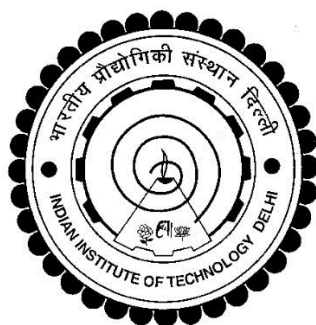


STUDIES ON NATURAL ANTIOXIDANTS FOR EDIBLE OIL PRESERVATION

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CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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STUDIES ON NATURAL ANTIOXIDANTS FOR EDIBLE OIL PRESERVATION

by

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Centre for Rural Development and Technology

Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

OCTOBER 2016

DEDICATED TO ...

MY BELOVED PARENTS

For their continuous effort to encourage me.....

The Almighty who blessed me with the ability and strength to accomplished it.

CERTIFICATE

This is to certify that the thesis entitled “**Studies on Natural Antioxidants for Edible oil Preservation**” being submitted by **Mr. Swapnil G. Jaiswal** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy** is a record of bonafide research work carried out by him under my guidance and supervision in conformity with the rules and regulations of Indian Institute of Technology, Delhi. The research report and results presented in this thesis have not been submitted, in part or in full, to any other university or institute for the award of any degree or diploma.

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(Swapnil G. Jaiswal)

ABSTRACT

Today, interest of peoples towards the consumption of natural antioxidant rich food is growing day by day due to their health promotional activities. On account of this, production of safer and antioxidant rich food is the need of the hour. This can be possible by developing green solvent free extraction processes for antioxidants and knowing chemistry of antioxidants in terms of their applicability in proper food system. Therefore, responsibility on researchers, scientists and food industrialist is increased to provide natural antioxidant rich food to the consumer.

In relation to this, present study has been focused on choosing lipid soluble antioxidant sources for edible oil preservation which is a base of all lipid based food materials. In context to raw material selection, preference has been given to spices (present abundantly) and industrial wastes (big source of natural antioxidants). In spices, ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) were selected due to its lipid soluble nature and good source of antioxidants. Industrial wastes of rice bran processing industry (rice bran oil distillate) and turmeric processing industry (spent turmeric oleoresin) were utilized for recovery of natural antioxidants. In terms of extraction, non-conventional green extraction techniques (supercritical fluid extraction, pressurized liquid extraction and liquid CO₂ extraction) has been used over conventional to get pure extracts rich in antioxidants. On the other hand GRAS solvents has been used for further recovery and purification of antioxidants from resinous part of spices and industrial wastes. After extraction, separated and antioxidant rich extracts were applied in edible oil preservation. Three edible oils namely linseed, sunflower and peanut were selected for stability study.

In the case of ginger, both solvent and supercritical fluid extraction (SFE) methods were used for comparing results of extraction yield and [6]-gingerol content. Result of this study indicate that solvent extraction method gives higher yield of oleoresin (3.85%) in comparison to SFE method. But, concentration of [6]-gingerol was found to be higher in SFE extracts (~40%) at the optimum condition of 250 bar pressure, 20 g/min CO₂ flow rate and 40°C temperature. Pale yellowish colour ginger oil (GO) was also obtained at 100 bar pressure with the yield of 0.93%. GC-MS analysis of ginger oil shows detailed data of chemical composition which contains some predominant pungent compound such as zingiberene, nerolidol, curcumiene

sesquibabinene, rishithin, shogaol and gingerol. Phenolic acid profile for seven phenolic acids namely gallic acid, vanillin, myricetin, rutin, protocatechuic acid, quercetin and kaempferol were also investigated in extracts. After extraction study, solvent extracts of ginger (GE), selected SFE extracts of ginger with ~25% (GE1) and ~40% (GE2) and ginger oil (GO) were used for total phenol (TPC), total flavonoid (TFC), antioxidant and oxidative stability study. Highest TPC and TFC values was observed for GE followed by GE2. Besides lowest IC₅₀ values for highest DPPH and ABTS were observed for GE2 followed by GE and GE1. Same trend of results was observed for percent inhibition activity in both DPPH and ABTS methods. Stability study of all four extracts in selected three oils give positive antioxidant index and comparable results with synthetic antioxidants. Linseed oil was found to be more benefitted with highest antioxidant index. GE and GE2 were found to be best antioxidant in increasing shelf life of all three oils and also observed to be comparable with BHA and BHT.

