MAXIMIZING THE BENEFIT OF SOME FOOD INDUSTRY BY-PRODUCTS USING BIOTECHNOLOGY AND NANO-TECHNOLOGY FOR PRODUCTION OF SOME FUNCTIONAL BAKERY PRODUCTS

By

AYMAN ABDEL AZIZ MOHAMMAD

B.Sc. Agric. Sci. (Food Technology), Cairo University, 2001 M.Sc. Agric. Sci. (Food Technology), Cairo University, 2010

A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Agricultural Science (Food Science and Technology)

Department of Food Science Faculty of Agriculture Ain Shams University

2016

Approval Sheet

MAXIMIZING THE BENEFIT OF SOME FOOD INDUSTRY BY-PRODUCTS USING BIOTECHNOLOGY AND NANO-TECHNOLOGY FOR PRODUCTION OF SOME FUNCTIONAL BAKERY PRODUCTS

By

AYMAN ABDEL AZIZ MOHAMMAD

B.Sc. Agric. Sci. (Food Technology), Cairo University, 2001 M.Sc. Agric. Sci. (Food Technology), Cairo University, 2010

This thesis for Ph.D. degree has been approved by:

Dr.	Salah	Kamel Els	sam	ahy					
	Prof.	Emeritus	of	Food	Science	and	Technology,	Faculty	of
	Agric	ulture, Sue	z Ca	nal Un	iversity				
Dr. Ibrahim Rizk Sayed Ahmed Rizk									
	Prof.	Emeritus	of	Food	Science	and	Technology,	Faculty	of
	Agric	ulture, Ain	Sha	ms Uni	versity				
Dr.	El-Sa	yed Ibrahi	m Y	ousif A	Abou El S	oud			
	Prof.	Emeritus	of	Food	Science	and	Technology,	Faculty	of
	Agric	ulture, Ain	Sha	ms Uni	versity				

Date of Examination: 9/1/2016

MAXIMIZING THE BENEFIT OF SOME FOOD INDUSTRY BY-PRODUCTS USING BIOTECHNOLOGY AND NANO-TECHNOLOGY FOR PRODUCTION OF SOME FUNCTIONAL BAKERY PRODUCTS

By

AYMAN ABDEL AZIZ MOHAMMAD

B.Sc. Agric. Sci. (Food Technology), Cairo University, 2001 M.Sc. Agric. Sci. (Food Technology), Cairo University, 2010

Under the supervision of

Dr. El-Sayed Ibrahim Yousif Abou El Soud

Prof. Emeritus of Food Science and Technology, Department of Food Science, Faculty of Agriculture, Ain Shams University, (Principal Supervisor)

Dr. Abdel Fattah Abdel Kareem Abdel Fattah

Lecture of Food Science and Technology, Department of Food Science, Faculty of Agriculture, Ain Shams University

Dr. Attia Abdel Fattah Yaseen

Researcher Prof. of Food Science and Technology, Department of Food Technology, National Research Center.

ABSTRACT

Ayman Abdel Aziz Mohammad. "Maximizing the Benefit of Some Food Industry By-Products Using Biotechnology and Nano-Technology for Production of Some Functional Bakery Products". Unpublished Ph.D. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2016

The aim of this study was to prepare nano and fermented-nano powders of wheat bran, wheat germ, rice bran, carrot pomace and pomegranate peels by superfine grinding of raw and fermented materials. Physico-chemical and functional properties as well as phenolic acids profiles of raw and prepared materials were evaluated. In addition, prepared extracts were used to evaluate the phytochemical contents, antioxidant activity and cytotoxic activity of the prepared materials. Moreover, wheat flour (72%) was substituted with nano wheat bran, nano wheat germ, fermented-nano rice bran, fermented-nano carrot pomace and fermented-nano pomegranate peel at the levels of 5, 15 and 25% to prepare functional formulas. Rheological properties of these formulas were investigated using the Mixolab compared to wheat flour 72%, wheat flour 82% and whole-meal flour as control samples. Also, the prepared formulas were used to manufacture functional pan and balady breads and the quality characteristics of produced bread were evaluated.

