In the previous chapter, RBS did not take place at core temperature of 85°C in water bath heating. Although cooking loss was comparable between samples cooked to core temperature of 82 and 85°C, cooking to core temperature of 82°C would gain more energy saving. Generally, cooking to core temperature of 82°C has been applied in the commercial steaming process alone which RBS has been sporadically found around 2-3% (Smith and Northcutt, 2003). This study illustrated that MW pre-heating for 7 min followed by steaming to 82°C could effectively eliminate RBS incidence without significant loss in yield. MW heating rapidly increased an internal temperature of meat sample, leading to a greater extent of denaturation of blood remaining in vessel and conversion of bloody

spots to brown (Gowen, Abu-Ghannam, Frias, and Oliveira, 2006; Datta and Rakesh, 2013; Suman et al., 2016). Therefore, the combined heating appeared to be an alternative process for RBS reduction with less energy consumption.

Table 5.1 Effect of microwave pre-heating followed by steaming to various core temperatures on cooking qualities and RBS formation of marinated chicken breast (n = 9).

Core temperature (˚C)	**Time of MW heating (min)**	**Cooking time (min)**	**Cooking loss (%)**	**RBS (%)**
80	0	26.2±1.8^{c}	26.5±2.0^{b}	44.4
	6	17.0±1.5^{ef}	26.3±2.8^{b}	33.3
	7	13.6±1.6^{g}	26.9±2.3^{ab}	22.2
82	0	28.8±2.4^{b}	28.1±1.8^{ab}	33.3
	6	17.7±0.8^{e}	27.6±0.3^{ab}	11.1
	7	15.1±1.6^{fg}	27.8±1.1^{ab}	0
85	0	32.1±2.4^{a}	28.5±1.1^{ab}	0
	6	23.2±1.2^{d}	28.4±0.9^{ab}	0
	7	19.5±1.0^{e}	29.7±0.1^{a}	0

Different letters in the same column indicate significant difference (P<0.05).

5.4.4 SR-FTIR spectroscopy

The area of sample for SR-FTIR measurement is shown in Figure 5.2. Cooked sample showed lower α-helical content in concomitant with higher β-sheet content when the combined MW heating and steaming was applied ($P<0.05$, Table 5.3). At each core temperature, α-helical contents of samples subjected to steaming alone were higher than that cooked by the combined heating regime ($P<0.05$), suggesting lesser degree of denaturation. The quantity of both β-turn and aggregated β-sheet was comparable at varied cooking treatments. In the combined heating process, β-sheet content slightly increased with increasing core temperature, indicating that degree of protein denaturation increased with increasing core temperature. Based on RBS results, MW heating for 7 min and followed by steaming to core temperature 82°C completely eliminated RBS problem. It might explain by the IR difference in the spectra. The spectra were processed by subtracting spectra of cooked blood remained in vessels from the spectra of raw sample. The net results reflected the effect of heating method on blood denaturation (Figure 5.3). The prominent band at 1657 cm^{-1} corresponding to α-helical content (Yu, Morton, Clerens, and Dyer, 2016) showed the highest negative band in the steamed sample and lowest negative band in the sample heated by the combined MW heating for 7 min and steaming at core temperature of 82°C. While a band at 1625 cm^{-1} reflecting the β-sheet content showed highest positive band in the sample heated by the combined MW heating for 7 min and steaming. This indicated that higher extent of blood denaturation occurred in the sample heated by the combined MW heating for 7 min followed by steaming. Higher heating rate of MW resulted in higher degree of blood denaturation. Moreover, the vibration bands around 2925 and 2855 cm^{-1} are assigned to symmetric and asymmetric bending of methylene (Calabrò and Magazù, 2012). Both

bands of methylene group were clearly observed in spectra after steaming, while their intensity after MW heating decreased in proportion to the cooking time. The higher intensity of the methylene group might be associated with lipid oxidation. Steaming alone could result in higher lipid oxidation compared to the combined heating. The characteristic band at 1746 cm^{-1} is assigned to C=O stretching of carbonyl group (Sinanoglou, Cavouras, Xenogiannopoulos, Proestos, and Zoumpoulakis, 2018), which showed higher intensity in steamed sample. Calabrò and Magazù (2012) reported that an increase in intensity of carbonyl and methylene group was due to Maillard reaction because both functional groups are typical products in Maillard browning. This suggested that steaming resulted in more brownish appearance of cooked products. Generally, commercial steamed products are expected to have high lightness based on consumer preference (Abdulhameed, Yang, and Abdulkarim, 2016). Therefore, the combined heating probably contributes to more desirable color appearance. Based on SR-FTIR, the combined MW heating and steaming induced denaturation of blood protein to a greater extent than steaming alone.

