UNIVERSITI PUTRA MALAYSIA

FOAMING CHARACTERISTICS OF ULTRASOUND-TREATED WHEY PROTEIN SOLUTIONS AND THEIR USE AS AERATING AGENT IN ANGEL CAKE MANUFACTURE

TAN MEI CHING

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By

TAN MEI CHING

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

November 2014

To my father and mother…

Thank you for your loving support.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement of the degree of Doctor of Philosophy

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The effect of ultrasound on foam properties of whey protein and its application on mixed batter and baked cakes were studied. Full factorial design was used to study the aeration and rheological properties of whey protein foam before and after its suspension was treated with ultrasound. This involved three factors, *i.e.* protein concentration of 10%, 15% and 20%, ultrasound amplitude of 20%, 40% and 60%, and sonication time of 5, 15 and 25 minutes. The increase of ultrasound amplitude and sonication time gave positive effects to overall quality of foam measured in terms of aeration and rheological properties. At the highest amplitude of 60% and longest sonication time of 25 minutes, foam from 20% concentration showed greatest increase in stability (35%), consistency index (18%), storage modulus (17%), loss modulus (26%) and viscosity (21%). The ANOVA results showed that protein concentration, ultrasound amplitude and sonication time gave significant effects ($P < 0.0005$) on all the aeration and rheological properties of whey protein foam. A general master-curve relating shear stress or viscosity of foam at any protein concentration, and treated at any ultrasound amplitude and sonication time at the desired shear rate was obtained with a high coefficient of determination, $R^2 = 0.9959$.

Cake batter mixed with ultrasound treated foam at 60% amplitude for 25 minutes showed decreased density (5%), increased consistency index (57%), storage modulus (33%), loss modulus (21%) and viscosity (31%), resulting in final cake product with increased volume (18%), decreased density (18%), hardness (65%), chewiness (64%), gumminess (64%) and resilience (3%) when compared with control cake

using untreated whey protein. The appearances of cake formulated with ultrasound treated whey protein were comparable to those from egg white protein in terms of volume and density. In addition, it gave better textural properties of lower hardness, gumminess and chewiness.

The X-ray tomography and X-ray microtomography techniques were able to show the significant effect of ultrasound treatment on aerated foam and cakes through 2D and 3D images and bubbles size distribution analyses. Both techniques proved that the quantity of microbubbles with size range of 0 to 0.0025 mm^3 in the foam system had increased 46% while microbubbles with size range of 0.01 to 0.1 mm^2 in cake system had increased 38% after ultrasound treatment at 60% amplitude and 25 minutes sonication of protein suspension before foaming. The relationships of aeration, rheological and textural properties between foam, batter and cake were found to have high correlation coefficients. In conclusion, ultrasound treatment can improve the functional properties of whey protein foam, and the treated whey protein foam can effectively replace egg white in the making of high quality egg free angel food cakes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

CIRI-CIRI PEMBUSAAN PROTEIN *WHEY* DENGAN RAWATAN-ULTRABUNYI DAN PENGGUNAANNYA SEBAGAI AGEN PENGUDARAAN DALAM PEMBUATAN KEK *ANGEL*

Oleh

TAN MEI CHING

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Pengerusi : **Profesor Madya, Chin Nyuk Ling, PhD, Ir.**

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Kesan ultrabunyi terhadap busa protein *whey* dan aplikasinya pada adunan yang dicampur dan kek yang dibakar telah dikaji. Reka bentuk full factorial telah digunakan untuk mengkaji pengudaraan dan reologi busa protein *whey* sebelum dan selepas campurannya dirawat dengan ultrabunyi. Ini melibatkan tiga faktor, iaitu kepekatan protein pada 10%, 15% dan 20%, amplitud ultrabunyi pada 20%, 40% dan 60%, dan masa personikan selama 5, 15 dan 25 minit. Kenaikan amplitud ultrabunyi dan masa personikan memberikan kesan positif kepada keseluruhan kualiti busa yang disukat dari segi pengudaraan dan reologi. Pada amplitud tertinggi, 60% and masa personikan terpanjang, 25 minit, busa dengan kepekatan 20% menunjukkan peningkatan terbesar dalam kestabilan busa (35%), indeks kekonsistenan (18%), modulus penyimpanan (17%), modulus pelepasan (26%) dan kelikatan (21%). Keputusan ANOVA menunjukkan kepekatan protein, amplitud ultrabunyi dan masa personikan memberikan kesan yang ketara (P < 0.0005) pada semua sifat pengudaraan dan reologi busa protein *whey*. *Master-curve* umum yang mengaitkan tegasan ricih atau kelikatan busa pada sebarangan kepekatan protein, dan dirawat pada sebarangan amplitud ultrabunyi dan masa personikan pada kadar ricihan yang diingini telah diperolehi dengan pekali penentuan yang tinggi, R^2 = 0.9959.