The results showed that superfine grinding could effectively pulverize the fiber particles to nano-scale. Chemical analysis revealed higher protein in wheat germ, higher fat and ash in rice bran and higher fiber in pomegranate peels. Color investigation showed higher lightness value (74.02) for wheat bran, higher redness value (14.24) for pomegranate peels and higher yellowness value (32.53) for wheat germ. Carrot pomace had the highest water holding capacity (7.49 g/g), as well as swelling capacity (6.12 ml/g). As particle size decrease, the functional properties were significantly (p<0.05) affected. The water and oil holding capacity decreased, while swelling capacity, water solubility index and emulsifying activity increased. Phenolic acids profiles of WB, WG and RB was nearly similar and ferulic acid dominated these profiles. Most of

phenolic acids in these materials were bound. While, the majority of phenolic acids in CP were found to be free. Gallic and protochatchuic acids dominated the phenolic acids in CP. Each gram of PP contained 851.48, 274.68 and 1744.69 µg free, conjugated and bound phenolic acids, respectively. Gallic acid dominated the free and bound forms, while catachine dominated the conjugated form. Ultrafine grinding and fermentation apparently increased the free, conjugated and bound forms of most identified phenolic acids.

The results of antioxidants analysis (phenols, flavonoids and carotenoids) and antioxidant activity conducted on successive extracts showed that ultrafine grinding did not significantly affect these parameters in WB, WG, RB or CP. Only antioxidants and antioxidant activity of NPP significantly increased. Also, fermentation process did not significantly alter these parameters in WB, WG or RB, while antioxidants and antioxidant activity of FNCP and FNPP significantly increased. Cytotoxic activity of the prepared extracts from ultrafine ground samples against cancer cell growth increased compared to raw materials. For instance, IC₅₀ value of NPP extract decreased to 1.64 mg/ml. Also, the extracts of fermented-nano materials showed lower IC₅₀ values compared to the extracts of raw and nano forms which indicated that fermentation process increased the anticancer activity of tested materials. The highest effect of fermentation process on the anticancer activity was in FNCP.

Fibers improved the nutritional value of bread but altered the rheological properties of dough, the quality and sensory properties of the final bread product. Regarding the obtained results functional bread suitable for cancer patients could be produced using wheat flour incorporated NWB up to 25%, NWG and FNRB up to 15% and FNCP and FNPP up to 5% with longer shelf life than control samples

Key words: Food by-products, biotechnology, nanotechnology, functional properties, antioxidant activity, anticancer activity, rheological properties and bread.

ACKNOWLEDGMENT

All praises are due to God, who blessed me with those kind professors and colleagues, who gave me the support to produce this thesis.

I would like to express my deep and sincere gratitude to my supervisors **Prof. Dr. El-Sayed I. Y. Abou El Soud** Prof. of Food Sci., Food Sci. Dept., Fac. of Agric., Ain Shams Univ. and **Prof. Dr. Attia A. E. Yaseen** Head of Food and Nutrition Division, National Research Center, who took me on the process of learning and challenged me to set my benchmark even higher. Their knowledge and logical way of thinking has been of great value to me. Their understanding, encouragement and personal guidance provided a good basis for the present thesis.

My sincere thanks also go to **Prof. Dr. Abdel Hfeez A. Shouk** Prof. of Food Sci. and Technol., Food Technol. Dept., National Research Center; **Dr. Mohamed G. E. Gad Allah** Assistant Prof. of Food Sci. and **Dr. Abdel Fattah A. Abdel Fattah** Lecture of Food Sci., Food Sci. Dept., Fac. of Agric., Ain Shams Univ., who gave me the confidence and guidance to explore my research.

I gratefully acknowledge the research and technical staff of the Central Lab of Food and Nutrition Division, National Research Centre for their assistance and support in using the various laboratory and pilot plant equipments. Also, I gratefully acknowledge the National Research Center fund (**Project No. 10090002**) for their financial support to this work. Grateful appreciation is also extended to **all staff members of Food Technology lab**, Food Technology Department, National Research Center, Dokki, Egypt.

