BIOPROSPECTING SUITABILITY OF SELECTED BASIDIOMYCETES FOR BIOCOLORATION AND BIOREMEDIATION POTENTIAL

GODFREY NATTOH

A Thesis Submitted in Partial Fulfilment for the Degree of Master of Science in Molecular Biology and Biotechnology in the Pan African University Institute for Basic Sciences Technology and Innovation

2014

DECLARATION

This thesis is my original work and has not been submitted to any other university for examination.

Signature:…..... Date:….....

Godfrey Nattoh , MB300-0008/12

This thesis has been submitted for examination with our approval as the University supervisors.

Signature:..... Date:.....

Prof. Erastus Gatebe,

KIRDI

Signature:..... Date:.....

Dr. Julius Mathara,

JKUAT

Signature:..... Date:.....

Dr. Fredrick Musieba

KIRDI

DEDICATION

To

My entire family and friends

With great love

ACKNOWLEDGEMENTS

My sincere acknowledgment goes to the Almighty God for enabling me finish this work. Second, my gratitude is to the African Union (CAU) for the full scholarship to study master's degree at PAUISTI. My supervisors were not only my friends but also my strength. I thank Prof Erastus Gatebe for his advice and encouragement, Dr. Julius Mathara for his input and guidance, and Dr Fredrick Musieba for guidance and mentorship as well. You all played a role that I won't thank you enough.

Many thanks to PAUISTI community, classmates, my course coordinator Prof. Naomi Maina and Prof. Gabriel Magoma your advice were invaluable. Besides, am grateful to the Commission of African Union (CAU) for the stipend and funds toward this project, KAPAP for additional funds through Dr. Musieba and KIRDI in spawn development. I would like to appreciate the management and board of directors KIRDI, through DR. Kenneth Chelule for facilitating part of this work.

All the scientist, technical staff and interns/students at KIRDI mushroom laboratory, JKUAT food Biochemistry Laboratory, GK laboratory JKUAT, Mushroom IBR Lab JKUAT, Food product development workshop JKUAT, and ICIPE scientist more so to Dr. Baldwyn Torto and MR. Chesettoh, thanks for helping with interpretation of LCQ-TOF-MS data. *I am a living testimony of the few who are part of sponsored success*. Mine is a sponsored success. I give glory to God. Thanks a lot.

TABLE OF CONTENTS

ABSTRACT

Prospects for utilizing edible fungi (Basidiomycetes) in cross-cutting industrial applications have not been fully explored. This study sought to bioprospect for bioactive molecules with colouring potential from golden yellow *Pleurotus citrinopileatus* and an exotic pink *P. djamor* and determine their application in food and non food (textile industry). Data obtained were analysed statistically using 1-way ANOVA, with multiple comparison done with Tukey test at α=0.05.These mushrooms were grown on seven selected substrates namely wheat straw, rice straw, sugarcane baggase, bean straw, banana fibres, maize stovers, sawdust and yields determined. Beans straw and rice straw reported earnest pin head formation with a significant biological efficiency of 72%, 70% for *P. citrinopileatus* and 68%, 62% for *P. djamor* respectively. These substrates reported a significant mean basidiocap number and pin head abortion from the five substrates (p<0.05). There were no statistical difference in growth parameters between wheat straw and sugarcane baggase for both species (p>0.05). Sawdust reported poor growth parameters. Extraction was done in four phenological states and subjected to phytochemicals screening, determination of total polyphenols content, and Total Flavonoid content, Total carotenoids, β-Carotene, Lycopene, antioxidant activities using ABTS assay, DPPH assay, and Ferricyanide reducing power. Standard methods were used for qualitative analysis; Folin Ciocalteu assay for Total polyphenols, Aluminium chloride reduction method for total flavonoid. Phytochemical components identified included saponin, terpenoids, flavonoids, and polyphenols. Primordials exhibited increased concentration of phytochemicals, which correlated positively (r≥0.9, p<0.05) with total phenolics (19.025±0.847; 13.803±0.797mg GAE/g dwt), total flavonoids (14.824±0.89; 10.619±0.785mg QE/g dwt) and total antioxidant properties. Antioxidant properties were concentration dependent with ethanol extracts exhibiting statistically significant higher values than water extracts. A Liquid Chromatography quadrupole time of Flight Mass Spectrometry was used for profiling metabolites across these phenologies. ChemSpider™ database was used for putative identification of compounds based on their empirical formula and accurate mass. A total of 21 compounds were identified based on their empirical formula and accurate mass. Extracts from primordials have potentiated health benefits. Formulated Mushroom powder was incorporated into yoghurt to assess psysicochemical and acceptability profiles based on organoleptic tests. Yoghurt formulated with mushroom powder at 1.5% (w:v) and 3% w:v) were statistically better in acceptance than 5% (p>0.05). The 5% formulated had significant colour properties but poor viscosity and pH with extended setting time (18hrs). Mushrooms have a potential in fortifying dairy products. One set of mushroom powder was

subjected for fastness study using mordanted fabric. Fabrics exhibited varied colour shades depending on mordants used. Copper sulphate showed significant intensity of colouration with lower fastness than Alum for both species dye bath (p>0.05). Unmordanted fabrics exhibited significant colour fast. Spent substrates were tested for degradation and discolour Levafix Blue CA based on optical density. Discolouration of levafix by spent substrates was concentration dependent for each treatment. Biodegradation by 50gm SMS exhibited significant difference between the two species, this signified the potential of spent substrates in mycoremediation.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Mushroom is a fruiting body of a fungus and can be either epigeous or hypogeous and large enough to be seen by naked eye (Chang and Miles, 1992; Chang & Chiu 1992). Edible mushrooms belong to genus *Pleurotus spp, lentinula spp, Agaricus spp,* and *Ganoderma spp*. Pleurotus spp are the most commonly grown owing to cheap technological requirement (Sanchez, 2010). Mushrooms constitute over 140,000 species of plants on earth, yet only 10% are known (Quintero *et al.,* 2008). Despite attempts to identify applications in folk medicine for various mushroom species (Chang, 2006; Chang, 2008), little effort has been focussed in determining their industrial application such as food and textile colourants. Recent studies have shown that mushrooms harbour therapeutic properties because of their constituents that are antitumor, antioxidant, antihyperlipidemic (Griensven, 2009; Hu *et al*., 2006; Lindequist *et al*., 2005; Preeti *et al*., 2012). Many studies attest to the fact that neglected plants can be the alternative solution to food insecurity in African (Aberoumand, 2009; Aberoumand and Deokule, 2009; Afolayan and Jimoh, 2009; Belcher *et al*., 2005; Lulekal *et al.,* 2011). Farming of edible mushroom is an emerging activity for sustainable foods in the developing nations and a potential source of employment to the youths, especially if more effort would identify several industrial applications in most edible mushrooms that are not grown here in Africa.

Pleurotus species occur in different shades of colour and this could be utilized in pigment production (Zhou and Liu, 2010). The pigment produced depends on the type of mushroom, its age, and the type of mordant used for dye extraction (Vankar, *et al.,* 2009; Samanta and Agarwal, 2009; Richard *et al.,* 2009). Currently, most consumers of food, textile, cosmetics, and paint products prefer organic dyes to synthetic ones because consumers associate organic dyes with less health risks (Vankar, *et al.*, 2009; Samanta and Agarwal, 2009). Fibres dyed with organic dyes have been shown to be more stable and tend to last longer; organic paints are environmental friendly and easily biodegradable as opposed to synthetic dyes (Ahlawat and Singh, 2009; Machado, *et al.* 2006). This warrants the need to identify and determine the potential different dyes extracted from *Pleurotus citrinopileatus* and *P. djamor*. These species occurs in golden yellow and pink colouration. From our literature search no existing study

has documented phenological variations of colours, their application, and bioactive molecules from these species.

Increased industrial activities exert stress on the environment through generation of wastes that are persistent and difficult to eliminate (Bennet, *et al*., 2002; Gupta, *et al.,* 2005; Sasek and Cajthaml, 2005). For instance synthetic dyes used in colouring foods, textiles, and pharmaceutical products requires chemical processes prior to its use. Most of these are normally released to the environment partially treated (Fernandez-Luqueno, *et al.*, 2010; Steffen, *et al.*, 2007). These have a persistent effect and bioaccumulation properties in the ecosystem and can lead to biomagnifications in the food chain hence adverse health effects (Eramo, 2009). Existing chemical and physical methods of treatment uses lots of power making it costly (Mohana *et al.,* 2008; Moosvi, *et al.*, 2005). This calls for the need to identify alternatively cheap environmental friendly ways of removing hazardous synthetic materials released into the environment. Spent mushroom substrate have been shown to contain a consortium of fungal and bacterial microbes that produce enzymes like manganese peroxide, lignin, which have potential for biodegradation of organopollutants like PAH, petroleum spells, fungicides, persistent pesticides (Ahlawat & Singh 2009; Bennet et al 2002; Emuh 2010; Hamman, 2004; Machado et al. 2006; Kaushik & Malik 2009; Lopez et al. 2006). Most pollutants from textile and leather industries are yet to be investigated with SMC hence the baseline to test levafix molecules.

