Faculty of Science
Microbiology Department

Fungal Production of Antioxidant Using Soybean Residue: Nutritional and Medical Application

A Thesis
Submitted for the degree of doctor of philosophy of science
(Microbiology)

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APROVAL SHEET

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Approved

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Approved Faculty of Council **Approved of University**

/ / 2015 **/ / 2015**

بسم الله الرحمن الرحيم

اللهُ نُورُ السَّمَاوَاتِ وَالأَرْضِ مَثَلُ نُورِهِ
كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ
الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِن
شَجَرَةٍ مُّبَارَكَةٍ زَيْتُونَةٍ لاَ شَرْقِيَّةٍ وَلاَ غَرْبِيَّةٍ
يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ
عَلَى نُورٍ يَهْدِي اللهُ لِنُورِهِ مَن يَشَاءُ وَيَضْرِبُ
اللهُ الأَمْثَالَ لِلنَّاسِ وَاللهُ بِكُلِّ شَيْءٍ عَلِيمٌ

صدق الله العظيم

سورة النور الايه رقم ٣٥-٣٦

اهداء

إلى والدي

الحبيب ...

وإلى أمي الغالية ...

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Dedication

To my parents

That seeded my curiosity and desire for knowledge and thanking them for their unlimited effort and invocation that is unquestionable honored.

To my husband

To my sons

EYAD & YOUSSEF

To my Sister and Brothers

Abstract

Okara is the residue obtained from ground soybean after removing the water- extractable fraction used to produce tofu or soymilk. The aim of this study was to evaluate the potential use of okara and to improve the health beneficial properties of soybean waste manufacture product (Okara).

In this study, three fungal isolates were isolated and subjected to the assessment of okara to prove their virulence of pathogenicity, only one fungal isolate was found to be non-toxic and safe to use, this fungal isolate was identified as *Trichoderma harzianum* using traditional mycological method of identification and nucleotide sequence analysis of the inter transcribed spacer region.The screening for the production of antioxidant using okara as substrate was carried out using *Trichoderma harzianum* in addition to a previously provided isolate of *Aspergillus oryzae*. The antioxidants were produced by solid-state fermentation using different solvents (water, ethanol, methanol, acetone and diethyl ether) for extraction of antioxidants in a period of 10 days. In comparison with non- fermented okara, the protein and ash contents increased in all fermented okara samples than non-fermented one; the level of protein increased from 6.2 to 11.1% and from 6.0 to 10.1% using *Aspergillus oryzae* and *Trichoderma harzianum*, respectively. Also, the ash contents increased from 1.03 to 2.6% and from 0.72 to 1.6% using *Aspergillus oryzae* and

Trichoderma harzianum. While, the fat content reduced from 3.0 to 1.4% and from 3.1 to 1.3 %, using *Aspergillus oryzae* and *Trichoderma harzianum*, crude fiber content reduced from 3.4 to 1.5% and from 3.3 to 1.43 %, using *Aspergillus oryzae* and *Trichoderma harzianum*, respectively.

The water extract of the antioxidants revealed a significant increase in the total phenolic compounds ranged from 9.2 to 43.8 mg gallic/g of okara after 5 days of fermentation using *Aspergillus oryzae,* while the total phenolic compounds increased from 6.5 to 27.2 mg gallic/g of okara using *Trichoderma harzianum.* The extraction with diethyl ether revealed the lowest content of total phenolic compound which ranged from 1.5 to 8.7 mg gallic/g of okara using *Aspergillus oryzae* after five days of fermentation. By using DPPH assay and their reducing power the antioxidants activity reveald an increase in all fermented okara samples in comparison with non- fermented one. The HPLC analysis of the water and ethanol extracts of the fermented okara using *Aspergillus oryzae* reveald a higher content of phenolic compounds in water extract; the cholorogenic (60117.6 mg/100g),caffeine (67876.86 mg/100g) and coumarin (45940.7 mg/100g).Also, the fermented okara with *Aspergillus oryzae* had a higher content of isoflavons in water extract ;formentin(863.9 mg /100g),then genistein (434.7 mg /100g) and diadzein(476.1 mg /100g), but in ethanol extract the biochanin was the highest

(831 mg /100g) ,followed by genistein(537.9 mg /100g) and diadzein (517.7 mg /100g). The antimicrobial activity of the fermented okara extracts using *Aspergillus oryzae* and *Trichoderma harzianum*, revealed a remarkable inhibition against all the tested and pathogenic bacterial and fungal species, using disc diffusion method. The assessment of the stability of phenolic extracts in fermented, non fermented okara , BHT and control was carried out on beef burger, the results revealed efficiency of the fermented okara.

Water & ethanol extracts of fermented okara using *Aspergillus oryzae* have been chosen and evaluated as a chemopreventive agent. Water extract of the fermented okara using *Aspergillus oryzae* was the most effective on Colon Carcinoma and Breast Carcinoma cell line, while the ethanol extracts of the fermented okara using *Aspergillus oryzae* was the most effective on Prostate cell line.

Key words: - Okara –*Aspergillus oryzae* –*Trichoderma harzianum* - phenolic compounds - reducing power –antioxidants - cell line – beef burger.

LIST OF ABBREVIATIONS

°C	Degree celsius
AOAC	Association of Official Analytical Chemists,
APHA	American Public Health Association
ABTS	2,2 azinobis 3,3 ethylbenzothiozoline 6-sulfonic acid
ANOVA	Analysis of Variance
AOA	Applied to asses
AS	Absorbance of sample
A St	Absorbance of standard
BHA	Butylated hydroxyl anisole
BHT	Butylatedhydroxyrotoluene
BLAST	Basic local alignment search tool
CFU	Colony forming unit
DPPH	2,2diphenyl -1,picrylhydrazyl
EFOA	Ethanol extract of fermented okara with *Aspergillus oryzae*
EFOT	Ethanol extract of fermented okara with *Trichoderma haraziaum*
ENF	Ethanol extract of non fermented okara
FAO	Food and Agricultural Organization
FC	Folin-Ciocalteau
g	gram
GRAS	Generally recognized as safe
HCT	Colon Carcinoma Cell Line

HPLC	High performance liquid chromatography
hr	Hour
Iμ	Microliter
i TOL	interactive tree of life
Kg	Killogram
LSD	Least Significant differences
m	mass of sample
m.equiv./kg	Milli Marketing/Kilogram
MCF7	Breast Carcinoma Cell Line
mg	Milli gram
mins	Minutes
MIRCEN	Microbial Resource center
mm	Mill meter
Mmol/I	Mill mole
N	Normility
NDGA	Nordihydroguaretic acid
NCBI	National center for biotechnology information
nm	Nano meter
P.V	Peroxide value
PC3	Prostat Carcinoma Cell Line
PDA	potato dextrose agar
PG	Propylgallate
pH	Potential hydrogen
ppm	Part per million

SD	standard deviation
SDS	sodium dodecyl sulphate
SLF	Submerged liquid fermentation
SRB	sulfo-rhodamine – B stain
SSF	Soild State fermentation
T.B.A	thiobarbituric acid value
T.V.B.N	total volatile bases nitrogen
TBHQ	Tertiary butyl hydroquinone
TCA	Trichloroacetic acid
TFS-M	Total phenolic content in methanol extract
TFS-W	Total phenolic content in water extract
TPC	total phenolic content
UFS	un fermented soybean
USDA	United States Department OF Agriculture
V	Volume
WFOA	water extract of fermented okara with *Aspergillus oryzae*
WFOT	water extract of fermented okara with *Trichoderma haraziaum*
WNF	water extract of non fermented okara

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