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Comparative Effect of Steam and Gamma Irradiation Sterilization of Sawdust Compost on the Yield, Nutrient and Shelf-Life of *Pleurotus Ostreatus* (Jacq.Ex.Fr) Kummer Stored In Two Different Packaging Materials

Kortei Julian Nii Korley

DEPARTMENT OF NUCLEAR AGRICULTURE AND RADIATION PROCESSING

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COMPARATIVE EFFECT OF STEAM AND GAMMA IRRADIATION STERILIZATION OF SAWDUST COMPOST ON THE YIELD, NUTRIENT AND SHELF-LIFE OF *PLEUROTUS OSTREATUS* (JACQ.EX.FR) KUMMER STORED IN TWO DIFFERENT PACKAGING MATERIALS

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Kortei Julian Nii Korley; Student ID 10361731

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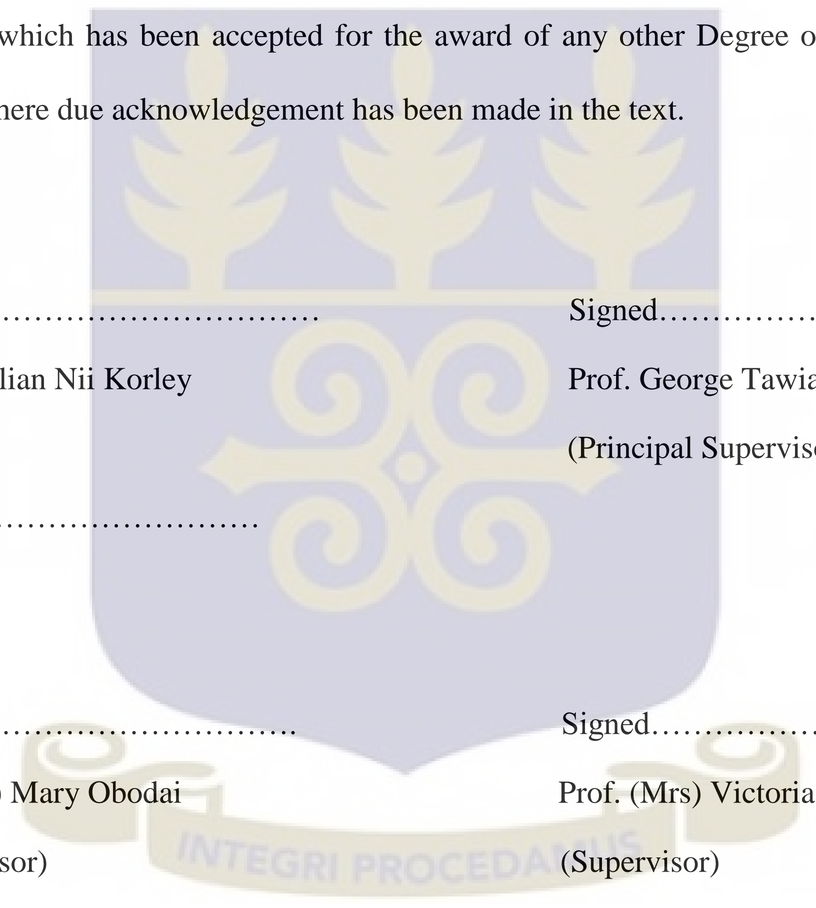
DECLARATION

This thesis is the result of research work undertaken by Kortei Julian Nii Korley in the Department of Nuclear Agriculture and Radiation Processing of the Graduate School of Nuclear and Allied Sciences, University of Ghana under the supervision of Prof. George Tawia Odamtten, Prof.(Mrs) Victoria Appiah and Dr.(Mrs) Mary Obodai and that to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other Degree of the University, except where due acknowledgement has been made in the text.



Signed…………………………… Signed…………………………

Kortei Julian Nii Korley Prof. George Tawia Odamtten

(Student) (Principal Supervisor)

Date…………………………

Signed…………………………. Signed…………………………

Dr. (Mrs) Mary Obodai Prof. (Mrs) Victoria Appiah

(Supervisor) (Supervisor)

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DEDICATION

This piece of work is dedicated to my late father Nii Abban Kortei II (Chief of Ofankor, Accra) who unstintingly prayed and encouraged me to be focused in life and in my academic career, to always persevere and move beyond my comfort zone to achieve greater things in life.

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TABLE OF CONTENT

TITLE PAGE………………………………………………………………………..i

DECLARATION………………………………………………………………...ii

DEDICATION……………………………………………………………...…...iii

ACKNOWLEDGEMENT…………………………………………………………iv

TABLE OF CONTENTS………………………………………………….……..v

LIST OF TABLES…………………………………………………………………ix

LIST OF FIGURES…………………………………………………………… xii

LIST OF PLATES……………………………………………………………..….xvii

ABSTRACT………………………………………………………………….. xviii

CHAPTER ONE……………………………………………………………...1

1.0 INTRODUCTION……………………………………………………..........1

1.1 Problem Statement and Justification……………………………………...3

1.2 Objectives…...……………………………………………………………7

CHAPTER TWO…………………………………………………………………..8

2.0 LITERATURE REVIEW………………………………………..………………8

CHAPTER THREE………………………………………………………………..45

3.0 MATERIALS AND METHODS…………...…………………………………45

CHAPTER FOUR……………………………………………………………………85

4.0 RESULTS………………………………………………………………………...85

EXPERIMENT 1…………………………………………………………………..85

PRELIMINARY RAPID APPRAISAL USING STRUCTURED QUESTIONARE TO OBTAIN DATA ON STERILIZATION, PRESERVATION, NUTRITIONAL QUALITIES AND CONSUMER PREFERENCE OF MUSHROOMS

EXPERIMENT 2……………………………………………………………………..95

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FUNGAL FLORA OF SORGHUM SPAWN USED FOR MUSHROOM CULTIVATION

EXPERIMENT 3……………………………………………………………………….101

COMPARATIVE INFLUENCE OF GAMMA IRRADIATION AND MOIST HEAT STERILIZATION TREATMENTS ON SOME PHYSICAL CHARACTERISTICS AND MYCELIAL SPAWN GROWTH ON SORGHUM

EXPERIMENT 4………………………………………………………………….....108

MYCOFLORA OF 'WAWA' SAWDUST (*TRIPLOCHITON SCLEROXYLON*) BEFORE AND AFTER GAMMA IRRADIATION AND COMPOSTING FOR 28 DAYS AT 28-30°C

EXPERIMENT 5………………………………………………………………….....109

RADIATION SENSITIVITY OF FUNGI IN 'WAWA' SAWDUST

EXPERIMENT 6…………………………………………………………………...…114

STUDIES ON THE MYCELIAL GROWTH ON THE VARIOUSLY- TREATED COMPOSTED 'WAWA' SAWDUST

(A) MYCELIAL GROWTH AND COLONIZATION TIME

(B) PRIMORDIA EMERGENCE, MYCELIAL DENSITY AND CONTAMINATION

EXPERIMENT 7………………………………………………………………………118

EFFECT OF PRETREATMENTS (IRRADIATION AND STEAM STERILIZATION) ON MOISTURE, CELLULOSE, HEMICELLULOSE, LIGNIN AND SILICA CONTENT OF COMPOSTED SAWDUST

EXPERIMENT 8………………………………………………………………………119

CHANGES IN SOME PHYSICAL CHARACTERISTICS DURING COMPOSTING OF 'WAWA' SAWDUST PRETREATMENT WITH STEAM AND GAMMA IRRADIATION BEFORE COMPOSTING

EXPERIMENT 9………………………………………………………………………121

YIELD AND YIELD ATTRIBUTES (TOTAL FRESH WEIGHT/ECONOMIC YIELD, NUMBER OF PRIMORDIA, TOTAL FRUIT BODIES, BIOLOGICAL EFFICIENCY, CAP AND STIPE DIMENSIONS AND MUSHROOM SIZE)

EXPERIMENT 10…………………………………………………………………..…127

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EFFECT OF RADIATION TREATMENT ON FOUR SELECTED THIN LAYER DRYING CURVE MODELS FOR MUSHROOM DRYING RATES AND THE CALCULATION OF EFFECTIVE MOISTURE DIFFUSIVITY

EXPERIMENT 11……………………………………………………………………….134

INFLUENCE OF GAMMA RADIATION ON THE COLOUR CHARACTERISTICS (L*,A*,B*) AND BROWNING INDEX (B.I) OF FRESH AND DRIED MUSHROOMS STORED FOR DIFFERENT PERIODS (0-12 MONTHS) IN TWO PACKAGING MATERIALS

EXPERIMENT 12……………………………………………………………………..140

DETERMINATION OF SOME SELECTED TEXTURAL CHARACTERISTICS OF THE NON-IRRADIATED AND IRRADIATED FRESH AND DRY MUSHROOMS AND REHYDRATED DRY MUSHROOMS

EXPERIMENT 13……………………………………………………………………..149

MICROBIOLOGICAL ASSESSMENT OF FRESH AND DRIED IRRADIATED MUSHROOMS STORED IN TWO PACKAGING MATERIALS FOR UP TO 12 MONTHS AT 28-30 ^{O}C

EXPERIMENT 14…………………………………………………………………......153

RADIATION SENSITIVITY (D_{10} VALUES) DETERMINATION OF *BACILLUS CEREUS* RESIDENT IN FRESH AND DRIED MUSHROOMS

EXPERIMENT 15……………………………………………………………………..155

RADIATION RESPONSE OF MEMBERS OF THE ENTEROBACTERIACEAE RESIDENT IN FRUITING BODIES OF *P.OSTREATUS* USING THE API 20E SYSTEM

EXPERIMENT 16…………………………………………………………………..……157

MYCOFLORA POPULATION ON DRY FRUIT BODIES OF *P.OSTREATUS* STORED IN EITHER POLYTHENE OR POLYPROPYLENE AND PACKS BEFORE AND AFTER GAMMA IRRADIATION FOR UP TO 12 MONTHS

EXPERIMENT 17……………………………………………………………………..165

RADIOSENSITIVITY CURVES (D_{10} VALUES) TOTAL MYCOFLORA RESIDENT IN DRIED MUSHROOMS STORED IN EITHER POLYPROPYLENE OR POLYTHENE PACKS AFTER GAMMA IRRADIATION FOR UP TO 12 MONTHS AT 28-30 ^{O}C

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EXPERIMENT 18……………………………………………………………………….167

DETERMINATION OF TOTAL PHENOLIC, TOTAL FLAVONOIDS AND ANTIOXIDANT CONTENT OF DRIED MUSHROOMS BEFORE AND AFTER IRRADIATION AND STORAGE IN EITHER POLYPROPYLENE OR POLYTHENE PACKS FOR UP TO 12 MONTHS AT 28-30 ^{O}C

(A) TOTAL PHENOLIC CONTENT

(B) TOTAL FLAVONOIDS

(C) DPPH FREE RADICAL SCAVENGING ACTIVITY (% INHIBITION)

(D) HALF MAXIMAL INHIBITORY CONCENTRATION (I.C $_{50}$)

EXPERIMENT 19……………………………………………………………………..176

DETERMINATION OF PROXIMATE (CHEMICAL) ANALYSES, METABOLIC ENERGY CONTENTS OF DRY MUSHROOMS BEFORE AND AFTER IRRADIATION AND STORAGE IN EITHER POLYPROPYLENE OR POLYTHENE PACKS FOR UP TO 12 MONTHS AT 28-30 ^{O}C

EXPERIMENT 20……………………………………………………………………..181

DETERMINATION OF ELEMENTAL MINERALS OF STORED IRRADIATED MUSHROOMS UP TO 12 MONTHS AT 28-30 ^{O}C

EXPERIMENT 21……………………………………………………………………..184

SENSORY ANALYSIS AND CONSUMER ACCEPTANCE TEST OF STEAMED IRRADIATED MUSHROOMS STORED FOR UP TO 12 MONTHS

DESCRIPTIVE TEXTURAL ANALYSIS

CHAPTER FIVE………………………………………………………………………189

DISCUSSION…………………………………………………………………………189

CHAPTER SIX……………………………………………………………………...243

CONCLUSION AND RECOMMENDATIONS…………………………………….243

REFERENCES………………………………………………………………………. .250

APPENDICES…………………………………………………………………………285

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LIST OF TABLES

Table 1: Some agricultural and industrial wastes used in the cultivation of edible mushrooms…………………………………………………………………………………5

Table 2: Scientific classification of *P.ostreatus*……………………………..………..10

Table 3: Environmental parameters for fruiting………………………………………15

Table 4: Treatments used in experiment to determine growth of mycelia on sorghum grain……………………………………………………………………………………...48

Table 5: Substrate compositions and their corresponding codes……………..……….53

Table 6: Selected thin layer drying models for *P.ostreatus*…………………………...57

Table 7: Mean D_{10} values of fungi associated with sorghum grains isolated from two (2) growth media ……………………………………………………………………….…...97

Table 8: Effect of pretreatment (gamma and moist heat) on the physical characteristics of sorghum grains (*Sorghum bicolor*)……………………………………………………..103

Table 9: Mean D_{10} values of fungi associated with sawdust isolated on the two indicated growth media………………………………………………………….…………..113

Table 10: Effect of the various indicated combination of gamma irradiation and sterilization on rate of mycelia growth, mycelia colonization time, period of appearance of primordial, mycelia density and contamination rate at 28-30 ^{o}C for 28 days….…. ..116

Table 11: Effect of pretreatments of sawdust on the lignocelluloses content on Air Dry Basis……………………………………………………………………………………118

Table 12: Moisture content and pH values of sawdust recorded during a 28 days composting period at varying time interval…………………………………………120

Table 13: Effect of steam sterilization and gamma irradiation on the pH and moisture contents of composted sawdust (*T.scleroxylon*) for 28 days…………………………...120

Table 14: Effect of pretreatments of composted sawdust on the yield of *P.ostreatus*….124

Table 15: Effect of pretreatment of composted sawdust (*T.scleroxylon*) on the fruiting pattern of *P.ostreatus*……………………………………………………………..….125

Table 16: Drying models and selection criteria for best fit for 2.0 g…………….........130

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Table 17: Effect of irradiation on the initial colour of fresh mushrooms……….........……136

Table 18: Effect of irradiation on the colour of fresh mushrooms after 5 days storage..136

Table 19: Colour change of fresh mushrooms due to effect of gamma radiation after 5 days of storage……………………………………………………………………136

Table 20: Effect of gamma radiation on the colour of dried mushrooms during 0-3 months storage …………………………………………………………………....137

Table 20 cont'd: Effect of gamma irradiation on colour of dried mushrooms during 6 - 12 months storage ………………………………………………………………………138

Table 21: Total change of colour due to irradiation during storage in the indicated packaging material for 12 months…………………………………………………...139

Table 22: Mean moisture content (%) of fresh, dried and rehydrated irradiated mushrooms…………………………………………………………………………..148

Table 23: Effect of irradiation on the microbial load of fresh mushroom fruit bodies of polypropylene pack (P2) stored for a period of 0 days…………………………………150

Table 24: Effect of irradiation on the microbial load of fresh mushroom fruit bodies of polythene pack (P1) stored for a period of 5 days……………………………………..150

Table 25: Effect of irradiation on the microbial load of dried mushroom fruit bodies of polypropylene pack (P2) stored for a period of 12 months……………………………151

Table 26: Effect of irradiation on the microbial load of dried mushroom fruit bodies of polythene pack (P1) stored for a period of 12 months……………………...………152

Table 27: Mean D_{10} values of *Bacillus cereus* on fresh and dried oyster mushrooms in storage packages……………………………………………………………………153

Table 28: List of members of the Enterobacteriaceae isolated from fresh and dry mushroom fruit bodies treated with 0-5 kGy of gamma irradiation and stored in indicated packaging material………………………………………………………………156

Table 29: Mean D_{10} values of fungi resident on mushrooms ……………...………166

Table 30: Effect of irradiation on total phenolic content of dried mushrooms stored in packaging materials for 12 months period……………………………………………169

University of Ghana http://ugspace.ug.edu.gh

Table 30 cont'd: Effect of irradiation on total phenolic content of dried mushrooms stored in packaging materials for 12 months period……………………………………………..170

Table 31: Effect of irradiation on flavonoid content of dried mushrooms stored in packaging materials for 12 months period…………………………………………...171

Table 31 cont'd: Effect of irradiation on flavonoid content of dried mushrooms stored in packaging materials for 12 months period ……………………………………….....172

Table 32: Percentage inhibition (%) of DPPH in dried and irradiated mushrooms stored in packaging materials for 12 months storage period……………………………………..173

Table 32 cont'd: Percentage inhibition (%) of DPPH in dried and irradiated mushrooms stored in packaging materials for 12 months storage period……………………..…….174

Table 33: Effect of irradiation on proximate composition of dried mushrooms in storage packs during storage period of 0-3months……………………………………………..….177

Table 33 cont'd: Effect of irradiation on proximate composition of dried mushrooms in storage packs during storage period of 6-12 months………………………...………178

Table 34: Effect of irradiation on the Metabolic Energy (Kcal/100g) of Mushrooms…180

Table 35: Effect of irradiation on the elemental composition of *P.ostreatus* during storage period of 0 months…………………………………………………………………...182

Table 36: Effect of irradiation on the elemental composition of *P.ostreatus* during storage up to 12 months……………………….……………………………………………183

Table 37: Mean scores of sensory analysis of gamma irradiated dried mushrooms stored in polythene and polypropylene packs…………………………………………………...186

Table 38: Mean scores of descriptive textural properties of mushroom sensory analysis…………………………………………………………………………………...188

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LIST OF FIGURES

Figure 1: Microscopic features of *Pleurotus ostreatus*……………………………………11

Figure 2: Life cycle of the *Pleurotus ostreatus*…………………………………………13

Figure 3: Structure of single cellulose molecule………………………………………..24

Figure 4: Demonstration of the hydrogen bonding that allows the parallel arrangement of the cellulose polymer chains……………………………………………………………25

Figure 5: Structures of some monomers of hemicelluloses……………………………..27

Figure 6: A schematic representation of the hemicelluloses……………………………27

Figure 7: Structural model of lignin……………………………………………………30

Figure 8: Schematic presentation of effects of pretreatment on lignocellulosic biomass……………………………………………………………………………….36

Figure 9: Mechanisms of degradation of cellulose by gamma irradiation……………....39

Figure 10: Texture Profile Analysis ……...……………………………………………...61

Figure 11: Method of sterilization of substrates used for mushroom cultivation………87

Figure 12: Mushroom farmer's satisfaction on the reliability of their sterilization methods in mushroom production…………………………………………………………...89

Figure 13: Consumer and public knowledge of the use of gamma irradiation in the sterilization of food to extend shelf-life……………………………………………...89

Figure 14: Aspects of production which needs attention according to respondents……90

Figure 15: Response of mushroom cultivators on the need to achieve better sterilization of compost and other ancillary methods used in the production of mushrooms………...90

Figure 16: Percentages of farmers who were able to preserve unsold mushrooms (Yes) and those unable to preserve (No)……………………………………………………91

Figure 17: Types of mushrooms most patronized by consumers………………………..91

Figure 18: Consumer preference to see their favorite mushroom produced throughout the year……………………………………………………………………………………92

Figure 19: Respondents view of the promotion of mushroom consumption……………92

Figure 20: Respondents view on the benefits of eating mushrooms……………………93

University of Ghana http://ugspace.ug.edu.gh

Figure 21: Source of mushrooms for use by consumers…………………………………...93

Figure 22: Choice of mushroom for consumption by consumers based on the listed criteria………………………………………………………………………………...94

Figure 23: What are the methods used to preserve mushrooms?………………………..94

Figure 24: Public opinion on labour and cost of mushroom production in Ghana……..95

Figure 25: Mycoflora population resident in steam and gamma irradiated sorghum grains on two indicated media…………………………………………………….…………98

Figure 26: Radiation sensitivity curves for mycoflora resident in sorghum grains cultured on two indicated growth media for 6 days at 28±30 °C……………………….…….99

Figure 27: Percentage occurrence of fungi isolated on Cooke's medium after pretreatment of sorghum grains with either steam or gamma irradiation……………………………99

Figure 28: Percentage occurrence of fungi isolated on DRBC medium after pretreatment of sorghum grains with either steam or gamma irradiation..…………………………..100

Figure 29: Effect of non-autoclaved and non-irradiated (0 kGy) sorghum grains on the daily mycelia growth of *P.ostreatus* strain EM-1…………………………………..……105

Figure 30: Effect of autoclaved and irradiated (5 kGy) sorghum grains on the daily mycelia growth of *P. ostreatus* strain EM-1…………………………………………..…105

Figure 31: Effect of autoclaved and irradiated (10 kGy) sorghum grains on the daily mycelia growth of *P. ostreatus* strain EM-1………………………………………………...106

Figure 32: Effect of autoclaved and irradiated (15 kGy) sorghum grains on the daily mycelia growth of *P. ostreatus* strain EM-1………………………………………………...106

Figure 33: Effect of autoclaved and irradiated (20 kGy) sorghum grains on the daily mycelia growth of *P. ostreatus* strain EM-1………………………………………………...107

Figure 34: Effect of autoclaved and irradiated (25 kGy) sorghum grains on the daily mycelia growth of *P. ostreatus* strain EM-1………………………………………………...107

Figure 35: Effect of autoclaved and irradiated (32 kGy) sorghum grains on the daily mycelia growth of *P. ostreatus* strain EM-1………………………………………………...108

Figure 36: Mycoflora population of sawdust during composting period enumerated on two indicated media at 30±2 °C for 7 days ……………………………………..…..111

University of Ghana http://ugspace.ug.edu.gh

Figure 37: Mycoflora population of sawdust after pretreatment with either steam or gamma irradiation and cultured on two indicated media at 30±2 ^{o}C for 7 days 111

Figure 38: Percentage occurrence of fungi of sawdust during composting plated on Cooke's medium...112

Figure 39: Percentage occurrence of fungi of sawdust during composting plated on OGYE. medium...112

Figure 40: Percentage occurrence of fungi after pretreatment of sawdust plated on Cooke's medium...113

Figure 41: Percentage occurrence of fungi after pretreatment of sawdust OGYE medium...113

Figure 42: Radiation sensitivity curves of fungi enumerated on OGYE and Cooke's media at 28-30 ^{o}C for 6 days...114

Figure 43: Temperature profile in the heap during composting of sawdust with 4 days turning interval over the period of 28 days..120

Figure 44: Effect of pretreatment (irradiation and steam) of sawdust on the yield of *P.ostreatus*...124

Figure 45: Functional relationship between fruiting bodies and economic yield........127

Figure 46: Variations of ambient air temperatures and relative humidity with time of day for solar drying of mushrooms (*P.ostreatus*)..130

Figure 47: Influence of irradiation on drying rate of oyster mushrooms..................130

Figure 48: Model fit for control (0.0 kGy) using Page's Model..........................132

Figure 49: Model fit for mushrooms exposed to 0.5 kGy using Page's Model..........132

Figure 50: Model fit for mushrooms exposed to 1.0 kGy using Page's Model..........133

Figure 51: Model fit for mushrooms exposed to 1.5 kGy using Diffusion Model.......133

Figure 52: Model fit for mushrooms exposed to 2.0 kGy using Diffusion Model.......134

Figure 53: Effective moisture diffusivity of dried mushrooms...........................134

Figure 54: Texture profile analysis of fresh mushrooms irradiated at 2 kGy............144

Figure 55: Texture profile analysis of dried mushrooms irradiated at 2 kGy............145

University of Ghana http://ugspace.ug.edu.gh

Figure 56: Influence of indicated doses of gamma radiation on hardness of mushrooms……………………………………………………………………………..145

Figure 57: Influence of indicated doses of gamma radiation on fracturability of mushrooms……………………………………………………………………………..146

Figure 58: Influence of indicated doses of gamma radiation on cohesiveness of mushrooms……………………………………………………………………………..146

Figure 59: Influence of indicated doses of gamma radiation on chewiness of mushrooms……………………………………………………………………………..147

Figure 60: Influence of indicated doses of gamma radiation on springiness of mushrooms……………………………………………………………………………..147

Figure 61: Influence of indicated doses of gamma radiation on gumminess of mushrooms……………………………………………………………………………..148

Figure 62: Influence of indicated doses of gamma radiation on adhesiveness of mushrooms……………………………………………………………………………..148

Figure 63: Influence of indicated doses of gamma radiation on resilience of mushrooms……………………………………………………………………………..149

Figure 64: Radiation sensitivity curves for *Bacillus cereus* on fresh mushrooms……..155

Figure 65: Radiation sensitivity curves for *Bacillus cereus* on dried mushrooms……..155

Figure 66: Mycoflora population of mushroom fruitbodies stored in polypropylene packs for up to 12 months and isolated on DRBC medium at 28-32 °C……………………...159

Figure 67: Mycoflora population of mushroom fruitbodies stored in polythene packs for up to 12 months and isolated on DRBC medium at 28-32 °C………………………....160

Figure 68: Mycoflora population of mushroom fruitbodies stored in polypropylene packs for up to 12 months and isolated on Cooke's medium at 28-32 °C……………………160

Figure 69: Mycoflora population of mushroom fruitbodies stored in polythene packs for up to 12 months and isolated on Cooke's medium at 28-32 °C………………………..161

Figure 70: Percentage initial occurrence of fungi on mushroom fruit bodies treated with indicated dosages of gamma irradiation and isolated on Cooke's medium at 28-30 °C……………………………………………………………………………………161

University of Ghana http://ugspace.ug.edu.gh

Figure 71: Percentage occurrence of fungi on mushroom fruit bodies stored for 3 months after irradiation in packaging materials and isolated on Cooke's medium at 28-30°C……………………………………………………………………………………162

Figure 72: Percentage occurrence of fungi on mushroom fruit bodies stored for 6 months after irradiation in packaging materials and isolated on Cooke's medium at 28-30°C……………………………………………………………………………………162

Figure 73: Percentage occurrence of fungi on mushroom fruit bodies stored for 12 months after irradiation in packaging materials and isolated on Cooke's medium at 28-30°C……………………………………………………………………………………163

Figure 74: Percentage initial occurrence of fungi on mushroom fruit bodies treated with indicated doses of irradiation in packaging materials and isolated on DRBC medium at 28-30 ^{o}C…………………………………………………………………………………...163

Figure 75: Percentage occurrence of fungi on mushroom fruit bodies stored for 3 months after irradiation in packaging materials and isolated on DRBC medium at 28-30°C……………………………………………………………………………………164

Figure 76: Percentage occurrence of fungi on mushroom fruit bodies stored for 6 months after irradiation in packaging materials and isolated on DRBC medium at 28-30°C…………..……………………………………………………………………...164

Figure 77: Percentage occurrence of fungi on mushroom fruit bodies stored for 12 months after irradiation in packaging materials and isolated on DRBC medium at 28-30°C……………………………………………………………………………………165

Figure 78: Radiation sensitivity curves for total fungi on mushroom fruit bodies immediately before and after and raised on Cooke's medium……………..…………..166

Figure 79: Radiation sensitivity curves for total fungi on mushroom fruit bodies immediately before and after and raised on DRBC medium……………….…………..167

Figure 80: Effect of storage and gamma irradiation on IC_{50} values of *P. ostreatus*…………………………………………………………………………...176

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LIST OF PLATES

Plate 1: Drum moist heat pasteurizing apparatus for pasteurizing compost bags………..52

Plate 2: Fresh mushrooms undergoing drying in the solar dryer in a tunnel…………….56

Plate 3: Solar dried and gamma irradiated *P. ostreatus* stored in polythene and polypropylene packs at 28-30°C…….…………………………………………………......59

Plate 4: API 20E test kit for detecting Enterobacteria (BioMerieux, France)………..….69

Plate 5: Fungal contaminations of sorghum grain spawn due to improper and partial pasteurization ……………………………………………………………………………99

Plate 6: Photographs of fungal species *Penicillium, Aspergillus, Fusarium, Rhodotorula* and *Trichoderma* ………………………………………………………………………100

Plate 7: Gamma irradiated raw sorghum soaked overnight before inoculation……………………………………………………………………………….102

Plate 8: Complete mycelia colonization of gamma irradiated sorghum……………………………………………………………………………...….107

Plate 9: Mycelia growth on contaminated (left) and non contaminated (irradiated) (right) sawdust………………………………………………………………………………….117

Plate 10: *P.ostreatus* fruiting bodies produced on differently treated substrates used in cultivation…………………………………………………………...…………………126

Plate 11: Complete biochemical reactions of *Klebsiella pneumonia, Citrobacter freundii* and API 20E……………………………………………………………………………156

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ABSTRACT

The conventional method of decontamination of the spawn substrate sorghum and compost of 'wawa' (*Triplochiton scleroxylon*) sawdust by the use of steam sterilization has attendant problems. In this thesis the possibility of using gamma radiation to decontaminate sorghum based spawn substrate and the compost before cultivation of the fruit bodies was determined as well as its effectiveness on selected nutritional, physical and organoleptic properties of the fruit bodies. A survey was carried out using the rapid appraisal method to determine the existing methods of sterilization, use of gamma radiation in food preservation, preference of mushrooms in Ghana by consumers and nutritional and medicinal attributes of the mushroom. The resident mycoflora in the sorghum grains and 'wawa' sawdust as well as in the fresh and dry fruitbodies of *P. ostreatus* was determined by using Standard Decimal Series Dilution technique on two media (Cooke's and DRBC). At the end of the prescribed incubation period of the compost bags, the following parameters were determined: surface mycelia density and contamination, total number of fruit bodies, number of primordial formed, total yield, Biological Efficiency, mycelia growth rate, average stipe length, average cap diameter and mushroom size at each dose applied. The bacterial loads were determined by the conventional microbiological techniques and identification was by the use of the API 20E system. The D_{10} dose requirement for reduction of fungi and *Baccillus cereus* were also carried out on the preserved fresh and dry mushroom using Fricke Dosimetry. To ascertain the influence of the different combinations of radiation and steam treatments and packaging material on the nutritional quality of the sporophore formed, proximate analysis and mineral elements composition (Na, Ca, K, P, N, Fe, Zn, Cu, Mg, Pb) were determined by using the International Standard Methods in the Mushroom Industry. Dry curves showing the influence of radiation on drying rate of oyster mushrooms were determined by five non- linear regression models (Lewis, Page, Henderson and Pabis, Diffussion and Wang and Singh by the estimation of R^2, X^2 and RMSE). The total phenolic contents, flavonoids and free radical scavenging activity DPPH (2, 2'- diphenyl-1- picrylhydrazyl) as sources of natural antioxidant of stored mushroom in polypropylene and polythene packs were determined using aqueous, ethanol and methanol extracts by Folin- Ciocalteau method. Finally, the influence of gamma irradiation on the colour

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characteristics were determined by the Hunter L* a* b* and Browning Index (BI) method while textural characteristics were determined mechanically by measuring Texture Hardness (kgf), Fracturability (N), Cohesiveness, Chewiness (N), Springiness (mm), Gumminess, Adhesiveness (kgf.s) and Resilience of fresh, dry and rehydrated mushrooms. The mechanical estimation to textural characters obtained were compared with mean scores on 9-point Hedonic scale of sensory acceptability attributes (appearance, colour, aroma, taste, mouthfeel and overall acceptability of coded samples in two packaging containers. The survey demonstrated the popularity of drum (moist heat) technique of sterilization in Ghana. Majority (64%) of the respondents were dissatisfied with the method of sterilization of compost and spawn substrate with 36% indicated the method was alright by them. Majority (82%) of the respondents have never heard of sterilization of food or mushroom by gamma irradiation technique. Because of the high contamination rate of the steam sterilized compost and spawn substrate, the farmers indicated the need to achieve better sterilization for these starting material for mushroom cultivation. Drying was selected as the most popular preservation method as the fresh fruiting bodies have short shelf-life and *P. ostreatus* was found to be the most preferred mushroom followed by the termite mushroom (*Termitomyces* sp.). The medicinal and nutritional value of mushrooms was underscored by this survey. Consumers seem to prefer mushroom on the basis of taste, appearance, texture, aroma or combination of these. A dose of 32 kGy was effective in decontamination of sorghum grains. The fastest rate of mycelia growth was 0.71 cm/day recorded by a single treatment of gamma radiation of 15 kGy on soaked raw sorghum. The slowest growth rate of 0.3 cm/day was recorded on raw non- irradiated and non- autoclaved sorghum grains (nI). The best treatment was the set of experiment which was steamed and irradiated at a dose of 25 kGy (S+I) which produced a growth rate of 11.8 mm/day and colonized completely in 7days. The slowest treatment combination was the set of experiment of non-irradiated sorghum treatment (nI) which produced a mycelium growth rate of 10.0 mm/day and used 13 days to completely colonize and produced poor mycelia density and about 80% contamination. Fungal counts on composted sawdust ranged from 4.72- 5.17 log CFU/g and 3.4- 4.1 log CFU/g on Cooke's and Oxytetracycline Glucose Yeast Extract agar respectively. Irradiation was more effective in reducing the fungal load by up to 3 log

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cycles than steam (1.1 log cycles) with D_{10} values ranging 5.64±1.12- 5.94±2.06 kGy. Ten encountered species belonging to 4 genera (*Aspergillus, Rhizopus, Fusarium and Mucor*) were predominated by *Aspergillus* (*A. alutaceus, A. fumigatus, A. flavus, A. niger, A. terreus, A. ustus*). Economic yield, biological efficiency, total fresh weight and flush weight of the fruiting bodies were recorded from the various interactions of irradiation and steam treatment of the compost showed significant differences ($P<0.05$). Autoclaved spawn + steam sterilized sawdust (A+S) of the 32 kGy treated set up recorded the highest yield of 1779g of total fresh weight (economic yield) and corresponding biological efficiency of 99.8%. Autoclaved spawn + non- steamed sterilized (A+nS) and irradiated spawn + non- steamed (I+nS) both recorded the least total fresh weight (economic yield, 0g) and biological efficiency (0%) respectively from the non-irradiated (0 kGy) set up. Microbes such as *Staphylococcus aureus, Coliforms* and *Salmonella* were not detected in the fresh and dry mushroom samples but aerobic mesophiles, *Bacillus cereus* and yeasts and moulds were encountered. The mean D_{10} values of *B.cereus* on fresh mushrooms were 3.21± 0.8 kGy (polypropylene bag), 0.76± 0.04 kGy (polythene). Low dose radiation of 1-2 kGy were effective in reducing the contaminants stored in polypropylene and polythene packs sufficiently to achieve the recommended levels of the International Commission for Microbiological Specification for Food (ICMSF). Polypropylene pack was preferred because of its rigidity and aesthetic quality. *P.ostreatus* contains significant amounts of ash, fat, protein, fibre, carbohydrate, metabolic activity as well as mineral elements (Ca, K, Mg, N, P, Na) and heavy metals (Zn, Mn, Pb, Fe, Cu) to merit its use in medicinal therapy. The drying characteristics of sliced mushroom exposed to low doses (0- 1.0 kGy) could be described by Page's Model while the Diffusion Model best fitted samples exposed to higher doses. The Effective Diffusivity (D_{eff}) (which describes the rate of moisture movement in foods) was enhanced by gamma irradiation. The textural profile of fresh and dry mushrooms (hardness, fracturability, chewiness, resilience, cohesiveness measured by instruments varied but data matched the same parameters using sensory analysis by trained panelists. Panelists found no difference between irradiated and the control samples. The colour change of fresh and dried mushrooms measured by L*a*b* values kept in two packaging materials varied little with radiation treatment and Browning Index (BI) was low showing a slow

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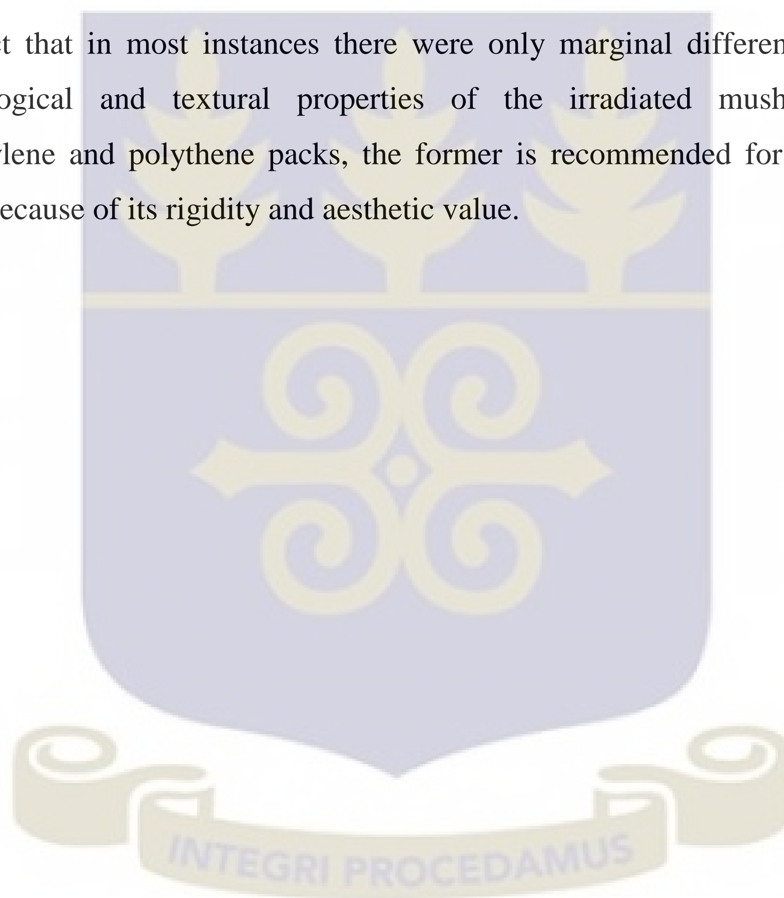
rate of enzymatic reaction after irradiation. Total phenolic contents of dry mushrooms kept in two packaging materials ranged from 0.56±0.01- 10.96±1.7 mgGAE/g, flavonoids ranged from 1.64±0.05- 8.92±0.6 mgQE/g; DPPH radical scavenging activity also ranged from 7.02±0.1- 13.03±0.04% and IC_{50} values ranged from 0.08- 0.16 mg/ml. A significant linear correlation was found between values for the total phenolic content and antioxidant activity. The high content of phenolic compounds indicates that these compounds contribute to the high antioxidant activity of *P. ostreatus* which can be promising candidate for natural mushroom source of antioxidant with high value. Owing to the fact that in most instances there were only marginal differences in nutritional, microbiological and textural properties of the irradiated mushrooms stored in polypropylene and polythene packs, the former is recommended for use as packaging material because of its rigidity and aesthetic value.



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CHAPTER ONE

1.0 INTRODUCTION

Africa's agricultural crop production systems generate large quantities of crop residues. More than 70% of the biomass of these crop residues could be put to new uses as raw materials for a wide range of new value-added products, but these are often discarded as waste. Various industrial activities similarly generate large quantities of waste products. Some of these are usable as raw materials for new value-added products. These too are discarded as waste or burnt off. In Ghana for instance, a survey conducted in 1994 revealed a total of 6,573,350 metric tones of agricultural wastes was generated (Sawyer, 1994). In recent times, one particularly effective form of bioconversion technology of agrowastes to useful protein is mushroom farming for food, and also for the production of mushroom derived substances (Obodai *et al.*, 2003). Mushroom production is so far the most profitable way of utilizing lignocellulose containing waste material (Carlile *et al.,* 2001). These wastes according to Mshandete and Cuff (2007), can be recycled into food to address the current food crisis problems in Africa. The bioconversion of agro-lignocellulose to useful food may assist in mitigating environmental pollution problems caused by the accumulation of recycled waste.

The use of fungi as dietary sources and in fermented beverages has been practiced since biblical times. Archaeological evidence traces association of edible wild mushroom to the inhabitants of Chile, almost 13,000 years ago (Rojas and Mansur, 1995); However, it was in China where the consumption of wild fungi was first reliably noted, several hundred years before the birth of Christ (Aaronson, 2000). Inspite of this age-old practice of

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consumption of fungi and their utilization in production of other food materials, their full potential was not explored until the latter half of the 20th century when it was boosted by the advent of the golden age of industrial microbiology. Since then, this diverse community of mycoflora represented by yeasts, mushrooms and filamentous fungi have been exploited in a miscellany of food products both for human and livestock consumption. The fungal kingdom possesses certain natural advantages in terms of their dietary supremacy over the rest of the Plant Kingdom. These are: (a) good protein content (20–30% of dry matter) having all the essential amino acids (yeasts are especially enriched in lysine) (Beluhan and Ranogajec, 2011; Wang *et al.,* 2001) thus capable of substituting meat. (b) chitinous wall to act as a source of dietary fibre (Kalac, 2012; Kalac, 2010), (c) high vitamin B complex , D and E content (Shao *et al.*, 2010; Kalac, 2009; Caglarirmak, 2009; Barros *et al.*, 2008), (d) low in fat (Pedneault *et al.*, 2008; Barros *et al.*, 2007) and (e) virtually free of cholesterol (Wasser and Weis, 1999; Cheung, 1998). Protein energy malnutrition has been identified as one of the biggest nutritional problems of the vulnerable group. Diseases such as Kwashiorkor, Marasmus and Anaemia are prevalent in Africa among children because protein is lacking in the daily dietary intake of the average Ghanaian child (FAO, 2013).

The Food and Agriculture Organization (FAO) of the United Nations recommended mushroom as supplementary food item to the growing population of the developing countries where cereals constitute staple diet (FAO, 2006). The lucrativeness of mushroom cultivation is further enhanced by their low cost of production, since most of them can be cultivated on agro wastes or other industrial waste products such as wastes from cereal straw, maize cob, cotton crop residues, forest sawdust; coffee bean residues,

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cashew-nut residues, sugar cane bagasse, banana leaves, brewery wastes, water hyacinth biomass, etc. (Obodai *et al.*, 2003; Philippoussis *et al.,* 2001). According to Weng and Chapple, (2010) the fungus accomplishes enzymatic degradation of the lignocellulosic portion of substrates by secreting saccharifying enzymes such as cellulases, hemicellulases and xylanases and oxidative enzymes such as lignin peroxidases (LiP), Manganese peroxidases (MnP) and Laccases.

Mushrooms also possess medicinal properties some of which have the potential to act as antitumor agents (Singh, 2013; Kawamura *et al*, 2000), antimicrobial agents (Morschhauser *et al.*, 2000; Sandven, 2000), antiviral agents (Gregori *et al.*, 2007; Zhang *et al.*, 2004), anti-HIV (Patil *et al.*, 2012), antioxidants (Yang *et al.*, 2002; Mau *et al.*, 2001), anti-inflammatory (Bobek and Galbavy, 2001; Nosalova *et al.*, 2001), immunomodulatory (Kurashige *et al.*, 1997; Svrcek *et al.*, 1996) to mention but a few.

Fungi could also be incorporated into animal feed (Akinfemi *et al.*, 2010), also used for bioremediation and degradation of xenobiotics (Buswell, 2001). The spent compost residues left after mushroom harvesting are also of value as soil conditioners (Wiafe-Kwagyan, 2014), or as feed for earthworm farming ventures (FAO, 2001).

1.1 Problem statement and Justification

The use of agrowastes as substrate for the cultivation of mushrooms is well- known world wide. Table 1 shows some agricultural and industrial wastes used in the cultivation of edible mushrooms (Howard *et al.*, 2003). The most basic concept of mushroom cultivation according to Gbogalade (2006) is the need to produce an environment in the substrate which is selectively preferential to the growth of the target species of mushroom, and less suitable for the survival of other types of microorganisms and pests.

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This involves sterilization (completely killing any other organisms which are present in the substrate likely to compete with the mushroom mycelia for utilization of the substrate as food) or pasteurization (killing off the majority of competitive organisms). Existing conventional technology available is the use of moist heat or steam for this process. But there are a number of disadvantages; it is usually very laborious, ineffective against all microbes, and lastly the throughput of the sterilization process is slow and few bags can be sterilized per unit time. Mushroom cultivation is a buisiness venture in Ghana. Although various methods of pretreatment have been reported (Martín and Thomsen, 2007; Bigelow and Wyman, 2002; Jeoh and Agblevor, 2001), few reports exist on the use of gamma radiation for the sterilization of mushroom compost and spawn substrate (Lam *et al.*, 2000; Martfnez *et al.*, 1995). Gamma radiation, if used on lignocellulosics, could serve both as a decontaminating agent (Mami *et al.*, 2013; Kim *et al.*, 2000; Gbedemah *et al.*, 1998) and a hydrolytic agent (Choi *et al.*, 2009; Byun *et al.*, 2008; Khan *et al.*, 2006) which causes a decrease in cell wall constituents or depolymerizes and degrades the fiber. An increase in organic matter digestibility has been reported due to cell wall degradation by irradiation (Al-Masri and Guenther, 1995). Production of healthy mushrooms is largely dependent on the mycelia vigor, strain and its ability to outgrow its fungal or bacterial competitors as mycelium quality is directly linked to period of fructification, number of fruiting bodies, the interval between flushes and basidiocarp size (Mshandete and Cuff, 2007).

Fresh mushrooms are perishable and can only be stored for a few days until they lose freshness and quality. There are many methods to extend the shelf-life of mushrooms (steaming, smoking, salting, drying etc).

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Table 1: Some agricultural and industrial wastes used in the cultivation of edible mushrooms

Agricultural and Industrial waste	Residues	Competing use
Grain harvesting Wheat, rice, oats barley and corn	Straw, cobs, stalks, husks	Animal feed, burnt as fuel, compost, soil conditioner
Processed grains Corn, wheat, rice, soybean	Waste water, bran,	Animal feed
Fruit and vegetable harvesting	Seeds, peels, husks, stones, rejected whole fruit and juice	Animal and fish feed, some seeds for oil extraction
Fruit and vegetable processing	Seeds, peels, waste water, husks, shells, stones, rejected whole fruit and juice	Animal and fish feed, some seeds for oil extraction
Sugar cane other sugar products	Bagasse	Burnt as fuel
Oils and oilseed plants Nuts, cotton seeds, olives, soybean etc.	Shells, husks, lint, fibre, sludge, press cake, wastewater	Animal feed, fertilizer, burnt fuel
Animal waste	Manure, other waste	Soil conditioners
Forestry-paper and pulp Harvesting of logs	Wood residuals, barks, leaves etc.	Soil conditioners, burnt
Saw-and plywood waste	Woodchips, wood shavings,sawdust	Pulp and paper industries, chip and fibre board
Pulp and paper mills	Fibre waste, sulphite liquor	Reused in pulp and board industry as fuel
Lignocellulose waste from communities	Old newspapers, paper, cardboard, old boards, disused furniture	Small percentage recycled, others burnt
Grass	Unutilised grass	Burnt

Data after Howard *et al*., (2003)

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Among the various methods employed for preservation, drying is one of the common methods used for mushrooms (Kumar *et al.*, 2013; Argyropoulos *et al.*, 2011; Kotwaliwale *et al.*, 2007). A current potentially attractive alternative method is exposure to ionizing radiation (Adu-Gyamfi and Appiah, 2012; Nimeria and Fan, 2005; Smith and Pillai, 2004). Recently, IAEA, (2005) has suggested that this method is highly effective in inhibiting physical changes associated with postharvest deterioration and maintaining a fresh product appearance. Food processing by employing radiation is well established as a physical, non-thermal mode of food preservation (cold-pasteurization) that processes foods at or nearly at ambient temperature (ASTM, 1998; CAST, 1996).

Irradiation of food products causes minimal modification in the flavor, color, nutrients, taste, and other quality attributes of food. However, the levels of modification (in flavor, colour, nutrients, taste etc.) might vary depending on the intrinsic nature of the raw material used, irradiation dose applied, and on the type of radiation source employed (gamma, X-ray, UV, electron beam) (Mexis *et al.*, 2009; Bhat and Sridhar, 2008; Bhat *et al.*, 2007). Gamma rays are short wave length, high energy photons, and have deep penetrating power. Gamma rays come from spontaneous disintegration of radioactive nuclides (Cobalt 60 or Cesium 137) as their energy source. During irradiation, the radioactive nuclides are pulled out of storage (water pool) into a chamber with concrete walls which keep any gamma rays from escaping (Park and Vestal, 2002). International Agencies like IAEA, FAO, WHO and Codex Alimentarius Commission have concluded that irradiation of any food commodity up to a dose of 10- 20 kGy poses no health risks (Diehl, 2002; WHO, 1981).

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1.2 Objectives

The overall importance of oyster mushrooms in the national nutrient supplementation and poverty alleviation economic strategies cannot be achieved without resorting to commercial cultivation using the best technological and easy to apply method. Currently, steam pasteurization treatment is used to decontaminate both the spawn substrate and the compost used in the cultivation of *Pleurotus ostreatus* in Ghana. The objectives of this study reported in this thesis were to:

- ascertain using a structured questionnaire the current knowledge of the state of the commercial cultivation of oyster mushroom in Ghana and the knowledge and acceptance of the possible use of gamma irradiation in food processing by the populace.
- update the resident fungi on sorghum (*Sorghum bicolor*) grains and 'wawa' (*Triplochiton scleroxylon*) sawdust.
- assess the comparative effect of steam (moist heat) and gamma irradiation on the mycoflora profile and phenology of resident fungal species of both the spawn run substrate (sorghum) and the compost ('wawa' sawdust, *Triplochiton scleroxylon*).
- compare the growth and yield of mushrooms grown on either irradiated or moist heat treated (steam sawdust compost) and spawn run of the sorghum grains.
- evaluate the microbiological quality of the fresh and dried mushrooms treated with either steam or gamma irradiation and stored in two packaging materials for varying periods.
- ascertain the comparative effect of gamma irradiation and steam sterilization on the drying characteristics, organoleptic and sensory qualities as well as the nutritional and elemental composition of the harvested mushrooms (fresh and dry samples) stored in the two packaging materials (polythene and polypropylene pouches).

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Oyster Mushrooms (*Pleurotus* spp.)

Pleurotus spp. are one of the choice edible mushrooms which can be cultivated in the tropics. They have gained importance only in the last decade and are now being cultivated in many countries in the subtropical and temperate zones. *Pleurotus ostreatus* is generally referred to as the oyster mushroom because the pileus or cap is shell-like, spatulate and the stipe is eccentric or lateral. Several other species of *Pleurotus* are now available for cultivation. These are *P. sajor-caju, P. florida* (probably a variant of *P. ostreatus*), *P. sapidus, P. eryngii, P. columbinus, P. cornucopiae, and P. abellatus*). (OECD, 2005).

The oyster mushroom has many advantages as a cultivated mushroom such as rapid mycelial growth, high ability for saprophytic colonisation, simple and inexpensive cultivation techniques and several kinds of species are available for cultivation under different climatic conditions. In addition, oyster mushroom is low in calories, sodium, fat and cholesterol, while rich in protein, carbohydrate, fibre, vitamins and minerals (OECD, 2005). These nutritional properties make this mushroom a very good dietary food. In addition, consumption of oyster mushroom has positive effects on the general human health because of a number of special nutritionally useful substances (Kues and Liu, 2000). Owing to these attributes, the production and consumption of this mushroom has increased tremendously and is ranked second to the button mushroom (*Agaricus campestris*). (OECD, 2005). *Pleurotus* spp. have a high ability to degrade lignocellulose and so are used in the elimination of the xenobiotic pollutants such as pentachlorophenol

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(PCP), dioxin and polycyclic aromatic hydrocarbons (PAHs). This suggests the possibility of new usage of this mushroom for environmental bioremediation (Kubatova *et al.*, 2001). Like the other mushrooms, oyster mushrooms have a wide range of temperature adaptability and substrate utilization. They are aggressive colonizers of many substrates, making them among the easiest edible fungi to cultivate. Spent mushroom compost can also be used as cattle feed, fertilizer or landfill (Kang, 2004; Poppe, 2000). *Pleurotus* spp. compost was used for bioaugmentation of tar-contaminated soils and for experimental remediation of PAH-contaminated samples (Kubatova *et al.*, 2001).

2.2 Taxonomy and Natural Distribution

2.2.1 Taxonomy and Nomenclature

Oyster mushrooms, *Pleurotus* spp., belongs to the genus *Pleurotus* (Quel.) Fr., tribe Lentineae Fayod, family *Polyporaceae* (Fr.) Fr. They are widely distributed throughout the Northern Hemisphere, such as Europe, North Africa, Asia and North America (Singer, 1986). To date, approximately 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently although some of these are considered identical to previously recognised species (OECD, 2005). The genus *Pleurotus,* which was first recommended as a tribe within the genus *Agaricus*, was proposed as a genus. Three genera of this group, *Pleurotus*, *Lentinus*, and *Panus,* were possible to be separated according to their anatomical characters of the sterile tissues of the hymenophores as being homogeneous taxonomic groups. Hilber (1982) recommended that crossing of monospore cultures is a valuable basis for *Pleurotus* studies. *Pleurotus ostreatus* (Jacq: Fr.) Kummer is the most cultivated species among the oyster mushrooms and the type species of the genus *Pleurotus*.

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According to OECD (2005), majority of mycologists have followed the proposition made by Singer (1986) which divides the genus *Pleurotus* into six sections: Sect. *Lepiotarii* (Fr.) Pilat, Sect. *Calyptrati* Sing., Sect. *Pleurotus* Sing., Sect. *Coremiopleurotus* (Hilber), Sect. *Lentodiellum* (Murr.) Sing. and Sect. *Tuberegium* Sing. *Pleurotus ostreatus* was placed in the Sect. *Pleurotus* based on the absence of veil and with the monomitic hyphal system (Table 2). (OECD, 2005).

Table 2: Scientific classification of *Pleurotus ostreatus*

KINGDOM	Fungi
DIVISION	Basidiomycota
CLASS	Hymenomycetes
ORDER	Agaricales
FAMILY	Polyporaceae
GENUS	Pleurotus
SPECIES	*Pleurotus ostreatus*

Source: OECD (2005)

2.2.2 Morphological description

Species identification within the genus *Pleurotus* is difficult because of the morphological similarities and possible environmental effects. Mating compatibility studies have demonstrated the existence of eleven discrete intersterility groups in *Pleurotus* to distinguish one species from the others. *P. columbinus, P. florida, P. salignus,* and *P. spodoleucus* are the synonyms or subspecies taxa for the species of *P. ostreatus*. (OECD, 2005). Recently, Wiafe-Kwagyan (2014) has used molecular characteristics employing PCR to distinguish between *P. ostreatus* and *P. eous*. The microscopic morphological features of *P. ostreatus* are illustrated in Fig.1.

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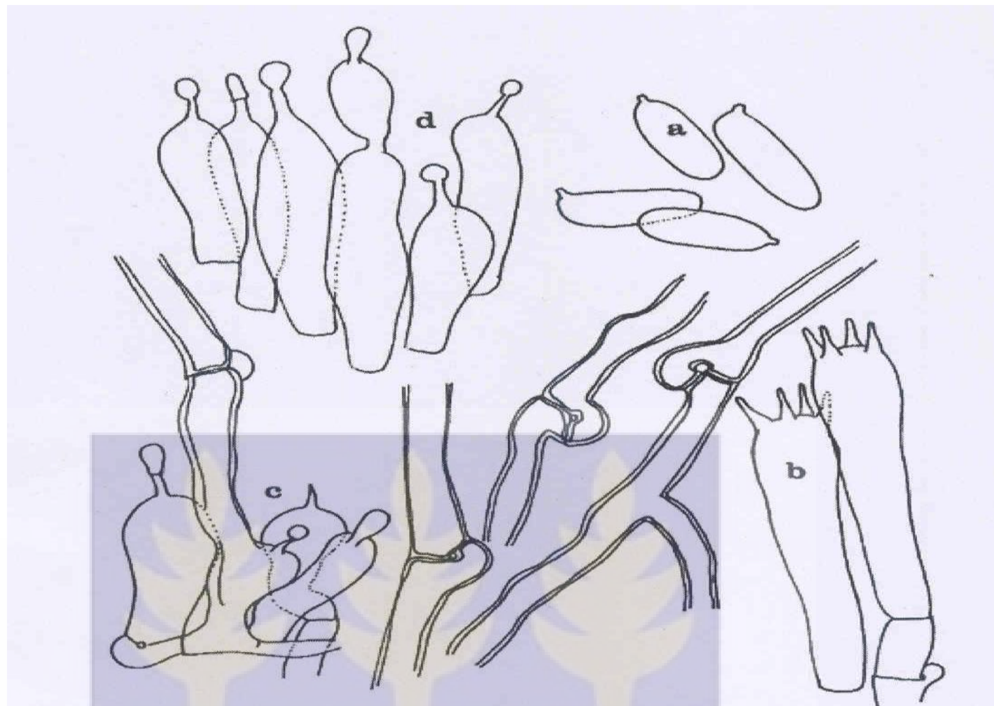

Fig. 1: Microscopic features of *P. ostreatus* (a: spores, b: basidia, c: cheilocystidia, d: pleurocystidia)

Source: OECD (2005)

2.3 Natural habitat

The geographic distribution of the oyster mushroom varies according to its species. For example, *P. pulmonarius* and *P. cystidiosus* are known to be distributed in the tropical and subtropical regions, while *P. eryngii* are found in southern Europe, North Africa and central Asia. It has many subspecies and similar taxa such as *P. fuscus* var. *ferulae* from China. *P. ostreatus* is widespread in the temperate zones such as Korea and Japan because it forms fruit-bodies at relatively low temperature compared to other *Pleurotus* species. Commonly grown on broad-leaf hardwoods in the spring and fall, cottonwoods, oaks, alders, maples, aspens, ash, beech, birch, elm, willows and poplars are especially favoured natural habitat for oyster mushroom. Although seen on dying trees, *P. ostreatus*

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is thought to be primarily a saprophyte, but behaves as a facultative parasite at the earliest opportunity. Occasionally, it grows on composting bales of straw and in Mexico, on the pulp residues from coffee production. The most abundant fruiting of this species is in low valley riparian habitats (Stamets, 1993).

2.3.1 Life cycle of *Pleurotus ostreatus*

The major events in the life cycle of *P. ostreatus* are described as follows (Fig. 2): A single basidiospore germinates to be a mass of homokaryotic mycelium, each cell of which contains a single haploid nucleus. The homokaryotic mycelia continue to grow until the hyphae fuse with the other hyphae which have compatible mating type. After fusion between compatible homokaryotic hyphae, reciprocal nuclear migration occurs and a heterokaryotic mycelium is formed. The subsequent growth involves the synchronous division (conjugate division) of the two nuclei in each compartment and their regular distribution as nuclear pair throughout the mycelium via clamp connections. Heterokaryotized mycelia with enough mycelia mass and appropriate environmental stimuli (cooling 10ºC– 21°C, relative humidity 85-90%, and light requirement 1000-2000 lux, CO_2 < 1000 ppm) can form the fruit bodies. During fruit body formation, nuclear fusion and meiosis occur only in the specialised basidia. Haploid nuclei migrate into a tetrad of basidiospores, external to the basidium. Each basidium has commonly four monokaryotic basidiospores. Occasionally five or more have been observed. These spores germinate into homokaryotic hyphae (Casselton, 1995).

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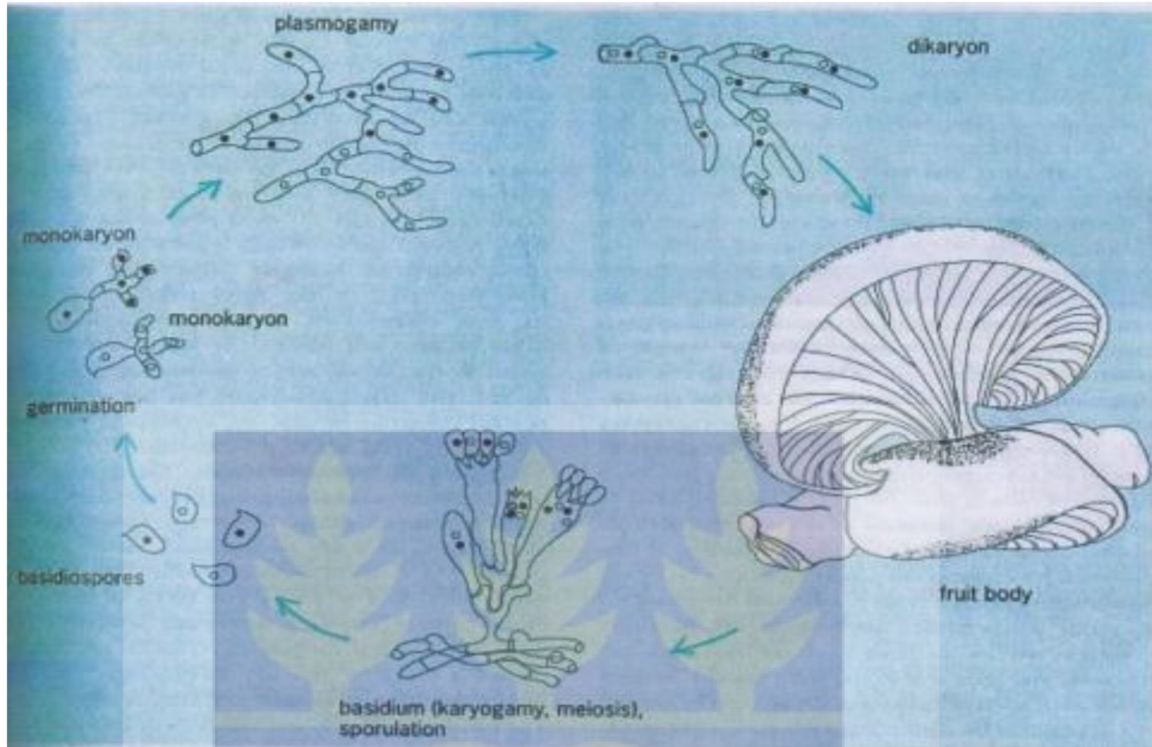

Fig.2: Life cycle of the *Pleurotus ostreatus* (Source: Martinez-Carrera, 1998)

2.4 Requirements for mycelial growth

The carbon sources suitable for mycelial growth are starch, glucose, fructose, maltose, mannose, sucrose, pectin, cellulose and lignin. Ethanol is also a source of carbon for mycelial growth; however, citrate, oxalate and other organic acids are not beneficial to the growth of the mycelium. The nitrogenous sources utilised by *Pleurotus* spp. are peptone, corn steep liquor, soybean cake powder, yeast powder, ammonium sulphate, asparagine, serine, alanine and glycine. The utilisation of urea is rather poor (OECD, 2005). The optimal temperature for growth of the mycelium is from 25-28 °C and the range of pH is from 5.5 to 6.5. The tolerance of mycelia for CO_2 is rather strong. The mycelia of *Pleurotus* spp. can still grow flourishingly at the carbon dioxide concentration

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of 15 to 20%. Only when the concentration of CO_2 is raised to 30% does the growth of mycelia rapidly decrease (Chang and Miles, 1989).

2.5 Requirement for fruit body formation

For fruit body formation, CO_2, light and temperature are key environmental factors. When the CO_2 concentration in the mushroom house or growing bags is higher than 600 ppm (0.06%), the stipe elongates and the growth of the caps will be prevented. The requirements for light are different for the various stages of growth. The growth of mycelium does not need any light and cultivation of the oyster mushroom in a dark place is better than in a bright place. The formation of primordia and the growth of fruiting bodies require light. The former requires light of 200 lux intensity for over 12 hrs. The growth of the fruiting body requires light of 50 to 500 lux intensity. The colour of the caps is closely related to the intensity of light, and if it is low, then the colour will be pale. The optimal temperatures for the development of fruiting bodies can range from 10 to 18 °C (Chang and Miles, 1989). Growers can choose a suitable strain for their own natural environment. Each *Pleurotus* species needs different environmental conditions for fruit body development as illustrated in Table 3.

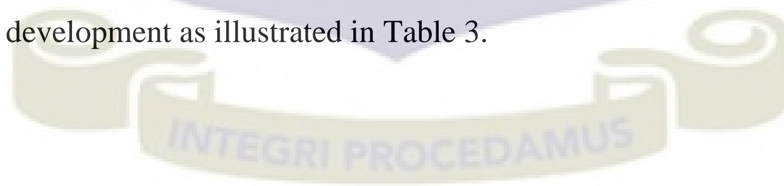

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Table 3. Environmental parameters for fruiting of *Pleurotus* spp.

Species	Temp. (°C)	Relative humidity (%)	CO_2 (ppm)	Light(lux)
P. pulmonarius	21-29	90-95	<1,000	500-1,000
P. cystidiosus	21-27	85-90	<2,000	500-1,000
P. djamor	20-30	85-90	500-1,500	750-1,500
P. eryngii	15-21	85-90	<2,000	500-1,000
P. euosmus	21-27	90-95	<1,000	750-1,500
P. ostreatus	10-21	85-90	<1,000	1,000-1,500
P. pulunonarius	18-24	85-90	400-800	1,000-1,500
P .tuberregium	30-35	85-90	<2,000	

(Source: Kang, 2004; Stamets, 1993)

The environmental and substrate conditions for the growth and fruiting of *P.ostreatus* in Ghana have been demonstrated by many workers (Wiafe-Kwagyan, 2014; Obodai and Odamtten, 2013; Obodai *et al.*, 2003, 2000; Obodai and Vowotor, 2002; Obodai, 1992).

2.6 Some Nutritional and Medicinal Properties of *Pleurotus ostreatus*

2.6.1 Nutritional properties

2.6.1.1 Active constituents

There are reports in pertinent literature on the chemical composition of *P. ostreatus* and related species. In most of the studies (Deepalakshmi and Mirunalini, 2014; OECD, 2013), the nutritional values of mushroom have been analysed in dried fruit bodies.

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Generally, fresh *Pleurotus* mushroom contain 85- 95% moisture (Khan, 2010). The fruiting body of *P. ostreatus* contains approximately 100 different bioactive compounds, which are mainly considered as a potential new source of dietary fiber. Whereas, fungal cell wall is rich in non-starch polysaccharides, such as β-glucan there are also other interesting functional components and phenolic compounds such as protocatechuic acid, gallic acid, homogentisic acid, rutin, myrictin, chrysin, naringin, α-tocopherol and γ-tocopherol, ascorbic acid and β-carotene each having its own outstanding therapeutic medical effects (Ferreira *et al.*, 2009; Wang *et al.*, 2001). Moreover, they are healthy foods, rich in protein, lipids, carbohydrates, vitamin and minerals content but low in calories and fat contents.

2.6.1.2 Proteins

Several proteins showing unique features have been isolated from mushrooms including lecithins, lignocellulolytic enzymes, proteases inhibitor and hydrophobins. Although mushrooms are rich sources of diverse proteins, not many of these proteins have been identified and even fewer have been characterized. The content of protein in *P. ostreatus* is reported to vary according to strains, physical and chemical differences in the growing medium (Akyuz and Kirbag, 2010), composition of the substrate, size of the pileus, and harvest time (Mshandete and Cuff, 2007). The protein content of *P.ostreatus* ranges from 13.1- 37.4 g/100 g dry weight fruit bodies (OECD, 2013; USDA, 2010; Obodai and Apetorgbor, 2008). Lectin a dimeric protein with a molecular weight of 40 kDa to 41 kDa respectively with antihepatoma and antisarcoma properties was isolated from the fresh fruiting bodies of *P. ostreatus* (Wang *et al.*, 2000).

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2.6.1.3 Carbohydrates and fibers

Carbohydrates constitute the major component of mushroom dry matter, (usually about 50-60%). The carbohydrate comprises various compounds-monosaccharide, their derivatives and oligosaccharides (commonly called sugars) and both reserve and construction polysaccharides (Glucans), which are important in the proper functioning of the alimentary tract (Kalac, 2012). Carbohydrates which are mainly present in *P. ostreatus* as polysaccharides are glycogen (and such indigestible forms as dietary fibers), cellulose, chitin, α- and β- glucans and other hemicelluloses like mannans, xylans and galactans (Hossain *et al.*, 2007; Manzi *et al.*, 2001).The glucans are present in different types of glycosidic linkages, such as branched (1→3), (1→6)-*β*-glucans and linear (1→3)-*α*- glucans.

2.6.1.4 Lipids

Pleurotus mushroom are low in fat content, but they contain some essential fatty acids. However, mushrooms are not considered as a significant source of essential fatty acids for fulfilling the requirements of human body. Oleic acid is the major monounsaturated fatty acid and linoleic acid is the major polyunsaturated fatty acid in *P. ostreatus*. According to Hossain *et al* (2007), *P. ostreatus* contains the monounsaturated fatty acids oleic acid (363µg/g dried mushroom) and the n-6 essential fatty acids linoleic acid (533 µg/g dried mushroom) at the higher concentrations (Hossain *et al.*, 2007). The n-3 essential fatty acid linoleic acid (11.6 µg/g dried mushroom) and arachidonic acid (10.8µg/g dried mushroom) were also found in *P. ostreatus*.

2.6.1.5 Vitamins

Mushrooms fruit bodies are rich in vitamins, mainly vitamin B_1, vitamin B_2, vitamin C and vitamin D_2 (Manzi *et al.*, 2004). The vitamin of group B are abundant particularly

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thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, nicotinamide, folic acid and cobalamin as well as other vitamins such as ergosterol, biotin, phytochinon and tocopherols (Mattila *et al.*, 2001). With respect to thiamine content, mushrooms are a bridge between yeast and other food products of vegetable origin. *P. ostreatus* contains more folacine, vitamin B_1, vitamin B_3 but less vitamin B_{12} than other mushroom species.

2.6.1.6 Mineral constituents

Pleurotus mushrooms have a miscellany of minerals and their fruiting bodies are characterized by high levels of mineral constituents. The fructifications of mushroom are characterized by a high level of well assailable mineral constituents (Mattila *et al.*, 2001). But the mineral level depends, among other things, on the species and age of the mushrooms, the diameter of the pileus and on the substratum (Demirbas, 2001). According to Vetter *et al* (1994), the distribution of these substances in the fructification varies and their content is usually greater in the pileus than in the stipes. The pileus or cap of *P. ostreatus* have greater contents of copper, iron, potassium, magnesium, phosphorous and zinc and the stipes have higher content of sodium (Vetter *et al.*, 1994; Watanable *et al.,* 1994). However, the bioavailability of these mineral contents in *Pleurotus* mushroom needs to be tested in animal and human studies.

2.6.1.7 Enzymes

A characteristic trait of the composition of mushrooms is the occurrence of a variety of enzymes, which are never or only rarely found in other organisms. The content of oxidative oxidases, fat splitting lipases, inverting enzymes proteolytic enzymes and activity of polyphenol oxidases have been demonstrated by some researchers (Espin and Wichers, 1999). According to Ratcliffe *et al* (1994), various species of mushroom show

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different enzymatic activity characterized by a higher activity of polyphenoloxidase compound in *P. ostreatus*. The effect of this enzyme is due to the catalysis of phenol compound oxidation, causing a rapid darkening of harvested mushrooms, which in turn reduces their sensory and nutritive properties. Conversely, the darkness of product decreases their keeping quality and hence their market value (Devece *et al.*, 1999).

2.7 Medicinal Properties of *Pleurotus ostreatus*

2.7.1 Antibacterial

Pleurotus has been explored to control simple and multiple drug resistant isolates of *Escherichia coli, Staphylococcus epidermidis*, *S. aureus* and species of *Candida, Streptococcus*, *Enterococcus* (Singh *et al.*, 2012; Wolf *et al.*, 2008). Cowan (1999), reported that phenolic and tannin constituents of *P. ostreatus* was capable of eliciting antibacterial activity as found in many medicinal plants with mechanisms of action characterized by cell memberane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesions (Cowan, 1999). The antimicrobial potency of the oil of the macrofungus extracted with petroleum ether and acetone, were observed to inhibit the gram positive and gram negative bacterial tested *in vitro*. This suggests that *P. ostreatus* has a broad-spectrum antibacterial activity (Iwalokun *et al.*, 2007). Organic extracts (methanol and chloroform) of *P. ostreatus* has been shown as effective against Gram-positive bacteria underscoring its potential as an antibacterial agent (Mirunalini *et al.*, 2012; Karaman *et al.*, 2010).

2.7.2 Antiviral

The goal of the antiviral chemotherapy is to find antiviral agents which are specific for the inhibition of viral multiplication without affecting normal cell division. Ei- Fakharany

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et al (2010) reported that a laccase has been purified from *P. ostreatus* mushroom, which is capable of inhibiting the entry of hepatitis C virus into peripheral blood cells and hepatoma HepG2 cells and its replication (Ei-Fakharany *et al.*, 2010). Isolation of a novel ubiquitin-like protein from *P. ostreatus* mushrooms manifested an inhibitory activity toward HIV-1 reverse transcriptase (Wang and Ng, 2000).

2.7.3 Antioxidants

Oxidative stress has been implicated as a primary factor in the progression of many degenerative diseases like cancer and hepatotoxicity. Nevertheless, antioxidants such as phenolic and flavonoid compounds are delaying and inhibiting oxidative processes. Generally, *Pleurotus* mushrooms are rich in vitamins and selenium content which are the important natural antioxidants in biological systems (Chang and Miles, 1989). Jayakumar *et al* (2010) reported that, an extract of *P. ostreatus* enhanced the Catalase gene expression and decreased the incidence of free radical-induced protein oxidation in aged rats, thereby protecting the occurrence of age-associated disorders that involve free radicals. The ethanolic extracts of the oyster mushroom *P. ostreatus* are reported to have potent antioxidant activity in both *in vitro* and *in vivo* studies. The ethanolic extract exhibit *in vitro* antioxidant activity by virtue of its scavenging hydroxyl and superoxide radicals, inhibiting lipid peroxidation, reducing power on ferric ions, chelating ferrous ions and quenching 2,3-diazabicyclo[2,2,2]oct-2-ene (DBO). It also showed a good *in-vivo* antioxidant activity by reducing the intensity of lipid peroxidation and by enhancing the activities of enzymatic and non-enzymatic antioxidants (Jayakumar *et al.*, 2010). Consequently, *P.ostreatus* serves as a good source for the development of antioxidant food additives (Mitra *et al.*, 2013).

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2.7.4 Immunomodulatory properties

Immunomodulatory properties alone with low cytotoxicity raise the possibility that mushrooms could be effective replacement in the cancer patients receiving conventional chemotherapy and radiotherapy treatment, to build up immune resistance and decreased toxicity. A large number of compounds like lectins, polysaccharides, polysaccharides-peptides, and polysaccharide-protein complex have been isolated from mushroom and many of these compounds have been found to have immunomodulatory effects (Wang *et al.*, 2000). Although modes of actions of these compounds are not clear, they are suggested to enhance cellular components of the immune system (Chihara *et al.*, 1992). It has been reported that water extract from fruit bodies and mycelia of *P. ostreatus* has a role in increasing the production of reactive oxygen species (ROS) from neutrophil and has immunomodulatory properties involving all immunocompetent cells (Shamtsyan *et al.*, 2004). However, the bioactivities of the polysaccharides depend on the binding ability on the lectin-like surface receptor of the immune cells.