Words fail me to express my appreciation to **my parents** for their support and help for me through my life and my study, as well as to **my dear brother** and **sisters** for their support in all my life. Also, I would thank **my wife** and **my Daughters** for their patience and help to complete my work.

CONTENTS

LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF ABBREVIATIONS	X
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	5
2.1. Functional foods	5
2.1.1. Prebiotics	7
2.1.2. Bioactive phytochemicals and functional foods	8
2.2. Comparative evaluation of antioxidant activity assay	10
2.2.1. Radical ABTS scavenging activity	12
2.2.2. Radical DPPH scavenging activity	12
2.2.3. Ferric reducing antioxidant power assay (FRAP)	13
2.3. Food by-products as potential natural sources of	
prebiotics and antioxidants	13
2.3.1. Cereal by-products as a source of antioxidants	14
2.3.2. Carrot pomace as a source of antioxidants	19
2.3.3. Pomegranate peels as a source of antioxidants	20
2.4. Antioxidants: their role in disease prevention	23
2.4.1. Anticancer potential of phytochemicals	24
2.4.1.1. Anticancer activity of wheat bran	26
2.4.1.2. Anticancer activity of wheat germ	27
2.4.1.3. Anticancer activity of rice bran	28
2.4.1.4. Anticancer activity of carrot pomace	30
2.4.1.5. Anticancer activity of pomegranate peel	31
2.5. Challenges facing use of food by-products	32
2.5.1. Solid-state fermentation of by-products	33
2.5.2. Ultrafine grinding of by-products	36
2.6. Rheological properties of wheat flour containing	
antioxidant sources	39
2.7. Application of food by-products in functional bakery	

products	45
3. MATERIALS and METHODS	49
3.1. MATERIALS	49
3.1.1. Wheat flours, wheat bran and wheat germ	49
3.1.2. Rice bran	49
3.1.3. Carrot and pomegranate	49
3.1.4. Yeast and other baking ingredients	49
3.1.5. Phenolic acids standards	49
3.1.6. Radical precursor and folin	49
3.1.7. Solvents and other chemicals	50
3.2. METHODS	50
3.2.1. Preparation of raw materials	50
3.2.1.1. Stabilization of wheat germ and rice bran	50
3.2.1.2. Preparation of carrot pomace	50
3.2.1.3. Preparation of pomegranate peel	51
3.2.2. Solid-state yeast fermentation	51
3.2.3. Preparation of nano and fermented-nano materials	52
3.2.4. Physico-chemical analysis	52
3.2.4.1. Transmission Electron Microscopy	52
3.2.4.2. Color measurements	52
3.2.4.3. Chemical composition	52
3.2.5. Functional properties	53
3.2.5.1. Water holding capacity	53
3.2.5.2. Swelling capacity	53
3.2.5.3. Water solubility index	53
3.2.5.4. Oil holding capacity	54
3.2.5.5. Emulsifying activity and emulsion stability	54
3.2.6. Determination of phenolic acids profile	55
3.2.6.1. Free phenolic acids	55
3.2.6.2. Conjugated phenolic acids	55
3.2.6.3. Bound phenolic acids	56
3.2.6.4. HPLC analysis of phenolic acids	56

3.2.7. Preparation of successive extracts molasses	57
3.2.8. Determination of major phytochemicals in prepared	
extracts	57
3.2.8.1. Determination of total phenolic content	57
3.2.8.2. Determination of total flavonoid content	58
3.2.8.3. Determination of total carotenoids	58
3.2.9. Determination of antioxidant activity of prepared	
extracts	59
3.2.9.1. Determination of radical DPPH scavenging activity	59
3.2.9.2. Determination of radical ABTS scavenging activity	59
3.2.9.3. Ferric reducing activity power (FRAP) assay	60
3.2.10. Cytotoxic effect of prepared extracts on human cell	
line (HCT 116)	60
3.2.11. Preparation of functional formulas	61
3.2.12. Mixolab properties of prepared formulas	62
3.2.13. Processing of pan bread	64
3.2.14. Processing of balady bread	65
3.2.15. Physical measurements of pan bread	65
3.2.16. Sensory evaluation of bread	66
3.2.16.1. Sensory evaluation of pan bread	66
3.2.16.2. Sensory evaluation of balady bread	66
3.2.17. Texture properties of bread crumb	66
3.2.18. Freshness of pan and balady bread	68
3.19. Statistical analysis	69
4. RESULTS and DISCUSSION	70
4.1. Particle size analysis	70
4.2. Chemical composition of raw materials as affected by	
fermentation and ultrafine grinding	72
4.3. Color quality as affected by fermentation and ultrafine	
grinding	74
4.4. Functional properties as affected by fermentation and	77
ultrafine grinding	77
4.4.1. Hydration properties	77

