

TABLE OF CONTENTS

	Page
ABSTRACT THAI	I
ABSTRACT ENGLISH	III
ACKNOWLEDGEMENT.....	V
TABLE OF CONTENTS.....	VII
LIST OF TABLES.....	XIII
LIST OF FIGURES	XVII
LIST OF ABBREVIATIONS	XXIII
CHAPTER	
1 INTRODUCTION	1
1.1 Rationale for the Research	1
1.2 Hypothesis of the Research.....	5
1.3 Objective of the Research	5
1.4 Expected Results of the Research	6
1.5 Scope of the Research	6
1.6 Organization of the Research.....	7
1.7 References.....	8
2 LITERATURE REVIEWS	12
2.1 Kombucha	12
2.1.1 Tea in Thailand.....	12
2.1.2 Kombucha Fermentation	17
2.2 Bacterial Cellulose.....	18
2.2.1 Biosynthesis.....	19
2.2.2 Bacterial Cellulose Production	21
2.2.3 Factor Affecting Bacterial Cellulose Production	24

TABLE OF CONTENTS (Continued)

	Page
2.2.4 Production of Bacterial Cellulose nanofibrils (BCNFs).....	28
2.2.5 Application of BC in Nutraceutical.....	29
2.3 Response Surface Methodology (RSM).....	32
2.4 References.....	33
3 PRE-OPTIMIZATION OF BACTERIAL CELLULOSE PRODUCTION: INVESTIGATING KEY FACTORS AFFECTING YIELD AND PROPERTIES	47
3.1 Abstract.....	47
3.2 Introduction.....	48
3.3 Materials and Methods.....	51
3.3.1 Experimental Design.....	51
3.3.2 Bacterial Cellulose Production	57
3.3.3 Culture Medium Characterization	59
3.3.4 Bacterial Cellulose Characterization.....	60
3.3.5 Statistical Analysis.....	62
3.4 Results and Discussion	63
3.4.1 Effect of Thai Tea Types on Bacterial Cellulose Yield and Characteristics.....	63
3.4.2 Effect of Different Types of Additives on Bacterial Cellulose Yield and Characteristics	85
3.4.3 Effect of Carbon Source Combinations on Bacterial Cellulose Yield and Characteristics	110
3.4.4 Effect of Process Parameters: pH, Harvesting Time, Tea Concentration, and Cultivation Method on Bacterial Cellulose Yield and Water Holding Capacity.....	136
3.5 Conclusion.....	151
3.6 References.....	152

TABLE OF CONTENTS (Continued)

	Page
4 OPTIMIZATION OF BACTERIAL CELLULOSE PRODUCTION FROM THAI RED TEA KOMBUCHA USING CENTRAL COMPOSITE DESIGN IN RESPONSE SURFACE METHODOLOGY	175
4.1 Abstract.....	175
4.2 Introduction.....	176
4.3 Materials and Methods.....	178
4.3.1 Experimental Design Using CCD of RSM	178
4.3.2 Laboratory Experimentation.....	181
4.3.3 Data Analysis and Model Fitting.....	183
4.3.4 Optimization and Solution Validation.....	183
4.3.5 Bacterial cellulose characterization.....	183
4.3.6 Statistical Analysis.....	183
4.4 Results and Discussion	183
4.4.1 Data Analysis of Experimental Design	183
4.4.2 Response Surface Analysis.....	187
4.4.3 Optimization of Formula, Validation, and Data Confirmation.....	198
4.4.4 Characterization of BC Product Resulted from Selected Formula.....	200
4.5 Conclusion.....	214
4.6 References.....	215
5 IMPACT OF HIGH-PRESSURE MICROFLUIDIZATION TREATMENT ON THE PROPERTIES OF BACTERIAL CELLULOSE DERIVED FROM THAI RED TEA KOMBUCHA	223
5.1 Abstract.....	223
5.2 Introduction.....	224
5.3 Materials and Methods.....	225

TABLE OF CONTENTS (Continued)

