

STUDIES ON SPECIALITY MUSHROOM SHIITAKE

SHALINEE



CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

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SHALINEE

CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

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DEDICATED

to

MY PARENTS

CERTIFICATE

*This is to certify that the thesis entitled '**Studies on specialty mushroom Shiitake**' submitted by **Ms. Shalinee** for the degree of **DOCTOR OF PHILOSOPHY** has been prepared under my guidance with the rules and regulations of Indian Institute of Technology Delhi, India. The research report and results presented in the thesis have not been submitted for any degree or diploma in any other institute or university.*

(Prof. Satyawati Sharma)

Date:

Centre for Rural Development and Technology

Place:

Indian Institute of Technology Delhi

Hauz Khas, New Delhi-110016

India

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ABSTRACT

The present scenario of rising population, malnutrition, depleting agricultural land, rise in environmental pollution and need for quality food production in our country is quite challenging. In this context, mushrooms emerge as a promising substitute that can resolve all these issues upto a certain extent. They are higher fungi, rich in protein (providing all the essential amino acids), low in fat, and contains relatively higher amounts of carbohydrates and fiber. Although India is endowed with favorable natural agro-climatic conditions and plenty of agro-wastes that could be exploited for cultivation of diverse mushroom species, yet our country does not have any significant status either as a mushroom producer or as a consumer. Cultivation of only three mushroom species is popular in the country i.e *Agaricus*, *Pleurotus* and *Volvariella*. Shiitake (*Lentinus edodes*), the most popular and third largest cultivated mushroom species in world is known for its immunomodulatory actions and therapeutic implications but has only limited popularity in our country. Despite sporadic research efforts to standardize its cultivation technology in India, it could not reach the commercial level, as available technology is not viable and henceforth it's cultivated and consumption is restricted. The present study was centered on designing a unified approach for the popularization of *L.edodes* by conducting extensive R & D in the area of its cultivation, nutraceutical and nutritional properties, value added product development and consequent awareness generation among people for its wider dissemination and acceptability.

Locally available saw dust substrate was initially screened for its potential to support the growth of shiitake mushroom. Out of all the selected substrates, mixed saw dust substrate supported maximum growth. The saw dust was supplemented with nitrogen sources namely

wheat bran, mustard cake and cottonseed cake at 0, 2, 3, 5, 10, 20%. The analysis of the substrates revealed good amount of nutrients that increased the mushroom yield. Maximum yield of 60.87% was attained in case of 2% cottonseed cake supplementation giving an average increase of 25% over the control. Mushroom fruit bodies obtained from supplemented substrates were found to be rich in proteins, amino acids and minerals (N, P, K, Fe, Zn, Mn) but low in fat and sugar content. Significant degradation of celluloses, hemicelluloses and lignin (quantitatively estimated) was found in spent (having 2% cotton seed cake supplementation) with good manurial values. The supercritical CO₂ extraction revealed increase in the extract yield upon addition of 5% methanol as co-solvent. Also, more no of compounds were present in the supercritical extract as compared to the solvent extract with the predominance of Linoleic acid (41.94%). Extraction and quantification of β -glucans in the fruit bodies was carried out by megazyme kit assay, HPLC, FTIR and NMR studies. Quantification by megazyme conceded highest β -glucan content i.e. 56.71 g/100g. FTIR, HPLC and NMR studies confirmed the presence of β -glucan in the isolated polysaccharides. Nutraceutically enriched fruit bodies were successfully utilized to make value added products like cookies and pickles. Cookies made with 10% shiitake powder were found to be highly acceptable with increased quantity of protein, minerals and β -glucan. Out of the two varieties of pickles developed i.e. sweet and sour and salty, the salty pikles were highly acceptable on sensory parameters. The mushroom based value added products were highly acceptable at the rural villages especially among children over the control cookies. The profit analysis indicated that processing of shiitake into value added products might offer great investment returns over fresh shiitake mushroom and will also augment farmer's income. Field surveys, group discussions, workshops and practical demonstrations were carried out in order to popularize shiitake mushroom. It was observed that there was a lot of interest among people to

adopt mushroom cultivation as an entrepreneurship. An integrated strategy for continual development through mushroom technology for sustainable rural development and to ensure nutritional security is thus presented here.

