Process Optimization and Shelf-Life Evaluation of Button Mushroom Sauce

काशी हिन्दू विश्वविद्यालय BANARAS HINDU UNIVERSITY

THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

Master of Science

in

Food Science & Technology

Supervisor

Dr. Durga Shankar Bunkar

Assistant Professor

Submitted by

Nilza Angmo

DEPARTMENT OF DAIRY SCIENCE AND FOOD TECHNOLOGY
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI- 221005
INDIA

ID.No. 19412FST015 **2021** **Enrolment No. 416763**

इंजी. दुर्गा शंकर बुनकर
Er. Durga Shankar Bunkar
सहायक प्राध्यापक
Assistant Professor
दुग्ध विज्ञान और खाद्य प्रौद्योगीकी विभाग
Deptt. of Dairy Science and Food Technology
कृषि विज्ञान संस्थान
Institute of Agricultural Sciences

बनारस हिन्दू विश्वविद्यालय
Banaras Hindu University
वाराणसी – 221005, उत्तर प्रदेश, भारत
Varanasi – 211005, U.P., India
E-mail: dsbunkar@bhu.ac.in
Mobile: +91-9389835175

Ref. No. **……** ***Date*** **……**

CERTIFICATE

To,
The Registrar,
Banaras Hindu University,
Varanasi-221005 (India)

Dear Sir,

I have great pleasure in forwarding the thesis entitled "**Process Optimization and shelf-life evaluation of Button mushroom sauce**" submitted by **Ms. Nilza Angmo, ID. No. 19412FST015, Enrolment No. 416763,** in partial fulfillment of the requirements for the degree of **Master of Science** in **Food Science and Technology**, in the Department of Dairy Science & Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

I certify that the entire scheme of investigation presented herein was planned and carried out solely by the candidate under my guidance. To the best of my knowledge, the data in the thesis are genuine and original.

Thanking you

Forwarded

Yours faithfully,

……
Dr. Durga Shankar Bunkar
(Supervisor)

(Head)

Process Optimization and shelf-life evaluation of Button mushroom sauce

By

Nilza Angmo

Thesis submitted in partial fulfillment of the requirements for the award of the degree of

Master of Science

in

Food Science and Technology

DEPARTMENT OF DAIRY SCIENCE AND FOOD TECHNOLOGY
INSTITUTE OF AGRICULTURAL SCIENCES
BANARAS HINDU UNIVERSITY
VARANASI – 221005 (INDIA)

ID. No. 19412FST015 **2021** **Enrollment No. 416763**

APPROVED BY MEMBERS OF ADVISORY COMMITTEE

Chairman

Dr. D. S. Bunkar
Assistant Professor
Department of Dairy Science and Food Technology
Institute of Ag. Sc., B.H.U., Varanasi – 221005

Members

Dr. Amrita Poonia
Assistant Professor
Department of Dairy Science and Food Technology
Institute of Ag. Sc., B.H.U., Varanasi – 221005

Dr. V.K. Paswan
Assistant Professor
Department of Dairy Science and Food Technology
Institute of Ag. Sc., B.H.U., Varanasi – 221005

External Examiner

ACKNOWLEDGEMENT

I bow my head with great reverence in the pious feet of ***Bharat Ratna Mahamana Pandit Madan Mohan MalaviyaJi****, a man of great vision and founder of the Banaras Hindu University, Varanasi, India, for his lifetime sacrifice and effort in establishing this temple of learning for the millions of student like me.*

I bow my head in great reverence to the omnipotent and omnipresent ***Lord Kashi Vishwanath*** *for their blessings and true love towards me and to every creature resending on this wonderful planet. It is my sublime privilege to express my deep sense of reverence and gratitude to my Supervisor,* ***Dr. Durga Shankar Bunkar,*** *Assistant Professor, Department of Dairy Science and Food Technology, for his inspiring and ingenious guidance, incisive and articulate criticism, cordial discussion, unending zeal and constant encouragement which assisted me to overcome every problem that come in my way during the period of this investigation and preparation of this manuscript. I shall always remain deeply indebted to him for his affectionate glances and generosity bestowed on me.*

Heartfelt Acknowledgement I would like to express to ***Prof. D. C. Rai*** *(Head of the Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, B.H.U. for ensuring the availability of the necessary facilities during this study. I am highly grateful to the member of my advisory committee Dr. Amrita Poonia, Assistant Professor, Department of Dairy Science and Food Technology, Dr. V. K. Paswan, Assistant Professor, Department of Horticulture for their direct and indirect, encouragement and the moral boost during the work.*

I am extremely thankful to my respected teachers, Prof. Anil Kumar Chauhan, Dr. Abhishek Dutt Tripathi, Assitant Professor, Dr. Arvind, Assistant Professor and I am thankful to all the faculty members of Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, B.H.U for their invaluable cooperation and encouragement during my entire course of investigation. They spend their valuable time for me, whenever I needed.

I feel paucity of words to express my gratefulness and warmest regard to all non-teaching staff ,Dr.Shambhu Sonkar, Mr Anand Kumar Singh, Mr. Ram Asare, Mr. Amaresh Singh, Mr. Chandrashekar and other members of Department of Dairy Science and Food Technology for extending their help during this strenuous period.

I have no words to express my feelings about the contribution of my respected father ***Shri Tashi Motup*** *for his blessings, sacrifice, affection, encouragement, moral and financial support throughout my life. I also express my heartfelt regard to my mother* ***Smt. Tashi Dolker*** *whose flawless love, blessings and guidance helper me to overcome all the barriers in life. I also owe to my grandparents* ***Shri Padma Tsultim, Tsewang***

Rigzin, Smt Sonam Kunzom, *and aunt* ***Phunchok Dolma*** *for their endless love, blessings and hope on me.*

I am thankful to my brother ***Mr. Jigmet Stobden*** *and sister* ***Ms. Rinchen Dolma*** *for their constant moral support, love and best wishes throughout my life. I am also thankful to all my family members for their blessings and encouragement without which, I could not have achieved my task.*

It is difficult to express this gratitude I owe to all my friends especially ***Rigzin Smanla, Tashu Bisht, Priyani Dhyani, Namrata Adhikari, Dasaroju Tharun Kumar, Sanya Sharma, Zakarya****, and all my batchmates for their love and affection which always animated me to face the challenge.*

Without the help of seniors none can learn the lesson of life so, heartful and special gratitude to my seniors ***Mr. Uday.***

Last but not least, I record my sincere thanks to all respectable people and teachers who helped & guided me but could not find separate mention. I still solicit their benediction to proceed at every step of a perfect destined life.

Date: …….
Place: *Varanasi* ***(Nilza Angmo)***

CONTENTS

ABBREVIATIONS

%	Percent
/	Per
@	At the rate of
ANOVA	Analysis of variance
AOAC	Association of Analytical Communities
C	Degree Celsius
CFU	Colony Forming Unit
cP	CentiPoise
et al.	et alia (and others)
Fig.	Figure
g	Gram (s)
i.e.	That is
Kcal	Kilo Calorie
KGy	Kilo Gray
L	Litre
Max	Maximum
Min	Minimum
ml	Milliliter
nm	nanometer
No.	Number
rpm	Revolution per minute
RSM	Research Surface Methodology
USDA	United States Department of Agriculture
UV	Ultraviolet
viz.	Namely
β	Beta

LIST OF TABLES

LIST OF FIGURES

Chapter I

INTRODUCTION

Mushroom is considered as one of the most valuable food in the world because of high value of protein containment, unique flavor and its texture **(Dunkwal *et al.,* 2007)**. Button mushroom can be substitute for meat as it contains high value of nutritional value as compare to many others vegetables nutritional value. In many developing countries, mushrooms are meant to meet the unbalance diet of the residing people as a value addition food **(Chang and Miles, 2004)**.

Under the kingdom of Basidiomycetes and Ascomycetes, 25% of the species are categorized under edible mushrooms, 50% under the inedible and the remaining 25% under the poisonous mushrooms **(Chang *et al.,* 1980)**. Mycelium, a vegetative part of fungi, comprises of a system of branching threads, cords like strands which diversify through soil, wood log, compost and /or lignocellulosic material, on which the fungus grows and after this growth period under a favorable suitable condition, the established mycelium produces a fruiting body which is what we call "Mushroom" **(Chang *et al.,* 2004)**.

Mushroom is a macrofungus that is big enough to be seen with the naked eye and can be Hypogeous or Epigeous, with its characteristic fruiting body features **(Chang *et al.,* 1996)**. Mushrooms are the fungi of high protein low fat of good economical value food **(Nazir A Munsbi *et al.,* 2020)**. Fungi are those group of organisms that lack chlorophyll, due to which they lack the ability to directly use the sun rays for the purpose of production **(Nithyatharani *et al.,* 2018)**.

Agaricus bisporus or button mushroom is counted among the one of the most commercially cultivated mushroom around the globe and it accounts for 1/3 of the worldwide edible mushrooms production **(Masoumi *et al.,* 2015)**. The contribution of the button mushroom towards the world mushroom production is about 40-45% **(Flegg, 1992)**.

out of 1200 species of mushroom, only 12 species are grown for food and medicinal purposes across tropical and temperate zones that includes the common mushroom (*Agaricus*), Shiitake (*Lentinus*), Oyster (*Pleurotus*), Straw (*sVolvariella*), Lion's Head or Pom Pom (*Hericium*), Ear (*Auricularis*), Ganoderma (*Reishi*), Maitake (*Grifola frondosa*), Winter (*Flammulina*), White jelly (*Tremella*), Nameko (*Pholiota*), and Shaggy Mane mushrooms (*Coprinus*) **(Kumar *et al.*, 2015)**.

(Bakowski *et al.*, 1986) The major minerals found in button mushroom are phosphorus, potassium and sodium followed by**)** Ca, Mg, Na, Fe, and Zn **(Guillamon *et al.*, 2010: Falandysz and Borovicka, 2013)**. **(Manzi *et al.*, 2001)** Also it a great source of vitamins (niacin, folate and B2) and essential amino acids. **(Beelman *et al.*, 2003)** Therapeutic compounds namely glycoprotenis, natural antibiotics, triterpenoids, enzymes and enzymes inhibitors are also present in mushrooms which strengthens the human immunity.

It is a low temperature crop which required 23±20℃ for its vegetative growth and 16 ± 20℃ for its fruiting **(Singh, 2011)**. The shelf life of the button mushroom is very short as it starst deteriorating within a day after its harvest and it is because of its high moisture content nature. The moist content in the button mushroom is around (85-90%) hence its very perishable in nature. The deterioration of the button mushroom is developed due to the enzymatic action of polyphenol oxidase on phenolic substance which leads to development of brown color **(Dunkwal *et al.*, 2007)**. **(Shukla and Singh, 2007; and Dekhordi, 2010)** Enzymatic action and microbiological activities can be reduced by adopting some of the dehydrating drying techniques.

Processing techniques such as canning and drying have been adopted on the commercial scale basis to prevent the immediate deterioration and to extend the shelf life of the button mushroom **(Kumar *et al.*, 2008)**. But, as per **(Shukla and Singh, 2007),** drying is one of the easiest ways to reduce the moisture content and to increase the shelf life of the button mushroom.

Mushrooms consist of high molecular weight (HMW) and low molecular weight (LMW) natural bioactive compounds. Under HMV compound metabolites such as peptides and proteins are included largely and LMV compound consists of metabolites such as sesquiterpenes and other terpenes, steroids, anthraquinone and benzoic acid derivatives, and quinolones **(Alves *et al*., 2012)**. According to **(Boa *et al*., 2004),** as from the nutraceutical and pharmaceutical point of view, the Button Mushrooms are of great importance due to the presence of biologically active phytochemicals in them.

Due to the low fat and high protein content with no cholesterol, mushrooms are accepted as a food source with good biological value **(Dutta *et al*., 2007)**. Button mushroom is also known for waste management crop as it used to grow on agriculture origin lignocellulosic materials, forest waste and garden waste **(M. Shanaz *et al*., 2020)**.

Wild *A. bisporus* mushroom that is isolated from Northeast of Portugal **(Cruz *et al*., 2008),** Turkey **(Ozturk *et al*., 2011),** China **(Shang *et al*., 2013)** and Northeastern Iran **(Soltanian *et al*., 2016),** have been investigated for the antibacterial activities against pathogenic bacteria.

Following are the major aims of this study:

1) To optimize and develop Button Mushroom Sauce.
2) To study the nutritional, physico-chemical and textural properties of Button Mushroom Sauce.
3) To study the shelf life of Button Mushroom Sauce at different storage temperatures.

Chapter II

REVIEW OF LITERATURE

(Chang *et al.*, 2004) In their studies, defined mushrooms as "the fruiting body of the macro fungi" however, *Agaricus* as the leader in production and technology and divided mushrooms into the following four categories:

1) Edible mushrooms e.g., *Agaricus bisporus*
2) Medicinal mushrooms e.g., *Ganoderma lucidum*
3) Poisonous mushrooms e.g., *Amanita phalloides*
4) Other mushrooms include those in a disordered class whose properties remain less well defined.

The Comparative Contribution of Different Mushroom Species in the Total Production:

According to **(VP Sharma *et al.*,2017)**, Button mushroom, Oyster mushroom, Paddy straw mushroom, milky mushroom and Shiitake mushroom are on the commercial scale of cultivation in India, out of which *Agaricus bisporus, Pleurotus spp.,* and *Volvariella volvacea* are contributing for about 96% of the total mushroom production.

As per **(Sharma *et al., 2015)***, mushrooms can be consumed in various different forms like fresh, dried, powdered, canned, etc.

Origin

The cultivation of button mushrooms (*Agaricus bisporus*) started in the 16th century. But, however on a commercial scale, the cultivation was begun in Europe around 17th Century. Many farms were established for the production of button mushrooms and this variety still dominates the world in production and consumption. For more than four decades, India has been producing mushrooms with an abundance of agricultural wastes for the domestic market. In the 1990s, commercial production increased, and several high relative export market farms were established with the help of global technology. However, small farms continue to produce the majority of mushrooms. **(Source: National Horticulture Board).**

Botanical Description

The vegetative mycelium comprises of many inter-woven septate hyphae. The form of reproduction stage is established by the small knob like swellings at distinctive parts of interwoven mycelial strands. And these swellings increase in size and break through the surface of the substratum as in small balls i.e., constituting the button stage. The fruiting body, a matured basidiocarp is whitish in colour and includes thick short stipe with an annulus. The pileus that appears as a hat like expansion is being supported by the stipe and a number of radiating gills or lamella are present beneath the pileus, and are pink when young while as are purple-brown when mature **(Source: National Horticulture Board).**

History

(Chang *et al*. 2004) Mushrooms were historically placed under the Division of lower plants in Thallophyte by Linnaeus due to the reason that, they were lack of true seeds, true leaves, true flowers, true stems and true roots. However, modern studies have established that mushroom biota have features of their own, together with other fungi which are greatly distinct to place them under the fungal Kingdom of Myceteae. During the reign of Louis XIV, the growing of Agaricus arose in France, the vicinity of Paris as per the records available. The commencement of export of the fresh mushrooms, took place in 1947 as they were first commercially grown in Ireland in the mid-1930s. In 1999 – 2000, China produced 637.3 thousand MT, followed by the United States, the Netherlands, and France and thus, jumped to become the largest producer of this important button mushroom.

(Singh *et al*., 2020) As per the historical records of the intentional cultivation of some important edible mushrooms, it is estimated that *Auricularia auricular*, was the first mushroom that was cultivated around 600 A.D. Later, *Flammulina velutipes* was also cultivated, around 800-900A.D in China. *Lentinula edodes* have been estimated to be cultivated for the first time between 1000-1100A.D. It is assessed that *Volvariella volvacea* around 1700 and *Tremella fuciformis*, around 1800 in China, have been cultivated for the first whereas in case of *Agaricus bisporus*, the first

serious attempt was initiated by Himachal Pradesh at Solan, in the year 1961 with a scheme entitled as "Development of mushroom cultivation in "Himachal Pradesh".

An experimental basis cultivation of button mushroom was initiated by CSIR and state government at Srinagar in J&K, in 1964.