	Page
5.3.1 Bacterial Cellulose Production	2266
5.3.2 Bacterial cellulose nanofibrillation	227
5.3.3 Characterization of BC and BCNFs	227
5.3.4 Statistical Analysis.....	229
5.4 Results and Discussion	229
5.4.1 Moisture Content and Water Holding Capacity	229
5.4.2 Particle Analysis.....	231
5.4.3 BC Morphology.....	234
5.4.4 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis.....	237
5.4.5 X-Ray Diffraction (XRD) Analysis.....	240
5.4.6 Thermogravimetric (TGA/DTG) Analysis	243
5.5 Conclusion.....	246
5.6 References.....	247
6 EFFECTS OF BACTERIAL CELLULOSE NANOFIBRILS ON JELLY CANDY PROPERTIES AND BIOACTIVE COMPOUND PROFILES DURING SIMULATED DIGESTION	253
6.1 Abstract.....	253
6.2 Introduction.....	254
6.3 Materials and Methods.....	255
6.3.1 Experimental Design.....	256
6.3.2 Jelly Candy Production	257
6.3.3 Selection of the Optimal Jelly Candy Formulation Incorporating BC.....	260
6.3.4 In Vitro Gastrointestinal Digestion Simulation	260
6.3.5 Analysis of Jelly Candy Product.....	261
6.3.6 Statistical Analysis.....	264

TABLE OF CONTENTS (Continued)

	Page
6.4 Results and Discussion	265
6.4.1 The effect of different BC treatment and its concentration on the characteristics of JC	265
6.4.2 Selection of the best JC formulation.....	282
6.4.3 Investigation of Bioactive Compound Profiles During In Vitro Gastrointestinal Simulation.....	283
6.5 Conclusion.....	300
6.6 References.....	301
7 CONCLUSION AND RECOMMENDATION	309
7.1 Conclusion.....	309
7.1.1 Pre-Optimization of Bacterial Cellulose Production: Investigating Key Factors Affecting Yield and Properties	309
7.1.2 Optimization of Bacterial Cellulose Production from Thai Red Tea Kombucha Using Central Composite Design in Response Surface Methodology.....	310
7.1.3 Impact of High-Pressure Microfluidization Treatment on The Properties of Bacterial Cellulose Derived from Thai Red Tea Kombucha	310
7.1.4 Effects of Bacterial Cellulose Nanofibrils on Jelly Candy Properties and Bioactive Compound Profiles During Simulated Digestion.....	310
7.2 Recommendations	311
APPENDICES	313
APPENDIX A.....	314
APPENDIX B	317
APPENDIX C	325

TABLE OF CONTENTS (Continued)

	Page
BIOGRAPHY.....	328

LIST OF TABLES

Table	Page
2.1 Nutritional value of green tea, black tea, and some spices used for additive in red Thai tea	14
2.2 The unique characteristic of BC and Its potential application	20
2.3 Examples of some studies of BC production using static cultivation methods.....	23
3.1 Thai tea type variations and their compositions used as substrates in kombucha fermentation for BC production.....	52
3.2 Types of additives incorporated into the fermentation medium for BC production in Thai tea-based kombucha fermentation	53
3.3 Carbon source combinations tested for BC production in in Thai tea-based kombucha fermentation.....	55
3.4 Experimental design of harvesting period for BC production	56
3.5 The change of pH and degree of °Brix during kombucha fermentation of different types of Thai tea for BC production.	65
3.6 The change of sugar composition during kombucha fermentation with different types of teas.....	68
3.7 Summary of BC color investigation of kombucha with various types of tea before and after purification.....	74
3.8 The details of data decomposition during the TGA process of BC samples from kombucha fermentation with different types of tea.....	82
3.9 Mechanical properties data analysis using nano-indenter of BC from kombucha fermentation of BTC, RTC, and commercial product (NDC).....	84
3.10 Changes in pH and °Brix before and after RTC kombucha fermentation with different type of additives.....	87
3.11 Changes in sugar composition in RTC kombucha broth with different types of additives before and after fermentation	89

LIST OF TABLES (Continued)

Table	Page
3.12 Crystallinity index and average crystallite size of dried BC from Thai red tea kombucha fermentation with different type of additives.....	103
3.13 The details of data decomposition during the TGA process of BC samples from RTC kombucha fermentation with different types of additives.....	105
3.14 Mechanical properties data analysis using nano-indenter of BC from kombucha fermentation of RTC-C, RTC-EtOH, RTC-VC, and NDC.	109
3.15 Changes in pH and °Brix degree during RTC kombucha fermentation with different carbon source combinations.	112
3.16 The change of sugar composition during RTC kombucha fermentation with different types of carbon source combinations.....	117
3.17 Crystallinity index and average crystallite size of dried BC from RTC kombucha fermentation with different carbon source combinations.....	129
3.18 Thermal decomposition of BC produced from RTC kombucha fermentation using different carbon source combinations, as analyzed by thermogravimetric analysis (TGA).....	132
3.19 Mechanical properties of BC from kombucha fermentation of RTC-SD, RTC-SGlu, and RTC-C analyzed using a nano-indenter.....	135
3.20 The change of pH and °Brix during RTC kombucha fermentation with different initial pH condition.....	137
3.21 The change of pH and °Brix during RTC kombucha fermentation time.....	142
3.22 The change of pH and °Brix (before and after) RTC kombucha fermentation with different tea concentration.....	146
3.23 The change of pH and °Brix (before and after) RTC kombucha fermentation with different cultivation methods.....	149
4.1 The factors used in the design of experiment for BC production from RTC kombucha fermentation and its value.....	179