सार

वर्तमान परिदृश्य में बढ़ती आबादी, कुपोषण, कृषि भूमि की कमी, पर्यावरण प्रदूषण में वृद्धि और गुणवत्ता वाले खाद्य पदार्थों की उत्पादन की आवश्यकता काफी चुनौतीपूर्ण है। इस संदर्भ में, मशरूम एक आशाजनक विकल्प के रूप में उभरते हैं जो इन सभी मुद्दों को कुछ हद तक हल कर सकता है। ये उच्च कवक हैं जो की प्रोटीन में समृद्ध (सभी आवश्यक अमीनो एसिड से युक्त हैं), वसा में कम, और अधिक मात्रा में कार्बोहाइड्रेट और फाइबर से परिपूर्ण हैं। भारत देश में अनुकूल प्राकृतिक कृषि-जलवायु स्थितियों का होना और अनेक प्रकार के कृषि-अपशिष्टों की सम्पन्नता, विविध मशरूम प्रजातियों की खेती के लिए प्रयाप्त है। यह दुर्भाग्यपूर्ण है की बावजूद हर तरह की उपलब्धता के, हमारे देश का अब तक कोई भी स्थान न तो मशरूम की खेती के आधार पर और न ही उसकी खपत के आधार पर बन सका। देश में केवल तीन मशरूम प्रजातियों की खेती लोकप्रिय है। शिटाके (लेंटिनस एडोइस) दुनिया में सबसे लोकप्रिय और तीसरी सबसे मशहूर मशरूम प्रजातियों में से एक है। यह मशरूम अपने इम्यूनोमॉड्यूलेटरी कार्यों और चिकित्सकीय गुणों के लिए जाना जाता है। किन्तु हमारे देश में इसकी बहुत ही सीमित उत्पादन है। साथ ही इसकी खपत भी अधिक नहीं है। वर्तमान अध्ययन शिटाके की लोकप्रियता को बढ़ाने के लिए केंद्रित था, जिसमें इसकी खेती, न्यूट्रास्यूटिकल और पौष्टिक गुणों की वृद्धि और इसके व्यापक प्रसार के लिए, लोगों के बीच जागरूकता पैदा करने के क्षेत्र में व्यापक अनुसंधान किया गया। स्थानीय लकड़ी के बुरादों का चयन किया गया था। यह पाया गया की सभी चयनित सबस्ट्रेट्स में से, मिश्रित लकड़ी का बुरादा सबसे अधिक शिटाके उत्पादन का समर्थन करता है। मिश्रित बुरादे को अलग अलग प्रकार के नाइट्रोजन स्रोत जैसे की गेहूं का चोकर, सरसों का केक और कपास केक के साथ 0, 2, 3, 5, 10, 20% स्तर पर डाला गया। सबस्ट्रेट्स के विश्लेषण ने पोषक तत्वों की अच्छी मात्रा का खुलासा किया जो मशरूम की उपज में वृद्धि करने में सहायक साबित हुआ। सबसे ज़्यादा 60.87% उपज 2% कपास केक के साथ पाया गया जो की कंट्रोल (जिसमें किसी भी नाइट्रोजन स्रोत का उपयोग नहीं किया गया था) उससे औसतन 25% से ज़्यादा था। मशरूमों (जो तीनों तरह के पूरक के प्रयोग से उगाया गया) में यह पाया गया की वे प्रोटीन, एमिनो एसिड और खनिजों (एन, पी, के, आयरन, जिंक मैंगनेसियम) में समृद्ध है जबकि वसा और चीनी में