Table 2.1: Global Mushroom Production for the Year 2018

COUNTRY	PRODUCTION (tons)	AREA HARVESTED (hectare)	YIELD (hectogram/hectare)
China	6,675,364.00	21,387.00	3,121,223.00
Italy	70,673.00	NA	NA
USA	416,050.00	NA	NA
Netherlands	300,000.00	64.00	46,875,000.00
Poland	280,232.00	NA	NA
Spain	166,250.00	550.00	3,023,222.00
Iran	81,406.00	NA	NA
Canada	138,412.00	NA	NA
France	83,013.00	NA	NA
UK	98,500.00	NA	NA
Germany	73,231.00	342.00	2,141,257.00
Ireland	65,300.00	NA	NA

Source: FAOSTAT, Food and Agriculture Organization

Table 2.2: Status of mushroom production in India

S.No.	TOP STATES STATE	2015-2016 PRODUCTION (000 Tons)	SHARE (%)
1	Uttar Pradesh	357.20	81.84
2	Tripura	27.00	6.19
3	Kerala	20.30	4.65
4	Orissa	10.89	2.50
5	Himachal Pradesh	8.91	2.04
6	Nagaland	6.07	1.39
7	Punjab	6.02	1.38
8	Tamil Nadu	0.08	0.02
	PAGE TOTAL	436.47	

Source: National Horticulture Board (NHB)

Table 2.3: State-wise different mushroom production in India during 2016

STATES	BUTTON MUSHROOM	OYSTER MUSHROOM	MILKY MUSHROOM	OTHER MUSHROOMS	TOTAL
Andhra Pradesh	3000	500	15	0	3515
Arunachal Pradesh	20	5	0	1	26
Assam	20	100	5	0	125
Bihar	950	1500	150	0	2600
Chhattisgarh	20	200	35	89	344
Delhi	3000	50	20	0	3070
Goa	4200	20	0	0	4220
Gujarat	10000	1200	0	0	11200
Haryana	15000	50	50	0	15100
Himachal Pradesh	9000	110	30	10	9150
Jammu Kashmir	565	15	50	0	630
Jharkhand	200	20	0	0	220
Karnataka	700	320	160	0	1180
Kerala	0	500	300	0	800
Maharashtra	10000	2000	50	0	12050
Madhya Pradesh	10	5	0	0	15
Manipur	0	10	0	50	60
Meghalaya	25	2	0	0	27
Mizoram	0	50	0	0	50
Nagaland	0	75	0	250	325
Odisha	126	6310	0	9550	15986
Punjab	16000	2000	0	0	18000
Rajasthan	100	1000	0	200	1300
Sikkim	1	2	0	0	3
Tamil Nadu	6500	2000	1500	0	10000
Tripura	0	100	0	0	100
Uttarakhand	8189	1228	819	0	10236
Uttar Pradesh	7000	100	0	0	7100
West Bengal	50	1500	0	500	2050
Andaman & Nicobar	0	300	0	0	300
Total	94676	21272	3184	10650	129782

Source: ICAR-DMR, Solan official data.

Major button mushroom producing states of India 2016

(Mehta *et al.*,2011) reported that, mainly two types of mushroom growers are there in India: one is those who are growing button mushroom round the year under controlled conditions whereas the second is the seasonal mushroom growers during winter in the north western part of India. White button mushroom production in India, accounts for approximately 8500 metric tons of button mushroom production from the seasonal growing units from Punjab and Haryana. And presently, the highest production of *Agaricus bisporus* is estimated from Punjab followed by Haryana and Maharashtra.

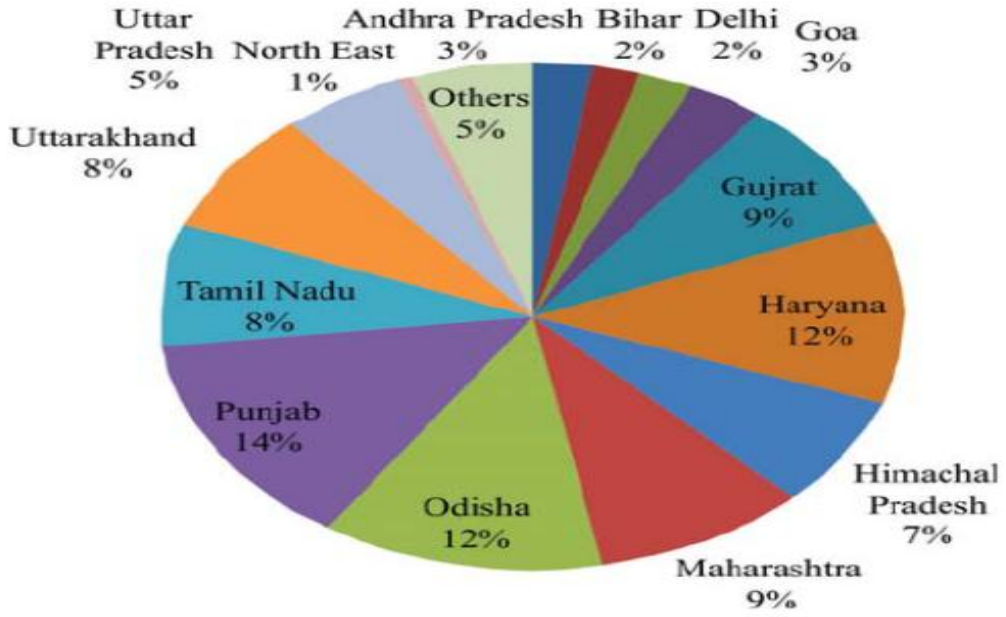


Fig. 2.1 Major Button Mushroom Producing states of India 2016

NUTRITIONAL PROPERTIES

(Kratika S., 2015) Button mushroom *(Agaricus bisporus)* is one of the major varieties, commonly grown and consumed in India. It belongs to the Family of Agaricaceae and Class Basidiomycetes, native to Europe and North America. Out of its two types i.e., white and brown, white type is commonly grown in India. **(Brugnari *et al.*, 2016)** White button mushrooms are higher in carbs, proteins, and

lipids than other commonly eaten mushrooms. When mushrooms are cultivated on different substrates, they have varying nutritional properties, according to **(Assadi *et al*., 2019).**

Table 2.4 Nutritional Value of *Agaricus bisporus* per 100 gram

Energy	**113KJ (27 Kcal)**
Water	92.45 g
Carbohydrates	4.1 g
Fat	0.1 g
Protein	2.5 g
Thiamine (Vit B1)	0.1 mg
Riboflavin (Vit B2)	0.5 mg
Niacin (Vit B3)	3.8 mg
Pantothenic acid (Vit B5)	1.5 mg
Vitamin C	0 mg
Calcium	18 mg
Phosphorus	120 mg
Potassium	448 mg
Sodium	6 mg
Zinc	1.1 mg
Vitamin D (D2 + D3)	0.2 µg
Sugar	1.98 g

Source: USDA Nutrient Database

PROTEINS AND AMINO ACIDS

(Braaksma *et al*., 1996) reported the principal kinds of amino acids found in *A. bisporus* include aspartic acid, serine, glycine, proline, threonine, glutamine, valine, cysteine, alanine, leucine, isoleucine, lysine, histidine, arginine, tyrosine, and norleucine. And moreover, the crude protein content ranges from 19–38% on a dry weight basis in *A. bisporus*. In the past, research has concentrated on determining the total protein and amino acid composition of *A. bisporus*. As per **(Sadiq *et al*., 2008),** the protein content of 11.01%, were found in *A. bisporus*. However, **(Mohiuddin *et al*., 2015)** discovered that *A. bisporus* has 17.7–24.7 percent protein. In contrast, **(Manikandan *et al*., 2016)** found that *A. bisporus* has a protein content of 29.1%. These findings demonstrate that the protein concentration of *A. bisporus* changes

depending on the growth substrate used.. **(Ragussi *et al*., 2019)** isolated "Ager- itin," a ribotoxin from *Agrocybe aegerita* and also founded that this ribotoxin possesses multiple biological activities, like antibacterial, antiviral, endonuclease, nuclease, and cytotoxic activities, that can be employed in plants via a transgenic techniques to enhance resistance against bacteria, viruses, and fungi.

LIPIDS

(Cruz *et al.*, 2008) *Agaricus bisporus* comprises of a good amount of linoleic acid but only a small amount of crude fats is present. **(Hanif *et al.*, 2008)** the components of fatty acids present in *A. bisporus* are caprylic, erucic, eicosanoic,linoleic, palmitic and stearic acid that accounts for about 44.19% of the fatty acids totally extracted. **(Kivrak *et al*., 2011)** Palmitic acid (12.67–14.71%) and linoleic acid (61.82– 67.29%) are the two main fatty acids present in *A. bisporus*, out of the detected 13 fatty acids.

VITAMINS

According to **(Caglarirmak 2009)** riboflavin, folic acid, niacin, and thiamin are abundant in *A. bisporus*, while vitamin C levels is low. **(Godoy *et al*., 2008)** From a fresh specimen of *A. bisporus*, the mean values of vitamins B1 and B2 were computed, yielding vitamin B1 (Thiamine) and B2 (Riboflavin) of 0.03 and 0.25 mg 100 g1, respectively. **(Ahlavat *et al*., 2016)** reported that this species has a sufficient amount of vitamin D (984 IU g1). However, **(Martins *et al*., 2012)** discovered that the low vitamin D concentration of the species might be due to its growing in darkness. UV light can boost vitamin D synthesis, and Ergo-sterol, a precursor to vitamin D2, is also found in the fungal cell wall, according to the study of **(Teichart *et al*., 2008)**.

CARBOHYDRATES AND FIBERS

(Reis *et al*., 2012) founded that the carbohydrate content in *A. bisporus* mainly comprises of the two sugars namely mannitol and trehalose are the two most abundant

sugars. **(Wehrens *et al.*, 2016)** out of which, Mannitol represents the most richful sugar in *A. bisporus*.

According to **(Vetter 2007)** chitin, an insoluble fiber that improves the gut health and promotes the immune functioning and when it is present in higher amount, it contributes towards the health of A. bisporus. **(Cheung 2010)** stated that the dietary fibers: mannans, glucans and hemicelluloses also present there in good amounts in that of the fungal cell wall which contributes to the medicinal properties of the mushrooms as a result. **(Osalina *et al.*, 2013)** reported that the chitin content in A. bisporus is around almost two times more than that of P. ostreatus.

MINERALS

(Falandysz *et al.*, 2013) Mushrooms are high in phosphorus (P) and potassium (K), as well as calcium (Ca), zinc (Zn), iron (Fe), magnesium (Mg), and sodium (Na), all of which are found in *A. bisporus* and so contribute to the health benefits of mushrooms. **(Mohiuddin *et al.*, 2015)** found mineral concentrations of 54.6–163.4 for copper (Cu), 56.2–91.1 for magnesium (Mg), 37.2–61.9 for sodium (Na), 143.6–396 for iron (Fe), and 36.6–58.0 for zinc (mg kg1) in *A. bisporus* samples grown in different locales (Zn). In *A. bisporus*, however, **(Ahlavat *et al.*, 2016)** computed Na (500.8 mg kg1) and Se (1.34 mg kg1). According to **(Lu *et al.*, 2009)**, Se is an important micronutrient in *A. bisporus* for both animals and humans.

IMPORTANT BIOACTIVE COMPOUNDS

(Klaus *et al.*, 2011) (Jurikova *et al.*, 2009) The relevance of glucans (β-Glucans) contained in mushrooms for the prevention and treatment of numerous diseases, including high blood pressure, diabetes mellitus, and other antibacterial, antiviral, and anti-inflammatory activities, was concluded in their investigations. When compared to therapeutic mushrooms like *Ganoderma lucidum* and *Phellinus linteus*, *A. bisporus* and *A. brasiliensis* had a larger level of total glucans, according to the study.

Polyphenols and other compounds with antioxidant activity

A variety of phenolic acids (gallic acid, caffeic acid, coumaric acid, cinnamic acid, ferulic acid, protocatechuic acid) were found in the sporocarp of *A. bisporus* **(Jia *et al.*, 2013).** A research was undertaken by **(Duru *et al*., 2011)** to determine the total phenolic compounds found in *A. bisporus* mushrooms, in which the TPC was determined to be 316–384 mg pyrocatechol equivalent per 100 g of fresh weight, whereas the total flavonoids content was reported as 379–669 QEs100 g-1 of fresh weight. **(Dashti *et al*., 2015)** *A. bisporus* fruiting bodies also contain ergothioneine, which varies from 1.2 to 1.8 mgg-1 in *A. bisporus* fruiting bodies.

HEALTH PROMOTING ACTIVITIES

Antioxidant activity

According to **(Dashti *et al.*, 2015),** frequent consumption of *A. bisporus* can protect the organism from free radicals due to its strong anti-oxidant potential, which is attributed to the presence of ergothioneine and phenolic compounds. **(Jaworska *et al.*, 2015)** If mushrooms are thermally processed soon before eating, their anti-oxidative action will be reduced by 45–79% due to the loss of phenolic components, flavonoids, Lascorbic acid, and carotenoids. When mushrooms were blanched, however, the loss was significantly greater. As a result, such a technique is not advised.

Anticancer activity

The presence and interaction of polysaccharides, lectins, and phenolic chemicals in white button mushroom fruiting bodies resulted in their anticancer activities **(Jagadish *et al*., 2009)**. In-vitro studies on neoplastic cell lines and animal studies have both demonstrated that the extract of *A. bisporus* has an anticancer effect. Its extracts also reduced the mushrooming of HL-60 leukaemic cells by triggering apoptosis in the cells. **(Phung *et al*., 2008)** While it reduced proliferation more than the raw extract, it did so more effectively than the boiling extract. According to **(Benzie *et al*., 2002),** when Lectins is isolated from the fruiting body of white button

mushrooms, it causes lung cancer and colorectal carcinoma cells to become more sensitive to chemotherapeutic drugs, inhibiting cancer cell proliferation and strengthening the cellular anti-oxidative defence mechanism. **(Jeong *et al.*, 2012)** ABP-1 and ABP-2 are two polysaccharide fractions isolated from the *A. bisporus* mushroom that influence cancer development.

Antimicrobial activity

According to **(Ozturk *et al.*, 2011),** *A. bisporus* extracts are effective against gram-positive bacteria such as Micrococcus luteus, Micrococcus flavus, Bacillus subtilis, and Bacillus cereus, as well as fungus such as Candida albicans and Candida tropicalis. According to **(Pal *et al.*, 2013),** methanol *A. bisporus* extract has antibacterial efficacy against bacteria such as Escherichia coli, Proteus, Pseudomonas aeruginosa, and Staphylococcus aureus, as well as fungus such as *Aspergillus niger*. Because of their barrier property in the development of microorganisms such as *Staphylococcus aureus, Salmonella spp., Escherichia coli, Shigella spp., Listeria monocytogenes,* and *Yersinia enterocolitica* in dairy produce, **(Reis *et al.*, 2014)** mentioned the use of *A. bisporus* extract as natural preservatives in yoghurts. According to **(Delgado *et al.*, 2016)**, aqueous enzymatic extracts of *A. bisporus* have antiviral potential as well as inhibitory efficacy against HCV protease.

Anti-inflammatory activity

(Komura *et al.*, 2010) reported that the isolated fucogalactan, fucomannogalactan, and mannogalactan of *A. bisporus* var. *hortensis* have anti-inflammatory and analgesic effects. Heterogalactan has been shown to have the ability to prevent sepsis in mice **(Ruthes *et al.*, 2011)**. **(Rattman *et al.*, 2012)** Sepsis is the leading cause of death in critical care units, and it is caused by an excess of pro-inflammatory mediators produced in response to bacterial infections. The anti-inflammatory effects of fucogalactan extracted from A. bisporus fruiting bodies were demonstrated in a research work on "mouse". According to this study, it has a potent anti-inflammatory impact that can be utilised to combat sepsis.

Codex Alimentarius

Codex Alimentarius: The product of salts, spices, soups, sauces, salads, and protein, according to the Codex Alimentarius, contains ingredients that are added to the meal to enhance its scent and taste. These are further categorised and discussed as follows:

Salt and salt replacements (12.1)

As salt, this category comprises sodium chloride, table salt, iodized and fluoridated iodized salt, and dendritic salt; as salt substitutes, this category includes spices with lower sodium content that are meant to be used on food instead of salt.

