

**DEVELOPMENT OF FUNCTIONAL FOOD PRODUCT
USING MUSHROOM (*Calocybe indica*) MYCELIA
FERMENTED AGRO-WASTE**

MANDIRA KAPRI



**CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

JULY 2023

© Indian Institute of Technology Delhi (IITD), New Delhi, 2023

**DEVELOPMENT OF FUNCTIONAL FOOD PRODUCT
USING MUSHROOM (*Calocybe indica*) MYCELIA
FERMENTED AGRO-WASTE**

by

MANDIRA KAPRI

Centre for Rural Development and Technology

Submitted

**in fulfillment of the requirements of the degree of philosophy
*to the***



INDIAN INSTITUTE OF TECHNOLOGY DELHI

JULY 2023

DEDICATED TO GAUR-NITAI

CERTIFICATE

This is to certify that the thesis entitled "**Development of Functional Food Product using mushroom (*Calocybe indica*) mycelia fermented agro-waste**" being submitted by **Ms. Mandira Kapri** to the **Indian Institute of Technology Delhi** for the award of "**Doctor of Philosophy**" is a record of bonafide research carried by her. She has worked under our supervision and guidance and has fulfilled the requirements for the submission of the thesis. To the best of our knowledge, the results presented in this thesis have not been submitted in part or full to any other institute or university for the award of any degree or diploma.



(Dr. Satyawati Sharma)

Professor

Centre for Rural Development and Technology
Indian Institute of Technology,
New Delhi, Hauz Khas
New Delhi-110016, India



(Dr. Prem Prakash Srivastav)

Professor

AgFE Department
Indian Institute of Technology
Kharagpur, West Bengal
West Bengal 721302 India

Acknowledgements

It takes me immense pleasure to finally arrive at the moment where I can submit my Ph.D. thesis. Writing each word of this thesis has been fascinating and extremely rewarding. While I look back at the start of my journey, I would like to thank those who have contributed to the final result in many different ways:

To commence with, I take a special moment to thank ‘Gour Nitai’ who were there with me all the times, thick and thin, motivating and inspiring me to never give up. Also, there are a number of people whom I would like to thank for their love, support and efforts.

First of all, I would like to express my sincere and deepest gratitude to my supervisor Prof. Satyawati Sharma for her love, motivation, support and motherly affection. Her immense knowledge, positive criticism, constant encouragement and faith in me helped me sail through my bad times and successfully finish my research work. Her continual inspiration and sweet words of wisdom will always be cherished throughout my life.

I would also like to thank other faculty members: Prof. S. K. Khare, Prof. S. N. Naik, and Dr. Hariprasad P. for their guidance, positive support and facilities for my research work. Support from all the staff members at all times is duly acknowledged. I am especially grateful to Prof. P.P. Srivastav, IIT Kharagpur, for his genuine guidance, and facilities to help me improve the quality of my work.

I sincerely admire the contribution of all my seniors and other project members: Dr. Shalinee, Dr. Monika Jangir, Dr. Ritika Pathak, Dr. Monica Verma, Dr. Pratibha Yadav, Dr. Kanika Tokas and Mr. Ram Kumar for their help and encouragement throughout my research work.

I would like to extend my heartfelt thanks to my friends who made this journey a memorable one. Their constant support and encouragement always motivated me to keep moving throughout my research work. Friends who stood with me all the times, made my tough times lighter and dark times brighter: Dr. Garima Singh, Dr. Sonal Yadav, Aamir Abhay Tiwari, Umesh Singh, Himanshu Arora, Akansha Gupta and also many more whom I have not mentioned here personally including all my roommates from hostel life.

I gratefully acknowledge DST INSPIRE for the financial assistance in form of JRF and SRF fellowships by Govt. of India, New Delhi. I also extend my thankful gratitude to Ravi Saroj, Vijay Setia and Chaman Lal Setia Exports PVT.LTD for the financial help during my research work.

Last, but not the least, words prove a meager media to express my emotional love towards my family. The strongest pillars of my life, my parents, who believed in me since I was born, motivated me to pursue my dreams and always pushed me forward. I admire them for their trust, patience and hope in me. No word of thanks will be enough to express the support I received from my Dad (Late Debabrata Kapri), Mother (Mrs. Sibani Kapri) and brother (Mr. Rajkumar Kapri). They geared me up time to time and always believed in me. I owe a special token of affection and love for the new member of my family, my sister-in-law Nibha Gupta, who not only brightened up my days but also added joy in my life. My life, indeed, is indebted to all my family members for their love, patience, support and sacrifices. I would never have been able to achieve such heights without their blessings.