कम है। सेल्युलोज, हेमिसेल्युलोज और लिग्निन (मात्रात्मक रूप से अनुमानित) में महत्वपूर्ण गिरावट अच्छे मॅनूरियल (खाद बनाने के लिए) वैल्यू के होने की संभावना की पुष्टि हुई। सुपरक्रिटिकल CO₂ निष्कर्षण में 5% मेथनॉल को सह-विलायक के रूप में सबसे अच्छा पाया गया क्योंकि उसके उपयोग ने यील्ड की मात्रा को बढ़ा दिया। सुपरक्रिटिकल निष्कर्षण तकनीकी में सामान्य साल्वेन्ट निष्कर्षण की तुलना में ज्यादा मात्रा में बायोएक्टिव (जैवसक्रिय) कंपाउंड्स पाए गए जिसमें कि सबसे अधिक मात्रा में लिनोलिक एसिड (41.94%) पाया गया। β -ग्लुकान के निष्कर्षण और उसकी मात्रा की परख, मेगाज़ाइम किट, एच.पी.एल.सी (HPLC) एफ़. टी. आई. आर (FTIR), और एन. एम. आर (NMR) अध्ययनों द्वारा किया गया। सबसे ज्यादा β -ग्लुकान की मात्रा मशरूमों में 56.71 ग्राम / 100 ग्राम पाया गया।

सभी अध्ययन तरीकों ने β -ग्लुकान पालिसैकराइड की उपस्थिति की पुष्टि की। इन उगाये हुए मशरूमों से अचार और कुकीज़ बनाये गए। 10% शिटाके पाउडर से बने कुकीज़ प्रोटीन, खनिज और β -ग्लुकान की मात्रा में अत्यधिक स्वीकार्य पाए गए थे। अचार की दो किस्मों में से विकसित खट्टा-मीठा और नमकीन में से, नमकीन को ज्यादा स्वीकार्य पाया गया। गावों में भी सर्वेक्षण में बच्चों के बीच में भी शिटाके निर्मित मशरूम कुकीज़ अधिक पसंद किये गए। लाभ विश्लेषण से यह संकेत मिलता है कि मूल्यवर्धित उत्पादों में शिटाके मशरूम की प्रसंस्करण ताजा शिटाके की तुलना में अधिक निवेश रिटर्न कर सकती है और किसानों की आय भी बढ़ेगी। शिटाके मशरूम को लोकप्रिय बनाने के लिए क्षेत्र सर्वेक्षण, समूह चर्चा, कार्यशालाएं और व्यावहारिक प्रदर्शन किए गए और इस दौरान यह पाया गया कि मशरूम की खेती को उद्यमिता के रूप में अपनाने के लिए लोगों में बहुत रुचि थी। दीर्घकालिक (सस्टेनेबल) ग्रामीण विकास के लिए शिटाके मशरूम की खेती में निरंतर विकास एवं पोषण सुरक्षा सुनिश्चित करने के लिए यह इस शोध में ठोस रणनीति प्रस्तुत की गई है।

CONTENTS

Acknowledgments	i
Abstract	iii
Contents	viii
List of Figures	xiii
List of Tables	xvi
List of Plates	xix
Abbreviations	xxi
CHAPTER 1: INTRODUCTION	1-13
1.1. Background	1
1.2. Global scenario of mushroom production	3
1.3. Cultivation of <i>L.edodes</i> in India: status	8
1.4. Mushrooms as Bio-therapeutics	9
1.5. Bioactive Mushroom Polysaccharides (β - glucan)	10
1.6. Development of functional foods from mushrooms	11
1.7. Mushroom cultivation as a sustainable enterprise for rural sector	12
CHAPTER 2: REVIEW OF LITERATURE	14-44
2.1. Mushroom Production: the global scenario	14
2.2. Mushrooms: ecology and its types	15
2.3. Status of Indian Mushroom Industry	17
2.4. <i>L.edodes</i> (shiitake) mushrooms	20
2.4.1.Cultivation of shiitake	21
2.5. Mushrooms: pool of nutrients	27
2.5.1 Nutritional composition of mushrooms	27
2.5.2.Nutraceutical potential of mushrooms	29
2.6 Lentinan (β glucan) in shiitake mushroom: biological activities and structure	35