Seasonings, herbs, spices, and condiments (12.2)

It contains items that are typically derived from botanical sources and used to enhance the aroma and flavour of food. It's possible to dehydrate it whole or ground. Chilli seasoning, chilli paste, curry paste, curry roux, and dry cures or rubs are examples of spices that are mixed in powder or paste form. Meat tenderizers, onion salt, garlic salt, Asian seasoning mix (dashi), a topping to sprinkle on rice, and seasoning for noodles are examples of seasonings, whereas condiment sauces such as ketchup, mayonnaise, mustard, or relishes are not included in the term "condiments" as used in the Codex Alimentarius Food Category System.

Vinegars (12.3)

"Vinegar" refers to the liquids produced by the fermentation of ethanol from a compatible origin such as wine or cider. Cider vinegar, wine vinegar, malt vinegar, spirit vinegar, grain vinegar, raisin vinegar, and fruit vinegar are all examples of vinegars.

Mustard (12.4)

A flavouring sauce made from powdered, generally defatted mustard seed that is blended with water, vinegar, salt, oil, and refined and other spices to form a slurry.

Soups and broths (12.5)

The following are examples of final products:

(1) ready-to-eat soups and broths, which include canned, bottled, and frozen products containing vegetable, meat, or fish broth with or without other ingredients, such as bouillon broths, consommes, water- and cream-based soups, chowders, and bisques; and (2) ready-to-eat soups and broths, which include canned, bottled, and frozen products containing vegetable, meat, or fish broth with or without other ingredients, such as bouillon broths

(2) Soup and broth mixes: comprises concentrated soups that must be reconstituted with water and/or milk, with or without the inclusion of non-essential ingredients such as powders and cubes, as well as condensed soups.

Sauces and similar items (12.6)

It includes ready-to-eat sauces, gravies, dressings, and mixes that must be reconstituted before use, and it is divided into emulsified and non-emulsified categories, while the sauce mixes are separated into two categories: emulsified and non-emulsified:

(1) emulsified sauces and dips, at least in part, or in a fat or oil-in-water emulsion, such as salad dressing, fat-based sandwich spreads, salad cream, fatty sauces, and snack dips; (2) emulsified sauces and dips, such as sauces, gravies, dressing-based sauces and dips; (3) emulsified sauces and dips, such as sauces, gravies, dressing-based sauces and

(2) sauces, gravies, and dressings made with water, coconut milk, or milk, such as barbecue (BBQ) sauce, tomato ketchup, cheese sauce, and Asian thick Worcestershire sauce (tonkatsu sauce);

(3) Sauce and gravy mixes are concentrated powdered items that are blended with water, milk, oil, or another liquid to make a completed sauce or gravy, such as cheese sauce, hollandaise sauce, and salad dressing; and

(4) Clear sauces, such as oyster sauce and Thai fish sauce, are thin, non-emulsified clear sauces that can be produced with water and used as condiments or components rather than full gravies.

Salads and spreads for sandwiches (12.7)

This category includes ready-to-eat salads, milk-based sandwich spreads, non-standardized mayonnaise-like sandwich spreads, and dressing.

Yeast and related products (12.8)

The majority of the ingredients are baker's yeast and leaveners, which are used in the production of baked goods, as well as the Asian product koji, which is rice or wheat malted with Aspergillus oryzae.

Seasonings and condiments made from soy beans (12.9): Include products made from soybeans and other ingredients that are intended to be used as seasonings and condiments, such as fermented soybean paste (e.g., miso), which is made from soybeans, salt, water, and other ingredients through the fermentation process and includes dou jiang (China), doenjang (Republic of Korea), or miso (Japan), and soybean sauce, a liquid flavouring made from soybean fermentation, non-fermentation (e.g., hydrolysis), or vegetable protein hydrolysis. It consists of the following ingredients: (1) fermented soybean sauce, a clear non-emulsified sauce (2) nonfermented soybean sauce (also known as non-brewed soybean sauce), and (3) other soybean sauce (also known as non-emulsified soybean sauce).

Protein products derived from sources other than soybeans (12.10)

Milk protein, cereal protein, and vegetable protein analogues or substitutes for quality items such as meat, fish, or milk are some of the protein products generated from sources other than soybeans.

SAUCE

Sauces have a higher total solids content of at least 30% than ketchups, which have a total solids content of at least 28%, according to (Srivastava et al., 1994). Sauces are made from apples, papaya, walnuts, mushrooms, soybeans, tomatoes, and other fruits and vegetables. The following are the two types of sauces:

(I) Low-consistency thin sauces made primarily of vinegar extracts of flavouring ingredients,

(II) Thick sauces with a lot of body.

High-quality sauces are made by desiccating fruits, vegetables, spices, and herbs in cold water or boiling them in vinegar. The conventional commercial procedure, on the other hand, is to create cold or hot vinegar extracts of each type of spice and fruit separately, then blend these extracts appropriately to obtain the saucs that are subsequently matured.

BULKING AGENTS

(Krasnow *et al.*, 2011) mentioned that the combination of wheat flour and a clarified fat is called as "Roux". It is used in soups, sauces, and stews as a thickener and as a flavor additive for many dishes. And due to this ability it is very important to know that how cooking roux affects the physical characteristics of the ultimate final product. The flour and fat are combined together, then heated for a short time duration over a low to medium heat depending on the use of the final product. The roux is then heated to the stage where the raw flavor is cooked off, but not to the stage where the flour is toasted . The thickening power of the roux decreases as the temperature of the roux rises during cooking. The ability of thickening power of roux rely on the size of starch granule, the polysaccharide matrix, and the collaboration of these.

SPICES AND CONDIMENTS

Spices and condiments, according to **(Rosas *et al.*, 2016)**, are items that are used to improve the flavour and scent of food. They are mainly produced from biological scientific sources and may be dried either powdered or whole.

Vinegar

(Solieri *et al.*, 2009) vinegar is a classic acidic condiment originated from the French words "Vin Aiger" which meaning "sour wine."**(Cruess *et al.*, 1958)** Vinegar is a sharp liquid that is also used as a food preservative.

"Vinegar is a liquid suited for human consumption, manufactured from a suitable raw material of agricultural origin by a process of twofold fermentation, initially alcoholic and then acetous," according to Codex Alimentarius (1987).

Garlic *(Allium sativum)*

(Agarwal *et al.*, 1996) Allium, garlic's Latin name, comes from the Celtic word al, which meaning "flaming" or "pungent." It is a member of Liliaceae family. But **(Kuettner *et al.*, 2002)** besides getting used for the enhancement of aroma and taste that is as a condiment, **(Agarwal *et al.*, 1996)** garlic is additionally concerned with the inhibition of lipid peroxidation as well as within the functioning of cardiovascular system.

Ginger (*Zingiber officinale*)

(Park *et al.*, 2002) one among the foremost used spice, is originated from South east Asia and it is also used for the medicinal purposes. **(Shukla *et al.*, 2007)** rootstock of ginger is consumed as a slices which is preserved in syrup, as a fresh paste, as dried powder or candy, or as in tea flavouring.

Pepper (*Piper nigrum*)

One of the most popular spice of the world, is a flowering vine of Piperaceae family. It is native to India and Indonesia. It is cultivated for its fruit which is named as Peppercorns and this seasoning is typically dried and used as a spice and seasoning.

Value Additional Merchandise of Mushrooms

Various technologies are being developed for the manufacturing of products such as mushroom based soup powder, biscuits, nuggets, preserves, papad, noodles, sweets, and premade mushroom curry in retort pouches, according to **(G C Wakchaure 2011)**. The following are some of them:

Powdered Mushroom Soup: Button and oyster fungus were dried in the dehumidifying air cabinet drier to produce ready-to-eat mushroom soups of high quality, which was done at DMR.

Mushroom Biscuit: Maida, sugar powder, bakery fats (ghee), mushroom powder, coconut powder, baking soda, milk powder, and ammonium bichromate are combined to make fresh crispy mushroom biscuits. The entire components were coarsely ground with an electric mixer and then cleaned separately with a fine screen. Ghee and sugar were well combined using a dough kneader to form a homogeneous mixture, and then the remaining components were added for dry compounding mixing. The dough was then kneaded with water to make it cohesive and uniform. With the use of several steel dies, thin sheets of dough were formed and cut into the various forms of biscuits.

Mushroom Preserve: To make excellent quality mushroom murabba, mature fruits and vegetables are simmered whole or in parts in thick sugar syrup until soft and translucent.

Mushroom Nuggets: A paste is formed by mixing dried and coarsely powdered mushroom powder with Urad dal powder and adding the necessary amount of water. Spices and ingredients are added, and the resultant mixture is formed into round balls with a diameter of 2-4 inches. These balls are then sun-dried on a steel tray.

Mushroom Candy: Freshly picked mushrooms are washed and blanched in 0.05 percent KMS solution, then split longitudinally and treated with 1.5kg sugar per kg of blanched mushrooms for half an hour after draining. The sugar was divided into three equal portions, the third of which was boiled with 0.1 percent citric acid to bring the concentration to 70ºBrix. The mushrooms are removed from the syrup and drained after cooling, and the drained mushrooms are sorted to remove any defective or undesirable portions.The mushroom pieces are next dried for 10 hours in a cabinet dryer at 60°C. As soon as the mushroom candies get crispy, they are removed and put into polypropylene bags. As a result, these sweets may be kept for up to 8 months while still tasting delicious.

Mushroom Chips: Freshly selected White Button mushrooms are blanched in a 2 percent brine solution before being soaked overnight in a solution of 0.1 percent citric acid, 1.5 percent NaCl, and 0.3 percent red chilli powder. Drained mushrooms are dried for 8 hours in a cabinet dryer at 60°C. The mushrooms are then cooked in refined oil, yielding high-quality mushroom chips.

Ready-to-Serve Mushroom Curry: DMR created a technology for the manufacturing of "Mushroom curry in flexible-retortable pouches." The mushroom curry was packed in a 105-thick retortable bag with an outside layer of 80 polypropylene, a middle layer of 12.5 aluminium, and an inside layer of 12.5 polyester available on the market.

Mushroom Ketchup: Freshly picked, washed Button mushrooms are sliced and boiled in 50 percent water for 20 minutes before being ground into mushroom paste with the aid of a grinder mixer. Arrarote (0.2%), acetic acid (1.5%), and additional additives such as salt (ten percent), sugar (twenty-five percent), and onion (ten percent). Garlic 0.5 percent, ginger 3%, cumin 1%, black pepper 0.1 percent, red chilli powder 1%, ajinomoto 0.2 percent, and 0.065 percent sodium benzoate for preservation are all combined in the paste and boiled to bring the TSS to 35ºBrix. Finally, the Ketchup is sterilised and placed in bottles or jars.

Table 2.5: Export of white button mushrooms in dried form from India

Country	2014-15		2015-16		2016-17	
	Qty	Value	Qty	Value	Qty	Value
Germany	37.55	876.67	53.54	1,159.53	67.37	1,481.75
France	9.11	1,383.21	6.72	736.31	3.55	354.84
Hong Kong	0	0	0.63	86.14	1.57	141.53
United States	6.46	176.19	6.33	159.57	4.79	108.82
Israel	0.12	2.98	1.24	32.5	0.73	19.56
Switzerland	4.77	942.64	0.36	53.18	0.09	10.01
Canada	6.65	149.09	0	0	0	0
China P Rp	2.88	399.12	1.68	223.9	0	0
Russia	0	0	1.64	18.75	0	0
Sweden	0.08	13.43	0.05	4.86	0	0
Total	67.62	3,943.33	72.19	2,474.74	78.1	2,116.51

Table 2.6: Export of white button mushrooms in preserved form from India

Country	2014-15		2015-16		2016-17	
	Qty	Value	Qty	Value	Qty	Value
Switzerland	193.21	3,830.19	188.56	2,629.55	207.27	2,333.70
France	263.93	3,965.12	315.84	3,578.44	263.59	2,215.37
United States	3.9	67.27	381	149.87	442.16	226.63
Germany	23.3	258.44	55.26	312.2	27.9	134.06
China P Rp	94.81	1,448.89	15.59		22.5	122.94
Hong Kong	19.65	4.97	2	26.85	8.6	83.21
Spain	0	0	0	0	2.5	25.24
1qJapan	0.48	11.94	0.72	15.05	0.72	15.3
Sweden	1.4	24.7	3.2	37.25	1	9.3
Canada	190	35.74	0	0	0	0
Mexico	40.28	51.05	0	0	0	0
UK	1.5	32.19	0	0	0	0
Unspecified	0	0	2.84	43.01	0	0
Total	832.46	9,730.53	965.01	6,994.50	976.24	5,165.75

Source: DGCIS Annual Export

Chapter III

MATERIALS AND METHODS

This session of the research study has been held at "Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Different materials and methods that were used and implemented while during this investigation are described in this chapter, for the topic "Development of Button Mushroom Sauce".

3.1 MATERIALS AND EQUIPMENTS

3.1.1 Materials

1. Steel tray
2. Utensils
3. Knife
4. Stirrer
5. Sintex Plastic sheets
6. Aluminium foil
7. White Button Mushroom
8. Spices and Condiments

3.1.2 Equipments

1. Electronic Weighing Balance
2. pH meter
3. Hand refractometer

4. Hot Air Oven
5. Vortex Shaker
6. Texture Profile Analyzer
7. Centrifuge Machine
8. High Pressure Steam Sterilizer (Vertical Autoclave)
9. Spectrophotometer
10. Muffle furnace
11. Incubator
12. Water Bath
13. Refractometer
14. Tray dryer

3.1.3 Chemicals and other materials

Chemicals and solvents used in the study were of analytical grade. Glass bottles were used for the package and storage of samples. Other minor ingredients were used from the laboratory stocks.

3.2 METHODOLOGY

3.2.1 Raw material and extraction of dried mushroom powder

Button mushroom powder was extracted by using dried mushroom flakes, roux, oil, salt, garlic, clove, black pepper, acetic acid and clean drinking water. White Button Mushroom and all the above mentioned ingredients and spices were purchased from the local market.

Fig. 3.1 FLOW CHART FOR THE EXTRACTION OF DRIED WHITE BUTTON MUSHROOM POWDER

RECEIVING OF FRESH BUTTON MUSHROOM CLEANING AND WASHING

CUTTING MUSHROOM INTO THIN FLAKES

BLANCHING

COOLING FOR FEW SECONDS

SPREADING THE BLANCHED MUSHROOM FLAKES ON SINTEX PLASTIC SHEETS, ON STAINLESS STEEL TRAY

TRAY DRYING AT 50℃ FOR 19-20 HOURS

SIEVING

GRINDING OF THE OBTAINED DRIED MUSHROOMS

WHITE BUTTON MUSHROOM POWDER OBTAINED

Fig. 3.2 FLOW CHART FOR THE PREPARATION OF ROUX:

HEAT THE PAN

ADD 12.50 g BUTTER

ADD 12.50 g REFINED WHEAT FLOUR

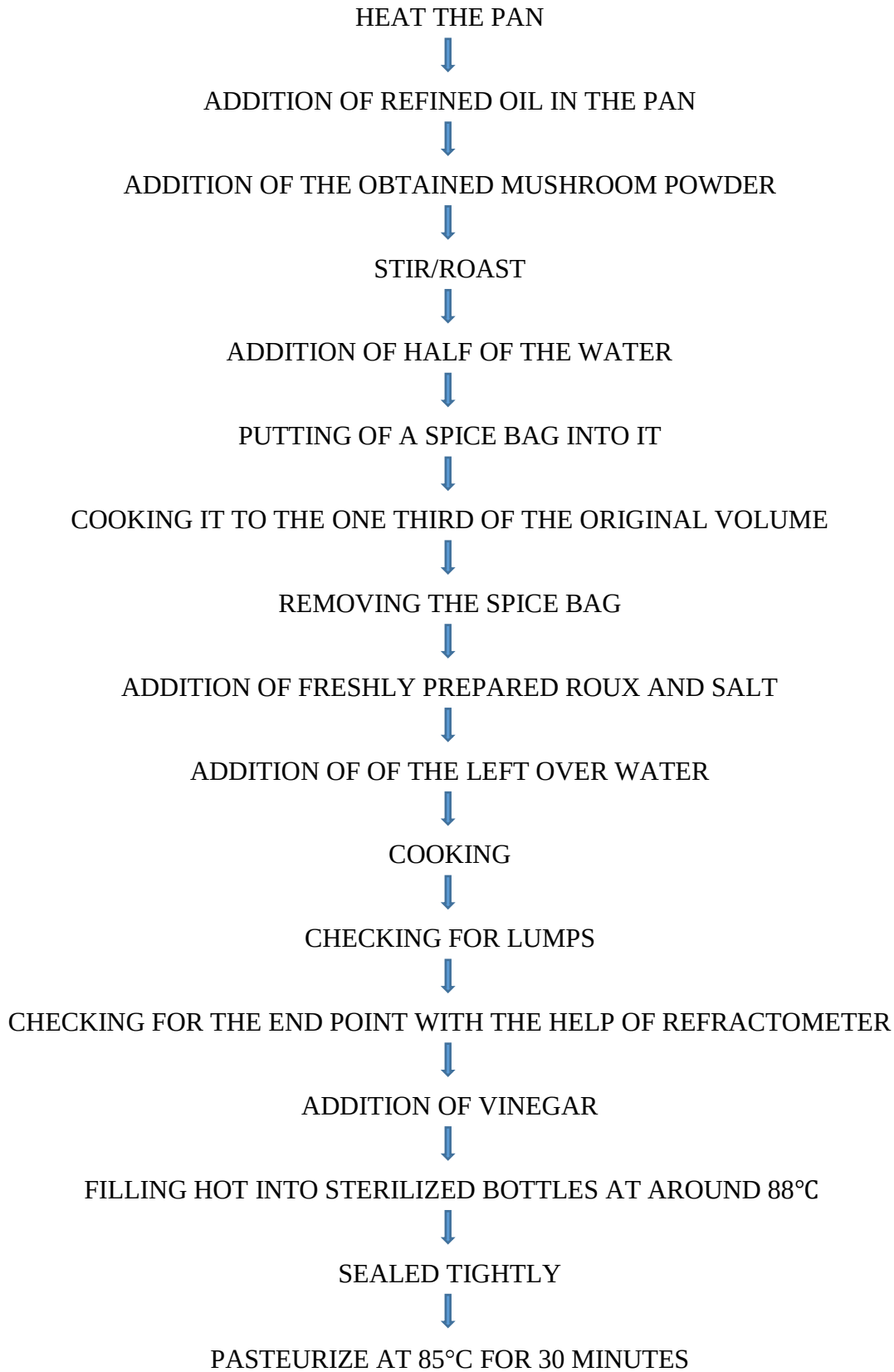
MIX IT WELL

CHECK FOR THE LUMPS FORMATION

COOLING

ROUX OBTAINED

Fig. 3.3 FLOW CHART FOR THE PREPARATION OF BUTTON MUSHROOM SAUCE



3.3 METHODS OF ANALYSIS

3.3.1 Determination of Moisture

The standard AOAC 2000 determined the moisture content of black rice. Two grams of sample was weighed in the moisture dish. The dish was then transferred to a hot air oven maintained at 105°C for 3 hours. Finally, the dish was transferred to a desiccator (containing calcium chloride) for cooling, followed by subsequent weighing. The heating, cooling, and weighing were continued till there was no difference in the last two subsequent weights. The percent moisture was calculated by using the following formula.

$$\text{Moisture (\%)} = \frac{W1-W2}{W1-W} \times 100$$

Where,

W = weight of empty dish with (g)

W1 = weight of dish with the sample

W2 = final weight of dish (g)

3.3.2 Determination of Total Ash

As per AOAC, 2000, in a pre-weighed silica crucible, a precisely weighed 5g sample was taken. The sample was ignited over a flame to completely burn the organic matter. After charring, the silica crucibles were placed in a muffle furnace and heated to 550°C for 5-6 hours or longer to produce grayish or whitesh ash. The crucibles were weighed after cooling in a desiccator. The ash content was calculated as under:

$$\text{Total Ash \%} = \frac{W2-W1}{W} \times 100$$

Where,

W = weight of the empty crucible taken

W1= weight of the crucible with ash taken

W2= Weight of crucible with a sample in grams of ash taken

3.3.3 Determination of Total Protein Concentration using Lowry Method

3.3.3.1 Reagents

A. 2%Na2co3 in 0.1 N NaOH (2g Na2CO3 in 100 ml 0.1 N NaOH)

B. 1% NaK Tartrate in H2O

C. 0.5% CuSO4.5 H2O in H2O

D. Reagent I: 48 ml of A, 1 ml of B, 1 ml C

E. Reagent II: 1-part Folin-Phenol [2 N]: 1 part water

F. Standard Bovine Serum Albumin (BSA) – 1 mg/g

3.3.3.2 Solution

A. **Stock solution**: Bovine serum albumin of 100 mg was weighed accurately and dissolved in 100 ml of distilled water in a standard flask (concentration 1 mg/ml).

B. **Working standard:** The stock solution of 10 ml is distilled to 100 ml with distilled water in a standard flask.

C. **Folin's Phenol Reagent:** Folin's Phenol reagent is mixed with distilled water in the ratio 1:2.

D. **Alkaline Copper reagent**

Solution A: 2% Sodium Carbonate in 0.1% N Sodium hydroxide.

Solution B: 0.5% Copper sulphate in 1% Sodium potassium tartarate.

Solution A, B, C is mixed in the proportion of 50:1:0.5.

E. **Unknown Preparation:** The unknown protein is made upto 100 ml with distilled water

Working standard of 0.2-1 ml was pipette out into clean test tube and labelled as S1-S5. Test solution of 0.2 ml was taken into test tube and labelled as T1. The volume was made upto 1ml of distilled water (Waterbog, 2009). Distilled water of 1 ml serve as blank to all the test tube. 4.5 ml of alkaline Copper sulphate reagent was added and incubated at room temperature for 10 minutes. In all the test tubes, 0.5 ml of Folin's-phenol reagent was added. The contents were mixed well and the blue colour developed was read at 640 nm on spectrophotometer after 15 minutes. From the standard graph the amount of protein in the given unknown solution was calculated.

Table 3.1: Absorbance of Standard protein solution (BSA)

Sample Name	Concentration (µg)	Absorbance (O.D.)
S1	200	0.221
S2	400	0.412
S3	600	0.609
S4	800	0.798
S5	1000	0.984

3.3.4 Determination of Crude Fibre

2g of sample was taken in a beaker and 200ml of pre-heated 1.25% sulphuric acid. The mixture was boiled for 30min while maintaining a constant volume of acid by adding distilled water. The Buckner flask was filled with Whatman filter paper and pre-heated by pouring hot water into the funnel. Boiled acid sample was filtered into the funnel. The residue was washed by boiling water and then transferred back into the beaker. After that, 200ml of pre-heated 1.25% sodium sulphate was added and boiled for 30min. It was again filtered and washed thoroughly with hot water and ethanol. The residue was dried at 65°C for 24h and weighed. The residue was finally transferred into crucible and placed in muffle furnace (400°C – 600°C) for 4h, then cooled and weighed.

$$\% \text{ Crude fibre} = \frac{Dry\ wt.of\ residue\ before\ ashing - wt.of\ residue\ after\ ashing}{wt.of\ sample} \times 100$$

3.3.5 Determination of total carbohydrates

The available carbohydrates were calculated by adding the value of moisture, crude protein, crude fat, fibre and ash which was then substracted from 100. The formula used was as under:

Carbohydrate % = 100 - (M.C% + CP% + CF% +EF% + Total Ash%)

3.3.6 Determination of Total Fat

Petroleum ether extractable fat was determined as per AOAC (2000) methods using soxhlet fat extraction apparatus.

Accurately weighed, ten gram of the sample was extracted with petroleum ether (40-60^{o}C) solvent for 12-16 hr. The solvent was evaporated at 80^{o}C and the fat content was weighed. The difference in the weight of the flask and the flask with fat after the complete evaporation of petroleum ether gives the weight of fat content in the sample.

$$\text{Total fat (\%)} = \frac{\text{Weight of the fat}}{\text{Weight of the sample}} \times 100$$

3.4 Bioactive compounds in button mushroom sauce:

3.4.1 Estimation of antioxidant by % DPPH Inhibition

Determination of the antioxidant potential of the Black rice was done by DPPH inhibition method as per the procedure given by Mimica-Dukic *et al*., (2004), with slight modification. 2g of the sample was taken in test tube and diluted 10 times the weight by ethanol and was dispersed thoroughly with the help of vortex. It was allowed to remain still overnight. To remove the suspended particle, the sample was centrifuged at 3000rpm for 10 minutes.

1 ml of the diluted sample was taken in an aluminum sheet covered test tube and 3 ml of freshly prepared DPPH solution (80mg/100ml ethanol) was added to it. The test tubes were allowed to remain in dark for 30 minutes. In a cuvette, 0.5 ml ethanol was taken and the UV-VIS spectrophotometer was calibrated. The sample was then taken in another cuvette and OD was measured against ethanol. The Optical Density of blank was also measured against ethanol. Absorbance was taken at 517 nm for blank and sample using ethanol as reference.

$$\%\text{DPPH Inhibition} = \frac{Absorbance\ of\ blank - Absorbance\ of\ sample}{Absorbance\ of\ sample} \times 100$$

3.4.2 Determination of the Total Flavonoids content

Principle

The principle of aluminium chloride ((AICI) colorimetric method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-4 hydroxyl group of flavones and flavonols. In addition, aluminium chloride forms acid liable complexes with the ortho-dihydroxyl groups in the A- or Brings of flavonoids (Mabry et al., 1970).

Procedure

Total flavonoid content was determined using aluminium chloride (AlCl3;) according to a known method, using quercetin as a standard. The sample (100mg) was added to 0.3mL distilled water followed by 5% sodium nitrite (NaNO2) (0.3ml). After 5 min. at 25°C, AlCl3 (0.3 mL, i0%) was added. After further 5min., the reaction mixture was treated with 2mL of 1mM NaOH. Finally, the reaction mixture was diluted to 10mL with distilled water and the absorbance was measured at 510nm using 3 cm cuvette UV-1800 spectrophotometer (Shimadzu, Japan). Quercetin (QE) (0 800 mg/L) was used to produce standard calibration curve. The result was expressed as mg quercetin (QE)/g of the extract (Ordonez et al., 2006).

3.4.3 Determination of the Total Phenolic content

Principle

The mechanism by which TPC is determined must be conditions of performed under basic approximately pH 10. This is because reductants are not limited to the family of phenolics, which will only react with FCR in a basic environment. Basic conditions will deprotonate a phenolic group creating a phenolate anion. This anion has the capability to reduce FCR, causing a color change from yellow to blue (Huang et al., 2005). The total phenolic content (TPC) assay using Folin-Ciocalteu Reagent allows for phenolic content to be quantified using a standard phenolic acid for comparison.

Procedure

The total phenolic content of the extracts was determined by the Folin-Ciocalteau method with some modifications. The five hundred microlitre of the sample was added to 2.5 ml of 0.2 N Folin-Ciocalteau reagent and placed for 5 minutes. 1 ml of 75 g/l of Na, CO, was then added. The above solution was then kept for incubation at room temperature for 2 hours. Absorbance was measured at 760 nm using UV-1800 spectrophotometer (Shimadzu, Japan). Gallic acid (0 - 800 mg/L) was used to produce standard calibration curve. The total phenolic content was 4pressed in mg of Gallic acid equivalents (GAE)/ g of extract.

3.4.4 Determination of Vitamin C (Ascorbic Acid)

Reagents

1) 3% Metaphosphoric acid - prepared by dissolving the 3 gm of $H3PO_4$ (stick) in 100 ml of distilled water.

2) Dye solution – 2,6-dichlorophenol indophenol (C12H7NCl2O2). Dissolve 52 mg of sodium salt of 2,6-dichlorophenol indophenol (DCPIP) and 42 mg of sodium bicarbonate in water and make the volume of 500ml.

3) Ascorbic acid standard – dissolve 10 mg of L-ascorbic acid in 3% (w/v) Metaphosphoric acid and makeup the final volume with metaphosphoric acid to 1 L.

Principle

The dye which is blue in alkaline solution and red in acid solution is reduced by ascorbic acid to a colourless form. Reaction is quantitative and is specific for pH range of 1-3.5.

Preparation of the sample

10 ml of the sample was taken and made up the volume to 10 ml with 3% (w/v) Metaphosphoric acid. Filtered with Whatman Paper No.1.

Standardization of the dye

20 ml of the standard ascorbic acid solution was taken. Fill a micro burette with the dye.

Titrated with the dye solution to a pink colour which persisted for 15 seconds.

Dye factor = 0.5 / Titre value

Ascorbic (Acid mg /100ml) = T.V

3.5 Shelf - life evaluation

Samples were stored at different temperatures for the study of its shelf life at 5°C, 15°C, 30°C and samples were withdrawn at 10 days intervals while during storage and were analyzed.

3.5.1 Free Fatty Acid (FFA)

The method prescribed by (**Deeth *et al.*, 1975)** was used to estimate the FFA content of the sample. The method consisted of accurate weighing of 0.5 g of sample into 60 ml stoppered test tube. 10ml of the extraction mixture (Iso propanol: Petroleum ether: 4N H2SO4 in the ratio of 40:10:1) was added and mixed thoroughly. This was followed by the addition of 6 ml of Petroleum ether and 4 ml of distilled water. The test tube was stoppered and tempered at 40ºC for 10 mins. The contents

were vigorously shaken for 20 secs. The two layers were allowed to separate for 10-15 mins and an aliquot of upper layer (5-8 ml) was withdrawn and titrated against 0.02N methanolic KOH solution using 1% methanolic phenolphthalein indicator. A blank, in which mushroom sauce sample was replaced with distilled water, was used to obtain the background titration.

FFA% = ml. of alkali × Normality of alkali × 28.2/ Wt. of sample

3.5.2 Thiobarbuturic Acid (TBA) Value

The extent of oxidation of fat in sauce sample was measured in terms of TBA value. The extraction method of **Strange *et al.*, (1997)** was followed with slight modifications.

For TBA value determination about 2g of sample was taken and blended with 50ml of 20% TCA (Tri-chloroacetic Acid) and 50ml of distilled water and left undisturbed for 10 minutes. Then the contents were filtered through Whatman no. 1 filter paper. The filtrate (5ml) was pipette out in test tube and added with 5ml of 0.0001M Thiobarbituric acid. Color was developed by incubating the tubes in boiling water bath for 30 minutes at 100°C. The contents were cooled to room temperature and absorbance was determined at 532 nm. Blank determination were made using distilled water in place of sample. TBA value was expressed as absorbance at 532 nm.

3.5.3 Determination of Microbial Population (AOAC,2000)

3.5.3.1 Preparation of the samples (Serial dilution)

1ml of sample was taken and transferred to test tube with 9ml of normal saline solution (0.0.9% NaCl). The samples were serially diluted up to 109 dilutions. The test containing samples were homogenized for proper mixing.

3.5.3.2 Total plate count

Total plate count (TPC) was used for determination of bacterial count in the sample.

3.5.3.3 Method

Sterilization

The prepared media was heated for 15 minutes in an autoclave maintain at 15psi for sterilization at 121° C. All glassware's and necessary item were properly autoclaved to avoid contamination.

Pouring

Pouring was done in the laminar- air flow chamber. The flame was lighted and Petri dishes were slightly opened near the flame and the media was lighted and petri dishes and kept for Solidification.

Inoculation of sample

Inoculation was done aseptically in laminar air flow chamber by taking 1 ml of Bael probiotic drink sample suspended in saline solution from 109 of dilution and transferred to the petri dishes with label 10^{-4} of nutrient agar media, similarly, all the samples were transferred to the respective Petri dishes of nutrient agar media. Duplicate samples were taken for each dilution and a control of nutrient agar media was not kept without inoculation. The inoculation petri dishes were incubated in incubation for 24 hours at 37±1° C Temperature. Total plate count was counted after 24 hours.

TPC (CFU/ml) = No. of colonies / dilution factor × 0.1

Where,

CFU = colony forming Unit

Amount plated = 0.1ml

3.5.3.4 Yeast and mold count determination

Potato Dextrose Agar (PDA) was used to determine the Yeast and Mold in mushroom sauce sample. The prepared media was heated for 15 mins in an autoclave,

maintained at 15 psi for the sterilization at 121℃. All the glasswares and the required items were properly autoclaved to avoid contamination. Pouring was done in the laminar-air flow chamber. The flame was lighted and petri dishes were slightly opened near the flame and the media was poured in the petri dishes and kept for solidification. Inoculation was done aseptically in the laminar-air flow chamber by taking 0.1 g of the sample suspended in saline solution from 10-2 transferred to petri dishes with label 10-2 of Potato Dextrose Agar media. Similarly, all the samples suspended in saline solution were transferred into the respective petri dishes of Potato Dextrose Agar media was also kept without inoculation. The inoculated petri dishes were incubated in incubator for 72 hours at 25℃ temperature. Colony was counted after 72 hours.