2.7.5 Antihypercholesterolic

The ethanolic extract of dried fruiting bodies of *P. ostreatus* showed an effective evidence of the anti-hyperlipidaemic activity when added to the diet of normal wistar male rat and a strain with hereditary hypercholesterolaemia. In this study, addition of the dry oyster fungus to diet significantly increased, by more than two-fold, triacylglycerol (TAG) level in plasma of both groups of rats compared with their respective controls. In contrast, the ethanolic extract did not significantly change TAG levels (Opletal *et al.*, 1997). Alam *et al* (2009) reported the enhancing effect of oyster mushroom on lipid profile, liver and kidney functions in hypercholesterolic rats. Feeding of hypercholesterolemic rats with 5% powder of *P. ostreatus* and *P. sajor-caju* reduced the

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plasma total cholesterol (TC) level (by 37% and 21% respectively) and triglycerides (TG) level (by 45% and 24% respectively) due to presence of active substance Lovastatin (Alam *et al.*, 2009).

2.7.6 Anticancer

Different types of extract from *P. ostreatus* have been demonstrated as potential anticancer agents in different cancer cell lines and experimental animals. However, clinical evidence of anticancer activities of *P. ostreatus* mushrooms has not been clearly established. Gu and Sivam, (2006), screened the anticancer activity of *P. ostreatus* against human androgen-independent prostate cancer PC-3 cells. They found that a water-soluble extract prepared from the fresh *P. ostreatus* produced the most significant cytoxicity and induced apoptosis in PC-3 cells as dose dependent manner. Lavin *et al* (2006) reported the activity of an aqueous polysaccharide extract from the edible mushroom *P. ostreatus* which induced anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells. Owing to the presence of newly identified low molecular weight α-glucan with promising anti-tumorigenetic properties, they demonstrated its direct effect on colon cancer cell proliferation via induction of programmed cell death (Lavin *et al.*, 2006). The hot water extract of *P. ostreatus* also showed suppression in proliferation of MCF-7 human breast cancer cells (Martin and Brophy, 2010). The methanol extract of *P. ostreatus* has been tested on some breast and colon cancer cells and proven positive (Jedinak and Silva, 2008).

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2.8 Physical and chemical characteristics of lignocellulosic biomass used in cultivation of mushrooms

2.8.1 Composition

The term "lignocellulosic biomass" is used when referring to higher plants, softwood or hardwood. Lignocellulose is the most abundant renewable biomass with a worldwide annual production of $1x10^{10}$ metric tonnes (Sánchez and Cardona, 2008). Lignocellulosic biomass is composed of cellulose, hemicellulose and lignin, as well as other minor components.

Cellulose is a major structural component of plant cell walls, and it provides mechanical strength and chemical stability to plants. Solar energy is absorbed through the process of photosynthesis and stored in the form of cellulose (Raven *et al.*, 1992). Hemicellulose is a copolymer of different C_5 and C_6 sugars which also exist in the plant cell wall. Lignin is a polymer of aromatic compounds produced through a biosynthetic process and forms a protective layer for the plant walls. In nature, the above substances grow and decay during the year. It has been estimated that around $7.5x10^{10}$ tonnes of cellulose are consumed and regenerated every year (Kirk-Otmer, 2001). It is thereby the most abundant organic compound in the world. Apart from the three basic chemical compounds that lignocellulose consists of; water is also present in the complex. Furthermore, minor amounts of proteins, minerals and other components can be found in the lignocellulose composition as well. The composition of lignocellulose highly depends on its source. There is a significant variation of the lignin and (hemi) cellulose content of lignocellulose depending on whether it is derived from hard-wood, softwood, or grasses.

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Figs.3, 4 and 5 summarize the composition of lignocellulose encountered in the most common sources of biomass including what is used for bioconversion to bioprotein by mushrooms.

2.8.2 Cellulose

Cellulose is the β-1,4-polyacetal of cellobiose (4-O-β-D-glucopyranosyl-D-glucose). Cellulose is more commonly considered as a polymer of glucose because cellobiose consists of two molecules of glucose. The chemical formula of cellulose is $(C_6H_{10}O_5)$ n and the structure of one chain of the polymer is presented in Fig.3

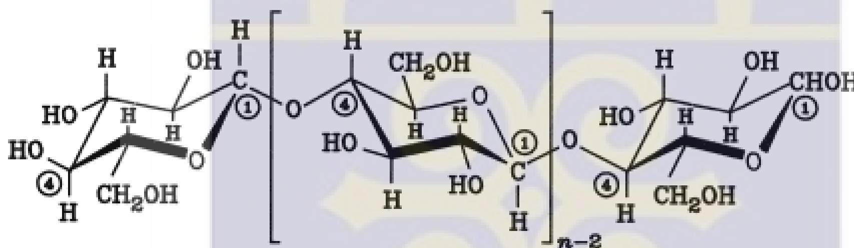

Fig. 3: Structure of single cellulose molecule

Many properties of cellulose depend on its degree of polymerization (DP), i.e. the number of glucose units which make up one polymer molecule. The DP of cellulose can extend to a value of 17000, even though more commonly a number of 800-10000 units is encountered (Kirk-Otmer, 2001). For instance, cellulose from wood pulp has a DP between 300 and 1700.

The nature of bond between the glucose molecules (β-1, 4 glucosidic) allows the polymer to be arranged in long straight chains. The latter arrangement of the molecule, together with the fact that the hydroxides are evenly distributed on both sides of the monomers, allows for the formation of hydrogen bonds between the molecules of cellulose. The

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hydrogen bonds in turn result in the formation of a compound which comprised several parallel chains attached to each other (Fig.4) (Faulon *et al.*, 1994). Cellulose is found in both the crystalline and the non-crystalline structure. The coalescence of several polymer chains leads to the formation of microfibrils, which in turn are united to form fibres. In this way cellulose can obtain a crystalline structure.

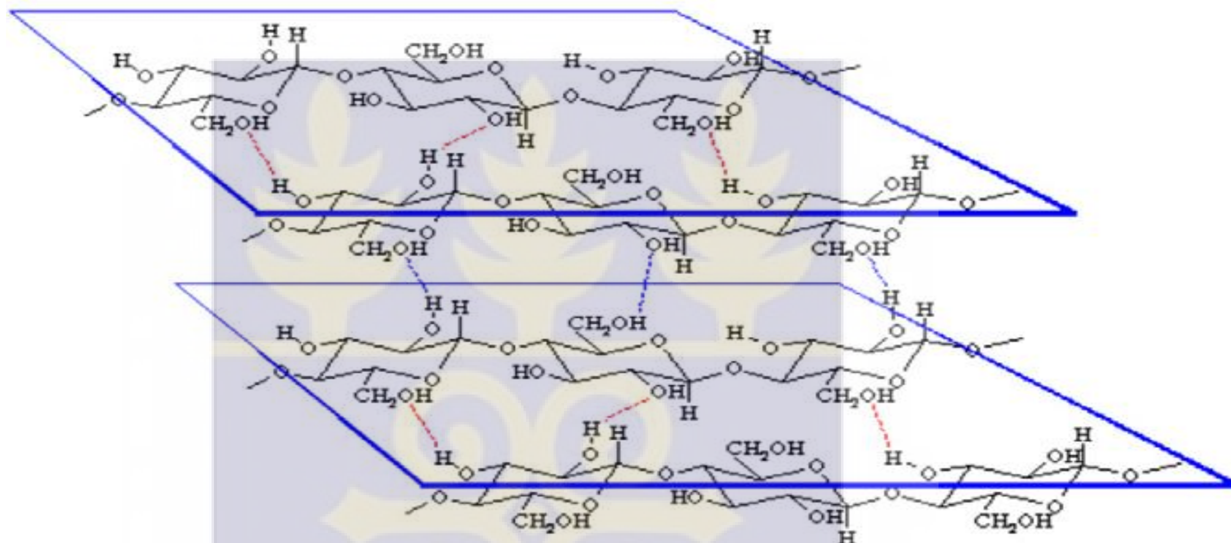

Fig. 4: Demonstration of the hydrogen bonding which allows the parallel arrangement of the cellulose polymer chains (Faulon *et al.*, 1994)

Cellulose is a relatively hygroscopic material absorbing 8-14% water under normal atmospheric conditions (20 °C, 60% relative humidity). Nevertheless, it is insoluble in water, where it swells. Cellulose is also insoluble in dilute acid solutions at low temperatures. The solubility of the polymer is strongly related to the degree of hydrolysis achieved. As a result, factors which affect the hydrolysis rate of cellulose also affect its solubility. At higher temperatures it becomes soluble, as the energy provided is enough to break the hydrogen bonds which hold the crystalline structure of the molecule. Cellulose is also soluble in concentrated acids, but severe degradation of the polymer is caused by hydrolysis. In alkaline solutions extensive swelling of cellulose takes place as well as

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dissolution of the low molecular weight fractions of the polymer (DP < 200) (Krassig and Schurz, 2002). Cellulose does not melt with temperature, but its decomposition starts at 180°C (Thermowood Handbook, 2003).

2.8.3 Hemicelluloses

The term hemicellulose is a collective term. It is used to represent a family of polysaccharides such as arabino-xylans, gluco-mannans, galactans, and others which are found in the plant cell wall and have different composition and structure depending on their source and the extraction method. The most common type of polymers which belongs to the hemicellulose family of polysaccharides is xylan. The molecule of a xylan involves 1->4 linkages of xylopyranosyl units with α-(4-O)-methyl-D-glucuronopyranosyl units attached to anhydroxylose units. The result is a branched polymer chain which is mainly composed of five carbon sugar monomers, xylose, and to a lesser extent six carbon sugar monomers such as glucose. Important aspects of the structure and composition of hemicellulose are the lack of crystalline structure, mainly due to the highly branched structure, and the presence of acetyl groups connected to the polymer chain (Kirk-Otmer, 2001). Hemicelluloses are hetero polysaccharides consisting of short branched chains of hexoses, e.g. mannose units in mannans and pentoses such as xylose units in xylans (Fig.5) (Kuhad *et al.,* 1997).

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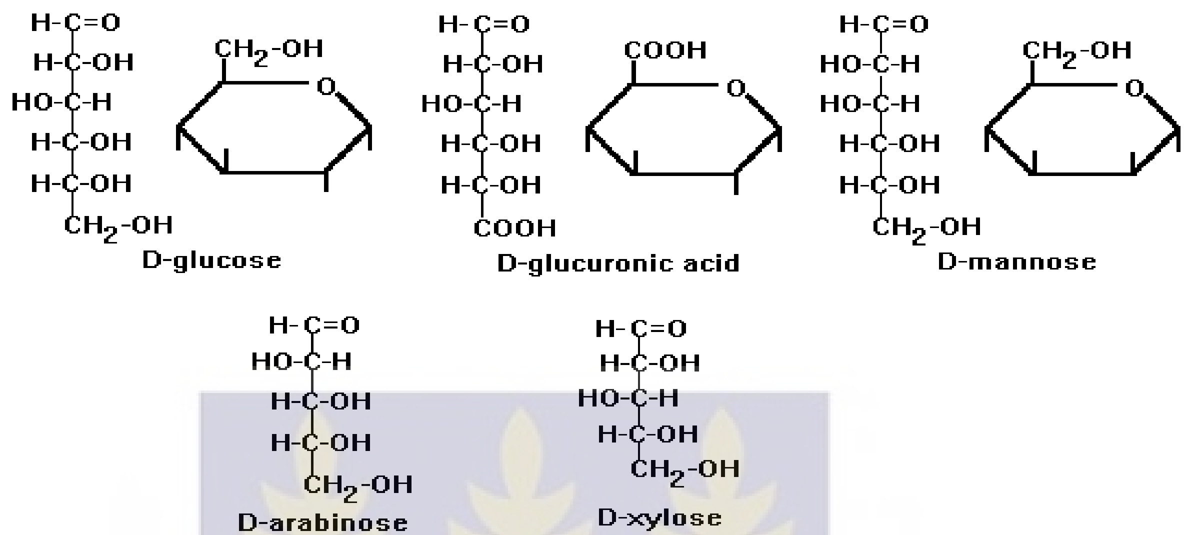

Fig.5: Structures of some monomers of hemicelluloses. Source: (Kuhad *et al*, 1997)

Usually, all of the pentoses are present. There may even be small amounts of L- sugars. There are hexoses as well as acids formed by oxidation of sugars (Fig.6). Mannose and mannuronic acid tend to be present, and there can be galactose and galacturonic acid. The pentoses are also present in rings which can be 5-membered or 6-membered. Xylose is always the sugar present in the largest amount

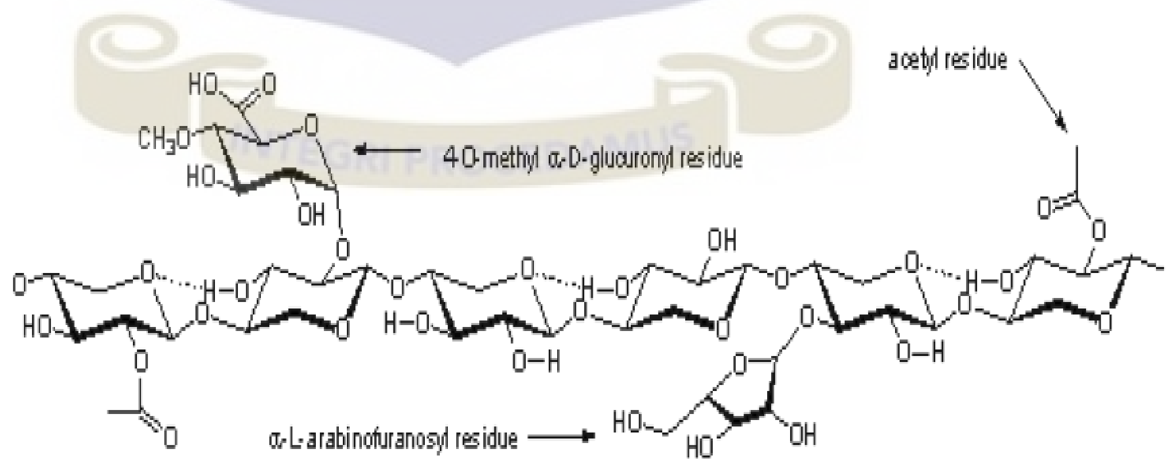

Fig.6: A schematic representation of the hemicellulose Source: (Kuhad *et al.*, 1997)

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Hemicellulose extracted from plants possesses a high degree of polydispersity, polydiversity and polymolecularity (a broad range of size, shape and mass characteristics). However, the degree of polymerization does not exceed the 200 units whereas the minimum limit can be around 150 monomers.

Hemicellulose is insoluble in water at low temperatures. However, its hydrolysis starts at a temperature lower than that of cellulose, which renders it soluble at elevated temperatures (Thermowood Handbook, 2003). The presence of acid highly improves the solubility of hemicellulose in water.

2.8.4 Lignin

Lignin is the most complex natural polymer and the second most abundant renewable biopolymer in nature. It is an essential part of the plant cell wall, imparting rigidity and protecting the easily degradable cellulose from attack by pathogens. It is an amorphous three-dimensional polymer with phenylpropane units as the predominant building blocks. More specifically, p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol consist of an aromatic ring and 3-carbon side chains are the ones most commonly encountered (Brown, 1985). Lignin is aromatic, 3-dimensional and amorphous. It is synthesized from phenyl propanoid precursors by polymerization in higher plants. In the lignin molecule (Fig.7), the precursors form 3 types of subunit: hydroxyphenol- (H-type), guaiacyl- (G-type) and syringyl subunits (S-type). A typical finding for the lignin polymer is that there is no single repeating bond between the subunits but a random distribution of at least 10 different types of bond, the most common being the β-aryl ether (*β*-O-4) bond (Argyropoulos and Menachem, 1997).

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Due to its complicated structure and non hydrolysable bonds, lignin is more difficult to break down than cellulose or hemicellulose. The molecular mass (MW) of lignin is high, about 100 kDa or more, which prevents its uptake inside the microbial cell (Eriksson *et al.,* 1990). Thus, the biological degradation of macromolecular lignin must occur through the activity of extracellular enzymes of resident microorganisms. The substrate material for degradation by composting is stacked up in large piles, the microenvironmental conditions (Temperature, pH, humidity, moisture content etc.) differ at different depths of the pile.

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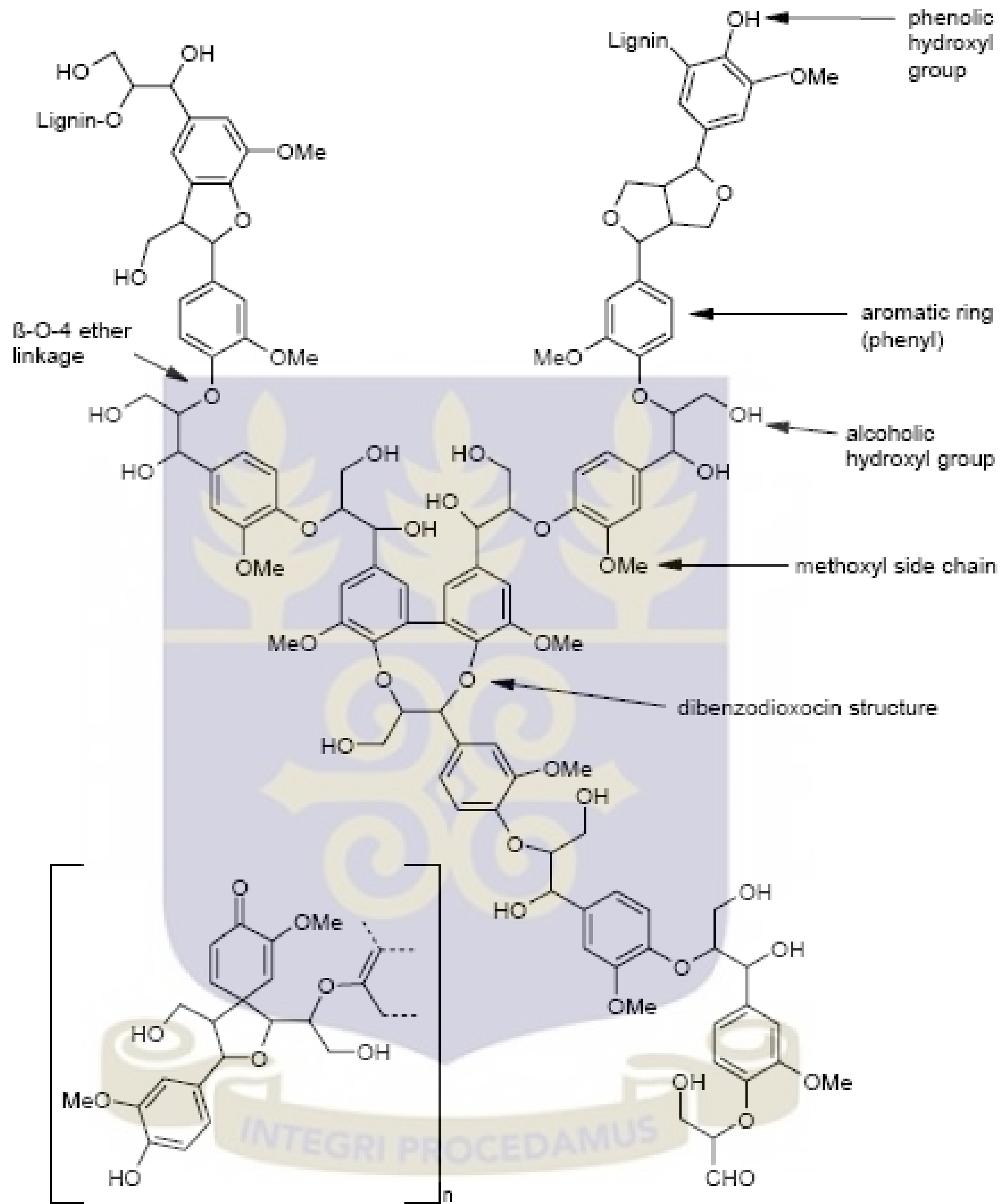

Fig.7: Structural model of lignin (Brunow, 2001)

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2.9 Composting

Composting is a thermogenic, solid state fermentation process, carried out by a succession of microbial populations beginning with mesophilic bacteria, actinomycetes and fungi followed by thermophiles and ending again with mesophiles (Johri *et al.*, 1999). Composting process creates stable, soil-enriching humus and concentrates the Nitrogen (N), Phosphorous (P), Potassium (K), Calcium (Ca) and Magnesium (Mg) contents (Eneji *et al.*, 2001). During the composting period, the phenology of microorganisms including bacteria, actinomycetes, fungi and protozoa is different at different stages.

Aerobic composting involves a process of biological decomposition and stabilization of organic substrates under conditions which allow multiplication and activity of thermophilic microorganisms as a result of biologically produced heat, to produce a final product that is stable, free of pathogens, pests and plant seeds, useful in agriculture and forestry as manure (Saravanan *et al.*, 2003; Balasundaran, 1999). High temperature within waste heap undergoing composting has been considered as consequence of microbial activity, whereby heat is liberated through respiration of microbes and built up within the pile (Tiquia and Tam, 2000). Composting is often a pre-requisite for a substrate which is to be used for mushroom cultivation.

2.9.1 Raw materials used for composting

A wide variety of waste materials such as sewage sludge, organic refuse and leaves, industrial wastes resulting from brewing, fermentation, herbal medicine industry and food processing, tree barks, agricultural residues, abattoir residues and animal manure can be composted (Johri *et al.,* 1999). In the mushroom industry agricultural lignocelluloses is

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the main raw material for composting to use as substrate for the cultivation of a variety of edible mushrooms.

2.9.1.1 Microorganisms involved

Macdonald *et al* (1981), noted that the composting process is brought about by several organisms such as bacteria, fungi, actinomycetes and protozoa and may also involve invertebrates such as nematodes, potworms, earthworms, mites and various other organisms. Hudson (1986) described succession in the aerobic process, noting that the composition of active microflora of composting wastes normally shifts from predominant mesophile in the early stages of thermogenesis to thermophiles at the peak of the heating cycle. Several studies have reported the presence of thermophilic bacteria in hot compost (Beffa *et al*., 1998).

Composting of agricultural waste as a process in environmental bioremediation biofertilizer application, nutrient recycling and preparation of substrate for mushroom cultivation has been recommended by Obodai and Odamtten (2013) as a means of enhancing substrate for mushroom cultivation in Africa.

During the composting process several thermophilic yield enhancing organisms such as *Humicola, Torula, Actinomycetes, Streptomyces, Pseudomonas* and *Bacillus* were encountered. Generally, underlisted fungal genera are also encountered in a compost: *Alternaria, Aspergillus, Botrytis, Chaetomium, Cladosporium, Coprinus, Dactylium, Epicoccum, Fusarium, Geotrichum, Monilia, Mucor, Mycelia Sterilia* (sterile mycelium), *Mycogyne, Neurospora, Papulospora, Penicillium, Rhizopus, Scopulariopsis, Sepedium, Trichoderma, Trichothecium, Verticillium* and *Yeasts* (Stamets, 1993). Sandhu and Sidhu (1980) found *Aspergillus fumigatus, A.terreus, Mucor pusillus, Penicillium cyclopium, P.*

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citreo-viride, P.expansum, Rhizopus microsporus, Trichoderma viride and Coprinus associated with the composting of sugar cane bagasse. During composting, the fungal succession in the compost was influenced by such factors as chemical reaction, aeration, temperature and nutritional factors (Chang-Ho, 1982).

2.9.1.2 Thermophilic microorganisms involved in composting

Thermophiles are found in materials or situations that have been naturally or artificially heated such as compost piles or sun heated soils (Tansey and Brock, 1972). The number of mesophilic microorganisms increase in the first few days of composting but decrease drastically when the temperature rises to 50 -70 °C. According to Cooney and Emerson (1964) thermophilic microorganisms require a maximum temperature for growth at or above 50 ^{o}C and a minimum temperature for growth at or above 20 ^{o}C. Thermophiles are reported to contain proteins which are thermostable and resist denaturation and proteolysis (Kumar and Nussinov, 2001).

Specialized proteins known as chaperones are produced by these organisms, which help, after their denaturation to refold the proteins to their native form and restore their functions (Everly and Alberto, 2000). The cell membrane of thermophiles is made up of saturated fatty acids, which provides a hydrophobic environment for the cell and keeps the cell rigid enough to live at elevated temperatures (Herbert and Sharp, 1992).

Several biochemical tests can be used for the identification of microorganisms involved in composting. Biochemical tests such as catalase production and fermentation of glucose, which are easy to perform, can be used for the preliminary differentiation of microorganisms (Barrow and Feltham, 1993). A number of biochemical tests such as Methyl Red test, Voges Proskauer test, citrate utilization, catalase production, starch

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hydrolysis, tryptophan hydrolysis etc. have to be performed to characterize microorganisms. Johri *et al* (1999) reported that there is a higher rate of breakdown of soluble proteins in thermophilic fungi as compared to that of mesophiles. Thermophilic fungi are known to produce thermostable proteases, lipases, amylases, cellulases, xylanases, lactases, trehalases and other extracellular enzymes (Satyanarayana *et al.*, 1992).

2.9.1.3 Enzyme production by thermophiles involved in composting

Several thermophiles were observed to grow on starch, cellulose, hemicellulose, lignocellulose, lignin and pectin, but their ability to degrade lignin is doubtful (Johri *et al.*, 1999). The capacity of the microorganisms to assimilate organic matter depends on their ability to produce the enzymes needed for the utilization of the substrate. Extracellular enzymes of thermophiles, particularly cellulases and xylanases are glycoproteins which are associated with varied amounts of carbohydrate decomposition (Yoshioka *et al.*, 1987). Since no single organism produces all the enzymes necessary for the degradation of all types of organic waste materials, there is a need to use a consortium of microorganisms which can act synergistically for the rapid conversion of organic waste materials. Kumar *et al* (2007) found certain species of thermophilic fungi *Aspergillus nidulans, Scytalidium thermophilus* and *Humicola* species in paddy straw compost and found them effective in converting paddy straw into nutritionally rich compost, thereby leading to economical and environmentally friendly disposal of crop residue. Wiafe-Kwagyan (2014) also found several fungi belonging to the genera *Aspergillus, Cladosporium, Curvularia, Fusarium, Penicillium, Rhizoctonia, Rhizopus,*

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Rhodotorula, Trichoderma, Byssochlamys, Geotrichum involved in composting of rice straw for bioconversion by *P. ostreatus* and *P. eous*.

2.10 Pretreatment of lignocellulosic agricultural waste for commercial and semi-commercial use

Pretreatment is a crucial process step for the biochemical conversion of lignocellulosic biomass into e.g. bioethanol. It is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes which convert the carbohydrate polymers into fermentable sugars (Mosier *et al.,* 2005). Pretreatment has been recognised as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion and several recent review articles provide a general over-view of the process (Alvira *et al.,* 2010; Carvalheiro *et al.,* 2008).

Both the cellulose and hemicellulose fractions are polymers of sugars, and thereby a potential source of fermentable sugars, or other processes that convert sugars into products. Hemicellulose can be readily hydrolysed under mild acid or alkaline conditions. The cellulose fraction is more resistant and therefore requires more rigorous treatment. After initial biomass processing by milling, the production of fermentable sugars is usually approached in two steps:

1. A pretreatment process in which the cellulose polymers are made accessible for further conversion. In this step hydrolysis of hemicellulose may occur (depending on the process conditions) as well as separation of the lignin fraction (for production of chemicals, combined heat and power production or other purposes);
2. Enzymatic cellulose hydrolysis, using cellulase enzyme cocktails produced on location or acquired from enzyme manufacturers.

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Obstacles in the existing pretreatment processes include insufficient separation of cellulose and lignin (which reduces the effectiveness of subsequent enzymatic cellulose hydrolysis), formation of by-products that inhibit ethanol fermentation (e.g. acetic acid from hemicellulose, furans from sugars and phenolic compounds from the lignin fraction), high use of chemicals and/or energy, and considerable waste production. Pretreatment involves the alteration of biomass so that (enzymatic) hydrolysis of cellulose and hemi-cellulose can be achieved more rapidly and with greater yields. Possible goals include the removal of lignin and disruption of the crystalline structure of cellulose (Fig.8). The following criteria lead to an improvement in (enzymatic) hydrolysis of lignocellulosic material: Increasing of the surface area and porosity, modification of lignin structure, removal of lignin (Partial) depolymerization of hemicellulose, removal of hemicelluloses and reducing the crystallinity of cellulose. In an ideal case the pretreatment employed leads to a limited formation of degradation products

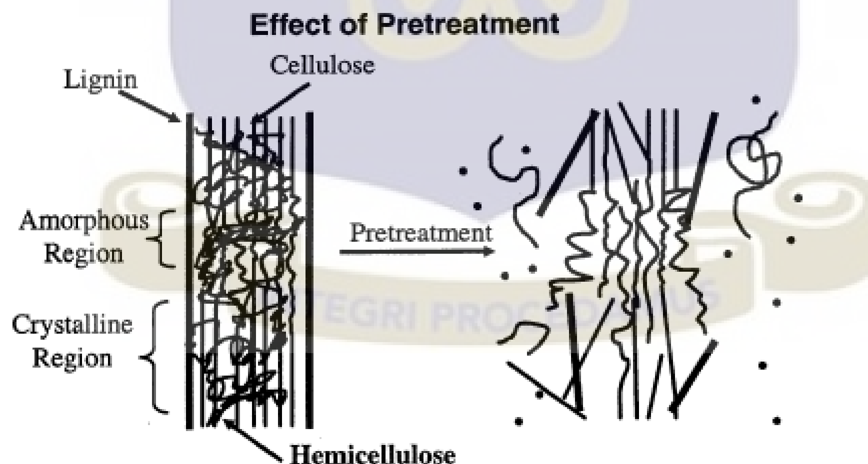

Fig.8: Schematic presentation of effects of pretreatment on lignocellulosic biomass (Hsu *et al.*, 1980)

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which inhibit enzymatic hydrolysis and fermentation, and is also cost effective. However, these are actually the most important challenges of current pretreatment technologies. In the following sub sections the most common pretreatment techniques of biomass are described as follows:

2.11 Use of Gamma Irradiation

Gamma irradiation employs controlled amounts of ionizing (having sufficient energy to create positive and negative charges) radiation to destroy bacteria, pathogens, and pests in food and agricultural products, greatly reducing the threat of foodborne disease. It is also being used for the degradation of polysaccharides.

Ionizing radiation includes gamma rays (from radioactive isotopes cobalt-60 or cesium-137), beta rays generated by electron beam or “E-beam,” and X-rays. None of these irradiation sources has sufficient energy to be capable of inducing radioactivity; however, they do have enough energy to remove electrons from atoms to form ions or free radicals. The freed electrons collide with chemical bonds in the microbial DNA molecules, thereby breaking them and rendering the microbe dead.

Microorganisms, whether pathogenic or comprising the normal microflora of foods, exhibit differences in their responses to ionizing radiation. The key factors which control the resistance of microbial cells to ionizing radiation are the size of the organism (the smaller the target organism, the more resistant it is to ionizing radiation), type of organism (i.e., cell-wall characteristics and gram positive or gram negative in nature), number and relative “age” of the cells in the food sample, and absence or presence of oxygen. The physical and chemical composition of the food also affects microbial responses to irradiation. Radiation sensitivity (killing effect of radiation) in

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microorganisms is measured by their D_{10} values which is the dose required to reduce the population by 90%. Lower D_{10} values indicate greater sensitivity of the organism to radiation.

The freed electrons of water in the product collide with chemical bonds in the microbial DNA molecules, thereby breaking them and thus killing the microbe. The amount of ionizing radiation absorbed is termed radiation absorbed dose and is measured in units of rads (1 rad=100 erg/g) or grays (1 Gy=100 rads), with 1 gray equal to 1 Joule/kg and 1,000 grays equal to 1 kiloGray (kGy). The level of microbe reduction is dependent on the dose absorbed in kilogray (kGy) by the target food (Olson, 1998). Gamma rays have high penetrating power into food than beta rays; and is used preferably in treatment of food to extend shelf life.

2.11.1 Effectiveness and Benefits of Irradiation

Apart from the obvious improvements in food safety through killing of pathogens, irradiation provides other benefits. Some of these contributions include increasing shelf life of meats (Thayer, 1993; Murano *et al.*, 1998) and fruits and vegetables (Thayer and Rajkowski, 1999); improving quality of fruits and vegetables (Thayer and Rajkowski, 1999); providing a suitable alternative to chemical treatments (e.g., methyl bromide and ethylene oxide), especially for decontamination of fruits and vegetables (Thayer and Rajkowski, 1999); and providing economic savings due to reduced incidence of illness. The joint FAO/WHO/IAEA Committee together with Codex Alimentarius Commission in 1980 approved as safe for human consumption, products treated with up to 10 kGy of gamma irradiation. Since then over 40 countries have approved application of gamma

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irradiation on a commercial or semi-commercial scale for decontamination of both fresh and dried foods and food ingredients for human consumption.

2.12 Gamma Irradiation as a Pretreatment Method for Lignocelluloses used as Substrate for Cultivating Mushrooms

2.12.1 Cleavage of the glycosidic bonds

Doses applied are higher than that for preservation. Doses ranged from 15- 100 kGy. The breakdown of lignocellulosic biomass involves the formation of long-chain polysaccharides, mainly cellulose and hemicellulose, and the subsequent hydrolysis of these polysaccharides into their component 5- and 6-carbon chain sugars. Cell wall polysaccharides were partially degraded, particularly cellulose and hemicelluloses (Fig.9). Lignin, as shown in Fig. 7, is more radiation resistant. It has been shown that the lignin plays a protective role in the cell wall by absorbing and scattering radiation energy (Ershov, 1998).

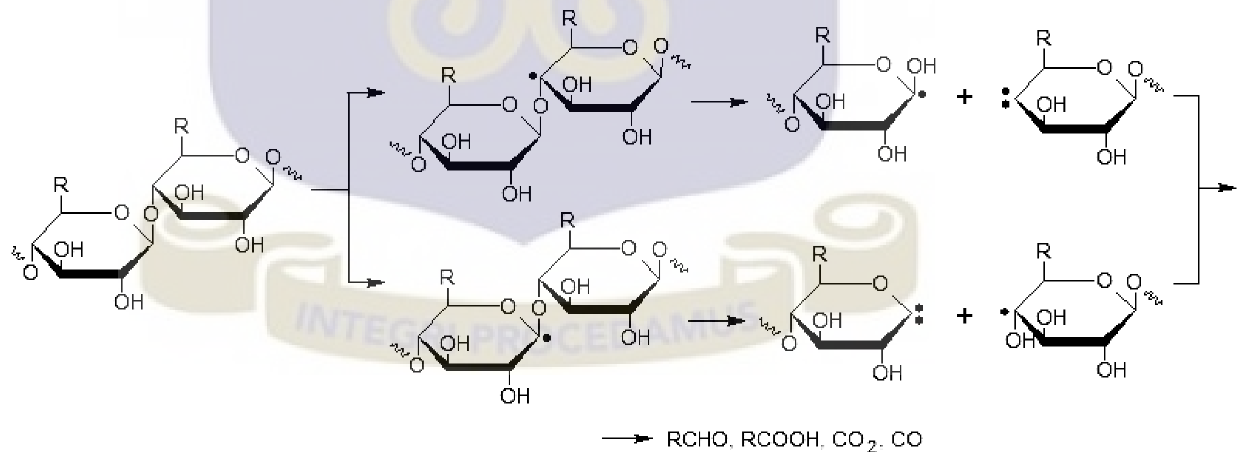

Fig.9: Mechanisms of degradation of cellulose by gamma irradiation, (adapted from Ershov, (1998)).

The main effect of irradiation on hemicellulose and cellulose is to cause degradation by splitting of the glycosidic bond with subsequent formation of reducing groups, such as

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reducing sugars (e.g., glucose). Each glucose residue of cellulose has two inter- and intramolecular hydrogen bonds. These bonds stabilize the long and parallel chains of cellulose. Gamma irradiation affects these bonds and causes the binding van der Walls force to weaken, which results in the degradation of cellulose and increasing degradability of the cell wall constituents (Choi *et al.*, 2009). In this thesis, steam sterilization was used to compare the efficiency of the conventional degradation method with radiation treatment. The mechanism of steam depolymerization of lignin has, on the other hand not been clearly elucidated.

2.13 Methods of Presevation of Mushrooms

2.13.1 Drying

The oldest and simplest method of preserving mushrooms is by drying. This removes bound water from the product (Kumar *et al.*, 2013; Tulek, 2011; Bala *et al.*, 2009; Kotwaliwale *et al.*, 2007). The method varies from sun or air drying to machine and oven drying (Rahart, 2000). According to Oei (1991), drying is based on the principle of limited water availability. Mushrooms could be set on drying trays or racks and placed under the sun or in dehydrator or oven at a temperature range of between 38-66°C to protect the mushrooms from insects and flies attack. The mushrooms are dried to low moisture level and then placed in air tight bags or jars for storage. The drying method preserves mushrooms for very long periods of time with little or no deterioration in flavour but changes the mushroom's texture. Drying even intensifies the mushroom flavour of many species (Rahart, 2000). According to Horubała and Wiśniewska (1978), the species most suitable for drying are: *Boletus edulis* (Bull.:Fr.) (edible); *Pleurotus sp.* and *Morchella esculenta* (L.:Fr.) Pers.: St-Am. (morel) (edible). Because of rapid

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darkening of the pileus during traditional drying, a temperature of 40°C is used at the start (Woźna *et al.*, 1996), followed by 50- 60°C towards the end. Correctly dried mushrooms are characterized by pleasant flavour and crispness, their water content not exceeding 12% (Achremowicz *et al.*, 1984). The Food Research Institute of the Council for Scientific and Industrial Research (CSIR), Ghana has designed a tunnel solar dryer (Plate 2, Materials and Methods section) which is suitable for efficient dehydration and preservation of agricultural produce and can be used for drying mushrooms like the oyster mushroom *Pleurotus* spp.

Fresh mushrooms store well from one to three days under ambient conditions because of their high moisture content and high transpiration rate (Mahajan *et al.*, 2008). It is therefore necessary that they are sold soon after harvest, or preserved to maintain its wholesomeness. Solar drying has been suggested to improve their shelf-life stability and enhance the economic potential of the produce. The reduced moisture content of the produce stabilizes its organoleptic qualities by lowering the rate of chemical reaction and consequently, its susceptibility to microbial attack.

Gamma irradiation technology has proved effective in sterilizing (pasteurizing) to extend the shelf life of food including fruits, vegetables and mushrooms. Pretreatment with gamma irradiation prior to drying have been shown to boost drying rates by altering the structure of the mushroom tissues (Wang and Du, 2005; Wang and Chao, 2002). Application of gamma irradiation prior to drying mushrooms may also result in changes which will alter the drying characteristics as well as the final quality of the dried product. Although drying kinetics of okra (Doymaz and Gol 2005) and different species of mushrooms have been reported in previous studies (Tulek, 2011; Wakchaure *et al.*, 2010;

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Addo *et al.*, 2009), the influence of gamma irradiation as a pretreatment of *Pleurotus osteatus* on the drying kinetics of the solar–dried mushroom has not been investigated. This thesis therefore studied the influence of gamma irradiation pretreatment of *P.ostreatus* on drying kinetics of the oyster mushroom in a tunnel solar dryer, designed by the Food Research Institute of the Council for Scientific and Industrial Research. Five different drying models (Table 5) were used to evaluate the drying characteristics of the slices exposed to the different doses of gamma irradiation and the results are presented here in this thesis.

2.13.2Canning

Canning is the most common process for preserving mushrooms. Washed and cleaned mushrooms are placed in cans containing 2.5 % sodium chloride and 0.25–0.5 % citric acid. The cans are then sealed and sterilized in an autoclave for one hour at 100-120ºC. Since mushrooms have insufficient acid, they are susceptible to *Clostridium botulinum* contamination and require pressure canning to be safely canned. In canning, the mushrooms are sterilized and sealed from contaminants (Rahart, 2000) at 12D. However, there is a change in taste with canning, but the products last very long. During canning, the mushrooms are graded and sorted. Spots and blemishes are removed. The adherence of black peat of the casing soil during cultivation is completely washed away. The water for washing the mushrooms sometimes contains 0.1% citric acid or 0.3% sodium metabisulphite to prevent the mushrooms from turning brown (Oei, 1991).

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2.13.3 Smoking

Mushrooms are smoked to reduce moisture and to acquire a characteristic flavour. In Ghana the mushrooms are parboiled prior to exposure to smoke on a heater. There is no information on the effect of the treatment on organoleptic qualities but the smoked mushrooms are used in soups over a prolonged period of time without a reported health hazard.

2.13.4 Brining

Brining is a conservation method based on the principle of limiting free water. The high concentration of salt in the solution prevents the growth of micro-organisms. The large number of molecules in the solution increases osmotic tension. Spores cannot germinate because no water is available to them, although there is water all around them. The salt concentration of the solution is about 18% per litre of water, 180g to 250g salt must be added to make the solution. The water is boiled and stirred until the salt is dissolved. The brine has to cool before use (Oei, 1991).The mushrooms are blanched in a 5% salt solution for five minutes after the water has come to a boil. The mushrooms are drained and cooled. They are then arranged layer by layer and each layer covered with the cooled brine and the container is closed. All the mushrooms are covered with brine all the time. Before use, the mushrooms need to be desalted (Oei, 1991) by washing in clean water.

2.13.5 Freezing

Freezing is the best processing method for preserving the natural taste, aroma and consistency of mushrooms (Łobaszewski and Paczyńska, 1995). In general, it is accepted that the nutritive value of frozen products exceeds that of sterilized food. It requires,

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however, good transport lines with cooled containers to expeditiously dispatch the produce to the market centres. Frozen mushrooms can be kept for at least three months. Freezing as conservation method preserves mushrooms for many consumers. The quick freezing method gives a whiter product, thus improving the appearance of the mushrooms. The mushrooms are transported through a tunnel where they are cooled with nitrogen vapour to -25ºC. However, transportation from farm to market requires special equipment (Oei, 1991).

2.13.6 Use of chemicals

Fresh mushrooms can be preserved for about 10 days at room temperature by steeping in a solution containing 2.5 % common salt, 0.2 % citric acid, 0.1 % ascorbic acid, 0.1 % sodium bicarbonate and 0.1 % potassium metabisulphite. This method of preservation can be used at places where facilities for canning, freezing and dehydration do not exist. The long- term effect of this treatment on the product is not well documented to date.

In this thesis, the results of applying low doses of gamma radiation 0.5, 1, 1.5, 2 kGy (fresh) and 1, 2, 3, 4, 5 kGy (dried) for the preservation of fresh and dried fruit bodies of oyster mushroom stored in two packaging pouches (polythene and polypropylene) are presented.

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample Area for Questionaire Administration

The study areas chosen for the collection of data were Greater Accra, Central and Eastern regions where majority of the producers and consumers of mushrooms are located. Accra is the capital city of Ghana and it is in close proximity with the Eastern and Central regions. Majority of the studies were conducted in Accra because the national irradiation facility is located at the Ghana Atomic Energy Commission at Kwabenya which was used for this study. Data from mushroom cultivators were collected from mushroom farms in the above mentioned regions of Ghana. Questionaires (Appendix 1) were administered to obtain information on methods of sterilization, aspect of production which needs much attention, general perception about production, popularity of gamma irradiation technique etc. A total of 50 volunteers were interviewed.

Consumers were interviewed from the following locations: Ofankor, Fra-fraha, Cape Coast Polytechnic, Somanya, Nkawkaw, Food Research Institute- CSIR, Shiashie, Graduate School of Nuclear and Allied Sciences, Kwabenya, University of Ghana, Abelenkpe and Dome market Questionaires (Appendix 1) were administered to obtain information on the benefits of consumption of mushrooms, methods of preservation, qualities looked out for in mushrooms, where mushrooms are obtained from etc. A total of 120 volunteers were interviewed.

3.2 Sample material for spawn run

The sorghum grains were prepared according to the method of spawn preparation outlined by Narh *et al* (2011). The cereal grains used were obtained from the Madina

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Market in Accra, Ghana. The grains (4000g) were separately washed and steeped overnight in water (7570.82 ml). They were then thoroughly washed separately with tap water to ensure that dust and other particles had been removed, drained, tied in a wire mesh and steamed for 45 min in an autoclave (Priorclave, Model PS/LAC/EH150, England) at 105°C to ensure that the steamed grains were cooked but intact. Broken grains are more prone to contamination (Narh *et al.*, 2011).

3.2.1 Moist heat sterilization

The grains were sterilized in an autoclave (Priorclave, Model PS/LAC/EH150, England) at 121°C for 1hr.

3.3 Irradiation

Sorghum grains were soaked overnight and packaged as described above and then irradiated at doses 0, 5, 10, 15, 20, 25 and 32 kGy at a dose rate of 1.6 kGy per hour in air from a Cobalt- 60 source (SLL 515, Budapest, Hungary). Doses were confirmed using the Fricke's dosimetry system at the Radiation Technology Centre of the Ghana Atomic Energy Commission, Accra, Ghana.

3.3.1 Enumeration of mycoflora on sorghum grains

The serial dilution plate technique was used in estimating fungal populations. About 10 g fresh weight of sample was placed in 250 ml Erlenmeyer flask containing 100 ml sterile distilled water. The mixture was shaken at 140 rev/min in a Gallenkamp Orbital Shaker for 30 min. Aliquot (1ml) of the suspension was placed in sterile universal bottles (McCartney tubes) containing 9 ml of 0.1% peptone as diluent, and was serially diluted up to 1:10^{3}. The fungal population was enumerated on modified Cooke's medium

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(Cooke, 1954) and Dichloran Rose Bengal Chloramphenicol (DRBC) oxoid agar incubated at 30-32°C for 6 days for colonies to appear.

3.3.2 Characterization and Identification of fungal isolates

Fungal isolates were examined under stereo-binocular microscope (Leica DM500, Germany) using the needle mounts technique. Their identification was performed according to their colour, morphological and cultural characteristics. All the isolates were identified up to the species level using keys and manuals (Samson *et al.*, 2004; Larone 1986; Barnett and Hunter, 1972). The percentage (%) occurrence of fungi was calculated by the formula according to Sreenivasa *et al* (2010) and population was calculated as $\log_{10}$ CFU/g sample.

$$\text{Percentage of fungal species} = \frac{\text{Number of specific fungal colonies isolated}}{\text{Total number of fungal colonies isolated}} \times 100\ \%$$

3.4 Determination of Radiation Sensitivity

3.4.1 D_{10} value determination

The D_{10} value is the reciprocal of the slope of the exponential part of a survival curve. This value may also be obtained from the equation below. The data were subjected to regression analysis. The surviving fractions, $\log_{10}$ (N/N_0) of microorganisms, was calculated and used as relative changes of their actual viable cell counts. The D_{10} values were calculated by plotting $\log_{10}$ (N/N_0) against dose (D) according to the equation (Mohan *et al.*, 2011).

$$D_{10} = \frac{\text{Radiation Dose (D)}}{\log_{10}(No - N)}$$

where

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No is the initial viable count;

N is the viable count after irradiation with dose *D*;   *D* is the radiation dose applied

3.5 Spawn preparation

Sorghum grains were air-dried to cool on a wooden frame with a wire mesh. To the sorghum grains, 3 % (w/w) of calcium carbonate ($CaCO_3$) was added and thoroughly mixed manually. About 265g of grains were packed into autoclavable medicinal bottles and then transferred into transparent heat resistant polypropylene bags (24cm x 38 cm). These were then plugged with cotton wool and covered with plain sheets. The sheets were held in place with rubber bands (Plate 7).

3.5.1 Preparation of stock culture

One-week-old pure cultures of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer, strain EM-1, were obtained from the National Mycelium Bank at the CSIR- Food Research Institute in Ghana. Each of the bottled sterilized grains were aseptically inoculated with one $1cm^2$ of the one-week-old tissue culture of the experimental strain grown on Malt Extract Agar (OXOID™ Ltd., Basingstoke Hampshire, England) using a flamed and cooled scalpel in a Laminar Flow Hood. Thereafter, the cultures were incubated for 16-21 days without illumination in an incubator (Tuttlingten™ WTC Binder, Germany) set at 28°C.

Table 4: Treatment permutations used in sorghum spawn experiment

Code	Interpretation
S + A	Steamed and autoclaved
S + I	Steamed and irradiated
I + I	Irradiated

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3.6 Mycelia Growth of Spawn

3.6.1 Growth rate of mycelia and mycelia colonizing time

Mycelia growths were measured by weekly markings of longest and shortest growths on the spawn bags, then average length taken. Average length = $\frac{\text{longest + shortest lengths}}{2}$

The rate of mycelia growth was then calculated as ; $\frac{\text{Average length}}{\text{Time}}$

Mycelia colonizing time was calculated as the time taken to undergo complete colonization.

3.6.2 Mycelia density

The mycelia density was graded by colonizing vigour or intensity as described by Obodai *et al.*, (2003).

3.7 Compost Preparation

The substrate consisted of 'wawa' (*Triplochiton scleroxylon*) of 89%, 1% of $CaCO_3$ and 10% rice bran. Moisture content was adjusted to 65-70% (Buswell, 1984). The mixture was mixed thoroughly, heaped to a height of about 1.5m and 1.5m base and covered with polythene and then made to undergo fermentation for 28 days. Turning was done every 4 days to ensure homogeneity (Obodai *et al.,* 2003).

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3.7.1 Daily compost temperature readings

Temperature readings of the compost were taken using a digital thermometer (Jenway, 332, U.K) with a probe. Probe was inserted in the middle region of the compost pile and average of three (3) readings recorded in the mornings at 8.30 GMT.

3.7.2 Determination of moisture content of wawa sawdust (*Triplochiton scleroxylon*)

The moisture content was determined by the gravimetric method of AOAC, (1995). One (1) gram of each substrate (W2 and W3) was measured separately into previously weighed moisture can. It was then dried in the oven at 105°C for 3 hrs, cooled in a dessicator and re-weighed. The cooled sample was returned to the oven for further drying. Drying, cooling and weighing were repeated at 1 hr intervals until no further reduction in the weight was obtained (constant weight). The weight of moisture loss was determined and expressed as a percentage of the sample analyzed. It was calculated using this formula;

$$\%\ \text{Moisture content} = \frac{W2-W3}{W2-W1} \times 100$$

Where ; W1 = weight of empty can

W2 = weight empty can + sample before drying.

W3 = weight of empty can + sample after drying.

3.7.3 Determination of pH

This was done according to AOAC (1995). Two (2) grams of composted sawdust sample was weighed into a conical flask containing 10 ml distilled water and allowed to stand for 2 hours. A standard pH meter (3510 Jenway, U.K) was used to determine the Hydrogen ion concentration.

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3.7.4 Bagging

Composted sawdust was compressed into 0.18m x 0.32m heat resistant polyethylene bags. Each bag contained approximately 1kg. There were six (6) replicates per treatment.

3.8 Enumeration of fungi of sawdust

Enumeration of fungi by decimal serial dilution technique was repeated as described in the sorghum procedure. Media used were Oxytetracycline Glucose Yeast Extract (OGYE) and Cooke's.

3.9 Gamma irradiation of sawdust

Bagged composted sawdust substrates were treated with gamma radiation doses of 0, 5, 10, 15, 20, 25 and 32 kGy at a dose rate of 1.6 kGy per hour in air. The absorbed dose was confirmed by Fricke's dosimetry. Each treatment was replicated six times.

3.10 Determination of radiation sensitivity (D_{10} values)

This was carried out as described on page 47 section 3.4.1

3.11 Sterilization/Pasteurization

Bagged composted sawdust substrates were pasteurized with moist heat at a temperature of 100 ^{o}C for 2.5 hours using a metal drum pasteurization equipment as shown in Plate 1.

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Plate 1: Drum moist heat pasteurizing apparatus for pasteurizing compost bags (Note the gas cylinder in the background provided gas for heating the drum).

3.12 Inoculation and incubation

The bags were inoculated with about 5g of spawn grains and so resulted in treatment permutations as follows; Autoclaved spawn and Steamed sawdust (A + S), Irradiated spawn and Steamed sawdust (I + S), Autoclaved spawn and Irradiated sawdust (A+I), Irradiated spawn and Irradiated sawdust (I+I), Autoclaved spawn and non-sterilized sawdust (A + nS), Irradiated spawn and non- sterilized sawdust, Irradiated spawn and non- sterilized sawdust (I + nS) (Table 5).

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Table 5: Substrate compositions and their corresponding codes

Substrate Code	Substrate Composition
S + S	Steamed spawn and Steamed sawdust
I + S	Irradiated spawn and Steamed sawdust
S + I	Steamed spawn and Irradiated sawdust
I + I	Irradiated spawn and Irradiated sawdust
S + n S	Steamed spawn and non-sterilized sawdust
I + n S	Irradiated spawn and non- sterilized sawdust

Compost bags were incubated at ambient temperature (28- 32 ^{o}C) for the spawns to thicken for a period of 4 weeks.

3.13 Growth rate of mycelia and mycelia colonizing time

Mycelia growths were measured by weekly markings of longest and shortest growths on the compost bags, then average length taken. Average length = longest + shortest lengths/2. The rate of mycelia growth was then calculated as described on page 49.

Mycelia colonizing time was calculated as the time taken to undergo complete colonization of the substrate.

3.13.1 Mycelia density

The mycelia density was graded by colonizing vigor or intensity as described by Obodai *et al.,* (2003).

3.13.2 Time for primordial appearance and number of contaminated bags

Time taken for the appearance of first flush of primordia was recorded. The number of contaminated bags were recorded and expressed as:

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$$\text{Contamination (\%)} = \frac{\text{Contaminated bags}}{\text{Total number of bags}} \times 100$$

3.14 Cropping and Harvesting

Primordia or pin heads were allowed to develop to fruiting bodies and were picked. These were harvested by grasping the base of the stalk and pulling them by hand from the substrate, they were then taken away and weighed the same day. The oyster mushrooms were harvested when the in–rolled margins of the basidiophores began to flatten (Tisdale *et al.*, 2006). Humidity was kept as high as possible (80-85%) by watering twice a day using a spraying gun. Parameters recorded were:

Stipe length = length of cap base to end of stalk

Average cap diameter = longest + shortest cap diameters/2

Dates of each harvest were also recorded.

Total number of flushes (flush number) produced per each bag till the end of four weeks.

The distribution of the yield per flush was tabulated to observe changes in yield over the course of multiple flushes. Seven aspects of crop yield were evaluated according to some authors (Amin *et al.*, 2008; Tisdale *et al.*, 2006; Morais *et al.*, 2000) as follows:

(i) Mushroom size (MS) = Total fresh weight of mushrooms harvested divided by their total number of mushrooms

$$\text{(ii) Biological efficiency (BE)} = \frac{\text{Weight of fresh mushrooms harvested (g)}}{\text{Weight of dry substrate (g)}} \times 100\ (\%)$$

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(iii) flush number = batch of mushroom fruitbodies harvested

(iv) crop period (sum of incubation and fruiting periods)

(v) Fresh weight = weight of fresh mushroom fruit bodies

Economical Yield (g/kg wet sawdust) = Total fresh weight of mushrooms. N.B- Dry weight of sawdust- 400g wet weight of substrate- 1000 g/ 1 kg

Economical or mushroom yield values were calculated as previously reported by Morais *et al*, (2000) as weight of fresh mushrooms harvested (g)/ fresh substrate weight.

Average weight of individual mushrooms was determined as a quotient of the total fresh weight mushrooms harvested by their total numbers according to Phillipoussis *et al* (2001).

3.15 Drying experiments (Solar drying method)

The oyster mushrooms (6.8±0.51 mm average thickness) were dried using a tunnel solar dryer designed and fabricated by the CSIR- Food Research Institute, Ghana (Plate 2). Prior to solar drying, the mushrooms were pretreated with gamma radiation, as described under section 3.17. Mushrooms, weighing forty grams (40g) (in triplicates) were spread in a single layer on a wire mesh and loaded into the solar tunnel dryer. Drying was conducted between the hours of 0900 and 1700 hrs each day. Moisture loss during drying was determined by measuring the loss in weight of samples at 30 min interval, with an electronic balance (Kern 510, Kern and Sohn, GMbH, Germany). Sampling and weighing were done until a constant weight was attained (Akonor and Tortoe, 2014). Both experimental and control samples were dried simultaneously under the same weather

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condition. At the beginning and ending of each experimental run, moisture content of mushrooms was determined by standard methods (AOAC, 1990). Mean drying temperature and relative humidity over the drying period were 53.2±6.4 °C and 30.7±5.8 % respectively. Dried mushrooms were sealed air-tight and stored in high density polypropylene containers. Plate 3 shows the two different types of packaging materials used for storing the dehydrated and irradiated mushrooms.

Plate 2. Fresh mushrooms undergoing drying in the solar dryer in a tunnel

3.15.1 Mathematical modeling

Moisture ratio of mushrooms for thin layer drying was calculated as follows:

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad \text{..................(a)}$$

However, due to varying relative humidity and temperature during drying and the fact that M_e is very small, compared to M_o and M_t, it could be neglected,thus simplifying equation (a) according to Goyal *et al*., (2007) and Yaldyz and Ertekyn (2001) as:

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$$MR = \frac{M_t}{M_0} \text{........................(b)}$$

Experimental data for moisture ratio vs. drying time were fitted to 5 drying models (Table 6), commonly used to describe the thin layer drying kinetics of perishable fruits and vegetables, by Non-linear regression (Statgraphics Centurion 15.1). The criterion for selecting the best model to describe the drying curves was the coefficient of determination (R^2). Also, the reduced chi square (χ^2) and the Root Mean Square Error (RMSE) were used to determine the goodness of fit between predicted and experimental data. High R^2 and low χ^2 and RMSE correspond to a better goodness of fit (Akpinar *et al*., 2003). The χ^2 and RMSE were calculated from the following formulae:

$$\chi^2 = \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{N-Z} \text{................(c)}$$

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}\left(MR_{pre,i} - MR_{exp,i}\right)^2\right]^{1/2} \text{......................(d)}$$

Table 6: Selected Thin Layer Drying Models for fitting drying data for dried mushrooms

Model name	Model
Lewis (Goyal *et al*, 2007)	MR = exp (-kt)
Page (Yaldyz and Ertekyn, 2001)	MR = exp (-kt^n)
Henderson & Pabis (Tulek, 2011)	MR = a exp(-kt)
Diffusion model (Tulek, 2011)	MR = a exp(-kt) + (1 – a) exp(-kbt)
Wang and Singh (Akpinar *et al*, 2003)	MR = 1 + at + bt^2

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3.15.2 Effective Moisture Diffusivity

Generally, diffusion is assumed as the dominant transport mechanism during drying and the rate of moisture movement is therefore described by an effective diffusivity value, D_{eff}, which is related to MR by equation (f) (Wang *et al*, 2007).

$$lnMR = ln\frac{8}{\pi^2} - \frac{\pi^2 D_{eff} t}{4L^2} \quad \text{......................(e)}$$

The effective moisture diffusivity was obtained by plotting the experimental drying data in terms of lnMR against time, t (min). From equation (f), a plot of lnMR against drying time, t, gives a straight line with slope, K, where

$$K = \frac{\pi^2 D_{eff}}{4L^2} \quad \text{...................(f).}$$

Where:

a,b	Drying coefficient	
k,n	Drying constants	min^{-1}
MR	Moisture ratio	
K	Slope	
L	Half thickness of slices	m
t	Drying time	min
M_t	Moisture content after time t	kg water/kg dry matter
M_o	Initial moisture content	kg water/kg dry matter
M_e	Equilibrium moisture content	kg water/kg dry matter
N	Number of observations	
z	Number of constants in the model	
MR_{exp}	Experimental moisture ratio	
MR_{pre}	Predicted moisture ratio	
D_{eff}	Effective diffusivity	m^2/s

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Plate 3. Solar dried and gamma irradiated *P.ostreatus* stored in polythene (left) and polypropylene (right) packs at 28-32 ^{o}C

3.16 Determination of colour

The colours of irradiated fresh mushrooms were measured immediately after harvest (0 days) and after storage period (5 days) at room temperature and in polythene and polypropylene containers. Also irradiated dried mushrooms were measured after drying (0 month) and during the storage period (3, 6, and 12 months) with a Minolta CR-310 (Minolta Camera Co. Ltd, Osaka, Japan) colorimeter. The colorimeter has a beam diameter of 8 mm, three response detectors set at 0 viewing angle and a CIE standard illuminant C with diffuse illumination. This illuminant is accepted as having a spectral radiant power distribution closest to reflected diffuse daylight.

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The colorimeter was calibrated with a reference white porcelain tile (L_o =97.63; a_o=0.31 and b_o=4.63), before the determinations. The colour space parameters L (lightness, ranging from zero (black) to 100 (white), a* (ranging from +60 (red) to -60 (green) and b* (ranging from +60 (yellow) to –60 (blue) were measured in triplicates and the means reported. Chroma (C) measures colour saturation or intensity and the hue angle (H^o) describes the relative amounts of redness and yellowness where 0 °- 360 ° is defined for red/magenta, 90 ° for yellow, 180 ° for green and 270° for blue color or purple, or intermediate colors between adjacent pairs of these basic colors. A lower hue value indicates a redder product. Hue angle and chroma were calculated from a* and b* values according to the following formulae (Wrolstad and Smith, 2010):

$$\text{Hue angle } (^o) = \arctan (b^*/a^*)$$

$$\text{Chroma} = \sqrt{(a^{*2} + b^{*2})}$$

The color difference if pretreated samples, ΔE, was calculated in relation to the control sample (Saricoban and Yilmaz, 2010) as follows;

$$\Delta E = [(L_0-L^*)^2 + (a_0-a^*)^2 + (b_0-b^*)^2]^{1/2}$$

where L_0, a_0 and b_0 are values for initial. L*, a* and b* are values for the final/pretreated sample. The browning index was calculated using L*, a*, b* according to Mohammadi *et al*, (2008);

$$\text{Browning Index (B.I)} = \frac{100\,(x - 0.31)}{0.17}$$

where:

$$X = \frac{(a^* + 1.75\,L^*)}{(5.645\,L^* + a^* - 3.012\,b^*)}$$

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3.17 Instrumental Texture Measurement

Texture Analyser (Stable Micro Systems, UK— Model: TA-XT2i) was used for texture analysis of all the samples. Texture analysis was carried out under following instrument parameters: pre-test speed—10 mm/s; test speed—2 mm/s; post-test speed—10 mm/sec; time lag between two compressions—2 sec; strain—30% of sample height; trigger force—0.05 N; data acquisition rate— 200 pps; 10 mm hemispherical plastic probe; load cell—25 kg with 1 g least count. Hardness, springiness, cohesiveness, and chewiness of the samples were calculated using the expressions as shown in Fig.10.

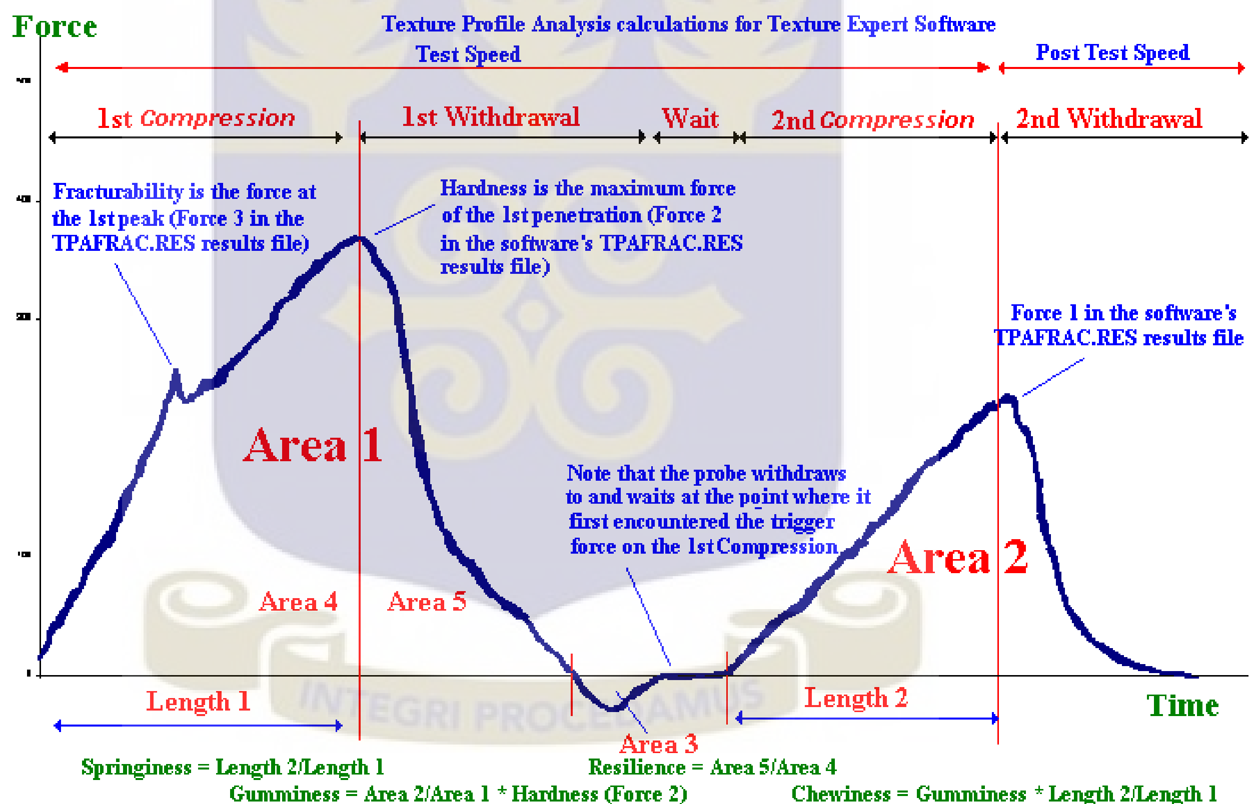

Fig.10. Texture Profile Analysis (TPA) (Source: Anonymous, 2005)

The textural properties: hardness, adhesiveness, springiness, cohesiveness, and chewiness were calculated after areas (1) to (5) (see Fig. 10):

Hardness (kgf) = $F1$

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Adhesiveness (kgf.s) = A3

Springiness = (t2/t1)

Cohesiveness (dimensionless) = A2/A1

Chewiness (kg.f) = (F1) (t2/t1) (A2/A1)

where F1 is the maximum force, i.e., the force in the first peak, A1 and A2 are the areas of the first and second peaks, respectively, and T1 and T2 are the time intervals for the first and second peaks, respectively. The area of the negative peak, that should be visible between vertical lines 3 and 4 (the vertical lines are auxiliary to compute the textural parameters), represents adhesiveness, and would be visible only when the food has measurable adhesiveness, which was sparingly recorded.

3.17.1 Determination of weight loss (g)

Fresh weights of mushroom fruit bodies of all samples in triplicates were determined using a weighing balance for both unpackaged and packaged storage conditions. The fruit bodies of *P.ostreatus* were grouped by weight of about 30 grams. The weights of each sample were measured and recorded in grams and their differences noted (Giri and Prasad, 2013).

3.17.2 Rehydration of mushrooms

Rehydration of mushrooms was determined as described by Giri and Prasad, (2013) with slight modifications by immersing 5 gm dried samples in distilled water at 90- 100°C temperature. The water was drained and the samples weighed at 2 min intervals until constant weight was attained. Moisture content was about 42.3- 49.7%. Triplicate samples were used.

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3.18 Microbial Analysis

The following microbiological analyses were conducted on irradiated and non irradiated fresh and dried mushrooms. Materials and Reagents used in microbial analyses:

Oxytetracycline Glucose Yeast Extract (OGYE) Agar, Cooke's Agar, Violet Red Bile Agar, Dichloran Rose Bengal Chloramphenicol (DRBC), Blood Agar, Plate Count Agar (PCA), Brilliant Green Agar, Xylose Lysine Deoxycholate (XLD Agar), Hydrogen peroxide, Agar, dextrose (glucose),

-Preparation of media

All media reconstitution was done based on guidelines in medical laboratory manual for tropical countries, as outlined by Cheesbrough (1994).

3.18.1 Total viable count

Ten grams of the mushrooms was added to 90 ml of 0.1% peptone water to form a stock solution which was agitated and then incubated at 37°C for 15 minutes. The stock solution was serially diluted using 10 fold serial dilution up to 10^5 into 10 other sterile McCartney bottles containing 9 ml peptone water. Through the pour plate method 1 ml of the suspension was added to 9 ml of peptone water in each McCartney tube. This was mixed to obtain a homogeneous mixture by rotation and poured into 90 mm sterile Petri dishes in a laminar flow. The media were allowed to cool and set and then incubated at 37°C for 18- 24 hours in a microbiological incubator (Heratherm IMC 18, U.K). Appropriate plates (30- 300 colonies) were selected for counts using the colony counter (QCounter ® model 530, USA). Based on the dilution factor the count was expressed as:

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A x 10^B CFU/g

where A is the colony counted, B is the dilution factor and cfu/g is colony forming unit per gram.

3.18.2 Total coliform count

Using plate count technique, (with the formed five times ten (10^5) serial dilution factors). One (1) ml each from various dilution factors was suspended in sterile Petri dishes and 9 ml of molten Violet Red Bile Agar was added, mixed well and then allowed to cool and incubated at 37°C for 24 hours. Colonies were counted after 1 day and expressed as standard form.

3.18.3 Moulds and yeasts count

This was done using the decimal pour plate technique, from the serial dilution made of the stock solution. Incubation was done at 28-32 °C for up to 7 days and colonies appearing were counted.

3.18.4 Detection and enumeration of *Staphylococcus*

Serial dilution was made as described above. Plating was done on molten Baird-Parker medium enriched with egg-yolk emulsion. Incubation was done at 37°C for 48 hours.

3.18.5 Detection and enumeration of *E.coli*

After serial dilution, incubation was done on molten Violet Red Bile Agar (Oxoid, England) at 37°C for 24-48 hours. The colonies appearing were counted and recorded as standard form.

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3.18.6 Detection and enumeration of *Bacillus*

After serial dilution, plating was done on *Bacillus cereus* select Agar (Oxoid, England) and incubated at 30-37 ^{o}C for 24 hours before counting.

3.19 Isolation and Identification of Coliform Bacteria

With the help of sterile inoculating loop, plate technique was used to pour the sample from the stock solution onto the Blood agar and McConkey medium and incubated at 37°C for 24 hours. Physiological characteristics of the colonies formed were examined separately. Pure colonies were subcultured onto Nutrient agar (Oxoid, England) medium and Eosine methyl blue (EMB) –(Merck, Darmstadt- Germany) medium and incubated at 37°C for 24 hours. Colonies were Gram stained using crystal violet stain and observed under the light microscope at x100 with oil immersion for physiological characteristics and cellular morphology. Based on these, catalase, oxidase motility, indole, urea and citrate tests were performed to identify organisms from coliform group. In the Gram stain, the cells were first heat fixed and then stained with a basic dye, crystal violet, which are taken up in similar amounts by all bacteria. The slides were then treated with an I_2-KI mixture (mordant) to fix the stain, washed briefly with 95% alcohol (destained), and finally counterstained with a paler dye of a different colour (safranin).

3.20 Isolation and Identification of *Salmonella*

One milliliter of the stock solution was added to 20 ml of double strength selenite broth, agitated and incubated at 37°C for 48 hours. One millilitrel from the selenite broth was inoculated onto BGA (Merck, Darmstadt- Germany) and XLD agar medium (Liofilchem, Italy) and incubated again at 37°C for 24 hours after which the organism’s physiological

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characteristics were examined. Urea, methyl red (MR) and Voges-Proskauer (VP) biochemical tests were conducted to identify the organism.

Urea medium screened out urease-producing organisms. Urea agar was inoculated heavily over the entire surface of the slant. Incubation was done overnight at 35–37°C. Urease-positive cultures produced an alkaline reaction in the medium, evidenced by a pinkish-red color. Urease-negative organisms did not change the colour of the medium, which was pale yellowish-pink.

The methyl red test detected production of acids formed during metabolism using mixed acid fermentation pathway using pyruvate as a substrate. The pH indicator Methyl Red was added to one tube and a red color appeared at pH's lower than 4.2, indicated a positive test (mixed acid fermentation is used). The solution remaining yellow (pH = 6.2 or above) indicated a negative test, meaning the butanediol fermentation was used.

The Voges Proskaur test used alpha-naphthol and potassium hydroxide to test for the presence of acetylmethylcarbinol (acetoin). After both reagents were added, the tube was shaken vigorously then allowed to stand for 10 minutes. A pinkish-red color indicated a positive test, indicating that the 2, 3-butanediol fermentation pathway was used.

INTEGRI PROCEDAMUS

CULTURE MEDIA

(a) Plate Count Agar (Oxoid, England)

Peptone from casein…………….5g

Yeast extracts…………………..2.5g

D (+) glucose…………………..1.0g

Agar……………………………14g

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Distilled water…………………1000ml

b) Dichloran Rose Bengal Chloramphenicol (DRBC) Agar (Oxoid, England)

c) Cooke's medium (Formulation)

Glucose………………………………………10g

Peptone……………………………………….5g

KH_2PO_4……………………………………….1g

$MgSO_4.7H_2O$……………………………….0.5g

Rose Bengal…………………………………0.035g

Streptomycin………………………………..0.35g

Agar………………………………………..15g

Distilled H_2O………………………………..1000 mL

d) OGYE medium (Merck, Germany)

e) Plate Count Agar (PCA) (Oxoid, England)

A mixture of plate count agar and purified agar consisting of 22.5g and 10g respectively was thoroughly dissolved in one litre of distilled water and boiled in a water bath at 98°C for 15 minutes. Pouring was done in a laminar flowhood into McCartney bottles and stored in a refrigerator at a temperature of 8± 2°C.

f) Nutrient Agar (NA) (Oxoid, England)

Twenty eight (28) grams of Nutrient agar and 10g of purified agar was completely dissolved in one litre of distilled water in beaker, boiled in water bath at 98°C for 15

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minutes after which 10 mL was aliquoted into McCartney bottles each and autoclaved at 121°C for 15 mins. They were then stored at 8 ± 2 °C until they were used.

g) Xylose Lysine Deoxycholate (XLD) medium (Oxoid, England)

Fifty three and half (53.5) grams of XLD agar was mixed with 10 g of purified agar and dissolved in one litre of distilled water in a beaker. The mixture was then put into a water bath for 15 mins at 98 °C. Aliquot of 10 mL of the samples were put into McCartney bottles each for sterilization at 121 °C for 15 minutes and stored there after.