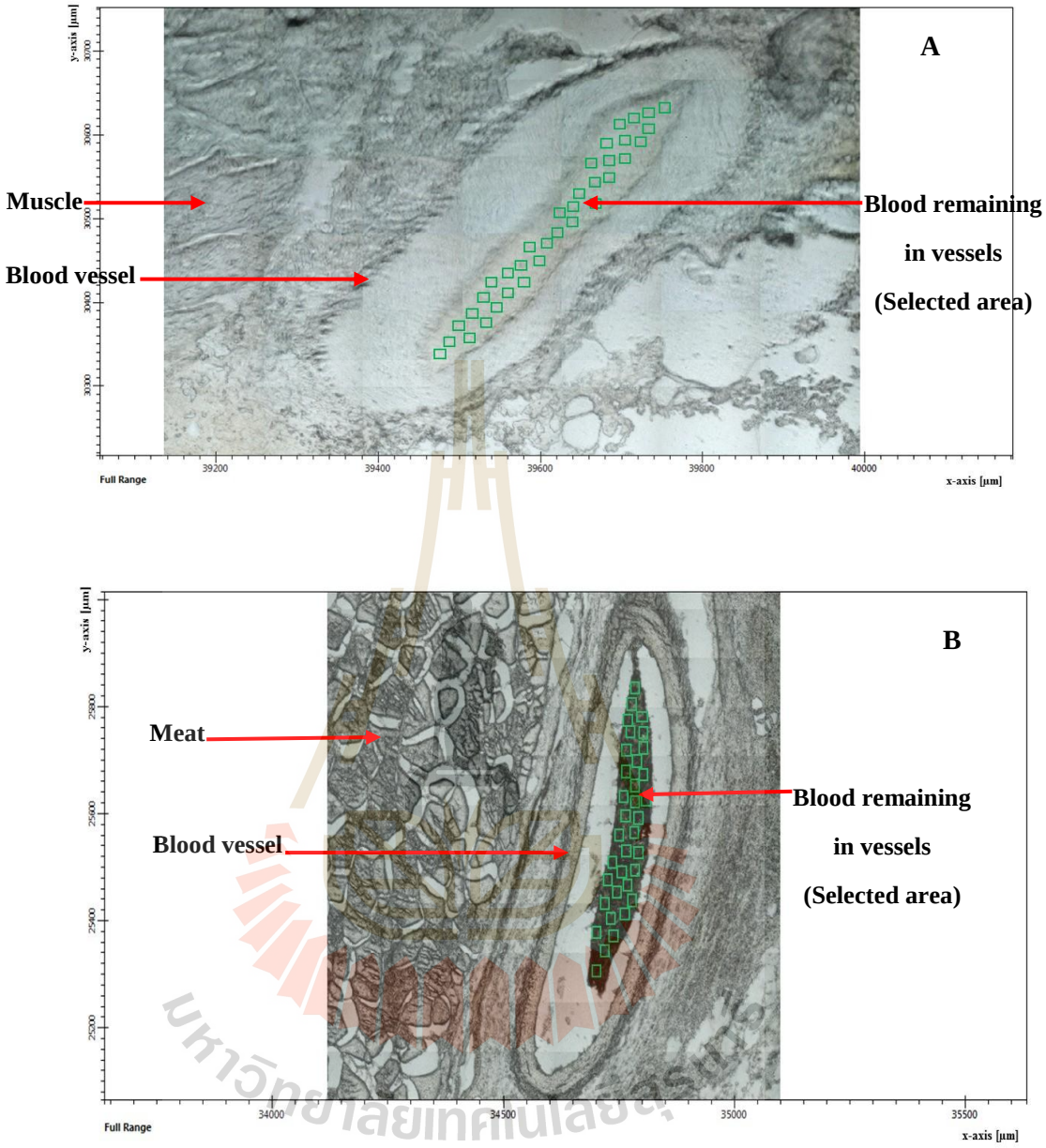

Figure 5.2 Micrograph of blood remaining in vessel of (A) raw meat and (B) sample cooked by the combined heating to 82°C obtained from a 36x objective microscope equipped to a synchrotron IR.