LIST OF TABLES (Continued)

Table	Page
4.2 Design of experiment for the optimization of BC production from RTC kombucha fermentation	180
4.3 The data of wet yield, dry yield, and WHC from the laboratory experiment	184
4.4 Summary of data analysis from the laboratory experiment	186
4.5 The resume of fit summary report of wet yield analysis.....	189
4.6 ANOVA for Quadratic Model of wet yield analysis	189
4.7 The resume of fit summary report of dry yield analysis.....	191
4.8 ANOVA for Quadratic Model of dry yield analysis	192
4.9 The resume of fit summary report of dry yield analysis.....	195
4.10 ANOVA for Quadratic Model of WHC	196
4.11 Summary of the criteria and constraints for optimizing the medium formulation	199
4.12 Software-Generated Optimal Formula and Predicted BC Production	199
4.13 Software-Generated Data Confirmation Output.....	200
4.14 CI and crystallite size of BC samples from various of medium formulations i.e. RTC-V1, RTC-V46, and RTC-V53.....	207
4.15 Detail parameter of TGA/DTG analysis of BC samples from optimized kombucha.....	210
4.16 Mechanical properties data analysis using nano-indenter of BC from kombucha fermentation of RTC-V1 and RTC-C.....	213
5.1 Moisture content and WHC of BC pulp and microfluidized BC.....	229
5.2 Results of polydispersity index analysis of BC from different mechanical treatments	231
5.3 Resume of the diameter size of the dried BC samples with different mechanical treatment and drying methods.	235
5.4 Crystallinity index and average crystallite size of dried BC with various mechanical treatment and drying methods	242

LIST OF TABLES (Continued)

Table	Page
5.5 Detail parameter of TGA/DTG analysis of BC samples with different mechanical treatments and drying methods.....	245
6.1 JC formulation based on different BC treatment and its concentration.....	258
6.2 Summary of L, a, b* Values from JC with different type and concentration of BC addition.....	267
6.3 The data of JC hardness as the effect of the type of BC, concentration of BC, and their interaction.	273
6.4 The data of JC adhesiveness as the effect of the type of BC, concentration of BC, and their interaction.	275
6.5 The data of JC springiness as the effect of the type of BC, concentration of BC, and their interaction.	276
6.6 The data of JC cohesiveness as the effect of the type of BC, concentratio of BC, and their interaction	277
6.7 The data of JC gumminess as the effect of the type of BC, concentration of BC, and their interaction	278
6.8 The data of JC chewiness as the effect of the type of BC, concentration of BC, and their interaction	279
6.9 The data of JC resilience as the effect of the type of BC, concentration of BC, and their interaction.....	281
6.10 Analysis result for selected formula determination using EDM.....	283

LIST OF FIGURES

Figure	Page
2.1	Formation of several types of compounds, including BC, in the fermentation of kombucha drinks 18
2.2	The structure of the cellulose chain composed of glucose monomers..... 18
2.3	Schematic diagrams of BC biosynthesis by bacteria..... 21
2.4	Schematic diagram of kombucha fermentation showing the production of BC and other compounds..... 22
2.5	Schematic representation of the microfluidization process used to prepare the nano delivery system 29
3.1	The change of pH and degree of °Brix during kombucha fermentation of different types of Thai tea for BC production..... 66
3.2	The change of sugar composition (sucrose, glucose, and fructose) of kombucha broth before and after fermentation 67
3.3	Wet yield (g/L), dry yield (g/L), and WHC (g water/g cellulose) from BC produced from kombucha with different types of Thai tea 69
3.4	The appearance of BC from WT sample in a medium fermentation and after drained 72
3.5	The appearance of BC (a) BC after harvesting, (b) sliced BC after harvesting and boiling in RO water, (c) sliced BC after purification using NaOH and RO water, (d) BC sheet after purification using NaOH and RO water, and (e) dried BC sheet. 73
3.6	The SEM images of BC samples: (a) NDC; (b) GTC; (c) BTC; (d) RBTH; and (e) RTC. The images are shown at varying magnifications: (1) 10,000x; (2) 30,000x; (3) 50,000x; and (4) 100,000x..... 75
3.7	Graph of poly distribution diameter size of BC samples from NDC, RTC, GTC, BTC, and RBTH..... 76