Turmeric rhizomes were extracted by solvent, SFE and pressurized liquid extraction (PLE) method. Oleoresin yield of solvent extraction method was observed to be higher than both SFE and PLE. But PLE method was found to be suitable for both recovery of oleoresin and turmeric oil by adopting two step separation process. Moreover yield of curcuminoids obtained after optimization of PLE solvents was also observed to be higher than SFE and solvent extraction methods. Turmeric oil (TO) obtain after liquid CO₂ extraction was analysed in GC-MS for its chemical composition. α , β -turmerone (~52%) and Ar-turmerone (13.35%) were observed to be principle chemical constituents. Turmeric oleoresin obtained by solvent (TE1), SFE (TE2) and PLE (TE3) were studied for phenol, flavonoid, antioxidant and stability study. Curcuminoid powder (C) obtained crystallization was characterized through different analytical techniques namely HPLC, HPTLC, NMR and XRD. Results of stability study of TO, TE1, TE3 and C in all three oils were found to be satisfactory at 100-300 ppm concentration. Antioxidant effect of TE1 and C was observed to be higher than BHA and BHT. In terms of oil, sunflower oil was found to be more benefitted by addition of TO, TE1, TE3 and C.

Industrial wastes, rice bran oil distillate (RBDO) and spent turmeric oleoresin (SPTO) were selected for the recovery of γ -oryzanol and curcuminoids recovery. Crystallization and column chromatographic separation process was employed for recovery γ -oryzanol from RBDO. On the other hand, two-step process including liquid CO₂ separation of turmeric oil from SPTO and further crystallization of curcuminoids from remaining oleoresin has been employed in the case of SPTO. Crystallized γ -oryzanol rich concentrate was further studied for phenol,

flavonoid and antioxidant activity. Values TPC and TFC for γ -oryzanol rich concentrate were found to be 2.7 ± 0.18 mg GAE/g and 1.28 ± 0.06 mg QE/g respectively. Results of stability study in all three oils at 100-300 ppm concentration were also gives positive antioxidant index. γ -oryzanol rich concentrate in all three oils give comparable antioxidant index with BHA and BHT except TBHQ.

Overall, present study conclude that extraction of biologically active compounds by using green extraction techniques help to isolate pure extracts without any impurities and also saves time, energy and higher cost of solvents. In addition to this, antioxidant compounds separated from industrial wastes were also proved to be beneficial for value addition in food products. Moreover extracts of ginger and turmeric extracted by SFE and PLE methods also have the potential to protect oil from oxidation. In future, use of synergistic effects of above extracted natural antioxidants (at higher concentration) in above mentioned oils may provide fruitful information by retarding oxidation period of oil.

CONTENTS

Sl. No.	Title	Page No.
	Certificate	i
	Acknowledgement	ii
	Abstract	iv
	Contents	vii
	List of Figures	xi
	List of Tables	xv
	List of Schemes	xvii
	Symbols	xviii
	Abbreviations	xx
Chapter 1	Introduction	1-15
1.1	Background	1
1.2	Spices and industrial by-products/wastes: Source of natural antioxidants	3
1.2.1	Scenario of spices and industrial wastes: India and International	5
1.2.2	Global demand for natural antioxidants	5
1.3	Health potential of spice antioxidants	6
1.4	Role of natural antioxidants in lipid based materials	8
1.4.1	Mechanism of action	8
1.4.2	Use of spices and industrial wastes for preservation of edible oils	10
1.5	Research gaps and aim of the study	12
1.6	Objective of the study	13
1.7	Organisation of the thesis	13
Chapter 2	Literature Review	16-52
2.1	Chemical classes of natural antioxidants	17
2.2	Sources of natural antioxidants	22