Yeasts and molds (CFU / ml) = No. of colonies / Dilution factor × 0.1

Where,

CFU = Colony Forming Unit

Amount Plated = 0.1 g

3.7 Design of Experiments (DOE)

Statistical design of experiments refers to a scientific approach to their planning so that appropriate data can be analyzed by statistical methods are collected, resulting in a valid, meaningful, and objective conclusion.

The capabilities of Design of Experiments (DOE) help to improve processes. DOE can screen the factors to be determined and which are important for explaining the process variation. After screening the factors, one can understand how factors interact and drive the process. DOE then finds the factor setting that produce optimal process performance. The factors taken into consideration were mushroom powder, garlic, butter, refined wheat flour, by keeping in view the objective of the investigation. The plan of work is discussed here under:

Table 3.2: Independent variables used for the optimization

Independent Variables	Symbol Code	Upper limit	Lower Limit
Mushroom Powder	A	70	20
Garlic	B	**7**	4
Butter	C	15	10
Refined Wheat Flour	D	15	10

Colour, flavour, texture, taste and overall acceptability were selected as responses for the process of optimization**.** The effect of independent variable on these responses has to be evaluated. A combination of 21 number of (experiments) trials were generated in Design Expert using Response Surface Methodology during investigation.

Table 3.3: Experimental design for analysis and optimization of mushroom sauce

Run	Mushroom Powder	Garlic	Butter	Refined Wheat Flour
1	45.00	5.50	12.50	12.50
2	45.00	5.50	15.00	12.50
3	45.00	5.50	12.50	12.50
4	45.00	4.00	12.50	12.50
5	45.00	5.50	12.50	12.50
6	20.00	5.50	12.50	12.50
7	45.00	5.50	12.50	12.50
8	45.00	5.50	12.50	10.00
9	70.00	7.00	15.00	10.00
10	45.00	7.00	12.50	12.50
11	45.00	5.50	12.50	12.50
12	20.00	7.00	15.00	15.00
13	70.00	4.00	15.00	15.00
14	20.00	7.00	10.00	15.00
15	70.00	5.50	12.50	12.50
16	45.00	5.50	10.00	12.50
17	45.00	5.50	12.50	15.00
18	20.00	4.00	15.00	10.00
19	20.00	4.00	10.00	10.00
20	70.00	7.00	10.00	10.00
21	70.00	4.00	10.00	15.00

Salt and acetic acid were constant with the rate of 6 % and 1% respectively of the total weight of the product. The experiments were done and responses were fitted in the design. After each individual experiments, responses were analyzed to assess the effect of independent variables on them.

3.7.3 Process Optimization

Numerical optimization technique of the software Design-Expert version 8.0.7.1 was used for the simultaneous optimization of the multiple response. The desired goal of each factor and responses was chosen, wherein the goals were applicable to either the factor or the response. The possible goals or constraints presented were: Maximize, Minimize, Target, In-range and None (for response only) and set to an exact value (for factor only). In order to search a solution maximizing multiple responses, the goals were combined into an overall composite function called the desirability function. Desirability is an objective function that ranges from 0 to 1. The Design-Expert software seeks to maximize the function. The goal seeking begins at a random start point and proceeds up to a steepest slope to a maximum. By starting from several points in the design space, the chances improve for finding the best local maximum. To compare the effects of black rice and black gram on color and appearance, body and texture, taste and flavor and overall acceptability on the basis of sensory analysis of the product were carried out.

3.7.4 Analysis of Button Mushroom Sauce:

The moisture, ash, protein, fat, fiber, carbohydrate, vitamin C, DPPH, Total Flavonoid content, Total Phenolic content, Free Fatty acids, Thiobarbituric acid value and microbial population of mushroom sauce was analyzed. Moisture, ash, carbohydrate, protein, fat, fiber, DPPH, TFC and TPC content were determined according to the methods described by Rangana (1994), AOAC (2000) and mentioned in section **3.3.** TBA value, FFA value and microbial analysis was done according to the methods described by AOAC (2000).

All analyses were performed in triplicate, and the results are expressed as the mean values.

Determination of pH

pH is the measurement of the logarithm of inverse of hydrogen ions in the solution.

$$pH = -\log [H+]$$

Where, H+ = Hydrogen ion Concentration (g/lit)

The pH values were determined with the help of electronic pH meter. The electronic pH meter was calibrated using 7 pH and 4 pH standard buffer solutions. The function selector switch was set to pH and reading of digital display was allowed to get stable before it was noted.

Determination of TSS

The total soluble solids of button mushroom sauce were determined using the hand refractometer.

TSS is an index of soluble sugars present in fruits and vegetable. The TSS content of the samples was determined using the refractometer (Bellingham and Stanley, UK) and the results was expressed as percentage by weight. Sample was thoroughly blended in a blender, and it was filtered through a Whatman filter paper. A few drops of the clear homogenate was taken on the prism of the refractometer and direct reading was observed on the screen as per the method of AOAC (1994).

Titratable Acidity

Acidity of the batter were determined using the method as recommended by Rangana (2001). 5g sample was dissolved in 50 ml of distilled water and out of this 20 ml aliquot was taken and titrated with 0.1 N NaOH using a 2-3 drops of phenolphthalein solution as indicator. The end point was judged by the appearance of pink color. The titre value was noted and result was calculated as per cent total acids using the following equation.

$$\text{Acidity (\%)} = \frac{Vol.of\ NaOH\ used \times Normality\ of\ NaOH \times Vol.made\ upto \times eq.wt.of\ prominent\ acid \times 100}{wt.of\ sample\ taken \times vol.of\ aliquot\ taken \times 1000}$$

FIG. 3.4: PROCEDURE FOR THE EXTRACTION OF DRIED WHITE BUTTON MUSHROOM POWDER

BLANCHING OF WHITE BUTTON MUSHROOM

SINTEX PLASTIC SHEETS SPREADED ON STAINLESS STEEL TRAYS

DRIED BUTTON MUSHROOMS

SIEVING OF GRINDED MUSHROOMS

MUSHROOM POWDER

FIG. 3.5: PROCEDURE FOR THE MAKING OF BUTTON MUSHROOM SAUCE

MUSHROOM POWDER FOR SAUCE MAKING

STIRRING ANG MIXING ON HEATED PAN

ADDITION OF WATER AND SALT, PUTTING OF A SPICE BAG AND ADD ACETIC ACID

PREPARATION OF ROUX

BUTTON MUSHROOM SAUCE

SAUCE PACKED IN TIGHTLY SEALED CLEAN BOTTLE

3.8 Viscosity

The viscosity of the sauce was measured using Brookfield Viscometer. Sauce sample of 400-600ml at 25°C temperature was taken in a beaker and placed under viscometer. LV-03 (63) spindle was used which was lowered to dip into the sample up to its immersion mark on the spindle shaft. The viscosity was measured by sensing the torque required to rotate the spindle immersed in the sample and was displayed by the viscometer.

3.9 Texture Profile Analyzer

3.9.1 Texture Profile Analysis of prepared mushroom sauce

Texture Profile Analysis is a popular double compression test for determining the textural properties of foods. During a TPA test, sample was compressed twice using a texture analyzer to provide insight into how samples behave when chewed. The TPA test was often called the "two-bite test" because the texture analyzer mimics the mouth's biting action. The textural properties, i.e., hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience, were determined with the help of TA.XT plus Texture Analyzer, stable Microsystems, the UK using Texture Exponent Lite Software. The analyzer was linked to a computer that recorded the data via a software program and the probe-32 was used which is available at The Department of Dairy Science and Food Technology, B.H.U, Varanasi.

Table 3.4: Texture Profile Analyzer settings

Test mode	Measure Force in Compression
Pre-Test speed	2.00 mm/sec
Test speed	2.00 mm/sec
Post-test speed	5.00 mm/sec
Target Mode	Distance
Distance	80 mm
Time	5.00 sec
Trigger type	Auto (Force)
Trigger Force	0.0050 Kg
Tare mode	Auto

3.9.2 A typical textural profile curve

The data obtained in the compression test were used for the determination of the following textural parameters. The curve of TPA is shown in appendix II, III, IV, and V.

3.9.2.1 Hardness: The Hardness value is the peak force that occurs during the first compression. The hardness does not occur at the point of deepest compression, although it typically does for most products. Within the TPA macro, this parameter was displayed as force 2. Units are kg, g, or N.

3.9.2.2 Adhesiveness: It is the area above the curve for the first negative peak.

3.9.2.3 Springiness: Springiness is how well a product physically springs back after it has been deformed during the first compression and has been allowed to wait for the target wait time between strokes. The spring back is measured at the down-stroke of the second compression.

3.9.2.4 Cohesiveness: The ratio of positive force area during the second compression cycle (dimensionless) Cohesiveness is how well the product withstands a second deformation relative to its resistance under the first deformation.

3.9.2.5 Gumminess: Gumminess is related to primary parameters of hardness and cohesiveness and is obtained by multiplication of these two parameters.

3.9.2.6 Chewiness: Chewiness applies only to solid products and is calculated as Gumminess • Springiness (which is Distance2/Distance1)

3.9.2.7 Resilience: Resilience is how well a product "fights to regain its original height". Resilience is measured on the withdrawal of the first penetration before the waiting period is started. Resilience can be measured with a single compression; however, the withdrawal speed must be the same as the compression speed.

3.10 Sensory Evaluation of Mushroom Sauce

Using organoleptic methods, the sauce was assessed for their coloration, flavor, texture, and general acceptability. In addition, a 1-9point hedonic rating test was used to determine the degree of acceptability of button mushroom sauce. Sensory evaluation was performed by the ten semi-trained panelists from The Department of Dairy Science and Food Technology, BHU, Varanasi. The panelists were asked to rate the sample on a 1-9 point scale for color, flavor, texture, and overall acceptability, with 9 representing extreme liking, 8 representing very liking, 7 representing moderate liking, 6 representing slight liking, 5 representing neither liking nor disliking, 4 representing slight dislike, 3 representing moderate dislike, 2 representing very dislike, and 1 representing extremely dislike.

Chapter IV

RESULTS AND DISCUSSION

In this research work, RSM model has been developed, for obtaining an optimal response. Design-Expert software of version 8 was used in formulating a model of RSM of 4 factors of variables with 21 runs of experiments. It has been developed for the "Optimization and Development of Button Mushroom Sauce" and to determine the effect of independent variables on the responses and to measure their variability. To obtain an optimal response, variance analysis was enacted for each of the responses to acquire the appropriateness of the picked model.

4.1 INTERACTIVE EFFECT OF BUTTON MUSHROOM SAUCE ON VARIOUS SENSORY PARAMETERS

4.1.1 EFFECT ON COLOR

The value of response for color varied from 6.6 to 8.8 for the mushroom sauce enriched with mushroom powder, roux and garlic. The following equation describes the sensory score of Color:

$$\text{Colour} = +6.95 + 0.75 \times A + 0.15 \times B - 0.010 \times C + 0.000 \times D - 0.038 \times A \times B - 0.038 \times A \times C + 0.21 \times A \times D + 0.16 \times B \times C + 0.063 \times B \times D + 0.11 \times C \times D + 0.99 \times A^2 + 0.19 \times B^2 - 0.16 \times C^2 - 0.059 \times D^2$$

The p-value 0.0039 was significant for colour and the model F-value of 10.81 suggests that the model is significant. R-squared was found to be 0.9619, showing that 96.19 % of the variability could be explained by the model in the response. The "Pred R-Squared" of 0.9323 is in reasonable agreement with the "Adj-Squared" of 0.8729. The estimated coefficient of the colour showed that butter had a negative effect on the score of colour of sauce than mushroom powder, refined wheat flour and garlic.

Table 4.1: Coefficient estimate for Color

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	6.95	1	0.086	6.74	7.16	
A-Mushroom Powder	0.75	1	0.19	0.30	1.20	5.00
B-Garlic	0.15	1	0.19	-0.30	0.60	5.00
C-Butter	-0.010	1	0.083	-0.21	0.19	1.00
D-Maida	0.000	1	0.19	-0.45	0.45	5.00
AB	-0.038	1	0.21	-0.55	0.47	5.00
AC	-0.038	1	0.093	-0.26	0.19	1.00
AD	0.21	1	0.21	-0.30	0.72	5.00
BC	0.16	1	0.093	-0.065	0.39	1.00
BD	0.063	1	0.21	-0.45	0.57	5.00
CD	0.11	1	0.093	-0.11	0.34	1.00
A^2	0.99	1	0.16	0.59	1.39	2.05
B^2	0.19	1	0.16	-0.21	0.59	2.05
C^2	-0.16	1	0.16	-0.56	0.24	2.05
D^2	-0.059	1	0.16	-0.46	0.34	2.05

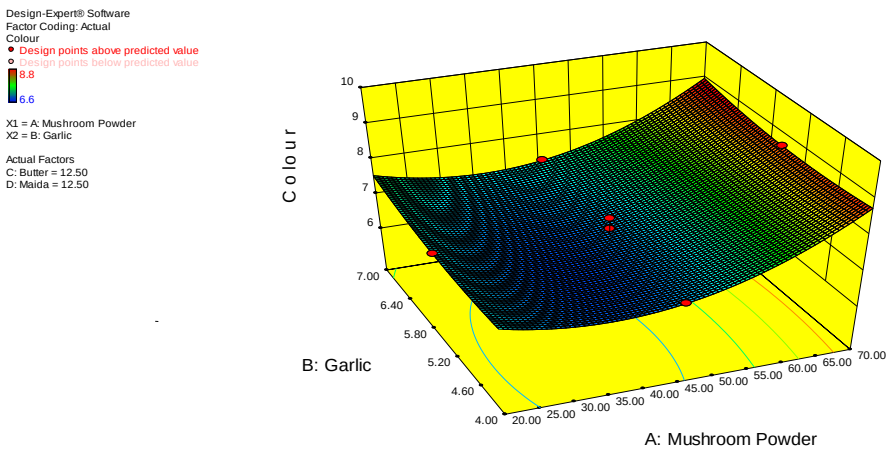


Fig. 4.1: Response surface of Color as affected by the level of Mushroom Powder and Garlic.

Figure 4.1 shows that there was a positive increase in the sensory score of colour when there is a gradual increase in the

Factor 1 and 2 but there was a decrease in the score of the colour when the factor 3 and 4 were slightly increased.

Table 4.2: RSM Design layout

No. of Trial Runs	Factor 1 A.Mushroom Powder gm	Factor 2 B.Garlic Gm	Factor 3 Butter gm	Factor 4 Maida gm	Response 1 Colour	Response 2 Flavour	Response 4 Texture	Response 4 Taste	Response 5 Overall acceptability
1	45.00	5.50	12.50	12.50	6.7	8	7.9	7.9	7.9
2	45.00	5.50	15.00	12.50	6.8	7.9	7.8	7.8	7.8
3	45.00	5.50	12.50	12.50	6.9	8.2	7.9	7.9	7.9
4	45.00	4.00	12.50	12.50	7	7	7.5	7	7
5	45.00	5.50	12.50	12.50	7.1	7	7.4	7	7
6	20.00	5.50	12.50	12.50	7.2	7.1	7.3	7.1	7
7	45.00	5.50	12.50	12.50	6.6	6.8	7	7	6.9
8	45.00	5.50	12.50	10.00	6.9	7	6.9	6.9	6.9
9	70.00	7.00	15.00	10.00	8.5	8.8	8.2	8	8.2
10	45.00	7.00	12.50	12.50	7.3	7.9	7.9	7.9	8
11	45.00	5.50	12.50	12.50	7.4	6.5	6.9	6.9	6.8
12	20.00	7.00	15.00	15.00	7.5	6.8	6.9	6.8	7
13	70.00	4.00	15.00	15.00	8.6	8.9	7.5	7.7	7.9
14	20.00	7.00	10.00	15.00	6.9	7	6.9	7	7
15	70.00	5.50	12.50	12.50	8.7	8.8	7.7	8	8.3
16	45.00	5.50	10.00	12.50	6.8	7.7	7.7	7.7	7.5
17	45.00	5.50	12.50	15.00	6.9	7.5	7.5	7.5	7.7
18	20.00	4.00	15.00	10.00	7	7.2	7.3	7.2	7.3
19	20.00	4.00	10.00	10.00	7.5	6.9	6.9	7	7
20	70.00	7.00	10.00	10.00	8.5	8.7	8.9	8.9	8.9
21	70.00	4.00	10.00	15.00	8.8	8.1	7.9	8.5	8.5

4.1.2 Effect on Flavour

The value of responses for the score of effect on flavour varied between 6.5 and 8.9 for the mushroom sauce enriched with mushroom powder, roux and garlic. The following equation describes the sensory score of flavour.