1.2. Problem statement and justification

Industries manufacturing food, cosmetics, pharmaceuticals, and textiles products utilises lots of dyes. The underlying reason these synthetic dyes are used is perhaps to add value to products by either improving or appealing to consumers or scaling up market value (Zhou and Liu, 2010). Most of these dyes are derived from synthetic formulations, which are manufactured chemically with a few others obtained from the natural ways (Downham, and Collins, 2000). One successful story in Kenya relates to Bixa plant (Collins, 1991). Collins ascribes that this plant is the source of annato dyes that has received international attention for its application in various industrial applications. The unfortunate story is that we export the plant to China, India, and UK (Stiles, 1991). These synthetic dyes are also heavily used in various non-food industries like textiles, however, studies show that natural dyes are gentler on textiles than the synthetic ones (Samanta, and Agarwal, 2009). One successful story in Kenya relates to Bixa plant (Stiles, 1991). This plant is the source of annato dyes that has

received international attention for its application in various industrial applications (Collins, 1991; Stiles, 1991). The unfortunate story is that we export the plant to Japan, Europe, and USA (Collins, 1991; Stiles, 1991). However from health and safety point of view, consumers are becoming aware, conscious and sceptical of the negative effects associated with synthetic formulations (Aberoumand, 2011). Such consumers shun items with synthetic formulation for alternatives with organic origin to remain healthy (Chattopadhyay, *et al.*, 2008). The general belief is that dyes added to any food should improve the nutritional and health benefit of the food rather than being a potential health hazard (Hallagan *et al.*, 1995; Hari *et al.*, 1994). Natural products with health promoting bioactive molecules have been proposed as potential substitute for synthetic bio-colorants (Aberoumand and Deokule, 2009). Besides, synthetic formulations pose a serious challenge to the ecosystem because of inefficient treatment (Amaral *et al*., 2004). Mushrooms exhibit different hues, which suggest their potential to synthesis dyes (Rai and Tidke, 2005). This necessitates the need to bioprospect for biocolorants from renewable sources like coloured mushrooms and test bioactive molecules in the extracts before testing applications in selected products.

Synthetic dyes used in industries are mostly released to the environment without exhaustive treatment processes. This is largely so because efficient treatment consumes power hence increasing production costs. Most of these industries resort to partial treatments hence contaminating the ecosystem. Alternative possible treatment systems that are sustainable and safe should be sought. Some studies have established the ability of spent mushroom substrates to biodegrade petroleum and oil wastes, polycyclic Aromatic Hydrocarbons (PAH) contaminated soils, and other wastes associated with heavy metals (Eramo, 2009; Khalid et al., 2008). Mushroom industries generate lots of wastes as spent mushroom substrates that are said to have a consortium of microorganisms secreting enzymes with biodegradation ability. This study bioprospects the potential of spent mushroom compost to biodegrade synthetic textile and leather dyes: *Levafix Blue CA- LBCA*.

1.3. General objective

To bioprospect for compounds of industrial importance from selected Basidiomycetes (*Pleurotus citrinopileatus* and *P. djamor*) for biocolouration (food and non-food products) and biodegradation of synthetic dyes (Levafix)

1.4. Specific objectives

1. To compare suitability of selected substrates for growing *Pleurotus citrinopileatus* and *P. djamor* mushroom
2. To determine qualitative, quantitative, total antioxidant activity, and metabolomic profiles of the dye extracts from *P. citrinopileatus* and *P. djamor* across phenological states.
3. To determine colour, acidity, setting time and acceptability of yoghurt incorporated with *Pleurotus citrinopileatus* and *P. djamor* mushroom floor.
4. To assess dye fastness of *Pleurotus citrinopileatus* and *P. djamor* mushroom dye on selected mordanted fabrics.
5. To determine the potential of mushroom spent substrate in degrading Levafix Blue CA synthetic dye.

1.5. Hypothesis

1. Extracts of *Pleurotus citrinopileatus* and *P. djamor* mushrooms do not contain bioactive secondary metabolites
2. Mushroom dyes will not enhance the organoleptic properties of selected local foods (yoghurt).
3. Selected fabrics dyed with *Pleurotus citrinopileatus* and *P. djamor* dyes will not be colour fast.
4. Spent mushroom substrate of *Pleurotus citrinopileatus* and *P. djamor* mushroom don’t have the potential for degrading Levafix Blue CA dyes.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1. Organic substrates

Cultivation of mushroom requires agricultural residues that offer appropriate Biomass (Sanchez, 2010). This is because these fungi have the ability of converting such organic wastes into biomass. Examples of these are the grasses from the wild (Das, *et al.*, 2000) agricultural wastes like wood (Tisdale *et al.*, 2006) sorghum leave, stalks and leaves of banana, and wheat straw (Dwivedi and Singh, 1994). The type of organic substrate utilised in growing process have a significant effect on the yield of mushroom because it affects mushroom biological processes (Chang, 1999; Dubey, 1999; Jain and Vyas, 2002). The most common type of substrate used in Kenya is the wheat straw supplemented with wheat bran (Kimenju et al., 2009). Most mushrooms grow well in solid substrate in their natural habitat, which justifies the need to identify appropriate substrates that simulates their natural ecosystem. Most studies selected locally available substrate from various environments and test their production parameters for specific species of mushrooms (Andrade, *et al.*, 2008; Gupta and Sharma, 1994; Montini, *et al.*, 2006). Selecting a suitable substrate in growing the coloured Basidiomycetes is an important factor for commercialisation potential. Such information guides the entrepreneurs to use optimal substrate with superior production. This justified our attempt to randomly select and screen seven substrates for their suitability to growing these species. Substrates tested included sugarcane baggase, wheat straw, rice straw, bean straw, sawdust, maize stovers, and banana fibres because they are the most abundant and easily accessible at low costs. The variety of agricultural waste disposed in the environment is immense; one cannot design a study to include all the waste in growing particular specie of mushroom because of spawn costs and the time it takes to obtain the fruiting bodies (Nyochembeng, *et al.*, 2008).

2.2 Phenological Distribution of Bioactive molecules

Edible fungi are rich sources of health promoting molecules like polyphenols, flavonoids with radical scavenging properties (Ferreira, *et al.*, 2009). The value added health benefits of edible basidiomycetes are being ascertained because they also posses protein, minerals, vitamins, unique taste, and flavor (Reis *et al.*, 2011; 2012). Several studies have shown that edible fungi in the genera *Pleurotus*, *Agaricus*, and *Lentinula* exhibit antibacterial properties

(Hirasawa *et al.,* 1999; Oyetayo, 2009), anti-allergic properties, reduces blood cholesterol levels (Jeong *et al.,* 2010), anti-tumor (Ferreira *et al.,* 20107), immunomodulatory effects (Valko *et al.,* 2007), and antioxidant properties (Mohamed *et al.,* 2011; Soobrattee *et al.,* 2005). These have elicited interests to bioprospect for natural pharmacological and nutraceutical antioxidants (Simic1988). Natural products with potent secondary metabolites would scavenge for such radicals (Soobrattee *et al.,* 2005; 2006). These molecules have been shown to reduce burdens of certain diseases (Scalber et al 2005). Studies by Barros *et al.,* (2007) demonstrate that different parts of a mushroom have varied health promoting properties. Previous studies have shown that mature fruiting bodies have a reduced number of bioactive molecules associated with radical scavenging properties (Wandati *et al.,* 2013; Ferreira, *et al.,* 2009). Bioactive molecules quantity drops with age of fruiting body due to their involvement in defense mechanism (Barros *et al,* 2007). Radical scavenging properties are largely attributed to phytochemicals such as flavonoids and polyphenols; that contribute largely to plant ecophysiology and survival against biotic and abiotic stressors (Akula and Ravishankar, 2011). However, little effort has been done to identify appropriate phase for collecting fruiting bodies with optimal quantity of these biomolecules.

2.3 Mushroom dyes and application

Mushrooms have diverse coloration because they contain flavonoids and polyphenolic compounds. According to Rai and Tidke (2005) 'these dyes are found in naturally collected or artificially growing fruit bodies, pure culture mycelia, and culture filtrate of mushrooms' (p.457). Research ascertains that international markets have great demands for natural organic dyes owing to their outstanding qualities for medicines, colorant of foods, and cosmetics applications (Kumaresan *et al.,* 2011a). Both synthetic and natural dyes are used in medicines and cosmetics to improve buying attribute to the consumers (Samanta and Agarwal 2009). These applications can also be expanded to other applications like textile and food industries. Espindola *et al.* (2007) reported that *Pleurotus* mushroom had a potential candidate to degrade red 40 dyes used in many food industries.

Pigmentation is thought to confer plants with protective mechanisms against UV damages, attack from bacterial invasion, or may be crucial in attracting insects (Velisek and Cejpek, 2011; Zhou and Liu, 2010). One characteristic of mushrooms is that they don't contain pigments that are predominant in higher plants like chlorophyll, anthrocyanins among other pigments (Bhattacharya and Shah, 2006). The pigment produced depends on the biosynthetic pathway (Samanta, and Agarwal, 2009). This makes mushrooms an interesting research area

owing to their diverse coloration that can be applied across various industrial applications (Mibei *et al.,* 2009). Food manufacturing industries utilize a lot of synthetic dyes in their production lines (Hari *et al.,* 1994). It follows that challenges of wastes treatment abound (Amaral *et al.,* 2004).

2.4 Mushroom Biocolorants

Natural pigments are present in virtually every plant material on the surface earth (Samanta, and Agarwal, 2009). Plants exhibit coloration either from their appearance, or due to their biological functions, structural, or chemical composition, and as a result of their physical properties like solubility (Anitha and Prasad, 2007; Das *et al*., 2007). Pigments in every living organism are necessary for their development (Delgado-Vargas *et al.,* 2000). Natural pigments are considered useful in colouring foods, natural fibres like wool, silk, and cotton (Adeel *et al*., 2009; Samanta, and Agarwal, 2009). However, most industries rely heavily in synthetic dyes that are associated with adverse health effects on humans and the ecosystem (Delgado-Vargas, et al., 2000). Several studies have attempted to identify and extract natural dyes from various plant sources for industrial applications in food coloration, cosmetics, textile colouring among others (Das et al., 2007; Kumaresan et al., 2011b; Mahangade et al., 2009). Natural dyes are not only friendly to the environment but also cheap and easily available (Samanta, and Agarwal, 2009).

2.5 Fortification of daily product with mushroom

Despite numerous potential health benefits in mushroom species reported by Chang (2006) and Barros et al., (2007a, b, c) little effort has been done to develop mushroom products. Studies have shown that Reish mushroom (*Ganoderma* spp) makes healthy tea and other value added products with immense health benefit (Sanodiya *et al.,* 2009; Wasser, 2002). Most studies recommend development of nutraceuticals and other value added products (Dureja et al., 2003). However, to date no existing study has attempted to incorporate mushroom in daily product like yoghurt. Bioprospecting studies in this area would demonstrate viability of using mushroom as a fortifying agent.