2.2.1	Spices	22
2.2.1.1	Ginger	22
2.2.1.2	Turmeric	24
2.2.2	Industrial waste	25
2.3	Extraction of natural antioxidants	26
2.3.1	Conventional extraction methods	27
2.3.2	Non-conventional extraction methods	28
2.4	Natural versus Synthetic antioxidants	37
2.5	Advanced analytical techniques for antioxidants	39
2.6	Legal implications on the usage of antioxidants	43
2.7	Applications of natural antioxidants	46
2.8	Measurement of antioxidants effectiveness in edible oil by rancimat method	49
Chapter 3	Materials and Methods	53-71
3.1	Materials	54
3.1.1	Collection of spices and industrial wastes	54
3.1.2	Chemicals and reagents	54
3.2	Extraction methods and experimental set up	55
3.2.1	Solvent extraction method	55
3.2.2	Liquid CO ₂ extraction method	56
3.2.3	Pressurized liquid extraction method	57
3.2.4	Supercritical fluid extraction method	59
3.3	Separation and purification methods for industrial wastes	61
3.3.1	Rice bran oil distillate	61
3.3.2	Spent turmeric oleoresin	62
3.4	Qualitative and quantitative analysis of antioxidants	64
3.4.1	TLC, PTLC, HPTLC and spectrophotometric analysis	64
3.4.2	High Performance Liquid Chromatography	65
3.4.3	GC and GC-MS	66
3.4.4	NMR, SEM, XRD	67
3.5.5	Phenol, flavonoid and antioxidant study	68

3.5	Rancimat set up for stability study of antioxidants	69
Chapter 4	Studies on natural antioxidants from ginger	72-100
4.1	Extraction methods for natural antioxidants	73
4.1.1	Solvent extraction	73
4.1.2	Supercritical fluid extraction	74
4.2	Chemical investigation of natural antioxidants	80
4.2.1	Analysis of ginger oil by GC-MS	80
4.2.2	Analysis of [6]-gingerol by HPLC	83
4.2.3	Analysis of phenolic acids by HPLC	85
4.3	Determination of antioxidant potential	88
4.3.1	Total phenol and flavonoid content	88
4.3.2	Antioxidant activity	89
4.4	Stabilization of edible oils by using ginger extracts	92
4.4.1	Fatty acid profiling of edible oils by using GC	92
4.4.2	Stability study	95
4.5	Summary and comparison of the study	99
Chapter 5	Studies on natural antioxidants from turmeric	101-136
5.1	Extraction methods for natural antioxidants	101
5.1.1	Solvent extraction	101
5.1.2	Supercritical fluid extraction	102
5.1.3	Pressurized liquid extraction	104
5.2	Separation and purification of curcuminoids	107
5.2.1	Separation of essential oil from spent turmeric oleoresin	109
5.2.2	Crystallization of curcuminoids	113
5.3	Chemical investigation of curcuminoids	114
5.3.1	Spectrophotometric analysis	114
5.3.2	Thin layer chromatography	115
5.3.3	HPTLC analysis	116
5.3.4	Preparative thin layer chromatography	118
5.3.5	Column chromatographic separation	119

	5.3.6 HPLC analysis	121
	5.3.7 ¹ H NMR	124
	5.3.8 XRD analysis	126
	5.3.9 SEM analysis	126
5.4	Determination of antioxidant potential	128
	5.4.1 Total phenol and flavonoid content	128
	5.4.2 Antioxidant activity by DPPH and ABTS methods	130
5.5	Stabilization of edible oils by using turmeric oleoresin extracts	133
Chapter 6	Studies on natural antioxidants from industrial wastes	137-152
6.1	Separation and purification of oryzanol from rice bran oil distillate	137
	6.1.1 Crystallization process	138
	6.1.2 Column chromatography	139
6.2	Qualitative and quantitative analysis of oryzanol	140
	6.2.1 TLC	140
	6.2.2 HPLC	141
6.3	Determination of antioxidant potential	145
	6.3.1 Total phenol and flavonoid content	145
	6.3.2 Antioxidant activity by DPPH and ABTS methods	147
6.4	Stabilization of edible oils by using γ -oryzanol	149
Chapter 7	Conclusions and Future scope	153-160
	References	161-179
	Appendix	180-184
	Bio data	185-188