Flavour = + 7.61 + 0.83 × A + 0.11 × B + 0.12 × C – 0.030 × D

The p-value 0.0027 was significant for the flavour and the model F-value of 6.49 implies that the model is significant.

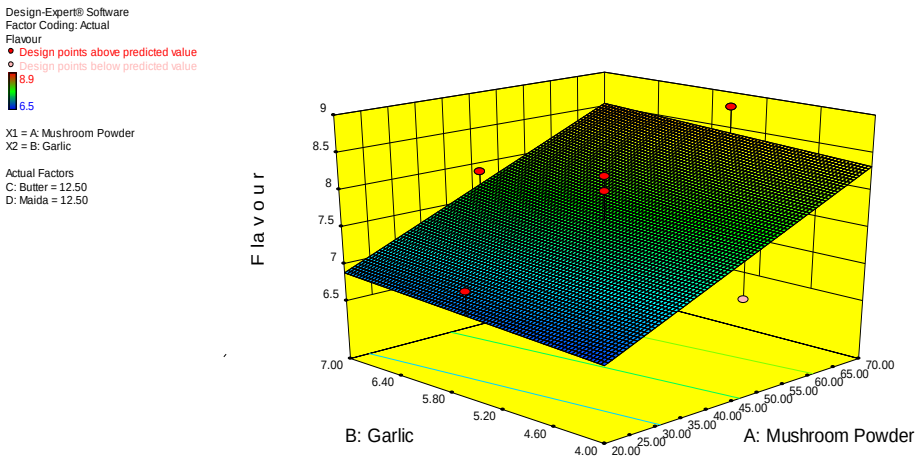
R-Squared was found to be 0.6187 indicating that 61.87 % of the variability in the response could be explained by the model and the "Pred R-Squared" of 0.4333 is in reasonable agreement with the "Adj R-Squared" of 0.5233.

The coefficient of estimation of flavour shows that maida (refined wheat flour) had a negative effect whereas mushroom powder, garlic and butter had a positive effect on the score of texture of sauce.

Table 4.3: Coefficient Estimate for Flavor

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	7.61	1	0.11	7.37	7.85	
A-Mushroom Powder	0.83	1	0.17	0.48	1.18	1.00
B-Garlic	0.11	1	0.17	-0.24	0.46	1.00
C-Butter	0.12	1	0.17	-0.23	0.47	1.00
D-Maida	-0.030	1	0.17	-0.38	0.32	1.00

Fig. 4.2: Response surface of Favour as influenced by Mushroom Powder and Garlic



From the above figure, it can be concluded that there is a possitive effect on the flavour score when there is a increase in the concentration of mushroom powder but there is a slight negative effect of garlic when slightly increased.

4.1.3 Effect on Texture

The value of responses for the score of effect on texture varied from 6.9 to 8.9 and the following equation describes the sensory score of Texture:

Texture = + 7.52 + 0.49 × A + 0.17 × B – 0.060 × C -0.15 × D

The p-value 0.0095 was significant and the model F-Value of 4.83 implies that the model is significant.

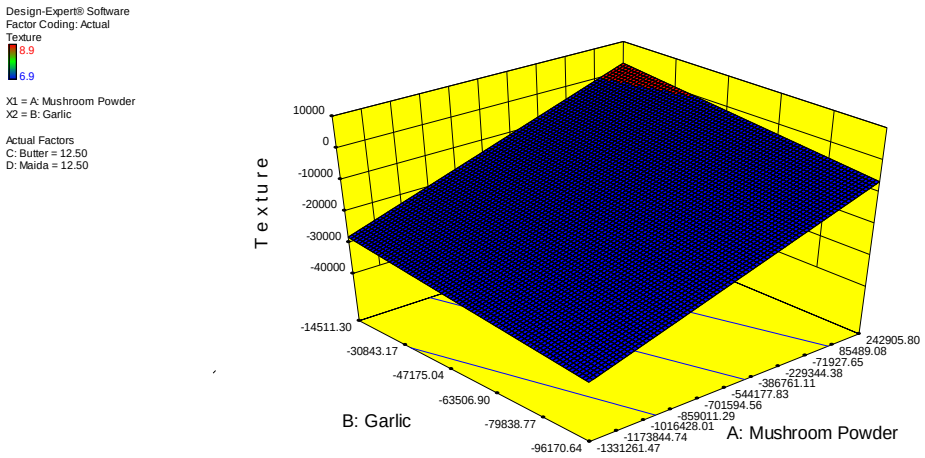
R-Squared was found to be 0.5473, indicating that 54.73 % of the variability in response could be explained by the model. The “Pred R-Squared” of 0.2461 is in reasonable agreement with the “Adj R-Squared” of o.4341

The coefficient of estimation of texture shows that butter and maida had a negative effect on the score of texture of the sauce.

Table 4.4: Coefficient Estimate for Texture

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	7.52	1	0.085	7.34	7.70	
A-Mushroom Powder	0.49	1	0.12	0.23	0.75	1.00
B-Garlic	0.17	1	0.12	-0.092	0.43	1.00
C-Butter	-0.060	1	0.12	-0.32	0.20	1.00
D-Maida	-0.15	1	0.12	-0.41	0.11	1.00

Fig. 4.3: Response surface of Texture as affected by Mushroom powder and garlic



It shows that there is a positive effect on the texture score when there is an increase in the level of mushroom powder and garlic.

4.1.4 Effect on Taste

The value of responses for the score of effect on taste varied from 6.8 to 8.9 for the mushroom sauce enriched with mushroom powder, roux and garlic. The following equation could describe the sensory score for taste:

Taste = + 7.51 + 0.60 × A + 0.12 × B – 0.16 × C – 0.050 × D

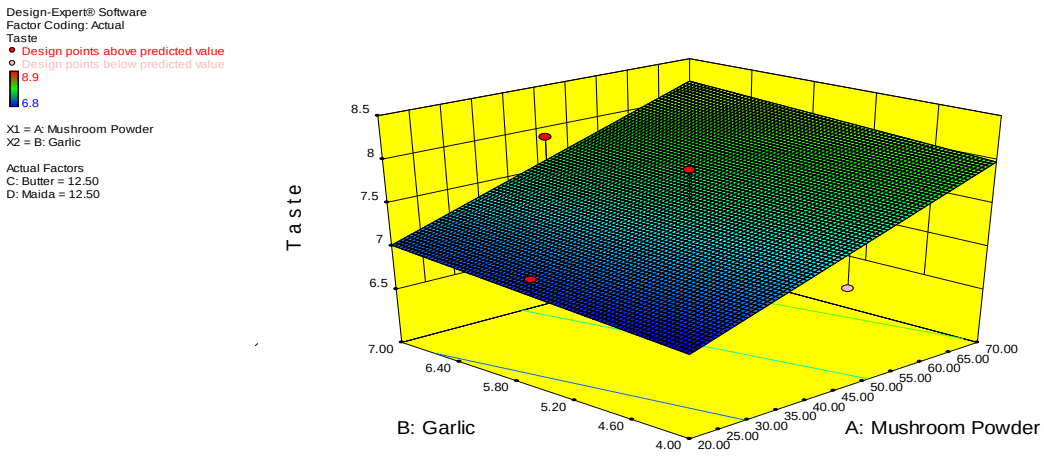
The p-value 0.0045 was significant and the model F-Value of 5.67 implies that the model is significant. R-Squared was found to be 0.5903, indicating that 59.03 % of the variability in response could be explained by the model. The "Pred R-Squared" of 0.2935 is in reasonable agreement with the "Adj R-Squared" of 0.4879.

The coefficient of estimation of taste shows that both butter and maida had a negative effect on the score of taste of sauce.

Table 4.5: Coefficient Estimate for Taste

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	7.51	1	0.091	7.32	7.70	
A-Mushroom Powder	0.60	1	0.13	0.32	0.88	1.00
B-Garlic	0.12	1	0.13	-0.16	0.40	1.00
C-Butter	-0.16	1	0.13	-0.44	0.12	1.00
D-Maida	-0.050	1	0.13	-0.33	0.23	1.00

Fig. 4.4: Response surface of Taste as affected by Mushroom powder and Garlic



The above figure shows that there is a positive effect on the score of taste of sauce when there is a slight increase in the concentration of mushroom powder and garlic.

4.1.5 Effect on the Overall Acceptability

The value of responses for the overall acceptability score of sauce enriched with mushroom powder, roux and garlic varied from 6.8 to 8.9. The following data as suggested by the software for the analysis can be fitted in a linear model as:

Overall Acceptability = + 7.55 + 0.65 × A + 0.14 × B – 0.070 × C – 0.020 × D

The p-value was significant and the model F-value of 5.63 implies the model is significant.

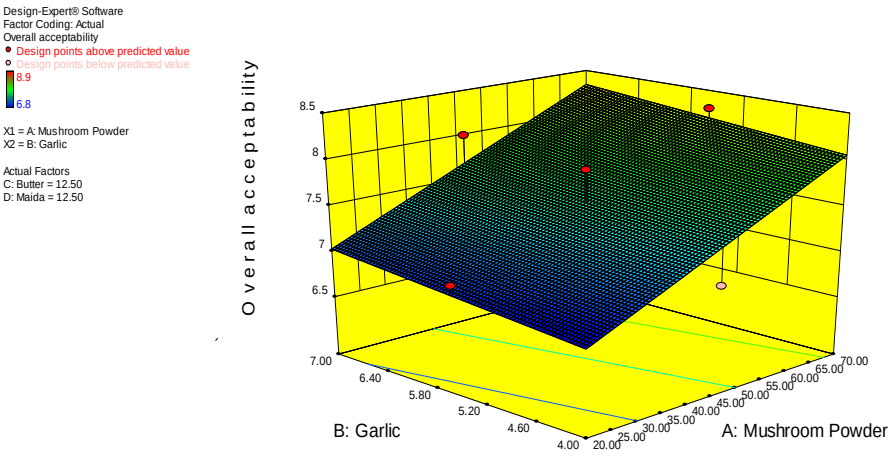
R-Squared was found to be 0.5847, indicating that 58.47 % of the variability in response could be explained by the model. The "Pred R-Squared" of 0.2907 is in reasonable agreement with the "Adj R-Squared" of 0.4808. Due to noise, there is only a 0.50 percent chance that a "Model F-Value" this big will occur.

The coefficient of estimation of the overall acceptability shows that the level of butter and maida both had a negative effect on the score of the overall acceptability of the sauce.

Table 4.6: Coefficient Estimate for the Overall Acceptability

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	7.55	1	0.097	7.34	7.75	
A-Mushroom Powder	0.65	1	0.14	0.35	0.95	1.00
B-Garlic	0.14	1	0.14	-0.16	0.44	1.00
C-Butter	-0.070	1	0.14	-0.37	0.23	1.00
D-Maida	-0.020	1	0.14	-0.32	0.28	1.00

Fig. 4.5 shows the plot of response surface for the overall acceptability as influenced by the concentrations of mushroom powder and garlic. It can be concluded that there is a significant positive effect on the increase of the levels of mushroom powder and garlic of the overall acceptability score of the sauce.



4.2 Optimization

As RSM Model was adopted for the optimization of Button Mushroom Sauce. The optimized product was made with the objective of "Optimization and Development of a shelf stable quality Button Mushroom Sauce".Certain ranges such as 20-70 g of mushroom powder, 4-7 g of garlic and roux (butter + mushroom) of 10-15 g were applied for the optimization respectively. However, 1 % of Acetic acid and 6 % of salt were kept constant. The most desirable product of 45 g mushroom powder, 5.50 g garlic, 12.50 g butter and 12.50 g maida ,was optimized with the help of desirability function method, respectively.

Table 4.7: Optimized Product

S.No.	Mushroom Powder	Garlic	Butter	Maida	Desirability
1	45	5.50	12.50	12.50	1

Table 4.8: Constraints fixed for the Optimization of Mushroom Powder, Roux and Garlic

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A.Mushroom Powder	Is in range	20	70	1	1	3
B.Garlic	Is in range	4	7	1	1	3
C.Butter	Is in range	10	15	1	1	3
D.Maida	Is in range	10	15	1	1	3

Table 4.9: Predicted score of the suggested optimized formulation of sauce by Design Expert 8

S.No.	Mushroom Powder	Garlic	Butter	Maida	Desirability
1	45	5.50	12.50	12.50	1
2	20	4.00	10.00	10.00	1
3	70	4.00	15.00	15.50	1
4	65.14	6.49	14.26	11.14	1
5	36.25	4.48	12.28	13.84	1

Figure 4.6: Contour representation of Response Surface for the Optimized sample of Sauce, for Desirability, Colour, Flavour, Texture, Taste and Overall Acceptability at constant roux level (Butter + Refined

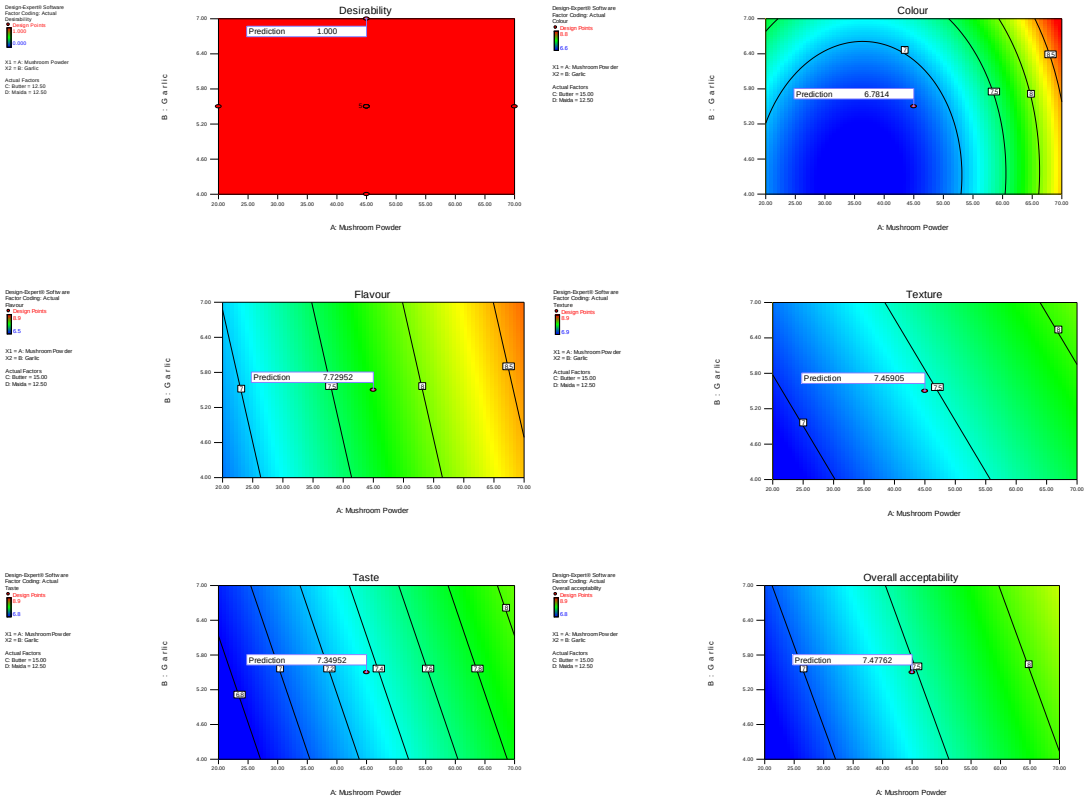


Table 4.10: The proximate physico-chemical properties of White Button Mushroom

Constituents	Mushroom
Moisture %	90.97
Total Ash %	0.99
Protein %	3.24
Crude Fibre %	1.10
Carbohydrate %	3.5
Fat %	0.29
Antioxidant Activity	51.14 %
Total Flavonoid Content	139 mg QE / 100 g
Total Phenolic Content	617 mg GAE / 100 g
Vitamin C	2.24 mg/100 gm

Data is represented as Mean ± Standard Deviation (n=3).