2.6.1. <i>Therapeutic activities of Lentinan</i>	36
2.6.2. <i>Extraction of Lentinan (β-glucan)</i>	39
2.7. Mushroom Value added products	41
2.8 Scope of Work	45
2.9 Objectives of the present work	46
CHAPTER 3: MATERIALS AND METHODS	47-80
3.1 Cultivation of mushrooms on various substrates	48
3.1.1 <i>Procurement of cultures and its maintenance</i>	48
3.1.2 <i>Procurement of raw materials</i>	48
3.1.3 <i>Substrate preparation and inoculation</i>	49
3.1.4 <i>Substrate supplementation</i>	49
3.1.5 <i>Harvesting and Data Collection</i>	50
3.2. Substrate analysis	53
3.3. Proximate analysis of mushroom fruit bodies	53
3.4. Estimation of amino acid content in fruit bodies	54
3.5. Estimation of fatty acid content in fruit bodies	55
3.6. Antioxidant profiling of mushroom fruit bodies	56
3.6.1 <i>Preparation of mushroom extracts</i>	56
3.6.2 <i>Determination of total polyphenol content (TPC)</i>	56
3.6.3. <i>Determination of total flavonoid contents (TFC)</i>	57
3.6.4. <i>FRAP (Ferric reducing antioxidant power) assay</i>	57
3.6.5. <i>2,2-Diphenyl-1-picrylhydrazyl radical-scavenging (DPPH) assay</i>	58
3.6.6. <i>Ferrous chelating antioxidant capacity</i>	58
3.7. Antimicrobial profiling of mushroom fruit bodies	59
3.7.1. <i>Preparation of methanolic and aqueous extract</i>	59
3.7.2. <i>Antimicrobial activity</i>	59
3.8. Supercritical studies of <i>L.edodes</i>	60
3.8.1. <i>Supercritical CO₂ extraction</i>	60
3.8.2. <i>Soxhlet extraction</i>	60

3.8.3. GC-MS Analysis	61
3.8.4. Identification of components	62
3.8.5. Antioxidant activities of the supercritical extracts	62
3.9 Quantification and estimation of beta glucans	63
3.9.1.Extraction and purification of polysaccharides	63
3.9.2. Yield of the polysaccharide extracts	63
3.9.3.Antioxidant activities of the isolated polysaccharides	65
3.9.4.Determination of total polysaccharide content	65
3.9.5 HPLC Studies	66
3.9.6 Megazyme assay for estimation of beta glucan	68
3.10 FTIR studies of polysaccharides	71
3.11. NMR studies of the polysaccharides	71
3.12. Development of value added products (cookies and pickles) from <i>L.edodes</i>	71
3.12.1 Procurement of raw materials (cookies)	71
3.12.2. Preparation of cookies	72
3.12.3. Composition of Pickles	73
3.13. Proximate analysis of the value added products	75
3.14 Functional properties of wheat flour and <i>L.edodes</i> powder	76
3.14.1. Bulk density (BD)	76
3.14.2. Water/oil absorption capacity	76
3.14.3. Swelling capacity	76
3.14.4 Physical properties of cookies	77
3.14.5. In vitro Starch Digestibility	77
3.14.6. Antioxidant activity of cookies	77
3.14.7 β glucan content in cookies	78
3.15 Sensory studies	78
3.15.1 Sensory evaluation of cookies	78
3.15.2 Sensory evaluation of pickles	78
3.16 Storage studies of cookies	79
3.17.Economics and Cost benefit analysis	79
3.18. Field level studies	79