List of Figures

Figure No.	Title	Page No.
1.1	Historical revolution in natural antioxidants through different stages	03
2.1	Ginger rhizome and ginger oleoresin	23
2.2	Turmeric rhizome, turmeric oil and turmeric oleoresin	25
2.3	Pressure-temperature phase diagram for CO ₂	32
2.4	Strong and weak points of natural and synthetic antioxidants	37
3.1	Schematic diagram of liquid CO ₂ apparatus	56
3.2	Schematic diagram of Pressurized liquid extraction unit	59
3.3	Schematic diagram of supercritical fluid extraction unit	60
3.4	Schematic flow chart of physical refining of crude rice bran oil	61
3.5	Powdered curcuminoids obtained after crystallization process	63
3.6	List of natural and synthetic antioxidants used for stability study	71
4.1	Effect of increasing pressure on changing colour of ginger extracts from pale yellow to dark brown colour (Left to right)	74
4.2	Yield of oleoresin obtained at changing pressure with keeping CO ₂ flow rate 5 g/min and temperature 30°C	77
4.3	Yield of oleoresin obtained at changing pressure with keeping CO ₂ flow rate 10 g/min and temperature 35°C	78
4.4	Yield of oleoresin obtained at changing pressure with keeping CO ₂ flow rate 20 g/min and temperature 40°C	78
4.5	GC-MS profiling of ginger oil (GO)	81

4.6	HPLC chromatogram for [6]-gingerol A. Standard B. Ginger extract	86
4.7	Phenolic acid profile A. Standards B. Ginger extract	87
4.8	DPPH % inhibition activity of ginger extracts	91
4.9	ABTS % inhibition activity of ginger extracts	91
4.10	GC-FID chromatogram for pea nut oil methyl ester	93
4.11	GC-FID chromatogram for sunflower oil methyl ester	94
4.12	GC-FID chromatogram for linseed oil methyl ester	94
4.13	Effect of temperature and concentration of added ginger extracts on stability of linseed oil	95
4.14	Effect of temperature and concentration of added ginger extracts on stability of sunflower oil	96
4.15	Effect of temperature and concentration of added ginger extracts on stability of pea nut oil	96
4.16	Effect of changing antioxidant and oil on Antioxidant Index (AI)	98
4.17	Effect of changing concentration of antioxidants on Antioxidant Index	99
5.1	Process flow chart for adopted two-step extraction process for recovery of turmeric oil and oleoresin from turmeric rhizome powder	103
5.2	Experimental set up of liquid CO ₂ unit for turmeric oil	104
5.3	Sample of turmeric oleoresins collected during SFE, PLE and soxhlet extraction process	107
5.4	Process flow chart for adopted two-step extraction process for recovery of turmeric oil and oleoresin from SPTO	108
5.5	GC-MS profiling of turmeric oil (A)	111
5.6	Spectrophotometric analysis of curcuminoids	115
5.7	Preliminary analysis of oleoresin extracts on TLC plate	116

5.8	HPTLC chromatogram and prepared standard curve for curcumin	117
5.9	Spotting of curcumin standard and samples on HPTLC plate	117
5.10	(A) PTLC separation of individual curcumin, (B) Collection of separated and purified individual curcumin band passing through silica glass column	119
5.11	(A) Experimental set up for column chromatography, (B) TLC plate for individual curcumin component obtained from chromatographic fractions	121
5.12	HPLC chromatogram for curcuminoids (A) Curcumin, (B) Demethoxycurcumin and (C) Bis-demethoxycurcumin	122
5.13	HPLC chromatogram for pure individual curcumin component	123
5.14	¹ H NMR spectrum of (A) Curcumin (B) Bis-demethoxycurcumin	125
5.15	XRD chromatogram for curcumin standard and crystallized curcumin	126
5.16	SEM analysis of turmeric powder (A) Before extraction and (B) After extraction	127
5.17	DPPH % inhibition activity of turmeric extracts	130
5.18	ABTS % inhibition activity of turmeric extracts	131
5.19	Effect of temperature and concentration of added turmeric extracts on stability of linseed oil	133
5.20	Effect of temperature and concentration of added turmeric extracts on stability of sunflower oil	134
5.21	Effect of temperature and concentration of added turmeric extracts on stability of pea nut oil	134
5.22	Effect of changing antioxidant and oil on Antioxidant Index (AI)	135
5.23	Effect of changing concentration of antioxidants on Antioxidant Index	136