3.20 Sterilization

a) Sterilization of culture media

Culture media were poured into medicinal flasks and autoclaved at 1.1 kg/steam pressure at a temperature of 121°C for 15 mins. They were stored in a refrigerator after cooling and melted when needed.

b) Sterilization of glassware

Measuring cylinders and Erlenmeyer flasks were autoclaved at 1.1 kg/steam pressure at 121°C for twenty minutes. Petri dishes and pipettes were put in canisters and sterilized in an oven (Gallenkamp 300 plus Series Oven) at temperature of 160 °C for at least six hours.

3.21 Determination of Radiation Sensitivity of *Bacillus cereus*

It was done as described on page 48

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3.22 Identification of *Enterobacteriaceae* associated with mushrooms using API 20E kit

Identification of isolates was done based on colony morphology of the organism on the medium. Physiological characteristics such as size, shape, colour, changes in media etc. were observed. Gram staining smear was prepared for Gram staining. Examination was done using light microscope at x100 with oil immersion. Isolates were grouped into Gram positive and Gram negative organisms. Both Gram positive and Gram negative organisms were sub- cultured on a nutrient agar (Oxoid, U.K) to obtain pure colonies. An Analytical Profile Index (API 20E) kit (BioMerieux, France) was used to identify Gram negative rods (Plate 4). Coagulase test was done using the slide method. A drop of distilled water was placed on a slide and two colonies of specific microorganism were dissolved on the slide. A drop of citrated plasma was added and mixed with a needle. Catalase tests were performed to detect production of the enzyme catalase by pouring 1 mL of 3% hydrogen peroxide over the surface of an agar culture. The catalase activity was checked through production of air bubbles (API 20E manual).

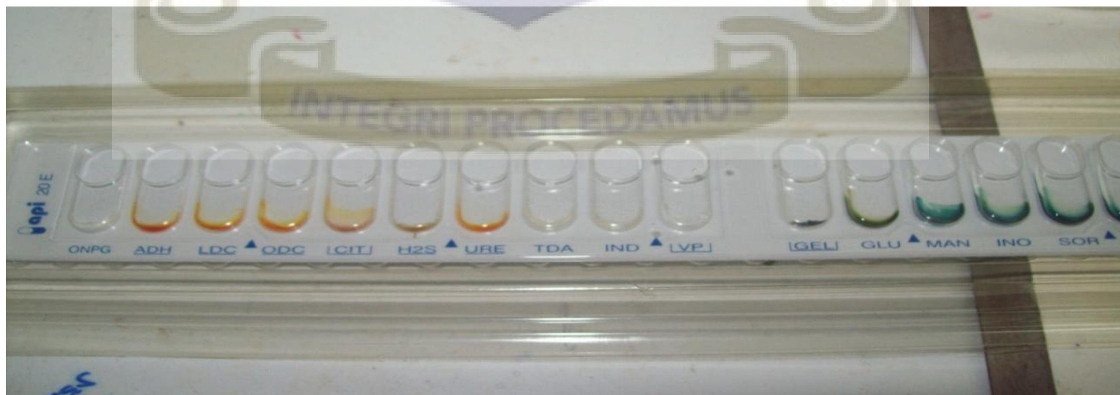

Plate 4. API 20E test kit for detecting Enterobacteriaceae (BioMerieux, France)

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3.23 Characterization and Identification of Fungi in the Substrate

Pure fungal cultures were observed while still on plates and after wet mount in lacto-phenol on slides under the compound microscope. The following standard characterization tests were performed in duplicate: Examination of fungus was carried out by observing the colony morphology, colour (pigmentation), texture and surface appearance etc. Microscopic examination was done by needle mount or wet mount method (Harrigan and McCance, 1990) and by observing sexual and asexual reproductive structures like sporangia, conidial heads, arthrospores and vegetative mycelium.

A wet mount was done for each fungal isolate. A drop of lacto-phenol was dropped on a clean slide aseptically, a piece of fungal hyphae under test was teased into it using 2 (two) sterile needles. The teasing was done carefully and slowly so as to make good spread of the fungal hyphae. The slides were then gently covered with a cover slip to avoid air bubbles. The slides were observed under low and high power objective for the cultural characteristics, pigmentation, sporangia, conidia, arthrospores, vegetative mycelium, septate and non-septate hyphae. Observed characteristics were recorded and compared with the established identification key of Barnett and Hunter (1972), Malloch (1997) and Samson *et al.,* (2004).

3.24 Effect of irradiation

The samples were packaged in polythene and polypropylene packs before sending to the gamma irradiation source at the Ghana Atomic Energy Commission, Kwabenya. The gamma source was from Cobalt-60 and at a dose rate of 1.6 kGy per hour in the inner chamber of the SLL-515 batch irradiator (Budapest, Hungary). The doses applied to the sorghum samples were 0, 5, 10, 15, 20, 25 and 32 kGy in air. The same doses were

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applied to sawdust compost samples. For the mushroom fruit bodies two sets of lower doses were applied 0, 0.5, 1, 1.5, 2 kGy and a set of 0, 1, 2, 3, 4, 5 kGy to investigate the effect of variation of radiation doses on resident microorganisms. After treatment, 1g sample of each irradiated sample was transferred into 250 ml Erlenmeyer flasks containing 100 ml of 0.1% peptone. Each flask was shaken at 140 rev/ min for 10 minutes on an orbital shaker. The samples were serially diluted up to 1: 10^5 and aliquot (1 ml) were plated on 20 ml of DRBC and incubated at 28 ± 2 °C for 5days. Surviving colonies of microflora were counted and were calculated as $\log_{10}$ CFU/g sample.

3.25 Dosimetry

Fricke dosimetry (Ferrous ammonium sulphate dosimeter) was used to estimate the absorbed doses. The Fricke dosimetry is based on the chemical process of oxidation of ferrous ions in acid aqueous solution to ferric ions by ionizing radiation. The method can be used to accurately determine absorbed doses using spectrophotometric measurement of the ferric ion concentration at 303 nm wavelength, in the peak of the absorption spectrum. The dosimeter solution was prepared by weighing 0.392 g of ferrous ammonium sulphate and 0.058g of sodium chloride which were dissolved in 10 ml of 0.8 N (0.4 mol/L) sulphuric acid. The solution was diluted to 500 ml using 0.8N (0.4mol/L) sulphuric acid in a calibrated flask at 25 °C. To measure the dose, capsules/ ampoules were cleaned by rinsing them with the dose meter solution, filled with the dosimeter solution and sealed.

It was then placed carefully in the calibration holder in the batch irradiator and finally irradiated in the holder for a fixed time. The optical density (OD) of the irradiated and

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non-irradiated Fricke dose meter solution was measured at a wavelength of 303 nm using a spectrophotometer (Labomed Inc UVD-3200, USA). Each value of OD was noted and the temperature of each solution during each measurement checked. The dose given to each capsule was calculated using the equation:

$$DGy = \frac{N\,(ODi - ODo)\,1.602 \times 10\text{-}7}{\rho G \varepsilon d(\,1 + 0.007\,(t\text{-}25)}$$

where $N = 6.022 \times 10^{23}$ (Avogadros number); $\rho = 1.024$ g/cm^3 (density of solution)

$G = 15.6 \times 10^{-2}$ eV^{-1} (the number of ferric ions formed by eV absorbed energy)

ε = 2195 L/mol/ cm (OD of 1mole/L of ferric ions in a 1-cm-long cuvette at 303nm- it is the extinction coefficient).

d = 1 cm (length of the cuvette used for OD measurement

t = temperature of the solution in ^{o}C at time of OD measurement; OD = optical density; ODo = optical density at initial time; ODi = optical density at time i

3.26 Determination of Total Phenolic Content using Folin-Ciocalteau Method

The Folin-Ciocalteau analysis was carried out according to the procedure in Singleton *et al.*, (1999). The contents of total phenolic compounds in the mushroom aqueous, ethanolic and methanolic extracts were determined by the Folin-Ciocalteau method. For the preparation of its calibration curve 1mL aliquots of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml Gallic acid solutions in methanol were mixed with 5ml of Folin-Ciocalteau reagent (diluted ten- fold) and 4 ml of sodium carbonate solution. Absorbance readings using the

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spectrophotometer were taken at 760 nm after incubating for 30 minutes at ambient temperature and calibration curve constructed (Appendix 4). One ml of the methanol mushroom extract (10g/l) was mixed with the same reagents as described above, and after 1 hour, the absorbance was measured for the determination of total mushroom phenolics. All determinations were performed in duplicates. Total content of phenolic compounds in mushroom methanol extracts in gallic acid equivalents (GAE) was calculated by the following formular:

$$C = c \times V/m$$

Where: C= total content of phenolic compounds in mg/g of mushroom extract, in GAE; c= the concentration of gallic acid established from the calibration curve in mg/ml (Appendix 4); v = the volume of extract in ml; m = the weight of mushroom methanolic extract in gram. It was expressed as mgGAE/g of mushroom sample.

3.27 Determination of Total Flavonoid using Aluminium Chloride Colorimetric Method

Total soluble flavonoids determination was carried out according to the method of Quettier *et al.*, (2000) and was expressed as quercetin equivalent (QE). Quercetin was used to obtain the calibration curve (Appendix 4) (standard solutions 6.25, 12.5, 50.0, 75.0, 100 μg/ml in 80% ethanol (v/v), 0.5 mL of a product (ethanolic solutions of extracts) was mixed with 1.5 mL 95% ethanol (v/v), 0.1 mL of 10% aluminium chloride ($AlCl_3$) (m/v), 0.1 mL of 1 mol/L potassium acetate and 2.8 mL water. A volume of 10% (m/v) $AlCl_3$ was substituted by the same volume of distilled water in blank. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. The total flavonoids content in the fractions was determined as

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μg quercetin equivalent by using the standard quercetin graph. The flavonoid content was expressed as mgQE/g of mushroom sample.

3.28 Total Antioxidant Activity Analysis using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Method

The DPPH assay was done according to the method of Brand-Williams *et al.*, (1995). The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and then stored at -20°C until needed. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol and an absorbance obtained using the spectrophotometer. Aliquot of 100 microlitres was allowed to react with 3900 μL of the DPPH solution for 30 minutes in the dark. Then the absorbance was taken at 517 nm. The standard curves (Appendix 4) were between 0.2 to 1.0 μL according to the method reported by Blois (1958). The experiment was carried out in duplicate. Radical scavenging activity was calculated as follows;

Activity= 1- As/Ac

Where: As – absorbance of sample, Ac- absorbance of control

$$\text{Percentage inhibition (\%)} = \frac{\text{A of control} - \text{A of sample}}{\text{A of control}} \times 100$$

3.29 Determination of Half Maximal Inhibitory Concentration (IC_{50})

Inhibitory concentration of 50% (IC_{50}) was calculated by plotting a graph of concentration and % inhibition. A linear regression was estimated to plot x-y and fit data with a straight line. y= mx+c where y=50. (Pournoradi *et al.*, 2006).

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3.30 Proximate Analysis and Physico-chemical Parameters

Mushrooms harvested were subjected to proximate analysis to determine their chemical composition. The chemical compositions determined included moisture, fat, fibre, ash, and protein and others are outlined below:

3.30.1 Determination of moisture content

Two crucibles were each washed, dried, weighed and 2 g of fresh mushrooms weighed into each of the crucibles. The crucibles were placed in a thermostatically controlled oven (Gallenkamp oven 300 plus series, U.K) and the temperature maintained at 105 ^{o}C for 5 hours, after which they were removed and placed in a dessicator to cool. They were then reweighed. The procedure was repeated until a constant weight was obtained. The moisture content was found by subtracting the final mass from the initial mass (AOAC, 1995).

3.30.2 Determination of neutral – detergent fibre (insoluble fibre) NDF.

The method outlined by AOAC (1997) was used in this experiment.Five hundred milligram (500mg) of ground sample was weighed (W1) and passed through a one milliliter (1mm) mesh or screen into 600 ml beaker (without spout). Fifty milliliters (50 ml) cold (room temperature) NDS (the NDF solution) was placed in a beaker on a refluxing unit and allowed to boil. Heat was adjusted to even boiling, keeping the sample particles suspended. Heat was reduced as boiling began, to avoid foaming. It was refluxed for 60 minutes from onset of boiling. Gooch crucible was placed on the filter manifold and rinsed with hot water. The residue was filtered on to the crucible using light suction. It was washed twice with hot water. Mixture was allowed to stand for 10 minutes, washed twice with hot water, twice with acetone and dried using suction. For

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recovery reasons, acetone washing was done separately. The crucible was air dried for 10-15min (some of the acetone will escape) and oven dried for 8 hours or overnight in a forced-air oven at 105°C. It was cooled in a desiccator and weighed (W2) to obtain yield of cell wall. Crucible was ashed at 510°C for 3 hours, allowed to cool and removed from furnace, put in an oven (set at 105°C) cooled and weighed (W3). The loss in weight was the ash free cell wall

$$\% \text{ NDF} = \frac{W_2 - W_0}{\% DM} \times 100\%$$

$$\% \text{NDF (DMB)} = \frac{\% \text{NDF}}{\% DM} \times 100\%$$

W_0 = Empty crucible weight. W_1= Weight of ground sample

W_2 = Weight of sample after cooling in dessicator W_3= Weight of reweighed sample

3.30.3 Determination of acid detergent fibre (ADF), lignin and silica.

ADF, lignin and silica were determined using the method outlined by AOAC, (1997). Half a gram (0.5g) of ground air dried sample was weighed (W1) and passed through a 1mm screen, into a Berzelius beaker. Fifty milliliters (50 ml) cold ADS (ADF solution) was added on a refluxing unit. The heat was reduced as boiling began to avoid foaming. It was refluxed for 60 minutes for the onset of boiling (the knob was adjusted to constant boiling). It was filtered on a previously weighed Gooch crucible (W2), which was set on a filter manifold using light suction. Residue was washed twice with hot water (90-100°C). The side of the crucible was rinsed in the same manner.

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It was repeatedly washed with acetone, until it was colourless. All lumps were broken up with a glass rod, so that the solvent came into contact with all the particles of fibre. Crucible and fibre (residue) were dried at 105°C overnight cooled and weighed (W3). The content of the crucible was covered or flooded with cooled (20°C) 72% H_2S0_4 and stirred with a glass rod to get a smooth paste, all lumps were broken. Crucible was kept at 20 – 23°C. Crucible was half filled, with the acid and stirred. Allowing the glass rod to remain in the crucible. It was refilled with acid (72% H_2S0_4) and stirred at hourly intervals as acid drained away. Crucible did not need to be kept full at all times, three additions sufficed. After three hours, as much acid as possible was filtered off using light suction (vacuum). Content was washed with hot water until free from acid (test with litmus paper), rinsed and removed with stirring rod. (NB; - cellulose was dissolved by the acid). Crucible was dried at 105°C, cooled in a desiccator and weighed (W4). Crucible was ignited with content in a muffle furnace at 510°C for three hours. Cooled to 105°C, in a desiccator and weighed (W5). Lignin was reported as the difference between the W5 and W4. ,N.B. W1= W2=empty crucible weight, DM = dry matter , W3= crucible + dry fibre (residue) W4= weight of dried dessicator, W5=Ignited crucible +content

Calculations;-

$$\% \text{ ADF} = \frac{W3 - W2}{W1} \times 100\%$$

$$\% \text{ ADF (DMB)} = \frac{\% \text{ADF}}{\% \text{DM}} \times 100\%$$

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i.e. % Hemicellulose (DMB) = % NDF (DMB) - % ADF (DMB)

$$\% \text{ cellulose} = \frac{W3 - W4}{W1} \times 100\%$$

$$\% \text{ cellulose (DMB)} = \frac{\%\text{Cellulose}}{\% \text{ DM}} \times 100\%$$

$$\% \text{ lignin} = \frac{W4 - W5}{W1} \times 100\%$$

$$\% \text{ lignin (DMB)} = \frac{\% \text{ lignin}}{\% \text{ DM}} \times 100\%$$

3.30.4 Determination of fat content

Procedure was carried out in accordance with AOAC (1995) with modifications. Two grams of dried sample was transferred into a 22 mm x 80 mm paper thimble and a small ball of cotton wool placed in the thimble to prevent loss of sample. A 250 ml round bottomed flask was washed and dried at 100 ^{o}C and weighed. Some antibumping granules were put into the flask. Fifteen millilitres (15 ml) petroleum spirit of boiling point 60 – 80 ^{o}C was added. A quick fit condenser was connected to a Soxhlet extracter and refluxed for 4 hours at high heat using a heating mantle. The flask was removed and the solvent evaporated on a steam bath. The flask with its contents was put into a dessicator to cool to room temperature. It was then weighed and the mass of fat determined by subtraction.

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3.30.5 Determination of ash content

Procedure was carried out in accordance with AOAC (1995) with modifications. Two crucibles were each washed, dried and weighed and 2 grams of the whole mushrooms weighed into the crucibles. They were then placed in a muffle furnace, pre heated to 600 ^{o}C for 2 hours. The crucibles were then removed, allowed to cool in air, placed in a dessicator to cool completely and re-weighed. The masses of the crucibles and their contents were found by subtraction.

3.30.6 Determination of protein content

The method reported by AOAC, (1995) was used in determining protein content. Two grams (2g) of dried sample was put into a digestion flask and half tablespoonful of selenium catalyst and a few anti-bumping agents added. Twenty five millilitres (25 ml) of conc. H_2SO_4 was added and the flask shaken to wet the sample thoroughly. The flask was slowly heated for 15 minutes. It was then cooled to room temperature. The sample was transferred into a 100 ml volumetric flask and made up to the 100 ml mark. Twenty five millilitres (25 ml) of 2% boric acid and 2 drops of mixed indicator were pipetted into 250 ml conical flask. The liquid was drained from the steam trap. The stopcock, which drains the steam trap, was then left open. The conical flask and its contents were placed under the condenser so that the condenser was completely immersed in the solution. About 15 ml of 40% NaOH was put into a decomposition flask. Steam was forced through the decomposition chamber by shutting the stopcock on the steam trap outlet. The boric acid comes into contact with ammonia and changes to blue-green. The distillation was made to continue for 3 minutes. The distillation was done for another 30 seconds, and the distillate titrated with 0.01M HCL.

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$$\text{The Total Nitrogen} = \frac{100 \times (V_A - V_B) \times 0.01410 \times 100 \times 6.25}{W \times 10}$$

Where, V_A = Volume in ml of standard acid.

V_B = Volume in ml of standard base used in the blank.

N_A = Normality of the acid (HCL).

W = Weight of sample.

% Protein = % total Nitrogen x 6.25

3.30.7 Determination of pH

The pH of the samples were measured using an electronic digital pH meter (Jenway 3510, U.K), in accordance with AOAC (1990). Buffer solution of pH 4 and 7 were used to calibrate the pH meter. Ten (10) grams of samples were weighed and homogenized using a laboratory Waring blender. Ninety (90) milliliters of distilled water was added to the samples in a conical flask and allowed to settle after shaking. Twenty (20) milliliters of the supernatant in a beaker was measured for pH value using the pH meter.

3.31 Determination of Metabolizable Energy Content

Fat, protein or carbohydrates can supply energy. Metabolizable energy was calculated using the following formula:

ME (Kcal /100g) = [(3.5 X CP) + (8.5 X CF) + (3.5 X NFE)] (Khan *et al.*, 2008)

Where, ME = Metabolic Energy; CP = % Crude Protein; CF = % Crude Fat; NFE = % Nitrogen Free Extract (carbohydrate)

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3.32 Determination of Trace Elements using Atomic Absorption Spectrometry (AAS 240FS, Australia)

The method of AOAC, (1990) was used to determine magnesium, manganese, sodium and potassium. Half a gram (0.5 g) sample was weighed using the Mettler Toledo balance into well washed and conditioned Teflon beakers. Six (6) ml of HNO_3 (30%) and 1ml of H_2O_2 (30%) were added to the sample. It was placed into a microwave. The following time and power were used. Two minutes (2 min) at 250 W, 2 min at 0 W, 6 min at 250 W, 5 min at 400 W, 5 min at 600 W. After 20 mins, 5 mins ventilation period was allowed before removing samples from the microwave. It was cooled in a water bath. Individual teflons were opened using tork wranche and digested samples transferred into test tubes. Volume of digested samples were recorded and often made up with distilled water (10- 25 ml) depending on the elements under investigation. Tissue was used as standards as well as some blanks and repeats were performed. Five millimeters (5ml) of HNO_3 and 1 ml H_2O_2 was added to mushroom and placed into a microwave. The following time and power were used. One (1) min at 250W, 1 min at 0W, 5 min at 250W, 5 min at 400W, 5 min at 650W, allowing 5 min ventilation period.

$$\text{Concentration of element} = \frac{\text{concentration x volume x nominal weight}}{\text{Nominal volume x weight}}$$

3.33 Determination of concentration of Sodium and Potassium ions using Flame Photometer (Sherwood, U.K model 420). The samples were first leached in distilled water for a day while being constantly shaken on an orbital shaker. Filtrates of the sample were then used for the analysis. The flame photometer was prepared for testing by first running the pump for 15 mins upon which the photometer was turned on. The system was

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then calibrated to check the efficiency of the photometer. This was done by taking 5ml of distilled water (blank test) and adding 100mg/l of lithium standard solution. The blank test was then aspirated with the system giving the reading- 1000.00 cals. After this reading, 2 ml of Lithium standard solution was added to each of the sample and then aspirated (AOAC, 1990).

3.34 Sensory Analysis

Sample preparation was done according to a modified method of Nyoagbe (2012). The dried mushroom samples were grouped according to storage package (polypropylene and polythene) as well as method of pretreatment (non-irradiated and irradiated). Equal amounts (18.76g) of all samples were cooked by steaming in a pressure cooker (Binatone®, England) in a slightly saline water medium (9g NaCl/1000ml) without spicing for a period of 30 minutes and used in the study. The samples were labelled by numerical coding and interpreted as follows:

808 - Control

512 - 1 kGy/ polythene

667 - 2 kGy/ polythene

891 – 3 kGy/ polythene

837 – 4 kGy/ polythene

491 – 5 kGy/ polythene

753 – 1 kGy/ polypropylene

371 – 2 kGy/ polypropylene

606 – 3 kGy/ polypropylene

412 – 4 kGy/ polypropylene

948 – 5 kGy/ polypropylene

INTEGRI PROCEDAMUS

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3.36.1 Sensory evaluation of steamed gamma irradiated mushrooms

To carry out sensory evaluation of the dried and gamma radiation pretreated mushroom samples, tasted panelists were sought by putting up notices for volunteers. It attracted a panel of 44; male (19) and female (25) trained panelists who are well educated (majority tertiary and above) and well conversant with agricultural products. Most of them were Food Scientists and Technicians from CSIR- Food Research Institute. The panelists were aged between 18 and 55 years. Using a structured questionnaire, the panelists independently assessed the samples for appearance, colour, aroma, taste, mouth feel and overall acceptability (Appendix 2). Water was provided to rinse the mouth each time a panelist carried out an evaluation. Organoleptic scores were made according to a nine point hedonic scale in which 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much and 1 = Dislike extremely, which were supposed to show the degree of likeness. Panelists indicated their rating for each sample by choosing the appropriate numerical score. The evaluation was carried out before lunch. Consumer acceptability test was used to assess acceptability limit.

3.36.2 Descriptive texture analysis

The same treatments of mushroom samples were used for the analysis by the same panel. The panelists independently assessed the samples for hardness, cohesiveness (integrity), adhesiveness (stickiness), chewiness (stringiness) and smoothness. Organoleptic scores were made according to a nine point hedonic scale as described above (section 3.36.1).

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3.37 General Experimental Precautions

1) It was ensured that there were no bubbles in the liquid in the pipette to obtain accurate volume dispensed into the test tubes.

(2) Glassware were cleaned with detergents and rinsed thoroughly with distilled water. They were then air dried before being sterilized in the autoclave.

(3) Test tubes were covered with aluminium foils to prevent direct contect of DPPH with sunlight to avoid degradation during antioxidant analysis

(4) The laminar flow was switched on 20 minutes before use. It was ensured that the spirit lamp had alcohol and work bench was thoroughly cleaned with alcohol before work begun.

(5) A lead shield was always put on to give protection and a dosimeter always carried along in the pocket to measure the dose of irradiation you might be exposed to.

(6) Laboratory coat was always put on anytime I entered the laboratory.

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CHAPTER FOUR

4.0 RESULTS

EXPERIMENT 1

PRELIMINARY RAPID APPRAISAL USING STRUCTURED QUESTIONAIRE TO OBTAIN DATA ON STERILIZATION, PRESERVATION, NUTRITIONAL QUALITIES AND CONSUMER PREFERENCE OF MUSHROOMS

Majority of the respondents (70%) use drum pasteurization (moist heat) while 26% treat the substrate with unspecified chemicals. The remaining 4% use other unspecified methods of sterilization (Fig.11). Majority of the respondents (64%) were not satisfied with the sterilization methods; 36% were in agreement with the sterilization procedure. The differences observed were significant ($p<0.05$) (Fig.12).

A significant ($p<0.05$) majority of 82% have not heard of the sterilization of mushroom compost by gamma irradiation. The remaining 18% are aware of its use in sterilization of compost before spawning (Fig.13). The survey also showed that 34% of the respondents believe that the sterilization process needs more attention in mushroom cultivation industry while 50% desired the inoculation process to be perfected to exclude contaminants (Fig.14). About 8% wanted more attention paid to packaging. The remaining were non-comittal. Majority (92%) of mushroom cultivators agreed to the need to achieve better sterilization of compost bags and other ancillary methods while an insignificant ($p>0.05$) minority of the cultivators disagreed (Fig. 15).

Because fresh mushrooms are perishable, shelf-life extension is vital to the industry. Exactly 54% of the respondents were able to preserve all their produce while 46% were

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unable to preserve all their produce (Fig.16). Majority of consumers interviewed (72%) intimated that they prefer eating oyster mushrooms (*Pleurotus spp*.) while 20% patronized the termite mushroom (*Termitomyces spp*.) (Fig.17). Domo (*Volvariella volvacea*) or the oil palm mushroom was the least patronized (8%). All consumers (100%) would like to see their favorite mushroom produced all year round and this constituted a significant ($p<0.05$) viewpoint (Fig.18). Furthermore, a significant ($p<0.05$) majority (90%) of the respondents were all for promotion of the consumption of mushroom (Fig.19) while a small percentage (10%) were not in agreement.

On the question of the medicinal and nutritional benefits derived from eating mushrooms, 54% stated that mushrooms have medicinal values; 34% agreed that mushrooms have nutritional attributes not excepting 10% who assigned other unspecified benefits of eating mushrooms. The remaining 2% said that mushrooms have both medicinal and nutritional values (Fig.20). It was shown by the survey that majority (64%) of mushroom consumers obtain them directly from the market place. Interestingly, 34% obtained their supply from back yard garden or from the farm (Fig.21) and the remaining 2% harvested them from the wild during the mushroom season.

The aesthetic appearance of mushrooms contributed to the choice of mushroom for consumption; 34% of the consumers considered appearance as important and 30% considered the taste as a determining factor while 12% considered both texture and taste as equally important. Only, 10% used texture as a criterion for choosing mushroom and 6% considered both texture and appearance concurrently before choosing a mushroom for purchase (Fig.22). Currently in the country, mushrooms are preserved by drying by 55% of the people while 32% use refrigerators for preservation. The remaining preserve

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mushrooms by smoking after blanching in brine (Fig.23). Exactly 45% of the respondents stated that mushroom production was laborious, while 10% believed that the process was expensive. About 45% found the process and costing normal (Fig.24).

(a) Producers

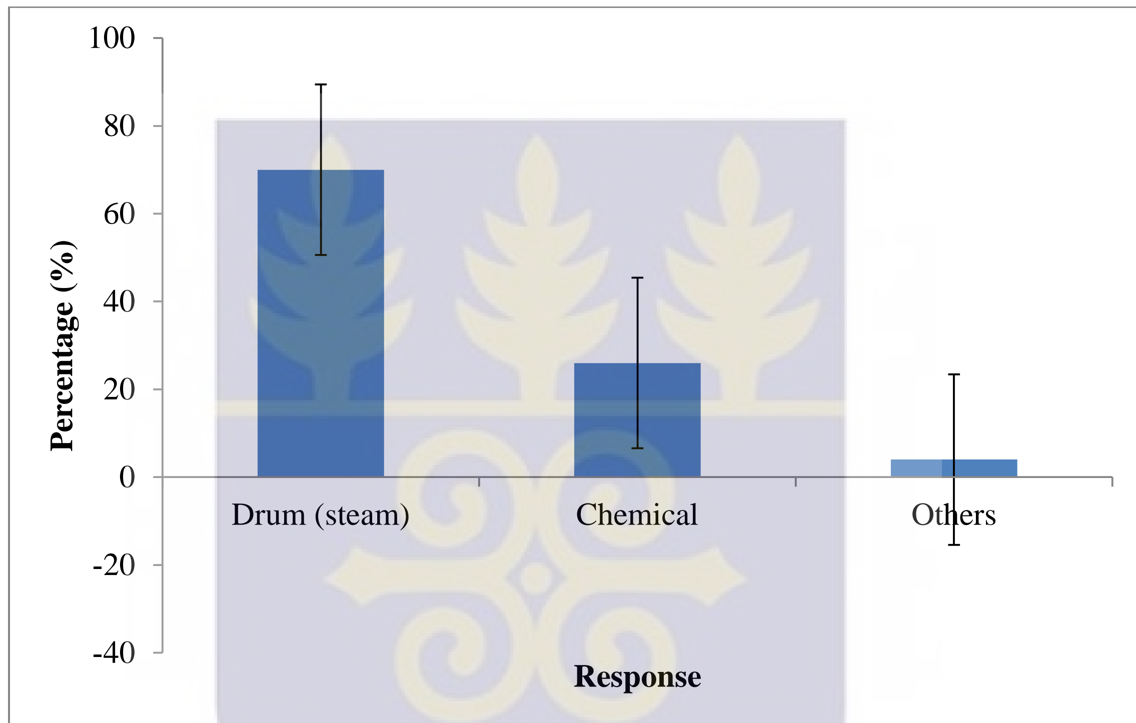

Fig.11. Method of sterilization of substrates used for mushroom cultivation

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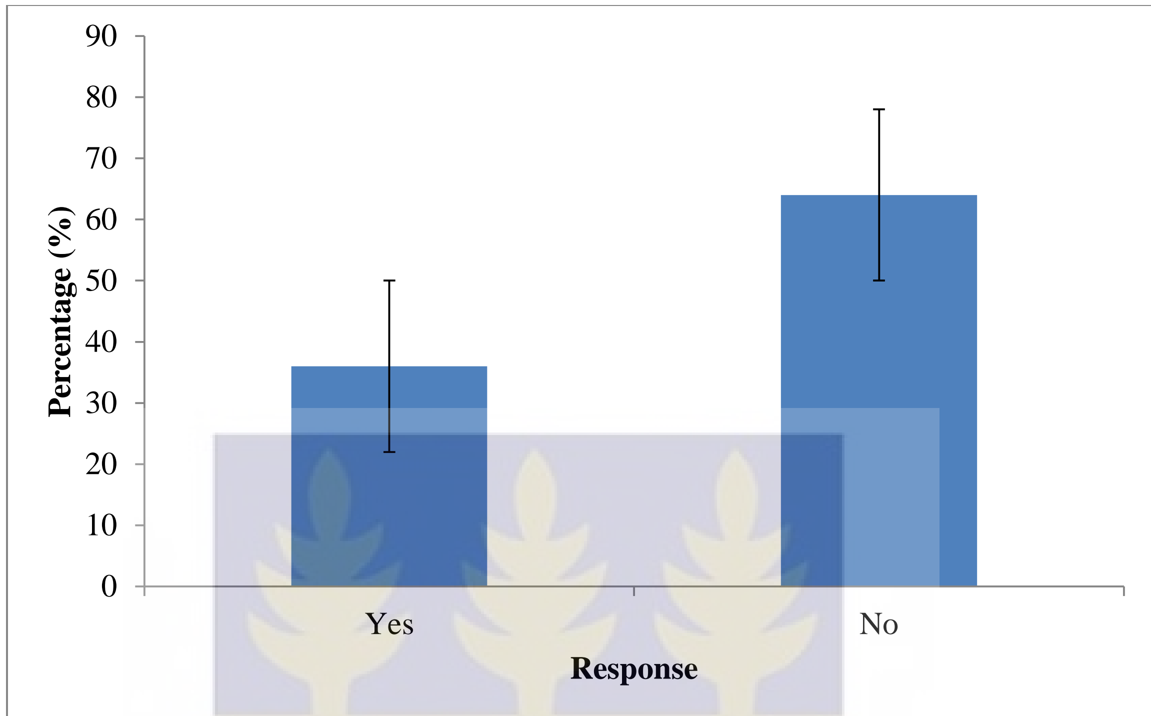

Fig.12. Mushroom farmer's satisfaction on the reliability of their sterilization methods in mushroom production

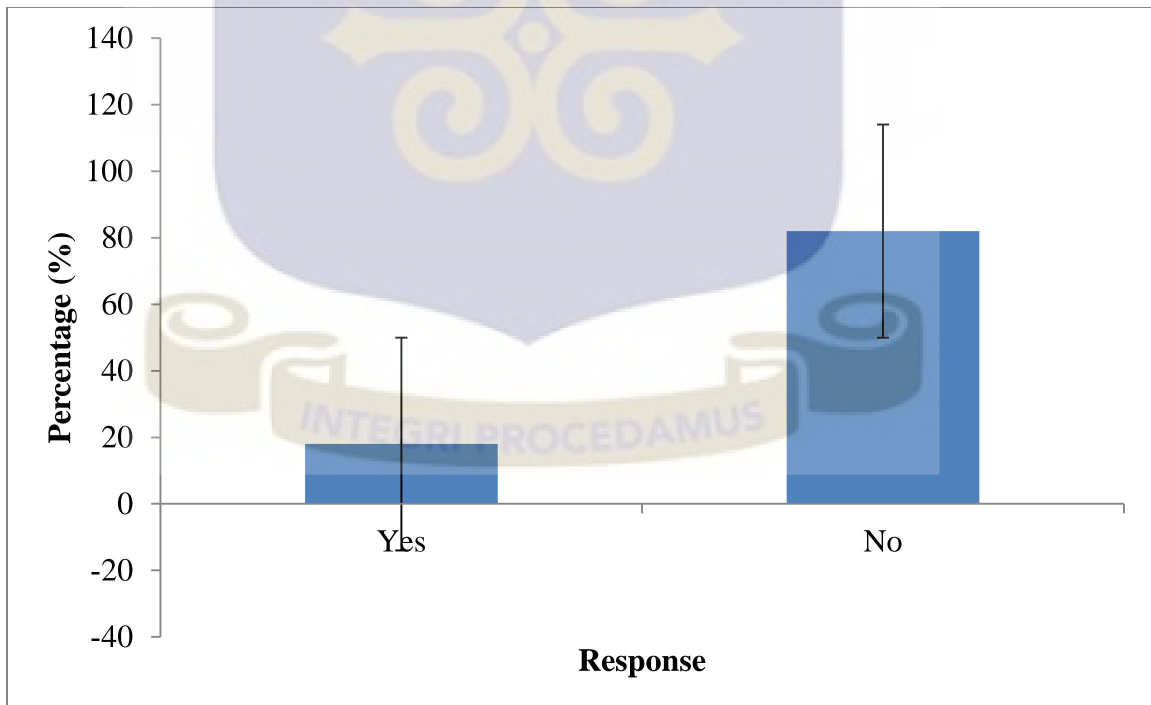

Fig.13. Consumer and public knowledge of the use of gamma irradiation in the sterilization of food to extend shelf-life

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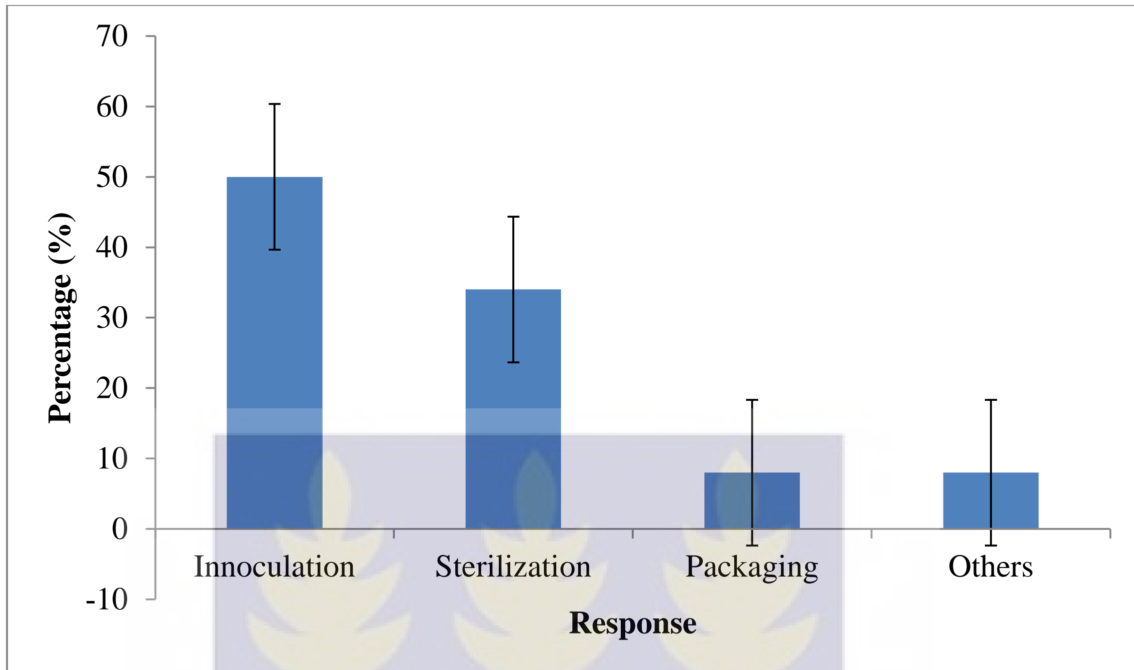

Fig.14. Aspects of production which needs much attention according to respondents

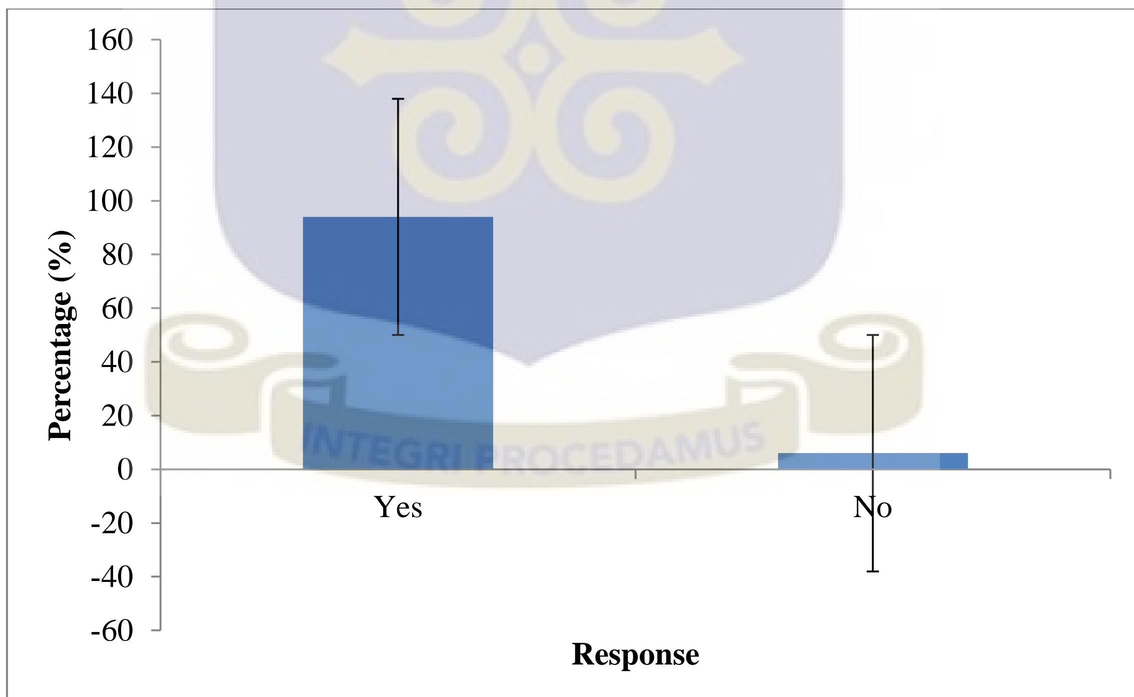

Fig.15. Response of mushroom cultivators on the need to achieve better sterilization of compost and other ancillary methods used in the production of mushrooms

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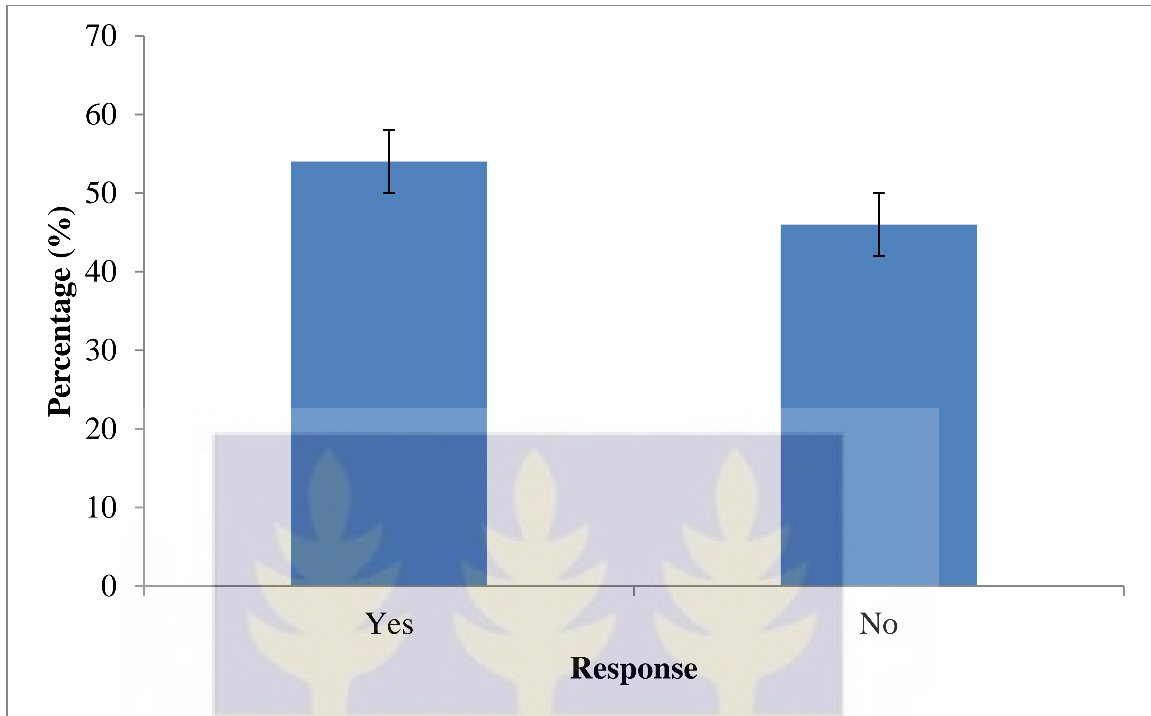

Fig.16. Percentages of farmers who were able to preserve unsold mushrooms (Yes) and those unable to preserve (No)

(b) Consumers

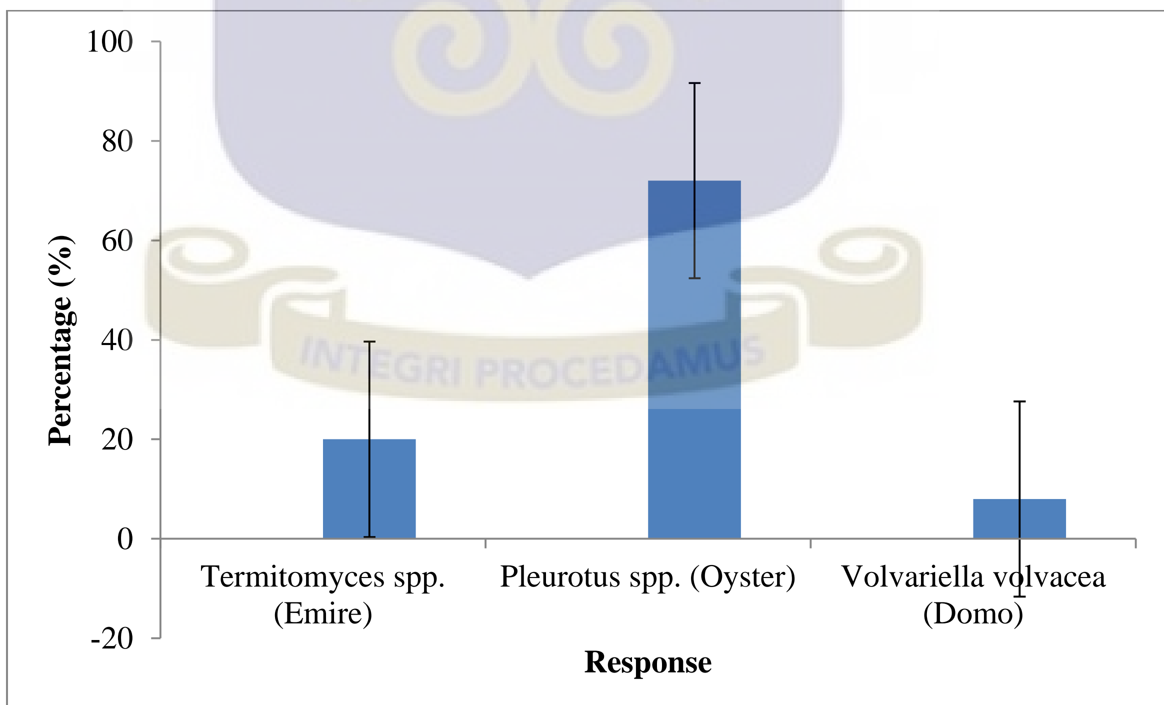

Fig.17. Types of mushrooms most patronized by consumers

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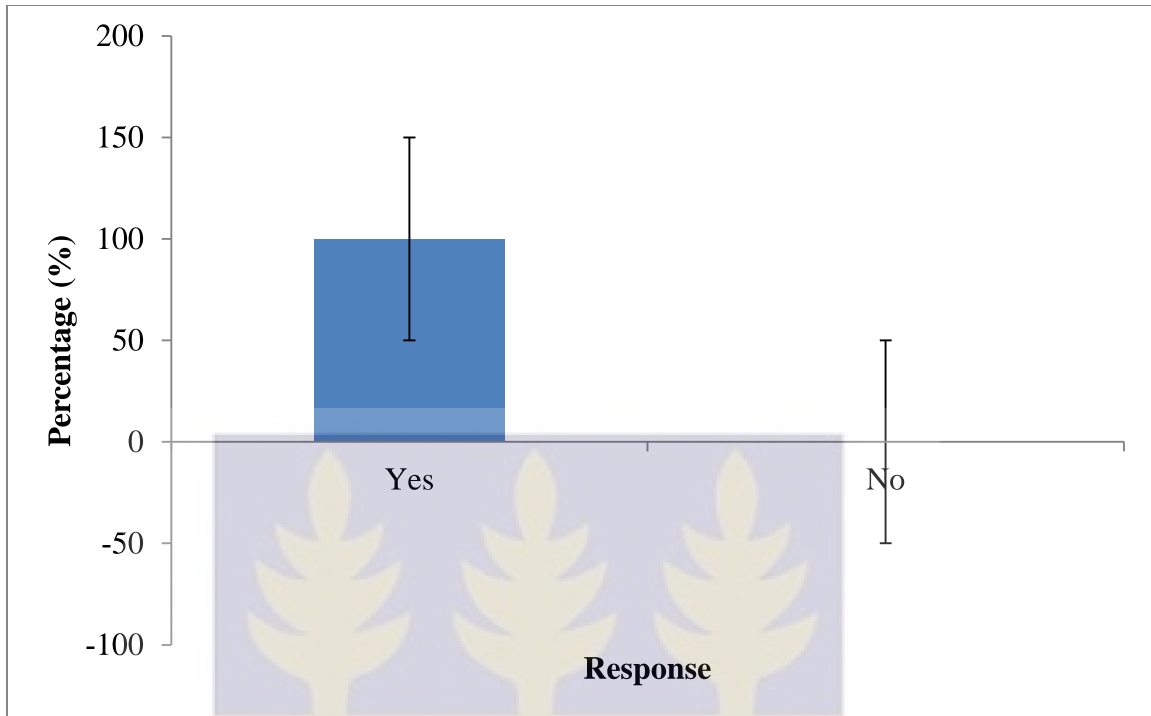

Fig.18. Consumer preference to see their favorite mushroom produced throughout the year

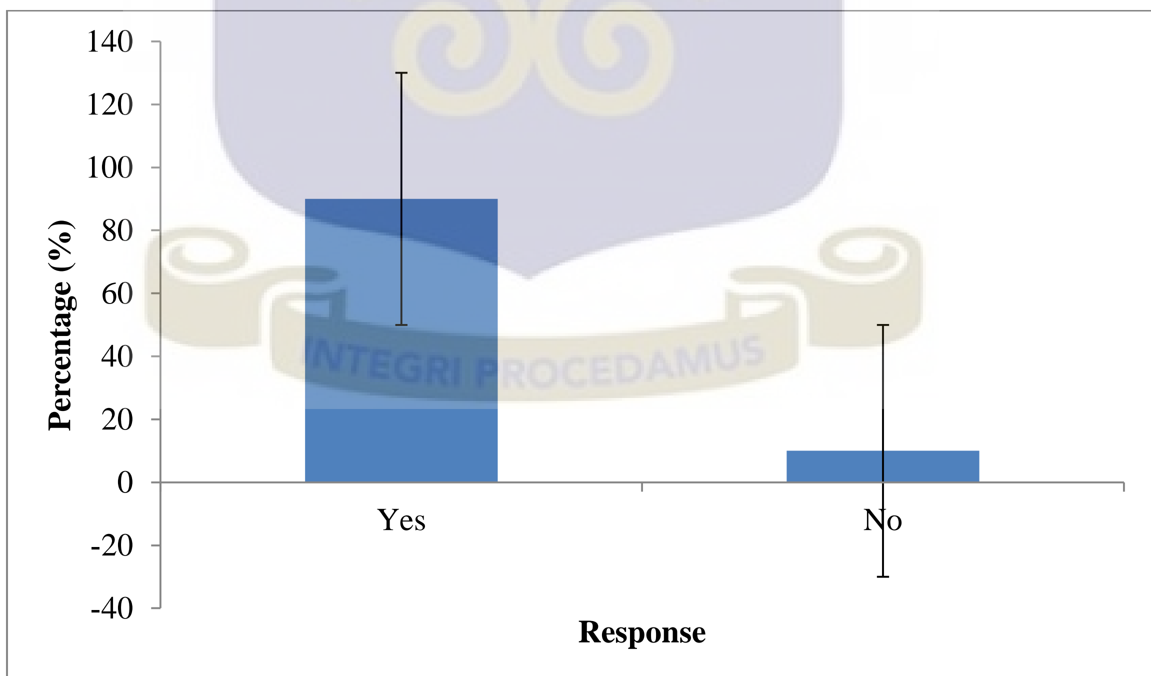

Fig.19. Respondents view of the promotion of mushroom consumption

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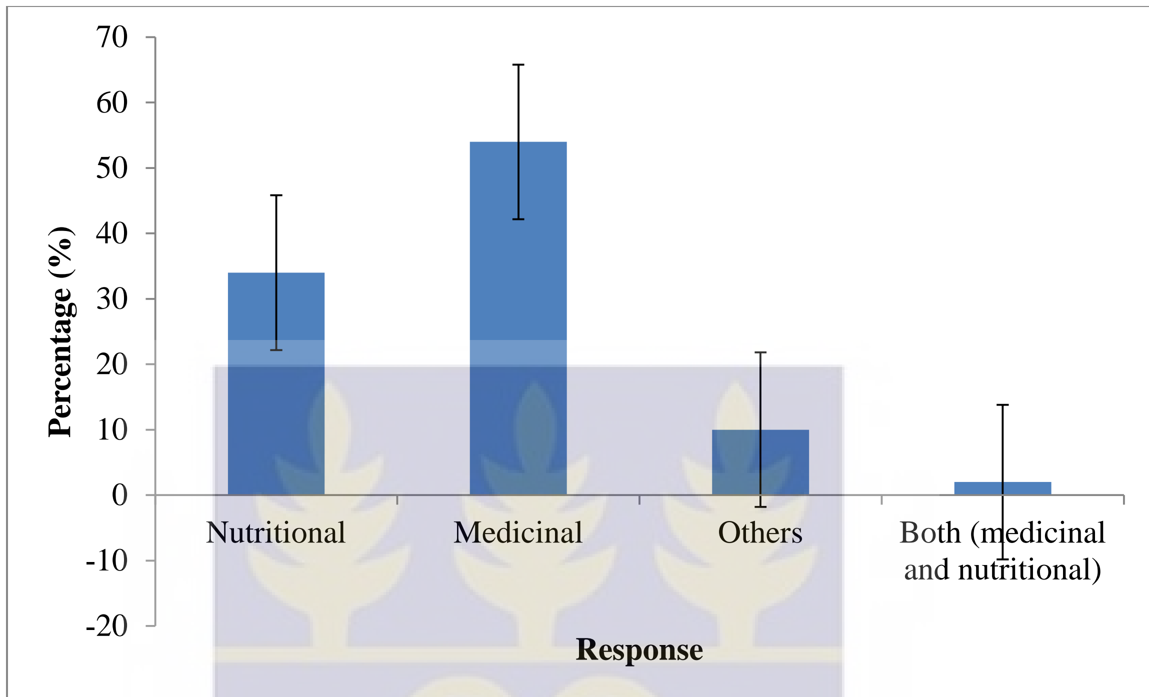

Fig.20. Respondents view on the benefits of eating mushrooms

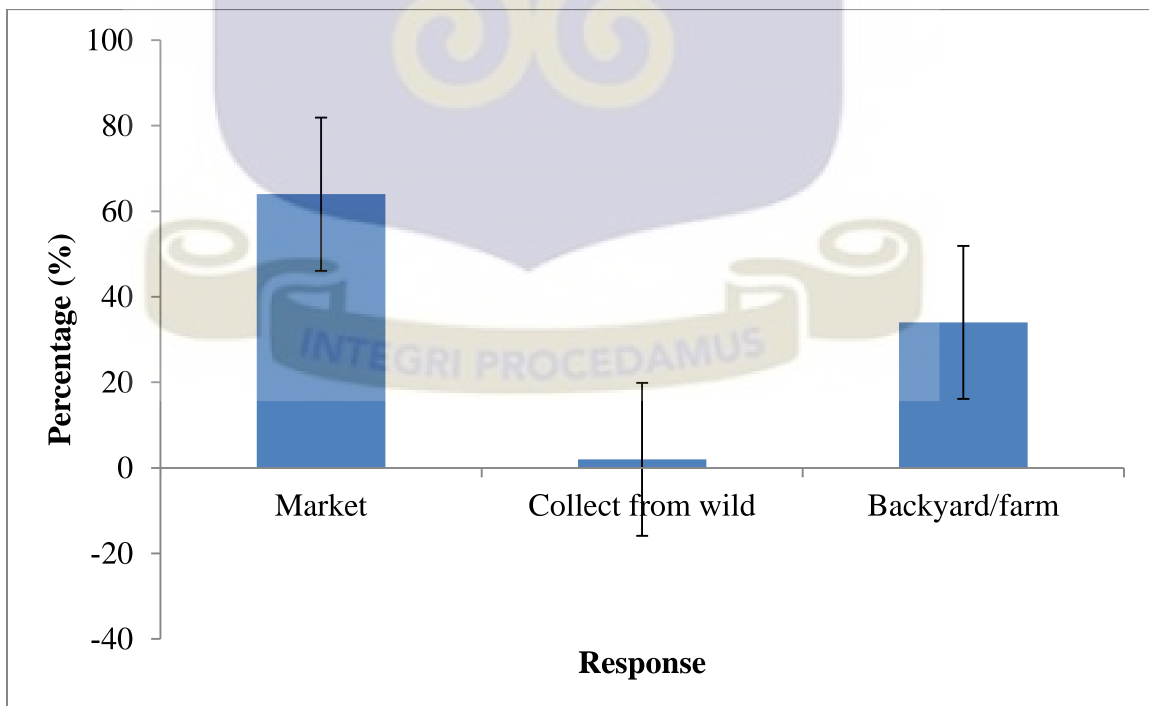

Fig.21. Source of mushrooms for use by consumers

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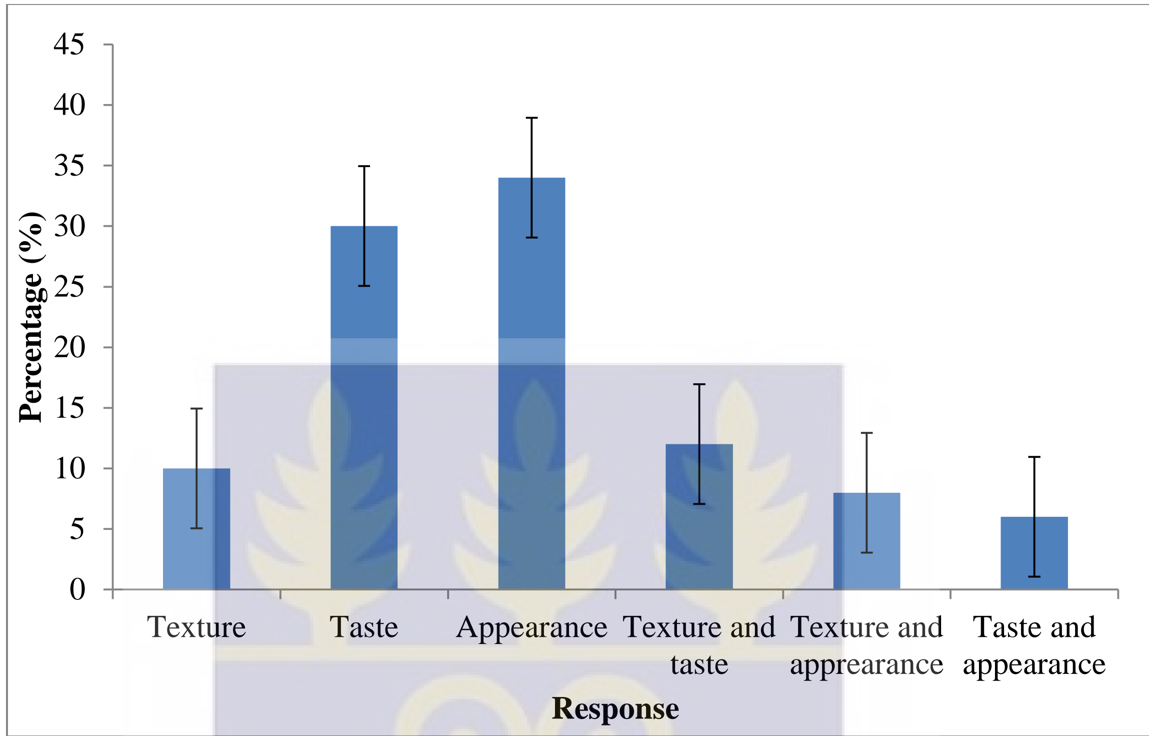

Fig.22. Choice of mushroom for consumption by consumers based on the listed criteria

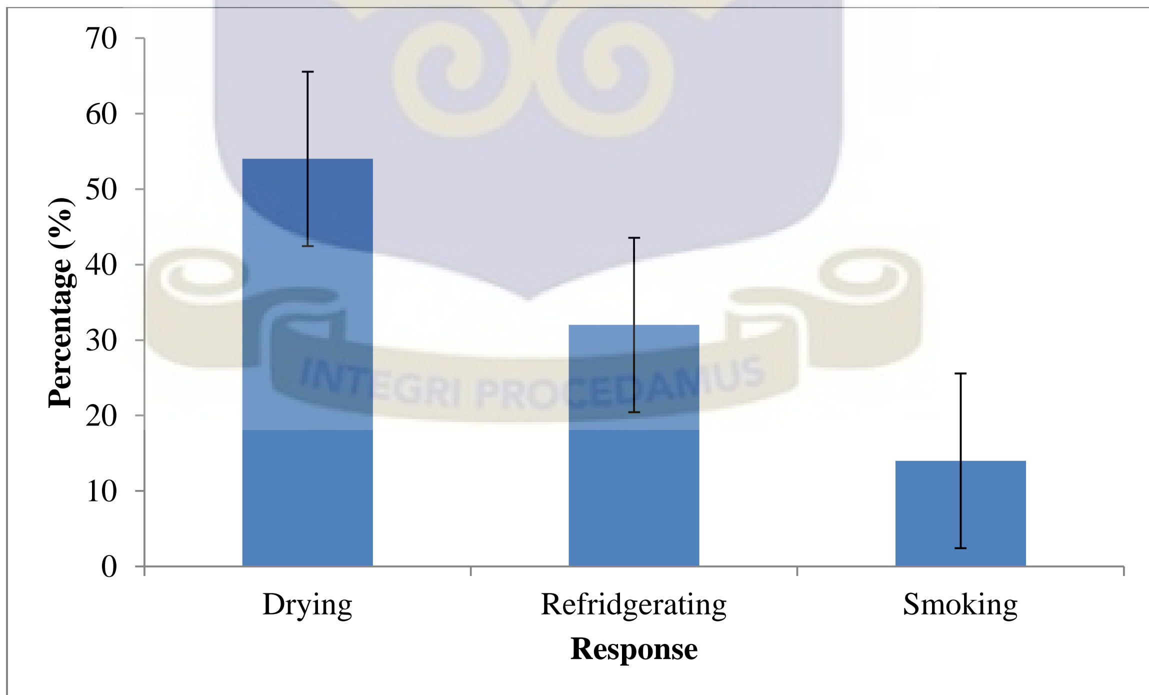

Fig.23. What are the methods used to preserve mushrooms?

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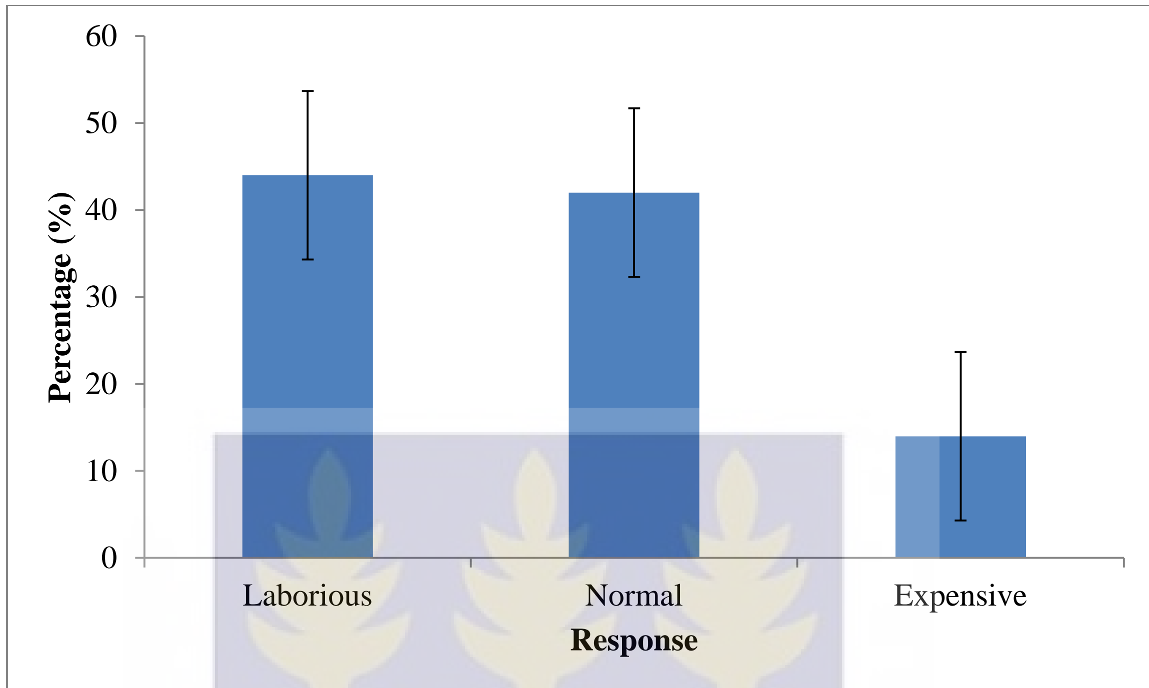

Fig.24. Public opinion on labour and cost of mushroom production in Ghana.

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EXPERIMENT 2

COMPARATIVE FUNGAL FLORA OF PRE- AND POST- IRRADIATED AND STEAM SORGHUM SEEDS SPAWN USED FOR MUSHROOM CULTIVATION

Fig.25 shows the influence of either steam sterilization or gamma irradiation on the resident mycoflora of sorghum seeds. Both methods were efficient in drastically reducing the mycoflora population. Steam sterilization reduced the initial load of 3.27-3.82 log_{10} CFU/g by 1.6-2.4 log cycles (Fig.25). The reduction in population by gamma irradiation was commensurate with the dose applied. The higher the dose, the greater the killing effect on the resident fungi. For example, a dose of 5 kGy reduced the mycoflora population cultured on Cooke's and DRBC by 1.2 and 1.6 log cycles respectively. On the other hand, increasing the dose to 32 kGy was significantly potent in killing the resident fungal population in the grains than steam sterilization treatment (Fig.25).

Fig.26 shows the radiosensitivity of the resident mycoflora in the grains plated on two media, Cooke's and DRBC. There was a good positive linear relationship between the dose applied and the resultant population at each dose (5- 32 kGy). The calculated decimal reduction dose or D_{10} was 7.9 ± 1.57 kGy and 6.4 ± 1.40 kGy respectively on Cooke's and DRBC media (Fig.26). The corresponding positive values were R^2=0.95 and R^2=0.748 respectively (Table 7). The effectiveness of steam sterilization in reducing total fungal population was comparable to radiation doses of 10 and 15 kGy. Doses beyond 15 kGy were significantly more efficacious than steam sterilization.

Figs.27 and 28 shows the changes in fungal flora after steam sterilization and gamma irradiation treatments. The non-treated grains haboured the following fungi: *Aspergillus*

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flavus (8.3-10%), *A.fumigatus* (16.7-26.25%), *A.alutaceus* (2.75-4.17%), *A.niger* (13.75-34.19%), *Fusarium oxysporum* (2.75-8.33%), *Cladosporium macrocarpum* (14.5%), *Penicillium spp.* (7.5-28.3%), *Rhodotorula* (1.25%) and *Trichoderma harzianum* (21.25%). *Aspergillus* species were predominant over the other species encountered and fungi of 6 genera (*Aspergillus, Cladosporium, Fusarium, Rhodotorula, Trichoderma* and *Penicillium*) were isolated (Figs.27 and 28) (Plate 6).

The residual fungal population cultured on Cooke's medium after pretreatment with 10 kGy included *A.fumigatus* (30%), *Penicillium spp*. (70%) and *Rhodotorula* (14%). After application of 15 kGy, *Rhodotorula spp*. dominated but was completely eliminated at 32 kGy (Figs 27 and 28) on DRBC medium only.

Steam sterilized grain harboured *Penicillium* (77.0%), *A. fumigatus* (18.0%), *A.alutaceus* (5.0%), *Rhodotorula* (11.5%), *A.niger* (11.5%). Improper heat pasteurization caused the contamination of sorghum grains in bags as shown in Plate 5. But gamma irradiated raw sorghum grains had the neat and contamination- free appearance as shown in Plate 7.

Moisture contents of the grains treated with 5-32 kGy in this study varied from 18.21-18.85% (Table 8). The pH range of the samples varied from pH 5.6±0.05- 6.36±0.04. Plate 6 show selected fungal species isolated belonging to the genera *Aspergillus, Cladosporium, Fusarium* and *Trichoderma*.

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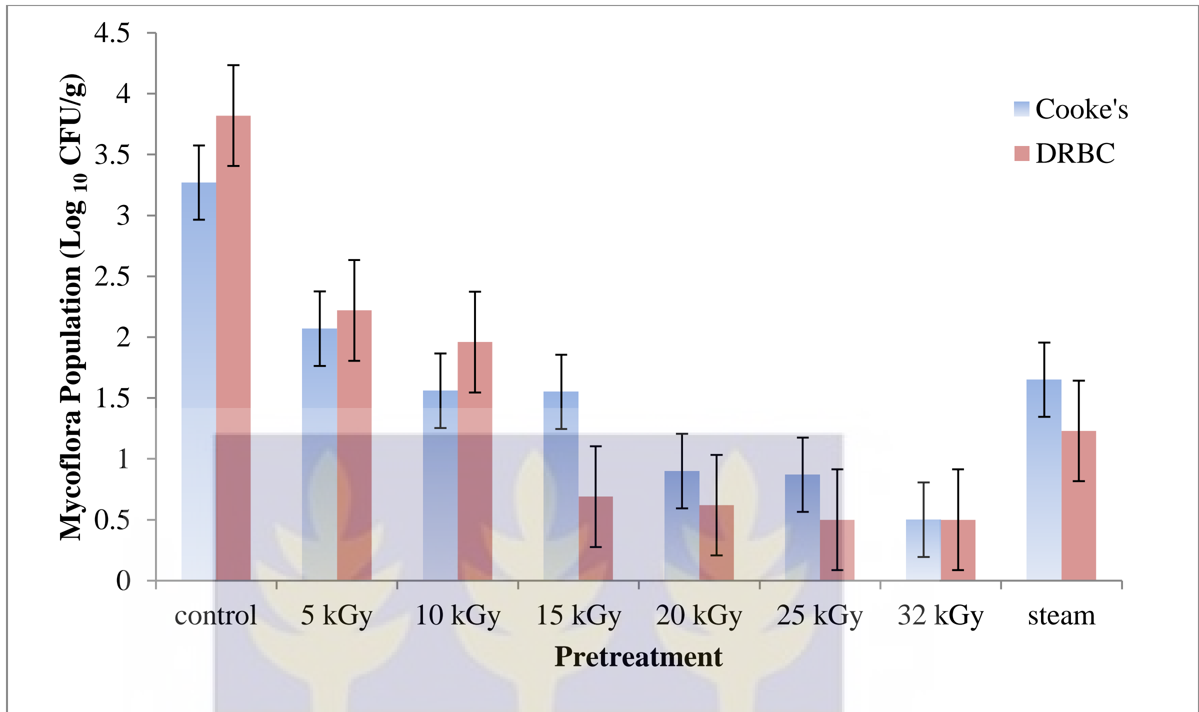

Fig.25. Mycoflora population resident in steam and gamma irradiated sorghum grains on two indicated media

Table 7. Mean D_{10} values of fungi associated with sorghum grains isolated from the indicated growth media.