LIST OF FIGURES (Continued)

Figure	Page
3.8	FTIR spectra of BC produced from different Thai tea kombucha fermentations..... 77
3.9	XRD spectra of BC produced from different types of tea kombucha fermentation 80
3.10	TGA (left) and DTG (right) thermographs of BC samples produced from kombucha fermentation using different types of tea 81
3.11	Changes in pH and °Brix before and after RTC kombucha fermentation with different type of additives..... 86
3.12	Changes in sugar composition (sucrose, glucose, and fructose) in RTC kombucha broth before and after fermentation with different types of additives..... 88
3.13	The appearance of BC sample from RTC kombucha fermentation with different type of additives: In fermentation process (a), before purification (b), after purification with sodium hydroxide (c), and after oven drying (d) 90
3.14	Wet yield (g/L), dry yield (g/L), and WHC (g water/g cellulose) from BC produced from RTC kombucha with different types additives..... 92
3.15	SEM image of BC (a) RTC-control; (b) RTC-SPI; (c) RTC-YE; (d) RTC-PC; (e) RTC-VC; (f) RTC-EtOH: (1) magnification of 10000 x; (2) magnification of 30,000x..... 96
3.16	Graph of poly distribution size of BC samples diameter from RTC kombucha fermentation with various types of additives..... 97
3.17	FTIR spectra of BCs from RTC kombucha fermentation with various types of additives..... 99
3.18	XRD spectra of BC from Thai red tea kombucha with different type of additives..... 101
3.19	TGA (left) and DTG (right) thermographs of BC samples from RTC kombucha fermentation with different types of additives. 104

LIST OF FIGURES (Continued)

Figure	Page
3.20	Changes in pH and °Brix degree during RTC kombucha fermentation with different carbon source combinations. 112
3.21	Change of sugar composition of RTC kombucha broth with different types of carbon sources combinations before and after fermentation..... 114
3.22	BC appearance at different stages of RTC kombucha fermentation with various carbon source combinations: (a) during fermentation, (b) before purification, (c) after purification with sodium hydroxide, and (d) after oven drying. 118
3.23	Wet yield (g/L), dry yield (g/L), and WHC (g water/g cellulose) of BC produced from RTC kombucha using different combinations of carbon sources. 120
3.24	SEM image of BC (a) RTC-control; (b) RTC-SF; (c) RTC-SD; (d) RTC-SG; and (e) RTC-SGly; (1) magnification of 10000 x; (2) magnification of 30,000x; (3) magnification 50.000x. 123
3.25	Graph of poly distribution size of BC samples diameter from RTC kombucha fermentation with various types of carbon source combinations 124
3.26	FTIR spectra of BCs from RTC kombucha fermentation with various of carbon sources combinations..... 126
3.27	XRD spectra of BC from RTC kombucha fermentation with different carbon sources combinations..... 128
3.28	TGA (left) and DTG (right) thermographs of BC samples from RTC kombucha fermentation with different combinations of carbon sources 131
3.29	Wet yield (g/L), dry yield (g/L), and WHC of BC samples from RTC kombucha fermentation with different pH conditions. 139

LIST OF FIGURES (Continued)