(Mandira)

Abstract

Mushrooms are edible fungi rich in nutraceuticals possessing various therapeutic benefits like anti-diabetic, anti-proliferative, hepato-protective, anti-hypertensive, anti-angiogenic, anti-depressive, etc. However, cultivation of mushrooms requires a specific substrate, extensive time and labour cost, and hence makes it unsuitable for the commercialization of mushroom fortified products. In this context, the cultivation of mushroom mycelia in agro-industrial wastes under a submerged fermentation process can be a promising alternative. Various agro-industrial wastes (ripe banana peel, green banana peel, orange peel, de-oiled groundnut cake, and de-oiled mustard cake) were screened as potential sources for the development of cost-effective functional food formulations. A novel submerged fermentation technique using *Calocybe indica* for enhancing the nutraceutical attributes of ripe banana peel powder (BPP) and de-oiled groundnut cake powder (DGCP) in combination was tested. An optimized aqueous media (through RSM) containing 6.6 g/l BPP and 7.5 g/l DGCP produced 5.3 g/l dry mycelial biomass of *C. indica* in 10 days. The fermented agro-waste (13.73 g/l, dry wt.) was found to improve its protein (9.37 to 17.06%), fibre (7.47 to 22.12%), total glucan content (11.16 to 27.62%, of which 24.67% were β -glucan content), minerals, total phenols, flavonoids (40.64 to 98.37 mg QE/g with its radical scavenging activity (IC_{50} , 94.86 to 28.43 mg/ml). A reduction in tannin, phytic acid (26.25 to 6.84 mg/100g) and oxalate was also observed after the fermentation process.

Nonetheless, the fermented agro-waste also revealed an increase in Ergosta-compounds, (3.57%) which include Ergosta-5,7,9(11),22-tetraen-3-ol,(3.beta.,22E)- (1.87%), Ergosta-5,7,22-trien-3-ol,(3.beta.,22e)- (1.24%) and Ergosta-7,22-dien-3-ol,(3.beta.,5.alpha.,22E)- (0.46%). Functional attributes were found to get significantly affected by a reduction in water holding capacity (34.29

to 30.97 ml/g), least gelation concentration (18 to 12.5%), bulk density (6.82 to 2.28 g/ml) and swelling volume (9.5 to 6.1 ml/g) with an increase in β -glucan (8 to 24.57%), dispersibility (78.27 to 92.92%), oil holding capacity (0.59 to 1.73 ml/g).

GC-MS analysis confirmed the enriched levels of bioactive compounds namely, (S)-(-)-Perillyl alcohol, α -Longipinene, isozonarol, widdrol, hydroxy valerenic acid, reynosin, β -Eudesmol, lupenone, etc. in fermented agro-waste mixture.

The mushroom mycelia grown in optimized agro-waste induced media was found to get enriched with protein (24.22 – 31.81%), fat (3.48 – 5.91%), fibre (5.46 – 11.35%), ash (4.80 – 6.02%) and β -glucan (22.05 – 34.62), various minerals, amino acids and vitamin D (194.81 μ g/g DW at 25 min to 264.17 at 20 min). The MTT assay of the mushroom revealed an enhanced anti-proliferative activity from 14.51 – 78% of HeLa cell lines when mycelium was cultivated in agro-waste. Nutraceutically enriched PoF-mixed-Ag was utilized for the preparation of two different types of halwa mixes namely suji substituted halwa mix and composite halwa mix. The composition of both the halwa mixes was optimized using hybrid PCA-RSM. The composite halwa was well accepted by the consumers, enriched with protein (22.80% wb), fat (5.75% wb) fibre (5.01% wb), ash (2.11% wb) and β -glucan (12.52 % W/W). The composite halwa was found to be a good source of TPC (476.33 mg GAE/100g), and TFC (521.45 mg QE/100g) and showed the lowest IC_{50} value of 24.52 mg/ml For DPPH scavenging activity. The value-added composite halwa mix was well appreciated by rural consumers (both adults and children). It was observed that mushroom mycelia fortified food products are well accepted by the consumers; however, the umami flavour of the mushroom is not well appreciated. An integrated strategy for the development of *C. indica* mycelia-fortified functional food products has been presented here

for promoting human health, protecting environment utilizing agro industrial waste materials and overall economic development.