Substrate	Regression equation (y)	R^2	D_{10} value (kGy)
(a) Cooke's	-0.025x + 0.993	0.945	7.9 ± 1.57
(b) DRBC	-0.018x + 0.602	0.748	6.4 ± 1.40

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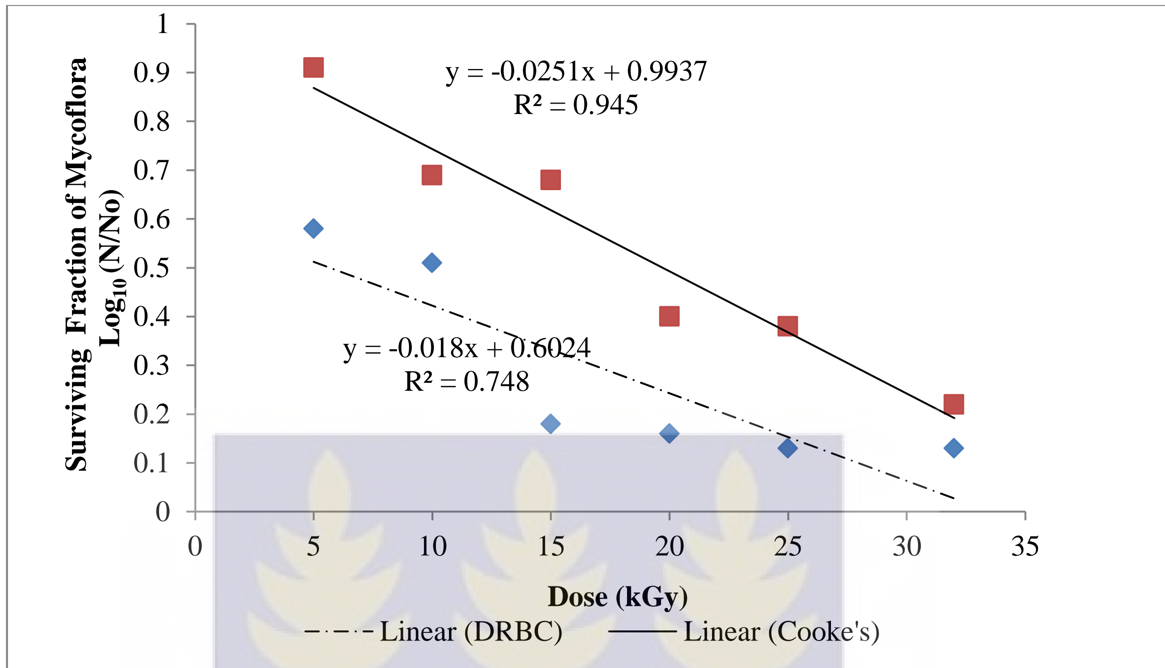

Fig.26. Radiation sensitivity curves for mycoflora resident in sorghum grains cultured on the indicated growth media for 6 days at 28±30 °C

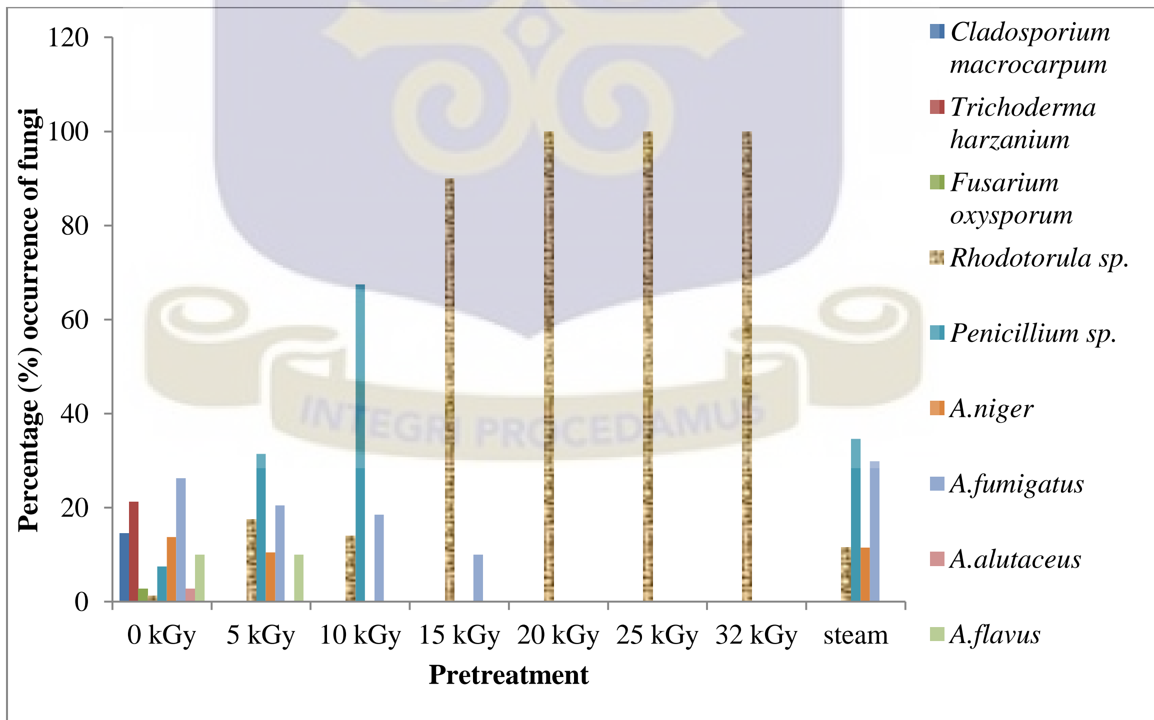

Fig.27. Percentage occurrence of fungi isolated on Cooke's medium after pretreatment of sorghum grains with either steam or gamma radiation

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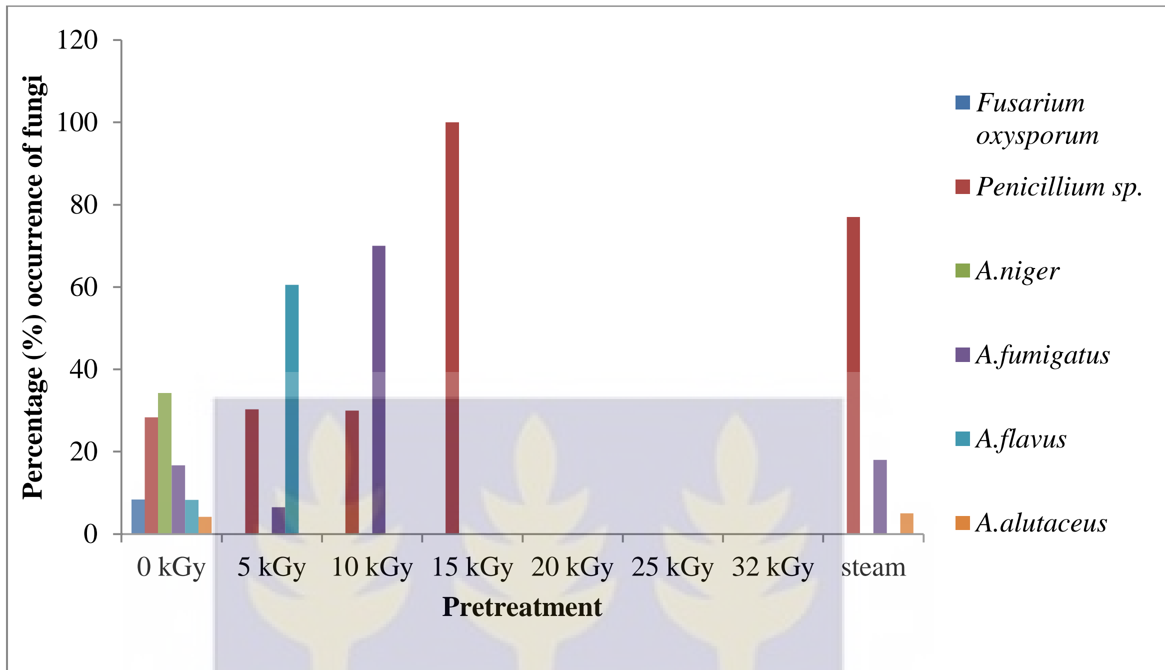

Fig.28. Percentage occurrence of fungi isolated on DRBC medium after pretreatment of sorghum grains with either steam or gamma radiation

Plate 5. Fungal contamination of sorgum grain spawn due to improper and partial pasteurization

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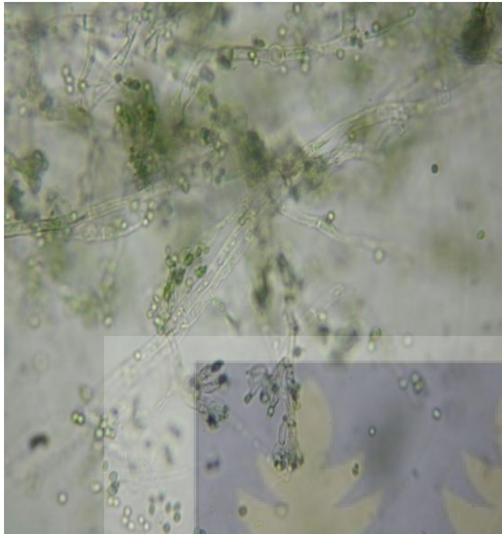
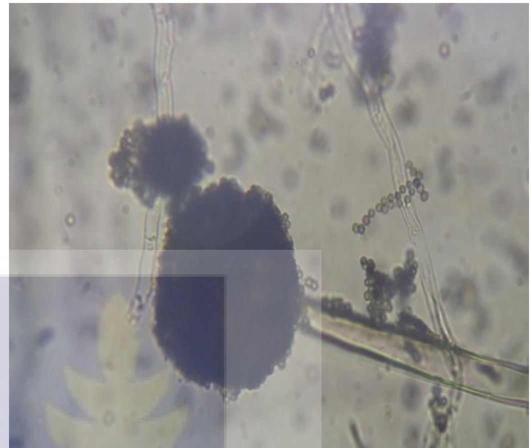
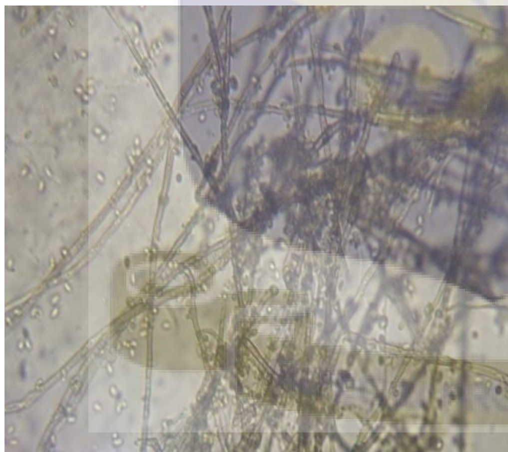


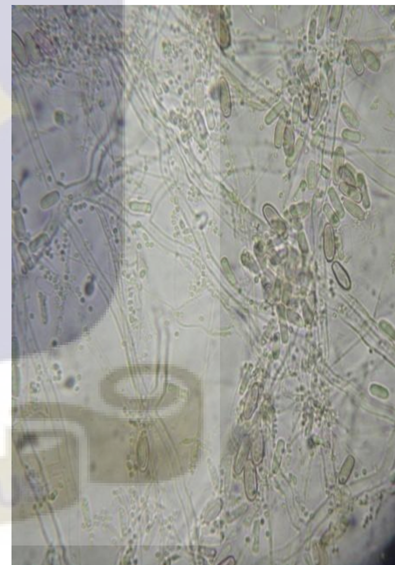
Penicillium spp.



Aspergillus niger



Trichoderma harzianum



Fusarium oxysporum

Plate 6. Photographs showing four fungi species resident in the substrate (x 400)

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EXPERIMENT 3

COMPARATIVE INFLUENCE OF GAMMA IRRADIATION AND MOIST HEAT STERILIZATION TREATMENTS ON SOME PHYSICAL CHARACTERISTICS AND MYCELIAL SPAWN GROWTH ON SORGHUM

The number of days from inoculation to total colonization of a substrate is related to the mycelial growth on the substrate. A faster growth rate results in a corresponding reduction in the days required for complete colonization of the substrate by mycelia (Narh *et al.*, 2011).

In this study, results obtained on the rate of growth of mycelium on autoclaved and irradiated sorghum grains are presented in Figs.29- 35 and Appendix 3. The fastest rate of mycelia growth was 0.71 cm/day. Samples treated with 5-32 kGy showed no significant ($p>0.05$) differences (Appendix 3). The shortest mycelia growth rate of 0.3 cm/day was recorded in the unirradiated and non-autoclaved sorghum grains.

Time taken for complete colonization

The shortest time for complete colonization of grains of sorghum after treatment combination with the use of radiation (5- 32 kGy) was 7days (Figs.29-35). The time taken for complete colonization of sorghum by *P.ostreatus* with treatment combination using 5, 10, 15, 20, 25, and 32 kGy, did not vary significantly ($p>0.05$) and were indeed the same (Figs.29- 35). On the other hand, the non autoclaved and non-irradiated sorghum grains (nS+nA, S+nI, nI +nI) were not completely covered even after 11 days (Fig.29) and growth stalled after 8 days.

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The pH of the substrate variously treated with a combination of steam sterilization or gamma irradiation varied from pH 5.18 ± 0.05 to 6.71±0.04 (Table 8). The moisture content also varied from 17.85±0.4 to 19.28±1.50% (Table 8) which is not ideal for fungal growth but may cause problems if moisture contents increases beyond 30%. Temperature ranged between 21.5±1.3 ^{o}C to 27.5±1.8 ^{o}C.

Contamination and Mycelia Density

Contamination of *P.ostreatus* spawn on the grains was severe in the control samples (50-80%) with lowest average growth rate of 0.3cm/day (i.e non-steam or non-irradiated) (Appendix 3). There was no contamination of sorghum grains which were given the combination pretreatment and irradiated with doses 10-32 kGy. In all these instances the mycelium was dense and white ramifying the entire bag. Only the non-pretreated (non-steam or non irradiated) differed in density. Plate 8 shows an example of complete mycelial colonization of the gamma-irradiated sorghum grains.

Plate 7: Soaked (overnight) and gamma irradiated raw sorghum

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Table 8. Effect of pretreatment (gamma radiation and steam) on the physical characteristics of sorghum grains (*Sorghum bicolor*)

Dose (kGy)	Treatment	Moisture content (%) (Mean ± S.D)	pH (Mean ± S.D)	Temperature (^{o}C) (Mean ± S.D)
0	nS + nA	18.85 ± 0.85	6.71 ± 0.04	24.1 ± 1.50
	S + nI	19.28 ± 1.50	5.88 ± 0.05	23.9 ± 1.50
	nI + nI	19.00 ± 1.20	6.51 ± 0.04	23.3 ± 1.50
5	S + A	18.69 ± 0.85	5.84 ± 0.05	24.1 ± 1.50
	S + I	18.34 ± 0.85	5.69 ± 0.05	24.2 ± 1.50
	I + I	18.53 ± 0.86	5.48 ± 0.05	22.3 ± 1.40
10	S + A	17.94 ± 0.64	5.18 ± 0.05	27.1 ± 1.80
	S + I	18.21 ± 0.78	5.71 ± 0.05	24.4 ± 1.50
	I + I	19.01 ± 1.20	5.98 ± 0.04	27.5 ± 1.80
15	S + A	17.89 ± 0.64	5.85 ± 0.05	24.5 ± 1.50
	S + I	18.93 ± 0.88	5.70 ± 0.05	24.4 ± 1.50
	I + I	18.27 ± 0.33	6.21 ± 0.06	24.1 ± 1.50
20	S + A	18.56 ± 0.45	5.64 ± 0.05	24.0 ± 1.50
	S + I	18.42 ± 0.82	5.43 ± 0.04	24.6 ± 1.50
	I + I	18.96 ± 0.88	5.88 ± 0.05	24.2 ± 1.50
25	S + A	18.84 ± 0.64	5.47 ± 0.04	24.5 ± 1.50
	S + I	19.00 ± 1.20	5.36 ± 0.04	23.6 ± 1.40
	I + I	18.77 ± 0.87	6.47 ± 0.06	23.9 ± 1.50
32	S + A	18.85 ± 0.64	5.76 ± 0.05	21.7 ± 1.30
	S + I	18.30 ± 0.33	5.73 ± 0.05	21.5 ± 1.30
	I + I	17.85 ± 0.64	6.08 ± 0.04	22.4 ± 1.30

Results are averages of 3 ± standard deviations

S+A= Steamed and Autoclaved

S+I= Steamed and Irradiated

I+I= Irradiated

nI+nI= Non irradiated (Raw)

nS+nA= Non autoclaved (Raw)

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GROWTH OF SORGHUM SPAWN

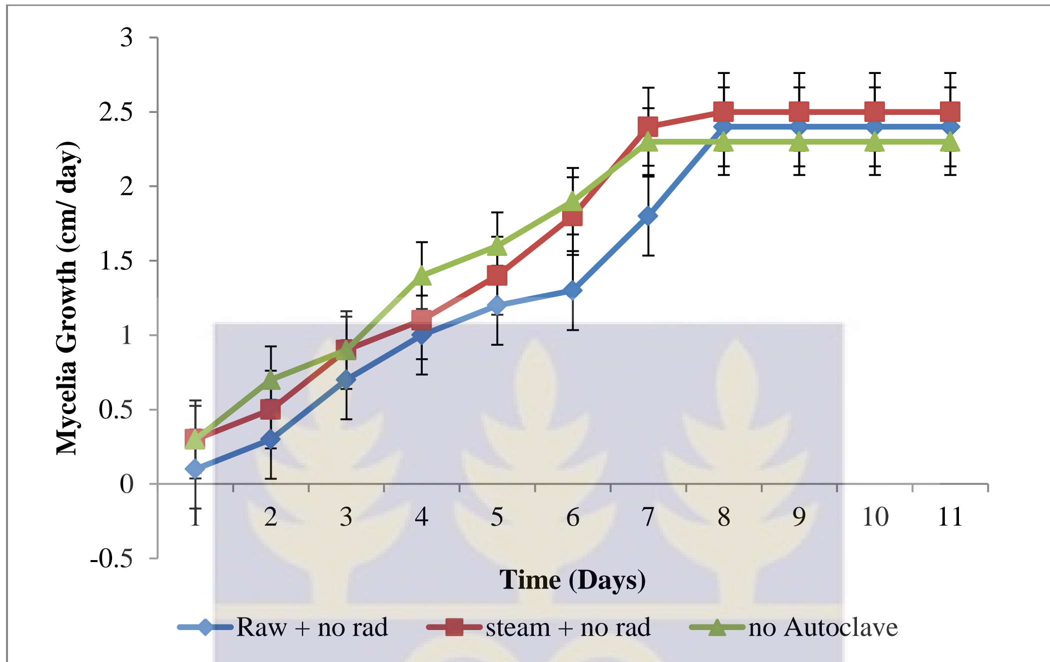

Fig. 29: The effect of non autoclaved and non- irradiated (0 kGy) sorghum grains on the daily mycelia growth of *P.ostreatus* strain EM-1.

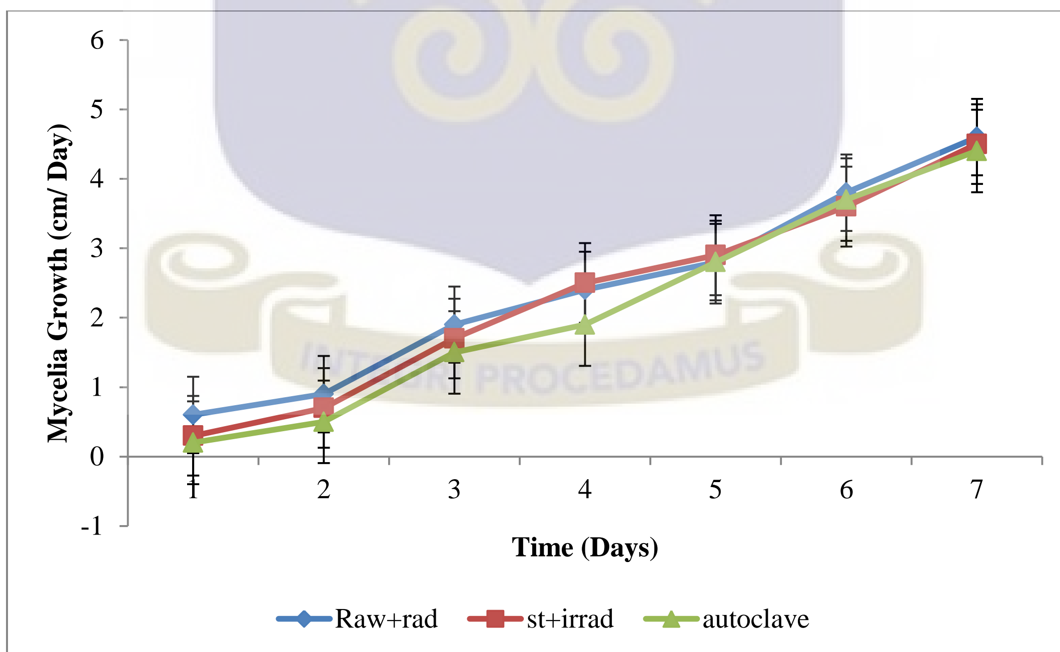

Fig. 30: The effect of autoclaved and irradiated (5kGy) sorghum grains (raw and steamed) on the daily mycelia growth of *P.ostreatus* strain EM-1.

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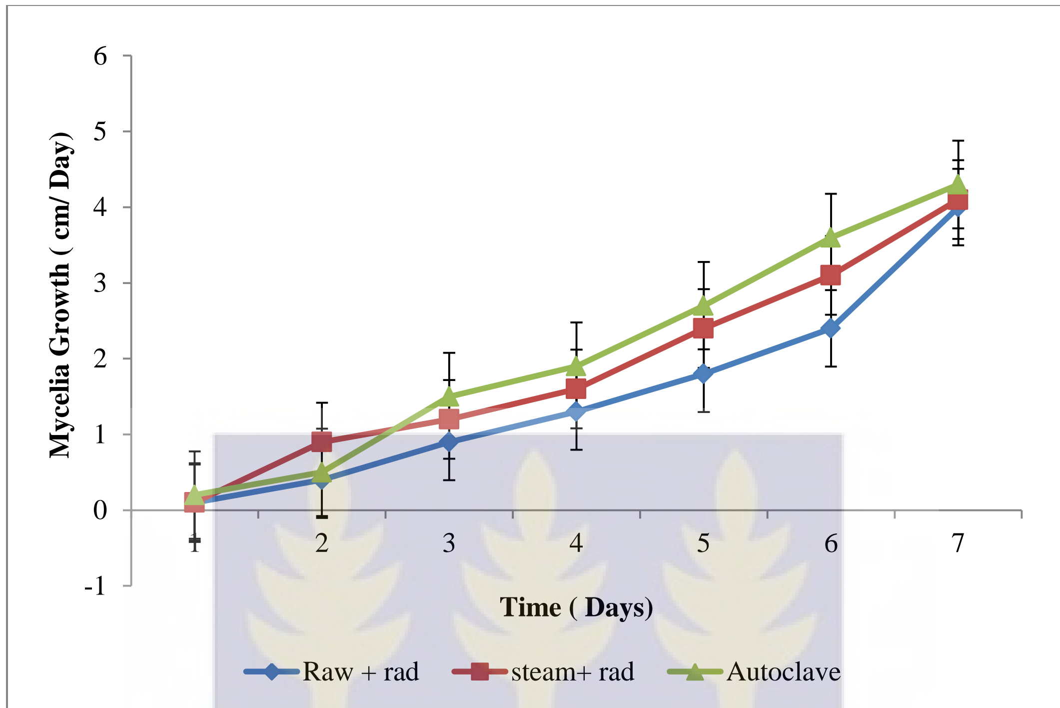

Fig.31: The effect of autoclaved and irradiated (10 kGy) sorghum grains (raw and steamed) on the daily mycelia growth of *P.ostreatus* strain EM-1

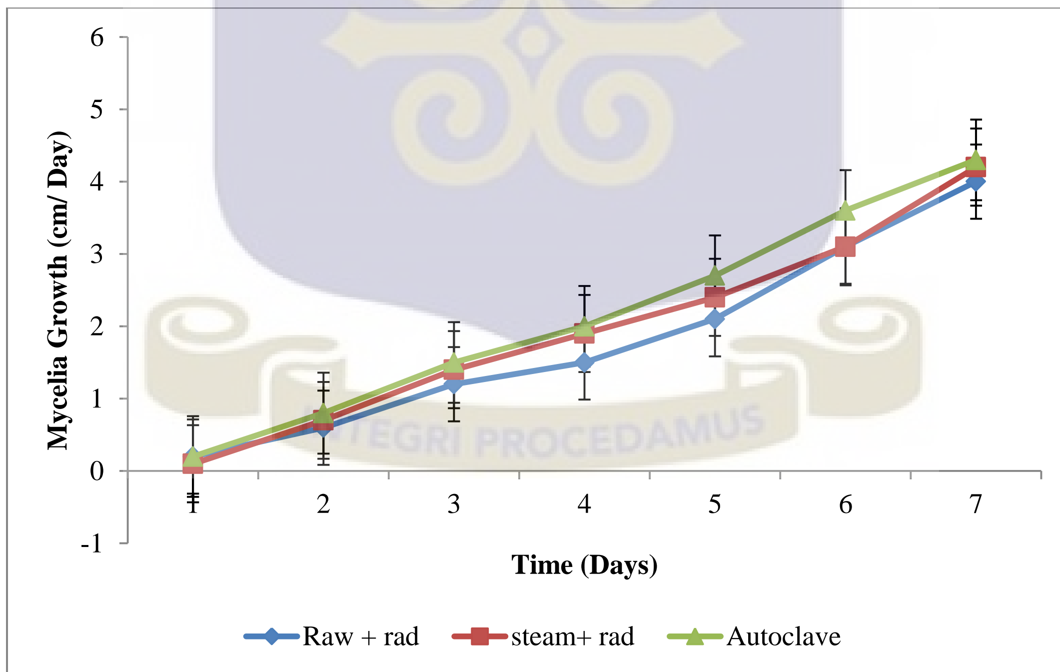

Fig.32: The effect of autoclaved and irradiated (15 kGy) sorghum grains on the daily mycelia growth of *P.ostreatus* strain EM-1.

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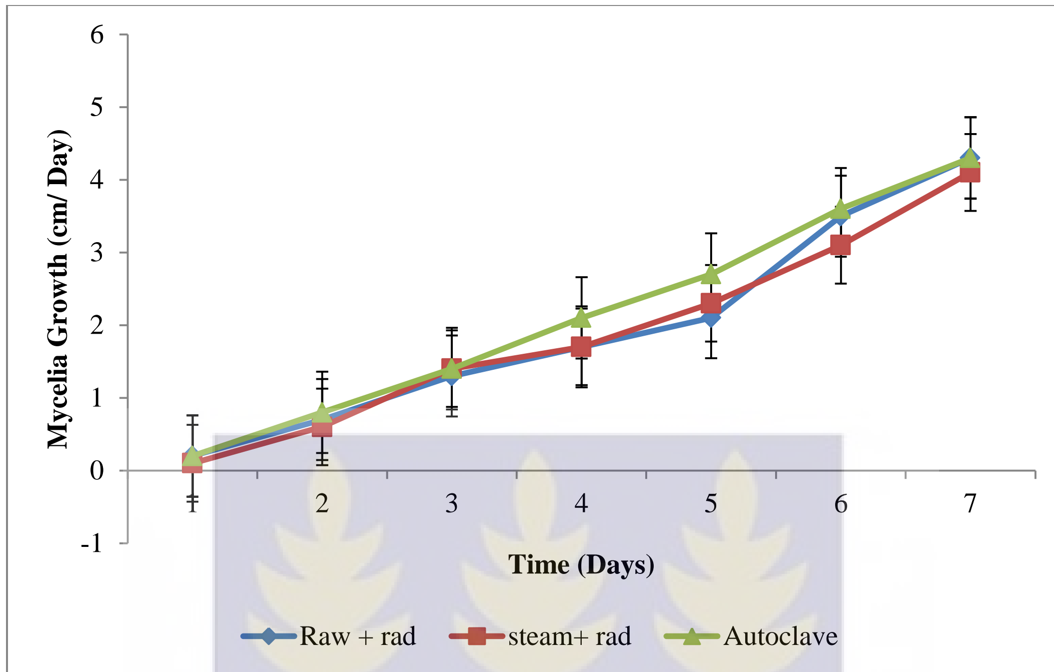

Fig.33: The effect of autoclaved and irradiated (20 kGy) sorghum grains on the daily mycelia growth of *P.ostreatus* strain EM-1.

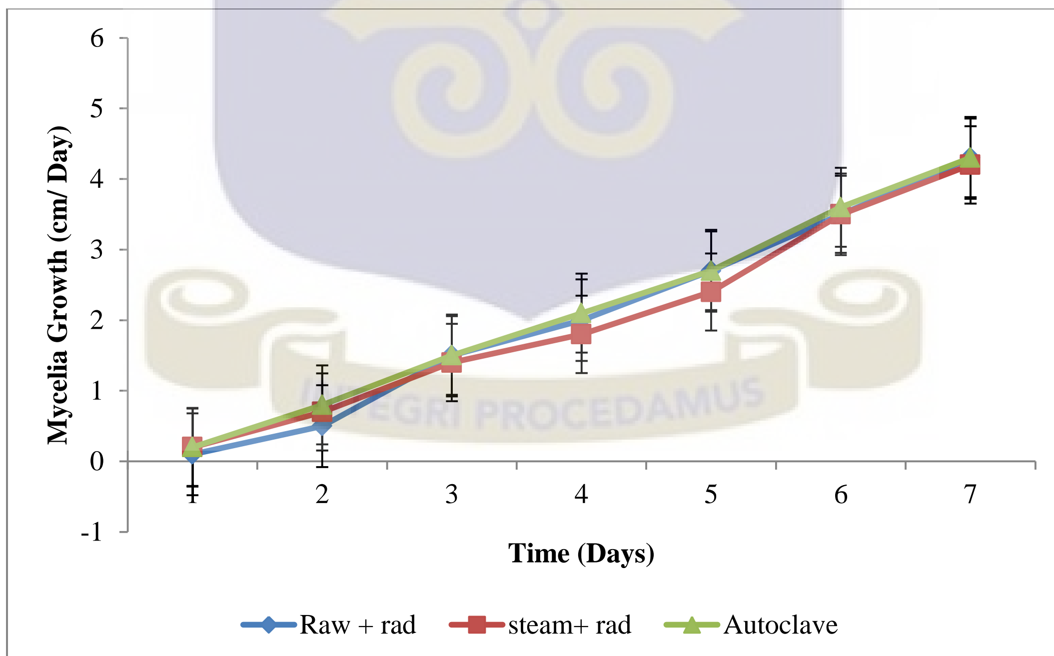

Fig.34: The effect of autoclaved and irradiated (25 kGy) sorghum grains on the daily mycelia growth of *P.ostreatus* strain EM-1.

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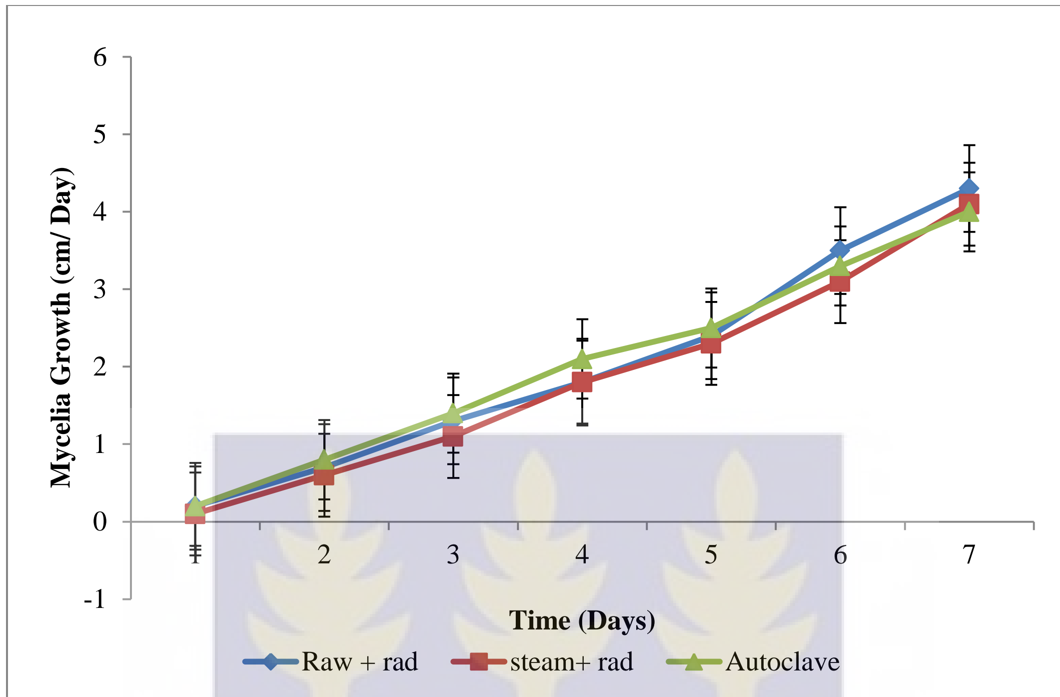

Fig.35: The effect of autoclaved and irradiated (32 kGy) sorghum grains on the daily mycelia growth of *P.ostreatus* strain EM-1.

Plate 8: Complete mycelia colonization of gamma irradiated sorghum

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EXPERIMENT 4

MYCOFLORA OF 'WAWA' SAWDUST (*TRIPLOCHITON SCLEROXYLON*) BEFORE AND AFTER GAMMA IRRADIATION AND COMPOSTING AT 30-32°C FOR 28 DAYS

(a) Mycoflora of 'wawa' sawdust and phenology during composting for 28 days

Fig.36 shows the variation in fungal population during the period of composting. Mycoflora population enumerated on Cooke's medium was about 1.0-2.2 log cycles higher than what was enumerated on OGYE (Fig 36). The differences observed in mycoflora population as composting progressed from 0-28 days were statistically significant ($p<0.05$) on the two enumeration media (Fig.36). The decrease in mycoflora population in sawdust after radiation treatment was commensurate with the doses applied. The higher the dose, the greater the reduction in mycoflora population (Fig.37) such that 32 kGy reduced the population by more that 4 log cycles while steam reduced load by about 2 log cycles. (Fig.37). The phenology of the resident fungi during composting of wawa sawdust and enumerating on two media is presented in Fig.38 (Cooke's) and Fig.39 (OGYE). Generally, ten (10) fungal species belonging to four genera (*Aspergillus, Rhizopus, Fusarium and Mucor*) were recorded on both media (Figs.38 and 39). Six *Aspergillus* species (*A. flavus, A. fumigatus, A. alutaceus, A. niger, A. parasiticus, A. terreus*) predominated. *Fusarium oxysporum, Mucor racemosus* and *Rhizopus stolonifer* were also isolated (Fig.38). The percentage contributions of each species to the total mycoflora are shown in the Figs 38 and 39. Species like *A.parasiticus* which persisted in the compost was initially high (30%) but declined to (>5%) in 28 days.

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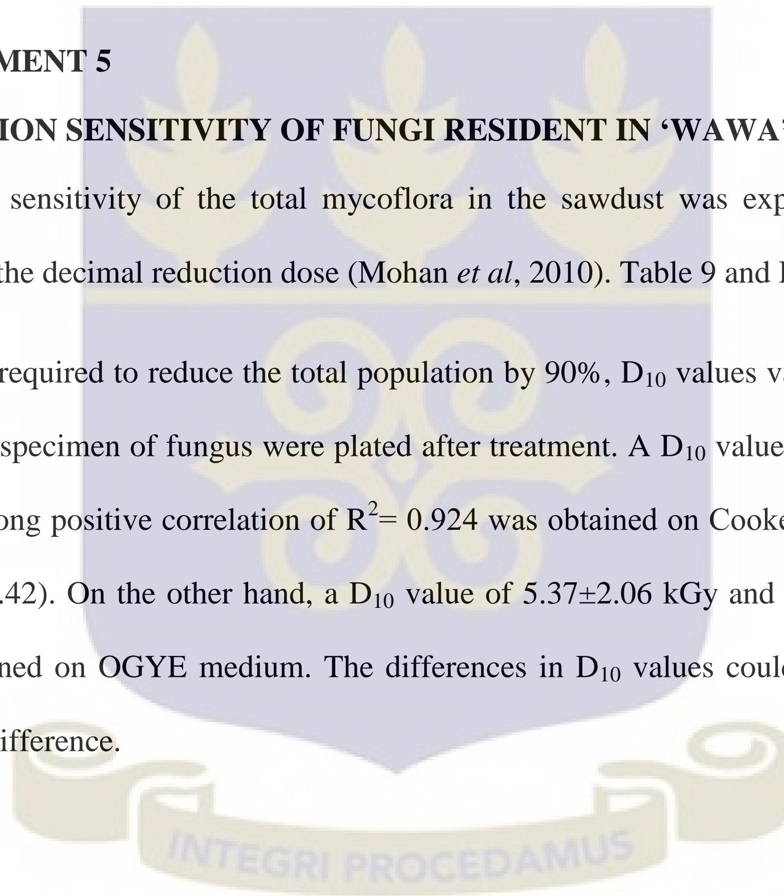
(b) **Mycoflora of 'wawa' sawdust after application of steam sterilization and gamma radiation and plated on two media.** Fig. 41 show results obtained. A dose of 15-32 kGy completely eliminated resident fungi (Figs 40 and 41), while there were some residual fungi such as *A.niger, A.flavus, Mucor racemosus, A.fumigatus, A.alutaceus, R.stolonifer,* and *Fusarium oxysporum* after steam sterilization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EXPERIMENT 5

RADIATION SENSITIVITY OF FUNGI RESIDENT IN 'WAWA' SAWDUST

Radiation sensitivity of the total mycoflora in the sawdust was expressed as the D_{10} values or the decimal reduction dose (Mohan *et al*, 2010). Table 9 and Fig.42.

The dose required to reduce the total population by 90%, D_{10} values varied on the media on which specimen of fungus were plated after treatment. A D_{10} value of 5.64±1.12 kGy with a strong positive correlation of R^2= 0.924 was obtained on Cooke's medium (Table 9 and Fig.42). On the other hand, a D_{10} value of 5.37±2.06 kGy and stronger R^2=0.971 was obtained on OGYE medium. The differences in D_{10} values could be due to media richness difference.

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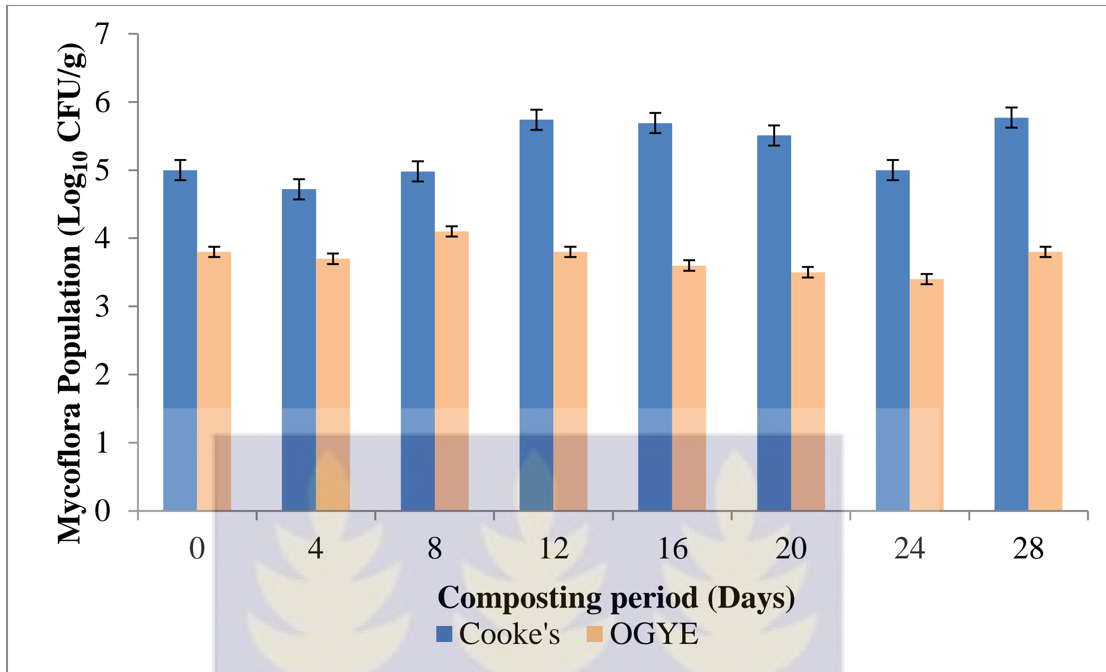

Fig.36. Mycoflora population of sawdust during composting period and enumerated on two indicated media at 30±2 °C for 7 days

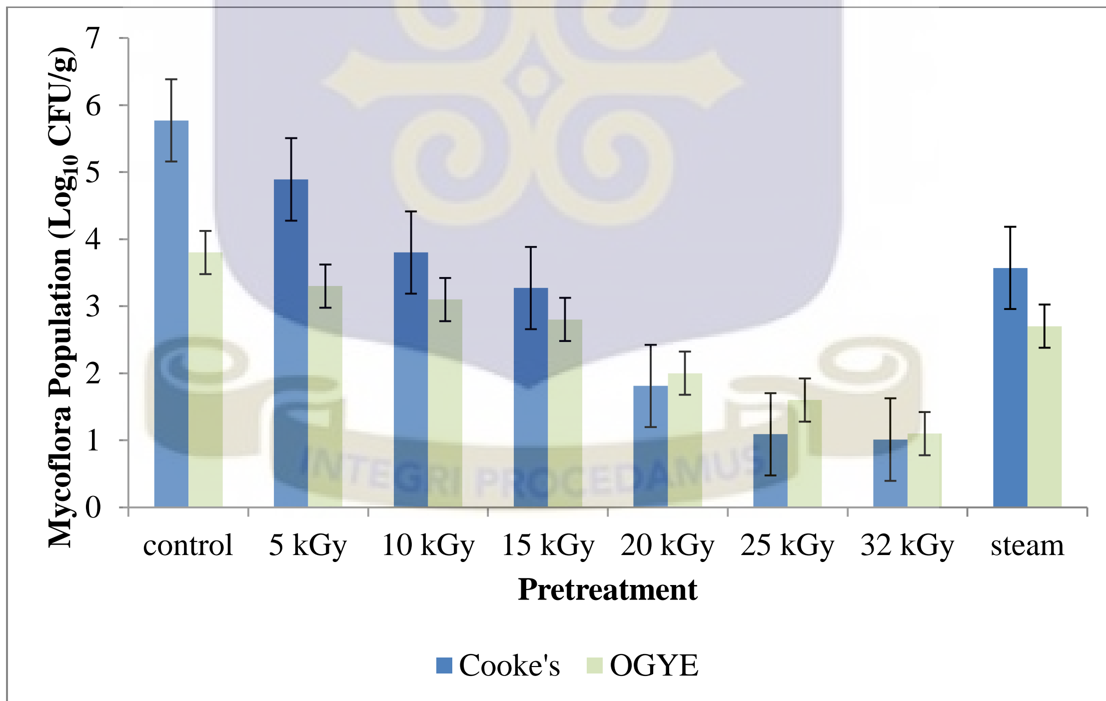

Fig.37. Mycoflora population of sawdust after pretreatment with either steam or gamma irradiation and cultured on two indicated media at 30±2 °C for 7 days

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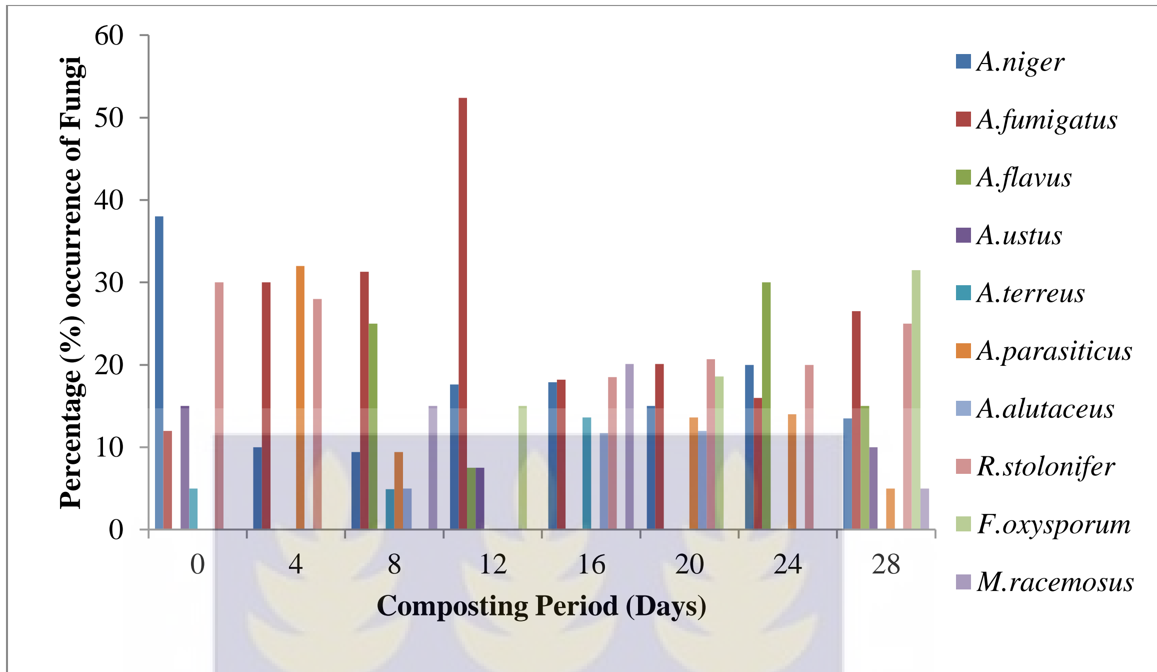

Fig.38. Percentage occurrence of fungi of sawdust during composting plated on Cooke's medium

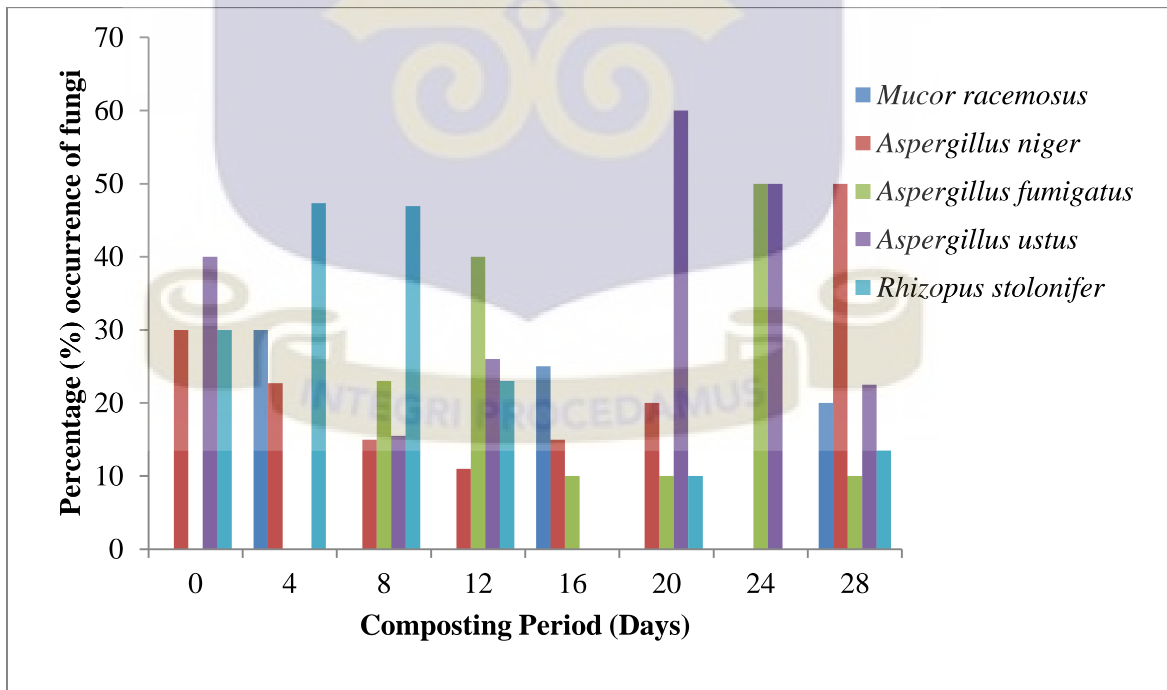

Fig.39. Percentage occurrence of fungi during composting of sawdust for 28 days plated on OGYE

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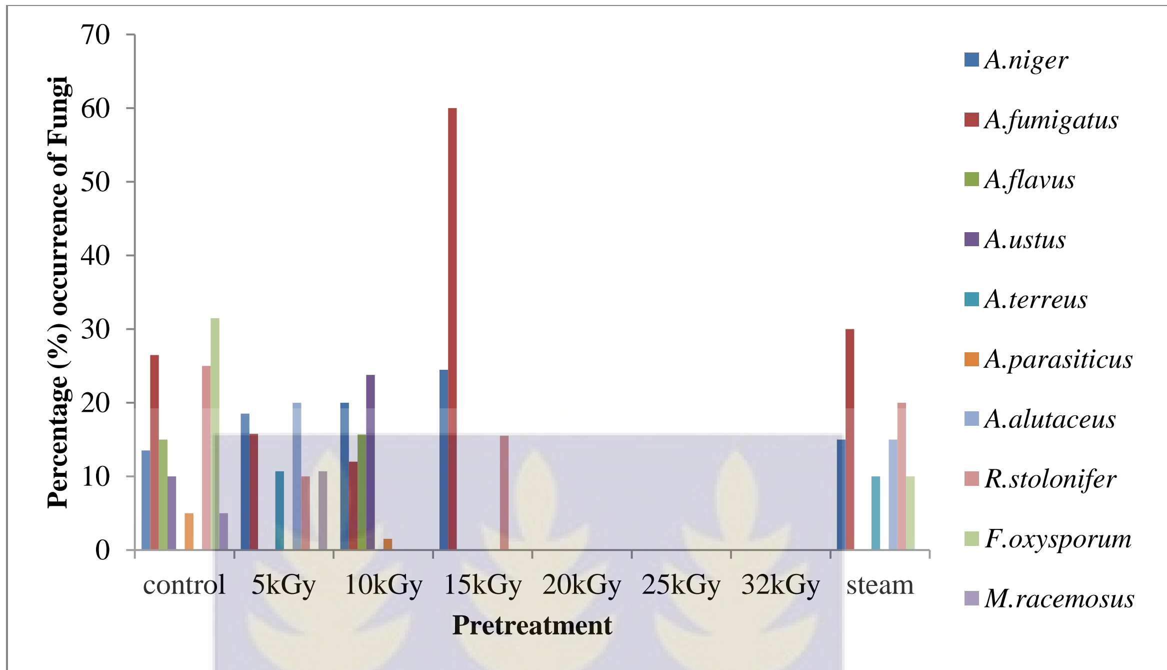

Fig.40. Percentage occurrence of fungi after the indicated pretreatment of sawdust plated on Cooke's medium

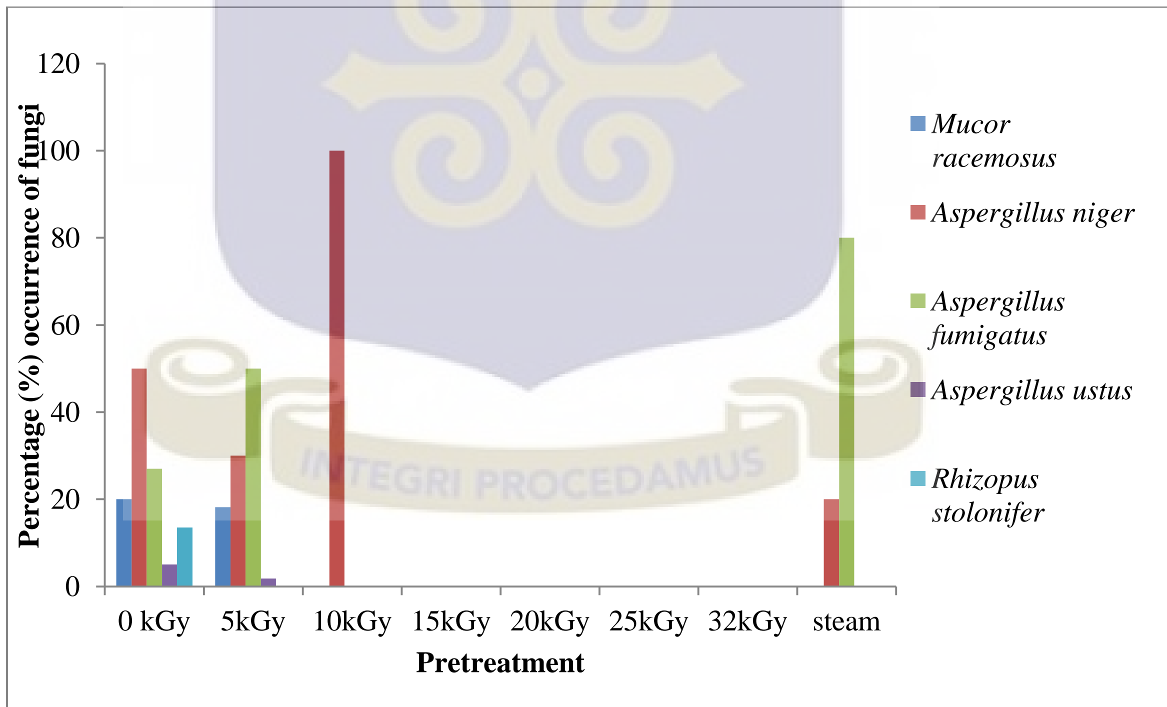

Fig.41. Percentage occurrence of fungi after the indicated pretreatment of sawdust plated on OGYE

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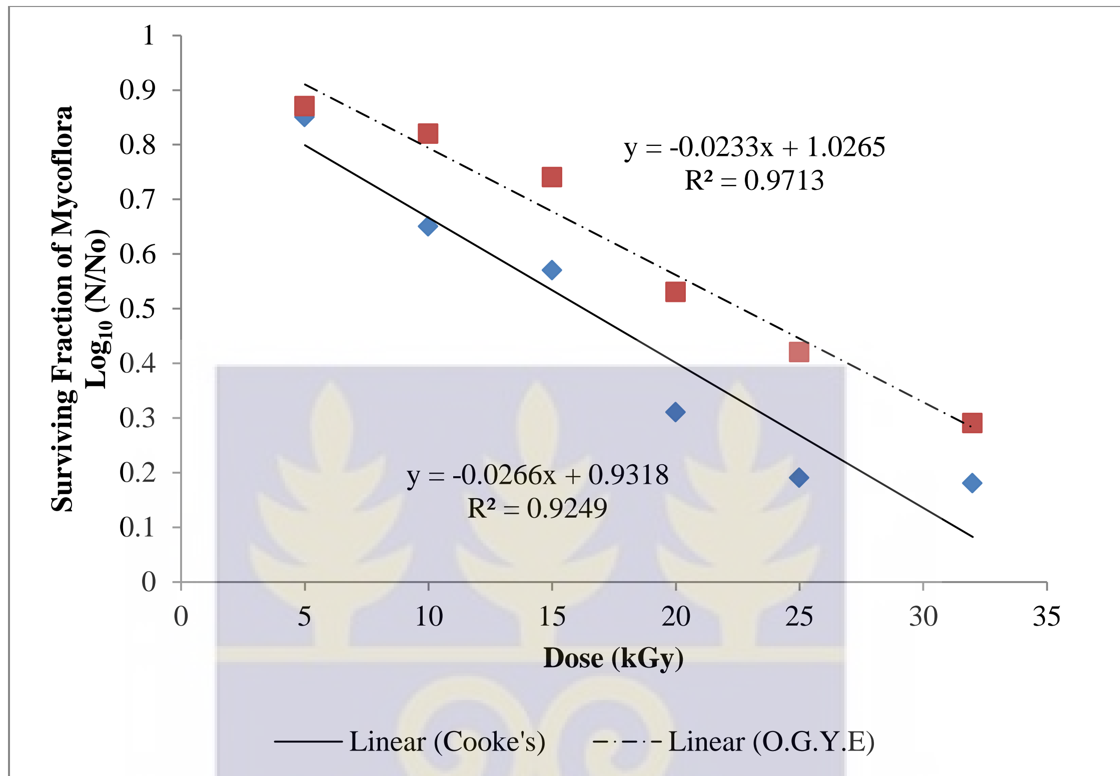

Fig.42. Radiation sensitivity curves for fungi in sawdust enumerated on OGYE and Cooke's media at 28-30 °C for 6 days

Table 9. Mean D_{10} values of fungi associated with sawdust isolated on the two indicated growth media.

Substrate	Regression equation (y)	R^2	D_{10} value (kGy)
(a) Cooke's medium	-0.026x + 0.931	0.924	5.64 ± 1.12
(b) OGYE	-0.023x + 1.026	0.971	5.57 ± 2.06

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EXPERIMENT 6

STUDIES ON THE MYCELIAL GROWTH ON THE VARIOUSLY- TREATED COMPOSTED 'WAWA' SAWDUST

(a) Mycelial growth and colonization time

The spawn run period is calculated as the number of days from inoculation to complete colonization of the compost bag by the mycelium (Obodai and Vowotor, 2002). The various substrate formulations are shown in Table 10. The substrate interactions had different nutrient composition and resulted in different growth responses shown in Table 10.

On the average, observable whitish mycelium began to grow after three (3) days on the bagged substrates from the grain of inocula. The mycelia grew down the substrates with time until complete colonization within an average of 4 weeks.

The linear growth of *P. ostreatus* mycelia down the substrates was similar for all the growing periods. There was no significant ($p>0.05$) difference between the different treatment permutations of the 5, 10, 15, 20, 25 and 32 kGy with a final average linear growth of 190.2- 248.2mm (Table 10). Substrate treatment combination of the unirradiated (0 kGy) and non-steam sterilized substrates supported the poorest linear growth of 0.7-1.7cm (Table 10). The differences observed were statistically significant ($p<0.05$).

Colonization time

The time taken to fully colonize the substrates and their rates of growth are presented in Table 10. The shortest period of colonization was 22 days by the (I+I) combination irradiated with 15 kGy while the longest time of colonization, 35 days was obtained on

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the (A+S) treatment irradiated with 24 kGy (Table 10). The (I+S) combination treated with 15 kGy gave the fastest rate of growth 28.3 mm/day while the slowest rate of growth 0.7mm/day was obtained by the (A+nI) combination which was unirradiated (Table 10).

(b) Primordia emergence

Time of primordial emergence is related to the vigour of the mycelium. A weak mycelium results in delay time of the primordia emergence. The period varied from non-emergence (0 kGy combination) to 24- 37days (Table 10)

(c) Percentage contamination and mycelia density

There was a direct relationship between substrate contamination and mycelia density on the substrate. For example, in the untreated combinations (0 kGy), contamination ranged from 83.8%, (I+nS) to 100% (A+nS; A+nI). In these instances, total linear mycelia growth was low 12-15 mm; mycelia density was zero and primordia did not form at all (Table 10). Generally, all the various substrate treatment combinations irradiated with 5, 10, 15, 20, 24 and 32 kGy of gamma irradiation resulted in good mycelia density which eventually produced primordial and mushrooms (Plates 9 and 10). This contrasts with unirradiated combination of substrate treatments which were the most contaminated resulting in no primordial formation and mushrooms yield (Table 10) (Plate 9).

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Table 10: Effect of the various indicated combination of gamma irradiation and sterilization on rate of mycelia growth, mycelia colonization time, period of appearance of primordia, mycelia density and contamination rate at 28-30 °C for 28 days

Dose (kGy) applied	Treatment	Rate of mycelia Growth (mm/day)	Mycelia Colonizing time (Days)	Time taken till appearance of primordia (Days)	No. of Contaminated bags (%)	Total Linear Mycelia Growth (mm)	Mycelia Density
0	(A + n S)	1.5 ^{a}	n.d	n.d	100	12.0 ^{ab}	_
	(I + n S)	1.7 ^{a}	n.d	n.d	83.3	13.0 ^{a}	_
	(A + n I)	0.7 ^{a}	n.d	n.d	100	12.0 ^{ab}	_
	(I + n I)	1.4 ^{a}	n.d	n.d	90	15.0 ^{b}	_
5	(A + S)	19.5 ^{b}	33 ^{d}	34 ^{d}	0	230.5 ^{c}	+ + + +
	(I + S)	21.1 ^{b}	30 ^{c}	33 ^{d}	8.3	214.6 ^{c}	+ + +
	(A + I)	20.5 ^{b}	31 ^{cd}	33 ^{d}	0	212.8 ^{c}	+ + + +
	(I + I)	18.5 ^{b}	33 ^{d}	35 ^{d}	0	239.9 ^{c}	+ + + +
10	(A + S)	17.5 ^{b}	32 ^{d}	33 ^{d}	20	190.2 ^{c}	+ +
	(I + S)	19.3 ^{b}	31 ^{cd}	32 ^{d}	0	203.8 ^{c}	+ + + +
	(A + I)	21.6 ^{c}	29 ^{c}	29 ^{bc}	0	220.1 ^{c}	+ + + +
	(I + I)	24.3 ^{d}	28 ^{c}	28 ^{b}	16.7	241.6 ^{c}	+ + +
15	(A + S)	24.0 ^{d}	31 ^{cd}	32 ^{d}	16.7	231.0 ^{c}	+ + +
	(I + S)	28.3 ^{e}	24 ^{a}	25 ^{a}	0	248.1 ^{c}	+ + + +
	(A+ I)	23.0 ^{c}	26 ^{b}	26 ^{b}	0	246.7 ^{c}	+ + + +
	(I + I)	23.1 ^{c}	22 ^{a}	24 ^{a}	0	247.8 ^{c}	+ + + +
20	(A + S)	23.7 ^{cd}	30 ^{c}	30 ^{c}	0	233.0 ^{c}	+ + + +
	(I + S)	24.1 ^{d}	31 ^{cd}	29 ^{bc}	16.5	248.6 ^{c}	+ + +
	(A + I)	23.4 ^{c}	26 ^{b}	28 ^{b}	0	248.8 ^{c}	+ + + +
	(I + I)	22.9 ^{c}	28 ^{b}	29 ^{bc}	0	246.6 ^{c}	+ + + +
24	(A+ S)	20.4 ^{b}	35 ^{e}	37 ^{e}	0	213.5 ^{c}	+ + + +
	(I+ S)	20.0 ^{b}	34 ^{e}	36 ^{e}	8.3	202.8 ^{c}	+ + +
	(A+ I)	22.6 ^{c}	32 ^{d}	36 ^{e}	0	236.7 ^{c}	+ + + +
	(I + I)	21.9 ^{b}	33 ^{d}	34 ^{d}	0	231.0 ^{c}	+ + + +
32	(A +S)	20.3 ^{b}	27 ^{b}	29 ^{b}	0	211.6 ^{c}	+ + + +
	(I + S)	21.5 ^{bc}	27 ^{b}	28 ^{b}	16.67	230.1 ^{c}	+ + +
	(A + I)	19.6 ^{a}	29 ^{c}	31 ^{c}	0	243.9 ^{c}	+ + + +
	(I + I)	19.1 ^{a}	31 ^{cd}	33 ^{d}	0	240.0 ^{c}	+ + + +

Means with same letters in a column are not significantly different (P>0.05)

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Key	Codes
+ + + + - Very thick mycelia	A+nS- autoclaved spawn+non steamed sawdust
+ + + - Thick mycelia	I+nS- irradiated spawn+non steamed sawdust
+ + - Poor mycelia growth	A+nI- autoclaved spawn+non irradiated sawdust
+ - Very poor mycelia growth	I+nI- irradiated spawn+non irradiated sawdust
- - No mycelia	A+S- autoclaved spawn+steamed sawdust
n.d – Not determined	I+S- irradiated spawn+steamed sawdust
	A+I-autoclaved spawn+irradiated sawdust
	I+I- irradiated spawn+irradiated sawdust

Plate 9. Mycelia growth on contaminated (left) and non contaminated (irradiated) sawdust (right). Note the line of growth and proliferation of mycelium on the right

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EXPERIMENT 7

EFFECT OF PRETREATMENTS (IRRADIATION AND STEAM STERILIZATION) ON MOISTURE, CELLULOSE, HEMICELLULOSE, LIGNIN AND SILICA CONTENT OF COMPOSTED SAWDUST

Effect of pretreatment on cellulose, hemicellulose, lignin, silica

Raw sawdust recorded values of 8.11%, 37.62%, and 16.85% for hemicelluloses, cellulose and lignin respectively. There was an observed increase in lignocelluloses contents after pretreatment. Application of low gamma radiation dosage of 5 kGy resulted in 10.89%, 39.24% and 17.56% for hemicelluloses, cellulose and lignin respectively. High dosage of 24 kGy also resulted in 11.26%, 40.15% and 19.81% for hemicelluloses, cellulose and lignin respectively. Lastly, steam recorded 8.24%, 39.33% and 19.78% for hemicelluloses, cellulose and lignin respectively (Table 11).

Table 11. Effect of pretreatments of sawdust on lignocelluloses content on Air Dry Basis

Sample	% Moisture	% D.M	% H.C (ADB)	% Cellulose (ADB)	% Lignin (ADB)	% Silica (ADB)
Steam	5.44	94.56	8.24	39.33	19.78	9.53
5 kGy	5.83	94.17	10.89	39.24	17.56	13.51
24 kGy	5.41	94.83	11.26	40.15	19.81	15.44
Raw	5.62	94.14	8.11	37.62	16.85	12.82

H.C- Hemicellulose D.M- Dry Matter

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EXPERIMENT 8

CHANGES IN SOME PHYSICAL CHARACTERISTICS DURING COMPOSTING OF 'WAWA' SAWDUST PRETREATED WITH STEAM AND GAMMA IRRADIATION BEFORE COMPOSTING

The initial temperature of the heap of sawdust compost was 32°C but increased 56-58 °C in 4-5 days.There was turning of the compost every 4 days throughout the 28 days of composting resulting in a near homogeneity in temperature ranging from 45-56 °C for the rest of the composting days. The pH of the sawdust ranged from pH 6.81-9.17 with a mean of pH 8.38; Moisture content varied from 58.2-65.8% in 28 days (Table 12) with a mean of 62.2±7.5%. During pretreatment with steam or gamma radiation, the moisture content changed to 62.3% and pH 7.45 with steam sterilization. On the other hand, moisture content varied from 62.7% and pH 8.83 to 61.8% and pH 8.33 at a dose of 32 kGy (Table 13).

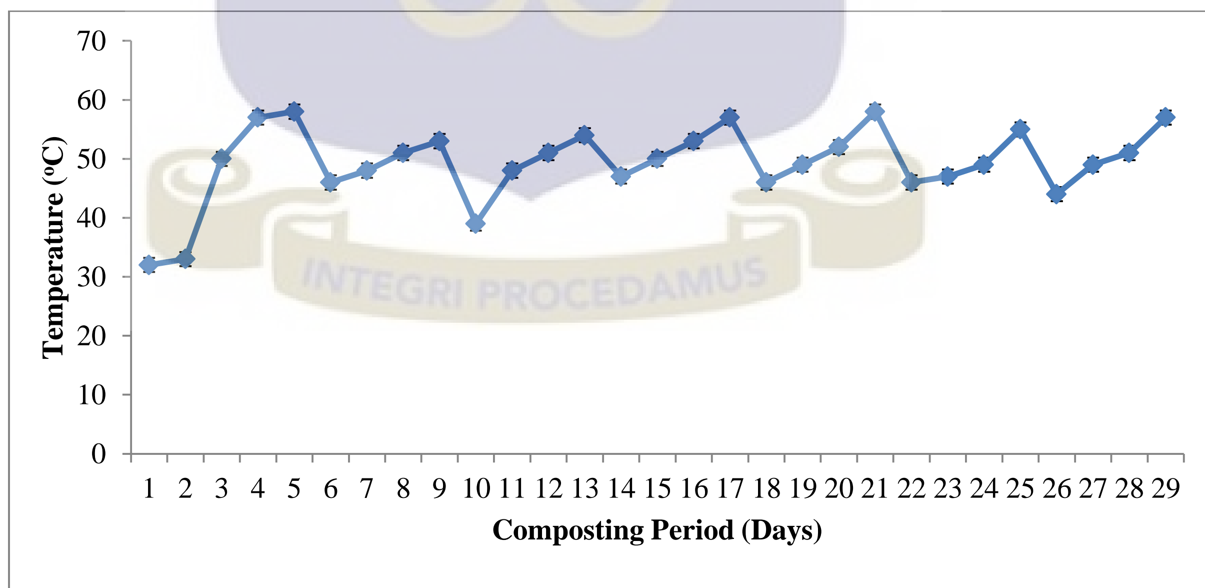

Fig.43. Temperature profile in the heap during composting of sawdust with 4 day turning interval over the period of 28 days

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Table 12: Moisture content and pH value of sawdust recorded during a 28 days composting period at varying time intervals

Composting Time(Days)	Moisture Content (%)	pH
0	65.1[b]	7.39[ab]
4	60.3[a]	8.54[b]
8	58.2[a]	6.81[a]
12	61.1[a]	8.60[b]
16	65.8[b]	8.75[b]
20	64.9[b]	9.17[c]
24	59.7[a]	8.93[b]
28	62.8[a]	8.85[b]

Means with same superscripts in a column are not significantly different (p>0.05)

Table 13: Effect of steam sterilization and gamma irradiation on the pH and moisture contents of composted sawdust (*T.sceroxylon*) for 28days

Pretreatment	Moisture Content (%)	pH
Steam	62.3[a]	7.45[a]
0 kGy	62.7[a]	8.83[b]
5 kGy	63.6[a]	8.13[b]
10 kGy	64.1[a]	8.29[b]
15 kGy	63.7[a]	8.39[b]
20 kGy	64.0[a]	8.08[b]
24 kGy	63.0[a]	8.36[b]
32 kGy	61.8[a]	8.33[b]

Means with same superscripts in a column are not significantly different (p>0.05)

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EXPERIMENT 9

YIELD AND YIELD ATTRIBUTES (TOTAL FRESH WEIGHT/ECONOMIC YIELD, NUMBER OF PRIMORDIA, TOTAL FRUIT BODIES, BIOLOGICAL EFFICIENCY, CAP AND STIPE DIMENSIONS AND MUSHROOM SIZE)

Table 14 shows the effect of various pretreatments of composted wawa sawdust on the yield attributes (Economic yield, Biological yield and Biological efficiency in relation to applied doses.

The fruit body is the fleshy edible part of fungi. Flushing (which is the amount of fruitbodies produced per batch) was observed for 8 weeks after incubation. The maximum weight of mushrooms produced per flush was 748g from (S+I) treatment with 24 kGy. The least number of mushrooms per flush 0g was from the (I+nS and S+nS) of the unirradiated 0 kGy set (Table 14). Generally, production of fruit bodies decreased with increasing flush numbers. There was a total of 4 flushes within 8 weeks of observation.

Total fresh weight/Economic yield

The total fresh weight of mushrooms or the Economic yield is the proportion of fresh mushrooms to the wet weight of the substrates recorded from four (4) flushes of cropping period of 4 weeks. There were statistically insignificant ($P>0.05$) variations in most instances in the total fresh weight or economic yield in the different permutations among the set of experiments (Fig.44). The maximum total fresh weight or economic yield was obtained on the steam sterilized sawdust compost (S+S) of the 32 kGy treated set of experiment (Table 14; Fig.44). The least total fresh weight of mushrooms yield of 0g was

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recorded on the permutations, steam sterilized spawn and non steam sterilized sawdust compost bag (S+nS) and irradiated spawn and non-steam-sterilized sawdust compost bag (I+nS) (Table 14) for each set of experimental dosage (0-32 kGy), there was no significant difference (P>0.05) between yields and the various permutations within the samples (Fig. 44).

Total number of primordial

There was significant variation (P<0.05) in the number of primordial formed. The maximum number of primordial 384 was recorded in the combination of irradiated spawn and irradiated compost bag (I+I) treated with 24 kGy set of combinations (Table 15). The surviving primordial eventually became fruit bodies.

Total number of fruit bodies

The maximum number of fruit bodies recorded was 379 by the irradiated spawn and irradiated compost bag (I+I) of the 32 kGy set (Table 15). On the other hand, no fruiting bodies were formed on the (S+nS) and (I+nS) control bags (Table 15). In the rest of the treatments the number of fruiting bodies formed were commensurate with the number of primordial formed ranging from 181 (I+I) 20 kGy to 379 (I+I) 32 kGy.

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Biological Efficiency; Biological yield

The biological yield which measures total fresh weight to the dry weight of substrate showed significant differences (P<0.05) with respect to the different combinations of treatments. The highest biological yield (0.99kg/kg of dry substrate) and biological efficiency (99.8%) were recorded on steam sterilized spawn and steam sterilized compost (S+S) of 32 kGy set. The lowest biological yield (0.0 kg/kg of dry substrate) and no

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flushes (0%) biological efficiency was recorded on the steam sterilized sawdust compost bag (I+nS).

Relationship between yield attributes and economic yield

There was a positive linear correlation (R^2=0.981) between economic yield and effective fruiting body formation (Fig.45). Plate 10 shows the fruiting bodies produced on different substrates used in cultivating *P.ostreatus*.