Figure	Page
3.30	Wet yield (g/L), dry yield (g/L), and WHC (g water / g cellulose) of BC samples from RTC kombucha fermentation with different harvesting period 143
3.31	Wet yield (g/L), dry yield (g/L), and WHC of BC samples from RTC kombucha fermentation with different tea concentration 147
3.32	Wet yield (g/L), dry yield (g/L), and WHC of BC samples from RTC kombucha fermentation with different cultivation method 151
4.1	Graph model of the effect of formula composition interaction to the wet yield of BC..... 190
4.2	Graph model of the effect of formula composition interaction to the dry yield of BC..... 194
4.3	Graph model of the effect of formula composition interaction to WHC of BC. 197
4.4	Wet yield (g/L), Dry yield (g/L), and WHC (g water/g cellulose) of purified BC from kombucha fermentation with different type of tea.....201
4.5	SEM image of BC from (a) RTC-C, (b) RTC-V1, (c) RTC-V46, and (d) RTC-V53. (1) 10k, (2) 30k, and (3) 50k magnifications.202
4.6	Graph of the polydispersity in fiber diameter for BC samples: (a) RTC-V1, (b) RTC-V46, (c) RTC-V53, and (d) RTC-C.....203
4.7	FTIR spectra of BCs from various of medium formulations i.e. RTC-V1, RTC-V46, RTC-V53, and RTC-C.....204
4.8	XRD spectra of BCs from various of medium formulations i.e. RTC-V1, RTC-V46, and RTC-V53.206
4.9	TGA (a) and DTG (b) thermograph of optimized and control BC samples209
5.1	Graph of polydistribution particle size from BC-Pulp and microfluidized BC suspension232

LIST OF FIGURES (Continued)

Figure	Page
5.2 SEM image of BC sample BC sheet (BCC), BCP, HPM1, HPM2, dan HPM3.....	235
5.3 Fiber size distribution of BC-C, BCP, and microfluidized BC samples (BCH-10, BCH-15, and BCH-20).....	236
5.4 FTIR spectra of BC-C, BCP, and microfluidized BC samples (BCH-10, BCH-15, and BCH-20), with "O" indicating oven-dried and "FD" indicating freeze-dried samples.....	238
5.5 XRD spectra from the BC samples with different mechanical treatments and drying methods.....	240
5.6 TGA (a) and DSC (b) results of dried BC from optimized RTC kombucha with various treatments and drying methods.	244
6.1 Image of JC products with the addition of BC (JCBC) and without the addition of BC (JC-Control). The numbers 5, 10, 15, and 20 indicate the concentration of wet BC (g) added to the formula.	266
6.2 The effect of different type and concentration of BC to the L* value characteristics of JC	268
6.3 The effect of different type and concentration of BC to the a* value characteristics of JC	269
6.4 The effect of different type and concentration of BC to the b* value characteristics of JC.....	270
6.5 Illustration of the graph obtained from the texture analysis of JC using a texture analyzer.....	271
6.6 Texture profile analysis result of JC with various BCNF and its concentration (a) hardness, (b) adhesiveness, (c) springiness, (d) cohesiveness, (e) gumminess, (f) chewiness, (g) resilience	272
6.7 Appearance of JC products (JC-BC and JC-NBC) with the addition of different bioactive compound materials.....	284

LIST OF FIGURES (Continued)

Figure	Page
6.8	Color analysis results of JC-BC and JC-NBC with the addition of different bioactive compound materials286
6.9	Texture properties analysis results of JC-BC and JC-NBC with the addition of different bioactive ingredients287
6.10	TPC of JC with and without BC, incorporating various additives (control, VE, BF, and TOM), across the product, gastric, and intestinal phases293
6.11	TFC of JC with and without BC, incorporating various additives (control, BF and TOM), across the product, gastric, and intestinal phases.....295
6.12	AA of JC with and without BC, incorporating various additives (VC, VE, BF, and TOM), across the product, gastric, and intestinal phases.....297

LIST OF ABBREVIATIONS

BC	=	Bacterial Cellulose
BCNF/s	=	Bacterial cellulose nanofibril/s
BF	=	Code for butterfly flower powder extract
BTC	=	Code for black tea <i>Chatramue</i> brand
GTC	=	Code for Thai green tea <i>Chatramue</i> brand
CI	=	Crystallinity index
HPM	=	High-pressure microfluidization
JC	=	Jelly candy
ND	=	Not detected
PC	=	Pure coffee
RBTH	=	Code for Chinese black tea <i>Three horse</i> brand
RTC	=	Code for Thai red tea <i>Chatramue</i> brand
RTC-SD	=	RTC-Sucrose-dextrose
RTC-SF	=	RTC-Sucrose-fructose
RTC-SGlu	=	RTC-Sucrose-glucose
RTC-SGly	=	RTC-Sucrose-glycerol
SCOBY	=	symbiotic culture of bacteria and yeast
SPI	=	Soy protein isolate
TFC	=	Total Flavonoid content
TPC	=	Total Phenolic content
TOM	=	Code for tomato powder extract
VC	=	Vitamin C
VE	=	Vitamin E
YE	=	Yeast extract
XRD	=	X-ray diffraction