3.19. Statistical analysis	79
CHAPTER 4: RESULTS AND DISCUSSION	81-189
4.1 Shiitake cultivation on locally available substrates	81
4.1.1. Spawn preparation	81
4.1.2. Mushroom production on available substrates	82
4.2. Shiitake cultivation using nitrogen supplements	85
4.2.1. Characteristics of substrates	85
4.2.2 Effects of supplementation on Yield and Biological efficiency	89
4.2.3Decadence of complex molecules and manurial value of spent	92
4.3. Analysis of <i>L.edodes</i> fruit bodies	98
4.3.1. Proximate analysis	98
4.3.2. Mineral content in the <i>L.edodes</i> fruit bodies	101
4.3.3. Amino acid content	104
4.3.4. Fatty acid content	106
4.3.5. Beta glucan content in the fruit bodies from best substrate combination	109
4.4. Antioxidant activities of <i>L.edodes</i> mushroom	110
4.4.1. Extract yield using different solvents	111
4.4.2 Total phenolic content (TPC) in the fruit bodies	112
4.4.3 Total Flavonoid content (TFC) in the fruit bodies	114
4.4.4 DPPH (2, 2-Diphenyl-1-picrylhydrazyl radical-scavenging) activity of the fruit bodies	116
4.4.5 FRAP (Ferric reducing antioxidant power) activity of <i>L.edodes</i>	119
4.4.6 ABTS (2,2-azobis-3-ethylbenzthiazoline-6-sulfonic acid) activity of <i>L.edodes</i>	120
4.4.7 Ferrous chelating activity of <i>L.edodes</i>	121
4.5. Supercritical fluid extraction studies	124
4.5.1. Yield by conventional and organic solvents	126
4.5.2 Composition profile (GC-MS analysis) of mushroom extracts	128
4.5.3. Antioxidant profiling of the extracts	135
4.6. Anti-microbial activity of <i>L.edodes</i> extracts	140
4.7. Extraction and Quantification of β Glucans	142

4.7.1. <i>Yield and composition of isolated polysaccharides</i>	143
4.7.2. <i>Estimation of glucans by Megazyme assay in L.edodes fruit bodies</i>	145
4.7.3. <i>EC50 values in the antioxidant properties of the polysaccharides</i>	147
4.7.4. <i>HPLC studies of the determination of β-glucan in the polysaccharides</i>	149
4.7.5. <i>FTIR analysis of the polysaccharides for the beta glucans</i>	152
4.7.6 <i>NMR studies of the polysaccharides for the beta glucans</i>	153
4.8. Value added products from L.edodes mushroom	156
4.8.1 Development of cookies	156
4.8.1.1. <i>Proximate and functional analysis of flour</i>	156
4.8.1.2. <i>Physical analysis of the cookies</i>	158
4.8.1.3. <i>Proximate analysis of cookies</i>	159
4.8.1.4. <i>Mineral content of the developed cookies</i>	160
4.8.1.5. <i>Starch digestibility characteristics of L.edodes cookies</i>	162
4.9. Antioxidant activity of the developed L.edodes cookies	165
4.10 .Beta glucan content in the developed cookies	167
4.11. Sensory analysis of the developed cookies	168
4.12. Storage attributes of the developed cookies with 10% LP	171
4.13. Development of L.edodes pickles	172
4.13.1. <i>Chemical analysis of the pickles</i>	173
4.13.2. <i>Sensory analysis of the pickles</i>	176
4.13.3. <i>Sensory studies of the developed pickles after storage</i>	177
4.14. Economics of L.edodes cultivation and value added products development	179
4.14.1. <i>Cost and returns from L.edodes production</i>	180
4.14.2. <i>Cost of production of L.edodes cookies and pickles</i>	183
4.14.3. <i>Cost benefit analysis of shiitake and its value added products</i>	185
4.15. Field studies among rural population	187
5. SUMMARY, CONCLUSIONS AND FUTURE PERSPECTIVES	190-201
REFERENCES	202-234
ANNEXURE	235
BIO-DATA	236-239

LIST OF FIGURES

Fig No.	Title	Page No.
1.1	Contribution of six major varieties of mushrooms to the world production	4
1.2	World Mushroom Production versus population growth	4
1.3	Major mushroom producing states in India	8
1.4	Mushroom cultivation- A consistent solution for sustainable development	13
2.1	Morphology of mushroom species	16
2.2	Some of the benefits of mushroom cultivation	16
2.3	Major mushrooms under cultivation along with their dominant cultivation areas in India	18
2.4	Different stages of shiitake mycelia growth	24
2.5	Structure of β -1,3 and β -1,6 linkages	31
2.6	Recurrent entity of Lentinan	36
2.7	Series of actions showing the antitumor potential of lentinan	37
3.1	Schematic diagram of the work plan	47
3.2	Schematic diagram showing steps for the supercritical fluid extraction technology of <i>L.edodes</i> mushroom	62
3.3	Scheme of isolation and purification of polysaccharide fractions by hot water (HWP)	64
3.4	Scheme of isolation of polysaccharides by microwave assisted extraction (MWEP)	67
3.5	Determination of β -glucan content in mushrooms by using megazyme kit assay	69
4.1	Days required for complete mycelium running in different saw dust	83