सार

मशरूम न्यूट्रास्यूटिकल्स से भरपूर खाद्य कवक है जिसमें विभिन्न चिकित्सीय लाभ होते हैं जैसे मधुमेह विरोधी, एंटी-प्रोलिफेरेटिव, हेपाटो-सुरक्षात्मक, एंटी-हाइपरटेंसिव, एंटी-एंजियोजेनिक, एंटी-डिप्रेसिव इत्यादि। हालांकि, मशरूम की खेती के लिए एक विशिष्ट सब्सट्रेट, व्यापक समय और श्रम लागत की आवश्यकता होती है, और इसलिए यह मशरूम फोर्टिफाइड उत्पादों के व्यावसायीकरण के लिए अनुपयुक्त है। इस संदर्भ में, जलमग्न किण्वन प्रक्रिया के तहत कृषि-औद्योगिक कचरे में मशरूम मायसेलिया की खेती एक आशाजनक विकल्प हो सकती है। विभिन्न कृषि-औद्योगिक अपशिष्टों (पके केले का छिलका, हरे केले का छिलका, संतरे का छिलका, बिना तेल वाली मूंगफली की खली, और बिना तेल वाली सरसों की खली) को लागत प्रभावी कार्यात्मक खाद्य निर्माण के विकास के संभावित स्रोतों के रूप में जांचा गया। पके केले के छिलके के पाउडर (बीपीपी) और तेल रहित मूंगफली केक पाउडर (डीजीसीपी) के संयोजन में न्यूट्रास्यूटिकल गुणों को बढ़ाने के लिए कैलोसाइब इंडिका का उपयोग करके एक नवीन जलमग्न किण्वन तकनीक का परीक्षण किया गया था। 6.6 ग्राम/लीटर बीपीपी और 7.5 ग्राम/लीटर डीजीसीपी युक्त एक अनुकूलित जलीय मीडिया (आरएसएम के माध्यम से) ने 10 दिनों में सी. इंडिका के 5.3 ग्राम/लीटर शुष्क मायसेलियल बायोमास का उत्पादन किया। किण्वित कृषि-अपशिष्ट (13.73 ग्राम/लीटर, सूखा वजन) में प्रोटीन (9.37 से 17.06%), फाइबर (7.47 से 22.12%), कुल ग्लूकन सामग्री (11.16 से 27.62%, जिसमें से 24.67% β -ग्लूकन सामग्री थी), खनिज, कुल फिनोल, फ्लेवोनोइड (40.64 से 98) में सुधार पाया गया। .37 मिलीग्राम क्यूई/जी अपनी कट्टरपंथी सफाई गतिविधि के साथ (आईसी50, 94.86 से 28.43 मिलीग्राम/मिलीलीटर)।

किण्वन प्रक्रिया के बाद टैनिन, फाइटिक एसिड (26.25 से 6.84 मिलीग्राम/100 ग्राम) और ऑक्सालेट में भी कमी देखी गई। बहरहाल, किण्वित कृषि-अपशिष्ट में एर्गोस्टा-यौगिकों में भी वृद्धि देखी गई,

(3.57%) जिसमें एर्गोस्टा-5,7,9(11),22-टेट्राएन-3-ओएल,(3.बीटा.,22ई)- (1.87%), एर्गोस्टा-5,7,22-ट्रायन-3-ओएल,(3.बीटा.,22ई)- (1.24%) और एर्गोस्टा- शामिल हैं। 7,22-डायन-3-ओएल,(3.बीटा.,5.अल्फा.,22ई) - (0.46%)। जल धारण क्षमता (34.29) में कमी से कार्यात्मक विशेषताएं काफी प्रभावित पाई गईं 30.97 मिली/ग्राम), न्यूनतम जमाव सांद्रता (18 से 12.5%), थोक घनत्व (6.82 से 2.28 ग्राम/मिली) और सूजन की मात्रा (9.5 से 6.1 मिली/ग्राम), β -ग्लूकन (8 से 24.57%), फैलाव क्षमता (78.27 से 92.92%), तेल धारण क्षमता (0.59 से 1.73 मिली/ग्राम) में वृद्धि के साथ।

जीसी-एमएस विश्लेषण ने किण्वित कृषि-अपशिष्ट मिश्रण में (एस)-(-)-पेरिलिल अल्कोहल, α -लॉनिपीन, आइसोजोनारोल, विड्रोल, हाइड्रॉक्सी वैलेरेनिक एसिड, रेनोसिन, β -यूडेस्मोल, ल्यूपेनोन आदि बायोएक्टिव यौगिकों के समृद्ध स्तर की पुष्टि की।