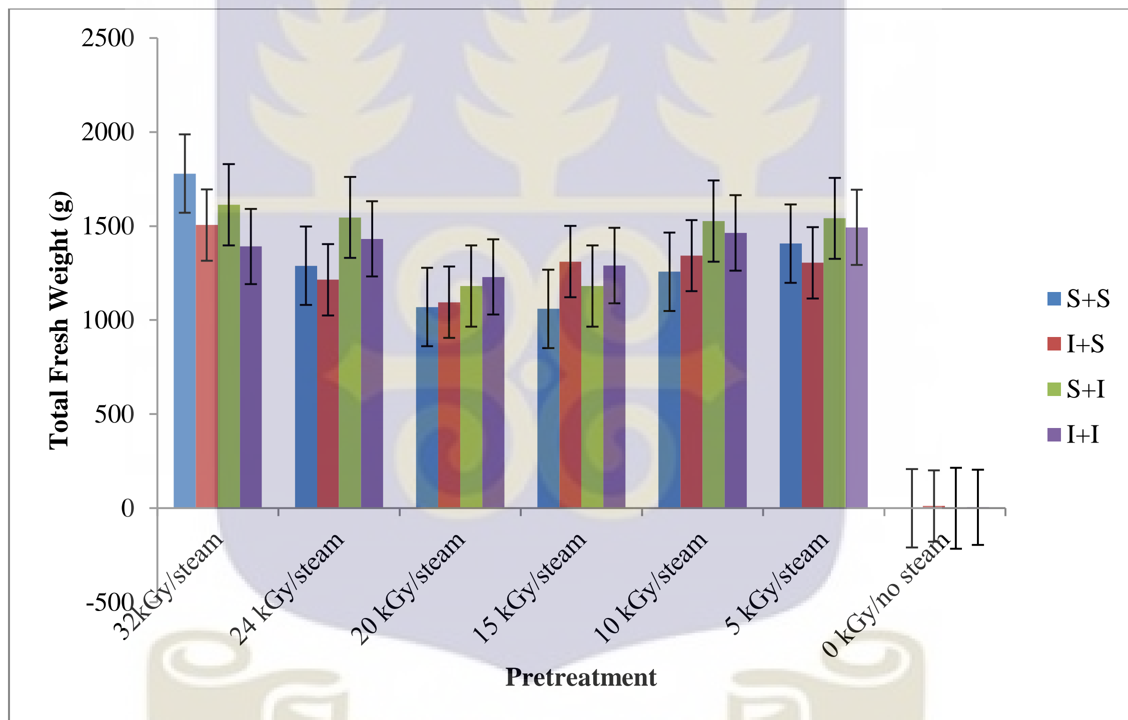

Fig.44: Effect of pretreatment (irradiation and steam) of sawdust on the yield of *P. ostreatus*

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Table 14: Effect of indicated pretreatments of composted sawdust on the yield of *P.ostreatus*

Substrate Code	Flush (g/kg wet sawdust)				Econ.Y (g/kg wet wt)	B.E (%)	Bio Yield (kg/kg dry wt)	Radiation Dose (kGy)
	1st	2nd	3rd	4th				
S + nS	-	-	-	-	-	-	-	0
I + nS	-	-	-	-	-	-	-	
S + nI	12	-	-	-	12 [a]	3.0	0.030 [a]	
I + nI	5.5	-	-	-	5.5 [a]	1.4	0.014 [a]	
S + S	352	450	355	250	1407 [d]	87.9	0.88 [d]	5
I + S	531	316	210	248	1305 [c]	81.6	0.82 [c]	
S + I	594	355	413	180	1541 [e]	96.3	0.96 [e]	
I + I	568	356	386	184	1493 [e]	93.3	0.93 [e]	
S + S	438	292.5	292.5	234	1257 [c]	78.9	0.79 [c]	10
I + S	452.5	402.5	289	199	1343 [c]	83.9	0.84 [cd]	
S + I	595.5	395.5	295.5	240	1527 [e]	95.4	0.95 [e]	
I + I	510.5	394	333	226	1464 [e]	91.5	0.92 [e]	
S + S	425	249	204	181.5	1060 [f]	66.3	0.66 [a]	15
I + S	539	309.5	257.5	205	1311 [c]	81.9	0.82 [c]	
S + I	443	330.5	244	163.5	1181 [b]	73.8	0.74 [b]	
I + I	472	323	310.5	184	1290 [c]	80.6	0.81 [c]	
S + S	472.5	246	205.5	145	1069 [f]	66.8	0.67 [a]	20
I + S	399	321	138.5	236	1095 [f]	68.4	0.68 [b]	
S + I	497	278.5	262.5	143	1181 [b]	73.8	0.74 [b]	
I + I	434.5	361	261	172.5	1229 [bc]	76.8	0.77 [c]	
S + S	468.5	474	243.5	103	1289 [c]	80.6	0.81 [c]	24
I + S	384	560	202.5	68	1215 [bc]	75.9	0.76 [c]	
S + I	748	484.5	131.5	182	1546 [e]	96.6	0.97 [e]	
I + I	653.5	237.5	332.5	209	1432 [de]	89.5	0.89 [e]	
S + S	664	487.5	426	201	1779 [g]	99.8	0.99 [e]	32
I + S	552	406	351	196.5	1506 [e]	94.1	0.94 [e]	
S + I	574	504	345	190	1613 [g]	99.8	0.99 [e]	
I + I	639	324	281.5	147.5	1392 [cd]	87.0	0.87 [d]	

N.B nI= nS Means with same letters in a column are not significantly different (P>0.05)

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Table 15: Effect of indicated pretreatment of composted sawdust (*T. scleroxylon*) on the fruiting pattern of *P.ostreatus*

Substrate Code	No.of primordia	No. of Fruit bodies	Average Stipe Length (mm)	Average Cap Diameter (mm)	Average Time b/n Flush Interval (Days)	Mush-room Size	Dose (kGy)
S + n S	4^{a}	-	-	-	n.d	-	0
I + n S	12^{a}	-	-	-	n.d	-	
S + n I	10^{a}	8^{a}	41^{ab}	44^{ab}	n.d	1.5^{b}	
I + n I	9^{a}	9^{a}	21^{a}	25^{a}	n.d	0.6^{a}	
S + S	258^{b}	216^{b}	57^{b}	60^{b}	9^{a}	6.5^{d}	5
I + S	254^{b}	210^{b}	56^{b}	59^{b}	10^{c}	6.2^{c}	
S + I	340^{d}	297^{c}	60^{c}	68^{bc}	10^{c}	5.2^{b}	
I + I	349^{d}	302^{c}	60^{c}	66^{b}	9^{a}	4.9^{c}	
S + S	286^{bc}	231^{b}	58^{b}	58^{b}	13^{e}	5.4^{b}	
I + S	280^{bc}	241^{bc}	59^{b}	60^{b}	13^{e}	5.6^{b}	
S + I	342^{d}	301^{c}	59^{b}	68^{bc}	12^{de}	5.1^{b}	
I + I	309^{cd}	256^{bc}	63^{c}	75^{c}	13^{e}	5.7^{b}	
S + S	259^{b}	259^{bc}	51^{b}	62^{b}	8^{ab}	4.1^{a}	15
I + S	272^{b}	198^{b}	53^{b}	64^{b}	9^{b}	6.6^{cd}	
S + I	307^{cd}	307^{c}	52^{b}	58^{b}	7^{a}	3.8^{a}	
I + I	288^{c}	214^{b}	56^{b}	60^{b}	9^{b}	6.0^{c}	
S + S	219^{b}	219^{b}	55^{b}	58^{b}	10^{c}	4.9^{b}	20
I + S	257^{b}	197^{b}	59^{b}	55^{ab}	11^{d}	5.6^{b}	
S + I	252^{b}	252^{bc}	57^{b}	57^{ab}	9^{b}	4.7^{a}	
I + I	229^{b}	181^{b}	57^{b}	56^{ab}	10^{c}	6.8^{cd}	
S + S	303^{cd}	223^{b}	50^{ab}	63^{b}	11^{d}	5.8^{d}	24
I + S	303^{cd}	320^{c}	67^{cd}	76^{c}	10.5^{d}	3.8^{a}	
S + I	359^{e}	285^{c}	52^{b}	73^{c}	14^{f}	5.4^{b}	
I + I	384^{e}	324^{d}	56^{b}	66^{b}	10.5^{d}	4.4^{a}	
S + S	382^{e}	305^{c}	51^{b}	73^{c}	12.5^{de}	5.8^{c}	32
I + S	336^{de}	275^{c}	55^{b}	73^{c}	15^{f}	5.5^{b}	
S + I	373^{e}	279^{c}	49^{ab}	72^{c}	13^{e}	4.3^{a}	
I + I	325^{d}	379^{e}	43^{ab}	50^{ab}	12.5^{de}	3.7^{a}	

N.B nI= nS Means with same letters in a column are not significantly different ($P>0.05$)

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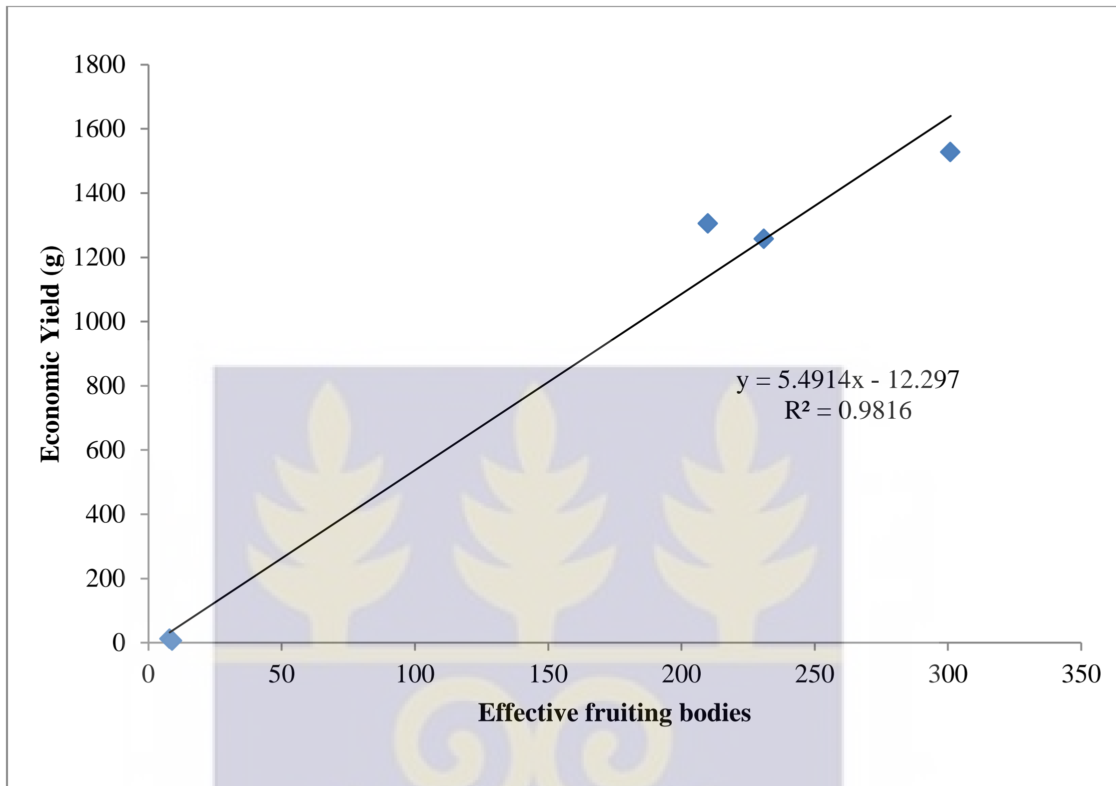

Fig.45: Functional relationship between effective fruiting bodies and economic yield

Plate 10. *P.ostreatus* fruiting bodies produced on differently treated substrate used in cultivation

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EXPERIMENT 10

EFFECT OF RADIATION TREATMENT ON FIVE SELECTED THIN LAYER DRYING CURVE MODELS FOR MUSHROOM DRYING RATES AND CALCULATION OF EFFECTIVE MOISTURE DIFFUSIVITY

During solar drying, humidity decreased from 48 to 19.8% and thereafter increased (Fig.46). Conversely, temperature continued to increase reaching peak at 59 to 60°C and thereafter declined to 47°C (Fig.46).

Drying curves showing the influence of radiation on drying rate of oyster mushrooms are presented in Fig.47. Irradiated slices of mushroom fruiting bodies dried faster than the control. The rate of moisture loss was directly proportional to increasing radiation dose (Fig.47). The drying curves also showed no constant water loss during the period of drying.

a) Non-linear regression modeling

Table 16 summarizes the results of the non-linear regression models using five thin layer drying models namely Lewis, Page, Henderson and Pabis, Diffussion and Wang and Singh. They were compared on the basis of their R^2, X^2 and RMSE values. All the five models gave a very good fit for their R^2 value (R^2>0.9). However Page and Difffussion Models best described the drying kinetics of the mushrooms under the different experimental conditions (Table 16). Drying kinetics of slices exposed to lower radiation doses (0.5-1.0 kGy) were similar to what was obtained in the control (Table 16). The Page Model (shown in bold) gave the highest R^2 value but the lower X^2 and RMSE (Table 16) and thus best suited its description.

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Interestingly, the Diffusion Model best predicted the drying behaviour of mushrooms treated with gamma irradiation between 1.0- 2.0 kGy (Table 16). The drying characteristics in terms of R^2, X^2 and RMSE (shown in bold figures) were different from the controls.

b) Experimental moisture ratios (MR)

Figs.48-52 show the moisture ratios of the experimental (observed) compared to the predicted values using the the Page Model in the case of samples treated with 0.5 and 1.0 kGy and Diffusion Models for samples treated with 1.5 and 2.0 kGy. These two models showed very good fit between the observed (experimental) and the predicted moisture ratios. This confirms the suitability of these models for describing drying of gamma irradiated oyster mushrooms.

Effective Moisture Diffusivity

The Effective Moisture diffusivity (D_{eff}) describes the rate of moisture movement in foods. D_{eff} values obtained varied from 1.88 x 10^{-08} m^2/s in the control to 2.44 x 10^{-08} m^2/s in the mushroom treated with 2.0 kGy of gamma radiation (Fig.53). The moisture diffusivity therefore increased with increasing dose of gamma radiation.

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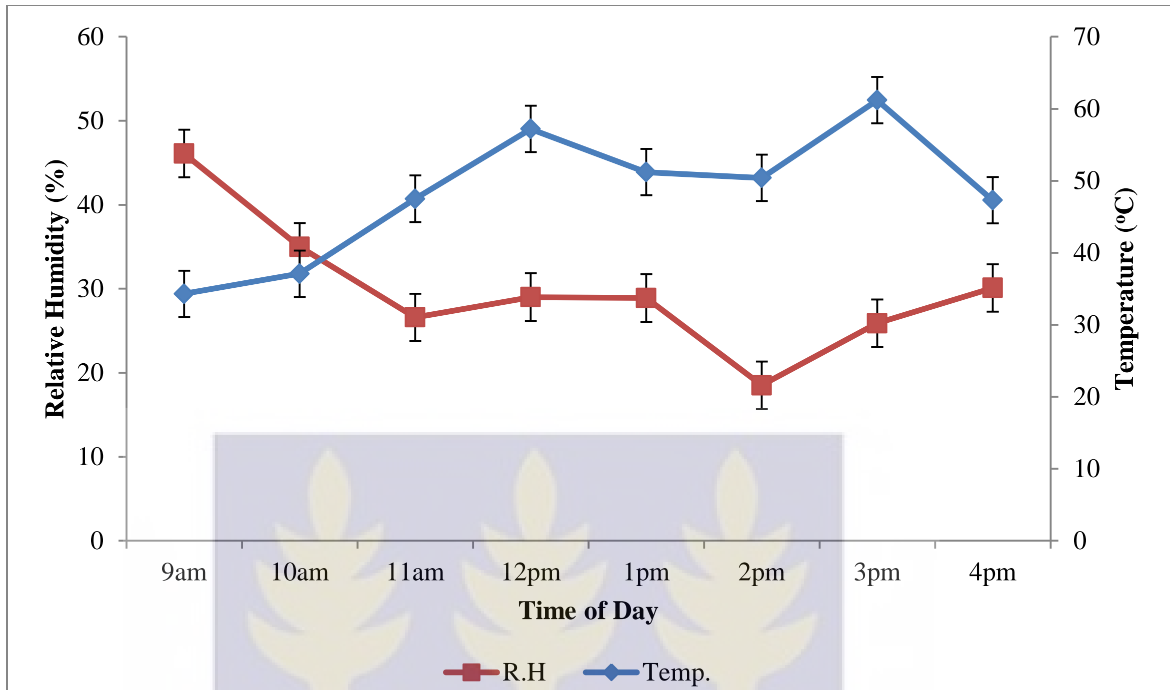

Fig.46. Variations of ambient air temperatures and relative humidity with time of day for solar drying of mushrooms (*P. ostreatus*)

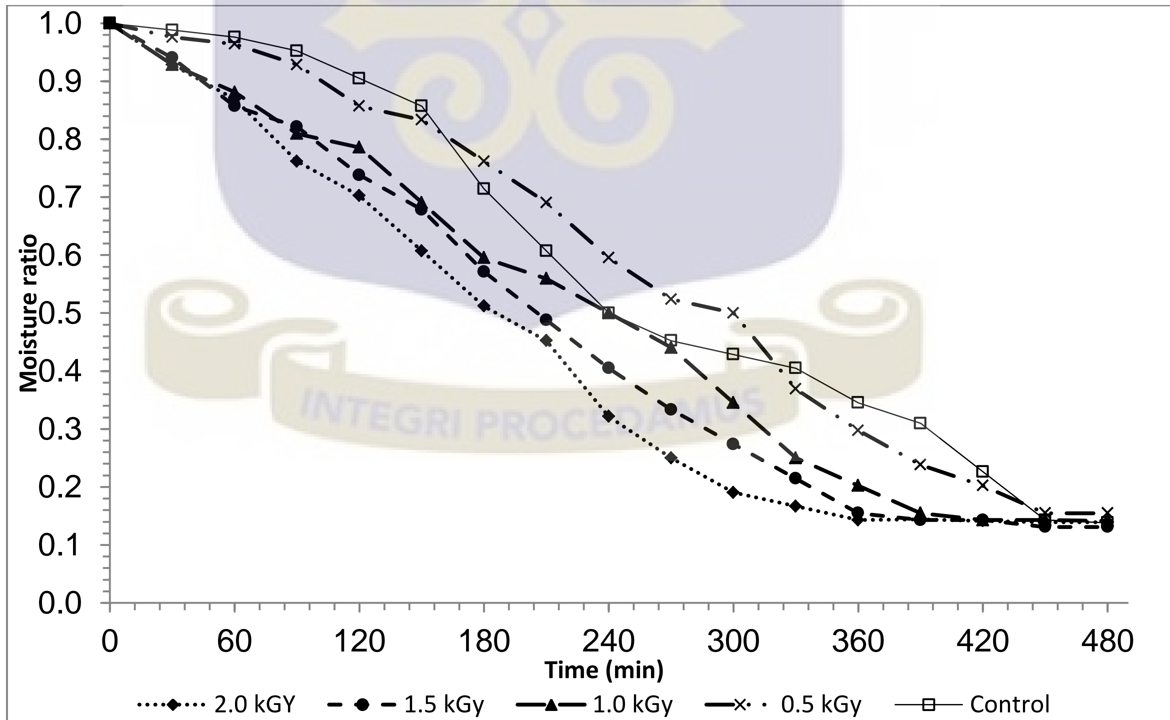

Fig.47. Influence of irradiation on drying rate of oyster mushrooms

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Table 16: Drying models and selection criteria for best fit for 2.0 kGy

Model	R^2	X^2	*RMSE*
0 kGy			
Lewis	0.9508	0.1287	0.0189
Page	***0.9878***	***0.0382***	***0.0014***
Henderson and Pabis	0.9304	0.0865	0.2755
Diffusion model	0.9862	0.0435	0.0020
Wang and Singh	0.9594	0.0693	0.0052
0.5 kGy			
Lewis	0.9446	0.1308	0.0195
Page	***0.9967***	***0.0184***	***0.0004***
Henderson and Pabis	0.9191	0.0932	0.0093
Diffusion model	0.9878	0.0389	0.0014
Wang and Singh	0.9771	0.0513	0.0028
1.0 kGy			
Lewis	0.9714	0.0982	0.0110
Page	***0.9925***	***0.0274***	***0.0008***
Henderson and Pabis	0.9191	0.0932	0.0093
Diffusion model	0.9891	0.0356	0.0012
Wang and Singh	0.9780	0.0518	0.0029
1.5 kGy			
Lewis	0.9781	0.0976	0.0109
Page	0.9938	0.0273	0.0007
Henderson and Pabis	0.9571	0.0669	0.0048
Diffusion model	***0.9938***	***0.0259***	***0.0007***
Wang and Singh	0.9731	0.0622	0.0041
2.0 kGy			
Lewis	0.9756	0.0935	0.0100
Page	0.9854	0.0403	0.0017
Henderson and Pabis	0.9667	0.0606	0.0039
Diffusion model	***0.9890***	***0.0367***	***0.0012***
Wang and Singh	0.9718	0.0709	0.0054

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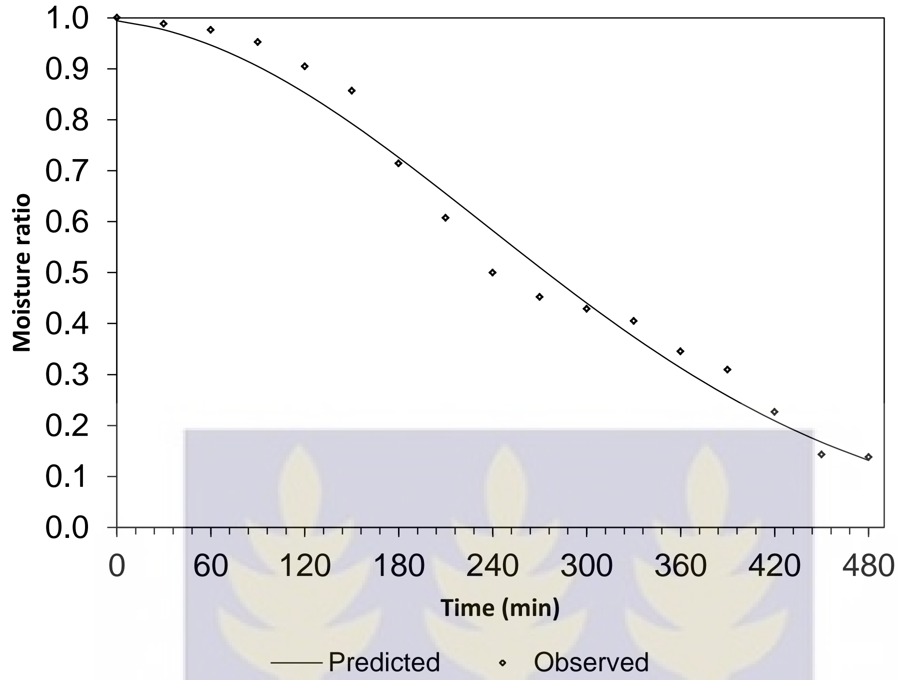

Fig. 48: Model fit for Control (0.0 kGy) using Page's Model

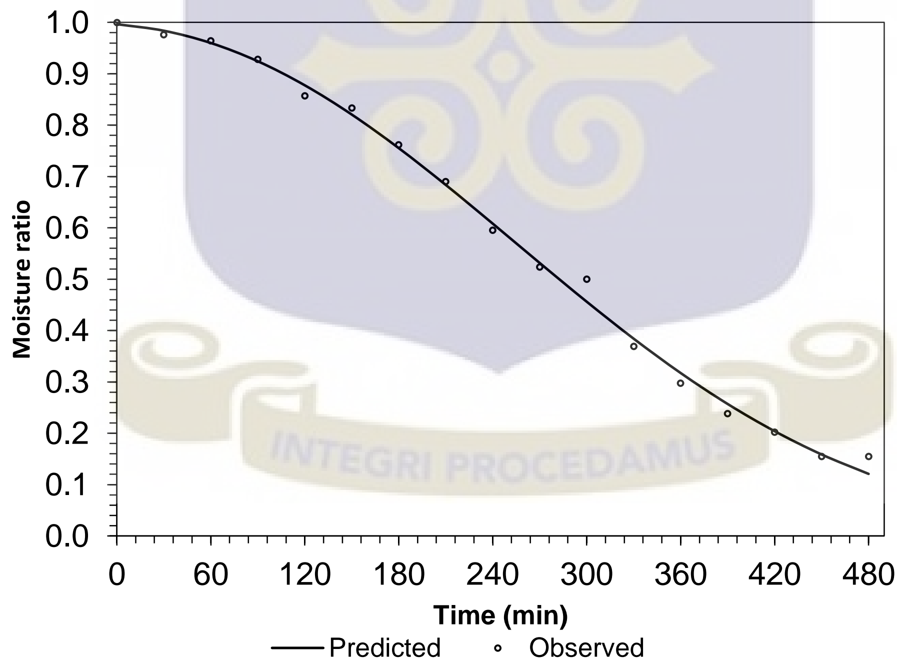

Fig.49: Model fit for mushroom exposed to 0.5kGy using Page's Model

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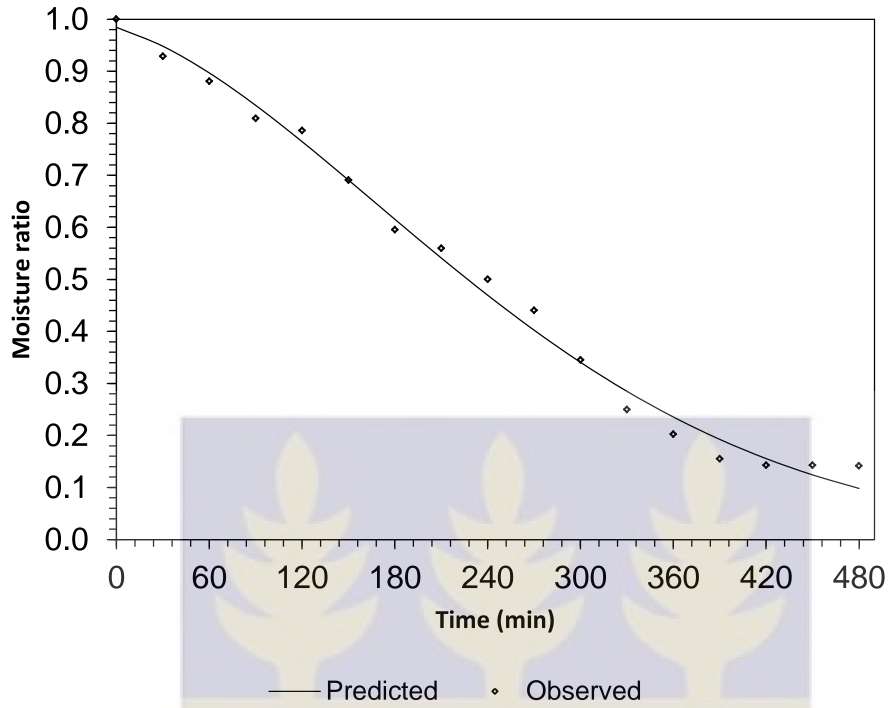

Fig.50: Model fit for mushroom exposed to 1.0 kGy using Page's Model

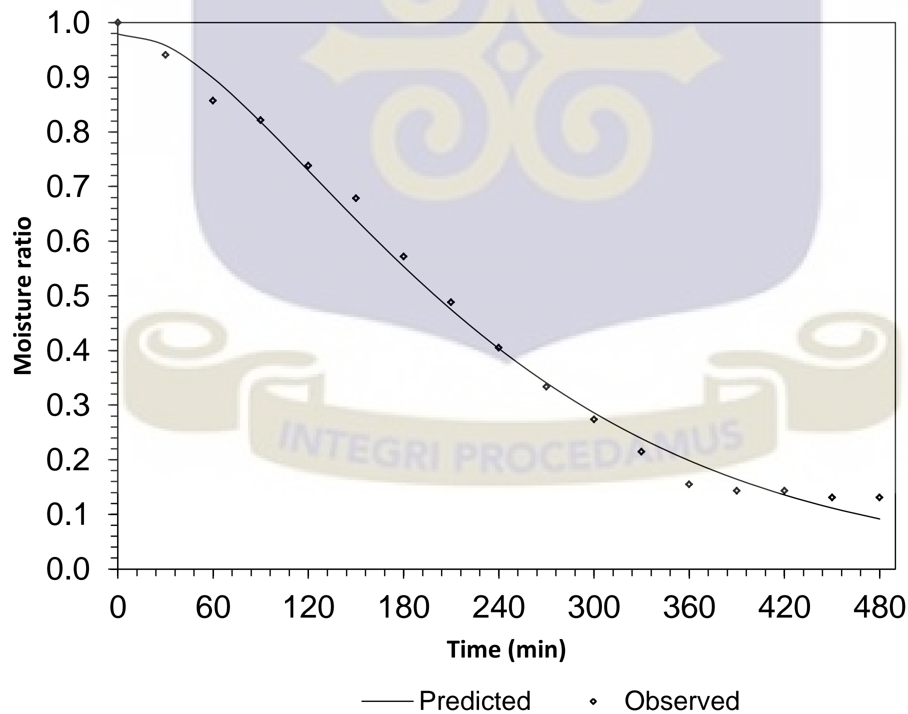

Fig.51: Model fit for mushrooms exposed to 1.5 kGy using Diffusion Model

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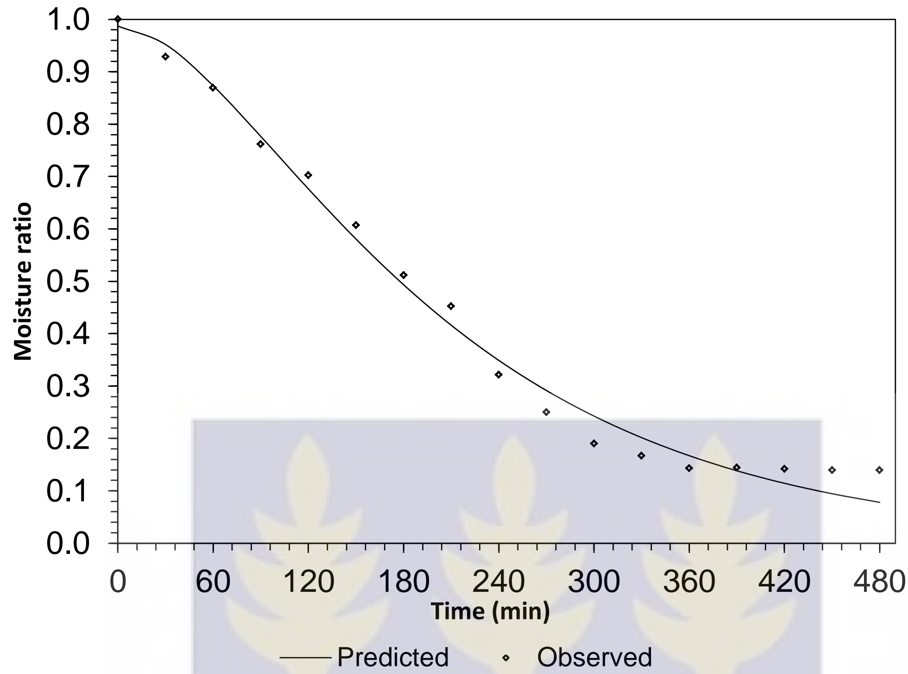

Fig. 52: Model fit for mushroom exposed to 2.0 kGy using Diffusion Model

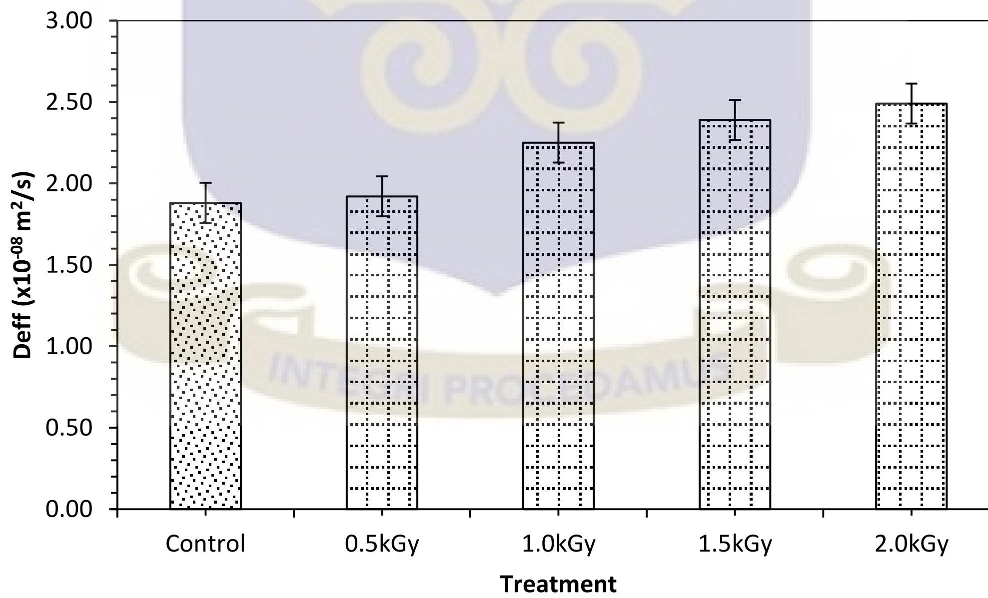

Fig.53: Effective moisture diffusivity of dried mushrooms

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EXPERIMENT 11

INFLUENCE OF GAMMA IRRADIATION ON THE COLOUR CHARACTERISTICS (L*,a*,b*) AND BROWNING INDEX (B.I) OF FRESH MUSHROOM STORED FOR DIFFERENT PERIODS (0-12 MONTHS) IN TWO PACKAGING MATERIALS

Freshly harvested oyster mushrooms treated with 0- 3 kGy of gamma irradiation recorded L*-value (whiteness) of irradiated fresh mushrooms ranging from 60.46- 61.30. The a*values (red/green) varied from 3.47- 3.91 and the b* values (yellow/blue) 18.13- 19.39 (Table 17). Statistical analysis showed that there was no significant difference (P>0.05) in the L*, a* and b* values initially after treatment with 0-3 kGy of gamma irradiation (Table 17).

The metric chroma (C) values varied from 18.53- 19.70; hue angle (H) ranged from 77.92- 79.85 and the browning index (B.I) registered between 35.3 and 41.2 (Table 17).

After five (5) days of storage, there was a general slight decrease in L*-values (whiteness) to 59.86- 61.18. There was an attendant increase in a* values while b* values decreased (Table 18) but showed no significant difference (P>0.05) with radiation dose.

There was a general slight decrease in metric chroma (C), the hue angle (H) range was within 90° and browning index (B.I) change was low indicating a slow rate of occurrence of enzymatic browning.

Table 19. shows that total change in luminosity (ΔL*) ranged from 0.07- 0.6. Total change in redness/greenness (Δa*-values) varied between -0.01 and 0.03 and change in yellowness/blueness (Δb*-values) ranged between 0.03 and 0.13; metric chroma ranged

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between 0.04 and 0.11. On the other hand, change in hue angle ranged -0.07- 0.29. The total colour change (ΔE) varied from 0.13- 0.61 (Table 19).

a) L*a*b* value and Browning index of dried mushrooms

The L*a*b* colour parameters and the Browning Indices of dried and irradiated mushrooms stored in either polythene or polypropylene pouches for up to 12 months are shown in Table 20.

b) Total changes in colour of dried and irradiated mushrooms during 12 months storage in two packaging materials

The overall colour changes in the irradiated mushrooms after 12 months storage in the packaging materials are presented in Table 21. The interaction of irradiation and type of packaging material showed a decrease in whiteness (ΔL*) values ranging from 1.6-4.1; Δb* values varied between -0.3 and 1.31 showing a shift from yellow to blue (Table 21). The Δa* values varied from -0.09 to -1.19 which connotes a change to green. The overall change in colour saturation (chroma) (ΔC) ranged from -0.26 to 0.85 which is considered statistically not significant ($P>0.05$).

The total colour difference ΔE which is a combination of L*a*b* values is a parameter extensively used to characterize the variation of colours depending on processing condition and packaging. ΔE values ranged from 1.59- 3.95. From the data, there seemed to be only marginal difference in the value of all parameters ΔE, ΔL*, Δa*, Δb* chroma and ΔE in both packaging materials.

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Table 17: Effect of irradiation on the initial colour of fresh mushrooms

Dose (kGy)	L-value	a*	b*	Metric Chroma	Hue Angle	B.I
0	60.46^{a}	3.47^{ab}	19.39^{a}	19.70^{a}	79.85^{bc}	41.2
1	60.77^{a}	3.91^{b}	18.48^{a}	18.89^{a}	78.05^{a}	35.3
2	60.91^{a}	3.89^{b}	18.17^{a}	18.58^{a}	77.92^{a}	35.3
3*	61.30^{a}	3.82^{b}	18.13^{a}	18.53^{a}	78.10^{a}	41.2

Means with same letters in a column are not significantly different (P> 0.05)

Table 18. Effect of irradiation on the colour of fresh mushrooms after 5 days storage

Dose (kGy)	L-value	a*	b*	Metric Chroma	Hue Angle	B.I
0	59.86^{a}	3.54^{a}	19.27^{a}	19.59^{a}	79.59^{ab}	41.2
1	60.70^{a}	3.98^{a}	18.35^{a}	18.78^{a}	77.76^{a}	35.3
2	60.72^{a}	3.90^{a}	18.09^{a}	18.51^{a}	77.83^{a}	35.3
3	61.18^{a}	3.79^{a}	18.10^{a}	18.49^{a}	78.17^{a}	41.2

Means with same letters in a column are not significantly different (P> 0.05)

Table 19: Colour change of fresh mushrooms due to effect of gamma radiation after 5 days of storage.

Dose (kGy)	Δ L*	Δ a*	Δ b*	Δ C	Δ Hue Angle	Δ E
0	0.60	-0.07	0.12	0.11	0.26	0.61
1	0.07	-0.07	0.13	0.11	0.29	0.13
2	0.19	-0.01	0.08	0.07	-5.08	0.21
3	0.12	0.03	0.03	0.04	-0.07	0.13

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Table 20: Effect of gamma radiation on the colour of dried mushrooms during 0- 3 months storage

Time	Package Material	Dose (kGy)	L*	a *	b *	Chroma	Hue Angle	B.I
0	Polythene	0	58.92^{c}	4.18^{a}	14.57^{e}	15.16^{e}	73.99^{d}	35.3
		0.5	56.66^{b}	4.64^{c}	12.65^{b}	13.47^{b}	69.86^{b}	29.4
		1	56.83^{b}	4.15^{a}	13.00^{c}	13.65^{c}	72.30^{d}	29.4
		1.5	58.09^{b}	4.17^{a}	13.82^{cd}	14.43^{cd}	73.20^{d}	29.4
		2	59.04^{c}	4.38^{b}	14.23^{d}	14.89^{e}	72.89^{d}	35.3
	Polypropy-lene	0	58.27^{bc}	4.64^{c}	14.22^{d}	14.96^{e}	71.93^{bc}	35.3
		0.5	53.29^{a}	4.88^{cd}	11.00^{a}	12.03^{a}	66.08^{a}	29.4
		1	58.64^{bc}	5.00^{d}	14.95^{e}	15.76^{f}	71.51^{bc}	35.3
		1.5	56.95^{b}	4.73^{c}	14.08^{d}	14.85^{e}	71.43^{bc}	35.3
		2	59.85^{c}	4.59^{c}	15.54^{ef}	16.20^{fg}	73.54^{d}	35.3
3	Polythene	0	56.93^{b}	4.19^{a}	14.58^{e}	15.17^{e}	73.97^{d}	35.3
		0.5	55.72^{b}	4.61^{c}	12.92^{c}	13.72^{c}	70.26^{b}	29.4
		1	55.40^{b}	4.19^{a}	12.88^{c}	13.54^{b}	71.98^{bc}	29.4
		1.5	57.03^{b}	4.17^{a}	13.82^{cd}	14.42^{cd}	73.20^{d}	35.3
		2	57.90^{b}	4.40^{b}	13.98^{d}	14.66^{e}	72.53^{d}	35.3
	Polyprop-ylene	0	56.37^{b}	4.68^{c}	14.52^{e}	15.26^{e}	72.14^{d}	35.3
		0.5	51.30^{a}	4.94^{d}	11.69^{ab}	12.69^{ab}	67.09^{a}	35.3
		1	57.29^{b}	5.30^{ed}	15.08^{e}	15.98^{f}	70.63^{b}	47.1
		1.5	56.41^{b}	4.79^{c}	14.24^{d}	15.02^{e}	71.40^{bc}	35.3
		2	58.28^{bc}	4.61^{c}	15.73^{ef}	16.39^{fg}	73.66^{d}	35.3

Means with same letters in a column are not significantly different (P> 0.05)

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Table 20 cont'd: Effect of irradiation on colour of dried mushrooms during 6-12 month storage

Time	Package Material	Dose (kGy)	L*	a *	b *	Chroma	Hue Angle	B.I
	Polythene	0	56.81^{b}	5.19^{d}	13.58^{cd}	14.54^{cd}	69.08^{b}	35.3
		0.5	54.69^{a}	5.61^{f}	12.92^{c}	14.09^{c}	66.53^{a}	35.3
		1	55.32^{b}	4.19^{a}	12.88^{c}	13.54^{b}	71.98^{bc}	29.4
		1.5	56.03^{b}	5.17^{d}	13.82^{cd}	14.76^{e}	69.48^{b}	35.3
		2	56.48^{b}	4.40^{b}	12.98^{c}	13.71^{c}	71.27^{bc}	29.4
6	Polyprop-ylene	0	55.16^{b}	5.68^{f}	14.52^{e}	15.59^{f}	68.63^{b}	35.3
		0.5	51.26^{a}	4.94^{d}	11.69^{ab}	12.69^{ab}	67.09^{b}	35.3
		1	56.19^{b}	5.30^{ed}	14.08^{d}	15.04^{e}	69.37^{b}	35.3
		1.5	56.28^{b}	5.79^{g}	13.24^{c}	14.45^{cd}	66.38^{a}	35.3
.		2	57.17^{b}	4.61^{c}	15.73^{ef}	16.39^{fg}	73.67^{d}	35.3
	Polythene	0	55.42^{b}	5.37^{ed}	13.26^{c}	14.31^{cd}	67.95^{b}	35.3
		0.5	53.19^{a}	5.68^{g}	12.52^{b}	13.73^{b}	65.59^{a}	47.1
		1	54.22^{a}	4.19^{a}	12.48^{b}	13.16^{b}	71.44^{bc}	29.4
		1.5	55.09^{b}	5.17^{d}	13.52^{cd}	14.47^{cd}	69.07^{b}	35.3
		2	55.40^{b}	5.40^{ed}	12.98^{c}	14.05^{c}	67.41^{b}	35.3
12	Polyprop-ylene	0	54.16^{a}	5.68^{f}	14.52^{e}	15.59^{f}	68.63^{b}	35.3
		0.5	51.26^{ab}	5.94^{gh}	11.69^{ab}	13.11^{b}	63.06^{a}	35.3
		1	55.19^{b}	5.80^{g}	14.08^{d}	15.23^{e}	67.61^{b}	35.3
		1.5	55.33^{b}	5.59^{f}	13.24^{c}	14.37^{cd}	67.11^{b}	35.3
		2	56.70^{b}	5.18^{d}	14.73^{e}	15.61^{f}	70.63^{b}	29.4

Means with same letters in a column are not significantly different (P> 0.05)

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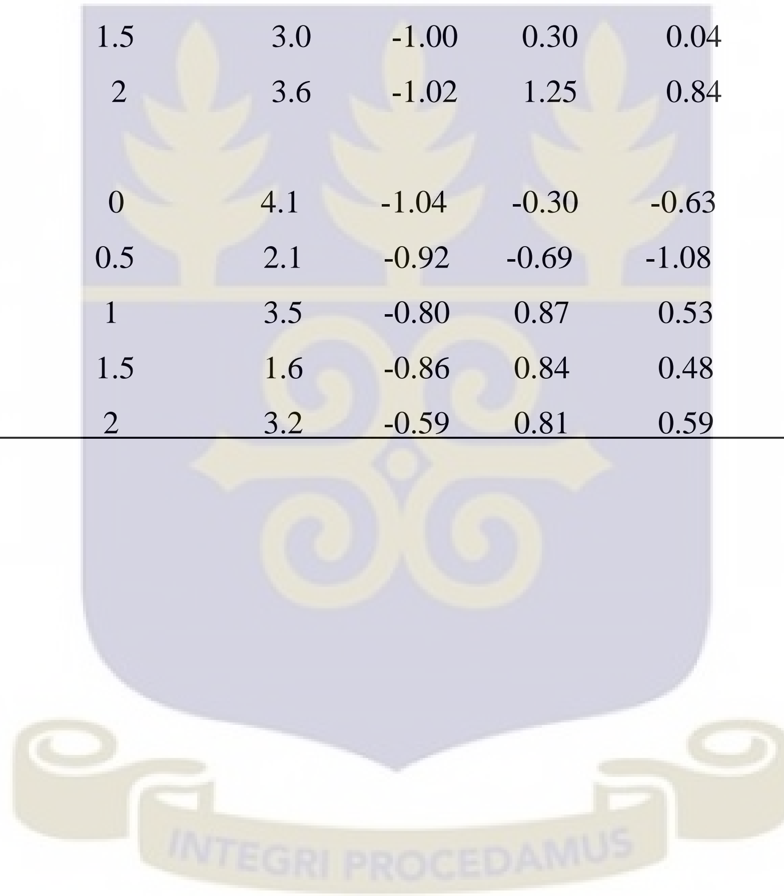
Table 21: Total change of colour of dried mushrooms due to irradiation during storage in the indicated packaging material for 12 months .

Package	Dose(kGy)	Δ L*	Δa*	Δ b*	Δ Chroma	Δ Hue	ΔE
Polythene	0	3.5	-1.19	1.31	0.85	6.04	3.54
	0.5	3.7	-1.04	0.13	-0.26	4.27	3.43
	1	2.6	-0.04	0.52	0.49	0.86	2.65
	1.5	3.0	-1.00	0.30	0.04	4.14	2.84
	2	3.6	-1.02	1.25	0.84	5.48	3.71
Polyprop-ylene	0	4.1	-1.04	-0.30	-0.63	3.30	3.95
	0.5	2.1	-0.92	-0.69	-1.08	3.02	1.75
	1	3.5	-0.80	0.87	0.53	3.90	3.52
	1.5	1.6	-0.86	0.84	0.48	4.32	1.59
	2	3.2	-0.59	0.81	0.59	2.91	3.20



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EXPERIMENT 12

DETERMINATION OF SOME SELECTED TEXTURAL CHARACTERISTICS OF THE NON-IRRADIATED AND IRRADIATED FRESH AND DRIED MUSHROOMS AND REHYDRATED DRY MUSHROOMS

The descriptions here are defined in the graphical representation (Fig.10) under materials and General Methods section. These represent textural attributes measured quantitatively by an instrument (Stable Micro Systems, UK— Model: TA-XT2i).

(a) The texture profiles of fresh and dried irradiated mushrooms treated (with 2 kGy) are shown in Fig. 54 (fresh mushrooms) and Fig. 55 (dried mushrooms). The maximum force applied at breaking point was 12N and 15N respectively. The moisture content of fresh, dried and rehydrated mushroom fruit body treated with varying doses (0-2 kGy) of gamma radiation is presented in Table 22. The fresh samples irradiated with 0-2 kGy had a moisture content varying from 80.8-83.7% whereas the dried samples had a moisture content ranging from 11.5-13.1% (Table 22). When the dried mushrooms were rehydrated, moisture content increased to between 42.3 and 49.7% in the irradiated samples.

Hardness of a sample which corresponds to the maximum force recorded during the first cycle of compression represents the force required between the molars for chewing food. It is a measure of the tensile strength of the samples. Generally tensile strength or hardness of the dry mushrooms were significantly ($P<0.05$) higher (63- 74 kgf) and there were no significant differences ($P>0.05$) between samples treated with 0- 1.5 kGy of gamma irradiation. At 2.0 kGy hardness of the dried mushrooms decreased significantly to 29 kgf (Fig.56).

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Fresh mushrooms recorded very low hardness values of 0.2- 0.4 kgf and rehydrated dried mushrooms also recorded similar low hardness values of 0.1- 0.2 kgf (Fig.56). The radiation treatment did not significantly change their hardness.

(b) Fracturability

Dried mushrooms irradiated with 0, 0.5 and 1.0 kGy of gamma irradiation required a force of 14.8- 15.9 N to fracture which did not differ significantly (P>0.05) from each other (Fig.57) At 1.5 and 2.0 kGy, the shearing force decreased to 12.9- 13.2 N. Fresh and rehydrated mushrooms require lower fracture forces of 0-1.7 N which decreased as radiation doses increased (Fig.57).

(c) Cohesiveness

Cohesiveness describes the ratio between the work done in the second compression and the work done in the first compression and reflects the ability of the product to stay intact or as one piece. The cohesiveness of dried mushrooms varied from 0.6-0.78 while that of rehydrated mushrooms increased to a value of 0.75- 0.86 (Fig.58). The differences observed with increasing doses were statistically significant (P<0.05). The cohesiveness of the fresh samples varied between 0.7 in the control to 0.65-0.80 in the sample treated with doses 0.5-2.0 kGy (Fig.58).

(d) Chewiness

This describes the energy required to disintegrate a solid material in order to swallow it. The dried mushrooms gave the highest chewiness at 0, 0.5, 1.0, 1.5 and 2.0 kGy application dose (Fig.59) ranging from 5N (at 0 kGy) to 16 N (1.0 kGy). The differences observed were statistically (P<0.05) significant. Fresh mushrooms showed a chewiness

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range of 0.1 N (1.5 kGy) to 2.0 N (doses 0, 1, 2 kGy). The same was true for rehydrated mushrooms (range of 0.2- 1.9 N). The differences observed between samples were statistically ($P<0.05$) significant (Fig.59).

(e) Springiness

This is the ratio between the times of two deformations and represents the ability to regain shape after the deformation stress is removed or reduced. Generally, dried mushroom untreated or irradiated recorded the lowest springiness except those treated with 1.5 kGy (Fig.60). Springiness values here varied from 0.23-0.31 mm. Fresh and rehydrated mushrooms recorded springiness of 0.12- 0.51 mm and 0.14- 0.50 mm respectively. The lowest value of springiness was recorded in the samples of fresh and rehydrated mushrooms irradiated with 1.5 kGy of gamma radiation (Fig.60). The differences observed in the fresh and rehydrated sample were not statistically significant ($P>0.05$).

(f) Gumminess

Gumminess is the Area 2/ Area 1 x Hardness (a product of hardness and cohesiveness). The gumminess of the dried unirradiated mushrooms and the radiation treated samples (0.5-2 kGy) was very high (30- 38). There was a commensurate decrease in gumminess of the dried mushroom with increasing gamma radiation dose (Fig.61). Gumminess of the fresh and rehydrated mushrooms was comparatively lower (1- 5) and did not change significantly ($P>0.05$) with increasing dosage of gamma irradiation.

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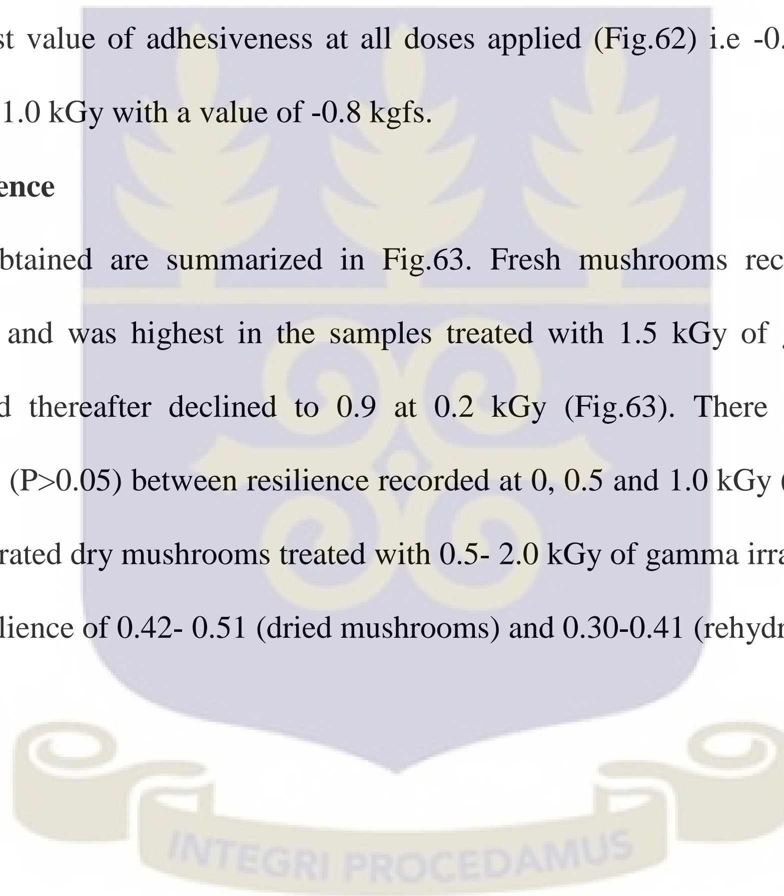
(g) Adhesiveness

Adhesiveness describes the work required to overcome the forces of attraction between sample and the probe surface. It is given by the value of the area corresponding to the negative force shown as A3 in Fig.10 (Materials and General methods).

Fig.62 shows the results obtained. Adhesivenesss was very low (-0.002 and -0.009) or almost negligible for dried and irradiated samples. The rehydrated mushrooms recorded the highest value of adhesiveness at all doses applied (Fig.62) i.e -0.2 to -0.8 kgfs but peaked at 1.0 kGy with a value of -0.8 kgfs.

(h) Resilience

Results obtained are summarized in Fig.63. Fresh mushrooms recorded the highest resilience and was highest in the samples treated with 1.5 kGy of gamma irradiation (1.71) and thereafter declined to 0.9 at 0.2 kGy (Fig.63). There was no statistical difference (P>0.05) between resilience recorded at 0, 0.5 and 1.0 kGy (0.85- 0.95). Dried and rehydrated dry mushrooms treated with 0.5- 2.0 kGy of gamma irradiation recorded a lower resilience of 0.42- 0.51 (dried mushrooms) and 0.30-0.41 (rehydrated mushrooms).



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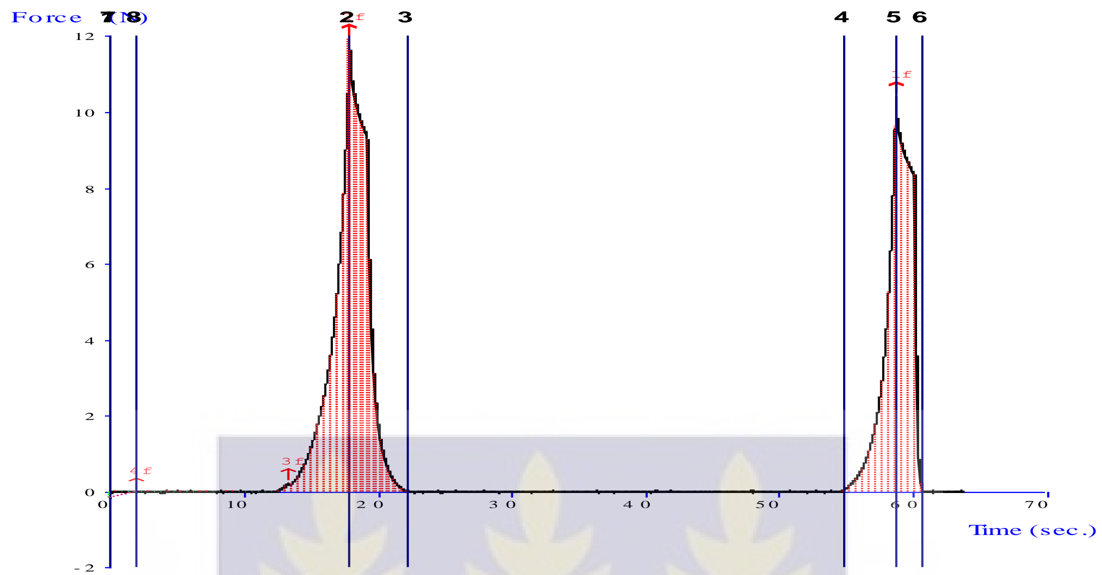

Fig.54: Texture profile analysis of fresh mushrooms irradiated at 2 kGy

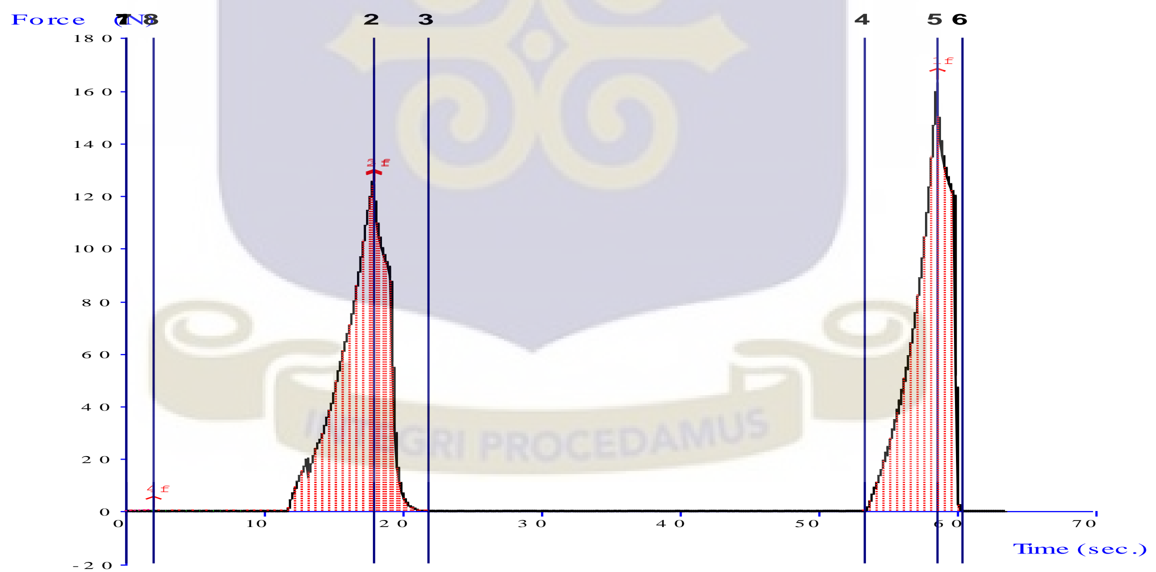

Fig.55. Texture profile analysis of dried mushrooms irradiated at 2 kGy

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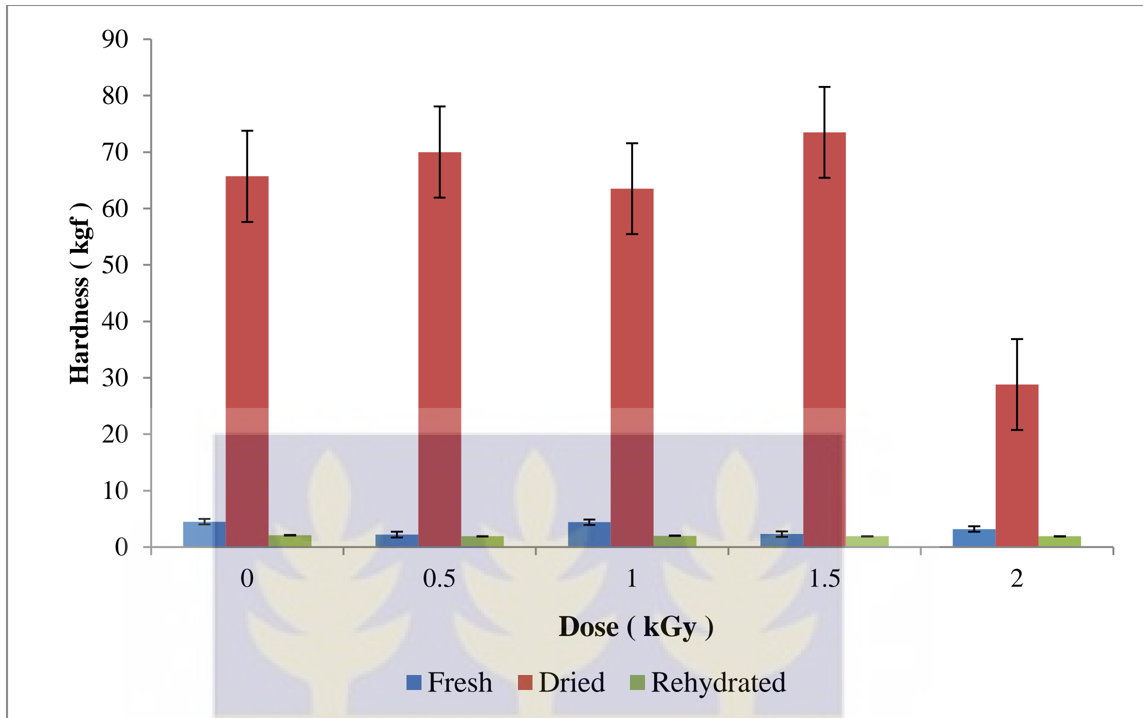

Fig.56. Influence of indicated doses of gamma radiation on hardness of fresh, dried and rehydrated mushrooms

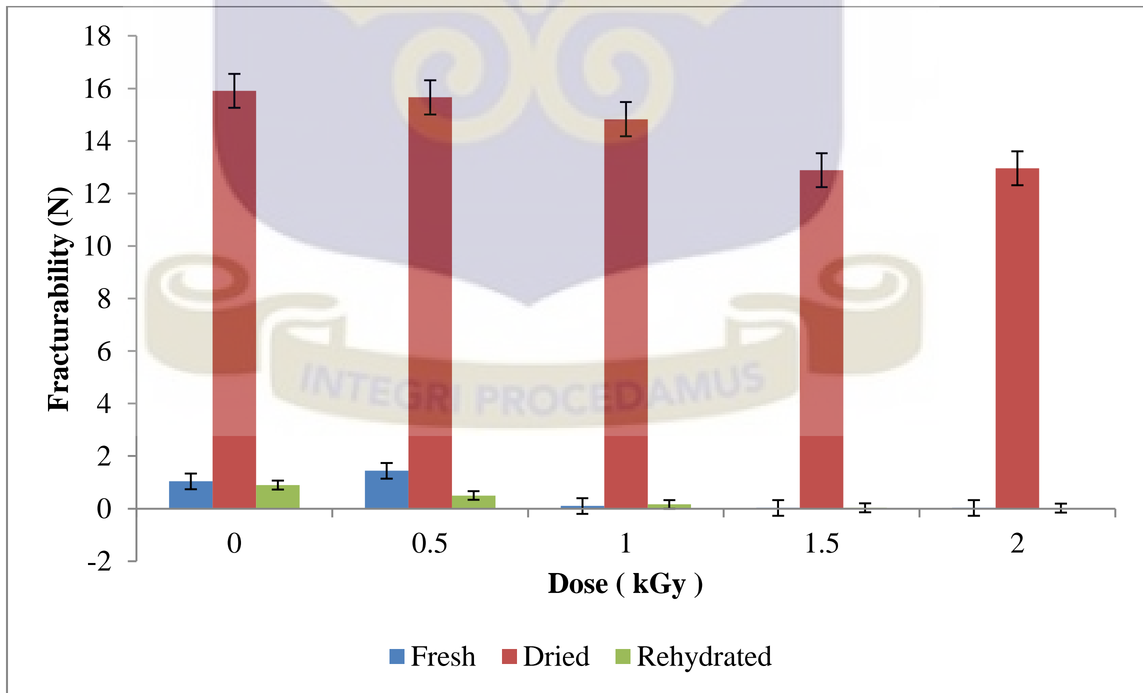

Fig.57: Influence of indicated doses of gamma radiation on fracturability of fresh, dried and rehydrated mushrooms

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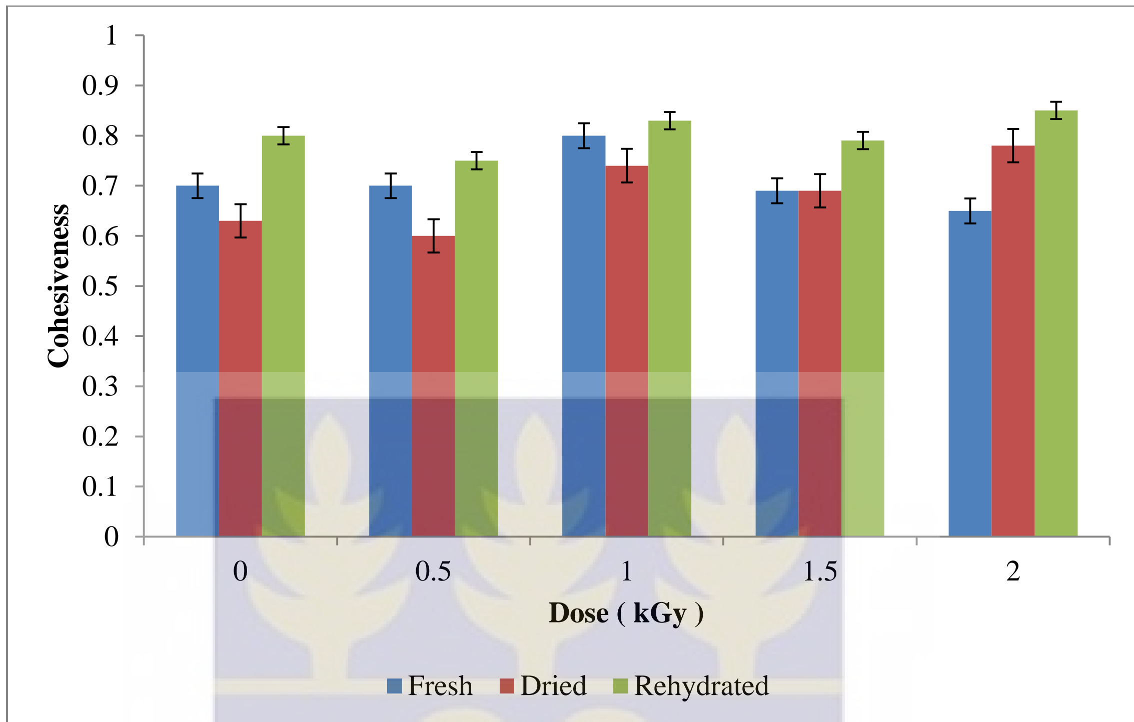

Fig.58: Influence of indicated doses of gamma radiation on cohesiveness of fresh, dried and rehydrated mushrooms

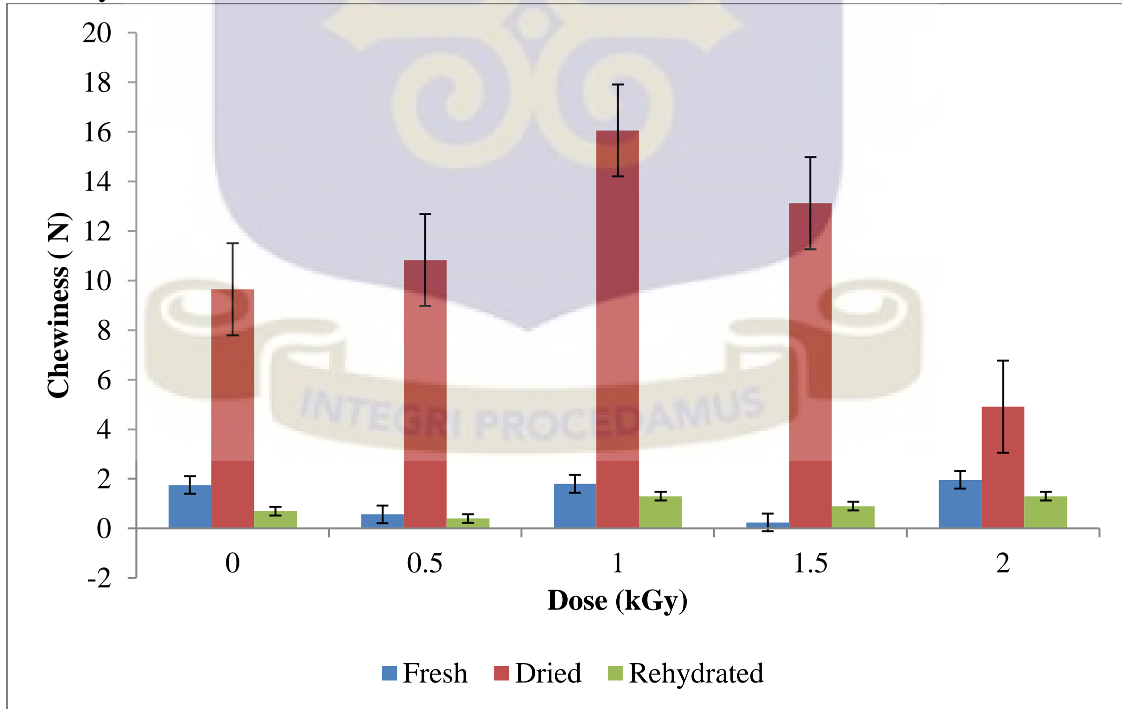

Fig.59: Influence of indicated doses of gamma radiation on chewiness of fresh, dried and rehydrated mushrooms

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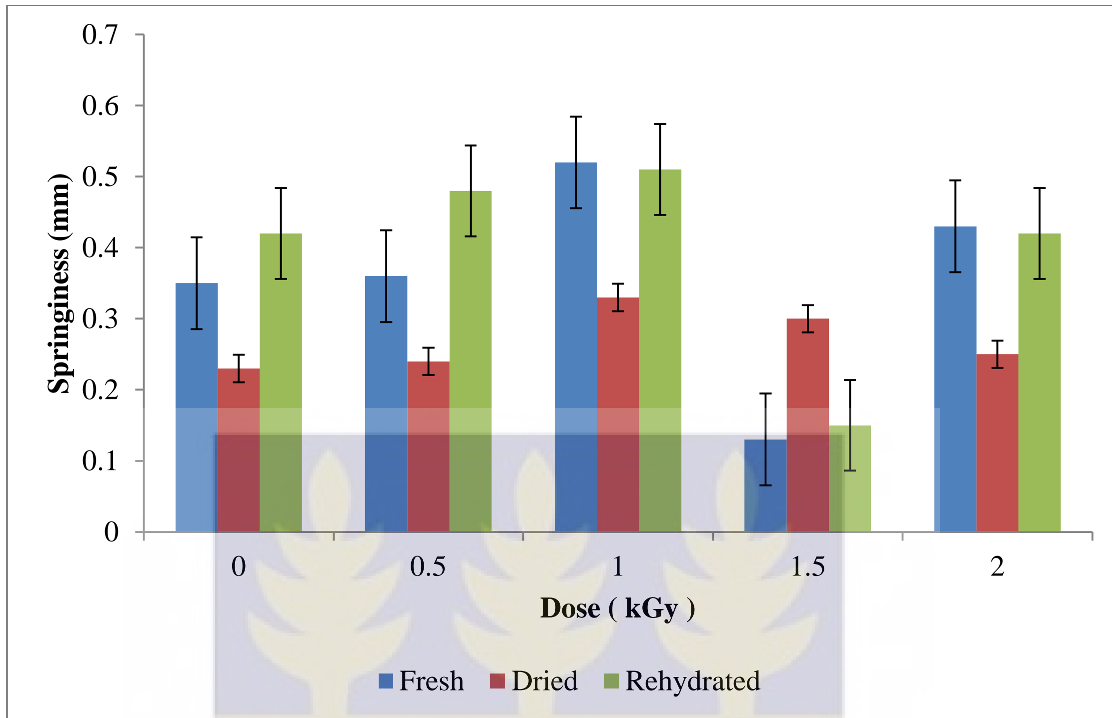

Fig.60: Influence of indicated doses of gamma radiation on springiness of fresh, dried and rehydrated mushrooms

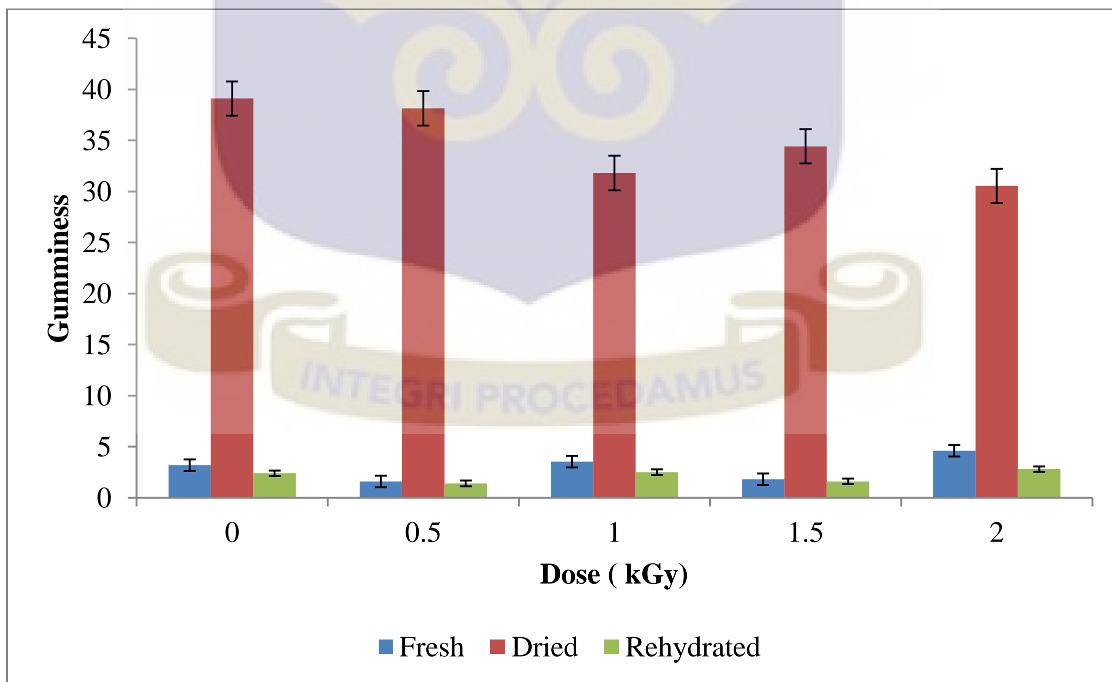

Fig.61: Influence of indicated doses of gamma radiation on gumminess of fresh, dried and rehydrated mushrooms

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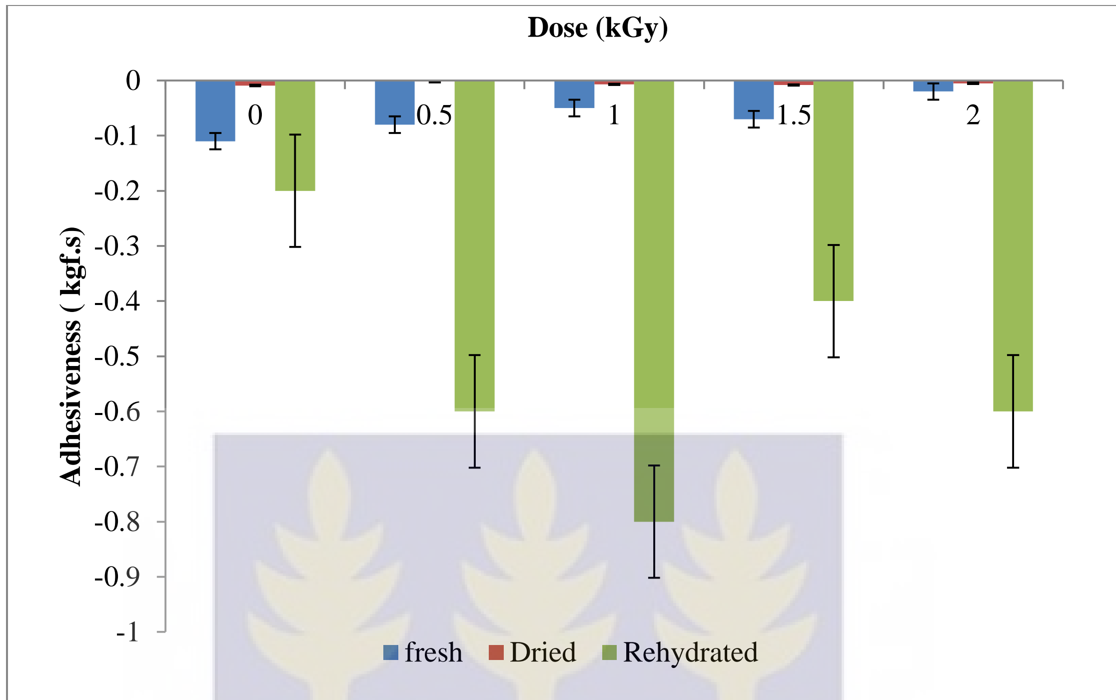

Fig.62: Influence of indicated doses of gamma radiation on the adhesiveness of fresh, dried and rehydrated mushrooms

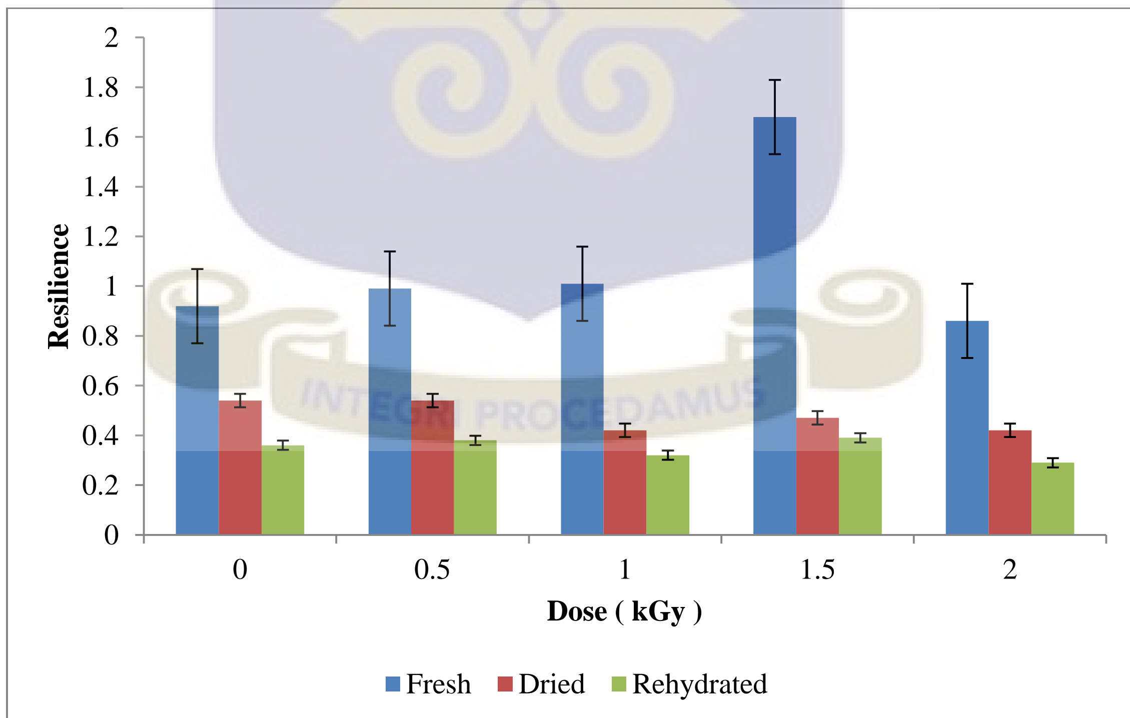

Fig.63: Influence of indicated doses of gamma radiation on the resilience of fresh, dried and rehydrated mushrooms

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The moisture content of fresh mushrooms ranged from 80.8 to 83.7%; dried mushrooms recorded 11.5-13.1% while rehydrated mushrooms varied from 42.3- 49.7% (Table 22).

