STUDIES ON NATURAL ANTIOXIDANTS FOR EDIBLE OIL PRESERVATION

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by

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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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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 CO_2 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 CO_2 flow rate and 40^oC 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, curcumienen

sesquisabinene, 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 IC_{50} 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 CO_2 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 CO_2 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.

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List of Schemes

Symbols

%	Percent
$	US dollar
^{o}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
Ɵ	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	*tert*-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-picryhydrazyl
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-d_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