अनुकूलित कृषि-अपशिष्ट प्रेरित मीडिया में उगाए गए मशरूम मायसेलिया को प्रोटीन (24.22 - 31.81%), वसा (3.48 - 5.91%), फाइबर (5.46 - 11.35%), राख (4.80 - 6.02%) और β -ग्लूकेन (22.05 - 34.62), विभिन्न खनिज, अमीनो एसिड और विटामिन डी (194.81 μ) से समृद्ध पाया गया। 25 मिनट पर जी/जी डीडब्ल्यू से 20 मिनट पर 264.17)। जब कृषि-अपशिष्ट में माइसेलियम की खेती की गई तो मशरूम के एमटीटी परीक्षण से हेला सेल लाइनों के 14.51 - 78% तक बढ़ी हुई एंटी-प्रोलिफेरेटिव गतिविधि का पता चला। न्यूट्रास्यूटिक रूप से समृद्ध PoF-मिश्रित-एजी का उपयोग दो अलग-अलग प्रकार के हलवा मिश्रणों की तैयारी के लिए किया गया था, अर्थात् सूजी प्रतिस्थापित हलवा मिश्रण और मिश्रित हलवा मिश्रण। दोनों हलवा मिश्रणों की संरचना को हाइब्रिड पीसीए-आरएसएम का उपयोग करके अनुकूलित किया गया था। प्रोटीन (22.80% wb), वसा (5.75% wb) फाइबर (5.01% wb), राख (2.11% wb) और β -ग्लूकन (12.52% w/w) से समृद्ध मिश्रित हलवा उपभोक्ताओं द्वारा अच्छी तरह से स्वीकार किया गया। मिश्रित हलवा टीपीसी (476.33 मिलीग्राम जीई/100 ग्राम), और टीएफसी (521.45 मिलीग्राम क्यूई/100 ग्राम) का अच्छा स्रोत पाया गया

और डीपीपीएच सफाई गतिविधि के लिए 24.52 मिलीग्राम/एमएल का न्यूनतम आईसी50 मूल्य दिखाया गया। मूल्यवर्धित मिश्रित हलवा मिश्रण को ग्रामीण उपभोक्ताओं (वयस्कों और बच्चों दोनों) द्वारा खूब सराहा गया। यह देखा गया कि मशरूम मायसेलिया फोर्टिफाइड खाद्य उत्पादों को उपभोक्ताओं द्वारा अच्छी तरह से स्वीकार किया जाता है; हालाँकि, मशरूम के उमामी स्वाद की अच्छी सराहना नहीं की गई है। सी. इंडिका मायसेलिया-फोर्टिफाइड कार्यात्मक खाद्य उत्पादों के विकास के लिए एक एकीकृत रणनीति यहां प्रस्तुत की गई है। मानव स्वास्थ्य को बढ़ावा देने, कृषि औद्योगिक अपशिष्ट पदार्थों का उपयोग करके पर्यावरण की रक्षा करने और समग्र आर्थिक विकास के लिए।

CONTENTS

Acknowledgments	iii
Abstract	xiii-xv
Contents	v-viii
List of Figures	ix-x
List of Tables	xi-xi
Abbreviations	
Chapter 1: Introduction	1-8
1.1. The worldwide scenario of mushroom cultivation	
Chapter 2: Review of Literature	9-83
2.1. Classification of agro-industrial waste and their processing techniques	
2.2. Bioactive ingredients present in agro-industrial wastes and their health benefits	
2.3. Agro-industrial waste-fortified commercial food products	
2.4. Limitations associated with utilization of raw agro-industrial waste	
2.5. Valorisation techniques of agro-industrial wastes	
2.6. Types of fungal valorisation techniques and their utilization for food product development	
2.7. Fermented agro-industrial waste-induced food products and their health benefits	
2.8. Effect of fermentation on the nutritional and functional attributes of agro-industrial wastes	
2.9. The life cycle of mushroom	
2.10. Different types of the mushroom cultivation process	
2.11. Traditional and valorised substrates used for the cultivation of mushrooms under the SSF process	
2.12. Challenges of Mushroom cultivation and upcoming strategies	
2.13. Mycelium physiology	
2.14. Mushroom mycelium cultivation status	
2.15. Enhancement of nutritional and therapeutic attributes present in mycelium and mushroom	
2.16. Nutraceuticals compounds present in mycelium and mushrooms along with their therapeutic effects	
2.17. Mushroom polysaccharide	
2.18. Therapeutic effects of the mushroom polysaccharides	
2.19. Food products developed from mushroom mycelium and fruit-bodies	
2.20. Umami flavour extracted from mushroom mycelium and fruit-bodies	
2.21. Scope of work	
2.22. Objectives of the present work	
Chapter 3: Materials and methods	84-114
3.1. Cultivation of mushroom mycelium on various substrates	
<i>3.1.1. Procurement of mushroom culture and its maintenance</i>	