2.6 Spent mushroom substrate

Spent substrate is the remnant remaining after mushroom growing (Sanchez, 2010). Disposal of spent mushroom substrates is a major concern for mushroom growers. It has been reported that some farmers would prefer burning these wastes (Singh *et al.,* 2003). These wastes (spent mushroom compost/substrate) can be employed in industrial applications. The compost is a rich source of xenobiotic degrading microbes largely made up of lignolytic fungi, actinomycetes, and bacteria (Sasek and Cajthaml, 2005; Lau *et a*l 2003). These have been shown to degrade pollutants or transform the pollutants into non-toxic or less toxic substances thereby lowering their bioavailability in the ecosystem (Semple *et al.,* 2001). However, current treatment industrial effluent is largely based on expensive chemical and physical methods (Mohana, *et al.,* 2008; Moosvi, *et al.,* 2005).

2.7 Biodegradation capacity of SMS

One of the underutilised applications of basidiomycetes is to degrade recalcitrant synthetic dyes effluent from the industries (Gao, *et al*., 2006). This is because chemical and physical methods currently in use are not cost effective (Khalid *et al*., 2008). Environmental conservation is an important strategy of ensuring sustainability and protection of the future generation (Adenipekun et al 2011). Therefore, every aspect of the environment is necessary for this to be realized. Use of synthetic dyes in industries manufacturing food, pharmaceutical products, cosmetics, papers, and textiles pose a serious problem to efforts of environmental conservation, which warrants alternative methods of reducing toxicity to the wastes prior to their release into the ecosystem (Chang *et al*., 2001; Gao *et al.,* 2006). The main rationale to this potential is the ligninolytic enzymes produced extracellularly by basidiomycetes (Aro et al 2005; Lau *et a*l 2003; Peng *et al*., 2008; Rigas *et al* 2007).

2.8. Lignolytic enzymes as potential degraders.

Unlike bacterial systems, fungal ones are distinct because their broad-spectrum activity that is not dependent on pollutant quantity (Asamudo *et al.,* 2005). Mushrooms are thought to play an important role in bioremediation because they have a variety of enzymes that transform contaminants into beneficial agents in the ecosystem (Hamman, 2004). Several studies have been done to elucidate mushrooms that produce extracellular enzymes like polygalactoronase, pectin lyase, and cellulase (Emuh, 2010). However, their ability to clean up the industrial effluent has not been fully studied (Rigas *et al.,* 2007) Most studies have

concentrated on degradation of agricultural wastes like sawdust and failing to at their potential to break down toxins in a waste waters and industrial effluents (Bennet *et al.,* 2002; Emuh, 2010; Hamman, 2004; Quintero *et al.,* 2008).

2.9. Industrial effluent and synthetic dyes

Across the world, there is quite a significant reserve for industrial wastes (Gao *et al.,* 2006). In fact a huge percentage of major polluting agents originate from industries utilizing synthetic materials for their manufacturing processes (Mohana *et al.,* 2008). These synthetic materials produce products like plastics, spent fuels, hydrocarbons, and dyes that are toxic to the environment (; Khalid *et al.,* 2008). Current methods of waste removal employing both physical and chemical approaches are not efficient because they consume energy and produce lots of sludge (Moosvi, *et al.,* 2005). For instance, textile industries utilises huge tons of levafix blue dyes for their manufacturing processes.

2.10. Levafix blue CA dyes

Levafix blue is a synthetic dyes with cellulosic reactive dye belonging to fluorotriazine (TFT) group (Dystar, 2013). These are preferred because of high performance, optimal dyeing, and excellent wet light fastness that achieve the highest levels of fixation even with repeated washing (Chung *et al.,* 1992). These dyes are easily applied using all the common methods of dyeing (Dystar, 2013). Their reproducibility makes them reliable for large scale application (Lorimer *et al*., 2001). They can also be combined with other dyes in the Levafix family or Remazol dyes (Pala *et al.,* 2003). These characteristics have been exploited by retailers and manufacturers in colouring garments of trending setting brands hence mostly used in the modern textile industries (Fewson *et al.,* 1998). Their molecules are held so tight that most conventional treatment methods do not reduce their toxicity by bond breakage prior to their environmental release hence the need to identify other biological methods to complement previous biological studies (Ismail *et al.,* 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.0 Methodology

3.1.1 Source of Pleurotus spp. mushroom cultures

Pleurotus citrinopileatus was provided by Dr. Fredrick Musieba of Kenya Industrial Research development Institute (KIRDI) while *P. djamor* was shipped from Plant Pathology Department, Penn State University, Pennsylvania State, USA. Starting cultures were obtained through tissue culture techniques outlined by (Stamets 2000) and cultures maintained on Potato Dextrose Agar (PDA) medium at 5° C.

3.1.2 Study site

The study was conducted at the Mushroom Technology Centre of the Kenya Industrial Research and Development Institute (KIRDI) and Jomo Kenyatta University of Agriculture and Technology (JKUAT) between October, 2013 and September, 2014.

3.1.3 Spawn production

Bird millet was used as carrier material for spawn production with a few modifications in the quantity of calcium carbonate from 10% to 1% (Isikhuemhen *et al.,* 2000). Eight (8kg) of these grains were soaked in 20 litres of clean tap water. These were cleaned twice with fresh batch of clean water. They were boiled at 55^{o}C for 1hr while turning until the grains softened. The grains were spread on a clean tiled laboratory bench and allowed to cool and drain excess water. Adjustment of pH to 7.4 was attained by mixing the boiled grains with 1% w/w AR grade $CaCO_3$ (Unilab Kenya Limited). The grain mixture were transferred into media bottles of capacity 1 litre at the rate of 800g per bottle and plugged with cotton wool, covered with aluminium foil before autoclaving at 121^{o}C for 20 minutes. The media bottles containing the sterile grains were transferred to the laminar airflow and left to cool. They were then shaken and turned to stop them from sticking on the sides. After which, they were inoculated with pure *Pleurotus* mushroom cultures mentioned in 3.1.1 above. Each bottle was inoculated with four Petri dishes of fully grown mycelia. The spawn bottles were incubated at room temperature on the laboratory bench for 14 days for complete colonization.

3.1.4 Substrate preparation and inoculation

Seven selected locally available substrates were evaluated for their suitability to grow exotic *Pleurotus djamor R22*; in comparison with their response to indigenous *Pleurotus citrinopileatus* Singer. These substrates included banana leaves (*Musa spp*), sugarcane baggase (*Sacchurum officinarum*), maize stovers (*Zea mays*), bean straw (*Phaseolus vulgaris*), rice straw (*Oryza sativa*), saw dust (*Eucalyptus spp*) and wheat straw (*Triticum aestivum*). Wheat straw was used as the control because it is the substrate commonly used for production of other oyster mushrooms (Philippoussis *et al.,* 2003). The substrates were processed and size reduced to 2-3 cm. They were soaked in water and allowed to drain excess water overnight and palm squeeze test applied. They were bagged in heat resistant polythene bags of dimension 15cm X 20cm, weighed (1kg) and tied with strings in readiness for sterilization in an autoclave at 121^{o}C for 20 minutes. Substrates were prepared in triplicates. Substrate bags were allowed to cool and spawned seeded at the rate of 7% w/w for the two species.

3.1.5 Pinning induction

Spawned bags were incubated in dark room at room temperature 13 days until the spawn run was complete (Wambua, 2004; Onyango *et al.,* 2011). Upon pinning bags were transferred to growing room (Upadhyay *et al.,* 2004; Singh *et al.,* 2008). Relative humidity was maintained between 60 - 90% using a spray pump twice every day. These bags were checked daily to monitor the colonization of mycelium and development of fruiting bodies (Royse *et al.,* 2004). Harvesting of early fruiting bodies that emerge from the bags were collected upon attaining a diameter of 6cm and weighed before taking them for further analysis. Harvesting of fruiting bodies was done three days after pin head formation.

3.1.6 Data collection

For each of the seven substrates, data collected was based on parameters: mycelium colonization of substrate, time taken from seeding to colonization (spawn run), pin head formation to maturity (1st flush), maturity after first harvest (2nd flush), mean basidiocap weight (MBW), mean basidiocap diameter (MBD), mean basidiocap number (MBN), abortion of pin head (APH), and biological efficiency (BE). Biological Efficiency (BE) was calculated based on the formula, BE = [Weight of fresh mushroom (grams)/Weight of wet substrate (grams)] x100.

3.2.1 Sample preparation at different phenological states

Fruiting bodies were picked at different phonological stages (Fig. 1). These samples were prepared for phytochemical analysis. Intact fruiting bodies of *Pleurotus citrinopileatus* and *P. djamor* mushrooms were dried in oven at 42^{o}C for 3days and milled to powder using electric miller. Ten (10g) of powder was placed in HPLC grade bottles and mixed with 100mL of analytical grade ethanol (Sigma Aldrich, Germany) and left in a 24 hour shaker set at 150 revolutions per minute at room temperature in darkness. The liquid was decanted and the residue re-suspended in 100mL of analytical grade ethanol (Sigma) for re-extraction for 24hours. The filtrate was filtered through Whatman paper no. 1(12.5cm) and stored at 4^{o}C awaiting further analysis (Barros *et al.,* 2008). The filtrate was reduced in volume (concentrated) using a vacuum rotary evaporator (40^{o}C). Samples were then analyzed for total polyphenols, total flavonoids, and radical scavenging properties as explained in 2.4 below.

3.2.2 Standard Qualitative (Phytochemical) Analysis

3.2.2.1 Cardiac Glycoside

The method described by Harborone (1973) was used. Two milliliters (2mL) of extract was added to one milliliter (1mL) of Acetic acid followed by the addition of one milliliter (1mL) of 10% Ferric Chloride (Sigma Aldrich, Germany) and three drops of concentrated Sulphuric acid along the sides of the test tubes. Brownish ring and green blue precipitate at the bottom of the test tube served as the indicator for the presence of Cardiac glycoside.

3.2.2.2 Phenol and Tannins

Four drops of Ferric chloride 10% was added to two milliliters (2mL) of extract for the examination of the blue or green colour precipitate due to the presence of phenol and tannins.