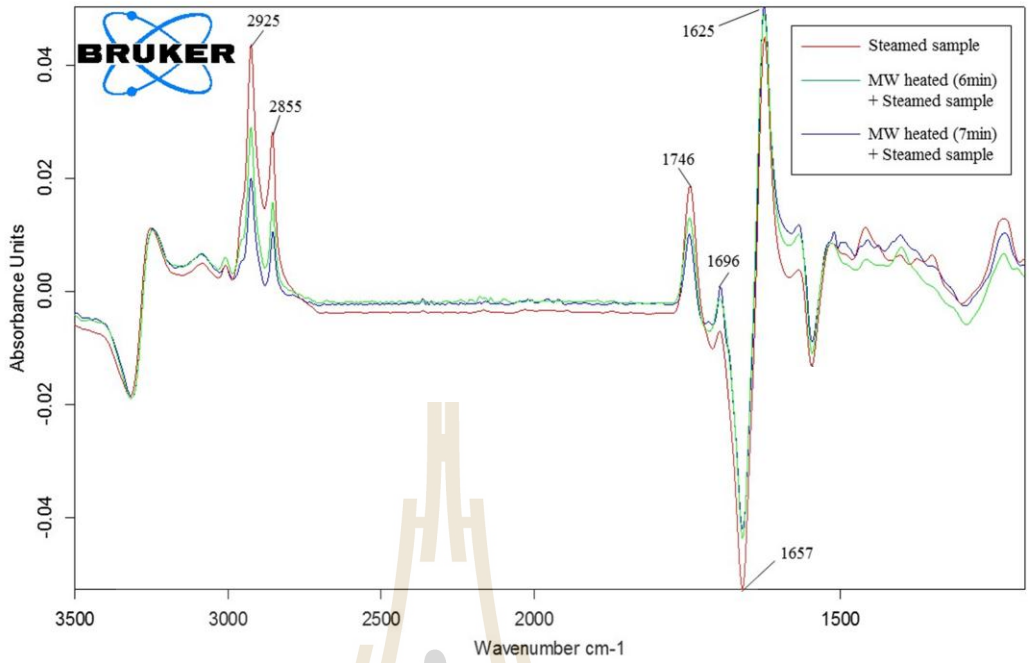

Figure 5.3 SR-FTIR difference spectra of chicken blood remaining in vessels at core temperature of 82°C, [(cooked sample)-(raw sample)] after baseline correction and vector normalization.

Table 5.2 Qualities of marinated chicken breast cooked by combined MW heating and steaming.

Core temperature (°C)	Time of MW heating (min)	pH	Moisture content (%)	WHC (%)	Shear force (kg)	L*	a*	b*
80	0	6.36±0.04	72.03±0.47^{a}	53.45±2.82	3.18±0.23	82.6±0.4^{a}	2.5±0.3^{ab}	14.1±0.3^{bc}
	6	6.33±0.02	70.14±0.46^{c}	56.33±1.80	3.19±0.15	82.2±0.7^{ab}	2.2±0.2^{cd}	13.7±0.3^{d}
	7	6.37±0.01	70.24±0.35^{c}	56.00±1.08	3.20±0.32	82.4±0.8^{ab}	2.3±0.2^{bc}	14.0±0.2^{bcd}
82	0	6.30±0.07	71.35±0.45^{ab}	54.20±1.60	3.03±0.09	80.8±0.2^{c}	2.6±0.3^{a}	15.3±0.1^{a}
	6	6.31±0.03	70.85±0.45^{bc}	55.95±3.14	3.12±0.12	81.7±0.5^{ab}	2.0±0.1^{d}	13.9±0.1^{cd}
	7	6.35±0.08	70.17±0.83^{c}	55.78±3.09	3.17±0.14	82.2±0.6^{ab}	2.1±0.2^{cd}	14.0±0.1^{bcd}
85	0	6.30±0.11	71.19±0.78^{b}	54.36±1.33	3.02±0.21	82.0±1.1^{ab}	2.2±0.3^{cd}	14.0±0.4^{bcd}
	6	6.33±0.02	71.08±0.29^{b}	55.68±3.16	3.10±0.15	82.4±0.7^{ab}	2.0±0.1^{d}	14.3±0.3^{b}
	7	6.37±0.01	70.21±1.15^{c}	55.90±3.50	3.12±0.12	81.4±0.6^{bc}	2.0±0.1^{cd}	14.1±0.2^{bc}

Different letters in the same column show significant difference (P<0.05).

Table 5.3 Relative content (%) of protein secondary structures of protein in marinated chicken breast cooked by the combined MW heating and steaming at various regimes.