<p>3.1.2. Procurement of raw materials</p> <p>3.1.3. Screening of the substrates for the best growth of <i>C. indica</i> mycelia (petri plate studies)</p> <p>3.1.4. Substrate analysis</p> <p>3.1.5. Utilization of the substrates for best <i>C. indica</i> mycelial growth (petri dishes studies)</p> <p>3.1.6. Measurement of linear growth rates of mushroom mycelium on different substrates</p> <p>3.1.7. Morphological studies of <i>C.indica</i> mycelium grown on different substrates (petri-dish studies)</p> <p>3.1.8. Effect of pH and substrate concentration on the growth of mushroom mycelium</p> <p>3.1.9. Experimental design for broth optimization and statistical analysis</p>	
<p>3.2. Characterization of agro-waste mixture prior to and after the fermentation process</p> <p>3.2.1. Proximate analysis</p> <p>3.2.2. Mineral analysis</p> <p>3.2.3. Estimation of amino acid</p> <p>3.2.4. Quantification of beta glucans (β-glucans)</p> <p>3.2.5. Determination of total phenolic content (TPC)</p> <p>3.2.6. Determination of total flavonoid content (TFC)</p> <p>3.2.7. Determination of total phenolic content (TPC)</p> <p>3.2.8. 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging (DPPH) assay</p> <p>3.2.9. Estimation of anti-nutritional attributes</p> <p>3.2.10. GC-MS analysis</p> <p>3.2.11. Identification of components</p> <p>3.2.12. Estimation of colour attributes</p> <p>3.2.13. Crystallographic analysis</p> <p>3.2.14. FTIR analysis</p> <p>3.2.15. Estimation of functional attributes</p> <ul style="list-style-type: none"> a. Swelling and solubility index b. Water holding capacity (WHC) c. Oil holding capacity (OHC) d. Swelling volume (SV) e. Least gelation concentration f. Bulk density <p>3.2.16. Analysis of SEM and estimation of porosity</p> <p>3.2.17. Isolation of exo-polysaccharides from the mycelial broth</p>	
<p>3.3. Comparative analysis of mushroom mycelium grown on agro-waste induced broth and on PDB (taken as control)</p> <p>3.3.1. Proximate analysis</p> <p>3.3.2. Mineral analysis</p> <p>3.3.3. Estimation of amino acid</p> <p>3.3.4. Quantification of beta glucans (β-glucans)</p> <p>3.3.5. Estimation of antioxidant activity</p>	

<p>3.3.9. <i>GC-MS analysis</i></p> <p>3.3.10. <i>Identification of components</i></p> <p>3.3.11. <i>Anti-cancer activity of C. indica mycelium</i></p> <p>3.3.12. <i>Vitamin D estimation of C. indica mycelia(grown in agro-waste and PDB)and in agro-waste mycelia mixture</i></p>	
<p>3.4. Development of functional food products from the fermented agro-waste mixture</p> <p>3.4.1. <i>Optimization of suji substituted agro-waste mixed halwa mix</i></p> <p>3.4.2. <i>Optimization of composite halwa mix</i></p> <p>3.4.3. <i>Proximate analysis of the value added products</i></p> <p>3.4.4. <i>Mineral content estimation</i></p> <p>3.4.5. <i>Estimation of antioxidant activity</i></p> <p>3.4.6. <i>Estimation of in-vitro digestibility of starch</i></p> <p>3.4.7. <i>Estimation of in-vitro digestibility of Protein</i></p> <p>3.5.8. <i>Sensory evaluation of the halwa cooked from developed halwa mix</i></p> <p>3.4.9. <i>Storage studies of the developed halwa mix</i></p>	
<p>Chapter 4: Result and discussion</p>	<p>116-200</p>
<p>4.1. Cultivation of mushroom mycelium on various substrates</p> <p>4.1.1. <i>Procurement of mushroom culture and its maintenance</i></p> <p>4.1.2. <i>Procurement of raw materials</i></p> <p>4.1.3. <i>Screening of the substrates for the best growth of C. indica mycelia (petri plate studies)</i></p> <p>4.1.4. <i>Substrate analysis</i></p> <p>4.1.5. <i>Utilization of the substrates for best C. indica mycelial growth (petri dishes studies)</i></p> <p>4.1.6. <i>Measurement of linear growth rates of mushroom mycelium on different substrates</i></p> <p>4.1.7. <i>Morphological studies of C.indica mycelium grown on different substrates (petri-dish studies)</i></p> <p>4.1.8. <i>Effect of pH and substrate concentration on the growth of mushroom mycelium</i></p> <p>4.1.9. <i>Experimental design for broth optimization and statistical analysis</i></p>	
<p>4.2. Characterization of agro-waste mixture prior to and after the fermentation process</p> <p>4.2.1. <i>Proximate analysis</i></p> <p>4.2.2. <i>Mineral analysis</i></p> <p>4.2.3. <i>Estimation of amino acid</i></p> <p>4.2.4. <i>Quantification of beta glucans (β-glucans)</i></p> <p>4.2.5. <i>Determination of total phenolic content (TPC)</i></p> <p>4.2.6. <i>Determination of total flavonoid content (TFC)</i></p> <p>4.2.7. <i>Determination of total phenolic content (TPC)</i></p> <p>4.2.8. <i>2,2-Diphenyl-1-picrylhydrazyl radical-scavenging (DPPH) assay</i></p> <p>4.2.9. <i>Estimation of anti-nutritional attributes</i></p> <p>4.2.10. <i>GC-MS analysis</i></p>	