Adunan kek yang dicampur dengan busa yang dirawat dengan ultrabunyi pada 60% amplitud selama 25 minit menunjukkan penurunan ketumpatan (5%), peningkatan indeks kekonsistenan (57%), modulus penyimpanan (33%), modulus pelepasan (21%) dan kelikatan (31%), mengakibatkan peningkatan isipadu (18%), penurunan ketumpatan (18%), kekerasan (65%), kekenyalan (64%), keperekatan (64%) dan

ketahanan (3%) pada produk kek berbanding dengan kek kawalan berumus protein *whey* tanpa ultrabunyi. Penampilan kek yang dirumuskan dengan protein *whey* yang dirawat dengan ultrabunyi adalah standing dengan kek yang dirumus dengan telur putih dari segi isipadu dan ketumpatan. Tambahan pula, ia memberikan ciri-ciri tekstur yang lebih baik, dengan kekerasan, keperekatan dan kekenyalan yang rendah.

Teknik tomografi sinar-X dan mikrotomografi sinar-X dapat menunjukkan rawatan ultrabunyi mempunyai kesan ketara pada pengudaraan busa dan kek melalui imej-imej 2D dan 3D serta analisis taburan saiz buih. Kedua-dua teknik membuktikan kuantiti mikrobuih dengan julat saiz 0 hingga 0.0025 mm^3 dalam sistem busa telah menigkat 46% manakala mikrobuih dengan julat saiz 0.011 hingga 0.1 mm^3 dalam sistem kek telah meningkat 25% setelah rawatan ultrabunyi pada 60% amplitud selama 25 minit dalam campuran protein sebelum pembusaan. Hubung kait antara ciri-ciri pengudaraan, reologi dan tekstur busa, adunan dan kek telah didapati dengan pekali penentuan yang tinggi. Kesimpulannya, rawatan ultrabunyi boleh menambah baik sifat-sifat kefungsian busa protein *whey*, dan protein *whey* yang dirawat boleh menggantikan telur putih dengan berkesan dalam penghasilan kek *angel food* tanpa telur yang berkualiti tinggi.

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I certify that a Thesis Examination Committee has met on **28 November 2014** to conduct the final examination of Tan Mei Ching on her thesis entitles "**Foaming Characteristics of Ultrasound-Treated Whey Protein Solutions and Their use as Aerating Agent in Angel Cake Manufacture**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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batter storage modulus, (c) loss modulus, (d) cake density, (e) cake volume, and (f) cake springiness

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CCD	Charge-coupled device
GRG2	Generalised Reducted Gradient 2
SEM	Scanning electron microscopy
SSE	Sum of square errors
SST	Total corrected sum of square
TEM	Transmission electron microscopy
TPA	Texture profile analysis
2D	Two-dimensional
3D	Three-dimensional

LIST OF NOMENCLATURES

a_A	Shift factor of amplitude
a_C	Shift factor of concentration
a_t	Shift factor of time
R^2	Goodnest of fit
K	Consistency index
n	Flow behavior index
σ	Shear stress
μ	Viscosity
$\dot{\gamma}$	Shear rate
G'	Storage modulus
G''	Loss modulus
X_A	Amplitude
X_C	Concentration
X_t	Time

CHAPTER 1

INTRODUCTION

Egg protein is the essential and commonly used ingredient in food formulations as a foaming ingredient for baking as it gives great contribution to the structure of the final cake product. It has the ability to develop a foam which is stable enough to hold the other ingredients until a solid foam of protein matrix is formed when it undergoes surface denaturation during the heat coagulation in the oven (Indrani and Rao, 2008), where limited surface denaturation is required to impart viscosity and rigidity to the interfacial film for foam stabilization (Kinsella, 1981). However, the bread and cake industry face the challenge of producing bakery products in conflict between health consciousness and a desire for taste gratification, when people restrict consumption of egg formulated food but aiming to achieve the comparable texture and appearance of egg formulated products. The problem occurred when whey protein with high nutritional values was widely applied in food products to replace egg protein but was unable to provide a comparable function as egg protein in food products due to poor foam stability (Yang and Foegeding, 2010). Hence, ultrasound treatment was proposed to enhance foam stability of whey protein through the early stage of protein denaturation induced by ultrasound acoustic wave in protein suspension before foaming to generate a stable foam, and mixed into batter followed by baking in the oven to undergo further protein denaturation until the solid foam of protein matrix is formed in the cake product.