Table 22. Mean moisture content (%) of fresh, dried and rehydrated irradiated mushrooms

Dose (kGy)	Moisture content (%)		
	Fresh	Dried	Rehydrated
0	80.8	12.4	43.2
0.5	81.3	11.5	45.6
1	83.7	12.5	48.6
1.5	81.5	13.1	42.3
2	80.9	12.8	49.7
S.D	1.3	0.4	1.1
Mean	81.6	12.5	45.9

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EXPERIMENT 13

MICROBIOLOGICAL ASSESSMENT OF FRESH AND DRIED IRRADIATED MUSHROOMS STORED IN TWO PACKAGING MATERIALS UP TO 12 MONTHS AT 28-30 ^{O}C

(a) Fresh mushrooms - Tables 23 and 24 show a summary of results obtained for samples stored in polypropylene and polythene packs respectively. The total aerobic mesophile count, *B.cereus* population and fungal counts for fresh mushrooms stored in polypropylene pack ranged from $1.5x10^4$ – $8.6x10^7$, $1.5x10^2$- $4.8x10^2$ and $8x10^1$- $9x10^4$ cfu/g respectively (Table 23). There was an average log reduction of 3.8, 0.5 and 3.1 respectively after exposure to gamma radiation. Total aerobic mesophile count, *B. cereus* and fungal counts for fresh mushrooms stored in polythene pack ranged $2x10^4$- $7.5x10^6$, $3.2x10^2$- $4x10^2$ and $1x10^2$- $5x10^4$ cfu/g (Table 24). Gamma radiation reduced these counts by 2.6, 0.10 and 0.7 log cycles respectively. Microbial counts increased after 5 days storage. High aerobic mesophilic counts may reflect poor handling. Interestingly, *Staphylococcus aureus*, Coliforms, *Salmonella spp*., and *E.coli* were not detected in the fresh samples. The non- irradiated (0 kGy) fresh mushrooms samples recorded lower moulds and yeasts counts in the range of 1.9- 4.95 log_{10} CFU/g (i.e $8.0x10^1$ – $9.0x10^4$ CFU/g) (Tables 23 and 24). Similar results were obtained in samples stored in polythene packs (Table 24)

(b) Dried mushrooms- Dried mushrooms stored in polypropylene packs recorded mean counts of total aerobic mesophiles, *B. cereus* population and fungal counts of $1.6x10^3$- $7.7x10^3$, $1x10^2$- $7x10^2$ and $3x10^1$- $2x10^3$ respectively (Table 25). The corresponding values of samples kept in polythene packs had the following mean counts: $1.67x10^3$-

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$6.3x10^4$, $2x10^2$-$5x10^3$ and $1x10^1$- $8x10^2$ for total aerobic mesophiles, *B.cereus* and fungal counts respectively (Tables 26). Data from this study show that there was a general modest increase in microbial counts over the storage period of 12 months. This slight increase was apparent after 6 months and 12 months. However, *S.aureus*, *Coliform*, *Salmonella* and *E.coli* were not detected in the dried samples as well (Tables 25 and 26) as after irradiation with 2 kGy of gamma radiation. No moulds were detected after irradiation.

Table 23: Effect of irradiation on the microbial load of fresh mushroom fruit bodies of *P.ostreatus* in polypropylene pack (P2) stored for a period of 0 days.

Time	Dose (kGy)	Aerobic Mesophiles	Coliforms cfu/g	*B. cereus* cfu/g	*S. aureus* cfu/g	Molds cfu/g	Yeasts cfu/g
0 Day	0	$8.6x10^7$	0	$4x10^2$	0	$2.6x10^1$	$9x10^4$
	1	$2.7x10^3$	0	$1x10^2$	0	0	$6x10^3$
	2	$1.5x10^4$	0	$1.5x10^2$	0	0	$8x10^1$
5 Day	0	$7.5x10^8$	0	$4x10^2$	0	$2.6x10^1$	$9x10^4$
	1	$3.0x10^3$	0	$3x10^2$	0	0	$6x10^3$
.	2	$2.0x10^4$	0	$5x10^1$	0	0	$8x10^1$

Table 24: Effect of irradiation on the microbial load of fresh mushroom fruit bodies of *P.ostreatus* in polythene pack (P1) stored for a period of 5 days.

Time	Dose (kGy)	Aerobic Mesophiles	Coliforms cfu/g	*B. cereus* cfu/g	*S. aureus* cfu/g	Molds cfu/g	Yeasts cfu/g
0 Day	0	$7.5x10^6$	0	$4.0x10^2$	0	$2.1x10^1$	$5x10^2$
	1	$3.0x10^4$	0	$3.0x10^2$	0	0	$1.0x10^4$
.	2	$2.0x10^4$	0	$3.2x10^2$	0	0	$1.0x10^2$
5 Day	0	$8.6x10^7$	0	$4.0x10^2$	0	$2.1x10^1$	$9x10^4$
	1	$2.7x10^5$	0	$1.0x10^2$	0	0	$6x10^3$
	2	$1.5x10^4$	0	$1.5x10^2$	0	0	$8x10^1$

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(B) EFFECT OF IRRADIATION ON MICROBIAL POPULATIONS OF DRIED MUSHROOMS BEFORE AND AFTER IRRADIATION AND STORAGE IN EITHER POLYPROPYLENE OR POLYTHENE PACKS FOR UP TO 12 MONTHS AT 28-30 ^{O}C

Table 25: Effect of irradiation on the microbial load of dried mushroom fruit bodies of *P.ostreatus* in polypropylene pack (P2) stored for a period of 12 months.

Time	Dose (kGy)	Aerobic Mesophiles	Coliforms cfu/g	*B. cereus* cfu/g	*S. aureus* cfu/g	Molds cfu/g	Yeasts cfu/g
0 Month	0	$7.7x10^3$	0	$7x10^2$	0	$1.7x10^1$	$1x10^2$
	0.5	$9.9x10^2$	0	$1x10^2$	0	0	$2x10^3$
	1.0	$4.9x10^2$	0	0	0	0	$3x10^2$
	1.5	$1.6x10^3$	0	$3x10^2$	0	0	$8x10^1$
	2.0	$3.8x10^2$	0	0	0	0	$3x10^1$
3 Month	0	$8.3x10^5$	0	$7x10^2$	0	$2.2x10^1$	$1x10^1$
	0.5	$9.9x10^2$	0	$1.0x10^2$	0	0	$2x10^3$
	1.0	$5.2x10^2$	0	0	0	0	$3x10^3$
	1.5	$3.1x10^3$	0	$3x10^2$	0	0	$8x10^1$
	2.0	$2.8x10^3$	0	0	0	0	$3x10^1$
6 Month	0	$7.3x10^5$	0	$5.0x10^3$	0	$2.2x10^1$	$1x10^1$
	0.5	$9.6x10^2$	0	$3.8x10^2$	0	0	$2x10^3$
	1.0	$4.9x10^2$	0	0	0	0	$3x10^1$
	1.5	$1.62x10^3$	0	$3x10^2$	0	0	$8x10^1$
	2.0	$3.8x10^2$	0	0	0	0	$3x10^2$
12Month	0	$7.7x10^4$	0	0	0	$1.7x10^1$	$1x10^1$
	0.5	$1.97x10^3$	0	$2x10^2$	0	0	$8x10^1$
	1.0	$1.77x10^3$	0	0	0	0	$5x10^1$
	1.5	$1.67x10^3$	$3x10^1$	$3x10^2$	0	0	$1x10^1$
	2.0	$2.08x10^3$	0	0	0	0	$7x10^1$

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Table 26: Effect of irradiation on the microbial load of dried mushroom fruit bodies of *P.ostreatus* in polythene pack (P1) stored for a period of 12 months.

Time	Dose (kGy)	Aerobic Mesophiles	Coliforms cfu/g	*B. cereus* cfu/g	*S. aureus* cfu/g	Molds cfu/g	Yeasts cfu/g
0 Month	0	$6.3x10^4$	0	$5x10^3$	0	0	$1.7x10^2$
	0.5	$1.97x10^3$	0	$2x10^2$	0	0	$8x10^2$
	1.0	$2.05x10^3$	0	0	0	0	$5x10^2$
	1.5	$1.67x10^3$	$3x10^1$	$3x10^2$	0	0	$1x10^1$
	2.0	$2.08x10^3$	0	0	0	0	$7x10^1$
3 Month	0	$7.8x10^4$	0	0	0	$1.7x10^1$	$1x10^1$
	0.5	$3.9x10^3$	0	$2x10^2$	0	0	$8x10^1$
	1.0	$4.27x10^2$	0	0	0	0	$5x10^1$
	1.5	$1.67x10^3$	$1.2x10^3$	$3x10^2$	0	0	$1x10^1$
	2.0	$2.08x10^3$	0	0	0	0	$7x10^1$
6 month	0	$7.7x10^5$	0	0	0	$4.2x10^3$	$1x10^1$
	0.5	$1.97x10^3$	0	$2x10^2$	0	0	$8x10^1$
	1.0	$3.8x10^2$	0	0	0	0	$5x10^1$
	1.5	$1.67x10^3$	$1.7x10^3$	$3x10^2$	0	0	$1x10^1$
	2.0	$2.1x10^3$	0	0	0	0	$7x10^1$
12 Month	0	$4.4x10^5$	0	0	0	$1.7x10^1$	$1x10^1$
	0.5	$1.97x10^3$	0	$2x10^2$	0	0	$8x10^1$
	1.0	$1.77x10^2$	$6x10^2$	0	0	0	$5x10^1$
	1.5	$3.5x10^3$	$3x10^1$	$3x10^2$	0	0	$1x10^1$
	2.0	$1.9x10^4$	0	0	0	0	$7x10^1$

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EXPERIMENT 14

RADIATION SENSITIVITY (D_{10} VALUE) DETERMINATION OF *BACILLUS CEREUS* RESIDENT IN FRESH AND DRIED MUSHROOMS

The killing effect of radiation treatment on microorganisms is generally expressed by the decimal reduction dose D_{10} values (Mohan *et al.*, 2010). Radiation sensitivity measured by the D_{10} values of *B.cereus* in fresh oyster mushrooms and stored in polypropylene and polythene packs were 3.21 ± 0.81 and 0.76 ± 0.04 kGy respectively (Table 27). Also, dried oyster mushrooms stored in polypropylene and polythene packs recorded corresponding D_{10} values of 2.40 ± 0.90 and 1.80 ± 0.85 kGy respectively. Mean D_{10} values of *Bacillus cereus* on both fresh and dried mushrooms were 1.98 and 2.10 kGy for polypropylene and polythene packs respectively. The differences observed were not statistically different (P>0.05). Figures 64 and 65 show the radiation sensitivity curves of *Bacillus cereus* in fresh and dried mushrooms from which the D_{10} values were derived.

Table 27: Mean D_{10} values of *Bacillus cereus* on fresh and dried oyster mushrooms in storage packages

Substrate	Regression equation	R^2	D_{10} value (kGy)
Fresh oyster mushrooms			
Polypropylene	y= -0.288x	0.358	3.21±0.81
Polythene	y= -0.064x	0.525	0.76±0.04
Dried oyster mushrooms			
Polypropylene	y= -0.318x	0.026	2.40±0.90
Polythene	y= -0.766x	0.576	1.80±0.85

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D_{10} values are means of 2 replicates ± S.E

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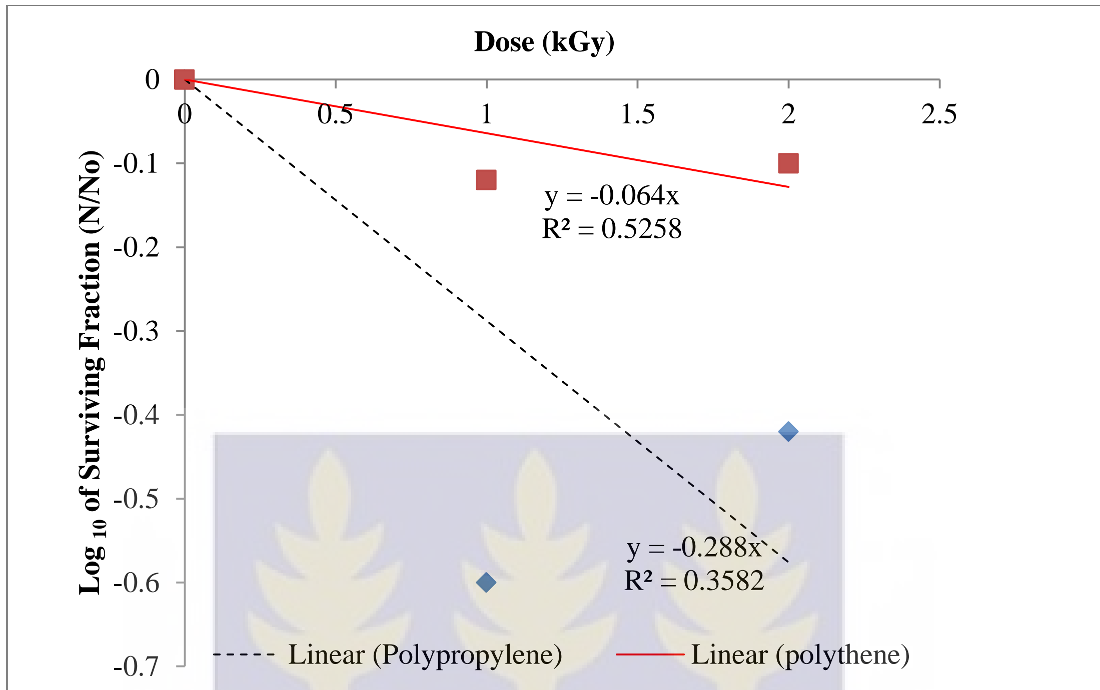

Fig.64: Radiation sensitivity curves for *Bacillus cereus* on fresh mushrooms

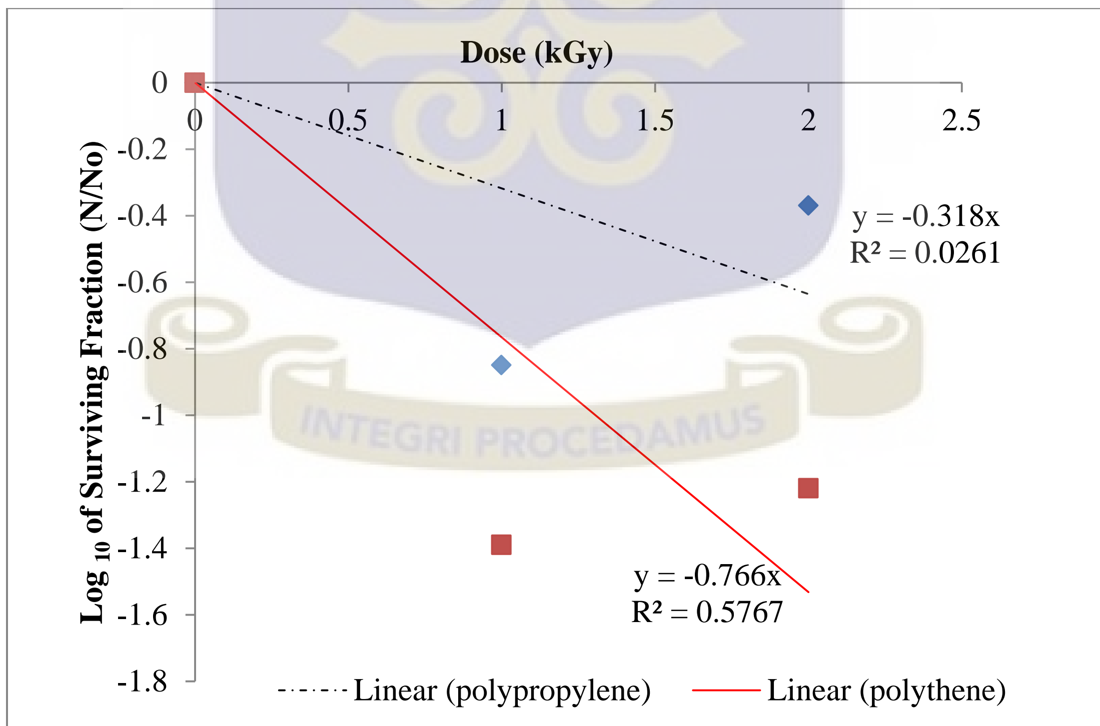

Fig.65: Radiation sensitivity curves for *Bacillus cereus* on dried mushrooms

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EXPERIMENT 15

RADIATION RESPONSE OF MEMBERS OF THE ENTEROBACTERIACEAE IN FRUITING BODIES OF *P.OSTREATUS* AND THEIR IDENTIFICATION USING API 20E SYSTEM

Members of the Enterobacteriaceae are generally facultative anaerobes, gram negative, non-spore formers. Most of these organisms are pathogenic, while others produce toxins responsible for food intoxication. Increasing doses of gamma radiation (0-5 kGy) caused commensurate decrease in species diversity. For example, bacterial species isolated from non-irradiated (0 kGy) mushroom fruit bodies stored in polypropylene packs were *Klebsiella pneumoniae, Citrobacter freundii, Proteus mirabilis, Serratia marscesens* and *Enterobacter spp*. After applying 1 kGy of gamma irradiation only *Klebsiella pneumoniae* remained and was also isolated from samples exposed to 5 kGy of gamma radiation (Table 28). The same trend was found for samples stored in polythene packs (Table 28). In the dried mushroom samples stored in either polypropylene or polythene pouches, only *Klebsiella pneumoniae* was persistent and was isolated in the bags treated with 5 kGy gamma irradiation. Plate 11 shows biochemical reactions between the kit reagent and some enterobacteria (*Klebsiella pneumoniae* and *Citrobacter freundii*).

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Table 28: List of members of the Enterobacteriaceae isolated from fresh and dry mushroom fruit bodies treated with 0-5 kGy of gamma irradiation and stored (12 months) in the indicated packaging materials.

Package	Applied Dose (kGy)	Microorganism (s)
Polypropylene	0	*Klebsiella pneumoniae, Citrobacter freundii* *Proteus mirabilis, Serratia marscesens*
	1	*Klebsiella pneumoniae, Citrobacter freundii*
	2	*Klebsiella pneumoniae*
	3	*Klebsiella pneumoniae, Enterobacter spp*
	4	*Klebsiella pneumoniae*
	5	*Klebsiella pneumoniae*
Polythene	0	*Pseudomonas aeroginosa,* *Klebsiella pneumoniae, Enterobacter spp.*
	1	*Klebsiella pneumoniae, Pseudomonas aeroginosa*
	2	*Klebsiella pneumoniae*
	3	*Klebsiella pneumoniae*
	4	*Klebsiella pneumoniae*
	5	*Klebsiella pneumoniae*

Plate 11: Complete biochemical reactions of *Klebsiella pneumonia* (Top) and *Citrobacter freundii* (Bottom) on API 20E kit

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EXPERIMENT 16

MYCOFLORA POPULATION ON DRY FRUIT BODIES OF *P. OSTREATUS* STORED IN EITHER POLYTHENE OR POLYPROPYLENE PACKS BEFORE AND AFTER GAMMA IRRADIATION FOR UP TO 12 MONTHS

(a) Mycoflora population

Results of the pre and post irradiation mycoflora of dried mushrooms kept in two packaging materials are presented in Figs. 66 and 67 (on DRBC) and Figs. 68 and 69 (Cooke's medium). Generally there was an average of 2.2 log cycles decrease in fungal population of non irradiated dry mushrooms by 5 kGy dose in most instances (Figs.66-69). In the non- irradiated samples there were significant differences (P<0.05) between the radiation treatments (1-5 kGy). The same trend was obtained on raising the fungi on Cooke's medium. Although there were increases in fungal population during storage in polythene and polypropylene bags for 3-12 months, the population counts were still low and within the acceptable limits counts (0.86-1.8 log_{10} CFU/g) especially in samples treated with radiation doses 2-5 kGy. Similar trends were recorded for spores incubated on Cooke's medium (Fig.69).

(b) Percentage occurrence of fungal species in the irradiated dried mushrooms

The phenology of the resident fungi in samples stored in polythene and polypropylene packs after radiation (0-5 kGy) for up to 12 months are presented in Figs. 70-73 (Cooke's medium) and Figs.74-77 (DRBC). The pooled data of fungi in the two media showed that 9 fungal genera *Aspergillus* (*A. niger, A. flavus, A. fumigatus, A. tamarii*), *Rhizopus* (*R. oligosporus*), *Mucor* (*M. racemosus*), *Fusarium* (*F. oxysporum*), *Penicillium* (*Penicillium*

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sp.), *Trichoderma* (*viride*) and *Rhodotorula sp*. were recorded in the packaging packs after irradiation. *Aspergillus* species predominated over the other species encountered. Notable were two potential toxin-producing *A. flavus* and *A. fumigatus* and *Penicillium sp*. Species like *A. flavus*, *A. fumigatus* and *Rhodotorula sp*. persisted on the fruit bodies from an initial >30% but increased to <50% in 12 months. The differences observed between the mycoflora of mushrooms stored in the two packaging materials were not statistically significant.

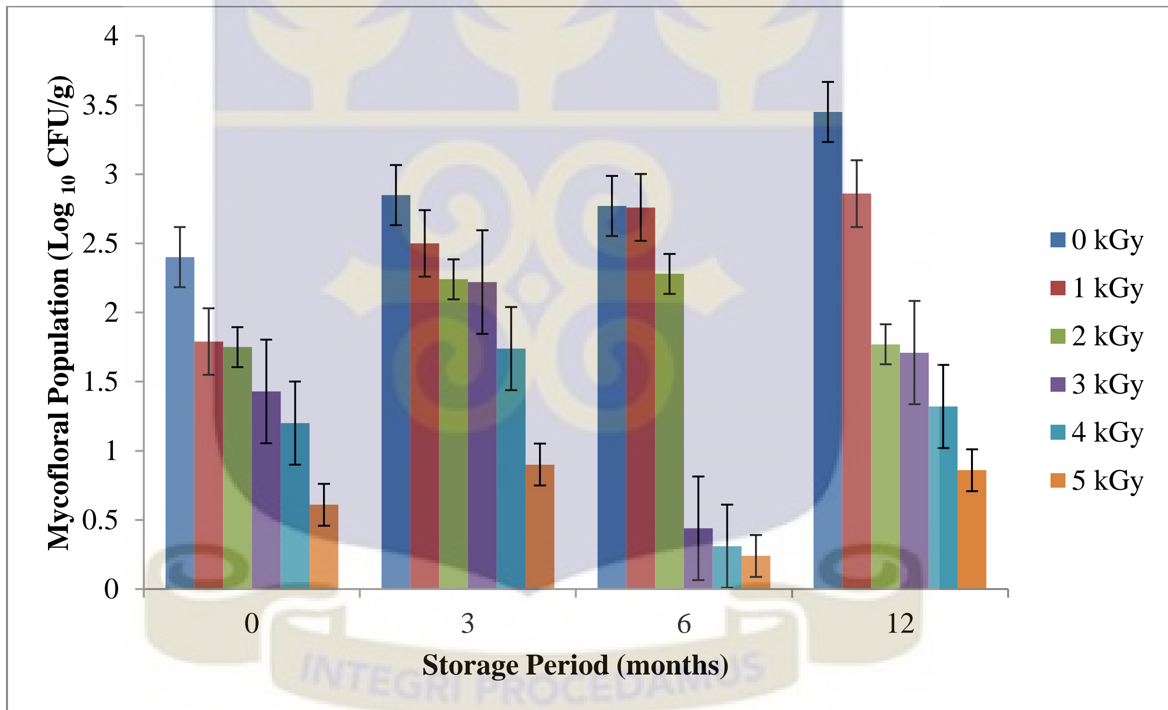

Fig.66. Mycoflora population of mushroom fruitbodies stored in polypropylene packs for up to 12 months and isolated on DRBC medium at 28-32°C

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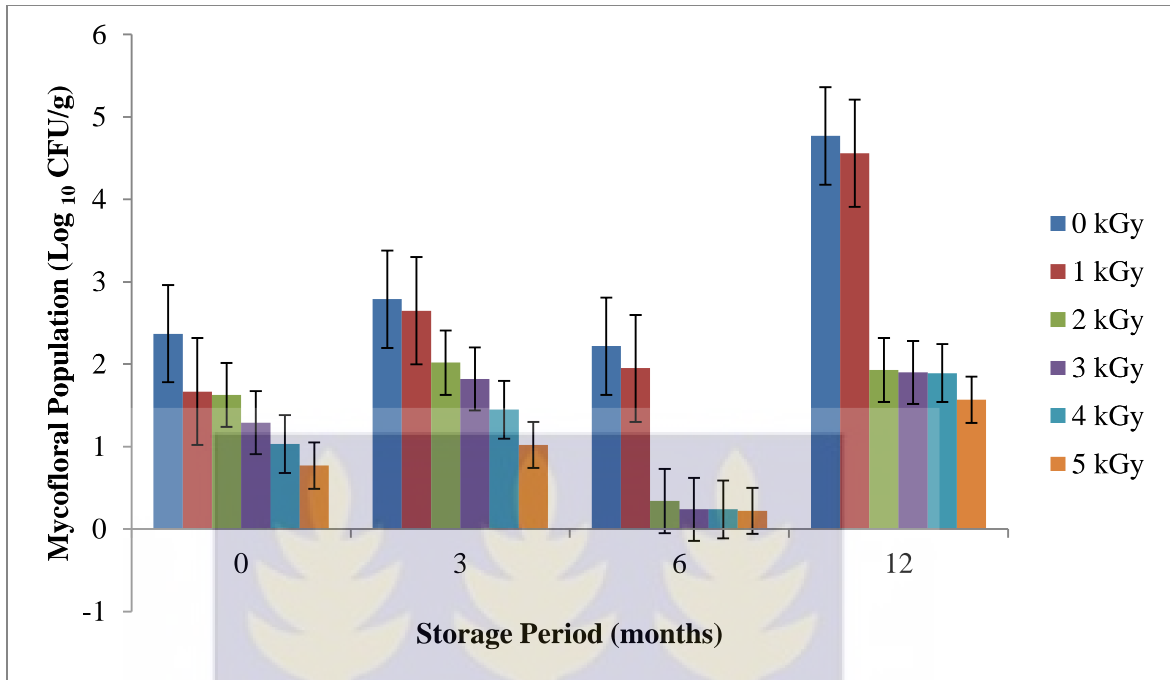

Fig.67. Mycoflora population of mushroom fruitbodies stored in polythene packs for up to 12 months and isolated on DRBC medium at 28-32°C

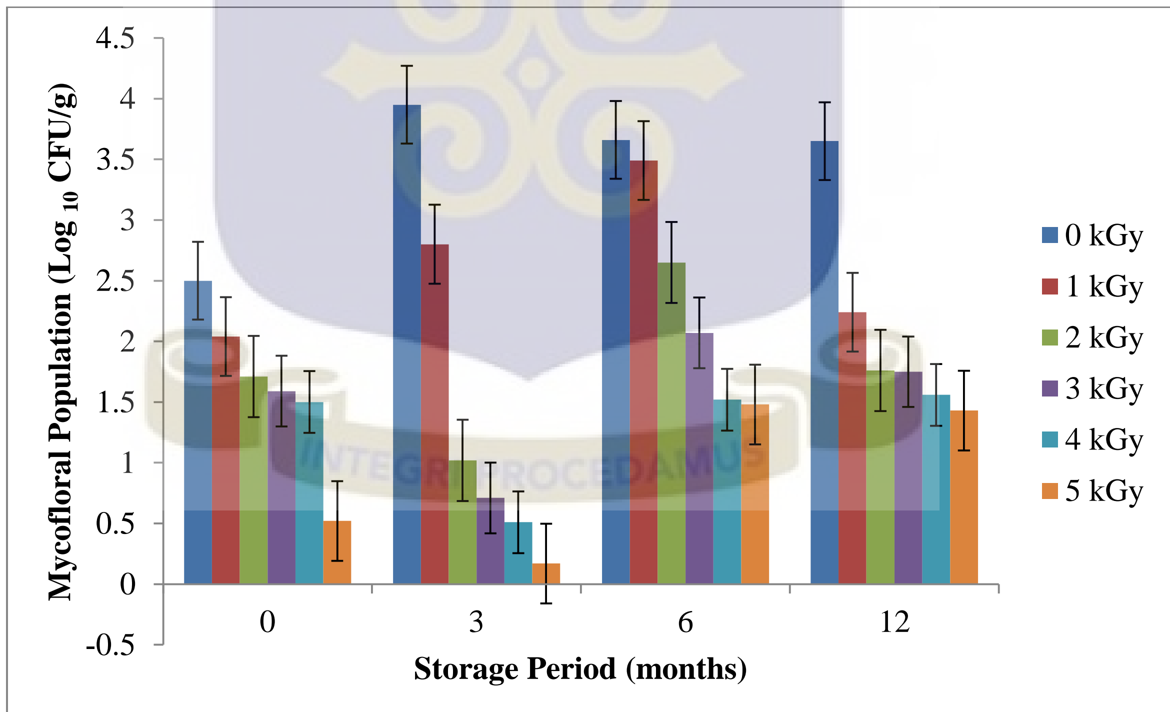

Fig.68. Mycoflora population of mushroom fruitbodies stored in polypropylene packs for up to 12 months and isolated on Cooke's medium at 28-32°C

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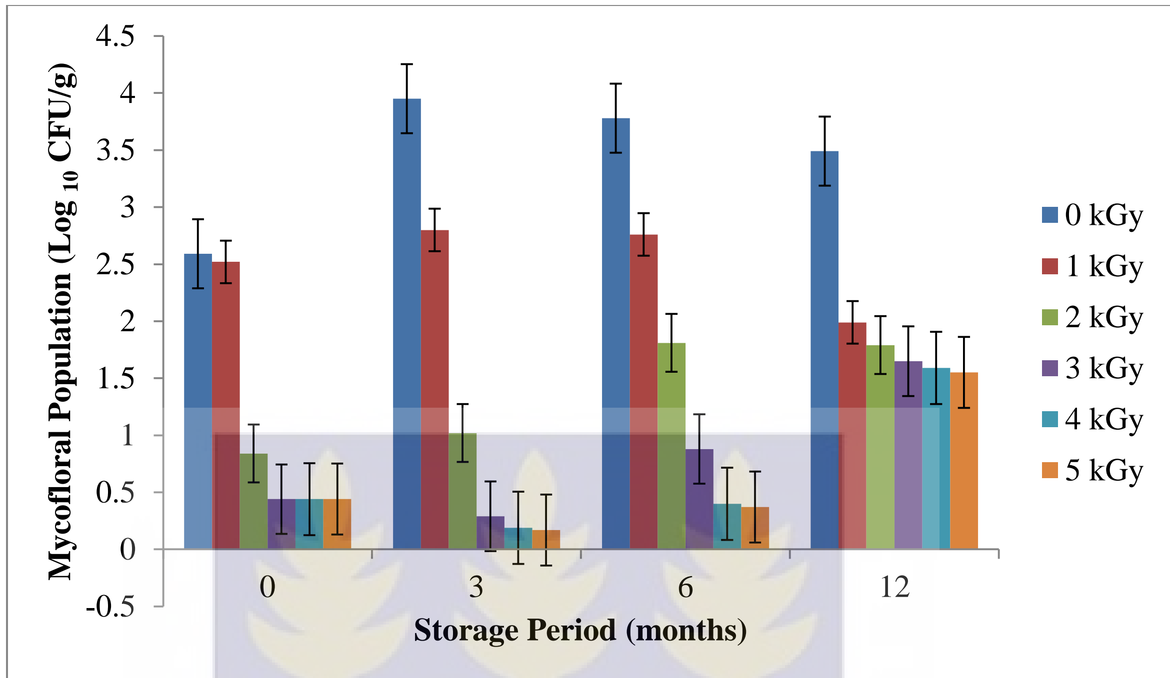

Fig.69. Mycoflora population of mushroom fruitbodies stored in polythene packs for up to 12 months and isolated on Cooke's medium at 28-32°C

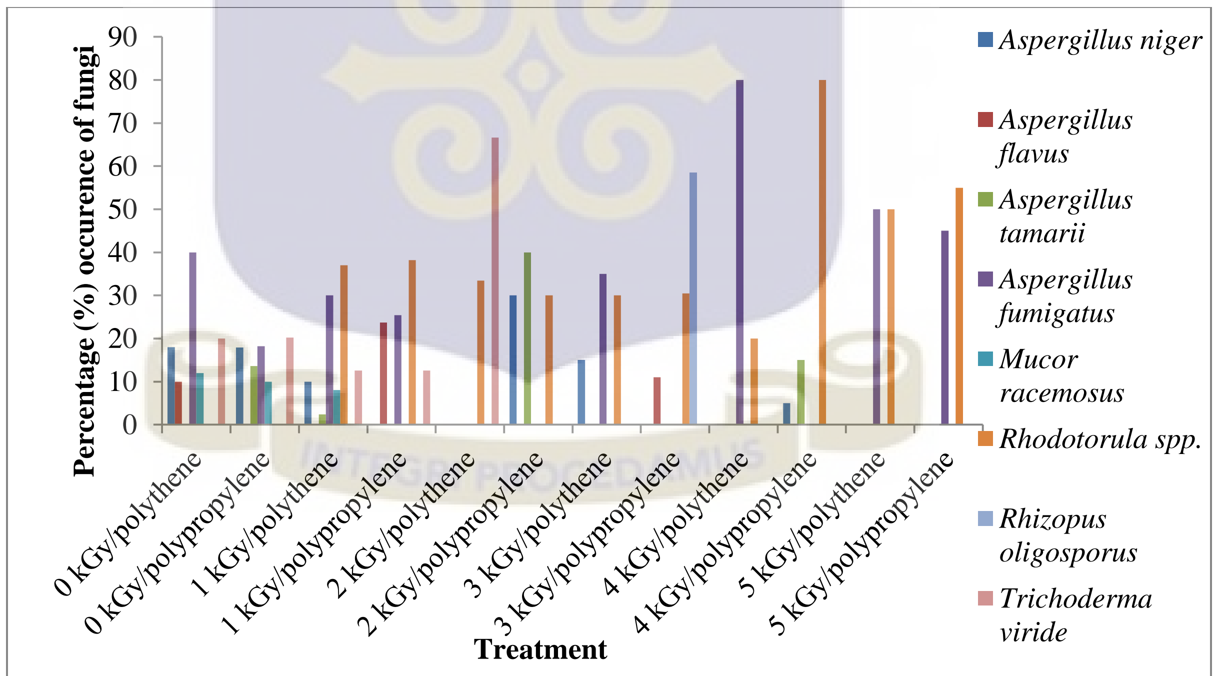

Fig.70. Percentage initial occurrence of fungi on mushroom fruit bodies treated with indicated dosages of gamma irradiation and isolated on Cooke's medium at 28-32°C

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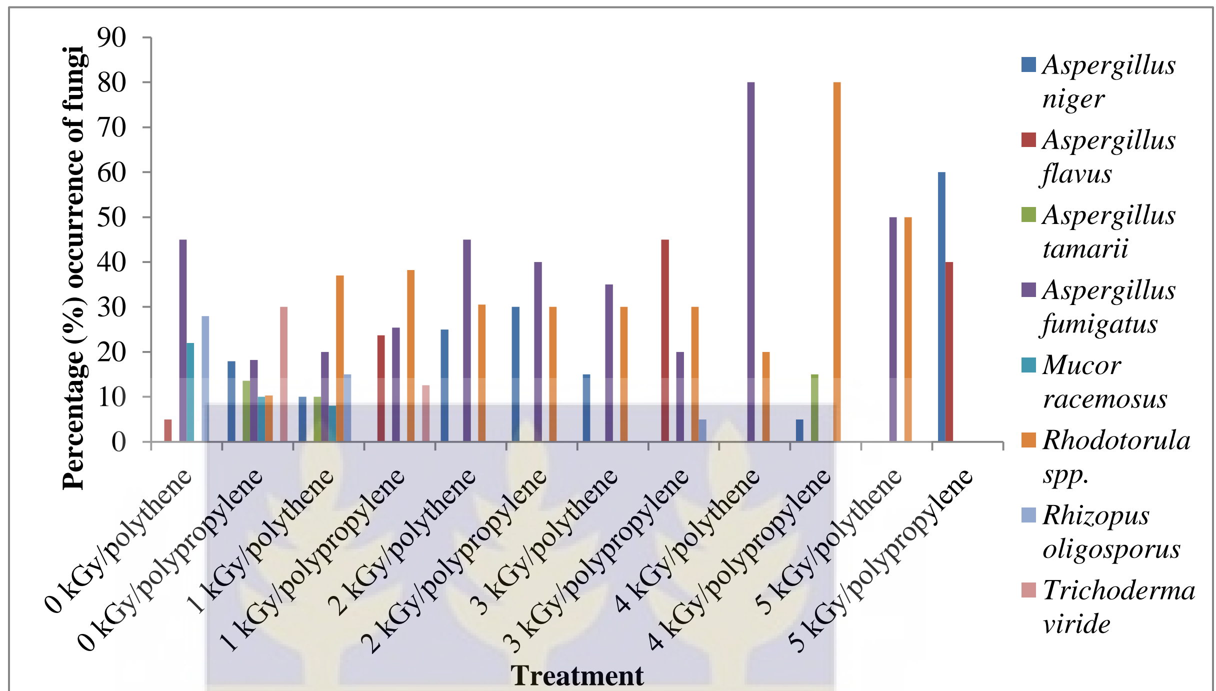

Fig.71. Percentage occurrence of fungi on mushroom fruit bodies stored for 3 months after irradiation in indicated packaging materials and isolated on Cooke's medium at 28-32°C

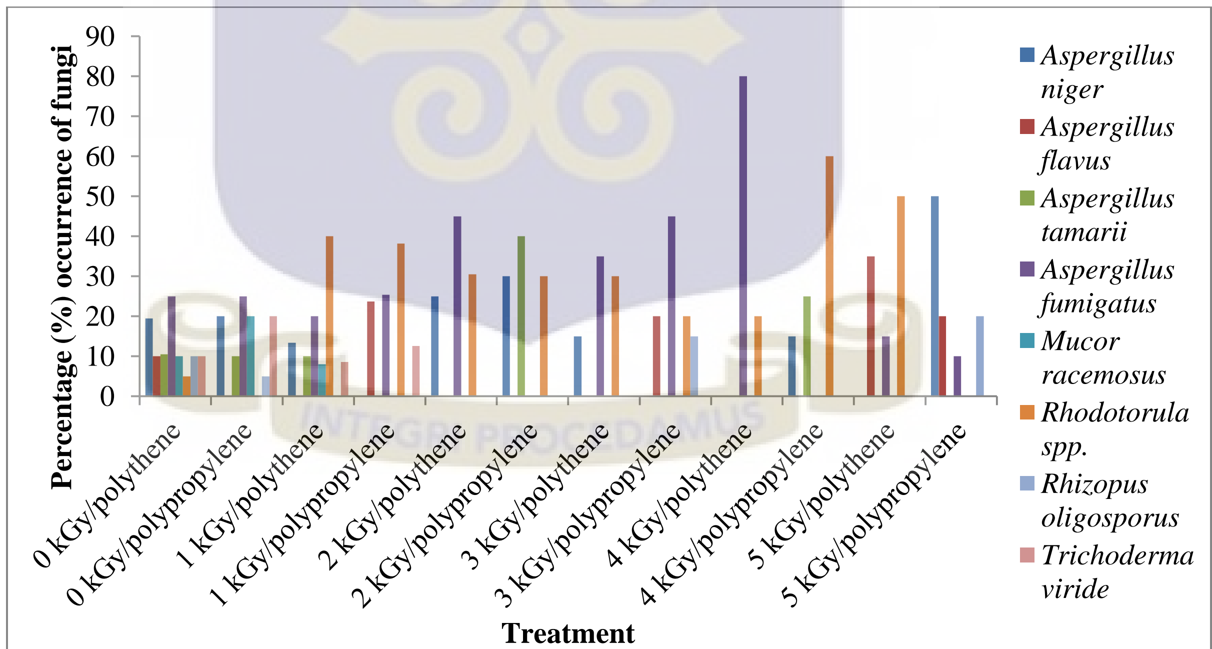

Fig.72. Percentage occurrence of fungi on mushroom fruit bodies stored for 6 months after irradiation in indicated packaging materials and isolated on Cooke's medium at 28-32°C

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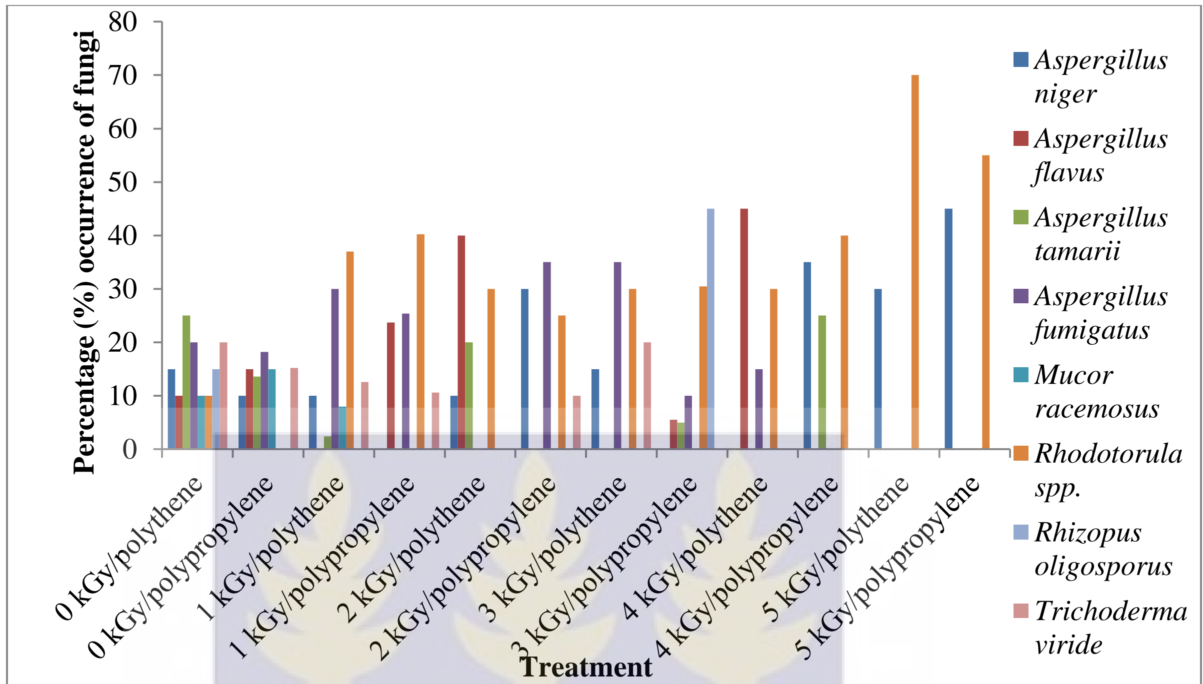

Fig.73. Percentage occurrence of fungi on mushroom fruit bodies stored for 12 months after irradiation in indicated packaging materials and isolated on Cooke's medium at 28-32°C

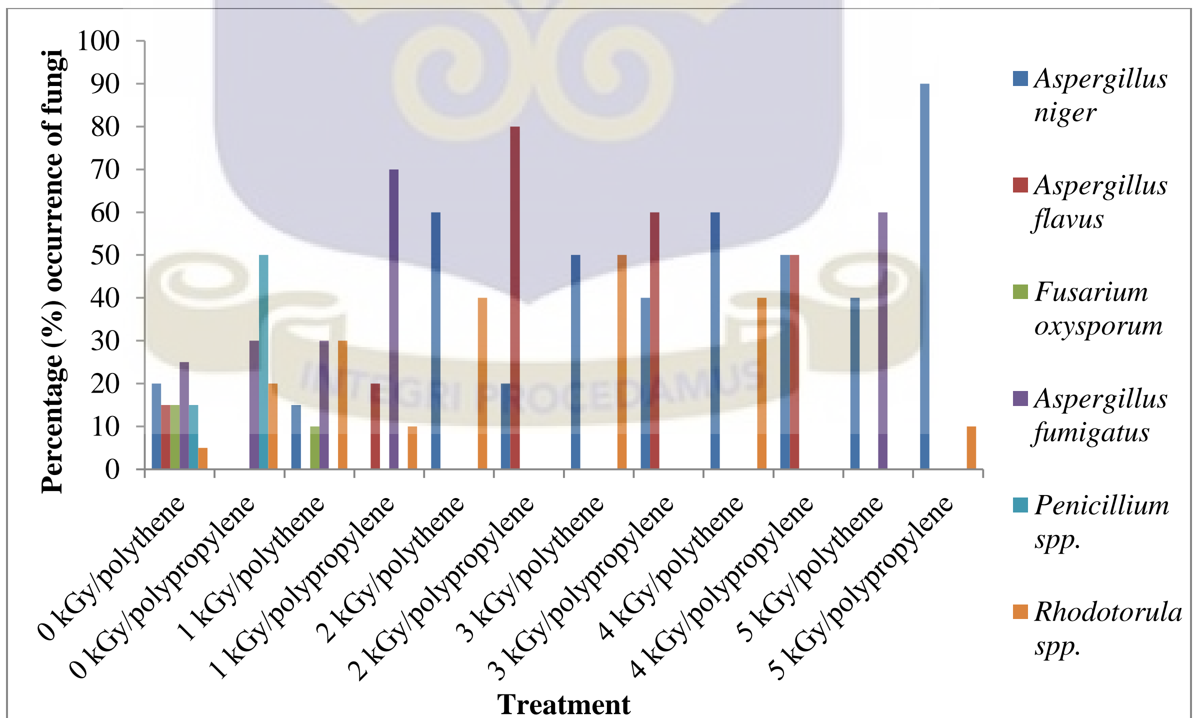

Fig.74. Percentage initial occurrence of fungi on mushroom fruit bodies treated with indicated dosages of gamma irradiation and isolated on DRBC medium at 28-32°C

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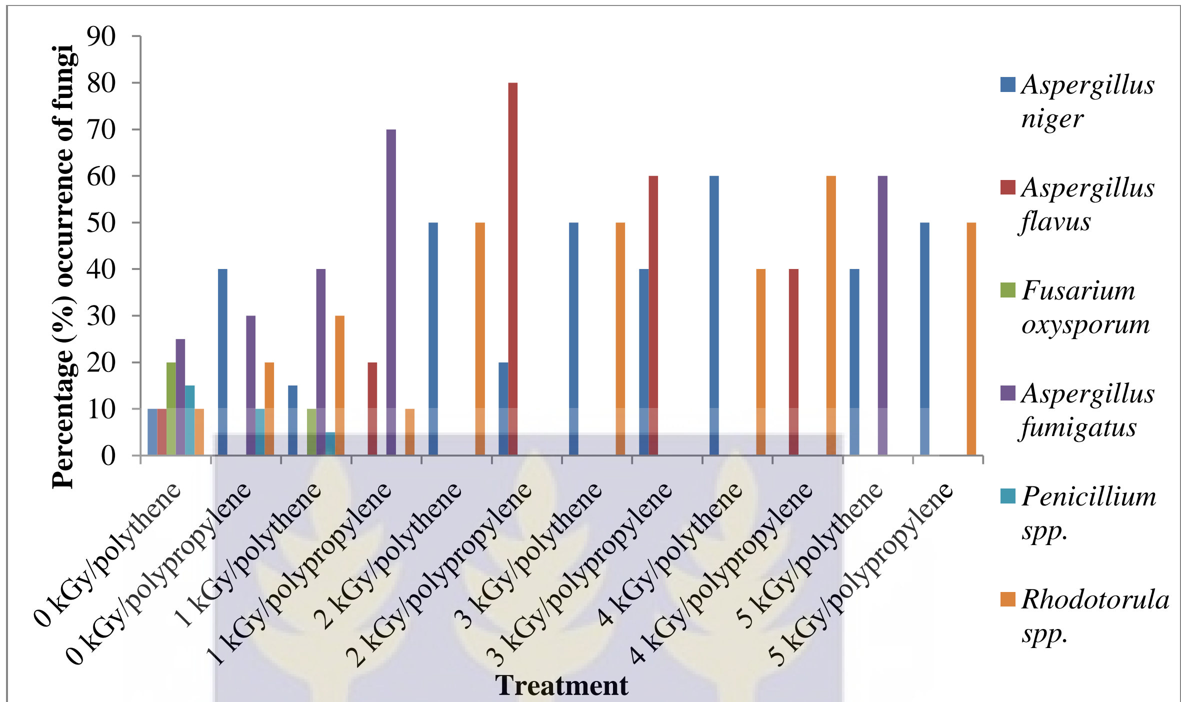

Fig.75. Percentage occurrence of fungi on mushroom fruit bodies stored for 6 months after irradiation in indicated packaging materials and isolated on DRBC medium at 28-32°C

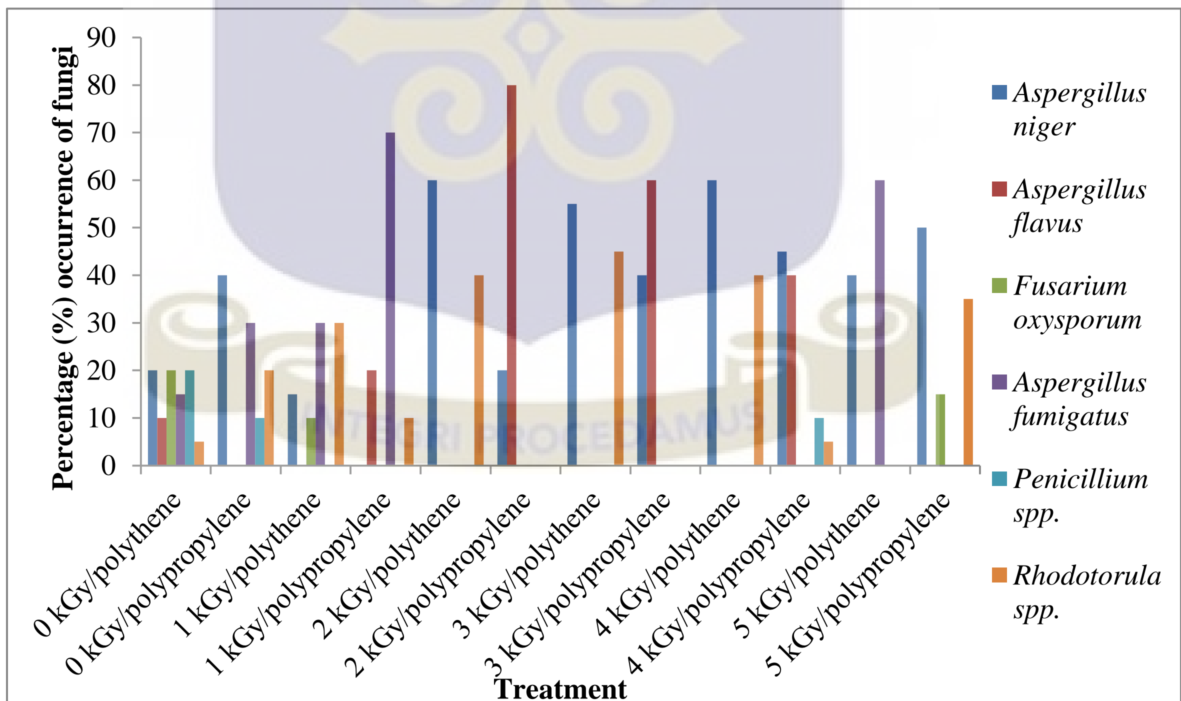

Fig.76. Percentage occurrence of fungi on mushroom fruit bodies stored for 6 months after irradiation in indicated packaging materials and isolated on DRBC medium at 28-32°C

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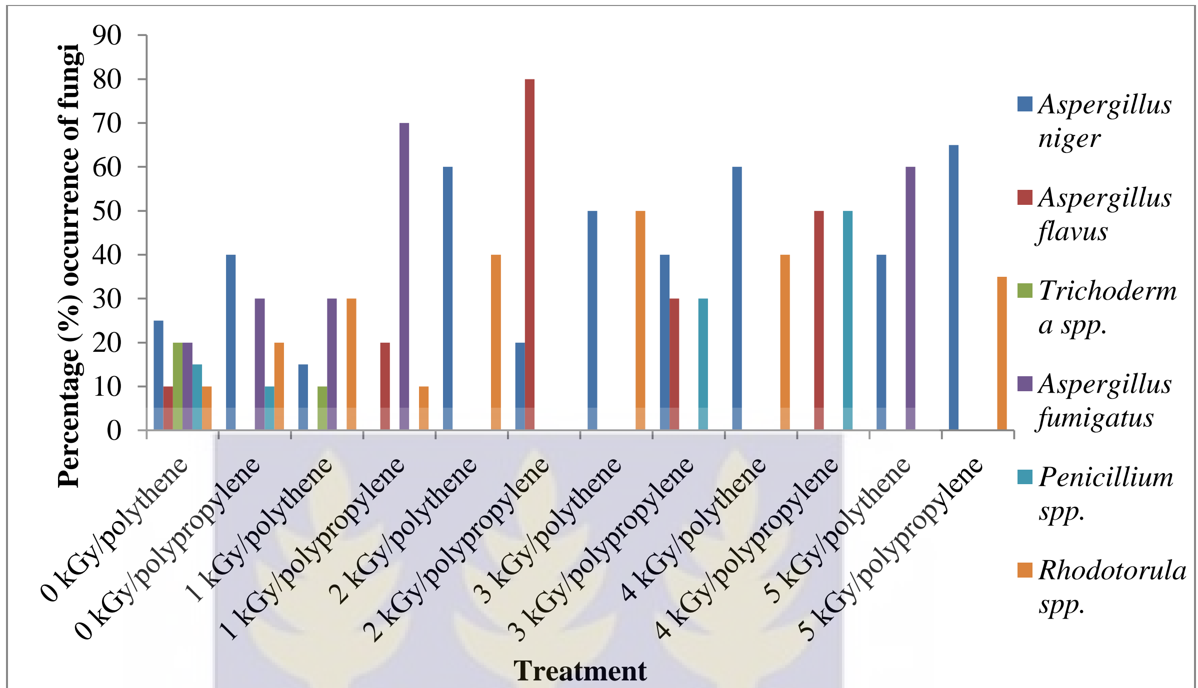

Fig.77. Percentage occurrence of fungi on mushroom fruit bodies stored for 12 months after irradiation in indicated packaging materials and isolated on DRBC medium at 28-32°C

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EXPERIMENT 17

RADIOSENSITIVITY CURVES (D_{10} VALUES) OF TOTAL MYCOFLORA RESIDENT IN DRIED MUSHROOMS STORED IN EITHER POLYPROPYLENE OR POLYTHENE PACKS AFTER GAMMA IRRADIATION AT 28-32 ^{O}C

Results obtained are presented in Figs. 78 and 79. The curves were linear and correlation ratio values R^2 obtained were positive ranging from R^2= 0.79- 0.95 (Table 29). From these curves the D_{10} values were calculated immediately after irradiation with respect to packaging material used. The D_{10} values ranged between 1.68- 2.78 kGy depending on the package material. (Figs. 78 and 79). The effective dose for killing fungi was close to what was found for *B.cereus* in both packaging packs i.e D_{10}= 0.76- 3.21 kGy (Table 27).

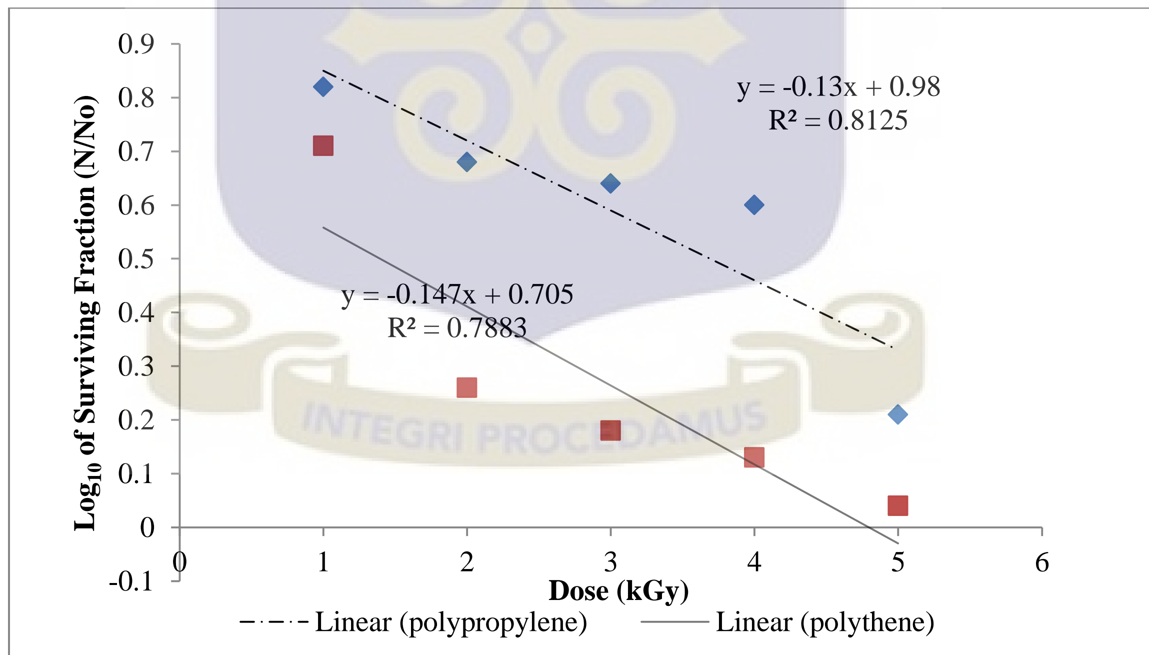

Fig.78: Radiation sensitivity curves of total fungi on mushroom fruit bodies immediately after irradiation and raised on Cooke's medium

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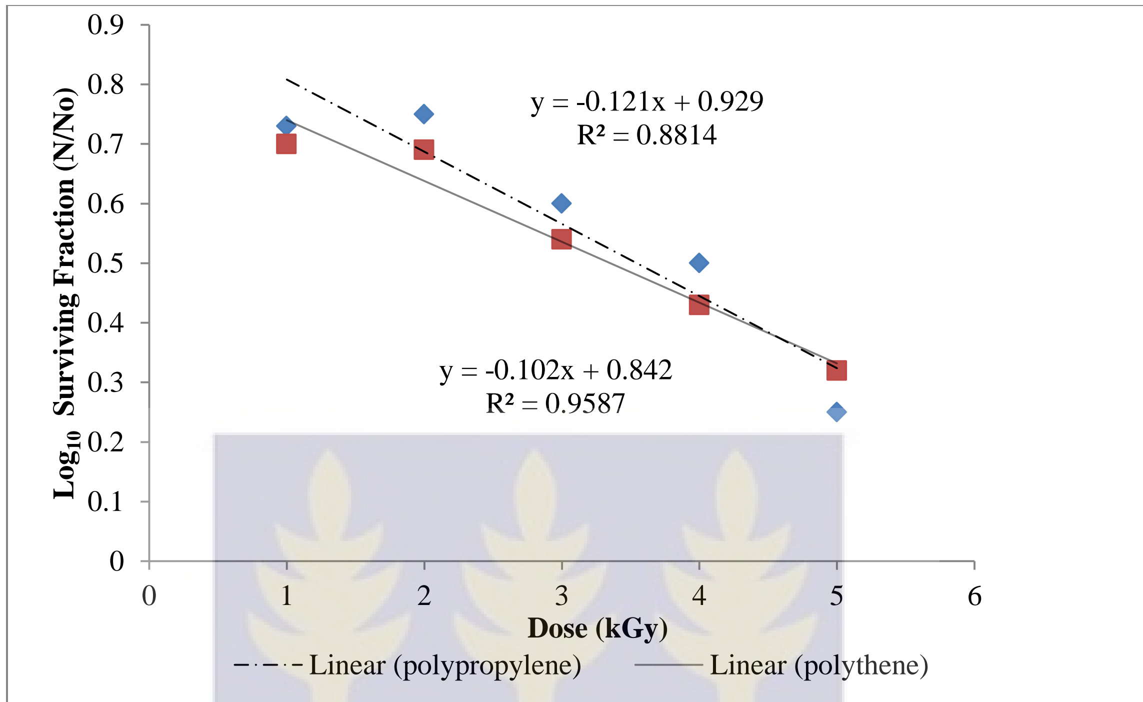

Fig.79. Radiation sensitivity curves of total fungi on mushroom fruit bodies immediately after irradiation and raised on DRBC

Table 29. Mean D_{10} values for fungi resident on mushrooms

Medium	Package	Regression Equation (y)	R^2 value	Mean D_{10} value (kGy)
Cooke's	Polypropylene	-0.13x + 0.98	0.812	2.61^b
	Polythene	-0.15x + 0.705	0.788	1.68^a
DRBC	Polypropylene	-0.121x + 0.929	0.881	2.78^b
	Polythene	-0.102x + 0.842	0.950	2.60^b

Means with different letters in a column are significantly different (P<0.05)

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EXPERIMENT 18

DETERMINATION OF TOTAL PHENOLIC, FLAVONOIDS AND ANTIOXIDANT CONTENT OF DRIED MUSHROOMS BEFORE AND AFTER IRRADIATION AND STORAGE IN EITHER POLYPROPYLENE AND POLYTHENE PACKS FOR UP TO 12 MONTHS AT 28-30 ^{O}C

(a) Total phenolic contents

The total phenolic content (TPC) was expressed in terms of Gallic Acid Equivalent (GAE) in milligram (mg) per gram of dry material. The calibration curves (absorbance vrs concentration) (Appendix 4) were used to determine total phenolic content. The total phenolic content of the various extracts are presented in Table 30. For 0- 3 months of storage, aqueous extract ranged from 1.96 - 10.96 mgGAE/g. Methanol and ethanol extracts ranged from 0.56 ± 0.01- 4.81 ± 0.4 mgGAE/g and 1.32 ± 0.02- 2.36 ± 0.2 mgGAE/g respectively. Data for 6-12 months storage ranged from 1.90- 10.60 mgGAE/g, 1.20-2.38 mgGAE/g and 0.50- 4.81 mgGAE/g for aqueous, ethanol and methanol extracts respectively. There was an apparent effect of 1 kGy irradiation dose generating higher quantity of TPC of 10.60- 10.96 mgGAE/g (Table 30). Irradiation dose of 0.5 kGy produced largest amounts of phenols for all the extracts while 2 kGy recorded the least for the extracts in some instances.

The total flavonoid content of the various extracts are summarised in Table 31. There were significant differences ($P \leq 0.05$) between flavonoid contents of doses applied and the type of solvent extracts. Post irradiation storage studies of 0-3 months recorded a range of 3.41-8.92, 1.64- 7.86 and 2.41-5.37 mgQE/g for aqueous, ethanol and methanol extracts respectively. A 6-12 months storage also recorded ranges 3.09-8.75, 1.64-7.86

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and 2.00-5.37 mgQE/g for aqueous, ethanol and methanol extracts respectively. There were marginal decreases in flavonoid contents over the storage period which caused significant ($P<0.05$) changes in aqueous and ethanolic extracts but caused no significant ($P>0.05$) change in methanolic extracts.

Analysis of variance (ANOVA) showed that gamma radiation and storage significantly ($P<0.05$) affected percent inhibition. Dried and gamma irradiated *P.ostreatus* stored for 0 and 3 months, ranged from 7.02 ± 0.10- 12.71 ± 0.02, 7.44 ± 0.16- 11.22 ± 0.15 and 8.79 ± 0.06- 13.03 ± 0.04 % mgGAE/g for the respective extracts. Storage months 6 to 12 also recorded ranges 7.02 ± 0.10- 12.71 ± 0.02, 7.44 ± 0.16 – 10.81 ± 0.15 and 8.79 ± 0.06- 13.03 ± 0.04% mgGAE/g (Table 32). Interaction of gamma radiation and storage period resulted in a general significant ($P<0.05$) decrease in percentage inhibition.

Fig 80. shows the amount of each extract needed for 50% inhibition (IC_{50}). IC_{50} mg ml^{-1} of the extracts were in the order aqueous < methanol <ethanol and corresponded to a range of 0.069- 0.073mg ml^{-1}, 0.22- 0.27mg ml^{-1} and 1.020- 1.071 mg ml^{-1} respectively over the 12 month storage period. IC_{50} of the standard compound quercetin was 0.00287mg ml^{-1}. The highest radical scavenging activity was shown by aqueous extract with IC_{50}= 0.069 mg ml^{-1} which is lower than that of quercetin ($P<0.05$). All the extracts showed significant difference ($P< 0.05$) from the standard compound (Fig.80).