<p>4.2.11. Identification of components</p> <p>4.2.12. Estimation of colour attributes</p> <p>4.2.13. Crystallographic analysis</p> <p>4.2.14. FTIR analysis</p> <p>4.2.15. Estimation of functional attributes</p> <p>a. Swelling and solubility index</p> <p>b. Water holding capacity (WHC)</p> <p>c. Oil holding capacity (OHC)</p> <p>d. Swelling volume (SV)</p> <p>e. Least gelation concentration</p> <p>f. Bulk density</p> <p>4.2.16. Analysis of SEM and estimation of porosity</p> <p>4.2.17. Isolation of <i>exo-polysaccharides</i> from the mycelial broth</p>	
<p>4.3. Comparative analysis of mushroom mycelium grown on agro-waste induced broth and on PDB (taken as control)</p> <p>4.3.1. Proximate analysis</p> <p>4.3.2. Mineral analysis</p> <p>4.3.3. Estimation of amino acid</p> <p>4.3.4. Quantification of beta glucans (β-glucans)</p> <p>4.3.5. Estimation of antioxidant activity</p> <p>4.3.9. GC-MS analysis</p> <p>4.3.10. Identification of components</p> <p>4.3.11. Anti-cancer activity of <i>C. indica</i> mycelium</p> <p>4.3.12. Vitamin D estimation of <i>C. indica</i> mycelia(grown in agro-waste and PDB)and in agro-waste mycelia mixture</p> <p>4.4. Development of functional food products from the fermented agro-waste mixture</p> <p>4.4.1. Optimization of suji substituted agro-waste mixed halwa mix</p> <p>4.4.2. Optimization of composite halwa mix</p> <p>4.4.3. Proximate analysis of the value added products</p> <p>4.4.4. Mineral content estimation</p> <p>4.4.5. Estimation of antioxidant activity</p> <p>4.4.6. Sensory evaluation of the halwa cooked from developed halwa mix</p> <p>4.4.7. Storage studies of the developed halwa mix</p>	
Chapter 5: Summery, conclusions and future prospective	201-210
References	211-241
Annexure	242-247