Core temperature (°C)	Time of MW heating (min)	α-helix (1654, 1660 cm^{-1})	β-sheet (1620, 1630 cm^{-1})	β-turn (1670, 1680 cm^{-1})	Aggregated β-sheet (1610, 1690 cm^{-1})
Raw	0	50.94±1.29^{a}	24.11±1.54^{f}	19.16±0.69^{d}	5.79±0.62^{ab}
80	0	38.74±0.68^{b}	33.61±0.50^{e}	22.04±0.60^{ab}	5.61±0.25^{ab}
	6	37.20±1.04^{c}	35.75±0.41^{cd}	21.50±0.49^{abc}	5.54±0.72^{a}
	7	35.42±0.32^{e}	35.98±0.49^{bcd}	22.35±0.77^{ab}	6.25±0.52^{a}
82	0	38.87±0.73^{b}	34.40±0.35^{de}	21.31±0.97^{bc}	5.42±0.55^{ab}
	6	36.31±0.89^{cde}	36.18±1.48^{abc}	21.22±0.69^{bc}	6.30±0.53^{ab}
	7	35.42±0.40^{e}	36.35±0.82^{abc}	22.57±0.22^{a}	5.66±0.38^{ab}
85	0	38.85±0.43^{b}	35.34±0.32^{cd}	21.44±0.31^{abc}	4.36±0.45^{c}
	6	36.90±0.69^{cd}	37.51±1.38^{ab}	20.71±0.57^{b}	4.88±0.44^{bc}
	7	35.67±1.04^{de}	37.76±0.84^{a}	21.60±1.28^{abc}	4.96±0.57^{bc}

Different letters in the same column show significant difference ($P<0.05$).

5.5 Conclusions

The combined MW heating and steaming reduced RBS incidence in a more effective fashion than steaming alone. Pre-heating by MW for 7 min, followed by steaming to reach a core temperature of 82°C completely eliminated RBS. SR-FTIR spectroscopy of blood remained in the vessel of marinated chicken breast cooked by the combined heating process showed lower α-helix and higher β-sheet structure of blood proteins, suggesting greater protein denaturation. The MW pre-heating reduced 28-48% cooking time as compared to steaming alone. Therefore, the combined MW

heating and steaming could be a promising process to alleviate RBS problem in cooked marinated chicken breast products.

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CHAPTER VI

SUMMARY

6.1 Conclusions

Red blood spots (RBSs) were caused by incomplete denaturation of hemoglobin (Hb) in the blood vessel. Among marinated ingredients studied, only NaCl absorbed into the center of chicken breast, while sodium tripolyphosphate (STPP) and glucose did not. NaCl had an effect on destabilizing Hb structure, resulting in a decrease in thermal denaturation temperature (T_d). The marinated ingredients appeared to have minimal effect on RBS formation. RBSs could be completely eliminated when marinated chicken breast was cooked to reach the core temperature at 85°C.

Dielectric properties (ε' and ε'') of chicken breast meat varied with marinade ingredients and sample temperature. At microwave (MW) frequencies of 915 and 2,450 MHz, STPP resulted in an increase of ε' values of marinated chicken breast, while NaCl increased ε'' values. NaCl resulted in non-uniform heating of MW heating due to the reduced penetration depth (d_p). The ε' value of chicken breast meat decreased as temperature increased >40°C, while the ε'' values increased with temperature.

The MW heating of 7 min and followed by steaming to core temperature of 82°C completely eliminated RBS incidence in cooked marinated chicken breast, while steaming alone required core temperature at 85°C for RBS-free incidence. The

combined heating reduced cooking time by 28-48%, resulting in energy saving. The combined MW heating and steaming of marinated chicken breast have proven to be an effective approach for eliminating RBS problem without significant loss of yield.

