





BIOCHEMICAL AND TECHNOLOGICAL EVALUATION OF FLAXSEED IN PRODUCTION OF SOME FUNCTIONAL FOODS

By

REHAM SAYED ABD EL-SALAM EISSA B.Sc. Agric. Sci. (Food Technology), Fac. Agric., Cairo Univ., (2004) M.Sc. Agric. Sci. (Food Technology), Fac. Agric., Cairo Univ., (2014)

> THESIS Submitted in Partial Fulfillment of The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

AGRICULTURAL SCIENCE (Food Technology)

Department of Food Technology Faculty of Agriculture Benha University

2019

CONTENTS

No.	Title	Page
INTROD	DUCTION	1
REVIEV	V OF LITERATURE	5
	ed and defatted flaxseed: general perspectives and	15
2. Physical	properties of flaxseed	8
3. Function	nal properties of flaxseed flours	10
4. Chemica	al composition and nutritional attributes of flaxseed	13
4.1. Proxin	nate composition of flaxseed flours	13
4.2. Minera	als content of flaxseed flours	15
U	cally bioactive compounds of flaxseed and its health	
5.1. Fatty a	acid composition of flaxseed	17
5.2. Flaxse	ed as a source of protein	20
5.3. Flaxse	ed as a source of dietary fiber	23
5.4. Flaxse	ed as a source of lignans (phenolic compounds)	26
6. Phytoche	emicals and antioxidant activity of flaxseed	28
7. Anti-nu	tritional factors of flaxseed	33

8. Utilization of flaxseed as foods	36
9. Flaxseed products	39
10. Flaxseed protein isolate (FPI) and its potential applications in foods	43
10.1. Functional properties of flaxseed protein isolate (FPI)	45
10.1.1. Solubility	45
10.1.2. Water holding capacity (WHC) and fat absorption capacity (FAC)	46
10.1.3. Emulsifying properties of flaxseed protein isolate (FPI)	47
10.1.4. Foaming capacity and stability	48
3. MATERIALS AND METHODS	49
3.1. Materials	49
3.1.1. Flaxseed sample	49
3.1.2. Chemicals	49
3.1.3. Carcinoma cell lines	50
3.1.4. Baking ingredients	50
3.2. Methods	50
3.2.1. Determination of physical properties of flaxseed	50

3.2.3. Preparation of flaxseed protein isolate (FPI)	53
3.2.4. Determination of physical and functional properties of flaxseed flour	54
3.2.5. Proximate chemical analysis of flaxseed flours	56
3.2.5.1. Determination of chemical composition	56
3.2.5.2. Determination of minerals content	57
3.2.5.3. Determination of amino acid profiles	57
3.2.5.4. Fatty acids compositions	57
3.2.5.5. Determination of physiochemical characteristics of flaxseed oil	58
3.2.5.6. Determination of dietary fibers and fiber fractions	58
3.2.5.6.1. Determination of soluble and insoluble dietary fiber	59
3.2.6. Fractionation of flaxseed flour protein from defatted meal	60
3.2.7. Determination of protein digestibility (<i>in vitro</i>)	62
3.2.8. Determination of anti-nutritional factors	63
3.2.9. Determination of phytochemical profiles (biological active compounds)	66
3.2.9.1. Determination of total phenolic content (TPC)	66
3.2.9.2. Fractionation of phenolic compounds	67

3.2.9.3. Determination of total flavonoid content (TFC)	68
3.2.9.4. Determination of tannins content	68
3.2.9.5. Determination of carotenoids	69
3.2.9.6. Determination of tocopherols	69
3.2.9.7. Determination of antioxidant activity by using DPPH radical scavenging method	70
3.2.9.8. Identification of flaxseed lignans by HPLC	71
3.2.10. Anticancer activity of flaxseed	72
3.2.10.1. Preparation of flaxseed lignans extracts	72
3.2.10.2. In vitro cytotoxicity (SRB assay)	72
3.2.11. Characterization of flaxseed protein (FPI)	73
3.2.11.3. Utilization of FPI as a partial substitute in pasta processing	75
3.2.12. Technological investigation	77
3.2.12.1. Production of flaxseed products	77
3.2.12.1.1. Preparation of flaxseed crackers (flaxseed as a flour replacer)	77
3.2.12.1.2. Preparation of flaxseed chocolate brownies (flaxseed as a fat replacer)	78
3.2.12.2. Proximate chemical analysis of flaxseed products	79

3.2.12.4. Sensory evaluation of flaxseed product	80
3.2.12.5. Microbiological analysis of flaxseed products	81
3.2.12.6. Shelf-life study of flaxseed products	81
3.2.13. Statistical analysis	83
4. RESULTS AND DISCUSSION	84
4.1. Physical properties of flaxseed	84
4.2. Physical properties of flaxseed flours	86
4.3. Functional properties of flaxseed flours	87
4.4. Proximate chemical analysis of flaxseed flours	90
4.4.1. Chemical composition of flaxseed flours	90
4.4.2. Minerals content of flaxseed flours	92
4.4.3. Amino acids composition of flaxseed flours	95
4.4.4. Fatty acids composition of flaxseed flours	97
4.4.5. The physicochemical characteristics of flaxseed oil	99
4.4.6. Dietary fiber content of flaxseed flours	99
4.4.7. Protein fractions of defatted flaxseed flours	101
4.4.8. Protein digestibility and anti-nutritional factors of flaxseed flours	101