	substrates	
4.2	Time required (days) for fruit body development after primordial initiation on different saw dust substrates	84
4.3	Biological efficiency of <i>L. edodes</i> fruit bodies harvested from SD supplemented with different nitrogenous sources	90
4.4	HPLC chromatogram for amino acid content of <i>L.edodes</i> fruit bodies harvested from saw dust supplemented (SD) with 2%CSC.	106
4.5	Extract yield (%) of <i>L.edodes</i> fruit bodies using different solvents	112
4.6	Total phenolic content (mg GAE/g dw) of <i>L.edodes</i> fruit bodies using different solvents from best substrate combinations in each treatment	113
4.7	Total Flavonoid Content (mg QE/g dw) of <i>L.edodes</i> fruit bodies using different solvents from best substrate combinations in each treatment	115
4.8	DPPH (2,2-Diphenyl-1-picrylhydrazyl radical-scavenging) activity of extracts (A) methanol (B) water; compared to the positive control of butylated hydroxyanisole (BHA) from <i>L.edodes</i> cultivated on different substrate combinations i.e SD+2% CSC, SD+3% MC and SD+5%WB	118
4.9	Effect on ferrous ions of extracts from (A) water; (B) Methanol; compared to the positive control of ethylenediaminetetraacetic acid (EDTA).	123
4.10	Yield % of shiitake extracts using different solvents i.e. SCE and Organic solvents	125
4.11	Chromatograms of the GCMS analysis of the extract obtained by (a) Conventional method using methanol (b) using ethyl acetate and (c) SCE with 5%MeOH	128-129
4.12	Molecular structures of the most abundant compounds found in <i>L.edodes</i> extracts	135
4.13	Antioxidant activity % (AA %) of <i>L.edodes</i> extracts obtained with SCE + co-solvent @ 5% addition and organic solvents.	137
4.14	Comparison between GAE and EC ₅₀ values for the shiitake fractions tested.	139

4.15	HPLC patterns of the extracted polysaccharides from a) Standard beta glucan b) Hot water (HWEP) c) Cold+Hot water extraction (CHWEP) d) Microwave assisted extraction (MWEP)	150-151
4.16	FTIR Analysis of the extracted polysaccharides (SA (microwave assisted extraction), SB (Cold+Hot water extraction) and SC (Hot Water assisted extraction)	153
4.17	¹³ C CP/MAS NMR spectrum of β-D-glucan isolated from <i>L.edodes</i> in D ₂ O	155
4.18	¹ H NMR spectrum of β-D-glucan isolated from <i>L.edodes</i> in D ₂ O	155
4.19	DPPH free radical-scavenging effect of LP incorporated cookies	167
4.20	Sensory scores of <i>Lentinus</i> powder fortified cookies at 0, 5, 10, 15, 20 and 25 % supplementation.	170
4.21	pH values of LSP (Pickle I) and LSSP (Pickle II) up to 25 days	174
4.22	Survey results regarding mushroom cultivation	188
5.1	A holistic sustainable approach for achieving nutritional security, strengthening mushroom industry and waste management through mushroom cultivation	199

LIST OF TABLES

Table No.	Title	Page No.
1.1	Mushroom production in different states of India (2010) in tons	5
2.1	Status of India in world mushroom production (in metric tons)	19
2.2	Trees found suitable for wood log cultivation	25
2.3	Effect of various supplements on the B.E of shiitake mushroom	26
2.4	Proximate composition (on dry weight basis) of some of the cultivated edible mushrooms	30
2.5	Major Nutraceutical potential of some common edible and medicinal mushrooms	31
2.6	Industrial uses of β -glucans	33
2.7	<i>L.edodes</i> extracts against pathogens (antimicrobial activity)	38
2.8	Methods commonly used in the estimation of purity of glucan preparations	40
2.9	Mushrooms based value added products	43
2.10	Overview of some mushroom dietary supplements available world wide	44
3.1	List of plant names locally available (saw dust) used for the cultivation of shiitake	49
3.2	Ingredients used in making shiitake pickles	74
4.1	Number of days required by different strains for mycelium and spawn production	82
4.2	Yield and Biological efficiency of <i>L.edodes</i> on selected sawdust substrates	85
4.3	Chemical composition of the raw substrates and supplements	86