3.2.2.3 Terpenoids

Three (3ml) of chloroform and four drops of concentrated sulphuric acid were added along the sides of the test tubes to 2 milliliters of extract and colour development observed. Reddish brown colour precipitate indicated the presence of terpenoids.

3.2.2.4 Alkaloids

Two milliliters (2mL) of Mayer's reagent (Sigma Aldrich, Germany) were added to 2mL of the extract and colour development observed. Development of pale precipitate was to indicate the presence of alkaloids.

3.2.2.5 Resins

Three (3mL) acetic anhydrite were added to two milliliters (2mL) of extract and the mixture gently dissolved by heating. Upon cooling, 5 drops of concentrated sulphuric acid were added and colour development observed. The bright purple coloration indicated resin presence.

3.2.2.6 Flavanoids

Four milliliters (4mL) 10% ammonia were added to two 2mL of sample extract and 1mL concentrated sulphuric acid added thereafter. Presence of flavanoids was determined by the disappearance of yellow coloration.

3.2.2.7 Anthraquinones

One milliliter (1mL) 10% ammonia and 1ml benzene were added to 1mL of the extract. Observation of pink or red or violet coloration on the lower layer/phase would have signified anthraquinone presence.

3.2.2.8 Saponins

Two (2mL) of extract was mixed with 4mL of distilled water and the mixture vigorously shaken for observance of persistent and stable.

3.2.3 The relative abundance of Phytochemicals

Upon initial identification of various phytochemicals in ethanol extracts of golden yellow *Pleurotus citrinopileatus Singer* and exotic pink *P. djamor* R22, their relative abundance were determined as previously described by (Cai et al., 2011). Data obtained were statistically analyzed with Chi - Square based on the goodness of fit test. This involved running a total of nine runs for each species and allocating values between high and low. Ho: The concentration was neither low nor high; H1: the concentration of phytochemicals was significantly different (p=0.1), with a degree of freedom (df) = 1, and the expected value (EV) of 4.5, while $\chi 2$ critical value set at 2.7055. Our data set met the conditions of Chi-

square with the exception of standard minimum EV = 4.5 instead of 5.0 given that a total of 9 sets of data for each test was selected.

3.2.4 Total Carotenoids content

These were determined as previously described by Ranganna (1999) with a few modifications in sample volume. Ten (10g) sample and 5g of celite 454 was extracted in 40mL stepwise cold acetone at room temperature in darkness until complete extraction was attained. Cleaning of extracts with distilled water was done carefully to prevent formation of emulsion. This was partitioned through separation funnel using petroleum ether. Trapping of water from the layer was done using on a funnel with anhydrous sodium sulphate. Absorbance was measured at 450nm with petroleum ether used as blank. Calculation of Total carotenoids in the mushroom extract was done using the formula: Total carotenoids (µg/mL) = (3.856 * Absorbance of sample * Volume (mL) * 100)/Weight of sample X 1000

3.2.5 Determination of β-Carotene and lycopene

These were determined based on protocol previously described by Nagata and Yamashita (1992), with a modification in sample volume. In brief, previously extracted methanol extracts of mushroom was vigorously shaken in a mixture of acetone and hexane (8:12; 20ml) for 1min. This was filtered with Whatman no. 4 and the optical density of the filtrate determined at various wavelengths of 453, 505, and 663 nm. Each test was done in triplicate and results expressed as means± SD. Contents expressed as mg carotenoid per gram of the extract. Calculations of β-Carotene and lycopene contents were determined according to the equations below.

$$\text{Lycopene (mg/100 ml)} = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$$

$$\beta\text{-carotene (mg/100 ml)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

(Nagata and Yamashita, 1992)

3.2.6 ABTS assay (Trolox equivalent antioxidant capacity assay)

The ability of coloured edible mushroom antioxidant potential to quench and scavenge for ABTS radicals was evaluated as previously described by Re (1999). Samples were analyzed in triplicates. The underlying principle is the ability of the extract to reduce the preformed ABTS radicals by decolorizing the blue green chromophore. A polar soluble vitamin E analogue, Trolox was used as standard for this evaluation, hence results expressed as Trolox Equivalent mg per grams dry weight (Linearity curve range 0.1- 0.8mM).

3.2.7 Reducing power (Ferricyanide Reduction Antioxidant Power - FRAP) Assay

The potential of mushroom reduction power was determined by following the previously described method of Oyaizu (1986). A concentration gradient for each phenological state was designed. Mushroom aliquot of 2.5millitres was mixed with equal volumes of 200mM sodium phosphate buffer (pH 6.5, AR) and 2.5millilitres 1% potassium ferricyanide (AR). This mixture was incubated for 20minutes at 50^{o}C before adding 2.5millilitres 10% trichloroacetic acid (w/v) and carrying out separation by centrifugation at 1200 revolution per minute for 10minutes. Five millilitres (5mL) of supernatant was pipette in a new labelled tube and volume doubled with deionised water (5 millilitres). Immediately, 1millilitre of 0.1% ferric chloride (AR) was added and optical density determined at 700nm using UV-Vis-SDD-10AV SHIMADZU spectrophotometer. BHA and α-Tocopherol were used as standards. Samples were analyzed in triplicates. Linear regression was used to determine mushroom extract that gave 50% absorbance (EC_{50}); data obtained were compared statistically using 1-way ANOVA.

3.2.8 Total polyphenols (Folin-Ciocalteu Reagent Assay)

Calorimetric method according to Singleton *et al.,* (1999) with a few modifications was used. Different concentrations of Gallic acid (Sigma Aldrich, Germany) standard were prepared (0.1mg/mL to 1mg/mL). Briefly, extract (0.5mL) was transferred in a 10mL test tube and 2mL ethanol (Sigma Aldrich, Germany) added. Folin-ciocalteu reagent, 1.25mL was added and after 5 minutes, 6.25 mL of 20% aqueous sodium carbonate added to the mixture. This was vortex mixed and incubated at room temperature for 40 minutes. One (1mL) of each preparation was diluted with 9mL of ethanol before reading absorbance at 725nm using UV-Vis-SDD-10AV SHIMADZU spectrophotometer against a blank containing all the reagents except the extract. Each experiment was done in triplicates. The total amount of phenol was calculated as Gallic acid equivalent from the curve calibrated with Gallic acid as the standard. This was expressed as mg GAE/g dry weight of mushroom material.

3.2.9 Total flavonoid content

Total flavonoid was determined using spectrophotometric method previously described (Cheung *et al.,* 2003) with a few modifications of sample volume. One millilitre (1 mL) of sample extract was aliquot to the test tube, 4ml of ethanol was added, and after 3minutes 0.3mL of 5% sodium nitrite ($NaNO_2$) (Sigma Aldrich, Germany) was added. The resulting

solution was mixed allowed to stand at room temperature for 3 minutes. To this, 0.3mL of 10% $AlCl_3$ (Sigma Aldrich, Germany) was added. After 5 minutes, 2mL of 1M NaOH was added and shaken to react. This was diluted by topping up to 10mL mark. A blank containing all the reagents except the extract was included in the set up. Ethanol (analytical grade-Sigma) was used to represent the sample. Each experiment was done in triplicates. Absorbance was taken at 415 nm by UV-Vis-SDD-10AV SHIMADZU spectrophotometer. Quercetin (Sigma Aldrich, Germany) standard was prepared in the range of 200ppm to 1000ppm, flavonoids content was expressed as mg Quercetin per gram dry weight (mg Qc/g DW).

3.2.10 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

Fresh solution of DPPH (Sigma Aldrich, Germany) was always prepared in ethanol. DPPH assay was based on Brand-Williams *et al.,* (1995) with a few modifications of sample volume. Briefly, 4mL of each extract concentration was used for this analysis; prepared as (1.5, 2.5, 5, 10, 20mg/mL) in ethanol, mixed with 1ml 0.1mM DPPH diluted in analytical grade ethanol. This 5mL capacity was shaken in a test tube and incubated in the darkness for 45 minutes at room temperature. Ascorbic acid was used as positive control. The decrease in absorbance was measured at 517nm by UV-Vis-SDD-10AV SHIMADZU spectrophotometer. Each experiment was done in triplicates. Antiradical activity was expressed as percentage inhibition. The percentage inhibition of the radicals due to antioxidant properties of the extract was calculated as: (%) Inhibition = $[(A_{blank} - A_{sample}) \text{ X } 100]/A_{blank}$.

3.2.12 Metabolomic profiling of *P. djamor* R22 and *P. citrinopileatus Singer* (LC-MS)

Phenological samples (two primordials phases, mature fruiting body, and spawn mycelium) of each species were prepared by dissolving 10gm of powdered extracts to 100ml ethanol and distilled water (1:1) and left overnight in a rotary shaker at 150rpm in darkness at room temperature for complete extraction. The resulting extracts were centrifuged at 10000rpm for 20min at 25^{o}C. The supernatant were filtered with a syringe fitted with 0.45 µm filter and stored in HPLC vials for a liquid chromatography–quadruple time of flight mass spectroscopy (LC–QTOF/MS) analysis. This untargeted metabolomics approach enables accurate and putative identification of compounds in positive electrospray ionisation (ESI) mode at both high and low collision energy (CE) scanned from 0 to 2000 mass spectra.

Identity was based on accurate mass, confidence fit, and empirical formula using Chemspider™ database.

3.2.13 Statistical analysis

Data obtained were analyzed statistically by SPSS 16 statistical software (SPSS Inc, Chicago, USA). Analysis of variance (1 way ANOVA) and Tukey HSD test were used to ascertain whether there were significant differences between the mean values ($p<0.05$). Pearson correlation was used to find if out the relationship between total phenols, total flavonoids, and radical scavenging activities

3.3.1. Processing mushroom samples

Fruiting bodies were collected, cleaned to remove dirt, soils, residues, or any compost. This was air dried in an oven set at 42^{o}C for 3 hours. The dried samples were ground to fine powder and stored at room temperature in an air tight food container awaiting further analysis with yoghurt.

3.3.2 Milk

Fresh milk from credible supplier, skim milk, sugar, and from Nairobi stores were purchased for this study.

3.3.3 Starter cultures

Probiotic starter culture was procured from credible company, with freeze dried lactic culture ABT-5, CHR-Hausen for use in this work.