4.4.9. Phytochemical profiles of flaxseed flours	104
4.4.10. Identification of phenolic compounds	107
4.4.11. Lignan fractions of flaxseed flours	110
4.4.12. Anticancer activity of ethanolic extracts of defatted and roasted defatted flaxseed	112
4.4.13. Characterization of flaxseed protein isolate (FPI)	118
4.4.13.1. Proximate chemical composition, yield and protein recovery of flaxseed protein isolate (FPI)	118
4.4.13.2. Amino acid composition of FPI	119
4.4.13.3. Functional properties of FPI	122
4.4.13.4. Protein solubility	124
4.4.14. Utilization of FPI as a partial substitute in pasta processing	126
4.4.14.1. Chemical composition of pasta samples substituted with FPI	127
4.4.14.2. Cooking quality of pasta prepared with FPI as a partial substitute	129
4.4.14.3. Dough rheological characteristics of pasta dough using Mixolab	132

4.4.14. 4. Sensory evaluation of pasta samples	135
4.4.15. Technological investigation of flaxseed products	137
4.4.15. 1. Flaxseed crackers as a flour replacer	137
4.4.15.1.1. Proximate chemical composition of flaxseed crackers blends	137
4.4.15. 1.2. Color measurement of flaxseed crackers blends	138
4.4.15.1.3. Effect of storage period at room temperature on sensory evaluation of flaxseed crackers blends	142
4.4.15.1.4. Effect of storage period at room temperature on the microbial quality of flaxseed crackers blends	145
4.4.15.1.5. Effect of storage period at room temperature on moisture content and hardness flaxseed crackers blends	147
4.4.15.1.6. Effect of storage period at room temperature on total phenolic content and antioxidant activity of flaxseed crackers blend	149
4.4.15. 2. Flaxseed brownies as shortening replacer	151
4.4.15. 2.1. Proximate chemical composition of flaxseed brownies	151
4.4.15. 2.2. Color measurement of flaxseed brownies	153
4.4.15.2.3. Effect of storage period at room temperature on sensory evaluation of flaxseed brownies blends	158

4.4.15.2.4. Fatty acid composition of produced brownies blends	160
4.4.15.2.5. Effect of storage period at room temperature on the microbial quality of flaxseed brownies blends	163
4.4.15.2.6. Effect of storage period at room temperature on the peroxide values of flaxseed brownies blends	165
4.4.15.2.7. Effect of storage period at room temperature on the thiobarbituric acid (TBA) values of flaxseed brownies blends	167
SUMMARY	170
CONCLUSIONS AND RECOMMENDATIONS	180
REFERENCES	
ARABIC SUMMARY	

LIST OF TABLES

No.	Title	Page
A.	Brownie formulas	83
1.	Physical properties of flaxseed.	85
2.	Physical properties of flaxseed flours	89
3.	Functional properties of flaxseed flours	89
4.	Chemical composition of flaxseed flours	93
5.	Minerals content of flaxseed flours (mg/100g sample).	94
6.	Amino acids composition of flaxseed flours (g/100g protein)	96
7.	Fatty acids composition of flaxseed (% of total lipid	98
8.	The physicochemical characteristics of flaxseed oil	100
9.	Protein fractions (%) of defatted flaxseed flours	102
10.	Protein digestibility and anti-nutritional factors of flaxseed flours	102
11.	Phytochemicals content (on dwt) and antioxidant activity (AOA) of flaxseed flours	105
12.	Phenolic compounds of defatted and roasted defatted flaxseed flours as mg/g	108
13.	Lignan fractions of defatted and roasted defatted flaxseed flours as mg/100g	111

14.	Inhibition percent of ethanolic extract of defatted flaxseed lignan on different carcinoma cell lines	114
15.	Inhibition percent of ethanolic extract of roasted defatted flaxseed lignan on different carcinoma cell lines.	114
16.	Chemical composition, yield and protein recovery (%) of flaxseed protein isolate (FPI)	119
17.	Amino acids profile of FPI (g/100g sample)	121
18.	Functional properties of FPI	125
19.	Protein solubility (%) of FPI at different pH values	125
20.	Chemical composition of pasta samples substituted with FPI	130
21.	Cooking quality attributes of pasta samples substituted with FPI	131
22	Rheological characteristics of pasta dough samples	134
23.	Sensory scores of pasta samples substituted with FPI.	136
24.	Chemical analysis and caloric values of flaxseed crackers blends	14(
25.	Color parameters (<i>L</i> , <i>a</i> and <i>b</i> values) of flaxseed crackers blends	141
26.	Sensory scores of flaxseed crackers blends during different storage periods	144
27.	Microbial quality (CFU/g) of flaxseed crackers blends during different storage periods at room temperatures.	146