List of Figures

Fig.No.	Title	Page no.
1.1.	Mushroom species cultivated majorly worldwide	4
1.2.	Major mushroom-producing states in India	5
2.1.	Various types of agro-waste processing techniques depending on the final product products	9
2.2.	Possible mechanism of anti-stress and anti-fatigue activity of induced fermented rice bran	23
2.3.	Possible mechanism for ergosterol-induced antitumor activity against cancer cells	25
2.4.	Different stages of the mushroom life cycle	34
2.5.	Physiological structure of mycelium	39
2.6.	A possible mechanism for ergosterol-induced antitumor activity against cancer cells	50
3.1.	Schematic diagram of the work plan	84
3.2.	<i>Calocybe indica</i> (strain 16AIEC) grown on PDA	85
3.3.	Various agro-wastes used for the growth of mushroom mycelium	86
3.4.	Mycelia grown on solo and mixed agro-wastes	88
3.5.	Mycelial growth at 12 th day on varied pH (6-9)	89
3.6.	Process for production of fermented Agro-waste-mycelial mixture	90
4.1.	Time required (days) for complete mycelia growth on various substrates	115
4.2.	Effect of different substrates on the growth pattern (in cm) of mushroom mycelium with respect to time (in days).	120
4.3.	Kinetics of mycelium growth on the selected agro-waste	121
4.4.	SEM micrographs (at magnification 5000 and EHT 20 kV) of <i>C. indica</i> mycelia grown on various substrate	122
4.5.	Mycelial growth obtained at 12 th day on mixed agro-wastes with varying pH	123
4.6.	Response surfaces showing the effect of BPP and DGCP overtime on the yield of <i>C. indica</i> under submerged fermentation	128
4.7.	Anti-oxidant activity of mixed raw and fermented agro-waste	136
4.8.	Anti-nutritional attributes: a. phytic acid, b. oxalates and c. tannin mixed raw and fermented agro-waste	138
4.9.	X-ray diffractogram of mixed raw and fermented agro-waste	146
4.10.	FTIR spectra of various substrates	149
4.11.	Swelling index of mixed raw and fermented agro-wastes	150
4.12.	Solubility index of mixed raw and fermented agro-wastes	151
4.13.	Functional attributes of the mixed raw and fermented agro-wastes.	152
4.14.	Functional attributes of the mixed raw and fermented agro-wastes	153
4.15.	SEM images of the mixed raw and fermented agro-wastes	155
4.16.	Polysaccharides are obtained from the agro-waste broth after the fermentation process	156

4.17.	SEM micrographs of mushroom mycelium grown on PDB and agro-waste	162
4.18.	Effect of mycelia extract in HeLa cell lines by MTT assay	164
4.19.	Effect of mycelia extract in HeLa cell lines by LDH leakage assay	165
4.20.	Fluorescent microscopic image after treatment with mycelia extract against HeLa Cell line by Propidium Iodide staining	165
4.21.	Scree plot for developed halwa mixes	171
4.22.	Correlation between PC 1 scores and (a) colour, (b) taste and (c) mouthfeel (1 st case)	172
4.23.	PCA Biplot	174
4.24	Correlation between PC 1 scores and (a) colour, (b) taste and (c) mouthfeel(2 ND case)	175
4.25.	Response surface plot showing variation of PC 1 score with changing the concentration of sooji and mushroom powder (1 st case)	176
4.26.	Response surface plot showing variation of PC 1 score with cahnging the concentration of ingredients (2 nd case)	178
4.27.	Desirability plot	180
4.28.	Total phenol content of the different halwa mixes	184
4.29.	Total flavonoid content of the different halwa mixes	185
4.30.	Total flavonoid content of the different halwa mixes	186
4.31.	Changes in moisture content of Sooji+MP Halwa mix and composite Halwa during storage	189
4.32.	Changes in FFA content of Sooji+MP Halwa mix and composite Halwa during storage	191
4.33.	Radar chart showing changes in sensory attributes during storage of a. 120 days, b. 150 days and c. 180 days.	195
4.34.	Various activities during the rural interaction and awareness programme	200

List of Tables

Fig.No.	Title	Page no.
2.1.	Bioactive ingredients present in agro-industrial wastes and their potential health benefits	11
2.2.	Agro-industrial waste-fortified commercial food products	14
2.3.	Functional food products obtained from valorised agro-industrial wastes	21
2.4.	Effect of fermentation on nutritional and functional attributes of agro-industrial wastes	29
2.5.	Traditional and valorised substrates used for mushroom cultivation	35
2.6.	Merits and demerits of mushroom cultivation (both mycelium and fruiting bodies)	38
2.7.	Summary of international status of mycelium production	41
2.8.	Summary of national studies on mycelium production	42
2.9.	Enhancement of nutrients during submerged cultivation of mushroom mycelium	44
2.10.	Augmentation of bioactive ingredients during submerged cultivation of mushroom mycelium	46
2.11.	Therapeutic attributes of Vitamin D	48
2.12.	Polysaccharides obtained from various mushrooms along with their therapeutic attributes	52
2.13.	Antimicrobial compounds present in different mushrooms	69
2.14.	Mushroom fruit-bodies-based commercialized functional foods	74
2.15.	Commercial mushroom-based products available in the market	76
2.16.	Compounds responsible for umami flavour in mushroom	78
3.1.	Decoded values of independent variables used in the CCRD experimental design	105
3.2.	Matrix of experimental central composite rotatable design (CCRD) for optimization of Sooji+MP formulation using PCA-RSM	106
3.3.	Decoded values of independent variables used in the Box-Behnken experimental design	107
3.4.	Matrix of Box-Behnken design for optimization of WB+MLP+GBR+MP formulation using PCA-RSM	108
4.1.	Physicochemical composition of the substrates promoted the best mycelial growth	117
4.2.	Mineral composition of the raw agro-wastes	119
4.3.	Kinetic studies of the mycelium on the selected agro-waste	120
4.4.	Matrix of central composite rotatable design (CCRD) for optimization of the growth parameters for C.indica cultivation	124