Table 4.11: The proximate physico-chemical properties of White Button Mushroom Sauce

Chemical Constituent	Sauce
Moisture	82.053±0.793 %
Fat	9.7±0.3 %
Protein	1.853±0.051 %
Ash	0.943±0.045 %
Crude Fibre	1.553±0.035 %
Carbohydrate	3.4±0.087 %
Vitamin C	0.516±0.030 mg/100 gm
Antioxidant activity	86.81±0.368 %
Total Phenolic Content	139±3.605 mg GAE / 100 g
Total Flavonoid Content	62.905±4.459 mg QE / 100 g
Titrable Acidity	1.44 % as Acetic acid
TSS	12.1±0.8 %
pH	4.7±0.152

Data is represented as Mean ± Standard Deviation (n=3).

As a result, a sauce that isn't tomato and soy must have a minimum of 8% TSS and 1% acetic acid, according to **FSSAI** guidelines. According to **FAO** (1995), a high TSS value indicated the existence of high sugar levels as well as other dissolved acids and minerals.

According to the **USDA**, the crude fibre level of the result was comparable to Portbello mushroom sauce.

Shelf Life study of sauce

The optimized product was packed in sterilized clean bottle jars and stored. Shelf life of the optimized product was studied at three different temperatures of: 5°C, 15°C and 30°C, respectively. These were studied at 7 days interval for 21 days, for the parameters of Free Fatty Acid, Thiobarbituric Acid and the Microbial load while during the storage temperature.

Table 4.12: Changes in the Free Fatty Acid content during the storage temperature

Days	0th	7th			14th			21st		
Temperatures °C		**5**	**15**	**30**	**5**	**15**	**30**	**5**	**15**	**30**
FFA(µ eq/g)	**0.01**	**0.02**	**0.05**	**0.12**	**0.05**	**0.08**	**0.22**	**0.1**	**0.19**	**0.35**

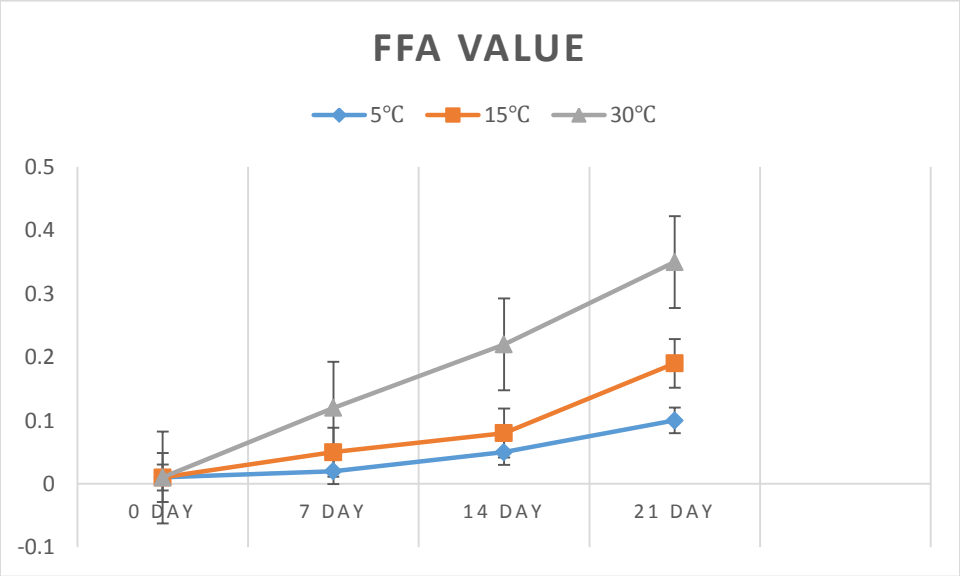


Fig. 4.7

Table 4.13: Changes in the Thiobarbituric Acid content during storage

Days	0th	7th			14th			21st		
Temperatures °C		5	15	30	5	15	30	5	15	30
TBA (mg MA/kg)	0.009	0.012	0.018	0.03	0.015	0.024	0.063	0.019	0.036	0.108

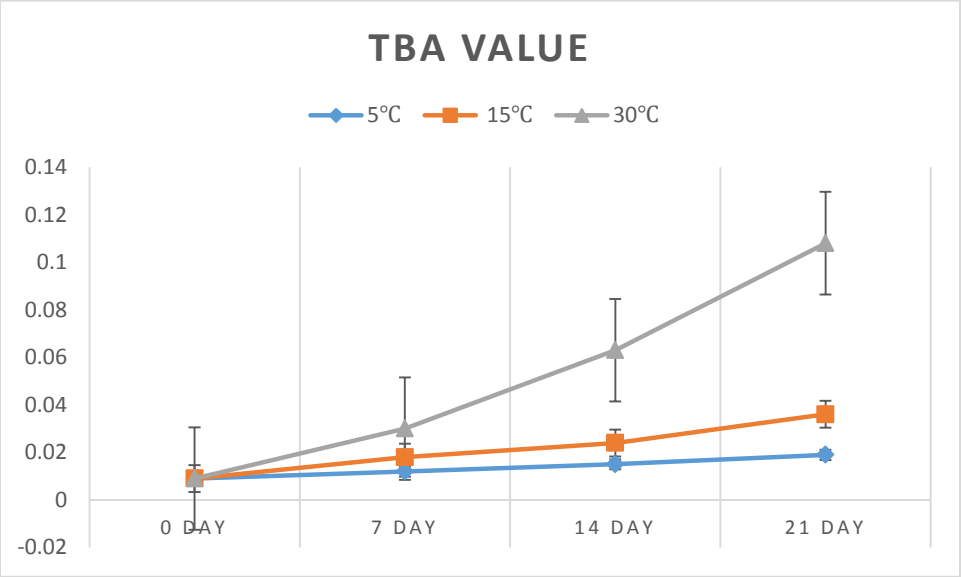


Fig. 4.8

Park *et al.*, 2015 published a study with comparable findings to the current one.

After 6 months, the TBA Value of Kimchi sauce increased significantly from 0.53 to 0.65, 1.01, and 0.68 mg/kg at 25, 35, and 45°C.

Study of the Microbial Load Storage

Table 4.14: Microbial growth during storage

Days	**0th**	**7th**			**14th**			**21st**		
Temperatures °C		**5**	**15**	**30**	**5**	**15**	**30**	**5**	**15**	**30**
Total Plate Count (10^3CFU/g)	0.03	0.05	0.1	0.98	0.09	0.3	2.3	0.12	0.5	3.16
Yeast and Mould (10^2CFU/g)	ND	ND	ND	ND	ND	ND	ND	ND	0.3	0.7

Initial microbial growth rate was found to be 0.03×10^3 CFU/g on 0^{th} day upto 0.12×10^3 CFU/g, 0.5×10^3 CFU/g and 3.16×10^3 at 5℃ 15℃ and 30℃, respectively on the 21^{st} day of observation. However in case of Yeast and Mold growth, 0.3×10^2 CFU/g to 0.7×10^2 CFU/g was noticed on the 21^{st} day of the observation. **Kumar and Ray** (2008) found similar results in their preservation trials of White Button Mushroom Chutney. After 60 days of storage at room temperature, the TPC increased from 0.53 103CFU/g to 1.70 103CFU/g.

Viscosity of Button Mushroom Sauce

The viscosity of the sample was measured in Brookfield Viscometer and the below table shows the obtaied results for viscosity of sauce:

Table 4.15: Viscosity Measurements

Viscosity	2,100 cP
Torque	17.0 %
Speed	20 RPM
Temperature	25.3°C
Time	00:02:00.0
SS	0.00 dyne/cm^3
SR	0.000 $1/s$
Density	0.0000 g/cm^3
Accuracy	60.00 cP

Texture Profile Analysis of Button Mushroom Sauce

Hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience, were determined for the textural properties of sauce by using Texture Exponent Lite Software. The analyzer was linked to a computer which recorded the data via a software program and the probe-32 was used which is available at The Department of Dairy Science and Food Technology, B.H.U, Varanasi.

Table 4.16: TPA Measurements

Textural Parameters	Values
Springiness	0.813±0.024
Cohesiveness	0.995
Gumminess	49896.727±243.680
Chewiness	40950.012±1386.810
Resilience	0.302±0.009

Data is represented as Mean ± Standard Deviation (n=2).

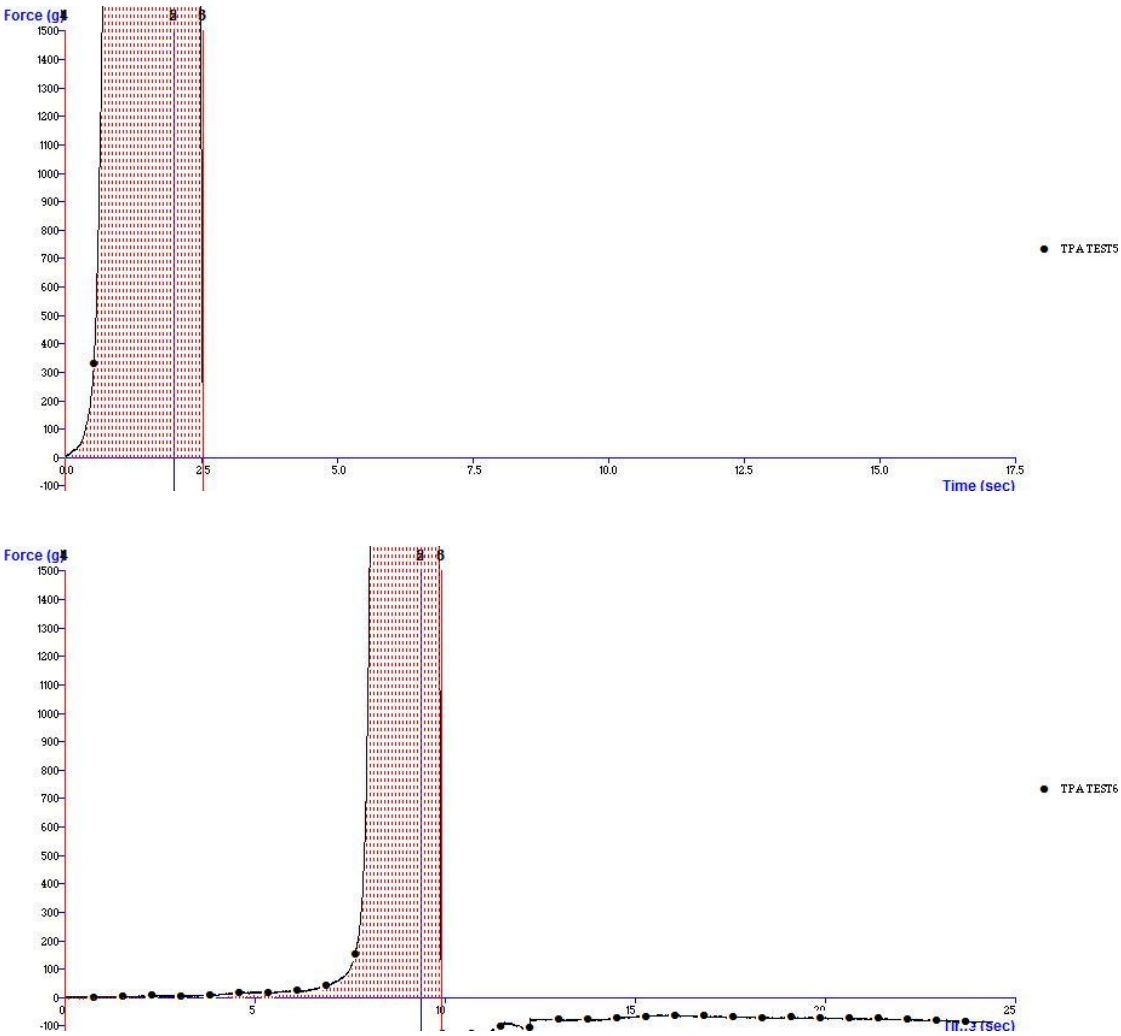


Fig. 4.9: TPA Graph

Table 4.17: Changes in the sensory traits of Sauce

Temperatures	5°C	15°C	30°C	5°C	15°C	30°C	5°C	15°C	30°C	5°C	15°C	30°C
Colour	8.5±0.1			8.3±0.1	8.3±0.2	8.4±0.2	8.3±0.3	8.2±0.4	8.1±0.3	7.3±0.1	7.3±0.3	7.1±0.1
Flavour	8.4±0.1			8.4±0.1	8.3±0.2	8.3±0.1	8.1±0.3	8.1±0.2	8±0.4	7.1±0.2	7.2±0.2	7.6±0.1
Texture	8.5±0.1			8.4±0.1	8.3±0.1	8.3±0.3	8.1±0.2	8.1±0.3	7.7±0.2	7.2±0.2	7.3±0.3	7.2±0.2
Taste	8.3±0.2			8.3±0.1	8.3±0.2	8.3±0.3	8.1±0.2	8±0.2	7.4±0.4	7.1±0.1	6.9±0.2	6.8±0.2
Overall Acceptibility	8.4±0.2			8.5±0.2	8.4±0.1	8.4±0.2	8.3±0.1	8±0.2	7.4±0.3	7.1±0.1	7.3±0.3	7.5±0.5

Data is represented as Mean ± Standard Deviation (n=10).

Chapter V

SUMMARY AND CONCLUSION

Development and Optimization of Button Mushroom Sauce was done with the help of Design Expert software by RSM Model. The independent factors of Mushroom Powder, Garlic, Butter and Refined Wheat Flour, were analyzed against the various responses of Sensory parameters viz., Colour, Flavour, Texture, Taste and the Overall Acceptability. A total of 5 optimized formulations were obtained from the predicted score of Design Expert, out of which 1was selected by the software with 1 desirability.

Results for the nutritional,physico-chemical and textural properties of Button Mushroom Sauce were obtained after discovering as 82.05 % moisture content, fat 9.7 %, crude fibre 1.55 %, Carbohydrate 3.4 %, TSS 12.1 %, ph 4.7, Titrable Acidity 1.44 %. The antioxidant activity of DPPH inhibition was obtained as 86.81 %. However, 139 mg GAE/100 g was obtained for the Total Phenolic content. Viscosity of the optimized Button Mushroom Sauce was found to be 2,100 Cp with 17.0 % Torque at 25.3. Springiness 0.813 %, Cohesiveness 0.995, Gumminess 49896.72, Chewiness 40950.012 and Resilience 0.302 was obtained by TA.XT plus Texture Analyzer for its textural properties.

Sensory Score

1. Positive efffect was observed on the sensory score of colour with an increase in the levels of mushroom powder and garlic.

2. With increase in the concentration of Wheat Refined Flour, negative effect on the flavour score was noticed.

3. Roux showed a negative effect on the texture score when increased.

4. There was an increase in the taste score when the levels of mushroom powder and garlic were increased.

5. Positive effect on the overall acceptability was found due to the levels of mushroom powder and garlic.

The Shelf Life Evaluation

The acceptability of the optimized product was done on the basis of the sensory analysis for its Colour, Flavour, Texture, Taste and Overall Acceptability.

The Thiobarbituric and Free Fatty Acid contents were studied at an interval of 7 days at 5°C, 15°C and 30°C, respectively for total of 21 days.

A significant increase in the Free Fatty Acid formation was observed from the initial value of 0.01 FFA (µ eq/g) to 0.1 FFA (µ eq/g), 0.19 FFA (µ eq/g), 0.35 FFA (µ eq/g) at 5°C, 15°C, 30°C respectively, on the 21st day.

In case of Thiobarbituric Acid formation, minimal increase was observed from 0.009 (mg MA/Kg) initially, to 0.19 (mg MA/Kg), 0.03 (mg MA/Kg), 0.108 (mg MA/Kg) respectively at 5°C, 15°C and 30°C on the 21st day.

Initial microbial growth rate was found to be 0.03×10^3 CFU/g on 0th day upto 0.12×10^3 CFU/g, 0.5×10^3 CFU/g and 3.16×10^3 at 5°C 15°C and 30°C, respectively on the 21st day of observation. However in case of Yeast and Mold growth, 0.3×10^2 CFU/g to 0.7×10^2 CFU/g was noticed on the 21st day of the observation.