6.1	TLC spots for oryzanol standard and eluted fractions of column chromatography	140
6.2	HPLC chromatogram for (A) γ -oryzanol standard, (B) chromatographic fractions of rice bran oil distillate, (C) crude rice bran oil and individual components of γ -oryzanol	141
6.3	Total phenol (TPC) and total flavonoid (TFC) content of crude rice bran oil and oryzanol rich concentrate	145
6.4	DPPH and ABTS % inhibition activity of crude rice bran oil and oryzanol rich concentrate	147
6.5	Comparative stability study of oryzanol with synthetic antioxidants in linseed oil	149
6.6	Comparative stability study of oryzanol with synthetic antioxidants in sunflower oil	150
6.7	Comparative stability study of oryzanol with synthetic antioxidants in pea nut oil	150
6.8	Effect of changing antioxidant and oil on Antioxidant Index (AI)	151
6.9	Effect of changing concentration of antioxidants on Antioxidant Index	152

List of Tables

Table No.	Title	Page No.
1.1	Industrial by-products/wastes: source of natural antioxidants	4
1.2	Use of herbs and spice extracts as a source of natural antioxidants in preservation of edible oils	11
2.1	Different antioxidant classes on the basis of functional group	18
2.2	Major functional group of antioxidants from natural sources	19
2.3	Role of enzymatic and non-enzymatic antioxidants in human body	21
2.4	Effect of changing state of solvents on physical properties	32
2.5	Extraction of antioxidant compounds from commonly used herbs and spices by using different extraction methods and process parameters	34
2.6	Dependency of antioxidant activity on different factors	38
2.7	Permissible limit of additives as antioxidant source allowed by Codex Alimentarius Commission for vegetable oils and fats	45
3.1	Specification of liquid CO ₂ apparatus	56
3.2	Gradient program of solvent system used for γ -oryzanol separation	62
3.3	Mobile phase used in TLC for separation of antioxidant compounds	64
3.4	Operational parameters used for rancimat study	70
4.1	Optimization parameters considered for ginger extraction	74
4.2	Percent recovery of ginger oleoresin obtained through SFE and solvent extraction	76
4.3	GC-MS profile of ginger oil	82

4.4	Recovery of [6]-gingerol rich oleoresin after batch SFE extraction and solvent extraction	84
4.5	Phenolic acid profile of ginger extracts	85
4.6	Total phenol content (TPC), total flavonoid content (TFC) and antioxidant capacities of ginger extracts	89
4.7	Fatty acid profile of selected edible oils for stability study	92
5.1	Percent yield of turmeric oleoresin obtained at varying extraction methods	105
5.2	GC-MS profile of turmeric oil obtained from turmeric powder and SPTO	110
5.3	Percent yield of curcuminoid obtained at varying extraction methods	113
5.4	Percent purity of curcuminoids in SFE, PLE and crystallized powder	118
5.5	Column chromatographic fractions of individual curcumin eluted at different interval	120
5.6	¹ H NMR spectral data of isolated curcumin components	124
5.7	Abbreviated name of turmeric extraction products obtained during different extraction methods	128
5.8	Total phenol content (TPC), total flavonoid content (TFC) and antioxidant capacities of turmeric extracts extracted by different methods	129
6.1	Chemical composition of individual component in γ -oryzanol	142

List of Schemes

Scheme	Title	Page
No.		No.
1.1	Mechanism of lipid oxidation	9
1.2	Action of antioxidants on oxidized lipid molecule	9