3.3.4 Production of flavoured probiotic yoghurt

Previous standardized methods for production of yoghurt were used (Hutkins, 2006; Isanga and Zhang, 2008). Briefly, sugar, skim milk, and mushroom powder (in different formulations) were added together and mixed. The solid constituents were mixed to pre-warmed fresh milk (45^{o}C). This was heated for 30mins at 90^{o}C while stirring vigorously for complete extractions. The mixture was double sieved and cooled to 45^{o}C before addition of probiotic yoghurt culture (frieze dried Lactic acid starter culture, ABT-5, CHR-Hansen, Denmark). Approximately 1.326g starter culture was added to 2litres of the skim milk-

mushroom-sugar mixture. This was incubated at 45^{o}C during which TTA, $_{P}$H, were tested at 2hrs, 4hours, 6hours, 8 hours and overnight intervals. The mixtures were refrigerated for 15hrs at (4-10^{o}C). These samples were kept refrigerated at 4^{o}C awaiting further analysis. Control samples without mushrooms extracts were included for side analysis.

3.3.5. Physicochemical analysis

3.3.5.1. Total titratable acidity (%TTA)

This was determined by titrating 10mls of sample with 0.1M NaOH until substance turned pinkish (reached pH = 8.2) which corresponded with the end point of phenolphthalein indicator. Confirmatory was one with a pH meter. Titratable acidity expressed as (titre value * M * 90 * 100) / (volume of sample*1000).

3.3.5.2. pH

A digital pH meter was calibrated with buffer standards of pH 4, 7.0, and 9.2 prior to use. Measurement was carried out at room temperature of 27.5^{o}C. Fifty millilitres (50mL) of each sample in a beaker was probed with pH meter probe stirred and left to indicate a constant value. This was rinsed thoroughly with distilled water before measuring subsequent samples, pH was monitored at an interval of 0, 2, 4, 6, 8, 22 hours.

3.3.5.3. Colour after fermentation

The colour of the product was evaluated and measured using Minolta colour difference meter (Model CR-200, Osaka, Japan) calibrated with a white and black standard tile. This involved measurement of L*, a* and b* coordinates. Hue angle (H°) was obtained by converting a* and b* values as earlier described (Mclellan et al. 1995).

3.3.5.4 Ash content

Five grams (5gms) of each sample was placed in a crucible and arranged in a labelled pattern in the furnace. This was determined at 550^{o}C according to AOAC (1995). Ash content was expressed as the inorganic residues left as percentage of the total weight of yoghurt incinerated.

3.3.5.5 Moisture content

Determination of moisture was based on association of official analytical chemists (AOAC, 1995). A triplicate of five grams (5gms) of sample was placed in a clean moisture dish and transferred in an oven set at 105^{o}C for 3hours. Reading was taken at a constant weight and expressed as percentage of dry weight.

3.3.5.5 Total solids

Weight of residues obtained from moisture content analysis was expressed as percentage total solids. Total solids (%) =100*[(weight of dish + dry yoghurt) – (weight of dish)]/weight of sample.

3.3.5.6. Viscosity

Viscosity of the yoghurt before and after fermentation was determined using capillary viscometer (B mode Japan, Osaka). Ten millilitres (10mL) was diluted with distilled water stirred and transferred in a viscometer suspended in a water bath maintained at 35^{o}C. Time obtained was converted to centipoises (Gassem, *et al.*, 1991).

3.3.6. Organoleptic analysis Basic sensory evaluation and hedonic testing

Acceptability of unflavoured yoghurt was evaluated by untrained panellist. This involves using untrained 17 panellists of students and university workers to sample the food items and give their rating on a scale of 1 to 9. The scale of categorizing the data ranged from extremely liked, to extremely dislike. These assessments were based on appearance, coloration, and aroma, texture, thickness, and overall acceptability of the food. Panellist were asked to evaluate the food items labelled in codes for the degree of liking using a point scale with scores from 1 to 9 variables. The 1 variable represent extremely liked while 9 will represent extremely disliked. Samples were randomly arranged with each individual having a score sheet for recording scores. The selected age ranged from (≥20, 21-25, 26-30, ≥30), gender (male and female) was also noted.

3.3.6. Statistical analysis

Analysis of variance (ANOVA) was used to evaluate the mean and standard deviations for each ratio preparation and compare these parameters between the different preparations using variance. Pearson correlation coefficient was used to determine sensory characteristics. Evaluation of significance difference between the experiment groups was assessed based on the $P < 0.05$.

3.4.1 Sample preparation

As discussed before mushrooms were cleaned to remove any dirt, soils, residues or any compost, these samples were dried in an oven set at 42^{o}C for 3 days (Guinot et al., 2008; Zin & Moe, 2008). The groups of samples were then ground to fine powder and stored at room temperature awaiting downstream processes (Samanta & Agarwal, 2009).

3.4.2 Selecting appropriate fibre for dying purposes

White silk and cotton were used in this study. They were divided into five groups; three groups that were pre-treated with mordants, one without mordants, and controls.

3.4.3 Fabric preparation

The fabric was scoured to eradicate starch It was weighed to a 2% equal weight of soda ash to the fabric was determined together with a detergent weighing 5.5% of the fabric. This salt was added and stirred in warm water after which the fabrics were added and the mixture stirred for 30minutes at 85^{o}C temperature (Guinot et al., 2008; Zin and Moe, 2008).

Bleaching: This process helped in removing natural materials that colours the substrate, such coloration originates from organic compounds that have double conjugated bonds - Bleaching therefore helps in breaking down the chromophores held together by these conjugated systems leading discoloration. In this case the process involved treatment of the fabric with 2mg of ascorbic acid in 3ml Na(HP04) in 200ml distilled water retained at 90^{o}C for 15minutes. After that the fabric were dried, washed and rinsed in sterile water waiting mordanting (Guinot *et al.*, 2008; Zin and Moe, 2008)..

3.4.4 Mordanting (Preparing fabrics before dyeing using mordant)

The mordants used included the alum [$KAl(SO_4)_2$. $12H_2O$], and copper sulphate (Kechi *et al.,* 2013; Vankar *et al.,* 2009; Samanta and Agarwal, 2009). The mordant powder were mixed with known volume of water (1:10) and heated to 85^{o}C for 20 minutes. These were cooled, filtered with 60 mesh cloth of nylon to obtain filtrate that was used in mordanting process. The fabrics were soaked in the mordant for 1hour prior to dyeing (Richard *et al.,* 2009). This would involve using 25% of the mordanting reagent and the textile (w/w) based on pre-mordanting method. Alumina was used as the first mordant while copper sulphate was used as second. A 1:20 material to liquor ratio was used for both mordant. Sample were taken out and squeezed. These were rinsed and squeeze to remove excess fixative using running water until the cold water runs clean prior to dyeing the fabric in the water colour.

3.4.5 Preparation of watercolour for extraction of dyes

The 50g of ground samples were submerged in polar solvents under darkness for dye extraction set at different extraction conditions (Kechi *et al.,* 2013; Samanta and Agarwal 2009). These conditions included room temperature, at 60°C, boiling at 90°C, soaking + stirring 150revolution per minute at room temperature, and soaking + stirring at 90°C. Effects of dyeing duration on development and strength of colour were evaluated for an interval of 1hour for 6hours. The optimal conditions of time and extraction conditions were combined to obtain the colour coordinates of the developed colour strength with a much richer coloration (Guinot *et al*., 2008; Zin and Moe, 2008). Each batch was divided into two groups based on mordanting process. Two mordant (alum and copper sulphate) were added individually and the third was a mixture while the fourth excluded a mordant.

3.4.6 Colour development

The colour of both species during extraction process was evaluated and measured using Minolta colour difference meter (Model CR-200, Osaka, Japan) calibrated with a white and black standard tile. This was done at 1hr, 3hr, 6hr, and 8hr interval. This involved measuring the L*, a* and b* coordinates. Conversion of recorded a* and b* values into hue angle (H°) was done as earlier described (Mclellan et al. 1995).

Hue angle (H°) = arctan (b/a) (for +a and +b values)

= arctan (b/a) + 180 (for -a and +b values)

= arctan (b/a) + 180 (for -a and –b values)

= arctan (b/a) + 360 (for +a and –b values)

3.4.7 Testing fastness of the dyed cloth

This involved checking the fastness of the dyed cloth upon washing with soap, rinsing, and drying under the sun and comparing the intensity of dyes before and after treatment. Polymer fabrics were washed with ordinary soap and exposed to full sunlight.

3.5.1 Preparation of spent mushroom substrate

The first stage involved collection and preparation of spent mushroom substrate from Oyster as per the method described by Law et al (2003). The compost were collected from fresh bags of recently harvested mushrooms and ground to smaller particles. These powders were measured in different grams (10gm, 25gm, 50gm, and 100gm) each for *P. citrinopileatus* and *P. djamor*.

3.5.2 Preparation of Levafix Blue CA and Brown VBR and Treatment of these dyes with SMC

Different concentrations of leather/textile dyes levafix pre-dissolved in methanol were prepared in different concentrations of 25mg, 50mg, 100mg, and 200mg. They were mixed with distilled water at different amounts of powdered SMC (10gm, 25gm, 50gm, 100gm) as described by Law et al (2003). Controls that lacked SMC were included in this set up; these helped in determining the extraction co-efficiencies and the stability of these synthetic dyes throughout the experimental procedure. The resultant mixtures were incubated at 250rpm for duration of 2days under darkness in room temperature condition. A total of 2 replicas were included for each point of sampling. Given that both *P. citrinopileatus* and *P. djamor* spent substrates were used, a total of 92 test-tubes were set up together and incubated for 2days before monitoring absorbance.

3.5.3 Measurement of Absorbance as a measure of degradation

Optical density was determined at each sampling point to evaluate decolouration of the synthetic dyes after two days of incubation. Spectrophotometric procedures are the preliminary though less accurate method of determining the effects of SMS on dye decolouration. Breakdown of dye molecules releases the bonds holding chromophore

together and this is manifested by decline in their intensity (measure of optical density). This was calculated as % absorbance = 100*(absorbance of untreated – absorbance of treated)/absorbance of untreated.