4.4	Substrate formulas (treatments) designed for <i>L.edodes</i> cultivation with nitrogen supplements	88
4.5	Percentage degradation of complex molecules in mushroom spent	95
4.6	Nutrient composition (pre and post) of the best substrates combination (based on their high yield)	97
4.7	Proximate nutrient composition of <i>L.edodes</i> fruit bodies harvested from SD supplemented with nitrogen sources	100
4.8	Mineral content of <i>L.edodes</i> fruit bodies harvested from SD supplemented with nitrogen sources	103
4.9	Amino acid composition (g/100g dw) of <i>Lentinus edodes</i> fruit bodies harvested from best substrate combination	105
4.10	Fatty acids content of <i>L.edodes</i> fruit bodies harvested from best substrate combination	108
4.11	Beta glucan content in fruit bodies of <i>L.edodes</i> from best combination of substrate mixture	110
4.12	Reducing power of extracts (FRAP) from different treatments using water and methanol as extractants	120
4.13	ABTS activity of aqueous and methanol extracts of <i>L.edodes</i>	121
4.14	Relative composition profile, in % peak area, of <i>Lentinus edodes</i> extracts obtained by conventional method (methanol and ethyl acetate and Supercritical fluid extraction (SCE) with 5% methanol co solvent	130-134
4.15	Antioxidant activities of the <i>L.edodes</i> extracts extracted by SFE and conventional method	139
4.16	Antimicrobial activity for the shiitake extracts, evaluated by Inhibitory Zone method	142
4.17	Chemical composition of the polysaccharides extracted by different methods	144
4.18	Total Polysaccharide, total polyphenols and glucan contents of <i>L.edodes</i> polysaccharide extract estimated by megazyme assay	147
4.19	EC ₅₀ values of polysaccharide extracts from various extraction methods in antioxidant properties	148
4.20	Quantification of β -glucan content in extracted polysaccharides using HPLC and Megazyme kit assay	152
4.21	Proximate and functional analysis of wheat flour (WF) and <i>L.edodes</i> powder (LP)	157
4.22	Physical analysis of cookies	159
4.23	Nutritional composition (% dry weight basis) of the cookies fortified with <i>Lentinus</i> Powder (LP)	161

4.24	Mineral composition (% dry weight basis) of the cookies fortified with <i>Lentinus</i> Powder (LP)	162
4.25	Starch digestibility of cookies formulated incorporating <i>L.edodes</i> powder as estimated by in vitro enzymatic hydrolysis (mean $\pm$ SD)	164
4.26	Antioxidant activity of the cookies	166
4.27	Effect of <i>Lentinus edodes</i> supplementation on the β -Glucan content of cookies	168
4.28	Storage characteristics of cookies with 10% LP	172
4.29	Elemental composition of Shiitake mushroom pickles	175
4.30	Sensory scores (on 9 point hedonic scale) for <i>L.edodes</i> pickles	177
4.31	Sensory characteristics of <i>L.edodes</i> mushroom pickles after storage	178
4.32	Costs and returns from <i>L.edodes</i> production under controlled and uncontrolled growth conditions	182
4.33	Investment involved in the production of shiitake cookies and pickles	184
4.34	Profit percentage analysis for the developed <i>Lentinus</i> cookies	186
4.35	Profit percentage analysis for the developed pickles	186