Symbols

%	Percent
\$	US dollar
°C	Degree Celsius
µg	Microgram
mg	Milligram
g	Gram
nm	Nanometre
µm	Micrometre
mm	Millimetre
cm	Centimetre
µL	Microliter
mL	Millilitre
L	Litre
SS	Stainless steel
rpm	Revolutions per minute
h	Hour
min	minute
s	Second
Θ	Theta
~	Approximately
GHz	Gigahertz
MHz	Megahertz

KHz	Kilohertz
w/v	Weight/Volume
dw	Dry weight
IC ₅₀	50% inhibition concentration
mM	Millimolar
ppm	Parts per million
Pa-s	Pascal-second
cm ² /s	Square centimetre per second
P	Pressure
T	Temperature
F	Flow rate
t	Time
>	Greater than

Abbreviations

SFE	Supercritical fluid extraction
PLE	Pressurized liquid extraction
UAE	Ultrasound assisted extraction
MAE	Microwave assisted extraction
PUFA	Polyunsaturated fatty acids
Liquid CO ₂	Liquid carbon dioxide
SC-CO ₂	Supercritical carbon dioxide
AO s	Antioxidants
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
TBHQ	<i>tert</i> -Butylhydroquinone
PG	Propyl gallate
EDTA	Ethylenediaminetetraacetic acid
AP	Ascorbyl Palmitate
EQ	Ethoxyquin
US	United States of America
FDA	Food and Drug Administration
GRAS	Generally Regarded As Safe
FSSAI	Food Safety Standards Authority of India
EFSA	European Food Safety Authority
FAO	Food and Agricultural Organization
WHO	World Health Organization

ADI	Acceptable Daily Intake
GMP	Good Manufacturing Practices
RBDO	Rice Bran Oil Distillate
RBDSBO	Refined bleached deodorized soya oil
SPTO	Spent Turmeric Oleoresin
NCR	National Capital Region
DPPH	1, 1-diphenyl-2-picrylhydrazyl
ABTS	2, 2-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid)
FRAP	Ferric reducing antioxidant power
HPLC	High performance liquid chromatography
TLC	Thin layer chromatography
HPTLC	High performance thin layer chromatography
PTLC	Preparative thin layer chromatography
CC	Column chromatography
FC	Flash Chromatography
CE	Capillary Electrophoresis
PLC system	Programmable logic control system
ABPR	Automated back pressure regulator
RRBO	Refined rice bran oil
CRBO	Crude rice bran oil
RP	Reverse phase
GC-FID	Gas chromatography-Flame ionization detector
GC-MS	Gas chromatography-Mass spectrometry
LC-MS	Liquid Chromatography-Mass Spectrophotometry
FTIR	Fourier transform infrared

FAME	Fatty Acid Methyl Ester
EtOH	Ethanol
MeOH	Methanol
IPA	Isopropyl alcohol
DMSO- <i>d</i> ₆	Deuterated dimethyl sulfoxide
NMR	Nuclear magnetic resonance
SEM	Scanning electron microscope
XRD	X-ray powder diffraction
TPC	Total phenol content
TFC	Total flavonoid content
GAE	Gallic acid equivalent concentration
QE	Quercetin equivalent concentration
IP	Induction period
OSI	Oil stability index
AOM	Active oxygen method
DSC	Differential scanning calorimetry
GMP	Good Manufacturing Practices
GO	Ginger oil extracted by liquid CO ₂ method
GE	Ginger oleoresin extracted by soxhlet method
GE1	Ginger oleoresin extracted by SFE method with 25% [6]-gingerol concentration
GE2	Ginger oleoresin extracted by SFE method with 40% [6]-gingerol concentration
TO	Turmeric oil extracted by liquid CO ₂ method

C	Crystallized curcuminoid powder obtained from turmeric oleoresin
TE1	Turmeric oleoresin extracted by soxhlet method
TE2	Turmeric oleoresin extracted by SFE method
TE3	Turmeric oleoresin extracted by PLE (EtOH+IPA) method
Cu	Curcumin
DMC	De-metoxycurcumin
BDMC	Bis-demethoxycurcumin
OMe	Methoxy group