4.4.2. Water solubility index (WSI)	80
4.4.3. Oil-holding capacity	82
4.4.4. Emulsifying properties	83
4.5. Phenolic acids profiles of raw, nano and fermented-nano	
materials	85
4.5.1. Phenolic acids profiles of cereal by-products	85
4.5.2. Phenolic acids profiles of carrot pomace	94
4.5.3. Phenolic acids profiles of pomegranate peels	97
4.6. Successive extraction yields of raw, nano and fermented-	
nano materials	100
4.7. Phytochemical analysis	102
4.7.1. Total phenolic content	102
4.7.2. Total flavonoids content	104
4.7.3. Total carotenoids content	104
4.8. Antioxidant activity of raw, nano and fermented-nano	
materials	106
4.8.1. DPPH radical scavenging activity	106
4.8.2. ABTS radical scavenging activity	107
4.8.3. Ferric ions reducing antioxidant power assay	109
4.9. Cytotoxic activity of raw, nano and fermented-nano	
materials	110
4.10. Correlation between antioxidants, antioxidant activity	
and cytotoxic activity of raw, nano and fermented-nano materials	112
4.11. Rheological properties of formulated dough	112
4.11.1. Mixing properties of wheat flour dough as affected by	11-
addition of nano and fermented-nano materials	114
	112
4.11.2. Pasting behavior of wheat flour dough as affected by	101
addition of nano and fermented-nano materials	121
4.11.3. Mixolab profiles of wheat flour dough as affected by	10
addition of nano and fermented-nano materials	124
4.12. Production of functional bread	127
4.12.1. Production of pan bread	127

4.12.1.1. Physical measurements of pan bread as affected by	
addition of nano and fermented-nano materials	127
4.12.1.2. Organoleptic characteristics of pan bread as	
affected by addition of nano and fermented-nano	
materials	130
4.12.1.3. Texture profile analysis of pan bread as affected by	
addition of nano and fermented-nano materials	132
4.12.1.4. Color properties of pan bread as affected by	
addition of nano and fermented-nano materials	138
4.12.2.3. Freshness properties of pan bread as affected by	
addition of nano and fermented-nano materials	142
4.12.2. Production of balady bread	146
4.12.2.1. Organoleptic characteristics of balady bread as	
affected by addition of nano and fermented-nano	
materials	147
4.12.2.2. Color properties of control and functional balady	
bread	150
4.12.2.3. Freshness properties of balady bread as affected by	
addition of nano and fermented-nano materials	153
5. SUMMARY AND CONCLUSION	157
6. REFERENCES	170
7. ARABIC SUMMARY	

LIST OF TABLES

No	TITLE	Pa
1	Prominent types of functional food	
2	Proximate chemical composition of raw, nano and fermented-nano-materials	7
3	Color attributes of raw, nano and fermented-nano materials	-
4	Phenolic acids profile of raw, nano and fermented-nano wheat bran	8
5	Phenolic acids profile of raw, nano and fermented-nano wheat germ	Ģ
6	Phenolic acids profile of raw, nano and fermented-nano rice bran	Ģ
7	Phenolic acids profile of raw, nano and fermented-nano carrot pomace	Ģ
8	Phenolic acids profile of raw, nano and fermented-nano pomegranate peels	Ģ
9	Phytochemicals of raw, nano and fermented-nano-materials	1
10	Antioxidant activity of raw, nano and fermented-nano-materials	1
11	Cytotoxic activity of raw, nano and nano-fermented materials	1
12	Correlation coefficient of antioxidants, antioxidant activity and cytotoxic activity of raw, nano and fermented-nano materials	1
13	Mixing properties of wheat flour dough as affected by addition of nano and fermented-nano materials	1
14	Pasting behavior of wheat flour dough as affected by addition of nano and fermented-nano materials	1
15	Mixolab profiles of wheat flour dough as affected by addition of nano and fermented-nano materials	1
16	Baking quality of pan bread as affected by addition of nano and fermented-nano materials	1