While determining the Sensory characteristic of Button Mushroom Sauce a vital decrease was observed in case of taste from the initial 8.3 to 7.1, 6.9, 6.8 at 5°C, 15°C, 30°C respectively, on the 21st day. But there was a little reduction in case of Colour from 8.5 to 7.3, 7.3, 7.1; Flavour from 8.4 to 7.1, 7.2, 7.6; Texture from 8.5 to 7.2, 7.3,7.2; and overall acceptability from 8.5 to 7.1, 7.3, 7.5, all at 5°C, 15°C, 30°C respectively on the 21st day of the sensory evaluation. Overall the Button Mushroon Sauce was acceptable as well as acceptable.

Conclusion

Since mushroom has relatively poor shelf life due to its high moisture content. However, converting it into sauce improved its nutritional profile as well as made it shelf stable becauce of the presence of salt, acetic acid and spice. Overall, the optimized product was satisfactory and acceptable after inquiring for the sensory characteristics from the panelists. The product was analysed for its storage study for 21 days at different temperature which was overall successful with gradual increase in the TBA value but fast increase in case of FFA content due to the presence of butter.

BIBLIOGRAPHY

Adams, L. S., Chen, S., Phung, S., Wu, X., & Ki, L. (2008). White button mushroom (*Agaricus bisporus*) exhibits antiproliferative and proapoptotic properties and inhibits prostate tumor growth in athymic mice. *Nutrition and Cancer*, ***60(6)***: 744-756.

Agarwal, K. C. (1996). Therapeutic actions of garlic constituents. *Medicinal Research Reviews*, ***16(1)***: 111-124.

Ahlawat, O. P., Manikandan, K., & Singh, M. (2016). Proximate composition of different mushroom varieties and effect of UV light exposure on vitamin D content in *Agaricus bisporus* and *Volvariella volvacea*. *Mushroom Research*, ***25(1)***: 1-8.

Alimentarius, C. (1987). CODEX regional standard for vinegar. *Codex Standard*, *162*.

Baars, J. J. P., Sonnenberg, A. S. M., Mumm, R., Stijger, I., & Wehrens, H. R. M. J. (2016). Metabolites contributing to taste *in Agaricus bisporus* (No. 2016-1). *Plant Research International.*

Barros, L., Cruz, T., Baptista, P., Estevinho, L. M., & Ferreira, I. C. (2008). Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food and Chemical Toxicology*, ***46(8)***: 2742-2747.

Braaksma, A., & Schaap, D. J. (1996). Protein analysis of the common mushroom *Agaricus bisporus*. *Postharvest Biology and Technology*, ***7(1-2)***: 119-127.

Çağlarırmak, N. (2009). Determination of nutrients and volatile constituents of *Agaricus bisporus* (brown) at different stages. *Journal of the Science of Food and Agriculture*, ***89(4)***: 634-638.

Chang, S. T., & Miles, P. G. (2004). *Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact*. CRC press.

CHERNO, N., OSOLINA, S., & NIKITINA, A. (2016). Chemical composition of *Agaricus bisporus* and *Pleurotus ostreatus* fruiting bodies and their morphological parts. *Food and Environment Safety Journal*, ***12(4).***

Cheung, P. C. K. (2010). The nutritional and health benefits of mushrooms. *Nutrition Bulletin*, ***35(4)***: 292-299.

Citores, L., Ragucci, S., Ferreras, J. M., Di Maro, A., & Iglesias, R. (2019). Ageritin, a ribotoxin from poplar mushroom (*Agrocybe aegerita*) with defensive and antiproliferative activities. *ACS Chemical Biology*, ***14(6)***: 1319-1327.

Corrêa, R. C. G., Brugnari, T., Bracht, A., Peralta, R. M., & Ferreira, I. C. (2016). Biotechnological, nutritional and therapeutic uses of Pleurotus spp.(Oyster mushroom) related with its chemical composition: A review on the past decade findings. *Trends in Food Science & Technology*, ***50***: 103-117.

Cruess, W. V. (1958). Commercial fruit and vegetable products: Chapter 21-Vinegar manufacture.

del Mar Delgado-Povedano, M., de Medina, V. S., Bautista, J., Priego-Capote, F., & de Castro, M. D. L. (2016). Tentative identification of the composition of *Agaricus bisporus* aqueous enzymatic extracts with antiviral activity against HCV: A study by liquid chromatography–tandem mass spectrometry in high resolution mode. *Journal of Functional Foods*, ***24***: 403-419.

El Sebaaly, Z., Assadi, F., Sassine, Y. N., & Shaban, N. (2019). Substrate types effect on nutritional composition of button mushroom (*Agaricus bisporus*). *Poljoprivreda i Sumarstvo*, ***65(1)***: 73-80.

Falandysz, J., & Borovička, J. (2013). Macro and trace mineral constituents and radionuclides in mushrooms: health benefits and risks. *Applied Microbiology and Biotechnology*, ***97(2)***: 477-501.

Furlani, R. P. Z., & Godoy, H. T. (2008). Vitamins B1 and B2 contents in cultivated mushrooms. *Food Chemistry*, ***106(2)***: 816-819.

García- Casal, M. N., Peña- Rosas, J. P., & Malavé, H. G. (2016). Sauces, spices, and condiments: definitions, potential benefits, consumption patterns, and global markets. *Annals of the New York Academy of Sciences*, ***1379(1)***: 3-16.

Ghahremani-Majd, H., & Dashti, F. (2015). Chemical composition and antioxidant properties of cultivated button mushrooms (*Agaricus bisporus*). *Horticulture, Environment, and Biotechnology*, ***56(3)***: 376-382.

Jagadish LK, Krishnan VV, Shenbhagaraman R, Kaviyarasan V. (2009). Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J.E. Lange) Imbach. before and after boiling. *African Journal of Biotechnology* ***8(4)***: 651-654.

Jaworska, G., Pogoń, K., Bernaś, E., & Duda- Chodak, A. (2015). Nutraceuticals and Antioxidant Activity of Prepared for Consumption Commercial Mushrooms *Agaricus bisporus* and *Pleurotus ostreatus*. *Journal of Food Quality*, ***38(2)***: 111-122.

Jeong, S. C., Koyyalamudi, S. R., Jeong, Y. T., Song, C. H., & Pang, G. (2012). Macrophage immunomodulating and antitumor activities of polysaccharides isolated from *Agaricus bisporus* white button mushrooms. *Journal of Medicinal Food*, ***15(1)***: 58-65.

Komura, D. L., Carbonero, E. R., Gracher, A. H. P., Baggio, C. H., Freitas, C. S., Marcon, R., ... & Iacomini, M. (2010). Structure of *Agaricus* spp. fucogalactans and their anti-inflammatory and antinociceptive properties. *Bioresource Technology*, ***101(15)***: 6192-6199.

Kozarski, M., Klaus, A., Niksic, M., & Jakovljevic, D. (2011). Helsper,. JPFG; van Griensven, LJLD Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus*, *Agaricus brasiliensis*, *Ganoderma lucidum* and *Phellinus linteus*. *Food Chemistry*, ***129(4)***: 1667-1675.

Krasnow, M. N., Hirson, G. D., & Shoemaker, C. F. (2011). Effects of cooking temperatures and starch source on the gelatinization and thickening power of roux. *Journal of Culinary Science &Technology*, ***9(4)***: 247-260.

Kuettner, E. B., Hilgenfeld, R., & Weiss, M. S. (2002). The active principle of garlic at atomic resolution. *Journal of Biological Chemistry*, ***277(48)***: 46402-46407.

Liu, J., Jia, L., Kan, J., & Jin, C. H. (2013). In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). *Food and Chemical Toxicology*, ***51***: 310-316.

Mehta, B. K., Jain, S. K., Sharma, G. P., Doshi, A., & Jain, H. K. (2011). Cultivation of button mushroom and its processing: an techno-economic feasibility. *International Journal of Advanced Biotechnology and Research*, ***2(1)***: 201-207.

Mohiuddin, K. M., Alam, M. M., Arefin, M. T., & Ahmed, I. (2015). Assessment of nutritional composition and heavy metal content in some edible mushroom varieties collected from different areas of Bangladesh. *Asian Journal of Medical and Biological Research*, ***1(3)***: 495-501.

NHB, D. (2019). National Horticulture Board.

Öztürk, M., Duru, M. E., Kivrak, Ş., Mercan-Doğan, N., Türkoglu, A., & Özler, M. A. (2011). In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *Agaricus* species with fatty acid compositions and iron contents: A comparative study on the three most edible mushrooms. *Food and Chemical Toxicology*, ***49(6)***: 1353-1360.

Parashare, V., Pal, S., & Bhandari, A. (2013). Antimicrobial and nutritional studies on *Agaricus bisporus* and *Pleurotus ostreatus*. *Acta biologica indica*, ***2(1)***, 310-315.

Park, E. J., & Pezzuto, J. M. (2002). Botanicals in cancer chemoprevention. *Cancer and metastasis reviews*, ***21(3)***, 231.

Rainieri, S., & Zambonelli, C. (2009). Organisms associated with acetic acid bacteria in vinegar production. In *Vinegars of the World* (***pp. 73-95***): Springer, Milano.

Reis, F. S., Martins, A., Barros, L., & Ferreira, I. C. (2012). Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: A comparative study between in vivo and in vitro samples. *Food and Chemical Toxicology* Roberts, J. S., Teichert, A., & McHugh, T. H. (2008). Vitamin D2 formation from post-harvest UV-B treatment of mushrooms (*Agaricus bisporus*) and retention during storage. *Journal of Agricultural and Food Chemistry*, ***56(12)***: 4541-4544, ***50(5)***: 1201-1207.

Rop, O., Mlcek, J., & Jurikova, T. (2009). Beta-glucans in higher fungi and their health effects. *Nutrition Reviews*, ***67(11)***: 624-631.

Ruthes, A. C., Rattmann, Y. D., Carbonero, E. R., Gorin, P. A., & Iacomini, M. (2012). Structural characterization and protective effect against murine sepsis of fucogalactans from *Agaricus bisporus* and *Lactarius rufus*. *Carbohydrate Polymers*, ***87(2)***: 1620-1627.

Ruthes, A. C., Rattmann, Y. D., Malquevicz-Paiva, S. M., Carbonero, E. R., Córdova, M. M., Baggio, C. H., ... & Iacomini, M. (2013). *Agaricus bisporus* fucogalactan: Structural characterization and pharmacological approaches. *Carbohydrate Polymers*, ***92(1)***: 184-191.

Saiqa, S., Haq, N. B., Muhammad, A. H., Muhammad, A. A., & ATA, U. R. (2008). Studies on chemical composition and nutritive evaluation of wild edible mushrooms. *Iranian Journal of Chemistry Engineering* ***27(3)***: 151-154

Sharma, K. (2018). Mushroom: Cultivation and Processing. *International Journal of Food Processing Technology*, ***5(2)***: 9-12.

Sharma, V. P., Annepu, S. K., Gautam, Y., Singh, M., & Kamal, S. (2017). Status of mushroom production in India. *Mushroom Research*, ***26(2)***: 111-120.

Shi, Y. L., Benzie, I. F., & Buswell, J. A. (2002). Role of tyrosinase in the genoprotective effect of the edible mushroom, *Agaricus bisporus*. *Life Sciences*, ***70(14)***: 1595-1608.

Shukla, Y., & Singh, M. (2007). Cancer preventive properties of ginger: a brief review. *Food and Chemical Toxicology*, ***45(5)***: 683-690.

Singh, R. P., & Mishra, K. K. (2008). *Mushroom cultivation*.

Srivastava, R. P., & Kumar, S. (1994). *Fruit and vegetable preservation: principles and practices*. CBS Publishers & Distributors Pvt. Limited.

Stojković, D., Reis, F. S., Glamočlija, J., Ćirić, A., Barros, L., Van Griensven, L. J., ... & Soković, M. (2014). Cultivated strains of *Agaricus bisporus* and *A. brasiliensis*: chemical characterization and evaluation of antioxidant and antimicrobial properties for the final healthy product–natural preservatives in yoghurt. *Food & Function*, ***5(7)***: 1602-1612.

Vetter, J. (2007). Chitin content of cultivated mushrooms *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*. *Food Chemistry*, ***102(1)*****:** 6-9.

APPENDICES

APPENDIX A

Sensory evaluation of Button Mushroom Sauce

SCORE CARD

DATE: …….. **TIME: ……..**

NAME OF THE PANELIST: ……..

Instructions: Given below are the samples of "***Button Mushroom Sauce"*** you are requested to judge the sample on the 9 points hedonic sale for the parameters listed below :

Sample	Color and appearance	Body &Texture	Flavor	Overall acceptance

Key:

Quality grade distribution		Score
1.	Dislike extremely	9
2.	Dislike very much	8
3.	Dislike moderately	7
4.	Dislike slightly	6
5.	Neither like nor dislike	5
6.	Like slightly	4
7.	Like moderately	3
8.	Like very much	2
9.	Like extremely	1

Signature

Remark

APPENDIX B

ANOVA for the Linear Response Surface Model for the sensory score of Colour:

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	10.42	14	0.74	10.81	0.0039
A-Mushroom Powder	1.12	1	1.12	16.33	0.0068
B-Garlic	0.045	1	0.045	0.65	0.4499
C-Butter	1.000E-003	1	1.000E-003	0.015	0.9080
D-Maida	0.000	1	0.000	0.000	1.0000
AB	2.250E-003	1	2.250E-003	0.033	0.8625
AC	0.011	1	0.011	0.16	0.7002
AD	0.072	1	0.072	1.05	0.3453
BC	0.21	1	0.21	3.07	0.1305
BD	6.250E-003	1	6.250E-003	0.091	0.7734
CD	0.10	1	0.10	1.47	0.2710
A^2	2.51	1	2.51	36.39	0.0009
B^2	0.093	1	0.093	1.35	0.2889
C^2	0.064	1	0.064	0.94	0.3707
D^2	8.862E-003	1	8.862E-003	0.13	0.7322
Residual	0.41	6	0.069		
Lack of Fit	1.411E-003	2	7.054E-004	6.849E-003	0.9932
Pure Error	0.41	4	0.10		
Cor Total	10.84	20			

APPENDIX C

ANOVA for the Linear Response Surface Model of Sauce for sensory score of Flavour

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	7.16	4	1.79	6.49	0.0027
A-Mushroom Powder	6.89	1	6.89	24.97	0.0001
B-Garlic	0.12	1	0.12	0.44	0.5173
C-Butter	0.14	1	0.14	0.52	0.4805
D-Maida	9.000E-003	1	9.000E-003	0.033	0.8590
Residual	4.42	16	0.28		
Lack of Fit	2.14	12	0.18	0.31	0.9476
Pure Error	2.28	4	0.57		
Cor Total	11.58	20			

APPENDIX D

ANOVA for the Linear Response Surface Model of Sauce for the sensory score of Texture

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	2.95	4	0.74	4.83	0.0095
A-Mushroom Powder	2.40	1	2.40	15.74	0.0011
B-Garlic	0.29	1	0.29	1.89	0.1877
C-Butter	0.036	1	0.036	0.24	0.6337
D-Maida	0.23	1	0.23	1.47	0.2422
Residual	2.44	16	0.15		
Lack of Fit	1.53	12	0.13	0.56	0.8016
Pure Error	0.91	4	0.23		
Cor Total	5.39	20			

APPENDIX E

ANOVA for the Linear Response Surface Model of Sauce for the sensory score of Taste

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	4.03	4	1.01	5.76	0.0045
A-Mushroom Powder	3.60	1	3.60	20.62	0.0003
B-Garlic	0.14	1	0.14	0.82	0.3772
C-Butter	0.26	1	0.26	1.47	0.2435
D-Maida	0.025	1	0.025	0.14	0.7101
Residual	2.79	16	0.17		
Lack of Fit	1.74	12	0.15	0.55	0.8087
Pure Error	1.05	4	0.26		
Cor Total	6.82	20			

APPENDIX F

ANOVA for the Linear Response Surface Model of Sauce for the sensory score of Overall Acceptability

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	4.47	4	1.12	5.63	0.0050
A-Mushroom Powder	4.23	1	4.23	21.27	0.0003
B-Garlic	0.20	1	0.20	0.99	0.3353
C-Butter	0.049	1	0.049	0.25	0.6262
D-Maida	4.000E-003	1	4.000E-003	0.020	0.8889
Residual	3.18	16	0.20		
Lack of Fit	1.96	12	0.16	0.54	0.8193
Pure Error	1.22	4	0.31		
Cor Total	7.65	20			