3.5.4 Extraction of leather /textile dyes

Upon treating the sampling points with SMC, the compost was removed by centrifugation to obtain the supernatant and the pellet. Bi-products in the supernatant were extracted with 10 volumes of hexane (HPLC grade) under 200rpm for two hours. This process was repeated to obtain two portions, which were pooled, and dried in a rotary evaporator and mixed with 1ml acetonitrile and filtered with 0.45um filter. For maximum accuracy, 1ml of HPLC grade methanol was added to re-dissolve the residue left on the 0.45µm filter. This process was done in parallel using LBCA without SMC to act as chromatogram subtraction reference in the HPLC analysis. The extraction efficiency was reported in percentage peaks reduction.

3.5.5 HPLC analysis

Reversed-phase HPLC, a HPLC technique with a non-polar stationary phase and an aqueous and moderately polar mobile phase was used. Column: Reverse phase, ODS 150mm x 4.6mm id, Mobile phase: Polar (acetonitrile (60%), water (40%), Flow rate: 1mL/min, Separating temperature: 30^0C, Elution mode: Isocratic Injection volume: 20µL; Detector: Refractive Index (RID), Working pressure: Below 150 kgf/cm^2. No standards were used hence the study was based on peaks obtained.

3.5.6 Assessment criteria

This experiment is based on two assessment criteria for achieving wastes removal. These are removal efficiency and removal capacity based on HPLC peaks. These depends on biosorption and biodegradation processes. Capacity was calculated as the quantity of wastes eradicated per unit mass of SMC, while efficiency is the percentage waste degraded and removed from the waste solution. Biosorption determines the amount of wastes bound in SMC while biodegradation is calculated by subtracting total residues wastes measured (i.e. waste residues level in supernatant and pellets bound to SMC from initial amount) added

.

CHAPTER FOUR

4.0 RESULTS

4.1.0 Substrate suitability

4.1.1 Growth parameters and substrates colonization

The growth rate of mycelium, mycelium surface density, period it takes for total colonization, and the period of first emergence of primordial of the two species across seven tested substrates are presented (Table 1). Briefly, the rate of mycelium formation between the two species were not significant (P>0.05) but statistical difference were noted between the seven substrates (p<0.05). Bean straw substrate recorded the earnest mycelium growth rate while sawdust reported the longest frequency. Comparatively, mycelium surface density varied significantly with bean straw reporting white and uniformly distributed. However, banana leaves and maize stovers substrates reported moderate surface colonization. Sawdust residue reported poor surface colonization. The period it takes for complete colonization of six substrates were significantly lower than the period recorded in sawdust (p<0.05). The duration it took from opening the bags to emergence of primordial ranged from 2 to 3 days for rice straw, wheat straw, bean straw and sugarcane baggase. Besides, days it took from opening of mushroom bags to emerging of first and subsequent primordia in maize stovers, banana leafs were 3 to 5 days, while sawdust took 10 days. Generally *Pleurotus citrinopileatus* showed better growth parameters than *P. djamor*, with the exception of sawdust substrates in *P. citrinopileatus* which did not colonize.

Table 1: The rate of Mycelium growth (mm/day), mycelium surface density, period for total colonization and duration it takes to form primordial of *Pleurotus citrinopileatus* (PC) and *P. djamor* (PD) cultivated in seven different organic substrates.

Treatment	Mycelia growth rate (mm/day)		Degree of mycelium surface density		Period for colonization (days)		Formation of 1st primordia (days)	
	P. citrinopileatus	*P. djamor*	*P. citrinopileatus*	*P. djamor*	*P. citrinopileatus*	*P. djamor*	*P. citrinopileatus*	*P. djamor*
Rice straw	11^{a}	6^{c}	+++	++	11^{a}	10^{a}	15^{a}	13^{aa}
Bean straw	13^{b}	12^{a}	++++	++++	10^{a}	10^{a}	13^{b}	14^{aa}
Sugarcane baggase	9^{c}	8^{b}	+++	+++	12^{b}	11^{a}	12^{c}	13^{aa}
Maize	5^{d}	4^{f}	++	++	13^{b}	14^{c}	17^{d}	16^{bb}

stovers								
Banana leaves	4^{d}	4^{f}	++	++	14^{c}	13^{b}	12^{c}	13^{aa}
Wheat straw	10^{a}	9^{b}	+++	+++	10^{a}	9^{a}	15^{a}	14^{aa}
Sawdust	$<1^{f}$	$<1^{d}$	+	+	>24	>26	nd	28^{cc}

Values with similar superscript letters signifies statistical similar at α=0.05; Nd denotes no data collected;

The degree of mycelium spread on the substrate: + denotes poor surface colonization; ++ denotes moderate spread of mycelium; +++ denotes good distribution of mycelium on substrate; ++++ denotes white and uniform mycelium colonization of the substrate.

4.1.2 Effects of substrates on pinning, flushes, Mean Basidiocap diameter, and mean Basidiocap weight

There was a statistical difference between the duration of pinning between the first and the second flush ($P<0.05$) in the tested substrates for both species (table 2). The second flush took a shorter duration for the emergence of basidiocaps. The order of earnest during the 2^{nd} flush in *Pleurotus citrinopileatus* was wheat straw<sugarcane baggase<bean straw, rice straw<banana leafs<maize stovers, no flushing from saw dust residues in this specie. The order for late emergence of primordia during the 2^{nd} flush in *Pleurotus djamor* was maize stovers>rice straw=banana leafs>sugarcane baggase >wheat straw>bean straw, no flushing from saw dust residues during this flush interval. The order of the size of fresh fruiting body harvest (Mean Basidiocap Diameter - MBD) was wheat straw>sugar baggase>rice straw>bean straw>maize stovers>banana leaves for *P. citrinopileatus*. The order of Mean basidiocap diameter for *P. djamor* was wheat straw>bean straw>rice straw>sugarcane baggase>banana leaves>maize stovers>saw dust. The mean basidiocap weight (MBW) between the flushes for all substrates, with the exception of sawdust residues were significantly different ($p<0.05$) (table 2). Comparatively, the first flush recorded statistically higher MBW than the second flush ($P<0.05$) for both *Pleurotus djamor* and *P. citrinopileatus* in all the six substrates with the exception of sawdust.

Table 2: Effects of substrate treatment on pinning time and mean basidiocap weight

Treatment	Pinning duration per flush (days)		Mean Basidiocap diameter (mm)	Mean Basidiocap Weight (g)	
	P.	*P. djamor*		*P. citrinopileatus*	*P. djamor*

	citrinopileatus									
	1st flush	2nd flush	1st flush	2nd flush	*P. citrinopileatus*	*P. djamor*	1st flush	2nd flush	1st flush	2nd flush
Bean straw	12±1[a]	11.67±0.57[aa]	13[c]	9.33±0.58[a]	88.0±9[aa]	84.33±9.1[a]	536.427±55.210[aa]	214.026±12.371[a]	487.031±49.010[aa]	196.341±16.727[c]
Rice Straw	13[b]	12.33±0.58[aa]	14[c]	13[b]	100.5±8.3[cc]	78.123±11.06[b]	481.647±21.608[cc]	198.426±14.093[a]	407.109±14.832[bb]	212.473±16.910[c]
Sugar Baggase	12[a]	10.11±0.58[aa]	13[c]	12[b]	122.5[bb]	65.0±7[c]	603.721±18.361[bb]	99.867±22.205[b]	423.591±25.617[bb]	134.712±22.642[a]
Maize stovers	18±1[c]	14.33±0.58[bb]	16[b]	15[c]	85.83±6.2[dd]	47±5.57[d]	362.457±11.316[ee]	153.472±41.398[a]	301.017±29.570[cc]	177.815±23.074[b]
Wheat straw	11.67±0.58[a]	8±1[dd]	13±1[c]	10[a]	134.67±5.5[ee]	93.0±9[a]	498.525±32.388[ff]	230.353±27.407[a]	310.860±37.248[dd]	203.175±13.642[c]
Banana leafs	15±1[d]	12.33±0.58[aa]	14.33±0.58[c]	13.33±0.577[b]	73.5±18.25[ff]	56.5±9.66[d]	281.749±32.103[gg]	177.164±19.832[a]	247.637±122.542[ee]	116.314±9.724[a]
Sawdust	Nd	Nd	33[d]	Nd	Nd	40.0[e]	Nd	Nd	89.207±5.21[ff]	nd

Values expressed as Means±SD, values with similar superscript letters signifies statistical similar at α=0.05; nd=denotes no data collected

4.1.3 Effects of substrates on MBN, BE, PHA

All the organic substrates tested in this study reported varied significance on Mean basidiocap number, Biological efficiency, and the frequency of pin head abortion (Table 3). The order of basidiocap number from the most fresh basidiocap was bean straw> rice straw>sugarcane baggase>maize stovers>banana leaves>wheat straw for *Pleurotus citrinopileatus*, no data was recorded in saw dust. The order of basidiocap number for *P. djamor* was bean straw> rice straw> maize stovers> sugarcane baggase> wheat straw> banana leaves> sawdust (table 3). The highest biological efficiency of *Pleurotus citrinopileatus* reported in bean straw (74.6%), wheat straw (72.4%), and sugarcane baggase (70.4%) were not significant statistically (p>0.05). Other BE of *P. citrinopileatus* included rice straw (67.7%), maize stovers (51.8%), banana leaves (45.6%), no basidiocap collected

from sawdust. Comparatively, Bean straw (68.3%) and rice straw (60.8%) of P. djamor were not significant (p>0.05). similarly, BE of sugarcane baggase (54.8%), Maize stovers (52.7%) and wheat straw (51.3%) were not different statistically (P>0.05), banana leaves (35.1%) and saw dust reported the lowest percentage yields in P. djamor. A similar frequency at which initially formed pin head of *P. citrinopileatus* retracts and fails to for primordia was highest in bean straw (7.103±2.162) and sugar cane baggase (6.864±1.438) (p>0.05). Besides, pin heads of rice straw, maize stovers, and wheat straw in *P. citrinopileatus* were not different statistically (p>0.05). The order of pin head abortion in *P. djamor* from the highest frequency was bean straw>rice straw>sugarcane baggase>maize stovers>wheat straw>banana leaves. Sawdust residues did not report any pin head abortion for both species (Table 3)