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Table 30: Effect of irradiation on total phenolic content of dried mushrooms stored in two packaging materials for up to 12 months

Incubation period (months)	Package material	Dose Applied (kGy)	Type of Extracts		
			Aqueous (mgGAE/g)	Ethanol (mgGAE/g)	Methanol (mgGAE/g)
0	Polythene	0	3.84 ± 0.4^{c}	2.00 ± 0.1^{b}	2.40 ± 0.1^{c}
		0.5	5.00 ± 0.3^{d}	2.36 ± 0.2^{bc}	2.36 ± 0.2^{c}
		1	4.63 ± 0.3^{c}	1.54 ± 0.18^{b}	4.81 ± 0.4^{d}
		1.5	2.78 ± 0.1^{b}	1.32 ± 0.02^{a}	2.83 ± 0.1^{c}
		2	1.96 ± 0.05^{a}	1.32 ± 0.02^{a}	1.96 ± 0.05^{b}
	Polypropy-lene	0	2.54 ± 0.1^{b}	1.32 ± 0.02^{a}	1.96 ± 0.05^{b}
		0.5	4.46 ± 0.3^{c}	1.96 ± 0.05^{bc}	1.93 ± 0.02^{b}
		1	10.96 ± 1.7^{e}	1.53 ± 0.04^{b}	1.35 ± 0.03^{a}
		1.5	4.46 ± 0.8^{c}	1.89 ± 0.07^{b}	0.56 ± 0.01^{a}
		2	2.50 ± 0.2^{b}	1.76 ± 0.05^{b}	1.40 ± 0.03^{a}
3	Polythene	0	3.74 ± 0.3^{c}	1.70 ± 0.05^{a}	2.38 ± 0.1^{c}
		0.5	5.11 ± 0.3^{d}	2.31 ± 0.2^{c}	2.36 ± 0.3^{c}
		1	4.60 ± 0.3^{c}	1.50 ± 0.14^{a}	4.80 ± 0.3^{d}
		1.5	2.75 ± 0.1^{b}	1.31 ± 0.02^{a}	2.83 ± 0.2^{c}
		2	1.94 ± 0.05^{a}	1.30 ± 0.01^{a}	1.92 ± 0.05^{b}
	Polypropy-lene	0	2.54 ± 0.1^{b}	1.32 ± 0.01^{a}	1.91 ± 0.02^{b}
		0.5	4.42 ± 0.3^{c}	1.93 ± 0.05^{c}	1.93 ± 0.03^{b}
		1	10.90 ± 1.7^{e}	1.50 ± 0.03^{a}	1.32 ± 0.02^{a}
		1.5	4.40 ± 0.6^{c}	1.84 ± 0.05^{b}	0.51 ± 0.02^{a}
		2	2.48 ± 0.2^{b}	1.73 ± 0.03^{b}	1.43 ± 0.03^{b}

Means with same letters are not significantly (P>0.05) different

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Table 30 cont'd: Effect of irradiation on total phenolic content of dried mushrooms stored in two packaging materials for 12 months

Incubation period (months)	Package material	Dose Applied (kGy)	Aqueous (mgGAE/g)	Ethanol (mgGAE/g)	Methanol (mgGAE/g)
			Type of Extracts		
	Polythene	0	3.70 ± 0.30[c]	1.20 ± 0.01[a]	2.26 ± 0.2[c]
		0.5	5.03 ± 0.3[cd]	2.36 ± 0.3[c]	2.27 ± 0.2[c]
		1	4.59 ± 0.40[c]	1.54 ± 0.13[b]	4.79 ± 0.3[d]
		1.5	2.70 ± 0.20[b]	1.27 ± 0.02[a]	2.83 ± 0.2[c]
		2	1.90 ± 0.05[a]	1.30 ± 0.01[a]	1.91 ±0.05[b]
6	Polypropy-lene	0	2.57 ± 0.10[b]	1.32 ± 0.01[a]	1.96±0.02[b]
		0.5	4.41 ± 0.30[c]	1.96 ± 0.05[b]	1.92±0.02[b]
		1	9.87 ± 1.60[e]	1.53 ± 0.03[b]	1.35±0.02[a]
		1.5	4.42 ± 0.60[c]	1.89 ± 0.05[c]	0.50±0.01[a]
		2	2.50 ± 0.20[b]	1.70 ± 0.02[bc]	1.26±0.02[a]
	Polythene	0	3.84±0.40[c]	2.00 ± 0.30[b]	2.41±0.1[c]
		0.5	4.81±0.20[cd]	2.38 ± 0.4[c]	2.36±0.4[c]
		1	4.63±0.30[c]	1.54 ± 0.03[b]	4.81±0.3[d]
		1.5	2.73±0.20[b]	1.32 ± 0.02[a]	2.83±0.2[c]
		2	1.94±0.06[a]	1.30 ± 0.02[a]	1.92±0.04[b]
12	Polypropy-lene	0	2.54±0.20[b]	1.32 ± 0.01[a]	1.96±0.05[b]
		0.5	4.42±0.30[c]	1.96 ± 0.05[b]	1.93±0.04[b]
		1	10.60±1.65[e]	1.51 ± 0.03[b]	1.30±0.02[a]
		1.5	4.46±0.70[c]	1.81 ± 0.05[b]	0.46±0.01[a]
		2	2.44±0.30[b]	1.73 ± 0.02[b]	1.22±0.03[a]

Means with same letters are not significantly ($P>0.05$) different

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Table 31: Effect of irradiation on flavonoid content of dried mushrooms stored in two packaging materials for 12 months

Incubation period (months)	Package material	Dose Applied (kGy)	Aqueous (mgQE/g)	Ethanol (mgQE/g)	Methanol (mgQE/g)
			Type of Extracts		
0	Polythene	0	7.67 ± 0.8^{c}	4.25 ± 0.2^{b}	3.93 ± 0.2^{c}
		0.5	8.92 ± 0.6^{d}	7.27 ± 0.4^{c}	3.81 ± 0.2^{c}
		1	8.07 ± 0.7^{c}	5.58 ± 0.2^{c}	4.61 ± 0.4^{d}
		1.5	3.70 ± 0.2^{a}	1.64 ± 0.05^{a}	3.51 ± 0.2^{b}
		2	6.73 ± 0.5^{bc}	7.88 ± 0.4^{d}	2.88 ± 0.1^{b}
	Polypropylene	0	3.09 ± 0.2^{a}	4.73 ± 0.2^{b}	4.18 ± 0.2^{d}
		0.5	8.75 ± 0.8^{d}	7.86 ± 0.4^{c}	5.37 ± 0.5^{e}
		1	7.92 ± 0.6^{c}	4.42 ± 0.2^{b}	2.50 ± 0.2^{a}
		1.5	7.92 ± 0.6^{c}	6.29 ± 0.4^{c}	2.41 ± 0.1^{a}
		2	3.46 ± 0.3^{a}	5.88 ± 0.3^{c}	4.90 ± 0.4^{e}
3	Polythene	0	7.53 ± 0.7^{c}	4.31 ± 0.2^{b}	3.84 ± 0.2^{c}
		0.5	8.88 ± 0.6^{c}	7.27 ± 0.4^{c}	3.81 ± 0.2^{c}
		1	8.00 ± 0.7^{c}	5.58 ± 0.2^{b}	4.58 ± 0.4^{d}
		1.5	3.74 ± 0.2^{a}	1.64 ± 0.05^{a}	3.72 ± 0.2^{c}
		2	7.03 ± 0.5^{c}	7.81 ± 0.4^{c}	2.88 ± 0.1^{b}
	Polypropylene	0	3.09 ± 0.2^{a}	4.69 ± 0.2^{b}	4.14 ± 0.2^{d}
		0.5	8.75 ± 0.8^{d}	7.86 ± 0.4^{c}	5.37 ± 0.5^{e}
		1	7.92 ± 0.6^{c}	4.42 ± 0.2^{b}	2.50 ± 0.2^{a}
		1.5	7.92 ± 0.6^{c}	6.29 ± 0.4^{c}	2.10 ± 0.1^{a}
		2	3.41 ± 0.3^{a}	6.02 ± 0.3^{c}	5.11 ± 0.4^{e}

Means with same letters are not significantly (P>0.05) different

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Table 31 cont'd: Effect of irradiation on flavonoid content of dried mushrooms stored in two packaging materials for 12 months

Incubation period (months)	Package material	Dose Applied (kGy)	Aqueous (mgQE/g)	Ethanol (mgQE/g)	Methanol (mgQE/g)
	Polythene	0	7.67 ± 0.8^{c}	4.33 ± 0.2^{b}	3.93 ± 0.2^{c}
		0.5	7.92 ± 0.6^{c}	7.27 ± 0.4^{c}	3.81 ± 0.2^{a}
		1	7.87 ± 0.7^{c}	5.51 ± 0.2^{b}	4.61 ± 0.4^{d}
		1.5	3.69 ± 0.2^{a}	1.66 ± 0.05^{a}	3.51 ± 0.2^{bc}
		2	6.73 ± 0.5^{c}	7.88 ± 0.4^{d}	2.88 ± 0.1^{b}
6	Polypropy-lene	0	3.09 ± 0.2^{a}	4.57 ± 0.2^{b}	4.18 ± 0.2^{d}
		0.5	8.75 ± 0.8^{d}	7.86 ± 0.4^{d}	5.37 ± 0.5^{e}
		1	7.92 ± 0.6^{c}	4.42 ± 0.2^{b}	2.50 ± 0.2^{a}
		1.5	7.88 ± 0.6^{c}	6.35 ± 0.4^{b}	2.00 ± 0.1^{a}
		2	3.46 ± 0.3^{a}	5.78 ± 0.3^{c}	4.93 ± 0.4^{e}
	Polythene	0	7.63 ± 0.8^{c}	4.25 ± 0.2^{ab}	3.93 ± 0.2^{cd}
		0.5	8.92 ± 0.6^{d}	7.27 ± 0.4^{c}	3.81 ± 0.2^{c}
		1	8.00 ± 0.7^{c}	5.52 ± 0.2^{c}	4.61 ± 0.4^{d}
		1.5	3.7 ± 0.2^{a}	1.64 ± 0.05^{a}	3.51 ± 0.2^{b}
		2	6.73 ± 0.5^{bc}	7.88 ± 0.4^{d}	2.88 ± 0.1^{b}
12	Polypropy-lene	0	3.05 ± 0.2^{a}	4.73 ± 0.2^{b}	4.18 ± 0.2^{cd}
		0.5	8.72 ± 0.8^{c}	7.86 ± 0.4^{d}	5.37 ± 0.5^{a}
		1	7.92 ± 0.6^{c}	4.42 ± 0.2^{b}	2.50 ± 0.2^{a}
		1.5	7.89 ± 0.6^{c}	6.29 ± 0.4^{c}	2.00 ± 0.1^{a}
		2	3.46 ± 0.3^{a}	5.81 ± 0.3^{c}	4.90 ± 0.4^{d}

Means with same letters are not significantly (P>0.05) different

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Table 32: Percentage inhibition (%) of DPPH in dried and irradiated mushrooms stored in packaging materials for 12 months storage period

Incubation period (months)	Package material	Dose Applied (kGy)	Type of Extracts Aqueous (%) (mgGAE/g)	Ethanol (%) (mgGAE/g)	Methanol (%) (mgGAE/g)
	Polythene	0	7.02 ± 0.10^{a}	10.06 ± 0.13^{bc}	10.09 ± 0.26^{b}
		0.5	9.65 ± 0.13^{bc}	10.47 ± 0.24^{c}	13.03 ± 0.04^{b}
		1	11.60 ± 0.05^{d}	9.36 ± 0.12^{bc}	12.82 ± 0.03^{b}
		1.5	9.46 ± 0.12^{bc}	9.36 ± 0.12^{bc}	10.30 ± 0.27^{b}
		2	9.26 ± 0.02^{b}	8.31 ± 0.04^{a}	10.60 ± 0.3^{b}
0	Polypropy-lene	0	9.65 ± 0.13^{bc}	8.95 ± 0.05^{bc}	10.40 ± 0.30^{b}
		0.5	8.24 ± 0.02^{a}	10.81 ± 0.25^{d}	11.21 ± 0.04^{b}
		1	11.12 ± 0.05^{c}	7.44 ± 0.16^{a}	9.09 ± 0.12^{a}
		1.5	12.71 ± 0.02^{c}	8.84 ± 0.05^{bc}	8.79 ± 0.06^{a}
		2	9.37 ± 0.12^{bc}	9.06 ±0.11^{bc}	10.30 ± 0.3^{b}
	Polythene	0	6.85 ± 0.11^{a}	9.12 ± 0.05^{b}	10.23 ± 0.25^{b}
		0.5	0.0 ± 0.3^{b}	11.11 ± 0.05^{d}	12.89 ± 0.10^{c}
		1	11.42 ± 0.05^{c}	9.36 ± 0.12^{b}	13.03 ± 0.20^{c}
		1.5	9.04 ± 0.02^{b}	9.27 ± 0.03^{b}	10.00 ± 0.13^{b}
		2	9.15 ± 0.02^{b}	8.30 ± 0.04^{b}	11.32 ± 0.05^{b}
3	Polypropy-lene	0	10.00 ± 0.02^{b}	11.15 ± 0.04^{de}	10.40 ± 0.30^{b}
		0.5	8.02 ± 0.01^{b}	11.22 ± 0.15^{d}	11.21 ± 0.04^{b}
		1	11.51 ± 0.02^{bc}	7.34 ± 0.14^{a}	9.12 ± 0.14^{a}
		1.5	11.60 ± 0.02^{bc}	9.12 ± 0.04^{b}	9.00 ± 0.11^{a}
		2	9.30 ± 0.04^{b}	9.11 ± 0.12^{b}	10.00 ± 0.02^{b}

Means with same letters are not significantly (P>0.05) different

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Table 32 cont'd: Percentage inhibition (%) of DPPH in dried and irradiated mushrooms stored in packaging materials for 12 months storage period

Incubation period (months)	Package material	Dose Applied (kGy)	Aqueous (%) (mgGAE/g)	Ethanol (%) (mgGAE/g)	Methanol (%) (mgGAE/g)
	Polythene	0	7.05 ± 0.10^{a}	10.06 ± 0.13^{bc}	10.09 ± 0.26^{b}
		0.5	9.65 ± 0.13^{bc}	10.47 ± 0.24^{c}	13.03 ± 0.04^{b}
		1	11.60 ± 0.05^{d}	9.36 ± 0.12^{bc}	12.82 ± 0.03^{b}
		1.5	9.46 ± 0.12^{bc}	9.36 ± 0.12^{bc}	10.30 ± 0.27^{b}
		2	9.26 ± 0.02^{b}	8.31 ± 0.04^{a}	10.60 ± 0.3^{b}
6	Polypropy-lene	0	9.65 ± 0.13^{bc}	8.95 ± 0.05^{bc}	10.40 ± 0.3^{b}
		0.5	8.24 ± 0.02^{a}	10.81 ± 0.25^{d}	11.21 ± 0.04^{b}
		1	11.12 ± 0.05^{c}	7.44 ± 0.16^{a}	9.09 ± 0.12^{a}
		1.5	12.71 ± 0.02^{c}	8.84 ± 0.05^{bc}	8.79 ± 0.06^{a}
		2	9.37 ± 0.12^{bc}	9.06 ± 0.11^{bc}	10.30 ± 0.3^{b}
	Polythene	0	7.00 ± 0.10^{a}	10.06 ± 0.13^{bc}	10.09 ± 0.26^{b}
		0.5	9.65 ± 0.13^{bc}	10.47 ± 0.24^{c}	13.03 ± 0.04^{b}
		1	11.60 ± 0.05^{d}	9.36 ± 0.12^{bc}	12.82 ± 0.03^{b}
		1.5	9.46 ± 0.12^{bc}	9.36 ± 0.12^{bc}	10.30 ± 0.27^{b}
		2	9.26 ± 0.02^{b}	8.31 ± 0.04^{a}	10.60 ± 0.3^{b}
12	Polypropy-lene	0	9.65 ± 0.13^{bc}	8.95 ± 0.05^{bc}	10.40 ± 0.3^{b}
		0.5	8.24 ± 0.02^{a}	10.81 ± 0.25^{d}	11.21 ± 0.04^{b}
		1	11.12 ± 0.05^{c}	7.44 ± 0.16^{a}	9.09 ± 0.12^{a}
		1.5	12.71 ± 0.02^{c}	8.84 ± 0.05^{bc}	8.79 ± 0.06^{a}
		2	9.37 ± 0.12^{bc}	9.06 ± 0.11^{bc}	10.30 ± 0.3^{b}

Means with same letters are not significantly (P>0.05) different

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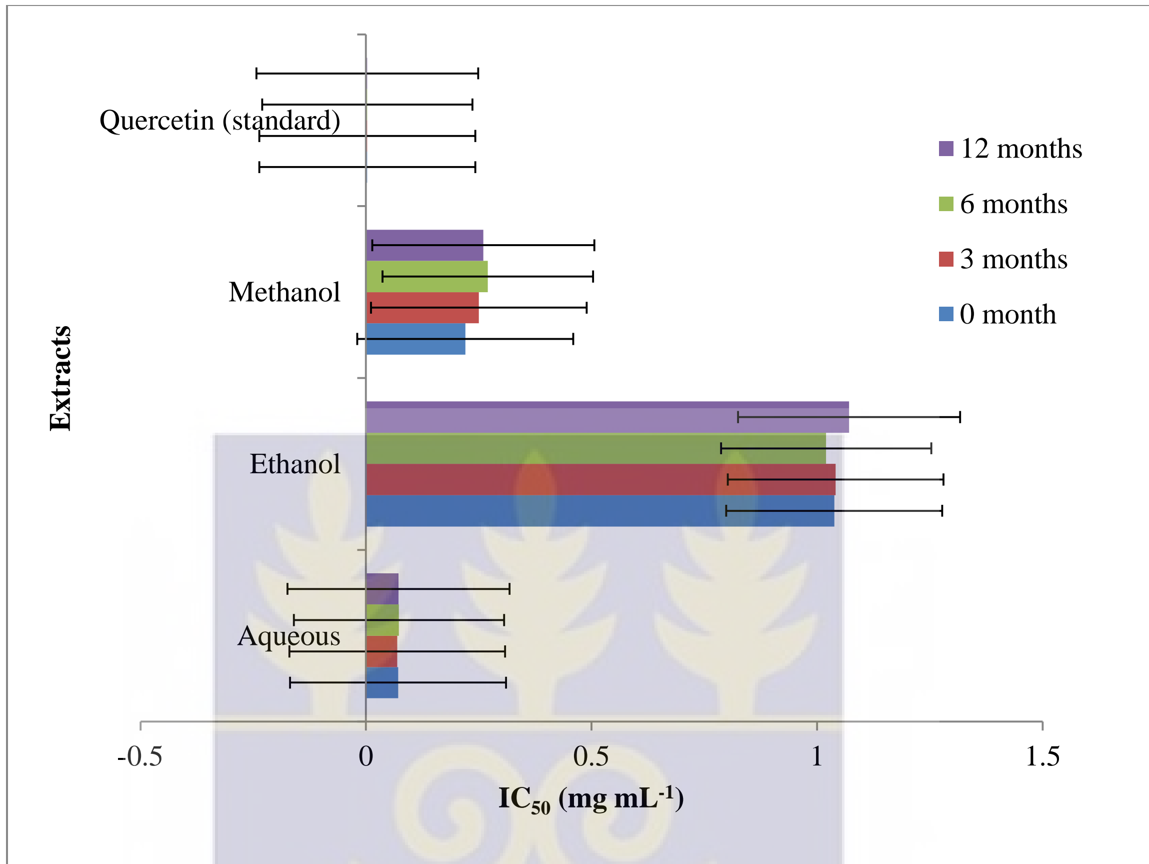

Fig.80. Effect of storage and gamma irradiation on IC_{50} values of *P.ostreatus*

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EXPERIMENT 19

DETERMINATION OF PROXIMATE (CHEMICAL) ANALYSES AND METABOLIC ENERGY CONTENTS OF DRY MUSHROOMS BEFORE AND AFTER IRRADIATION AND STORAGE IN EITHER POLYPROPYLENE OR POLYTHENE AND STORED AT 28-30 ^{O}C

The results of chemical analysis of gamma irradiated dried mushrooms stored in polythene and polypropylene packs are presented in Table 33. Protein content for 0 month storage ranged from 12.51- 15.25%. Post irradiation storage studies revealed a decreasing trend of protein content which was however not significantly different (P>0.05) irrespective of ionizing radiation dose and storage package used. After 12 months of storage, protein content ranged from12.54 - 15.20%.

Fat content ranged from 0.65- 1.24% for 0-3 month while that of 6- 12 months ranged 0.68- 1.24%. The average fat content of mushrooms is reported to be generally low, ranging from 0.6-3.2%. Gamma radiation had a significant (P<0.05) effect on the fat content of dried mushrooms during storage. Radiation dose 2 kGy had an apparent significant (P<0.05) effect on the fat content.

Moisture content ranged from 14.11- 15.80% for 0-3 month while 6-12 months storage ranged 14.11-16.11% and showed significant difference (P<0.05). Crude fibre content in this mushroom ranged 13.56- 15.22% Carbohydrate content ranged between 61.88- 65.51%

Ash content ranged between 7.08-8.31%. Packaging had no significant (P>0.05) effect on the proximate analyses.

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Table 33: Effect of irradiation on proximate composition of dried mushrooms in storage packs during storage period of 0- 3 months

Storage Time (months)	Package Material	Dose Applied (kGy)	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fibre (%)	Carbohydrate (%)
	polythene	0	14.51^{c}	7.11^{a}	1.24^{d}	15.22^{e}	13.72^{b}	61.88^{a}
		0.5	14.30^{b}	7.19^{b}	1.03^{b}	14.26^{d}	13.70^{b}	63.22^{bc}
		1	14.50^{c}	7.21^{b}	1.04^{b}	12.51^{a}	13.77^{b}	64.66^{c}
		1.5	14.21^{b}	7.39^{c}	1.15^{c}	13.38^{cd}	13.56^{a}	64.71^{c}
		2	15.74^{d}	7.31^{c}	0.68^{a}	13.27^{c}	15.22^{c}	63.00^{bc}
0	Polyprop -ylene	0	14.58^{d}	7.08^{a}	1.20^{d}	14.82^{d}	13.77^{b}	62.78^{b}
		0.5	14.30^{b}	7.14^{a}	1.03^{b}	14.11^{cd}	13.70^{b}	63.63^{bc}
		1	14.54^{c}	7.20^{b}	1.10^{bc}	12.51^{a}	13.78^{b}	64.74^{c}
		1.5	14.14^{a}	7.30^{c}	1.11^{bc}	13.40^{cd}	13.50^{a}	62.91^{b}
		2	15.77^{d}	7.29^{c}	0.65^{a}	13.37^{c}	15.38^{c}	63.09^{bc}
	polythene	0	14.59^{c}	7.11^{a}	1.21^{d}	15.22^{e}	13.70^{b}	61.42^{a}
		0.5	14.41^{bc}	7.19^{b}	1.02^{ab}	14.21^{d}	14.10^{bc}	63.22^{bc}
		1	14.58^{c}	7.21^{b}	1.05^{b}	12.51^{a}	13.47^{a}	64.61^{c}
		1.5	14.11^{a}	7.30^{c}	1.15^{c}	13.35^{cd}	13.56^{a}	63.50^{bc}
		2	15.80^{d}	7.31^{c}	0.71^{ab}	13.24^{c}	14.38^{bc}	63.00^{bc}
3	Polyprop -ylene	0	14.54^{c}	7.06^{a}	1.24^{d}	15.25^{e}	14.72^{bc}	60.68^{a}
		0.5	14.30^{b}	7.19^{b}	1.16^{c}	14.19^{d}	14.70^{bc}	63.22^{bc}
		1	14.50^{c}	7.29^{c}	1.04^{b}	12.51^{a}	14.09^{bc}	64.71^{c}
		1.5	14.11^{a}	7.35^{c}	1.12^{bc}	13.38^{cd}	13.56^{a}	62.55^{b}
		2	15.74^{d}	7.31^{c}	0.68^{a}	13.27^{c}	15.33^{c}	63.00^{bc}

Means with same letters in a column are not significantly ($P>0.05$) different

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Table 33 cont'd: Effect of irradiation and storage on proximate composition of dried mushrooms in storage packs during storage period of 6-12 months

Storage Time (months)	Package Material	Dose Applied (kGy)	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fibre (%)	Carbohydrate (%)
	polythene	0	14.51[c]	7.11[a]	1.24[d]	15.22[e]	14.69[bc]	61.39[a]
		0.5	14.30[b]	7.19[b]	1.03[b]	14.26[d]	14.75[bc]	63.22[b]
		1	14.50[c]	7.21[b]	1.04[b]	12.47[a]	14.12[bc]	64.71[b]
		1.5	14.11[a]	7.29[c]	1.13[c]	13.40[cd]	14.56[bc]	64.71[b]
		2	15.74[d]	8.31[d]	0.53[a]	14.27[d]	15.38[c]	65.50[c]
6	Polyprop-ylene	0	14.55[c]	8.11[d]	1.24[d]	15.25[e]	14.18[bc]	61.68[a]
		0.5	14.30[b]	7.08[a]	1.04[b]	14.26[d]	14.74[bc]	63.22[bc]
		1	14.20[b]	7.21[b]	1.04[b]	12.41[a]	14.77[bc]	64.71[c]
		1.5	14.11[a]	7.30[c]	1.16[c]	13.38[cd]	14.56[bc]	63.21[bc]
		2	15.71[d]	8.31[d]	0.68[a]	13.27[c]	14.32[bc]	63.00[c]
	polythene	0	14.51[c]	7.11[ab]	1.24[d]	15.20[e]	14.75[bc]	61.92[a]
		0.5	14.30[b]	6.16[a]	1.03[b]	14.22[d]	14.70[bc]	63.24[bc]
		1	14.50[c]	7.21[b]	1.17[c]	12.48[a]	14.37[bc]	64.71[b]
		1.5	16.11[d]	8.30[d]	1.15[c]	13.30[cd]	14.06[bc]	65.31[d]
		2	15.74[d]	8.51[d]	0.66[a]	13.25[c]	15.38[c]	63.22[b]
12	Polyprop-ylene	0	14.47[b]	7.14[ab]	1.21[d]	15.22[e]	14.72[bc]	61.58[a]
		0.5	14.22[a]	7.18[ab]	1.00[b]	14.26[d]	14.69[bc]	63.82[bc]
		1	14.50[c]	7.21[b]	1.04[b]	12.52[a]	14.67[bc]	64.60[c]
		1.5	14.12[a]	7.30[c]	1.11[c]	13.31[cd]	14.56[bc]	64.66[c]
		2	15.64[d]	8.18[d]	0.63[a]	13.19[bc]	15.39[c]	63.00[c]

Means with same letters in a column are not significantly ($P>0.05$) different

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(B) METABOLIC ENERGY

Generally, energy values of dried and gamma irradiated mushrooms stored in the different packaging materials ranged 247.8- 284.9 Kcal./100g of dried mushrooms and are presented in Table 34.

The initial energy values ranged 272.7-283.1 and 273.1- 280.0 Kcal/100g for polythene and polypropylene respectively. There was no significant ($p>0.05$) effect of storage packs on the metabolizable energy. In terms of effect of gamma radiation, dose 2 kGy showed an apparent effect ($p<0.05$) on fat content which affected the energy content indirectly since it constitutes an integral part of the energy equation. After 3 months, energy ranged from 273.7-282.1 and 274.4- 284.6 Kcal./100g for polythene and polypropylene respectively. There were significant differences ($p<0.05$) in energy values obtained.

Storage for 6 months, gave values ranging 247.8-283.7 and 273.4-280 kcal./100g for polythene and polypropylene respectively. In polythene package, irradiation caused significant difference ($p<0.05$). However, polypropylene showed no significant difference ($p>0.05$) to irradiation effect. For storage for 12 month values, ranged from 273.3-284.9 and 272.0- 282.3 Kcal./100g for both packages respectively. Energy values due to irradiation showed significant differences ($p<0.05$) in both polythene and polypropylene.

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Table 34: Effect of irradiation and storage on the Metabolic Energy (Kcal/100g) of Mushrooms

Storage Time (month)	Package material	Applied Dose (kGy)				
		0	0.5	1.0	1.5	2.0
0	P1	280.4^{b}	279.9^{b}	278.9^{b}	283.1^{c}	272.7^{a}
	P2	279.9^{b}	280.8^{b}	279.7^{b}	276.5^{bc}	273.1^{a}
3	P1	273.7^{a}	279.3^{b}	282.1b^{c}	279.5^{c}	276.9^{c}
	P2	274.4^{a}	282.6^{c}	284.6^{c}	275.3^{a}	279.9^{ab}
6	P1	278.6^{b}	281.7^{b}	247.8^{a}	282.9^{b}	283.7^{b}
	P2	279.8^{b}	280.0^{b}	278.7^{b}	277.5^{b}	273.4^{b}
12	P1	280.0^{b}	279.9^{b}	280.0^{b}	284.9^{c}	273.3^{a}
	P2	279.0^{b}	281.8^{b}	278.8^{b}	282.3^{c}	272.0^{a}

Means with same letters in a row are not significantly (P>0.05) different

P1- Polythene P2- Polypropylene

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EXPERIMENT 20

DETERMINATION OF ELEMENTAL MINERAL CONTENTS OF STORED IRRADIATED MUSHROOMS UP TO 12 MONTHS AT 28-30 ^{O}C

Results of elemental analysis of gamma irradiated *P.ostreatus* are presented in Tables 35 and 36.

Macro elements

Sodium contents ranged from 14.00 ± 0.7 - 14.90 ± 0.8 mg/100g. There were significant differences (P<0.05). Potassium content was found to be in the range of 30.20 ± 0.5 - 33.10 ± 0.6 mg/100g were significantly different (P<0.05). Magnesium content was found to be in the range 1.27 ± 0.15- 3.53 ± 0.04 mg/100g. There were significant differences (P<0.05) observed. Calcium content was found to be 11.00 ± 0.4 - 12.53 ± 0.4 ± 0.03mg/100g. There were statistical difference (P<0.05) observed. Phosphorus content was found to be 6.11 ± 0.30- 6.41 ± 0.35mg/100g. There were significant differences (P<0.05) observed.

Micro elements as heavy metals

Copper content was found to be in the range 0.00 ± 0.00 - 0.02 ± 0.001 mg/100g. Statistical differences (P<0.05) were observed. Zinc content was found to be in the range 0.01 ± 0.002- 0.03 ± 0.001mg/100g. There was significant differences (P<0.05) observed Iron content was found to be in the range 0.29 ± 0.01 - 0.37 ± 0.1 mg/100g. There were significant differences (P<0.05) observed. Manganese content was found to be in the range 0.03 ± 0.001- 0.04 ± 0.01 mg/100g. There were significant differences (P<0.05) observed. Lead content was found to be 0.00 ± 0.00 - 0.03 ± 0.001mg/100g. There were

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statistical differences (P<0.05). Nitrogen content was found to be 3.00 ± 0.03 - 3.60 ± 0.25 mg/100g. There were statistical differences (P>0.05) observed.

Table 35: Effect of irradiation on the elemental composition of *P.ostreatus* before storage (0 months)

Element (mg/100g)	Dose (kGy)				
	0	0.5	1.0	1.5	2.0
Calcium	11.00±0.3[a]	11.03±0.4[a]	11.85±0.5 [b]	11.32± 0.3[b]	12.20±0.4[c]
Potasium	31.91±0.5[b]	30.20±0.5[a]	30.72±0.5[a]	32.84±0.6[c]	33.10±0.6[c]
Magnesium	1.77±0.18[a]	1.27±0.15[a]	2.40±0.05[b]	2.40±0.05[b]	3.53±0.04[c]
Nitrogen	3.51±0.02[c]	3.56±0.02[c]	3.31±0.04[b]	3.00±0.03[a]	3.59±0.05[a]
Zinc	0.03±0.001[b]	0.03±0.001[b]	0.01±0.002[a]	0.01±0.002[a]	0.01±0.002[a]
Manganese	0.04±0.001[b]	0.03±0.001[a]	0.03±0.001[a]	0.04±0.001[b]	0.04±0.002[b]
Lead	0.02±0.001[b]	0.02±0.001[b]	0.02±0.001[b]	0.03±0.001[bc]	0.00±0.00[a]
Phosphorus	6.10±0.35[bc]	6.14±0.35[b]	6.11±0.30[a]	6.27±0.25[b]	6.32±0.25[b]
Iron	0.37±0.1[c]	0.31±0.01[b]	0.35±0.0[c]	0.29±0.01[b]	0.35±0.03[c]
Sodium	14.10±0.7[a]	14.10±0.8[a]	14.61±0.8[b]	14.00±0.7[a]	14.90±0.8[bc]
Copper	0.02±0.001[b]	0.02±0.001[b]	0.01±0.00[a]	0.02±0.001[b]	0.00±0.00[a]

Values are means of duplicate results ± S.E

Means with same letters in a row are not significantly (P>0.05) different

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Table 36: Effect of irradiation on the elemental composition of *P.ostreatus* during storage up to 12 months

Element (mg/100g)	Dose (kGy)				
.	0	0.5	1.0	1.5	2.0 .
Calcium	11.02±0.3^{a}	11.00±0.4^{a}	11.75±0.5 ^{b}	11.34± 0.3^{b}	12.53±0.4^{c}
Potasium	31.91±0.5^{b}	30.20±0.5^{a}	30.72±0.5^{a}	32.84±0.6^{c}	33.10±0.6^{c}
Magnesium	1.77±0.18^{a}	1.27±0.15^{a}	2.40±0.05^{b}	2.40±0.05^{b}	3.53±0.04^{c}
Nitrogen	3.61±0.02^{c}	3.51±0.02^{c}	3.31±0.04^{b}	3.00±0.03^{a}	3.59±0.05^{a}
Zinc	0.03±0.001^{b}	0.03±0.001^{b}	0.01±0.002^{a}	0.01±0.002^{a}	0.01±0.002^{a}
Manganese	0.04±0.001^{b}	0.03±0.001^{a}	0.03±0.001^{a}	0.04±0.001^{b}	0.04±0.002^{b}
Lead	0.02±0.001^{b}	0.02±0.001^{b}	0.02±0.001^{b}	0.03±0.001^{bc}	0.00±0.00^{a}
Phosphorus	6.41±0.35^{bc}	6.34±0.35^{b}	6.11±0.30^{a}	6.27±0.25^{b}	6.32±0.25^{b}
Iron	0.37±0.1^{c}	0.31±0.01^{b}	0.35±0.0^{c}	0.29±0.01^{b}	0.35±0.03^{c}
Sodium	14.10±0.7^{a}	14.50±0.8^{b}	14.67±0.8^{b}	14.00±0.7^{a}	14.90±0.8^{bc}
Copper	0.02±0.001^{b}	0.02±0.001^{b}	0.01±0.00^{a}	0.02±0.001^{b}	0.00±0.00^{a}

Values are means of duplicate results ± S.E

Means with same letters in a row are not significantly (P>0.05) different

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EXPERIMENT 21

SENSORY ANALYSIS AND CONSUMER ACCEPTANCE TEST AND DESCRIPTIVE TEXTURAL ANALYSIS OF STEAMED IRRADIATED MUSHROOMS STORED UP TO 12 MONTHS IN POLYPROPYLENE AND POLYTHENE PACKS AT 28-30 ^{O}C

The mean scores for the acceptability attributes (appearance, colour, aroma, taste, mouthfeel and overall acceptability) and descriptive texture attributes (hardness, cohesiveness, adhesiveness, chewiness and smoothness) of steamed gamma irradiated *P.ostreatus* are presented in Tables 37 and 38. Mean values after analysis of variance (ANOVA) showed some significant differences (P<0.05) in some attributes.

Appearance recorded mean scores of range 5.6 ± 0.4 - 7.4 ± 0.4 which corresponded to dried mushrooms irradiated at 2 kGy and stored in polythene packs (code 667) and non-irradiated mushrooms (0 kGy) (code 808) respectively. Appearance for all samples showed no significant (P>0.05) difference.

Colour which is perhaps the most important organoleptic attribute, recorded mean scores of range 5.4 ± 0.3 - 7.4 ± 0.4 which corresponded to dried mushrooms irradiated at 1kGy and stored in polypropylene packs (code 753) and non- irradiated dried mushrooms. These samples (codes 753 and 808) differed significantly (P<0.05) from the other samples.

Aroma recorded mean scores of range 5.6 ± 0.4 - 6.7 ± 0.2 which corresponded to dried mushrooms irradiated at 2 kGy and stored in polypropylene pack (code 371) and dried mushrooms irradiated at 0 kGy (code 808) respectively. There was no significant difference (P>0.05) recorded.

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Mouthfeel recorded mean scores of range 5.3 ± 0.5 - 6.6 ± 0.4 which corresponded to both dried mushrooms irradiated at 2 kGy and 5 kGy, stored in polypropylene packs (codes 371 and 948) and non- irradiated dried mushrooms respectively. There was no significant difference ($P>0.05$) recorded.

Taste recorded mean scores of range 5.3 ± 0.5 - 7.1 ± 0.3 which corresponded to dried mushrooms irradiated at 2 kGy and stored in polypropylene pack (code 371) and dried mushrooms irradiated at 0 kGy (code 808) respectively. However, there was no significant difference ($P<0.05$) recorded.

Overall acceptability recorded mean scores of range 5.4 ± 0.5 - 7.3 ± 0.3 which corresponded to dried mushrooms irradiated at 2 kGy and stored in polypropylene pack (code 371) and non-irradiated dried mushrooms.

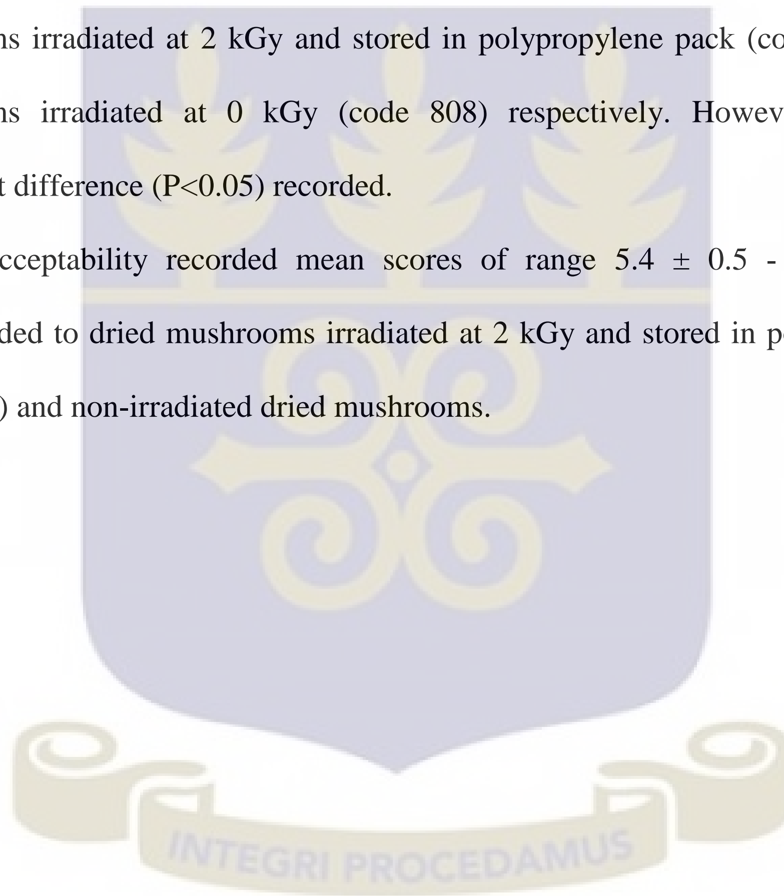

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Table 37. Mean scores of sensory analysis of gamma irradiated dried mushrooms stored in polythene and polypropylene packs

Samples	Appearance	Color	Aroma	Taste	Mouthfeel	Acceptability
371	5.9 ± 0.5^{a}	5.6 ± 0.5^{a}	5.6 ± 0.4^{a}	5.3 ± 0.5^{a}	5.3 ± 0.5^{a}	5.4 ± 0.5^{a}
412	5.9 ± 0.4^{a}	5.6 ± 0.5^{a}	6.1 ± 0.4^{a}	5.8 ± 0.3^{a}	5.7 ± 0.3^{a}	5.9 ± 0.4^{ab}
491	6.4 ± 0.3^{a}	5.8 ± 0.3^{a}	6.2 ± 0.4^{a}	5.7 ± 0.3^{a}	5.7 ± 0.4^{a}	5.8 ± 0.4^{ab}
512	5.7 ± 0.5^{a}	5.9 ± 0.4^{a}	6.1 ± 0.5^{a}	5.9 ± 0.4^{a}	5.7 ± 0.5^{a}	6.0 ± 0.5^{ab}
606	5.9 ± 0.3^{a}	5.4 ± 0.3^{a}	5.9 ± 0.2^{a}	5.4 ± 0.4^{a}	5.4 ± 0.3^{a}	5.5 ± 0.3^{a}
667	5.6 ± 0.4^{a}	5.9 ± 0.3^{a}	6.1 ± 0.4^{a}	5.8 ± 0.3^{a}	5.6 ± 0.3^{a}	5.6 ± 0.3^{ab}
753	6.5 ± 0.4^{a}	6.6 ± 0.3^{ab}	6.6 ± 0.2^{a}	6.3 ± 0.4^{a}	6.1 ± 0.4^{a}	6.8 ± 0.3^{bc}
808	7.4 ± 0.4^{a}	7.4 ± 0.4^{b}	6.7 ± 0.2^{a}	7.1 ± 0.3^{a}	6.6 ± 0.4^{a}	7.3 ± 0.3^{c}
837	5.8 ± 0.4^{a}	5.5 ± 0.3^{a}	5.9 ± 0.4^{a}	5.5 ± 0.4^{a}	5.4 ± 0.3^{a}	5.6 ± 0.4^{ab}
891	6.1 ± 0.4^{a}	5.9 ± 0.4^{a}	5.9 ± 0.4^{a}	5.7 ± 0.5^{a}	5.4 ± 0.3^{a}	5.7 ± 0.4^{ab}
948	6.1 ± 0.3^{a}	6.1 ± 0.3^{a}	5.8 ± 0.3^{a}	5.5 ± 0.5^{a}	5.3 ± 0.5^{a}	6.0 ± 0.4^{ab}

Means within a column with different superscripts are significantly different ($p<0.05$)

Codes to samples are provided on page 82 (Materials and General methods)

Mean scores of the descriptive texture analysis of irradiated and non-irradiated dried mushrooms stored in polythene and polypropylene packs are presented in Table 38.

Hardness recorded mean scores of range 5.6 ± 0.3 - 6.9 ± 0.4 which corresponded to both dried mushrooms irradiated at 1 and 3 kGy and stored in polythene packs (codes 891 and

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512) and the control (0 kGy coded 808) . However, there was no significant difference ($P>0.05$) observed in all the samples.

Cohesiveness (integrity) recorded mean scores of range 5.5 ± 0.3 - 7.4 ± 0.3 which corresponded to samples irradiated at 3 kGy and stored in polythene pack (code 891) and non-irradiated (0 kGy) sample (code 808) respectively. Non-irradiated samples significantly differed ($P<0.05$) from all samples.

Adhesiveness (stickiness) recorded mean scores of range 5.1 ± 0.4 - 6.6 ± 0.5 which corresponded to samples irradiated at 5 kGy and stored in polythene packs (code 491) and non-irradiated (0 kGy) (code 808) samples respectively. There was no significant difference ($P>0.05$) observed for all the samples.

Chewiness (stringiness) recorded mean scores of range 5.5 ± 0.3 - 7.3 ± 0.4 which corresponded to samples irradiated at 1 kGy and stored in polythene pack (code 512) and non-irradiated (0 kGy) (code 808) respectively. There was no significant difference ($P>0.05$) shown in all samples.

Lastly, smoothness recorded mean scores of range 5.4 ± 0.4 - 7.1 ± 0.4 which corresponded to samples irradiated at 2 kGy and stored in polypropylene pack (code 371) and non-irradiated (0 kGy) (code 808) respectively. There was no significant difference ($P>0.05$) shown in all samples.

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Table 38. Mean scores of descriptive textural properties of mushroom sensory analysis

Sample	Hardness	Cohesiveness	Adhesiveness	Chewiness	Smoothness
371	5.9 ± 0.4^{a}	5.6 ± 0.3^{a}	5.6 ± 0.4^{a}	5.7 ± 0.4^{a}	5.4 ± 0.4^{a}
412	5.9 ± 0.4^{a}	5.8 ± 0.4^{a}	6.0 ± 0.5^{a}	5.6 ± 0.5^{a}	5.7 ± 0.4^{a}
491	5.7 ± 0.3^{a}	5.8 ± 0.3^{a}	5.1 ± 0.4^{a}	5.7 ± 0.4^{a}	5.8 ± 0.4^{a}
512	5.6 ± 0.3^{a}	6.1 ± 0.3^{a}	5.5 ± 0.4^{a}	5.5 ± 0.3^{a}	5.6 ± 0.5^{a}
606	5.8 ± 0.4^{a}	5.8 ± 0.3^{a}	5.2 ± 0.3^{a}	5.7 ± 0.4^{a}	5.6 ± 0.4^{a}
667	5.9 ± 0.3^{a}	5.9 ± 0.4^{a}	5.7 ± 0.4^{a}	6.0 ± 0.3^{a}	6.3 ± 0.4^{a}
753	6.4 ± 0.4^{a}	6.1 ± 0.5^{a}	5.9 ± 0.5^{a}	6.5 ± 0.4^{a}	6.3 ± 0.3^{a}
808	6.9 ± 0.4^{a}	7.4 ± 0.3^{b}	6.6 ± 0.5^{a}	7.3 ± 0.4^{a}	7.1 ± 0.4^{a}
837	5.9 ± 0.3^{a}	5.9 ± 0.3^{a}	5.6 ± 0.5^{a}	6.4 ± 0.6^{a}	6.0 ± 0.4^{a}
891	5.6 ± 0.3^{a}	5.5 ± 0.3^{a}	5.3 ± 0.4^{a}	6.0 ± 0.3^{a}	5.6 ± 0.3^{a}
948	6.5 ± 0.4^{a}	6.4 ± 0.3^{a}	6.3 ± 0.4^{a}	6.2 ± 0.4^{a}	6.3 ± 0.4^{a}

Means within a column with different superscripts are significantly different ($p<0.05$)

Codes to samples are provided on page 82 (Materials and General methods)

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CHAPTER FIVE

DISCUSSION

Results obtained from the structured questionnaire survey (Fig.11) demonstrated the popularity of drum (moist heat) technique of sterilization both locally and internationally which agrees with findings by several researchers (Kortei, 2011; Obodai *et al.*, 2003; Owusu-Boateng and Dzogbefia, 2002; Gbedemah *et al.*, 1998). Majority (60%) of the respondents were not satisfied with their method of sterilization of compost while 30% found it alright. (Figs.12).

The outcome also gave an indication of a general unsatisfactory results attained from their methods and therefore their desire to improve the improper or incomplete sterilization resulting in partial or incomplete elimination of contaminants. Residual contaminants from incomplete pasteurization of compost and spawn substrates result in reduction in economic yield of mushrooms on compost substrates.

Interestingly, majority of the respondents (82%) had not heard about sterilization of mushroom compost using gamma irradiation (Fig.13) although gamma irradiation sterilizing technique has now gained some credence among mushroom farmers in Ghana. Inspite of this, some researchers (Kortei and Wiafe-Kwagyan, 2014; Gbedemah *et al.*, 1998) have reported success in the use of gamma radiation to decontaminate and depolymerize different lignocellulosics agrowastes for *P.ostreatus* cultivation thus achieving growth and yield results comparable to the moist heat (drum) technique. Thus gamma irradiation technique allows a greater quantity of sterilized compost bags per unit time, less laborious and better decontamination. This could probably allay the fears of

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majority of cultivators of mushrooms who expressed the need to achieve better sterilization technique for mushroom compost (Fig.15).

Shelf-life extension of mushroom is vital to the industry. The most common method for the preservation of mushrooms is drying since it is the most economical and the oldest method (Bala *et al.*, 2009). Data from this study showed that drying is the most popular preservation method of mushrooms in Ghana (Fig. 23). Several edible mushrooms are consumed by respondents but the most preferred was oyster mushrooms (*Pleurotus* spp.) (Fig.17). Afetsu (2014) reported similar results in a survey of mushroom consumption conducted in the Volta Region of Ghana which agrees with the results obtained in this study. The popularity of mushrooms to Ghanaians was shown in the survey where all respondents wanted an all year round supply (Fig.18).

Obodai *et al* (2010) attributed oyster mushroom's emerging popularity among Ghanaians to its probably easy method of cultivation. However, results obtained in this thesis (Fig.17) contrasts the report of Apetorgbor *et al* (2005) who found *Termitomyces* spp. as the most preferred mushrooms in the Eastern and middle belts of Ghana. Dijk *et al.*, (2003) made similar findings in South Cameroon where they very often consumed this mushroom. This difference in preference might be attributed to the availability of particular kinds of mushroom species, taste and/or medicinal attributes. Mushrooms have a myriad of benefits derived from its consumption. Mushrooms were found to have some medicinal values (Chipompha, 1994). The Asantes and Sefwis in Ghana believe that mushrooms lower blood pressure in hypertensive patients. The globular subterranean sclerotium of *P. tuber-regium* is chewed by local people to alleviate heart pains and the powder is taken in warm water to lower blood pressure in hypertensive patients (Sawyerr,

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2000). The medicinal value of mushrooms for Ghanaian consumers was evident from the questionaire survey (Fig.20). Completely colonized composted bags are readily available for sale at numerous mushroom farms and some research institutions such as the Food Research Institute of Ghana. With the current rate of deforestation caused by urbanization, bush fires and mining companies, the collection of wild mushrooms by the rural folk is greatly threatened leaving government protected areas (forest reserves) as the only remaining areas where non timberforest products can be collected (Meke *et al.*, 2000).

The depletion of our forest is a major cause of scarcity of most mushroom species (Apetorgbor *et al.*, 2005). Rigorous scientific research into bioconversion of lignocellulosic waste by mushroom is making it possible to cultivate different species of mushrooms all year round with specific emphasis on Jun-Cao technology which involves the use of plastic bag and grass as its substrate. This practice is advantageous since it is more efficient and does not require so much space. Small scale mushroom farms which have emerged in Southern Ghana as a result of the introduction of the National Mushroom Development Project aimed at promoting the economic welfare of rural communities (Sawyerr, 2000). Mushroom production is a demand driven enterprise and so requires the appropriate technologies to keep up with its supply. Consumers seem to prefer mushrooms on the basis of taste, appearance, texture or combination of these qualities (Fig.22). Although production of oyster mushroom is laborious, it is not very capital intensive (Fig.24).

Fungal flora

Results of the influence of gamma radiation and moist heat pasteurization on the relative abundance and total microbial population on the surface of the sorghum grains showed

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some significant ($p<0.05$) differences (Figs 25, 27 and 28). This observation could be attributed to the higher penetrating and killing effect of gamma radiation which cause injuries to the genetic apparatus of cells and ultimately death of microorganisms (Atique *et al.*, 2013; Adu-Gyamfi *et al.*, 2012; Adu-Gyamfi and Appiah, 2012).

Radiation sensitivity (the killing effect of radiation) in microorganisms is generally expressed by the decimal reduction dose or D_{10} value (Mohan *et al.*, 2011). From the calculated D_{10} values (Table 7), it was obvious that the fungal spores in the sorghum grains were radioresistant as values obtained were in agreement with published findings of Saleh *et al*. (1988) who reported that *A. alternata* was resistant to 1.15 – 1.39 megarad (11.5 – 13.9 kGy) and *A. flavus* was also resistant to 0.25 – 0.30 megarad (2.5 – 3.0 kGy) (D_{10} = 6.00 kGy) when isolated from corn seed. Conidia of *Aspergillus spp.* are multicellular, thick walled and for example *A.niger*, contain melanin and other pigments (Maity *et al.*, 2011). These pigments might have contributed to the increased resistance. Increasing doses beyond 10 – 20 kGy should augment the killing effect of gamma radiation on the fungal spores.

Steam sterilization was also effective in reducing the total fungal population. Results obtained agree with findings of Rico *et al* (2010) who observed a 1–2 log cycle reduction in initial microbial count of 10^6 CFU/g with steam, while gamma irradiation at 10 kGy resulted in a 5-log cycle reduction in same initial microbial count as they investigated the comparative effect of steam and irradiation on the physicochemical and microbiological properties of dried red pepper (*Capsicum annum* L.). Al-Bachir and Al-Dawi (2014) recently reported a 1– 2 log cycle reduction in total aerobic plate count with steam while

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a 4-log cycle reduction was recorded with 10 kGy dose of gamma radiation in the microbiological population of licorice (*Glycyrrhiza glabra* L.) root powder.

Phenology of mycofloral population

Various fungi isolated from non-pretreated (control) sorghum grains on both DRBC and Cooke's media included *Cladosporium macrocarpum, Trichoderma harzianum, Fusarium oxysporum, Rhodotorula spp., Penicillium spp., Aspergillus niger, Aspergillus fumigatus, Aspergillus alutaceus* and *Aspergillus flavus* (Figs 27 and 28).

The dynamics of a fungal community may be attributed generally to abiotic variables and nature of substrate (Thorman *et al*, 2003). Pretreatment of sorghum grains with either steam or radiation resulted in the disappearance and appearance of certain fungal species which was recorded as their percentage occurrence relative to the total number of species recorded. Antagonism between fungi according to Obodai and Odamtten, (2013) may be in the form of competition for nutrients, chemical antibiosis and lysis of mycelia. Antibiosis is the inhibition of one species of microorganism by the metabolic product of another. Although it is usually an inhibition of growth and sporulation, it may be lethal. Metabolites produced by antagonistic fungi penetrate the cell wall of the target and inhibit activity by chemical toxicity (Obodai and Odamtten, 2013). It is expected that the increasing radiation dose would augment the killing effect on fungal spores resident in the compost for bioconversion.

The vulnerability of microorganisms and their spores to gamma radiation has been well documented by many researchers (Adu-Gyamfi and Appiah, 2012; Diehl, 1995). Ionizing radiation produces chemical and physical changes in substrate which act in concert to inactivate microorganisms. The energy of ionizing radiation affects directly the microbial

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DNA (genome), causing the damage on fungal or bacterial cells. The ability of an organism to withstand a physical stress (gamma radiation and/or steam) depends on how quickly it is able to repair its damaged DNA as a result of denaturing (IAEA-TECDOC, 2005). Generally as expected on both growth media, fungal populations and species number decreased as gamma radiation doses increased. At gamma radiation dose 10 kGy, *A.fumigatus* (30%) and *Penicillium spp*. (70%) persisted. Only *Rhodotorula spp.* (100%) persisted at 15 kGy. Nonetheless, doses beyond 15 kGy recorded no fungi on DRBC. Similarly on Cooke's medium, *Rhodotorula spp.*(14.0%), *Penicillium spp*.(67.5%) and *Aspergillus fumigatus* (18.5%) persisted at 10 kGy. At 15 kGy, resident fungi *A.fumigatus* (10.0%) and *Rhodotorula spp*.(90%). Beyond 15 kGy no fungi survived except *Rhodotorula spp*. (100%). Steam sterilized sorghum grains harboured *Penicillium spp*. (77.0%), *Aspergillus fumigatus* (18.0%) and *Aspergillus alutaceus* (5.0%) enumerated on DRBC. While *Rhodotorula spp*. (11.54%), *Penicillium spp*. (34.62%), *Aspergillus niger* (11.54%) and *Aspergillus fumigatus* (29.9%) were enumerated on Cooke's medium. (Figs 27 and 28).

The variation in resistance of fungi to gamma radiation, steam, drought etc. may be attributed to several factors. The cell walls of some fungi may contain appreciable fractions of lipids (up to 20%) as in the case of some *Aspergillus* species. Some investigators postulated that filamentous fungi produce some metabolites, which may assist in the resistance to radiation. Secondly, the genetic composition may vary among fungi as well as their water content. Fungi may be susceptible or resistant to radiation depending on the free radicals produced by radiation. Furthermore, it is known that intracellular fungal components (sulfhydric compounds, pigments, amino acids, proteins

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and fatty acids) may probably be responsible for radioresistance of fungi (Aziz *et al.*, 1997). Aquino *et al.*, (2005) demonstrated a higher resistance of *A. flavus* to gamma radiation, which showed no growth after exposure to 10 kGy. Studies by Odamtten *et al* (1986, 1985) have shown that a combination of moist heat (60^oC for 5 mins) prior to radiation with 5 kGy synergistically increased the killing effect of gamma radiation on *A.flavus* spores.

The genus *Aspergillus* was the most dominant fungus among the fungi reported in this study. *Aspergillus* species are reported as a natural contaminant in cereals and also in many other agricultural commodities (Thakur *et al.*, 2006; Hocking, 2003). Sreenivasa *et al,* (2010) showed that sorghum was contaminated by nine species of *Aspergillus*. The predominant *Aspergillus* species isolated were *A. flavus* (72.7%) and *A. niger* (59.1%). Three *Aspergillus* species *A. alutaceus, A. versicolor* and *A. candidus* were recorded with a frequency of 20.5%. A low frequency of *A. sydowii* (2.3%) was also recorded. Surveys conducted worldwide also indicated that, *A. flavus* and *A. niger* are known to frequently contaminate peanuts and were able to produce mycotoxins such as aflatoxins (Syed *et al.*, 2013; Reddy *et al.*, 2010; Gassen, 1999). *A. flavus* contamination and aflatoxin production in sorghum are serious problems in most sorghum producing countries where the crop is grown under rain fed conditions (Klich, 2007). Fungi isolated in this present study were the same as reported by other researchers (Little *et al.*, 2012; Dass *et al.*, 2007; Ghiasian *et al.*, 2004) on sorghum grains. Future studies should examine their mycotoxigenic potential in-vitro and in-vivo.

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Effect of Moisture content (%) and pH on fungal flora

Low moisture content below a critical level (<30%), would decrease activities of microorganisms by restricting the germination capacity and make them dormant (Hubbe *et al.*, 2010). Moisture content which is too high (>65%) could cause oxygen depletion and losses of nutrients through leaching (Dougherty, 1998; Tiquia *et al.*, 1996). A higher contamination rate by bacteria has also been observed where there is excess moisture. Hence, a moisture content ranging between 30- 40% (Narh *et al.*, 2011) would be appropriate when cereal grains such as sorghum is being used as substrate for *P.ostreaus* EM-1 spawn production.

The pH values obtained in this study were within the optimal pH range for growth of *P.ostreatus* (Narh *et al.*, 2011). Obodai *et al.*, (2010) and Barcenas-Moreno (2011), indicated that pH range of 4- 6 favours good fungal growth and recolonization of substrates. The optimum pH for growth of fungi is related to species, strains, enzymatic activity, important vitamin influx into the mycelium and surface metabolic reactions (Narh *et al.*, 2011; Wheeler *et al.*, 1991). High pH tends to depress growth as well as antagonize fungi in compost reducing competition (Mandeel, 2005).

Rate of growth

The number of days from inoculation to the total colonization of a substrate is related to the mycelia growth rate on the substrate. A faster growth rate results in a corresponding reduction in the days required for complete colonization of the substrate by the mycelia (Narh *et al.*, 2011). The rate of growth of mycelium was recorded after mycelium inoculants grew down the sorghum grains after passage of time and resulted in varied responses (Appendix 3). The fastest rate of mycelia growth, 0.70 cm/day was recorded by

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a single treatment of gamma radiation of 15 kGy (I+I) on soaked raw sorghum. The various treatments under the 5, 10, 15, 20, 25 and 32 kGy sets of experiment showed no significant differences (P>0.05) (Appendix 3). This might be due to the fact that at 5 kGy dose level and beyond, there was effective decontamination of the sorghum grains which might have reduced the number of competitive microorganisms. According to Elhami and Ansari, (2008), the combined effect of metabolic activities and substrate oxygen concentration might be responsible for maximum mycelia growth. The results obtained in this study were in agreement with Fan *et al* (2000) who recorded 0.97 cm/day mycelia growth on sorghum. The slowest mycelium growth rate of 0.3 cm/day was recorded on raw non-irradiated and non autoclaved sorghum grains (nI) (Fig.29). This slow rate of growth of mycelium could be attributed to oxygen depletion and certain metabolites produced by competing microorganisms. Respiration rate is directly related to O_2 concentration of substrate (Mehravaran, 1993).

Time taken for complete colonization

Spawn running requires high humidity (80- 90%) and high temperature (25- 30°C) for the vegetative growth (Buah *et al.,* 2010). The shortest time of an average of 7 days was observed for the complete colonization of sorghum by *P. ostreatus* attained by treatment combinations which involved irradiation at 5 kGy to 32 kGy (Figs.30- 35). The time taken for complete colonization was not significantly different (P>0.05) within this dose range. The longest time of 11 days for complete colonization was observed for the non pretreated sorghum (nS+nA), (S+nI) and (nI+nI). Growth halted on the 8th day and rate was insignificant due to competition from contaminants which resulted in prolonged colonization time (Fig.29).

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Radiation treatment is known to affect the molecular size of polysaccharides, whether it is applied to a solution or in the solid state, although the decrease in molecular size is larger when the polysaccharide is in solution (IAEA, 1999) and cause a break up of polysaccharides into smaller units (Bouchard *et al.,* 2006; IAEA, 1999) for assimilation which speed up growth of mycelia. The number of days obtained during this study were comparable to data of Fan *et al,* (2000) who reported 9 days as fastest time for spawn run on sorghum grains at 24°C. Stanley and Awi- Awaadi, (2010) also recorded a slightly higher value of 10 days as fastest colonization time for *P. pulmoniarius* on white maize.

Contamination and Mycelium Density

Contamination of *P. ostreatus* spawn was apparent in non pretreated sorghum substrates which ranged between 50- 80% (Appendix 3). Pretreatment of sorghum substrates with 5 kGy recorded less contaminations of between 5- 10%. Contaminations appear in the spawn after an insufficient sterilization of the sorghum grains of cereal which act as substrate (Kurtzman, 2010). This circumstance permits contaminants to compete successfully with *P. ostreatus* for space and nutrients (Kurtzman, 2010). Several fungi have been found to produce a range of antibiotics, each produced under specific conditions which either restrict or inhibit some fungal competitors.

Among the various treatments, there were no notable differences in the density of mycelia (Appendix 3). The mycelia were dense on all the treatments except for non-pretreated (non steam or non irradiated). According to IAEA/RAS, (2001) and Bouchard *et al,* (2001), gamma radiation and moist heat are effective depolymerization agents for the breakdown of polysaccharides into simplest units of carbon, hydrogen and oxygen

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which were utilized by the mycelia. Narh *et al.,* (2011) observed no difference in mycelia density when they used moist heat to produce *P. ostreatus* strain EM-1 spawn on millet and sorghum.

Sawdust mycoflora

The dynamics of fungal community may be attributed generally to abiotic variables and nature of substrate (Thorman *et al.*, 2003). There was variation of the fungal species in the sawdust with respect to the period of composting. The total mycoflora population enumerated on Cooke's medium was comparatively higher than on Oxytetracycline Glucose Yeast Extract (OGYE) agar. This is expected as the nutrient composition is not the same in each medium used. The use of two different media also allows the isolation of a wider spectrum of resident mycoflora.

Generally, at the beginning of composting, there was a comparatively lower mycoflora population which suggested a minimal occurrence of conducive factors such as moisture content, relative humidity and a wide fluctuation in air temperature which could favour the growth and sporulation of fungi on the sawdust substrate (McTiernan *et al.*, 2003; Cruz *et al.*, 2002). Microbial activities however increased as the composting period progressed. There was an increase in temperature, moisture content, pH, relative humidity and electrical conductivity as a result of the activities of resident fungi which resulted in an increase in metabolic activities to produce enzymes to decompose the lignocellulosic materials. The enzymatic activities in compost piles are effective indicators for stress or adaptive practices of the microorganism to different environmental conditions, particularly to feed stock sources. Various hydrolytic enzymes can control the rate of decomposition of complex polymers during composting (Umsakul *et al.*, 2010).

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Obodai *et al* (2010) also suggested that environmental and nutritional conditions created during composting might have selectively favoured certain fungi to the detriment of others. This present trend in this thesis is in agreement with findings of some researchers (Hubbe *et al.*, 2010; Obodai *et al.*, 2010).

Effect of pretreatment with gamma radiation and steam on mycofloral population of sawdust substrate

Both irradiation and steam pasteurization of pretreatment were effective in reducing the mycofloral population of sawdust. The reduction in population of resident fungi in the sawdust was commensurate with the dose applied. The higher the dose applied, the greater the reduction in mycoflora population although doses 10 and 15 kGy did not differ significantly (P>0.05) in their effect on mycoflora. However, doses ranging from 20- 32 kGy significantly reduced the mycoflora population by 3.96- 4.76 log cycles (Fig. 37). Presumably, the killing effect of gamma radiation on the fungi followed two mechanisms of action; firstly by indirect action of producing free radicals through the ionizing energy and secondly by causing direct disruption of the DNA strand which result in injury to the cell (Abdel-Kader, 1986).

Steam/moist heat treated composted sawdust at 100°C reduced mycofloral populations by 2.20 and 1.10 log cycles on both Cooke's and OGYE media respectively. The effectiveness of moist heat in reducing mycofloral populations were comparable (P>0.05) to gamma radiation doses of 5, 10 and 15 kGy on both media. However, doses 20, 25 and 32 kGy were significantly more effective in reduction of fungal load by an average of 3.96- 4.76 log cycles. Reduction by these doses differed significantly (P<0.05) from moist heat treatment. Thus, gamma radiation treatment up to 32 kGy is a viable and

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effective pasteurization treatment of compost prior to spawning (Fig.37). It was observed that in steam sterilized compost, there were a residual fungi (*A.niger, A.flavus, A.fumigatus, A.alutaceus, Fusarium oxysporum, R. stolonifer* and *Mucor racemosus*) which may have presumably contributed to antibiosis against *P.ostreatus*. Indeed, recent studies by Wiafe- Kwagyan, (2014) have shown the antagonist potential of these fungi not excepting *Trichoderma sp*. against *P. ostreatus* and *P. eous.*

Percentage (%) occurrence of microorganisms during composting of sawdust substrate

Various microorganisms were involved in the composting of agricultural wastes and these include fungi and bacteria which are thermophiles (heat loving) due to the temperature rise above 30°C in the compost (Fig.43) mesophiles (moderate temperature loving) and tolerant mesophiles were also present. Results obtained imply relatively large numbers of fungi were involved in the decomposition of the *T.scleroxylon* sawdust compost to make it suitable for the growth of mushrooms as diversity encourages alteration of substrate, chemically or physically (Chiu *et al.*, 2001; Tripothi and Yader, 1992) for utilization by the mushroom.

The genus *Aspergillus* predominated over the others in all composting period of sawdust. Several members of this genus have been reported by several researchers (Pandey and Sinha, 2008; Thakur *et al.*, 2001) to be capable of hydrolyzing the β-(1-4)-glucosidic linkage in the cellulose chain. According to Obodai and Odamtten (2013), there is the production of metabolites in the compost during fungal growth which penetrate fungal walls and inhibit activity by chemical toxicity. Antagonism among fungi may be in the

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form of competition for nutrients, chemical antibiosis and lysis of mycelium. Antibiosis is the inhibition of one microbe by the metabolic product of another. Although it is usually an inhibition of growth and sporulation, it may be toxic. Lysis is destruction and decomposition of biological materials by enzymes of the antagonist. Fungal phenology observed in the compost may be partly attributed to antibiosis and lysis of mycelium. Fungal species occurrence was erratic probably because the environmental and nutritional conditions created during composting selectively favored certain fungi to the detriment of others.

Radiation Sensitivity (D_{10} values) of fungi on sawdust

Lower D_{10} values indicate greater sensitivity of the organism to radiation treatment. Gamma radiation doses achieved significant reduction in mycoflora during pasteurization of 'wawa' sawdust for mushroom production (Table 9). Saleh *et al*. (1988) reported D_{10} values of *Aspergillus niger* and *Curvularia geniculata* to be 4.20 kGy and 24.2 kGy respectively, isolated from air. Results in this study were within range of values reported by Addo (2008) for D_{10} values of fungi on irradiated flour (Hausa koko) (i.e sorghum flour for preparation of local porridge in Ghana). The differences in D_{10} values obtained on OGYE and Cooke's media could again be partly attributed to differences in the nutritional richness of the two media.

Mycelia growth

The spawn run period is calculated as the number of days from inoculation to complete colonization of the compost bag by the mycelium (Obodai and Vowotor, 2002). The various substrate formulations and their interactions had different nutrient compositions

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and so resulted in different growth responses as reported by other workers (Kortei, 2008; Vertayasuporn, 2006). On the average, three days after inoculation, observable whitish mycelia began to grow on the bagged substrates from the grains of inocula. The mycelia grew down the substrates with the passage of time until colonization was fully or partially completed within an average of four weeks.

Linear growth of *P.ostreatus* mycelia down the substrates was similar for all the growing time (weeks) and thus showed no significant (P>0.05) difference for treatment permutations of 5- 32 kGy with final average linear growths of 190.2- 248.8 mm (Table 10). However, a permutation of unirradiated 0 kGy and non- steam sterilized substrates performed extremely badly recording final average growth of 12.0- 15.0 mm (Table 10). This was statistically different (P>0.05) from all the treatment doses. This poor performance could be attributed to the presence of other competitive residual mycoflora such as *Aspergillus spp., Trichoderma viride, Mucor racemosus, Rhizopus stolonifer, Fusarium oxysporum* etc (Obodai *et al.*, 2010; Obodai, 1992) which might have suppressed the development of the mycelia of *P. ostreatus*.

Colonizing time and rate of growth

The time of colonizing and rate of growth of mycelia is directly related to adequate nutrient availability in the substrate to effectively utilize ligninocellulosic materials and is conjectured to have affected the growth and development of *P. ostreatus* mycelia (Kortei, 2008; Wong *et al.*, 2006). Variation in C: N ratio of medium is reported by Wong *et al.*, (2006) to affect growth performance of *P.ostreatus*.

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Primordia emergence

Time of primordial emergence is also related to the vigour of the mycelia. Thus weak mycelia results in delayed time of primordial emergence. The average time difference for primordial emergence was 0- 4 days after complete colonization of substrates. The time difference for emergence of primordia was within the same range as found by some other workers (Shah *et al.*, 2004; Obodai *et al.,* 2003) who carried out comparative study on the growth and yield of mushroom on different lignocellulosic by-products.

Mycelia density, percentage (%) contamination

Mycelia vigour is directly linked to optimal nutrients, pH, temperature and other physic-chemical properties of the substrate. Generally, all substrate combinations of the various dose experimental set up (5 kGy, 10 kGy, 15 kGy, 20 kGy, 24 kGy and 32 kGy) resulted in visually good mycelia which ultimately produced mushrooms. Conversely, the substrate combinations of the unirradiated/unsteamed 0 kGy set up resulted in the poorest mycelia growth and the largest number of contaminations (Table 10). Presumably, nutrient depletion and an increase in temperature and other metabolites due to antibiosis from the other resident fungi such as *Aspergillus*, *Cladosporium*, *Mucor*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Rhizopus* and *Trichoderma* (Obodai *et al.*, 2010) might have acted in concert to inhibit mycelia growth.

Effect of pretreatment on cellulose, hemicellulose, lignin and silica

The biochemical parameters monitored over the period of decomposition and after the pretreatments were lignin, cellulose, hemicelluloses and silica. The composted sawdust substrate treated with gamma radiation of 24 kGy had the highest concentrations of hemicelluloses, cellulose, lignin and silica as compared to the steam and unirradiated

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(Table 11). The change in biochemical properties of composted sawdust could be attributed to the weakening of the intermolecular bonds of the polysaccharides to make available the oligo, di and mono saccharides and xylans hence their increase. Ionizing radiation possesses the unique ability to enhance depolymerization and/or crosslinking reactions without the need to add exogenous toxic chemicals (Betiku *et al.*, 2009). The use of gamma radiation to sterilize compost will therefore have an advantage over steam sterilization.

Effect of pretreatment on lignocellulose content of sawdust

Pretreatment involves the alteration of biomass so that (enzymatic) hydrolysis of cellulose and hemi-cellulose can be achieved more rapidly and with greater yields. Possible goals include the removal of lignin and disruption of the crystalline structure of cellulose (Hubbe *et al.*, 2010). Biodegradation of untreated natural lignocellulosic biomass is very slow, giving rise to the low extent of degradation, often under 20% (Fan *et al.*, 1980). This low rate and extent of conversion hinder the development of an economically feasible hydrolytic process (Betiku *et al.*, 2009).

Increasing doses of gamma irradiation affected these bonds and caused the van der Walls forces to weaken resulting in the degradation of cellulose and increasing degradability of the cell wall constituents or depolymerizes and delignifies the fiber (Choi *et al.*, 2009; Eggeman and Ellander, 2005).

Effect of composting period on Temperature, pH and moisture profiles of sawdust

At any day of the composting period (0, 4, 8, 12, 16, 20, 24 or 28 days), the species of fungi which appeared, varied depending on the temperature, pH and moisture content of the medium. However, turning of compost was done every four days throughout the composting period resulting a near homogeinity in temperature ranging from 45- 56 ^{o}C

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(Fig.43). This turning process was neccessary to ensure homogeneity of the compost mixture. Results obtained in this study fell within range of high temperatures (55–75°C) reached by thermophilic fungi during composting as reported by McGregor *et al.,* (1981). This rise in temperature was responsible for reducing the number of pathogens.

The observed changes in pH could probably be due to rapid breakdown of soluble and easily degradable carbon sources (Beffa *et al.*, 1998). According to Tchobanoglous *et al,* (1993), initial pH range of 7- 7.5 is recommended as it aids in the production of lactic acid and acetic acids during initial degradation of biomass. Interestingly, after 20 days of composting the pH value of 9.17 was recorded. Hubbe *et al.*, (2010) stated that in the thermophilic stage of composting, the pH could rise to pH 9.0 resulting in the release of ammonia, and thereafter the pH returns to near neutral conditions as the compost become mature. The pH of the gamma irradiation treated substrates also drifted to basic side of pH scale (8.03- 8.83) (Tables 12 and 13).

The moisture content of the composted sawdust samples ranged from 58.2- 65.8% (Table 12). The irradiation pretreated sawdust had moisture content values of 61.8- 64.1% (Table 13). Moisture is important in composting processes for two reasons: it facilitates substrate decomposition through mobilizing microorganism activities and also provides better conditions for nitrogen fixation in the compost. A low moisture content below a critical level (<30%), would decrease activities of microorganisms by restricting their motility and make them dormant (Hubbe *et al.*, 2010).Under drier conditions, the ammonium and ammonia present generate a higher vapour pressure; thus conditions are more favorable for nitrogen loss. On the other hand, too high moisture content which is (>65%) could cause oxygen depletion and loss of nutrients through leaching (Dougherty,

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1998; Tiquia *et al.*, 1996). The moisture content values were within the range required for growth and development of mycelia of *Pleurotus ostreatus* (OECD, 2005).

Yield and yield attributes

In Experiment 8, yield and yield attributes (Total fresh weight, economic yield, biological efficiency, cap and stipe dimensions) were evaluated on pretreated composted sawdust. The fruit body is the fleshy edible part of fungi. Flushing (amount of fruit bodies produced per batch) was observed for 8 weeks after incubation. The maximum number of mushrooms produced per flush was 748g from (S+I) of the 24 kGy set. The minimum number of mushrooms per flush 0g was attained from the (S+nI) of the 0 kGy set. Generally, production decreased with increasing flush numbers. This could be attributed to the lignocelluloses depletion and accumulation of metabolites in the substrates (Kortei, 2011).

Total fresh weight/ Economical yield

The total fresh weight of mushrooms or economic yield is the proportion of the fresh mushrooms to the wet weight of the substrate. It was recorded from four flushes of cropping period. They were statistically significant (P<0.05) variation in the total fresh weights or economic yield with different permutations among the different sets of experiments (Fig.44). The maximum total fresh weight was 1779g recorded from steam sterilized spawn and steam sterilized sawdust compost bag (S+S) of the 32 kGy set of experiment (Table 14). The high yield could be attributed to the comparatively better availability of nitrogen, carbon and minerals from this substrate (Shah *et al.*, 2004). On the other hand, the minimum total fresh weight of mushrooms or mushroom yield was 0g

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(no fruiting) recorded by permutations; steam sterilized spawn and non steam sterilized compost bag (S+nS) and the irradiated spawn and non steam sterilized compost bag (I+nS). Generally, one can surmise that irradiated sawdust compost bags produced comparable yields as steam sterilized sawdust (Table 14) as reported by several other researchers (Mshandete *et al.*, 2011; Mondal *et al.*, 2010; Patil *et al.*, 2010).

Biological Efficiency and Biological yield

Biological yield refers to the measure of total fresh weight to the dry weight of substrate, while biological efficiency is expressed as a percentage of the proportion. The maximum biological yield and efficiency (0.99 kg/kg of dry weight and 99.8% B.E) were recorded by steam sterilized spawn and steam sterilized sawdust compost bag (S+S) and also the 32 kGy set of experiment. The minimum biological yield and efficiency (0g per flush, 0% B.E) were recorded by the permutations steam sterilized spawn and non steam sterilized sawdust compost bag (S+nS) and irradiated spawn and non steam sterilized compsting bag (I+nS) (Table 14). Interestingly, biological yield and efficiency of these substrates were within range recorded by Mshandete *et al* (2011), Hasan *et al* (2010) and Obodai *et al*, (2003). Gamma irradiation is therefore an equally efficient alternative to steam sterilization which can help boost flushing and Biological Efficiency on sawdust compost by *P.ostreatus*.

Total number of primordial

The maximum number of primordial was 384 recorded by combination of irradiated spawn and irradiated composted sawdust bag (I+I) of the 24 kGy set (Table 15). The minimum recorded by steamed spawn and non steamed composted sawdust bag (S+nS)

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was 4 from the 0 kGy set (Table 15). Thriving primordia ultimately becomes fruit bodies, if there is a balance of carbon to nitrogen (C: N) ratio (Hubbe *et al.*, 2010). This is what presumably existed in the gamma irradiation treated composted sewdust resulting in abundant primordial formation and good yield of fruit bodies.

Total fruit bodies

The maximum fruit bodies recorded was 379 by the (I+I) set of the 32 kGy set. Effective fruiting may be due to the presence of glucose, fructose and trehalose in the substrate as reported by Kitamoto *et al* (1995). Poppe (1973) showed that Indole Acetic Acid (IAA) increases the number of fruit bodies in mushrooms. Although the carbohydrate content (glucose, fructose, trehalose) content of substrate and the role of IAA on fruiting of *P.ostreatus* were not followed in this studies, it would be worthwhile to investigate their roles in production of fruitbodies in future studies.

Cap diameter and Stipe length

In the present study, the longest cap diameter (76 mm) and stipe length (67 mm) were from the combination of irradiated spawn and irradiated composted sawdust (I+I) of the 24 kGy set. The shortest cap diameter (25 mm) and stipe length (21 mm) were from the irradiated spawn and non-irradiated composted sawdust bag (I+nI) of the 0 kGy set. (Table 15). These results are in agreement with the findings of Raymond *et al* (2013), Ajonina and Tatah (2012), Mshandete, (2010) and Owusu-Boateng and Dzogbefia, (2005). The cap diameter and stipe length play important roles in the valuation of economic production and pricing of oyster mushrooms for sale. The bigger the fruit bodies, the higher the marketing value.

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Mushroom size

According to researchers (Kurtzman, 2010; Reyes *et al.*, 2009), interactions between environmental factors and nutrients in mushroom growth substrate have been reported to play important role in inducing formation of the fruiting bodies which resulted in mushroom size variations. Results obtained in this study were within the range reported by Raymond *et al* (2013). Furthermore, big mushroom sizes are advantageous to farmers if product for sale is to be priced by weight of fruitbodies instead of volume.

Relationship between yield attributes and economic yield

A positive linear relationship was observed between economic yield and effective fruiting body (Fig.45). This suggests that economic yield was directly proportional to the number and size of cap and stipe of effective fruiting body and more than 98% (R^2= 0.981) of variation in the economic yield may be explained by variation of number of effective fruiting bodies harvested.

Drying curves

Drying curves from the drying experiment is shown in Figure 47. Exposure to radiation influenced rate of moisture loss in the mushrooms during drying, such that irradiated slices dried faster than the "control" (0.0 kGy). The mushrooms exposed to γ-radiation, lost moisture directly corresponded to radiation dosage, with those exposed to high levels of gamma rays drying faster. (Fig.47).

There was an inverse relationship between relative humidity with the increase of ambient air temperature (Fig.46) depending on time of day. Results obtained in this study were

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within the range of values obtained by other researchers (Bala *et al.*, 2009; Bala and Mondal, 2001) who also used the solar drying of mushrooms in a solar tunnel dryer.

High rate of moisture loss in irradiated mushroom is advantageous and may be attributed to the breakdown of tissue structures. Upon exposure to γ-irradiation, chitin, which is the main structural carbohydrate in mushrooms depolymerizes, resulting in loss of firmness (Akram *et al.*, 2012). Consequently, resistance to moisture migration towards the surface of the product reduces. This observation agrees with the suggestion that food structure is influential in determining moisture transport within food materials (Labuza and Altunakar, 2007). The drying curves (Figs. 48- 52) showed no constant rate period, suggesting that diffusion is the dominant mode of moisture removal from the mushrooms (Srikiatden and Roberts, 2006). This observation agrees with earlier findings for other products such as leafy vegetables (Akonor and Amankwah, 2012), eggplant (Doymaz and Gol, 2011) and *Agaricus spp*. (white button mushrooms) (Wakchaure *et al*., 2010).

Non-Linear Regression modeling

Table 16 summarizes the outcome of the non-linear regression modeling using five thin layer drying models, and these were compared based on their R^2, X^2 and *RMSE*. All five models showed very good fit ($R^2 \geq 0.9$) to the experimental data. Nevertheless, the Page and Diffusion models were the best to describe drying kinetics of mushrooms under the different experimental conditions. Drying kinetics of slices exposed to lower radiation dosages (0.5 – 1.0 kGy) were similar to the “control”. Under these experimental conditions, the Page’s model gave the highest R^2 and lowest X^2 and *RMSE* and best suited its description.