4.5.	Estimated coefficients of the polynomial model in L-pseudo forms and corresponding ANOVA data representing the relationship between process parameters and response variables.	125
4.6.	Comparative illustration and validation of the numerical optimization process	128
4.7.	Physicochemical compositions of the mixed raw (Mixed-Ag) and fermented agro- wastes (PoF-mixed-Ag) (% on DWB)*	131
4.8.	Mineral compositions of the mixed raw (mixed-Ag) and fermented agro-wastes (PoF- mixed-Ag) (on DWB)*	132
4.9.	Amino acids content of the mixed raw (Mixed-Ag) and fermented agro- wastes (PoF- mixed-Ag) (on DWB)* (mg/100g)	134
4.10.	Bioactives in raw and mixed fermented agro-waste (GC-MS)	140
4.11.	Physicochemical composition of the mushroom fruiting bodies and mycelia grown on PDB and agro-wastes (% on DWB)*	157
4.12.	Mineral composition of mushroom fruiting bodies and mycelia grown on different substrates (on DWB)*	158
4.13.	Enhancement of Vitamin D2 in C.indica mycelium and fruiting bodies content under UV-B exposure	161
4.14.	Matrix of experimental central composite rotatable design (CCRD) for optimization of Sooji+MP formulation using PCA-RSM	166
4.15.	Correlation coefficient (r) among original responses* (1st case)	167
4.16.	Matrix of Box-Behnken design for optimization of WB+MLP+GBR+MP formulation using PCA-RSM	169
4.17.	Correlation coefficient (r) among original responses* (2nd case)	173
4.18.	ANOVA and their estimated coefficients for PC 1 scores (1st case)	176
4.19.	ANOVA and their estimated coefficients for PC 1 scores (2nd case)	178
4.20.	The set of constraints for different parameters for optimizing the formulation made from Sooji+MP	181
4.21.	The set of constraints for different parameters for optimizing the formulation made from WB+MLP+GBR+MP	181
4.22.	Sensory attributes for optimum formulation halwa and standard Sooji halwa	182
4.23.	Physico-chemical composition of various halwa obtained from different halwa mixes.	183
4.24.	Changes in moisture content of Sooji+MP and Composite Halwa with different storage condition	188
4.25.	Changes in FFA content of Sooji+MP and Composite Halwa with different storage condition	189
4.26.	Sensory attributes of Sooji+MP Halwa mix and composite halwa mix during 120 days of storage	192
4.27.	Sensory attributes of Sooji+MP Halwa mix and composite halwa mix	192

	during 150 days of storage	
4.28.	Sensory attributes of Sooji+MP Halwa mix and composite halwa mix during 180 days of storage	193
4.29	Summary of rural interactions and awareness programme	200

ABBREVIATIONS

FAO: Food and Agricultural Organization
SSF: Solid state fermentation
SmF: Submerged fermentation
EPS: Exopolysaccharide
VDR: Vitamin D receptor
LLC: Lewis Lung Carcinoma cells
MDA: Malondialdehyde
ROS: Reactive oxygen species
HYP: Hydroxyproline
TLR: Tolllike receptors
KCR: Kupffer cell receptor
GLUT4: Glucose transporter 4
RIPs: Ribosomal inactivating proteins
GABA: gamma-aminobutyric acid
GI: Glycaemic index
%: percent
°C: degree celcius
h: hour
min: minutes: seconds
PDA: Potato Dextrose Agar
g: gram
mg: milligram
µg: microgramL: Litre
mL: milliliter
ppm: Parts per millions
rpm: Revolutions per minutecm: centimeter
m: meter
nm: nanometer mm: millimeter
dw: Dry weight
N: Nitrogen
C: Carbon

K: Potassium

P: Phosphorus

Fe: Iron

Zn: Zinc

Mn: Manganese

WB: Wheat bran

FTIR: Fourier Transform Infrared Spectroscopy

HPLC: High Performance Liquid Chromatography

GC-MS: Gas Chromatography-Mass Spectrometry

ANOVA: analysis of variance

DMRT: Duncan's Multiple