17	Organoleptic characteristics of pan bread as affected by addition of nano and fermented-nano materials	131
18	Texture profile parameters of pan bread as affected by	
	addition of nano and fermented-nano materials	133
19	Color attributes of pan bread crust as affected by addition	
	of nano and fermented-nano materials	140
20	Color attributes of pan bread crumb as affected by	
	addition of nano and fermented-nano materials	141
21	Alkaline water retention capacity of pan bread as	
	affected by addition of nano and fermented-nano	
	materials	144
22	Organoleptic characteristics of balady bread as affected	
	by addition of nano and fermented-nano materials	148
23	Color attributes of balady bread crust as affected by	
	addition of nano and fermented-nano materials	151
24	Color attributes of balady bread crumb as affected by	
	addition of nano and fermented-nano materials	152
25		132
43	Alkaline water retention capacity of balady bread as	
	affected by addition of nano and fermented-nano	
	materials	155

LIST OF FIGURES

No	TITLE	Page
1	Examples of naturally occurring phenolic acids	9
2	Examples of naturally occurring flavonoids	10
3	Polyphenol-protein complex formation	40
4	Preparation of raw materials	51
5	Phenolic acid extraction procedure	56
6	Description of a typical curve obtained from the Mixolab	64
7	Calculations of texture profile analysis	67
8	Transmission electron micrographs of, nano and	
	fermented-nano powder	71
9	Hydration properties of raw, nano and fermented-nano	
	materials	78
10	Water solubility index of raw, nano and fermented-nano	
	materials	81
11	Oil-holding capacity of raw, nano and fermented-nano	
	materials	82
12	Emulsifying properties of raw, nano and fermented-nano	
	materials	84
13	HPLC chromatograms of typical fractions of wheat bran.	89
14	HPLC chromatograms of typical fractions of wheat germ	91
15	HPLC chromatograms of typical fractions of rice bran	93
16	HPLC chromatograms of typical fractions of carrot	
	pomace	96
17	HPLC chromatograms of typical fractions of	
	pomegranate peels	99
18	Successive extraction yield of raw, nano and fermented-	
	nano materials	102
19	Mixograms of wheat flours (left) and wheat flour	
	incorporated with nano wheat bran (NWB) (right)	118

20	Mixograms of wheat flour 72% incorporated with nano	
	wheat germ (NWG) (left) and fermented-nano rice bran	
	(FNRB) (right)	119
21	Mixograms of wheat flour 72% incorporated with	
	fermented-nano carrot pomace (FNCP) (left) and	
	fermented-nano pomegranate peel (FNPP) (right)	120
22	Mixogram profiles of wheat flour dough as affected by	
	addition of nano and fermented-nano materials	126
23	Photographs of pan bread as affected by addition of nano	
	and fermented-nano materials	129
24	Texture profiles of pan bread made from wheat flour	
	with different extraction (left) and wheat flour 72%	
	incorporated nano wheat bran (NWB) (right)	134
25	Texture profiles of pan bread made from wheat flour	
	72% incorporated nano wheat germ (NWG) (left) and	
	fermented-nano rice bran (FNRB) (right)	135
26	Texture profiles of pan bread made from wheat flour	
	72% incorporated fermented-nano carrot pomace	
	(FNCP) (left) and fermented-nano pomegranate peel	
	(FNPP) (right)	136
27	Loss of freshness in pan bread during storage as affected	
	by addition of nano and fermented-nano materials	145
28	Photographs of balady bread as affected by addition of	
	nano and fermented-nano materials	149
29	Loss of freshness in balady bread during storage as	
	affected by addition of nano and fermented-nano	
	materials	156