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The Diffusion model best predicted drying behaviour of mushrooms exposed to γ-radiation in excess of 1.0 kGy. Drying characteristics of mushrooms slices from this group were therefore dissimilar to the earlier group, which includes the "control". Drying characteristics of mushroom slices in this study were different from observations made in some previous studies. In these other studies, drying characteristics were best described by Logarithmic (Wakchaure *et al*., 2010; Tulek, 2011) and Wang and Singh models (Arumuganathan *et al*., 2009). Differences in variety and or processing conditions may account for these contrasting outcomes and underscores the role of variety, and model in determining best predicted drying behaviour. These models used in this thesis (Figs.48-52) showed very good fit between the experimental and predicted moisture ratios, confirming the suitability of these models for describing solar drying of γ-irradiated *Pleurotus ostreatus* mushrooms.

Effective Moisture Diffusivity

The effective moisture diffusivity (D_{eff}) describes the rate of moisture movement in food (Okos *et al*., 2007). D_{eff} varied between 1.88 and 2.44 x $10^{-08}m^2/s$ for the control and mushrooms treated with 2.0 kGy of ɣ -rays. The moisture diffusivity in the differently treated mushrooms showed an increase with increasing dosage of irradiation (Fig 53).

Differences ($p<0.05$) in effective diffusivities may be attributed to the extent of tissue disruption that may have occurred in mushrooms as a result of irradiation. Gamma irradiation causes breakage of fibrous structure and enlarges the pores therein (Akram *et al*., 2012) thus facilitating moisture removal. High diffusivity values as a result of increasing radiation exposure further emphasizes the enhancement of moisture removal by this processing technology. D_{eff} results obtained in this study were comparable to the

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generalized range of 10^{-9} – 10^{-12} for most foods (Labuza and Altunakar, 2007), higher than 9.62 – 1.56 x $10^{-09}m^2/s$ reported for oyster mushroom (Tulek, 2011) and 1.55 – 4.02 x $10^{-09}m^2/s$ reported for milky mushrooms (Arumuganathan *et al*., 2009) but lower than 9.21x $10^{-08}m^2/s$ to 1.49x$10^{-07}m^2/s$ for white button mushrooms (Wakchaure, 2010). These variations are likely to result from varietal and conditional differences adopted in the various studies. The increase in moisture diffusibility in irradiated mushroom fruiting bodies underscores its use in pre-treatment prior to drying for effective preservation in storage packs.

Effect of gamma radiation on the colour intensities of fresh mushrooms

According to Aktas *et al*., (2011), decreasing of the L* values in these applications supported the decreasing of b* values i.e occurring of slight browning which is attributed to non enzymatic browning (Kotwaliwale *et al*., 2010). Whiteness is known to be affected by physico-chemical properties and pretreatments (Matser *et al*., 2000).

There was a general decrease in metric chroma (C) values which suggests an increased activity of enzymes to degrade melanin caused by radiations. Goncalves *et al*., (2007) reported that lower (C) value indicates an increase in tonality of the mushroom colour. This observation contrasts findings of Sasnauska *et al*., (2011) who reported an increase in metric chroma values of apples during storage. The hue angle range was within the 90° region which suggests an apparent reddish yellow colour (Pedisic *et al*., 2009). Browning index was also low which gives an indication of a slow rate of occurrence of enzymatic browning (Tables 17- 19). Colour is an important parameter used by consumers in

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purchasing mushroom products and so slow rate of browning in irradiated mushrooms is useful.

The BI values obtained in this present, were within range reported by other researchers (Wan- Rosli and Aishah, 2011; Kotwaliwale *et al*., 2010) who investigated changes in textural and optical properties of oyster mushroom (*Pleurotus spp.*) during hot air drying such as colour, textural properties, cooking characteristics and fibre content of chicken patty added to oyster mushroom (*Pleurotus sajor-caju*) respectively. However, Mami *et al*., (2012) recorded higher BI values for effect of gamma radiation on colour of *Agaricus bisporus* during storage.

Total colour change in fresh and dried mushrooms

The overall colour change after 5 days and 12 months storage are presented in Tables 19 and 21. Generally, an interaction of gamma radiations and the two storage packs caused a decrease in lightness and browning of the dried mushroom samples.

Total colour difference ΔE, which is a combination of parameters L*, a* and b* values, is a colorimetric parameter extensively used to characterize the variation of colours depending on processing conditions (Maskan, 2001). In this study, ΔE values were calculated in relation to the initial (0 month) period of storage (control mushroom). A larger ΔE indicates greater colour change from the reference mushroom sample (Saricoban and Yilmaz, 2010). Generally, lower values of ΔE were recorded as the overall colour difference and this could be due to the absence of water in the capillary voids of the dried mushrooms which usually transport the enzymes to catalyze the oxidation of phenolic substrates into quinines (Jolivet *et al*., 1998). These products then undergo subsequent reactions leading to the formation of dark pigment melanin (Weijn *et al*., 2011). These ΔE values obtained in this present studies were lower than that reported

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by Aktas *et al* (2011) who found higher ΔE values when they studied the influence of pretreatments and different drying methods on color parameters and lycopene content of dried tomato.

Textural Characteristics

There are two ways of ascertaining textural characteristics. One is by mechanical estimation using sophisticated instruments. The second is by descriptive means using trained taste panelists. Influence of irradiation and storage on the texture of fresh, dried and rehydrated mushrooms were studied next by instrumentation in Experiment 12. Texture results from complex interactions between food components, and is affected by cellular organelles and biochemical constituents, water content as well as cell wall composition. Hence, external factors affecting these qualities can modify texture (Guiné and Barroca, 2011). The changes in texture occurring during the processing of mushroom materials or certain physiological events have been related with tissue and cell micro-structural changes (Marsilio *et al.,* 2000). The effect of processing treatments such as irradiation, dehydrating and rehydrating had significant ($P< 0.05$) effect on the textural parameters of the *Pleurotus ostreatus* mushrooms

Hardness

The hardness of samples taken from irradiated fresh, dried and rehydrated mushrooms using a texture meter are presented in Fig.56. This property corresponds to the maximum force recorded during the first cycle of compression, and represents the force required between the molars for chewing food, being in most cases related to the tensile strength of the sample. Hardness changes observed might be due to the hydrolytic effect of gamma radiations on the cell membranes. Mami *et al* (2013), Gbedemah *et al* (1998),

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Giri and Prasad, (2013) recorded higher values of between 96- 108N of hardness in their work. While Kotwaliwale *et al*, (2007) recorded lower values ranging between 6- 8N.

Fracturability

Not all products fracture; but when they do, the fracturability point occurs where the plot has its first significant peak (where the force falls off) during the probe's first compression of the product (Fig.10). This is suggestive of increased gamma radiation doses having an apparent effect of protein and polysaccharide degradation, hyphae shrinkage, central vacuole disruption and expansion of intercellular space (Zivanovic *et al.*, 2000). In this study, irradiated dried mushrooms required a force of 14.8- 15.9N to fracture while fresh and rehydrated mushrooms required lower forces of 0- 1.7N which decreased with increasing doses (Fig.57).

Cohesiveness

Cohesiveness represents the ratio between the work done in the second compression and the work done in the first compression, and reflects the ability of the product to stay as one. Results obtained in this work (Fig.58) are in agreement with some researchers (Parentelli *et al.*, 2007; Kotwaliwale *et al.*, 2007; Zivanovic *et al.*, 2000) who recorded an increase in cohesiveness after rehydration. This trend has been explained by an increase in chitin content and formation of covalent bonds between chitin and β-glucan, increasing rigidity of the hyphal wall (Zivanovic *et al.,* 2000).

Chewiness

Chewiness represents the energy required to disintegrate a solid material in order to swallow it. Dry mushroom gave the highest chewiness (5- 16 N). Fresh mushrooms

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recorded a lower chewiness ranging from 0.12 N (Fig.59). The same was true for rehydrated mushroom. Values recorded for fresh mushrooms in this study were consistent with results of Santos *et al* (2012).

Springiness

Springiness is the ratio between the times in the two deformations, and represents the ability to regain shape when the deforming stress is removed or reduced, i.e., expresses the percent of recovery of the sample. The ability of the fresh and rehydrated mushrooms to regain their shapes after the stress is reduced is suggestive of the presence of moisture in the cells of the mushrooms which causes turgidity to aid in strengthening the cell walls. The springiness results for fresh and rehydrated mushrooms in this current study were similar to data of Riebroy *et al.* (2010). Conversely, dried and irradiated mushrooms were depleted of moisture to a stage that capillary voids were created hence the recorded reduced springiness. This same trend was reported by Kotwaliwale *et al* (2007).

Gumminess

Gumminess is mutually exclusive with chewiness since a product would not be both a semi-solid and a solid at the same time. It is a product of hardness and cohesiveness (which is Area 2/Area1) (Figs.10 and 61). Increase in gamma radiations might have caused a weakening of the intermolecular bonds and so increased radiation dose decreased gumminess and the value was lower for fresh and rehydrated mushrooms (Gunes *et al.*, 2001; IAEA, 1999).

Adhesiveness

Adhesiveness represents the work necessary to overcome the forces of attraction between the sample and the probe surface, and is given by the value of the area corresponding to

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the negative force (A3 in Fig. 10). Adhesiveness was low or negligible for dried irradiated mushrooms while the rehydrated mushrooms recorded the highest values at all doses (Fig.62). The values obtained compare favourably with the report of Guine and Barroca (2011) and Riebroy *et al* (2010). It is not surprising that dry samples had low adhesiveness because of loss of water but rehydration restored this parameter at the different doses applied.

Resilience

Resilience is how well a product "tries stretching to regain its original position after deformation". The calculation is the area during the withdrawal of the first compression, divided by the area of the first compression. (Area 5/ Area 4 on Fig.10). The ability of fresh and irradiated mushrooms to exhibit high resilience (Fig.63) could be ascribed to its original and unaltered cell membrane as it has not undergone senescence (Beelman *et al.*, 1987). In this study, recorded values agreed with the data of Riebroy *et al*, (2010) for oyster mushrooms. Dehydration has a very pronounced effect on the structure of foods, due to the loss of a considerable amount of water, increasing density by shrinkage in the case of convective drying. Apparently, rehydrated mushrooms could not regain their original shape after subjection to stress probably because water molecules could not fill all the pore spaces of the cell membrane and so turgidity was minimal.

According to Ares *et al* (2007), mushrooms are only protected by a thin and porous epidermal structure, lacking the specialized epidermal structure of higher plant tissues. This epidermal layer permits a quick superficial dehydration which causes important quality losses (Singer, 1986). Post harvest senescence in a variety of horticultural commodities is accompanied by changes in cell membrane characteristics, which lead to

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loss of barrier function and loss of turgor (Mazliak, 1987). Softening of mushrooms and firmness loss during postharvest storage has been ascribed to membrane changes which are directly linked to moisture content of cells (Marsilio, 2000; Waldron *et al*., 1997; Beelman *et al*., 1987).

Microbiological Analysis of fresh and dried irradiated mushrooms before and after irradiation and storage in two packaging materials

Microbial counts showed an increase after 5 days storage in both packaging materials (Tables 23- 26). High aerobic mesophilic counts found in samples according to Najafi *et al* (2012), may reflect poor handling, inappropriate processing or a general lack of hygiene. The results obtained for total aerobic mesophile count were in agreement with findings of Kamal *et al*, (2011) who recorded average counts of 10^6 on fresh oyster mushrooms collected from Sutrapur Dakar city in India. In this present study *Staphyloccocus aureus*, *Salmonella spp, E.coli* and coliforms were not recorded. This contrasts the work of Beraha *et al* (1961) who recorded 27, 13, 13 and 7% respectively in the case of fresh cut mushrooms. Non- irradiated fresh mushrooms recorded lower fungal counts than Najafi *et al* (2012) and Seo *et al* (2010) who all worked on fresh cut vegetables and reported lower values of fungal counts than 1.4- 3.8 log cfu/g for their control fresh mushroom samples. The role of yeasts and moulds in the spoilage of mushrooms is well documented and their growth on foods can cause major problems. Some of moulds like *Aspergillus flavus* may produce aflatoxins which have carcinogenic, mutagenic, teratogenic and immunosuppressive effect on animal and humans alike (Tournas, 2005; Guengerich *et al.*, 1996). Mycotoxins in human foods can be a health hazard.

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Effect of gamma irradiation on microbial counts of dried mushrooms

Results from this study (Tables 25 and 26) indicate a general increase in microbial counts of unirradiated dry mushrooms during storage period of 12 months. The marginal increase in microbial load content was apparent in 6th and 12th months. Physical environmental factors such as moisture, pH and temperature in the packs can become conducive to support growth of microorganisms (Food Safety, 2003). As expected, dried mushroom samples recorded lower microbial counts than fresh mushrooms. This might be due to the processing activities such as solar drying which was augmented by gamma irradiation treatment which reduced mould counts by 1.4- 3.7 log cycles.

The presence of microorganisms in food is not necessarily an indicator of hazard to the consumers (Kamal *et al.*, 2010). *Bacillus cereus* can be detected in many raw foods of plant origin and in raw milk. According to Zahran *et al*, (2008) and NSW-FA (2009), microbial spores can survive cooking, and poor temperature control after cooking may result in germination of the spores and subsequent growth. *B. cereus* is of greatest concern in plant or cereal based ready-to-eat foods and cream based sauces. Ready-to-eat foods containing raw components may contain low levels of *B. cereus.* The International Commission for Microbiological Specification for Foods (ICMSF, 1996) stated that ready-to-eat foods with plate counts between 0 - 10^3 is acceptable; between 10^4- $\leq 10^5$ is tolerable and 10^6 cfu/g and above is unacceptable. The microbial aerobic plate counts recorded for both fresh and dry mushrooms kept in the two packaging materials can be considered as acceptable. Furthermore, coliforms were nil to low, and *Staphyloccocus aureus* could not be detected after 12 months storage and after irradiation up to 2 kGy.

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Microbial quality of samples kept in the two packaging material were not significantly ($P>0.05$) different.

Radiation sensitivity (D_{10} values) of *Bacillus cereus* on fresh and dried mushrooms

B.cereus is a gram positive, facultative anaerobic spore forming rod bacteria ubiquitous in water, soil and air (Giwa and Ibrahim, 2012). It is the aetiological agent of food poisoning causing diarrhoea, vomiting and indigestion through the formation of two types of enterotoxins (Schneider *et al.*, 2004). Endospores of *B.cereus* are resistant to heat, dessication, UV, high pressure and oxidation agents (Setlow, 2006). Gamma irradiation tried to inactivate *B.cereus*.

Radiation sensitivity (the killing effect of radiation) in microorganisms is generally expressed by the decimal reduction dose or D_{10} value (Mohan *et al.*, 2011). The mean D_{10} values on both fresh and dried mushrooms were 1.98 and 2.10 kGy respectively. The observed slight difference ($P<0.05$) in D_{10} values of *Bacillus cereus* on mushrooms stored in polypropylene and polythene (Table 27) were probably due to the densities of materials constituting the fabrics of the packaging materials and how they affected the penetration of the gamma radiation to the target microorganisms (da Silva Aquino, 2012). Likewise, the radiosensitivity of bacteria varies depending on the packaging atmosphere (N_2, O_2, CO_2) used and are also very sensitive to irradiation in the presence of oxygen (IAEA, 2005). In this present study, radiation was done in air inside the bags.

The D_{10} values of *B. cereus* obtained, agreed with data from Zahran *et al*, (2008) who reported D_{10} values of 1.9 kGy and 0.4 kGy for *B.cereus* and *Listeria monocytogenes* respectively on some chicken products. Abd El-Hady (1993) reported that D_{10} values of three strains of *Bacillus cereus* were 2.3, 2.2 and 2.0 kGy on beef. D_{10} values are

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important because they lead to an estimation of the dose required to inactivate any microorganisms in foods (Zahran *et al.*, 2008). Results of this study indicate that low doses of gamma irradiation up to 2 kGy were effective in reducing the *B.cereus* population stored in both packaging materials sufficiently to achieve recommended levels prescribed by the International Commission for Microbiological Specification for Foods (ICMSF, 1996).

Effect of gamma radiation on Enterobacteria of *P.ostreatus* fruit bodies identified using API 20E kit

Enterobacteriaceae comprise a large group of genetically and biochemically related bacteria (ILSI, 2011). Members of the Enterobacteriaceae family are generally facultatively anaerobic, gram negative non spore formers and range from 0.3 to 1.0 mm in width and 0.6 to 6.0 mm in length (Abbot, 2007).

Most of these organisms are pathogenic while others produce toxins responsible for food intoxication. With certain foods Enterobacteriaceae can also provide a measure of food quality and spoilage potential. Some Enterobacteriaceae are commonly found in the gastrointestinal tract of animals including humans. *Klebsiella pneumoniae* is part of the normal flora in the nose, mouth and intestines. It may cause lesions in almost every part of the body, pneumonia, chronic lung abscess, upper respiratory tract infections, sinusitis, endocarditis, septicemia, meningitis, gastroenteritis, peritonitis, liver and bilary tract disease, wound infections, salpingitis and skin and uterine tract infections (Mikat and Mikat, 1975).

The observed persistence of *Klebsiella pneumoniae* demonstrates its resistance to low gamma radiation doses. *Klebsiella* spp. are a gram-negative, nonmotile, usually encapsulated rod-shaped bacteria, also belonging to the family Enterobacteriaceae.

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Amako *et al* (1988) described the composition of its encapsulated wall to consist of a heavily packed accumulation of fine fibers which represented a polymer of capsular polysaccharide with approximate layer thickness of 160 nm. Sridha-Rao (2009) showed that *K. pneumoniae* has a very large capsule which is impregnated with water which might have contributed to its radiation resistance. According to Ali *et al* (2012), some bacteria can repair the damage of DNA and resist the effect of irradiation. The effectiveness of the process depends on the organism's sensitivity to irradiation, the rate at which it can repair damaged DNA, and especially on the amount of DNA in the target organism. Molins and Ricardo, (2001) stated that the survival of a microorganism may also depend on the dose and the nature of radiation used in the process. Generally, the decrease in population of *K. pneumoniae* in this study was probably due to the effect of energy produced from increasing doses of irradiation which might have broken the bonds in the DNA molecules, leading to inability of microorganisms to replicate and reproduce resulting in bacterial death (Gillard *et al.,* 2007). The overall decline and death of microorganisms is largely dependent on two factors namely unfavourable environmental conditions such as pH, moisture, temperature, access to nutrients and water activity content (Aw). Results obtained in this present study agrees with that of similar previous research by Nyoagbe, (2012) who reported a decrease in Enterobacteriaceae population on snails after treatment with gamma radiation up to 6 kGy.

Mycoflora population on fruit bodies (DRBC)

Response of fungi to radiation in pretreated and non-pretreated samples differed significantly ($P<0.05$). Post irradiation storage studies revealed a general modest increase in mycoflora population and ranged 0.17- 3.95, 1.48- 3.66 and 1.43- 3.65 $\log_{10}$ CFU/g

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after 3, 6 and 12 months of storage respectively. Although there were increases in fungal population during storage in both packaging materials, the resident mycoflora populations were still within the acceptable limit counts of 0.86- 1.8 log_{10} CFU/g especially in samples treated with doses 2-5 kGy and the difference between the two packaging materials were marginal and not statistically significant ($p>0.05$) (Figs. 66-69).

Percentage (%) occurrence of fungi on fruit bodies

Gamma radiation influenced the percentage (%) occurrence of fungal species. Storage after irradiation showed that non irradiated mushrooms haboured *A. niger*, *A. flavus*, *A. fumigatus*, *M. racemosus*, *Rhodotorula spp.*, *T. viride* and *A. niger*, *A. tamarii*, *A. fumigatus*, *M. racemosus*, *R. oligosporus*, *T. viride* in both polythene and polypropylene packs respectively. Gamma irradiation up to 5 kGy proportionately reduced the incidence of fungi up to 12 months storage although species like *A. flavus, A. fumigatus,* and *Rhodotorula sp*. persisted, (Figs.70-77) albeit in very low population. The mycotoxigenic potential of *A. flavus* and *A. fumigatus* was not tested but in the case of unlikely availability of favourable environmental conditions they could resume growth and impart mycotoxins like aflatoxin and fumigallin under extremely remote possibilities.

Radiation sensitivity curves of fungi on fruit bodies after irradiation (Cooke's and DRBC)

Radiation sensitivity (D_{10} values) of fungi on dried *P. ostreatus* ranged between 1.68 and 2.61 kGy for polythene and polypropylene respectively plated on Dichloran Rose Bengal Chloramphenicol (DRBC), D_{10} values of 2.60 and 2.78 kGy were obtained for same samples stored in polythene and polypropylene packs respectively plated on Cooke's medium (Table 29). Statistical differences ($P<0.05$) observed could be attributed to the

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differences in nutrient composition of the growth media and densities of packaging material used for storage.

Total phenolic content

Phenolic compounds are secondary metabolites which are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways of living organisms (Tura and Robards, 2002; Madsen and Bertelsen, 1995). Polyphenols have protective functions which have been previously attributed to free radical scavenging, metal chelating properties, capability of inhibiting or reducing different enzymes, such as telomerase cyclooxygenase or lipooxygenase (Naasani *et al.*, 2003) and most important of all as antioxidant compounds with the ability to trap free radicals and thus inhibit oxidative mechanisms. However, more recently, they play roles such as interacting with signal transduction pathways and cell receptors (Wiseman *et al.*, 2001).

Naturally occurring antioxidants can be found in whole grains, fruits, vegetables, teas, spices, herbs. Mushrooms have also been reported to have antioxidant activity which is correlated with their phenolic and polysaccharide compounds (Dubost *et al.*, 2007). The global economic value of mushrooms and their consumption is a combination of their value as food and their nutraceutical properties (Kortei, 2011; Ferreira *et al.*, 2010; Ferreira *et al.*, 2009). The major antioxidants found in mushrooms are phenolic compounds while other potential antioxidants eg. ascorbic acid, β- carotene, lycopene and γ- tocopherols have been found in small quantities (Yang *et al.*, 2002). Generally, irradiation dose of 0.5 kGy increased the production of phenolics and caused a significantly ($P<0.05$) higher contents with aqueous (1.96 ± 0.01- 10.96 ±1.7 mgGAE/g),

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ethanol extract (1.32 ± 0.02- 2.36 ± 0.2 mgGAE/g) and methanol exctract (0.56 ± 0.01- 4.18 ± 0.4 mgGAE/g). On the other hand, 2 kGy recorded the least phenols for these extracts. However, the values obtained in these studies for *P. ostreatus* were within range of total phenolic content of antioxidant activity (15.38 ± 0.56- 16.80 ± 0.15gGE/100g dry weight of irradiated pomegranate (*Punica granatum*) (Lele *et al.*, 2011) and within the same range as that for termite mushrooms *Termitomyces striatus* and *T. robustus* (Ola and Oboh, 2001; 2000). On the other hand, Unekwu *et al*, (2014) recorded a significantly ($P<0.05$) higher phenolics of 97.16-248.8 mgGAE/g for mushroom species they tested (*P.ostreatus, P.pulmonarius, Auricularia auricula, Hericium erinaceus, Termitomyces robustus* and *T.manniformis*). In contrast, Choi *et al.* (2006) and Mau *et al.* (2001) reported values of 0.55 mgGAE/g (*Lentinus edodes*), 8.7 mgGAE/g (*Auricularia fuscosuccinea*), 4.61 mgGAE/g (*A. mesenterica*) which were within the range recorded in this present study for *P. ostreatus*. Presumably, production of phenolics may be influenced by substrates, species and environmental conditions. Interestingly, results in this study compares favourably well with phenolics found in apples, oranges, banana, pineapples, pear and strawberry (Kottoh *et al.*, 2014; USDA, 1998). There were no significant statistical differences ($P>0.05$) between values recorded in mushrooms stored in polythene and polypropylene packaging materials.

Total Flavonoid content

These are classes of secondary plant metabolites with significant antioxidants and chelating properties. Antioxidant activity of flavanoids depends on the structure and substitution pattern of hydroxyl groups (Sherifafar *et al.*, 2009). There were significant differences ($P<0.05$) between flavanoid content with different doses of radiation and

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solvent extracts such that the extraction concentrations followed a descending order of aqueous> methanol> ethanol. Irradiation dose of 0.5 kGy recorded the highest amounts of flavonoids in general for all treatments while 2 kGy recorded the least flavonoids.

The observed decrease might be attributed to some changes in the molecular conformation as a result of low moisture levels in the dry samples which might have solidified or aggregated to immobilize the flavonoids. The radical scavenger is reduced after high doses of gamma radiation due to displacement from the B-ring to the 2.3-double bond, indicating a relatively small reactivity (because of the absence of high density spin on a given site) for these intermediate species (Marfak *et al.*, 2003; Zhao *et al.*, 2002). The existence of such intermediate species after irradiation apparently does not abolish the antioxidant properties and can maintain the radioprotective effects of flavonoids. This feature has already been observed in gamma-irradiated strawberry (Breifeller *et al.*, 2002). The present results are in good agreement with the presence of stabilized quinones, which have been observed after H-abstraction from the B-ring of flavonoids in radiolytic solutions (Pannala *et al.*, 2001). High doses of radiation can cause oxidative damage and impair flavour in food. However, the action of effective and radiostable natural antioxidants may prevent chemical oxidation of biomolecules in irradiated foods.

In terms of packaging material suitability, there was no significant (P>0.05) difference between polythene and polypropylene. Mujic *et al* (2010) reported flavonoid values of range 1.61- 5.04 mgCE/g for mushrooms *Lentinula edodes, Agrocybe aegerita and Hericium erinaceus* which were in agreement with present results. Lin *et al* (2013) also reported comparatively low flavonoid values of 2.18 mg QE/g for white variety of

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Auricularia fuscosuccinea, 0.78 mg QE/g for *T. fuciformis* and 0.49 mg QE/g for *Auricularia polytricha*. However, values obtained from this present work, were higher than what was reported by Iwalokun *et al*, (2007) and Mattila *et al*, (2001) who found no flavonoids in *P.ostreatus* as they investigated the comparative phytochemical, antimicrobial and antioxidant properties and also, contents of vitamins, mineral elements and some phenolic compounds in cultivated mushrooms. The antioxidant concentration in a food material according to Lele *et al,* (2011) also depends upon the time of evaluation (i.e analyzing immediately after the irradiation treatment or after certain period of time duration) may affect results. In this study, analysis immediately followed the irradiation treatment.

DPPH free radical scavenging activity

Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity (Chye and Wong, 2009). The process of reduction of free radicals through the mechanism of hydrogen donation from antioxidant compounds is well known. Free radicals used are synthetic DPPH which react with an antioxidant compound through the donation of electrons from an antioxidant compound to get a pair of electrons. DPPH radical compound with deep purple colour would fade to yellow if it is reduced by antioxidants into non radical DPPH (Molyneux, 2004). When the free electron of DPPH radical has been paired with electrons from traps compounds (antioxidants) it would reduce DPPH to radical (DPPH-H), and form stable compounds

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that are DPP Hydrazine (Molyneux, 2004). There were significant differences ($P<0.05$) recorded between the aqueous and ethanol extracts. Methanol extracts however, recorded no significant differences with increasing radiation dose. Packaging did not influence results significantly. Present data cannot adequately elucidate the influence of type of extract on yield and activity of DPPH in *P.ostreatus*. Future studies are required to answer this question.

Half maximal inhibitory concentration (IC_{50}), percentage inhibition of DPPH

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting a biological or biochemical function. The antioxidant activity with $IC_{50}<$ 10 micro grams/ml value corresponds to good antioxidant activity and are included in the category of powerful antioxidants class (Amoo *et al.*, 2012, Chye and Wong, 2009; Pourmoradi *et al.*, 2006). The highest radical scavenging activity was shown by aqueous extract (IC_{50}= 0.064 mg/ml) which was lower than quercetin ($P<0.05$) (Fig. 80). The high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity indicated that these compounds in mushrooms contribute to the strong antioxidant activity. Previous studies by Mujic *et al* (2011) showed a range of 0.02- 0.198 mg/ml for *L.edodes, H.erinaceous* and *A.aegerita* which is comparable to *P.ostreatus*. Qusti *et al* (2010) also reported comparable values of 0.22, 0.47, 0.54, 0.73 mg/ml for figs, snake cucumber, banana and white grapes respectively which are ranked high in antioxidants.

Although gamma irradiation can damage biomolecules directly by rupturing covalent bonds as a result of transfer of photon energy, and indirectly, by producing free radicals

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and other non-radical reactive oxygen species (Kempner, 2001) its adverse effect was not evident and so it was in this present results (Fig.80).

Nutritional Studies

The nutritional attributes of edible mushrooms are directly linked to their chemical composition. There is species variation in nutrients but is dependent on type of substrate, stage of development, environmental conditions and essentially the post harvest condition of storage (Adejumo and Awosanya, 2008; Manzi *et al.*, 2001). Likewise, the physical status of food (frozen or fresh, solid, liquid or powder) and also its composition influence the reactions induced by radiation (Arvanitoyannis, 2010).

Protein is an important constituent of dry matter of mushrooms (Chang and Buswell, 1996; Alofe *et al.*, 1995). The digestibility of mushroom protein can be as high as 72 to 83%. Protein content of mushrooms depends on the composition of the substratum, size of pileus, harvest time and species of mushrooms (Bano and Rajarathnam, 1982). According to Mostafarvi (2012), an interaction of ionizing irradiation and proteins could produce chemical reactions depending on the protein structure, state (native or denatured), physical status, amino acid composition, the presence of other substances and the radiation treatment. The most important changes include dissociation, aggregation, cross-linking and oxidation. Arvanitoyannis (2010) indicated that low and medium doses induce only a small breakdown of food proteins into lower molecular weight protein parts and amino acids which cause less chemical reactions than steam heat interactions. The average protein content of cultivated edible mushrooms ranges from 3.5-4% of their fresh weight (Oyetayo and Ariyo, 2013). Protein content range obtained for *P.ostreatus* in this study, generally fell within the range (12.51- 15.25%) (Table 33) reported by several

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authors (Oyetayo and Ariyo, 2013; Aisha and Wan-Rosli, 2011; Khan *et al.*, 2008; Regula and Siwulski, 2007) who worked on *Pleurotus spp*.

Egwim *et al*, (2011) reported low protein content values for *P. ostreatus* (14.03 ± 0.01%) and also investigated some selected wild edible Nigerian mushrooms and recorded higher protein contents of range 26.25±1.93- 60.38±0.20% for mushroom species *Cantharella cibarius*, *Laccaria amethysta, Clitocybe odora, Lepista nuda, Macrolepiotata procera, Lepista saeva, Lactarius deliciousus, Laccaria laccata, and Hericium erinaceus*. Al-Momany and Salih, (2011) found values of 16.0- 16.8% range for *Pleurotus spp*. amongst other edible fungi such as *Agaricus macrosporus* and *Tricholoma saponaceum* var *squamosum*. In terms of the amount of crude protein, mushrooms rank below animal meats but well above most other foods including milk (Chang, 1980). On a dry weight basis, mushrooms normally contain 19 to 35% proteins compared to 7.3% in rice, 12.7% in wheat, 38.1% in soybean and 9.4% in corn (Crisan and Sands, 1978; Li and Chang, 1982; Bano and Rajarathnam, 1988). A 100g serving can provide about 12.5-15.1% of the recommended dietary allowance (RDA) or recommended nutrient intake (RNI). This high protein content implies that this fungus can contribute significantly to the daily human protein requirements, usually about 23-56g (Chaney, 2006; FAO/WHO/UNU, 1991).

Mushrooms contain high moisture depending on the mushroom species and other parameters related to harvest, growth, culinary and storage conditions (Guillamón *et al.*, 2010). The moisture content of any food is an index of its water activity (Olutiola *et al.*, 1991), and is used as a measure of stability and susceptibility to microbial contamination (Uraih and Izuagbe, 1990). Dried mushrooms are not prone to rapid deterioration due to

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the absence of medium of transport of enzymes to facilitate chemical reactions which cause deterioration.

Although mushrooms are generally low in fat, they do contain essential unsaturated fatty acids and are also cholesterol free and as such considered essential and significant for human diet and health. Free radicals formation during irradiation has been proven to increase lipid oxidation (WHO, 1994). The range of fat content values obtained (0.63-1.24%) were comparable to the 1.23% – 0.53% reported by Musieba *et al* (2013) and Nurudeen *et al* (2013) respectively.

Crude fibre contents obtained in this present study (13.50- 15.33%) fell within range of results (3–32%) reported by some researchers (Musieba *et al.*, 2013; Egwim *et al*, 2011). On the average, a 100 g serving of mushrooms guarantees from 9 to 40 % of the daily recommendation of dietary fibre (Manzi *et al.,* 2001). Dietary fibre content was high (approx. 45 % of dry matter). The fairly high level of fibre in the mushroom was a desirable characteristic since fibre plays an important role in human diet. Manzi *et al.*, (2001), Manzi and Pizzoferrato, (2000) and Pizzoferrato *et al* (2000), observed that glycogen and chitin form the major constituent of fibre content of mushrooms. Gordon (2002), indicated that there is a "dietary fibre hypothesis" which suggests that fibre helps to prevent many diseases prevalent in affluent societies. Evidence from epidemiological studies suggest that increased fibre consumption may contribute to a reduction in the incidence of certain diseases like diabetes, coronary heart disease, colon cancer, high blood pressure, obesity, and various digestive disorders (SACN Report, 2008; Eriyamremu and Adamson, 1994; FAO, 1990). Dietary fibres alter the colonic environment in such a way as to protect against colorectal diseases. It provides protection

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by increasing faecal bulk, which dilutes the increased colonic bile acid concentrations which occur with a high-fat diet (Dillard and German, 2000). So there are health benefits derivable from the consumption of mushrooms including oyster mushrooms.

Carbohydrate values obtained in this study ranged from 50 to 65% which represents the bulk of fruiting bodies accounting for on dry weight basis. Similar results have been reported by other researchers for cultivated *Pleurotus* mushroom (Wani *et al.*, 2010; Kortei, 2008). *Pleurotus spp.* dry matter usually include 50- 60% carbohydrates composed of various compounds; monosaccharides, their derivatives and oligosaccharides (commonly called sugars) and both reserve and construction polysaccharides (glycans). Kalac, (2009) reported a decrease in mannitol and α, trehalose which are the main constituents of oligosaccharides as well as polyols respectively. In general, irradiation modifies mono and polysaccharides, but thermal treatment can produce more modifications (Fan, 2005). Sadiq *et al* (2008) reported carbohydrate values of *Agaricus bisporus* (56.47 ± 0.21 %) and *Agaricus bitorquis* (39.94 ± 0.17 %). The amount of carbohydrates determined in *Agaricus bitorquis* was comparable to results obtained (61.39- 65.50%) as average value for *P.ostreatus* in this present study. Carbohydrate that can be used by humans produces four calories per gram as opposed to nine calories per gram of fat and four per gram of protein. In areas of the world where nutrition is marginal, a high proportion (approximately 0.45- 0.9 kg) of an individual's daily energy requirement may be supplied by carbohydrate, with most of the remainder coming from a variety of fat sources (Roffey, 2008).

Ash content values of 6.16-8.5% obtained in these present studies were within range of values of 5.69- 7.82% as reported by Wiafe-Kwagyan, (2014), Obodai *et al* (2014) and

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Musieba *et al* (2013) respectively. However, Egwim *et al* (2011) recorded higher values of 20.55 ± 0.13 %.

Metabolizable Energy

Manzi *et al* (2001) emphasized that owing to their high water content and low caloric value, mushrooms could be considered as a dietetic food suitable for low-calorie diets. In terms of energy values, the present results indicated that dry mushrooms stored up to 12 months were of good quality, low in calorie content and their energy value varied significantly ($P<0.05$). Obodai *et al* (2014) and Obodai (1992), reported energy values within range of 272- 389 Kcal./100g and 381- 389 Kcal./100g respectively for *Pleurotus spp*. Mshandete and Cuff (2007) reported energy values of 313 Kcal/100g dry matter for *Coprinus cinereus*, 305 Kcal/100g dry matter for *Volvariella volvacea* and 302 Kcal/100 g dry matter *Pleurotus flabellatus*. Egwim *et al* (2011) obtained energy values of 305 Kcal/100g and 302 Kcal/100g for *Laccaria amethystea* and *Lepista nuda* respectively. These values fall within what was recorded in the dry mushroom samples stored in both packaging materials before and after irradiation. Recently, Wiafe-Kwagyan, (2014) also found energy value of *P.ostreatus* on differently formulated rice straw composts to vary from 305.81- 387.80 Kcal./100g.

Mineral contents

Mushrooms have a very effective bioaccumulation mechanism which enables them to readily take up some heavy metals from the ecosystem (Zhu *et al.*, 2011). The accumulation of heavy metals in mushrooms seems to be influenced by some environmental and fungal-related factors. According to Radulescu *et al* (2010), environmental factors such as organic matter content, pH and metal concentration in soil

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as well as fungal factors, the species, morphological part of the fruit body, developmental stages, age of mycelium, intervals between fructifications and biochemical composition play a role. Vetter (1994) reported that the occurrence and distribution of different toxic components in certain mushrooms does not only represent a theoretical mycological problem but also has practical environmental and toxicological implications. The mineral content of *P. ostreatus* treated with ionizing radiations did not vary significantly ($P<0.05$) according to their elemental bioaccumulative potentials in their growth environment (Vetter, 1994).

Macro elements in *P.ostreatus*

Sodium concentration levels in *P.ostreatus* from these studies ranged between 14.00± 0.7- 14.90±0.8 mg/100g. Applied gamma radiation doses showed significant differences ($P<0.05$) in some instances (Tables 35 and 36). The preponderance of Sodium in the fruitbodies could be attributed to the varying degrees of stimulatory effect of doses on dry matter due to activation of cellular and extracellular metabolic enzymes (Dawoud and Abu-Taleb, 2011). Oyetayo and Ariyo, (2013), reported values of range 4.03±0.02- 4.39±0.012 mg/kg. Regula and Siwulski, (2007) recorded values of range 3.7 ± 21.4 mg/kg and Obodai *et al* (2014) recorded values of 3.80 ± 0.01 mg/kg. According to Mallikarjuna *et al* (2013), Sodium is good for patients with hypertension; however, relatively less amounts are needed. Data from this study makes *Pleurotus ostreatus* a good source of Sodium for treatment of hypertension and is recommended for consumption.

Calcium contents of *P.ostreatus* ranged 11.02±0.3-12.53±0.4 mg/100g. There were significant differences ($P<0.05$) observed with respect to the different radiation doses

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applied (Tables 35 and 36). Applied dose 2 kGy showed significance ($P<0.05$) probably due to its ability to stimulate the tissues of dried fruit bodies and activate enzymatic activities according to nutrient composition. Results obtained in this study agreed with literature values of 13.03 mg/100g by Okechukwu *et al*, (2011). Oyetayo and Ariyo (2013) recorded values of 5.37±0.01- 8.87± 0.006 mg/100g; Alam *et al* (2008) recorded values of 35.9±3.8 mg/100g. Calcium aids in formation of strong bones and teeth (USDA, 2009) and is found in adequate quantities in the fruit body of *P.ostreatus.*

Potassium levels in this study were high and ranged between 30.20±0.5- 33.10±0.6 mg/100g and showed significant differences ($P<0.05$) with radiation treatment. Obodai *et al,* (2014) reported values of range 17.40±0.01- 17.60 ±0.01 mg/kg. Oyetayo and Ariyo, (2013) reported values of range 9.42±0.15-11.34±0.02 mg/100g. Musieba *et al* (2013) recorded 2.28±0.14 mg/100g. Potassium is important in human health and nutrition. It aids in the maintenance of normal fluid, mineral balance in the control of blood pressure. It also plays a role in making sure nerves and muscles, including the heart, function properly (Duyff, 2006). *Pleurotus spp*. contains 182 to 395 mg/100g (0.0182- 0.0395 mg/kg) which is 3-11% of the Daily Value (USDA, 2009). Recommended Daily Intake (RDI) of Potassium is 3100mg/day (Manzi *et al*, 1999).

Magnesium concentration in mushrooms from these studies ranged between 1.77±0.18- 3.53±0.04 mg/100g and showed significant differences ($P<0.05$) with radiation. Dose of 2 kGy had an apparent effect on the dry tissues of mushroom fruit bodies (Table 35). Presumably, the higher doses stimulated enzyme activities. Levels obtained in this study fell within range of previous studies which were 1.69±0.015- 3.57±0.01mg/kg (Oyetayo

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and Ariyo, 2013), 1.067-1.380mg/kg (Wiafe-Kwagyan, 2014) and 0.07mg/100g (Musieba *et al*., 2013).

Phosphorus contents of *P.ostreatus* fruit bodies ranged between 6.11 ± 0.3 -6.41 ± 0.35 mg/100g and showed significant differences (P<0.05) with radiation dose. Results obtained fell within range of values recorded by Ahmed *et al* (2013) who reported values 8- 9 mg/kg. Wiafe-Kwagyan, (2014) recorded a range of 5.22-14.5 mg/kg. Patil *et al.,* (2010) also recorded a range of 7.90-9.10 mg/100g. Since recommended daily intake (RDI) of P is 0.7g, *P. ostreatus* is high in P content, and can therefore contribute to human nutrition as good source of Phosphorus (Çağlarırmak, 2007).

Nitrogen concentration obtained ranged from 3.00±0.03-3.61±0.02 mg/100g and showed significant differences (P<0.05). Ahmed *et al* (2013) reported a range of 45-49 mg/kg. The body utilizes nitrogen for promoting protein synthesis, the creation of compounds and amino acids that influence growth, hormones, brain functions and the immune system. About 0.83 gram of protein per kilogram per day is considered sufficient to cover nitrogen requirements (WHO, 1982). Recently, Layman (2013) suggested a maximum intake of 2 to 2.5 g/kg of body weight per day.

Micro-elements as heavy metals in *P.ostreatus*

Some heavy metals such as copper (Cu), Iron (Fe), Manganese (Mn), Lead (Pb) and Zinc (Zn) were detected in the gamma irradiated fruit bodies of *P.ostreatus* in small quantities. Heavy metal concentration in mushroom is considerably higher than those in agricultural crop plants, vegetables and fruits. This connotes that mushrooms have a very effective mechanism which enables them to readily take up some heavy metals from the environment (Zhu *et al.*, 2010) due to their dense mycelia system which infilterates the

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substrates (Garcia *et al.*, 2005). According to Radulescu *et al*, (2010), the accumulation of heavy metals in mushrooms has been found to be affected by environmental factors such as organic matter content, pH and metal concentration in the soil as well as fungal factors such as species of mushroom, morphological development of the carpophores, age of mycelium, intervals between fructifications and biological composition of substrate.

Copper content obtained in *P. ostreatus* ranged between 0.00±0.00 (nil) – 0.02±0.001 mg/100g and showed significant differences (P<0.05) with increase in radiation doses. Levels of Cu obtained in this study were below the safe limit set by World Health Organization (WHO) (40 mg/kg) as copper in foods (WHO, 1982). Copper levels in mushrooms reported by some researchers were 4.71–51.0 mg/kg (Tuzen *et al.,* 1998), 13.4–50.6 mg/kg (Soysal *et al,* 2005), 12–181 mg/kg (Tuzen *et al.,* 2003) and 0.0018-0.08mg/kg (Wiafe-Kwagyan, 2014). Copper is an essential constituent of some metallo-enzymes and is required in haemoglobin synthesis in red blood cells which carry oxygen throughout the body. It helps keep bones and nerves healthy (Duyff, 2006) and aids in the catalysis of metabolic growth (Silvestre *et al*., 2000).

Zinc levels in *P. ostreatus* in this study ranged 0.01±0.001-0.03±0.001 mg/100g. There were significant differences (P<0.05) observed with the doses applied. Results obtained in this study were within the Recommended Daily Intake (RDI) of trace elements reported by ICMR, (1990). Soylak *et al*, (2005) recorded values of range 45.2–173.8 mg/kg; Tuzen (2003) recorded a range 33.5–89.5 mg/kg and Isıloğ lu *et al* (2001) also recorded a range of 29.3–158 mg/kg. Zn is an essential micronutrient associated with a number of enzymes that aids in the synthesis of ribonucleic acids and DNA polymerases (Sadiq *et al.*, 2008).

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Iron (Fe) concentrations in *P. ostreatus* obtained in this study ranged from 0.29±0.01-0.37 ± 0.01 mg/100g. Values obtained in this study were lower than results reported by Regula and Siwulski, (2007) who recorded 68.6±5.50 mg/kg, Tuzen (2003) recorded 146–835 mg/kg, Sesli *et al* (1999) recorded 31.3–1190 mg/kg, and Isıloğ lu *et al* (2001) also recorded 180–407 mg/kg. The main functions of iron include transport and storage of oxygen which aids in energy production and cell division. It helps the immune and central nervous systems. Iron is the only nutrient for which women have a higher daily requirement than men. The U.S. Recommended Daily Allowance (RDA) of iron for men is 10 milligrams and 15 milligrams for women. According to Mamashealth (2013), breastfeeding increases iron requirements by about 0.5 to 1 mg a day.

Manganese (Mn) concentrations of *P.ostreatus* ranged from 0.03±0.001-0.04±0.001 mg/100g. Ahmed *et al*, (2012) obtained a range of 2.3±0.1- 2.6±0.1 mg/kg, Soylak *et al*, (2005) obtained results ranging 14.2–69.7 mg/kg. Tuzen (2003), obtained results ranging from 12.9– 93.3 mg/kg. Sesli and Tuzen (1999) also obtained results ranging from 14.5–63.6 mg/kg. Results obtained in this study were within the Recommended Daily Intake (RDI) of trace elements reported by ICMR, (1990) and was also found to be below toxicity levels of 400–1000 mg/kg (WHO, 1982). Manganese plays an important role in enzymatic catalysis and is crucial to virtually all biochemical and physiological process (Sadiq, 2008).

Lead (Pb) concentrations of *P.ostreaus* ranged 0.00±0.00 (nil) -0.02±0.00 mg/100g. Results agreed with levels reported by Regula and Siwulski, (2007) who recorded 0.000±0.0000 (nil) mg/kg, Tuzen *et al* (1998) recorded a range of 0.75–7.77 mg/kg, Tuzen (2003) obtained values of range 1.43–4.17 mg/kg, 0.40–2.80 mg/kg, Radulescu *et*

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al (2010) and Wiafe- Kwagyan, (2014) recently recorded 0.004 mg/kg. According to FAO/WHO tolerable weekly intake of lead is 0.025 mg/kg body weight. Lead (Pb) is toxic even at trace levels (Dobaradaren *et al.,* 2010) and the impairment related to Pb toxicity in humans include abnormal size and haemoglobin content of the erythrocytes, hyper stimulation of erythropoisis and inhibition of haemoglobin synthesis. Lead levels found in these studies with *P.ostreatus* were very low and thus, these mushroom species are safe for consumption.

Mean sensory scores for irradiated steamed mushrooms

Gamma irradiation and storage of dried mushrooms in polypropylene and polythene packages at the doses used in the present study had no significant effects (P > 0.05) on the majority of the sensory attributes of the steamed mushrooms samples. Panelists gave similar preference scores for both irradiated and non-irradiated samples, which indicated that all were highly satisfactory as judged by appearance, aroma, taste, and mouthfeel (Table 37). Nonetheless, panelists showed some significant (P<0.05) preferences for colour and acceptability for non irradiated and 1 kGy irradiated samples probably because higher doses might have caused an activation of tyrosinase enzymes to undergo some biochemical reactions to produce melanin which made the colour not appealing enough to the panelists as suggested by some researchers (Ares *et al.*, 2007; Zasler *et al.,* 1992). The flavour experienced from eating mushrooms, or any other food, comes from a combination of taste, texture, temperature, spiciness, and aromatic qualities (Zasler *et al.*, 1992). Effects of irradiation on odour and flavour have been studied with sensory panels of varying degrees of training; however, few consumer studies have been conducted. According to Hallock (2007), the taste and smell of mushrooms are important for both

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the issues regarding gustation and olfaction important to the mycophagist. Also mouthfeel of all sample treatments observed was regular and was appreciated as no extraordinary mouthfeel was recorded. Product acceptability of mushroom in general varied ($p>0.05$) probably due to the diversity of preference of different mushroom species, method of cooking etc.

Descriptive Texture Analysis

This was carried out primarily to authenticate results obtained by the instrumental evaluation method. The mean scores of the descriptive texture analysis of irradiated and non-irradiated dried mushrooms stored in polythene and polypropylene packs are presented in Table 38. Texture is a sensory attribute which greatly influences taste perception and the marketability of a product. The observed high score of non-irradiated *P.ostreatus* for hardness might be due to the ability of high doses of gamma radiation to cause a weakening of covalent bonds to cause depolymerization (Ares *et al.*, 2007). Hardness, adhesiveness, chewiness and smoothness of all the variously treated samples were normal and so were liked by the panelist confirming the textural measurement on the instrument. However, cohesiveness of the control sample was preferred most over the others. This observation is an agreement with findings of some researchers (Nayga *et al.*, 2005; Fox, 2002) who carried out analysis to assess consumer attitudes to irradiated foods. Results have consistently shown that many consumers have misconceptions about the technology and believe that it makes food radioactive. When consumers are given information about the process and a chance to try irradiated products for themselves they are much more likely to accept the technology such as in this case reported in this thesis.

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CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Oyster mushrooms have been shown in this study as an important nutritional, as well as medicinal sources for the well-being of the Ghanaian populace. The cultivation of this mushroom has not caught on well in Africa especially in Ghana because of the paucity of scientific knowledge of its cultivation to reduce cost and the humdrum tasks in the production pipeline. This thesis provides baseline scientific information which when effectively disseminated to the farmers can boost the cultivation of *P. ostreatus* in Ghana.

1) A survey carried out using the rapid appraisal method showed among other things, the humdrum tasks of preparing the compost and spawn medium and the sterilization method, not excepting the short shelf-life of the harvested fruit bodies. Majority of the farmers were not aware of the possible use of gamma irradiation for the sterilization/ pasteurization of the spawn medium and the 'wawa' sawdust compost as well as the preservation of both fresh and dry fruitbodies although consumers prefer to see their favourite mushroom on the market throughout the year.

2) The resident fungi in sorghum grains (used as substrate for the spawn) and the 'wawa' sawdust (used as compost for bioconversion to fruit bodies of *P.ostreatus*) included fungi of eight genera (*Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, Rhodotorula, Trichoderma*). Members of the genus *Aspergillus* (*A. alutaceus, A. flavus, A. fumigatus, A. niger, A. parasiticus, A. terreus*) were observed. Increasing doses of gamma irradiation up to 32 kGy was able to commensurately reduce by 4-5 log cycles the population of the resident mycoflora. Radiation treatment was significantly ($P<0.05$) more efficacious at the higher (10- 32 kGy) doses than steam sterilization. During

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composting for 28 days prior to bagging for use as substrate for the cultivation of *P.ostreatus*, temperature in the compost increased to approximately 65°C in the heap which was turned every 4 days. The heat contributed to the elimination of some of the fungi leaving the thermophilic (heat loving) species.

Results of the radiation treatment of the compost and steam sterilization clearly demonstrated that gamma irradiation could be used as a substitute for steam sterilization for sorghum spawn production and the cultivation of the oyster mushroom.

3) There was a direct negative relationship between high substrate contamination to mycelia density, primordial and fruit formation, stipe length and cap diameter.

The drying characteristics of sliced mushroom fruiting bodies exposed to low doses (0.0, 0.5, 1.0 kGy) could be described by Page's model (with R^2= ranging 0.9878-0.9967; RMSE= 0.0004-0.0014) while the Diffusion Model best fitted the samples exposed to higher doses 1.5-2.0 kGy (with R^2= 0.9890- 0.9938 ; RMSE= 0.0007- 0.0012). The Root Mean Square Error RMSE is used to determine the goodness of fit between predicted and experimental data. Thus high R^2 and RMSE correspond to a better goodness of fit. The Effective Diffusivity (D*eff*) which describes the rate of moisture movement in food during drying was enhanced by gamma irradiation (increasing from 1.88×10^{-08} m^2/s in the control to 2.44×10^{-08} in the mushroom cap treated with 2.0 kGy)

4) The textural profile of fresh and dry mushrooms varied. Generally, hardness of dry mushrooms were significantly higher (63-74 kgf), did not vary significantly ($p>0.05$) at 0-1.5 kGy but decrease significantly ($p<0.05$) to 29 kgf at 2.0 kGy. Fresh mushrooms recorded low hardness (0.2-0.4 kgf) and did not change with increasing doses up to 2.0 kGy. Free and rehydrated dry mushroom had low fracturability (0- 1.7 N) which

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decreased with increasing doses. On the contrary, dried irradiated mushrooms recorded high fracturability (14.8- 15.94 N) and reduced to 12.9- 13.2 N at 2.0 kGy; Cohesiveness on compression varied from 0.6- 0.78 in dried samples and 0.75- 0.86 in rehydrated samples. Irradiation up to 2.0 kGy did not significantly ($p>0.05$) affect this parameter; Chewiness (which represents the energy required to disintegrate a solid material in order to swallow) was high as radiation dose increased in the dried samples (5N, control to 16N at 2.0 kGy). Fresh samples recorded a chewiness range of 0.1-0.2N with increasing dose and the same was true for rehydrated dry mushrooms. Increasing gamma irradiation reduced gumminess in most instances for fresh and rehydrated dry mushroom. Resilience measures how well a product tries to regain position after deformation.The fresh irradiated mushroom showed high resilience while dehydration had a pronounced effect on mushroom cap. Rehydration did not fully restore the dry mushroom to the original. Interestingly, sensory analysis using trained taste panelists confirmed the results of the mechanical measurement of textural profile with similar trends in the irradiated samples which were acceptable to the panelists.

6) Colour change of fresh and dried mushroom samples were estimated by the Hunter's L*a*b*, Metric Chroma, Hue Angle and Browning Index after storage varied. Storage of fresh unirradiated mushroom for 5days decreased the L* values attended by increase in a* and b* values but showed no variation with radiation such that metric Chroma (C), the Hue Angle (H) did not change with radiation and Browning Index (BI) was low showing a slow rate of enzymatic reaction after irradiation. ΔE which is a combination of L*a*b* values is used in the estimation of variation of colour depending on processing and

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packaging seemed to show only marginal differences in value of ΔE, ΔL*, Δa* Δb* and Chroma in sample stored in both packaging packs.

7) A gamma dose of up to 5.0 kGy significantly reduced mycoflora population by about 2- 3 log cycles in the dried fruit bodies stored for up to 12 months with a D_{10}= 1.68- 2.4 kGy (on Cooke's medium) and D_{10}= 2.6- 2.70 kGy (on DRBC medium). The differences in mycofloral quality of samples stored in the two packaging packs were not statistically significant (p>0.05). Fresh and dry fruitbodies did not contain *Coliforms, Salmonella* and *Staphylococcus aureus* but had low mycoflora population considered to be safe which was reduced by about 3 log cycles by 2 kGy of radiation. *Bacillus cereus* was detected in both fresh and dry mushroom samples. The mean D_{10} values for *B.cereus* on fresh mushrooms were 3.21±0.81 kGy (polypropylene) and 0.76±0.04 kGy (polythene). Dry mushrooms recorded D_{10} of 2.40±0.90 kGy (polypropylene) and 1.80±0.85 kGy (polythene). Low doses up to 2 kGy gamma irradiation were effective in reducing the contaminants to acceptable international standards.

8) Although the unirradiated fruitbodies were contaminated with *Klebsiella pneumonia, Citrobacter freundii, Proteus mirabilis, Serratia marcescens* and *Enterobacter*, they were killed by up to 5 kGy of gamma irradiation except *K. pneumoniae* which persisted albeit in low insignificant population.

9) Total phenolic contents detected in mushrooms ranged from 0.56±0.01- 10.96±1.7 mg GAE/g, flavonoids ranged from 1.64±0.05-8.92±0.6 mgQE/g, DPPH radical scavenging activity also ranged from 7.02±0.1- 13.03± 0.04% and IC_{50} values varied from 0.08- 0.16 mg/ml. A significant linear correlation was found between values for the total phenolic contents and antioxidant activity of mushroom extract. The high contents of phenolic

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compounds indicated that these compounds contribute to high antioxidant activity and *P.ostreatus* can be regarded as a promising candidate for natural mushroom sources of antioxidant with high value.

10) *P.ostreatus* contains significant amounts of ash, fat, protein, fibre carbohydrate, metabolic energy, as well as essential nutrient elements like Ca, K, Mg, N, P, Na and heavy metals (Zn, Mn, Pb, Fe, Cu) to merit its use in medicinal therapy and nutrition. The content of heavy metals fell below WHO recommended levels and indicate the ability of mushrooms to accumulate heavy metals in substrates if present.

11) In most instances listed above, there were only marginal differences in the nutritional, microbiological and textural properties of mushrooms stored in polypropylene and polythene containers. The only difference is the thicker and rigid texture of the polypropylene which makes the packaging robust and aesthetically more acceptable to the consumer.

CONCLUSIONS

The possible use of high doses of gamma irradiation up to 32 kGy for sterilization of spawn substrate and compost for bioconversion of compost to mushroom by *P. ostreatus,* has been amply demonstrated in this thesis. What remains is the cost- benefit analysis viz- a –vis the current steam sterilization which is capital intensive using firewood or gas. The use of low doses (up to 2- 5kGy) of gamma irradiation by local food industry and mushroom farmers to preserve the fresh and dry samples of fruitbodies could improve the hygienic quality, extend shelf-life and preserve nutrients and anti-nutrients contents in *P. ostreatus*. If this is done, this thesis could be a useful springboard to improving human

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health and poverty alleviation in this country through the promotion of mushroom cultivation.

RECOMMENDATIONS

1) It is important to follow up this study with a cost benefit analysis of the gamma irradiation treatment viz- a- vis the process of steam sterilization using steam drums. This analysis is in progress and will be reported in another paper in due course.

2) Combination treatment of heat and gamma irradiation has synergistic effect on killing of microorganisms by lowering the dose required for lethal effect. This should be tried as the lowering of dose required will be an added advantage to reduce cost of treatment for product preservation.

3) The polypropylene packaging is recommmended for use because of its advantage of robustness and better aesthetic presentation to the consumer.

4) To disseminate effectively the findings of this thesis, a Handbook Guide to the preparation of spawn medium (sorghum), compost bagging and their radiation treatment for the cultivation of *P. ostreatus* should be prepared to guide the mushroom cultivator.

5) Because of the location of the irradiation source (facility) in Kwabenya, Accra, the logistics of conveying bagged sorghum and composted bag substrates to the facility would be cumbersome if it is done on individual basis.The recommendation is that;

(a) There should be a spawn and compost preparation centre owned by a mushroom growers Cooperative and supervised by competent research scientists knowledgeable in

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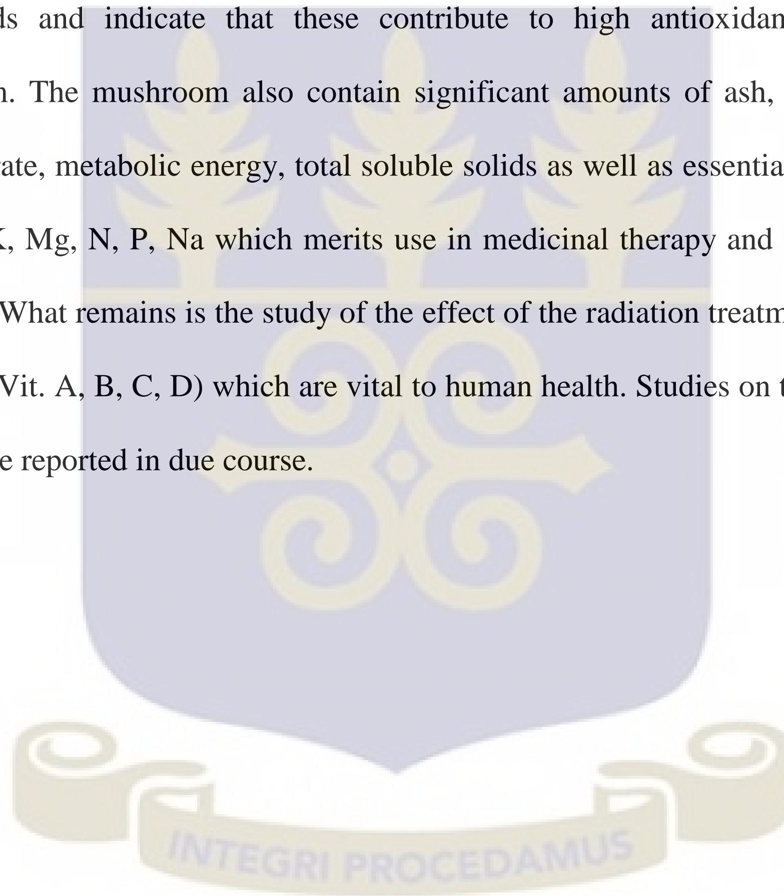
Mushroom Science. The spawn substrate and the compost will be bagged here and sent to the Radiation Facility and returned for sale to interested farmers for use.

(b) A small pilot scale trial can be carried out to test the feasibility of value chain suggested in this thesis.

6) The results of this thesis show that the oyster mushroom has high content of phenolic compounds and indicate that these contribute to high antioxidant activity of this mushroom. The mushroom also contain significant amounts of ash, fat, protein, fibre, carbohydrate, metabolic energy, total soluble solids as well as essential mineral elements like Ca, K, Mg, N, P, Na which merits use in medicinal therapy and to improve human nutrition. What remains is the study of the effect of the radiation treatment on the vitamin contents (Vit. A, B, C, D) which are vital to human health. Studies on this are in progress and will be reported in due course.



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APPENDIX 1

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1 A. STRUCTURED QUESTIONNAIRE FOR PRODUCERS OF MUSHROOMS

Background information

Name of volunteer…………………………………………………………………

Date of survey………………………………………………………………………

Place of survey

- Village/ town …………………………………………………
- District ………………………………………………………...
- Region …………………………………………………………

i) Gender

Male ☐ ☐ female ☐ ☐

ii) Age……………

Ethnic/socio-cultural group………………………………………………

Educational level

Nil ☐ Primary/middle/JHS ☐ Secondary/ SHS ☐ Tertiary ☐

PRODUCTION

5. Which kind of mushroom do you produce here?

Oyster ☐ Domo ☐ others (specify)…………………

6. Why do you produce this type?

Family trade ☐ Easy to produce ☐ Profitable ☐

7. What material do you grow your mushrooms on?

Rice straw ☐ sawdust ☐ Cassava peels ☐

8. Why do you grow mushrooms on these materials?

Cheap cost ☐ Availability ☐ That is what you were taught ☐

9. What method of sterilization do you use?

Drum (steam) ☐ Autoclave ☐ others ……………

10. Are you satisfied with it?

Yes ☐ No ☐

11. Do you wish to improve upon it to achieve better sterilization?

☐ ☐

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Yes ☐ No ☐

12. Have you ever heard of irradiation method of sterilization?

Yes ☐ ☐ No ☐ ☐

13.What quantity of mushrooms do you produce per batch?......................

14. Are you able to preserve some of the unsold mushrooms?

Yes ☐ ☐ No ☐ ☐

15. Which method of preservation do you use?

Drying ☐ Freezing ☐ ☐ Smoking ☐

16. How long do they last?

1 week ☐ ☐ 2 weeks ☐ ☐ 3 weeks or more ☐ ☐

17. How do you find the mushroom industry generally?

Laborius ☐ ☐ normal ☐ ☐ expensive ☐ ☐

18. Which aspect of mushroom production needs more attention?

Innoculation ☐ ☐ Sterilization ☐ ☐ Packaging ☐ ☐ Others (specify)……………

1B. STRUCTURED QUESTIONAIRE FOR MUSHROOM CONSUMERS

Background information

Name of volunteer……………………………………………………………………

Date of survey………………………………………………………………………

Place of survey

- Village/ town …………………………………………………
- District ……………………………………………………...
- Region ……………………………………………………….

iii) Gender

Male ☐ ☐ female ☐ ☐

iv) Age……………

v) Ethnic/socio-cultural group………………………………………

vi) Educational level

Nil ☐ Primary/middle/JHS ☐ Secondary/ SHS ☐ ☐ Tertiary ☐

Consumer

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1. What type of mushroom do you patronise the most?
   Domo ☐ oyster ☐ Ganoderma ☐

2. What benefits do you derive from it?
   Nutritional ☐ Medicinal ☐ others ☐

3. If nutritional, what does the body gain from it?
   ………………………………………………………………………………………

4. If medicinal, what does the body gain from it?

   ………………………………………………………………………………………

5. Where do you get them from?

   Market ☐ Collect from the wild ☐ Backyard garden/farm ☐

6. What dish do you use your mushrooms to prepare?
   Stew ☐ Soup ☐ Salad ☐

7. What do you consider to be the quality of good mushroom?
   Texture ☐ Taste ☐ Appearance ☐

8. How do you preserve your mushrooms?
   Drying ☐ Refridgerating ☐ Smoking ☐

9. Do you know if it could be grown by man?
   Yes ☐ No ☐

10. Do you wish your favourite mushroom be produced all-year round?
    Yes ☐ No ☐

11. Do you think consumption of mushroom should be encouraged

    Yes (why)……………………………………………………………………………
    ………………………………………………………………………………………
    ………………………………………………………………………………………

12. No (why)…………………………………………………………………………….

APPENDIX 2

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STRUCTURED QUESTIONAIRE FOR SENSORY EVALUATION OF THE VARIOUSLY TREATED MUSHROOMS

Name…………………………………………….. Date…………………………………

Product………………………………………………………………………………………

Please assess these samples and rate them under the listed attributes using the scale below. Remember to rinse your mouth with the water provided before moving on to the next sample.

Scale	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**
Interpretation	Like extremely	Like Very much	Like moderately	Like slightly	Neither Like nor dislike	Dislike slightly	Dislike moderately	Dislike Very much	Dislike Extremely

RATING

ATTRIBUTE	PRODUCT CODE										
Appearance											
Colour											
Aroma											
Taste											
Mouth feel											
Overall acceptability											

TEXTURAL ATTRIBUTES	SAMPLE CODES										
Hardness											
Cohesiveness (integrity)											
Adhesiveness (stickiness)											
Chewiness (stringiness)											
Smoothness											

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APPENDIX 3: Influence of pretreatment (Irradiation and steam) of sorghum (*Sorghum bicolor*) on mycelia growth during spawn run period

Dose (kGy)	Treatment	Av. Rate of growth (cm/Day)	Time for Complete Colonization (Days)	Contamina-tion (%)	Mycelia Density
0	No stm + no autoclave	0.3 [c]	11 [b]	60 [cg]	+
	Stm + non irrad.	0.3 [c]	10 [b]	50 [bf]	+
	Non Irradiated	0.3 [c]	11 [b]	80 [cg]	+
5	Stm + autoclave	0.68 [ab]	7 [a]	5 [de]	+
	Stm + irrad	0.70 [ab]	7 [a]	10 [f]	+ +
	Irradiated	0.70 [ab]	7 [a]	0	+ + +
10	Stm + autoclave	0.65 [ab]	7 [a]	5 [de]	+ +
	Stm + irrad.	0.66 [ab]	7 [a]	0	+ + +
	Irradiated	0.68 [ab]	7 [a]	0	+ + +
15	Stm + autoclave	0.63 [ab]	7 [a]	0	+ + +
	Stm + irrad.	0.68 [ab]	7 [a]	0	+ + +
	Irradiated	0.71 [ab]	7 [a]	0	+ + +
20	Stm + autoclave	0.68 [ab]	7 [a]	0	+ + +
	Stm + irrad	0.66 [ab]	7 [a]	0	+ + +
	Irradiated	0.68 [ab]	7 [a]	0	+ + +
25	Stm + autoclave	0.70 [ab]	7 [a]	0	+ + +
	Stm + irrad	0.66 [ab]	7 [a]	0	+ + +
	Irradiated	0.70 [ab]	7 [a]	0	+ + +
32	Stm + autoclave	0.70 [ab]	7 [a]	0	+ + +
	Stm + irrad	0.66 [ab]	7 [a]	0	+ + +
	irradiated	0.63 [ab]	7 [a]	0	+ + +

Means with same letters in a column are not significantly different (P> 0.05)

Degree of mycelial density when the mycelia fully colonised the substrate:
\+ poor running growth,
++ mycelium grew throughout the whole bag but was not uniformly white,
+++ mycelium grew throughout the whole bag and was uniformly white

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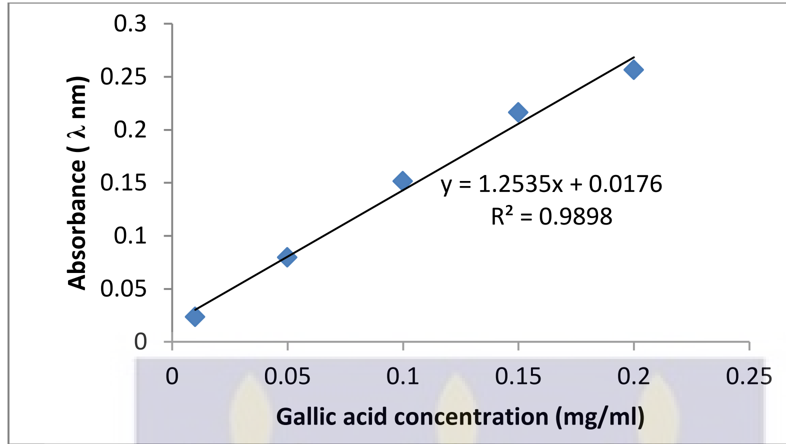
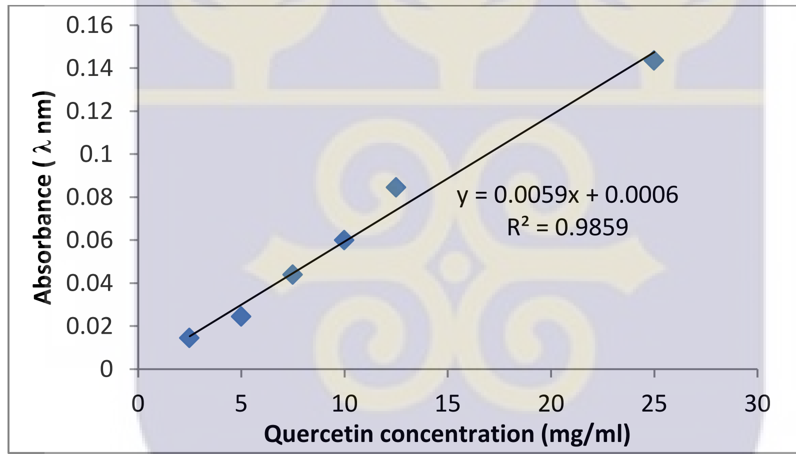
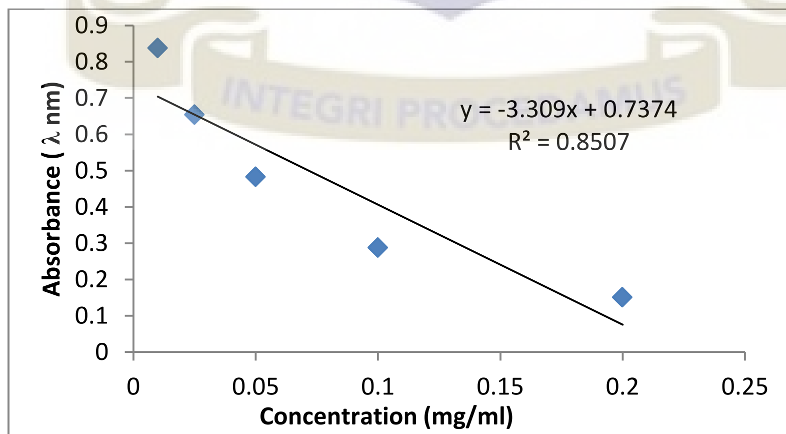
APPENDIX 4: Calibration curves for determination of antioxidants



Total phenolic contents (aqueous)



Flavonoids (methanol 60%)



DPPH (Ethanol 60%)

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APPENDIX 5

LIST OF PUBLICATIONS FROM THIS THESIS

1) Kortei, N.K, Odamtten G.T, Obodai M, Appiah V, Annan S.N.Y, Acquah S.A and Armah J.N.O. (2014). Comparative effect of gamma irradiated and steam sterilized composted 'wawa' (*Triplochiton scleroxylon*) sawdust on the growth and yield of *Pleurotus ostreatus* (Jacq. ex. Fr) Kummer.
Innovative Romanian Food Biotechnology, 2014, 14: 69-78.
http://www.bioaliment.ugal.ro/ejournal.htm

2) Kortei, N.K, Odamtten, G.T, Obodai, M, Appiah, V, Akuamoah, F, Adu-Bobi, A.K, Annan, S.N.Y, Armah, J.N.O and Acquah, S.A (2014). Evaluating the effect of gamma radiation on the total phenolic content, flavonoids and antioxidant activity of dried *Pleurotus ostreatus* (Jacq. ex. Fr) Kummer stored in packaging materials.

Advances in Pharmaceutics, 2014, Article I.D 262807, 1-8
http://dx.doi.org/10.1155/2014/262807

3) Kortei, N.K, Odamtten, G.T, Appiah, V, Obodai, M, Annan, T.A, Adu- Gyamfi, A, Akonor, P.T, Annan, S.N.Y, Acquah, S.K, Armah, J.O, Mills, S.W.O.(2014). Microbiological quality assessment of gamma irradiated fresh and dried mushrooms (*Pleurotus ostreatus*) and determination of D_{10} values of *Bacillus cereus* in storage packs.

European Journal of Biotechnology and Biosciences, 2014; 2 (1): 28-34.

ISSN: 2321-9122 www.biosciencejournals.com

4) Kortei, N.K, Odamtten, G.T, Obodai, M, Appiah, V, Wiafe-Kwagyan M and Narh-Mensah, D.L. (2015). Comparative effect of gamma irradiated and steam sterilized sorghum grains (*Sorghum bicolor*) for spawn production of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer.

Applied Science Report, 2015; 10(1): 12-21. DOI: 10.15192/PSCP.ASR.2015.10.1.1221

5) Kortei, N.K, Odamtten, G.T, Obodai, M, Appiah, V, Wiafe- Kwagyan, M. (2015). Evaluating the effect of gamma irradiation and steam sterilization on the survival and growth of sawdust fungi in Ghana.

British Microbiology Research Journal, 2015; 7(4): 180-192. DOI: 10.9734/BMRJ/2015/16521