1.1 Significance of this Study

This research works on investigating the effects of ultrasound during processing of highly aerated food. It studies the foaming process of protein concentrates which are to be used as alternative ingredients in the confectionery market from the health perspective in the eyes of the consumer and for cost effectiveness for the manufacturers. The successful application of ultrasonic usage to create foams from protein concentrates helps as an alternative ingredient of a less expensive material in cake making while curbing problems of consumers who cannot take or tolerate eggs in their diets. The ultrasonic treatment applied is at a higher power range and relatively lower frequencies. It has the capability of inducing acoustic cavitation and promotes bubble stability in aerated food. Bubble stability is a very dynamic process during food processing. Its assessment needs an accurate quantification method where in this research 3D imaging techniques were applied. The effects of using ultrasonic waves in aerated food system to create and control these dynamic bubbles in food systems were studied. Besides food structural properties, the ultrasonic treatment on aerated food also warrants opportunities of advancement in food processing methods which in this context, is favored because of its chemical and preservative free approach. The novelty of this research is in its use of ultrasonic wave and 3D imaging approach for highly aerated food.

1.2 Problem Statements

The application of whey protein in bakery products addresses the problem that whey protein has not yet perfectly replaced egg protein in cake formulation in order to achieve the properties of egg-protein cake in producing presentable appearance and texture. The research looks into the problem of controlling variations in bubble size and the presence of large bubbles during production of highly aerated food which result in a coarse and uneven texture, e.g. in cakes and snack bars. In the course of measuring bubble size distribution using 3D imaging, it addresses current problems of inaccurate imaging technique of measuring bubble size and distribution due to stereological techniques from where underestimates frequently occur due to 2D imaging.

1.3 Objectives

The general objective of this research was to study the effect of ultrasound on aeration and rheological properties of foam which was then used in cake batter for making cakes. The specific objectives are:

i. to determine the effect of ultrasonic wave treatment at various amplitudes and time on whey protein foam,
ii. to identify the effect of aeration properties at various stages from foam to batter and cake,
iii. to use 2D and 3D X-ray imaging techniques to investigate the microstructure of aerated food system,
iv. to study the mechanism of aeration in a highly aerated food system in terms of bubble size and distribution, and
v. to investigate the relationships between aeration, rheological and textural properties of foam, batter and cake.

1.4 Scope of Work and Thesis Outlines

The work conducted in this research focused on applying ultrasonically treated whey protein concentrate as the main ingredient in angel food cake formulation for baking. The aerated, rheological and textural properties of foam, batter and cake were evaluated quantitatively. Chapter 2 begins by introducing the mechanism of developing aerated structure in cake product, the roles of bubbles in aeration process, and the effect of aerated structure on cake quality. The advantages and disadvantages of using whey protein and egg white in cake baking, and the enhancement on whey protein's functional properties in cake baking are discussed. The ultrasound technique is then explained, its applications in previous studies and the mechanism of ultrasonic waves' effect on aeration are described. The theories of modelling and measurement analysis are also presented.

The materials and methods, equipment used and procedures for analyses conducted in the experiments are described in Chapter 3. The work started with protein suspension preparation, followed by ultrasound treatment, and preparation of the foam and batter, and cake production. The experimental design of ultrasound treatment at three levels of ultrasound amplitude and sonication time on protein suspension at three concentration levels is presented with aims to evaluate its foaming properties such as foaming capacity, stability, rheological properties including consistency index, flow behavior index, storage modulus, loss modulus, and viscosity, and to generate master-curve models of foam behavior. This chapter also describes the experimental design of the application of ultrasound treated foam at selected level of ultrasound amplitude, sonication time and protein concentration in the cake formulation where batter properties including batter density, consistency index, flow behavior index, storage modulus, loss modulus and viscosity, and cake properties including cake density, volume, hardness, springiness, chewiness, gumminess, cohesiveness and resilience were evaluated. The experimental design of imaging on foam and cake structure is drawn. It included methods for analyses and data analyses.

Chapter 4 details the set-up of X-ray microtomography imaging technique to scan the aerated structures of foam and small cubes of cake using Skyscan system, and X-ray tomography imaging technique to scan whole cake structure non-destructively. The preparation of foam sample holder is discussed and the schematic diagram illustrating the design of the sample holder is presented. The steps of constructing and analyzing those 2D and 3D images of foam and cake are described.

In Chapter 5, ultrasonically treated whey protein foams are characterized in terms of aeration and rheological properties, and compared with egg white protein. The images and analyses on the aerated foam structures using X-ray microtomography technique are presented. The modelling to predict shear stress and apparent viscosity of the foam whipped from protein suspension of various concentrations at variable ultrasound treatments in terms of amplitude and sonication time is presented.

Chapter 6 discusses the evaluation on batter and cake properties from foams produced in Chapter 5 using the angel food cake formulation. The aeration and rheological properties of aerated batter formulated with whey protein foam were measured and compared with those for egg white protein foam. The aeration and textural properties of cake, images and analyses of the aerated cake structures using X-ray microtomography and X-ray tomography techniques are discussed.

Chapter 7 presents the finding on the relationships between aeration, rheological and textural properties of foam, batter and cake. Lastly, Chapter 8 concludes the overall findings and contributions of the works. Recommendations are listed for further research.

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