LIST OF PLATES

Plate No.	Title	Page No.
2.1	Commonly cultivated and consumed mushrooms in India	19
2.2	Some of the <i>Lentinus</i> spp	22
2.3	<i>L.edodes</i> grown on (a) naturally on trees (b) artificially on wood logs and (c) on saw dust substrate	23
3.1	(A-N) Different stages of shiitake cultivation: A: Prepared spawn; B: Substrate wetting; C: Mixing of the substrates; D: Filling of the bags; E: Autoclaving of the bags; F: Inoculation of the bags; G: Spawn run; H: Growing mycelium; I: Mycelial coat formation; J: Hardening of mycelial coat; K: Cold shock treatment; L: Primordia initiation; M: Mushroom pin head formation; N: Fruit body formation	51-52
3.2	Megazyme assay: Characteristic pink color indicates presence of β -glucans in the sample while the sample.	70
3.3	Extraction of β -glucans from <i>L.edodes</i>	70
3.4	Cookies developed using <i>L.edodes</i> powder along with wheat flour at various ratios i.e. A=control, B=5%, C=10%, D=15%, E=20%, F=25%	73
3.5	Prepared Shiitake pickles	75
4.1	<i>Lentinus edodes</i> (OE 388) grown on PDA	81
4.2	<i>L.edodes</i> cultivated on (a): SD+5% WB; (b) SD+3% MC and (c) SD+2% CSC	92
4.3	Positive zone of inhibition observed with methanolic and aqueous extracts against (A) <i>Salmonella typhimurium</i> ; (B) <i>Klebsiella pneumonia</i> (C) <i>Staphylococcus epidermidis</i> (D) <i>Lactobacillus acidophilus</i>	140
4.4	Sensory evaluation done at (a) Lab level; (b) Field level at villages of Farrukhnagar, Haryana, India	171
4.5	Different activities in the rural villages for propagation of <i>L.edodes</i> (a): Workshops; (b) Training programmes; (c) Training programme on spawn making; (d) Shiitake and other mushroom products displayed during one of the training programmes in Krishi Vigyan Kendra, Shikhopur, Gurgaon, Haryana, India	189

ABBREVIATIONS

%	percent
°C	Degree Celsius
h	hour
min	minutes
s	seconds
SD	Saw dust
kJ	Kilo Joules
PDA	Potato Dextrose Agar
g	gram
mg	milligram
µg	microgram
L	Litre
mL	milliliter
ppm	Parts per millions
rpm	Revolutions per minute
/	per
cm	centimeter
m	meter
nm	nanometer
mm	millimeter
BE	Biological Efficiency
fw	Fresh weight
dw	Dry weight
LP	<i>Lentinus</i> Powder
WF	Wheat Flour
¹ H	Proton
¹³ C	Carbon-13

N	Nitrogen
C	Carbon
K	Potassium
P	Phosphorus
Fe	Iron
Zn	Zinc
Mn	Manganese
CSC	Cotton seed cake
WB	Wheat bran
MC	Mustard cake
NMR	Nuclear Magnetic Resonance
FTIR	Fourier Transform Infrared Spectroscopy
HPLC	High Performance Liquid Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
MTCC	Microbial Type Culture Collection and Gene Bank
kg	kilogram
Rs	Rupees
ANOVA	Analysis of Variance
DMRT	Duncan's Multiple Range Test
@	at
SCE	Supercritical Extraction
EtAc	Ethyl Acetate
MeOH	Methanol

ABBREVIATIONS

%	percent
°C	Degree Celsius
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min	minutes
s	seconds
SD	Saw dust
kJ	Kilo Joules
PDA	Potato Dextrose Agar
g	gram
mg	milligram
µg	microgram
L	Litre
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BE	Biological Efficiency
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N	Nitrogen
C	Carbon
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P	Phosphorus
Fe	Iron
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Mn	Manganese
CSC	Cotton seed cake
WB	Wheat bran
MC	Mustard cake
NMR	Nuclear Magnetic Resonance
FTIR	Fourier Transform Infrared Spectroscopy
HPLC	High Performance Liquid Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
MTCC	Microbial Type Culture Collection and Gene Bank
kg	kilogram
Rs	Rupees
ANOVA	Analysis of Variance
DMRT	Duncan's Multiple Range Test
@	at
SCE	Supercritical Extraction
EtAc	Ethyl Acetate
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