Table 3: Effects of substrate on yield and growth parameters

Treatment	Mean Basidiocap Number (Fresh weight/1kg		Biological efficiency (%)		pin head abortion (no./1kg)	
	Pleurotus citrinopileatus	*P. djamor*	*P. citrinopileatus*	*P. djamor*	*P. citrinopileatus*	*P. djamor*
Bean straw	23 ± 3.5^{a}	20.35 ± 4.0^{a}	74.647 ± 2.521^{a}	68.367 ± 5.901^{aa}	7.103 ± 2.162^{a}	10.667 ± 2.511^{aa}
Rice Straw	27 ± 9^{b}	19.50 ± 2.6^{a}	67.710 ± 1.102^{b}	60.827 ± 4.424^{aa}	3.568 ± 0.916^{b}	7.019 ± 1.056^{bb}
Sugar Baggase	18 ± 7^{c}	14.7±2.81c	70.433 ± 0.836^{a}	54.833 ± 4.251^{bb}	6.864 ± 1.438^{a}	6.431 ± 2.376^{cc}
Maize stovers	12 ± 2.5^{d}	15.7 ± 1.05^{c}	51.807 ± 1.316^{c}	52.667 ± 2.947^{bb}	4.103 ± 1.101^{b}	4.703 ± 1.528^{cc}
Wheat straw	7.3 ± 2.5^{e}	11.6 ± 2.11^{b}	72.367 ± 1.674^{a}	51.286 ± 6.725^{bb}	3.246 ± 0.535^{b}	2.186 ± 1.155^{dd}
Banana leafs	10 ± 1.5^{e}	10.67 ± 1.25^{d}	45.631 ± 3.760^{d}	35.067 ± 7.254^{cc}	2.176 ± 0.528^{c}	2.067 ± 0.571^{dd}
Sawdust	Nd	1.50 ± 1.0^{e}	Nd	8.903 ± 0.02^{dd}	Nd	nd

Values with similar superscripts signifies statistical similar at α=0.05; Nd denotes no data collected;

4.2 Phenological Distribution 0f Bioactive Molecules

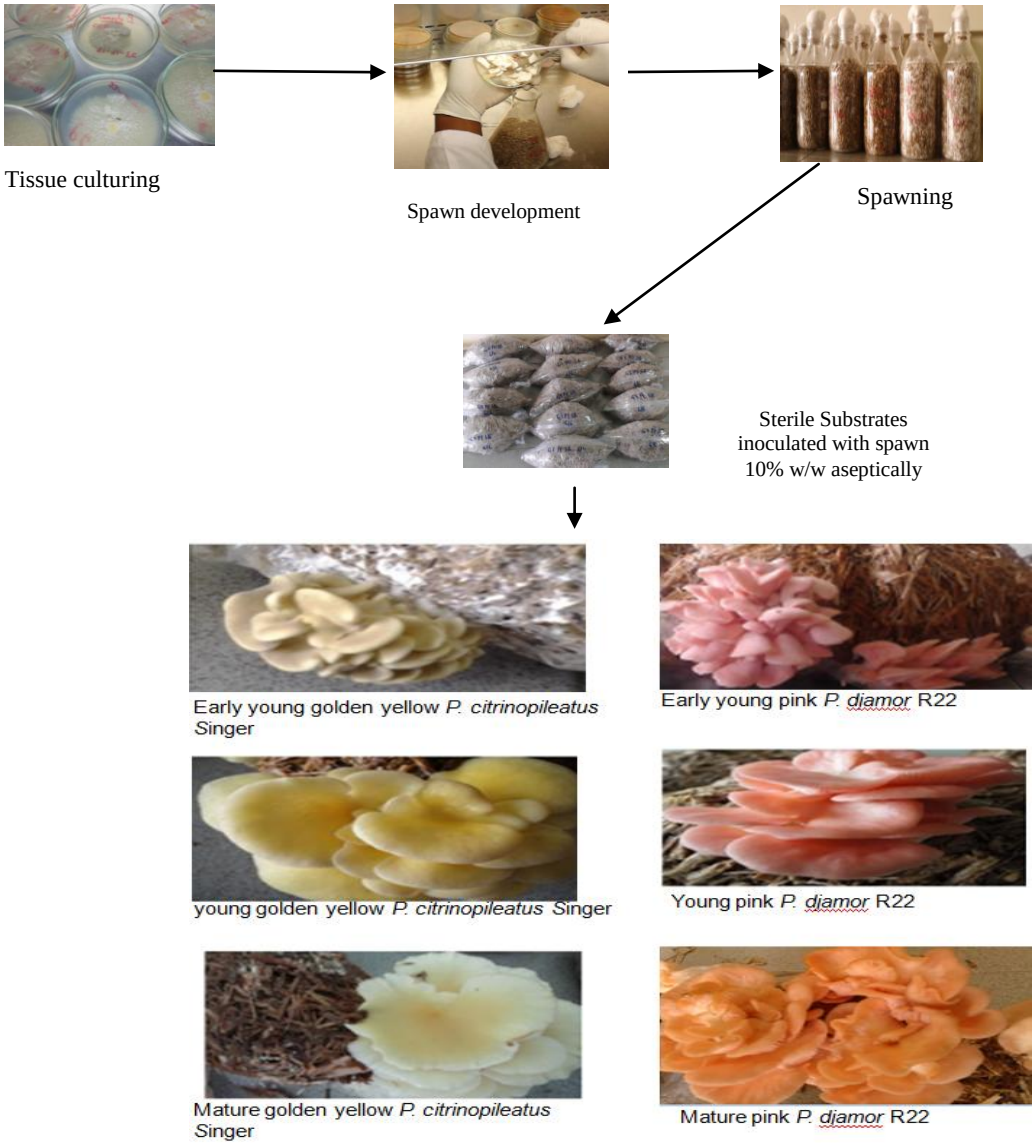

Fig. 1: processes of developing pure tissue cultures, spawning, substrate preparation/inoculation and phenological states of basidiomycetes

4.2.1 Relative Abundance of phytochemicals

The relative abundance based on goodness of fit test X^2 was used in allocating the phytochemicals for the two species. Primordial stage of both species exhibited comparable high concentrations of phenols, flavones, phytosterols, and terpenoids based on the set critical value (Table 1). Low quantities of saponins and tannins were detected. However, cardiac glycosides, resins, alkaloids, anthraquinones, were not detected across these phenological states (fig.2; fig.3).