APPENDIX

CURVE-FITTING OF THE SR-FTIR SPECTRA OF CHICKEN SAMPLE

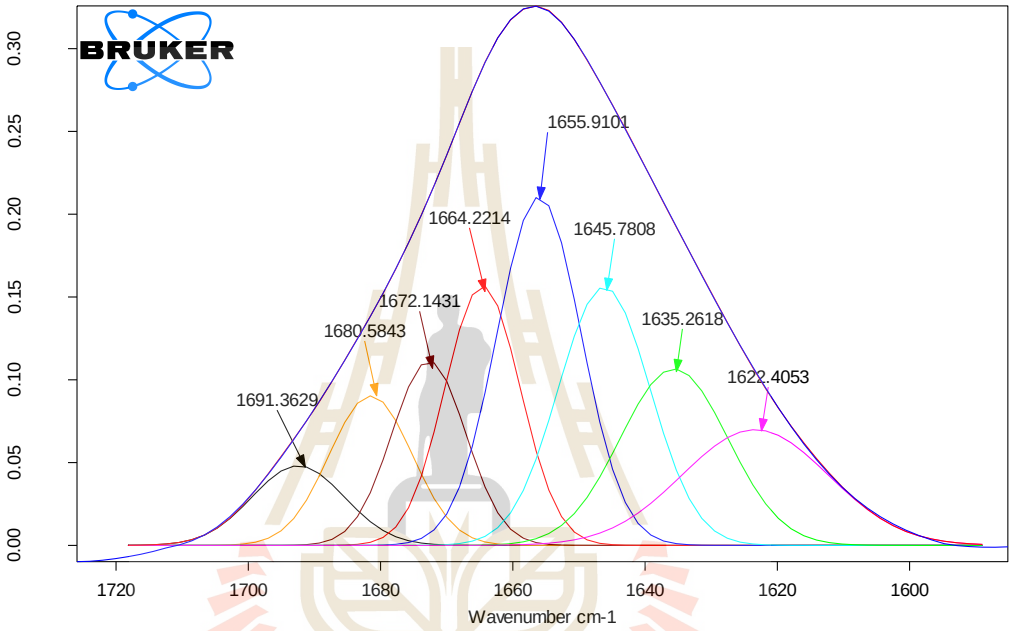

Figure 1A Curve-fitting of the SR-FTIR spectrum of raw sample

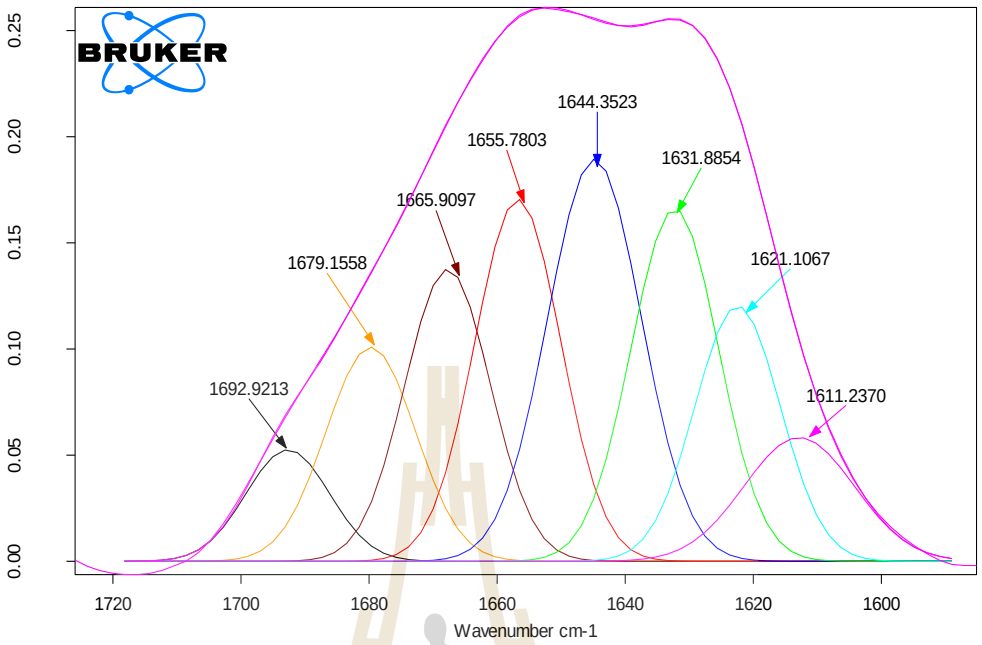

Figure 2A Curve-fitting of the SR-FTIR spectrum of cooked sample by steaming alone at core temperature of 85°C.

BIOGRAPHY

Matthanee Jantaranikorn was born in September 13, 1981, at Lopburi, Thailand. She studied for her high school diploma at Saraburi wittayakhom School, Saraburi, Thailand (1994-2000). In 2004, she received Bachelor's degree of Science (Food Technology) from Suranaree University of Technology. After graduation, she has worked with Charoen Pokphand Foods PCL. (Thailand). During working, she enrolled a Master's degree program in 2010. She was promoted to quality assurance manager in 2012. She obtained Master of Engineering (Energy management engineering) from Suranaree University of Technology in 2013. She got opportunity to study her Ph.D. program in Food Technology under financial support of Charoen Pokphand Foods PCL. (Thailand) in 2015. During her Ph.D. study, she received the first place in poster competition from The 21st Food Innovation Asia Conference (Bangkok, Thailand, 13-14 June 2019) under the title of "Effect of ingredients on red blood spot formation in cooked marinated chicken breast".