28.	Chemical analysis and caloric values of flaxseed brownies samples	156
39.	Color parameters (L^* , a^* and b^* values) of flaxseed brownies samples	157
30.	Sensory scores of flaxseed brownies blends during different storage periods	159
31.	Fatty acids composition of flaxseed brownies blends (% of total lipid)	162
32.	Microbial quality (CFU/g) of flaxseed brownies blends during different storage periods at room temperature	164

LIST OF FIGURES

No.	Title	Page
1.	HPLC chromatogram of phenolic extracts from roadted defatted flaxseed flour (RDFF)	109
2.	HPLC chromatogram of phenolic extracts from defatted flaxseed flour (DFF)	109
3.	The <i>in vitro</i> cytotoxicity effect of defatted flaxseed lignans extract on HCT	115
4.	The <i>in vitro</i> cytotoxicity effect of rostaed defatted flaxseed lignans extract on HCT	115
5.	The <i>in vitro</i> cytotoxicity effect of defatted flaxseed lignans extract on MCF-7	116
6.	The <i>in vitro</i> cytotoxicity effect of rostaed defatted flaxseed lignans extract on MCF-7	116
7.	IC ₅₀ of ethanolic extracts of defatted and roasted defatted flaxseed on different cell lines	117
8.	The solubility of flaxseed protein isolate as a function of pH	126
9.	Different pasta samples substituted with FPI	128
10.	Flaxseed crackers samples as flour replacer	139
11.	Effect of storage period on moisture content of crackers blends	148
12.	Effect of storage period on hardness of crackers blends	148

13.	Effect of storange period on TPC of crackers sample.	150
14.	Effect of storange period on antioxidant activity (% radical Scavenging activity) of crackers samples	150
15.	Flaxseed brownies samples as shortening replacer	155
16.	Effect of storage period at room temperature on the peroxide values of flaxseed brownies blends	166
17.	Effect of storage period at room temperature on the thiobarbituric acid (TBA) values of flaxseed brownies blends	168

ABSTRACT

The present study was carried out to evaluate the physical, functional, chemical properties, phytochemical profiles as well as antioxidant and anticancer activities of different flaxseed flours i.e. full fat flaxseed flour (FFF), roasted full fat flaxseed flour (RFFF), defatted flaxseed flour (DFF) and roasted defatted flaxseed flour (RDFF). The characterization of flaxseed protein isolate (FPI) from defatted flaxseed was studied. The incorporation of FPI into pasta (at 2.5, 5 and 7% of wheat flour) was assessed relative to pasta control (100% wheat flour). Increasing the percentage of FPI up to 7.5% decreased the overall acceptability scores compared with other pasta samples. The quality and technological characteristics of the flaxseed crackers as partially flour substitution and flaxseed brownies as partially shortening substitution (10, 20 and 30% substitution level) were also evaluated. Results revealed that flaxseed flours especially RFFF and RDFF have a good nutritional and functional profiles. Roasting process was found to be an effective method for reducing anti-nutrients content such as (phytic acid, cyanogenic glycoside and trypsin inhibitors) and improved protein digestability. Results showed that RDFF had significantly the highest content of phytochemical profiles and antioxidant activity. Cytotoxicity effect of lignans ethanolic extracts from defatted and roasted defatted flaxseed on colon carcinoma cell line (HCT) and breast carcinoma cell line (MCF-7) increased as the extract concentration increased. The RDFF had strong anticancer activity toward two tested cell lines (MCF-7 and HCT) with IC₅₀ value (29.0 and 31.4 μ g/ml, respectively). Substitution with RDFF increased significantly the content of protein, fat, ash

and fiber contents of the crackers relative to control. The microbial load obtained of produced flaxseed were under the acceptable limits for a period of 3 months from the date of manufacture. Total phenolic content (TPC) and antioxidant activity (AOA) of flaxseed crackers tended to increase significantly with increasing the addition level of flaxseed and decreased with increasing storage period at ambient temperature $(25\pm5^{\circ}C)$ when compared to control sample. Overall acceptability of fat-replaced brownies samples was decreased by increasing level substitution with RFFF relative to control. Results indicated that linolenic acid percentage (omega-3) was increased via increasing flaxseed level of brownies samples. The obtained microbial load of produced brownies was in acceptable value range without any adverse effect on the qualities of the brownies for a storage period up to 3 weeks at room temperature (25±5°C). Concerning fat quality parameters, it could be noticed that peroxide value increased gradually up to the end of the storage period in all brownies samples. In addition, mean score values for thiobarbituric acid (TBA) showed gradually increased in values for both control and flaxseed brownies samples with increasing storage period up to 3 weeks. TBA values increased with shortening substitution level was increased and the increase was considerably higher in brownies prepared with 30 % RFFF of shortening substitution.

Key words: Flaxseed, defatted and roasted flaxseed flour, bioactive compounds, antioxidant activity, anticancer activity, flaxseed protein isolate, crackers, brownies.