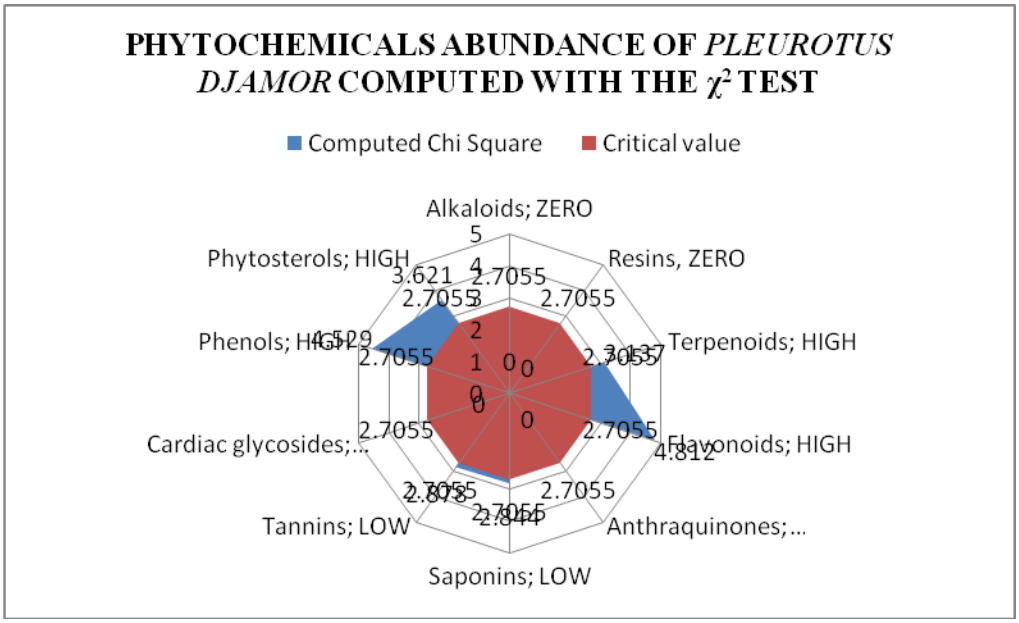

Figure 2: Qualitative Abundance of pooled *Pleurotus djamor* R22

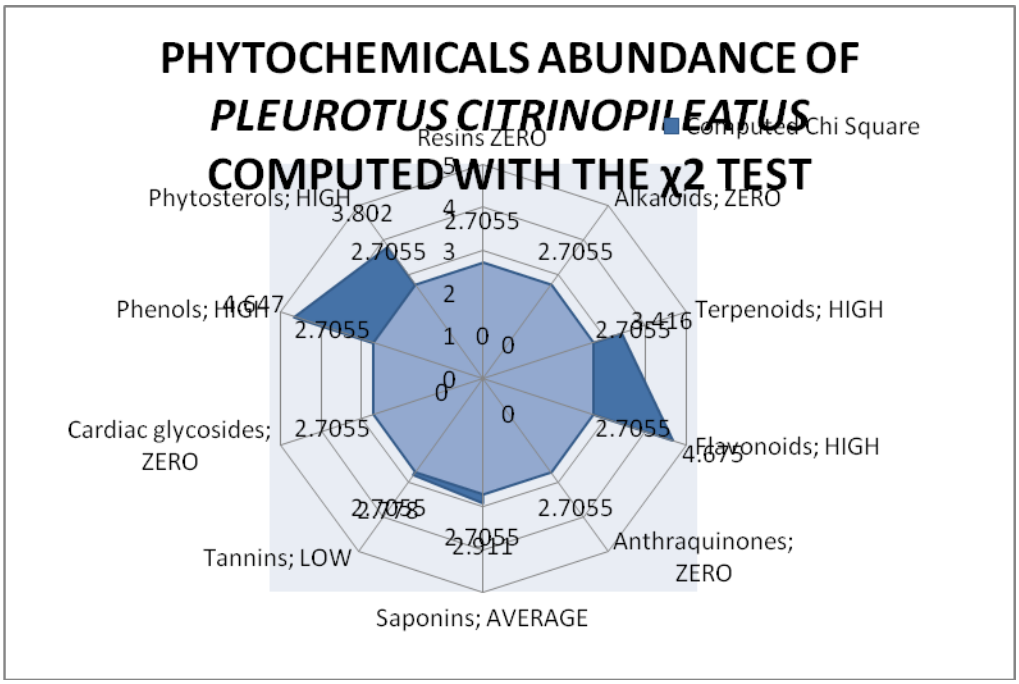

Figure 3: Qualitative Abundance of pooled *P. citrinopileatus* Singer

4.2.3 Total Polyphenol content (TPC),

Folin Ciocalteu *in vitro* assay was used in the determination of total polyphenols. Results depicted in Table 1 shows the different distribution of phenolic content across these phenological states. The two primordial phases recorded the highest total phenol contents for both species, with *Pleurotus djamor* R22 recording 14.369±0.495 mg GAE/g and 19.025±0.847 mg GAE/g while *Pleurotus citrinopileatus* Singer recording 10.018±0.601 mg GAE/g and 13.803±0.797 mg GAE/g for the 1st and 2nd primordial respectively. Although spawn mycelia phase recorded the lowest phenolic contents, it was not significantly different in *Pleurotus djamor* R22 as was the case in *Pleurotus citrinopileatus* Singer.

Table 4: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of *Pleurotus djamor* R22 and *Pleurotus citrinopileatus* Singer across phenological states

Pleurotus djamor R22			*Pleurotus citrinopileatus* Singer	
Phenology	Total Phenols (GAE mg /g of dry weight)	Total flavonoids QE mg/g dry weight	Total Phenols (GAE mg/g of dry weight)	Total flavonoids QE mg/g dry weight
SPM	8.211 ± 0.709^{a}	3.311 ± 0.730^{a}	6.713 ± 0.763^{ab}	3.922 ± 0.57^{ab}
EYFB	14.369 ± 0.495^{b}	11.470 ± 0.532^{c}	10.018 ± 0.601^{bc}	7.831 ± 0.576^{bc}
YFB	19.025 ± 0.847^{c}	14.824 ± 0.890^{d}	13.803 ± 0.797^{cd}	10.619 ± 0.785^{cd}
MFB	9.483 ± 0.686^{a}	9.737 ± 0.472^{b}	9.031 ± 0.436^{bc}	5.437 ± 0.079^{df}

Value expressed as Mean±SD

Value with different superscript in the same column denotes they are significantly different at $p< 0.05$

TPC – Total polyphenols content expressed as mg Gallic acid equivalent (GAE) per g dry weight

TFC – total Flavonoids content expressed as mg Quercetin Equivalent (QE) per g dry weight

SPM – Spawn mycelium (10 days)

EYFB – 1st primordial phase - early young fruiting bodies (16th day after substrate inoculation/2 days after pinning)

YFB – 2nd primordial phase - young fruiting bodies (17th day after inoculation/3 days after pinning)

MFB - mature fruiting bodies (21st day after substrate inoculation/7days after pinning)

Table 5: TPC and TFC for water extracts of pink *Pleurotus djamor R22* and *Pleurotus citrinopileatus singer*

Pleurotus djamor R22			*Pleurotus citrinopileatus singer*	
Phenological state	Total Phenols (mg/GAE g dwt)	Total flavonoids mg QE/g dwt	Total Phenols (mg/GAE g dwt	Total flavonoids mg QE/g dwt
Mycelia	4.708±0.047[a]	7.403±1.001[a]	6.516±0.221[a]	4.387±0.606[b]
1st period (EYFB)	10.908±1.052[b]	9.612±1.306[a]	8.112±0.017[b]	6.039±0.489[a]
2nd period (YFB)	12.655±1.019[b]	8.014±0.703[a]	8.726±0.801[b]	6.471±1.205[a]
3rd period (MFB)	7.317±0.921[c]	8.840±0.088[a]	4.512±0.310[c]	4.921±1.003[b]

Value expressed as Mean±SD

Value with different superscript in the same column denotes they are significantly different at $p< 0.05$

4.2.4 Total Flavonoid Contents

Spectrophotometric method was used to evaluate total flavonoid content in the two species across four phenological states. Results shown in Table 1 illustrates that flavones were significantly different across the four phases in both species with 2nd phase of primordial of both recording the highest values (14.824±0.890 and 10.619±0.785 mg QE/g; $p< 0.05$). In both species fruiting phase recorded a significantly higher value than the spawn mycelia phase. However, with the exception of mycelia phase *Pleurotus djamor* R22 recorded significantly higher contents than *Pleurotus citrinopileatus* Singer in the remaining three phases ($p< 0.05$).

4.2.5 Determination of β-Carotene and lycopene

The contents of β-carotene and lycopene exhibited considerable variation between the four phenological states of the two species. The β-carotene ranged from 0.002 to 0.262 µg/g dry weights while lycopene ranged from 0.001 to 0.026 µg/g dry weights (Table 2). The highest content of β-carotene was detected in the second phase of *P. djamor* R22 primordial while the relative highest lycopene was detected in the first primordial phase of the *P. citrinopileatus*.

Spawn mycelium of both species were deprived of these antioxidant properties. Contents of lycopene were lower than the β-carotene concentrations. Primordials of both species showed higher values of both lycopene and B carotene in than the fruiting phases

Table 6: Total Carotenoid, Contents of B-carotene and Lycopene of coloured edible

Phenological state	*Pleurotus djamor* R22			*Pleurotus citrinopileatus*		
	Total carotenoid µg/Ml	B- carotene µg/g dry weight	Lycopene µg/g dry weight	Total carotenoids µg/mL	B- carotene µg/g dry weight	Lycopene µg/g dry weight
SPM	0.067 ± 0.053^{ac}	0.004 ± 0.001^{a}	0.001 ± 0.0004^{aa}	0.028 ± 0.019^{aa}	0.002 ± 0.001^{ac}	0.002 ± 0.001^{a}
EYFB	2.910 ± 1.007^{bc}	0.195 ± 0.025^{b}	0.026 ± 0.003^{bb}	2.470 ± 0.004^{bb}	0.112 ± 0.011^{ba}	0.017 ± 0.002^{b}
YFB	5.632 ± 1.808^{ad}	0.262 ± 0.005^{c}	0.017 ± 0.0001^{cc}	3.926 ± 0.059^{cc}	0.137 ± 0.037^{cd}	0.021 ± 0.006^{c}
MFB	3.124 ± 0.725^{bc}	0.109 ± 0.004^{d}	0.008 ± 0.001^{dd}	3.003 ± 0.001^{dd}	0.023 ± 0.020^{da}	0.014 ± 0.001^{d}

mushrooms Pleurotus citrinopileatus and *P. djamor* (mg/g dry weight)

Value expressed as Mean±SD

Value with different superscript in the same column denotes they are significantly different at $p< 0.05$

4.2.6 TEAC assay (Trolox equivalent antioxidant capacity assay)

The main observation made in this method is that ABTS scavenging properties were maturity dependent. The potential of these mushrooms to scavenge for ABTS radicals ranged from 0.617±0.008 to 14.075±1.001 Trolox Eq. mg/g dry weight in *Pleurotus citrinopileatus,* while *Pleurotus djamor* R22 ranged from 1.019±0.004 to 16.209±0.011 Trolox E/g dry weight (Table 7). The 1st and 2nd primordials exhibited significantly higher values than both the fruiting phases and the spawn mycelia phase. Statistical significant relationship was observed between TEAC, β-carotene, and lycopene; this Pearson correlation points out the strong positive relationship between TEAC and TPC (r >0.9, p<0.9) (Table 5).

Table 7: Reduction Power and Content of TEAC assay of coloured edible mushrooms *Pleurotus citrinopileatus* and *P. djamor* (mg/g dry weight)

Phenological state	*Pleurotus citrinopileatus*		*Pleurotus djamor* R22	
	TEAC Assay (Trolox Eq. mg/g dry wt)	FRAP (EC_{50})	FRAP (EC_{50})	TEAC (Trolox Eq. mg/g dry wt)
SPM	0.617 ± 0.008^{aa}	0.004 ± 0.002^{a}	0.022 ± 0.004^{ab}	1.019 ± 0.004^{aa}
EYFB	11.842 ± 0.204^{bb}	1.218 ± 0.240^{b}	1.731 ± 0.291^{bc}	14.633 ± 0.061^{bb}
YFB	14.075 ± 1.001^{cc}	1.162 ± 0.194^{c}	2.464 ± 0.141^{ac}	16.209 ± 0.011^{cc}
MFB	8.336 ± 0.007^{dd}	0.073 ± 0.007^{d}	1.007 ± 0.025^{bd}	11.517 ± 0.058^{dd}

Values expressed as mean±SD, $n \geq 3$

Value with different superscript in the same column denotes they are significantly different at $p< 0.05$

4.2.7 Reducing power Assay

Our study demonstrates the existing phenological differences in the two species to reduce ferricyanide. Generally these results show that the two primordial phases have a better potential in the reduction power (Table 3). There was significance difference in the ability of each phenology to reduce ferricyanide for both species. The reduction power of *P. citrinopileatus* ranged from 0.004±0.002 to 1.218±0.240 while that of *Pleurotus djamor* R22 ranged from 2.464±0.141 to 0.022±0.004. Both spawn mycelia phases were deprived of these potential. Pearson correlation shows that reducing power relates positively with TPC, TFC, lycopene, and B-carotene ($r >0.9$; $p< 0.001$) in P. citrinopileatus as well as *Pleurotus djamor* R22 ($r>0.75$; $p< 0.001$) as represented in (Table 4).

Table 8: Pearson Correlation between Bioactive molecules and FRAP/TEAC/DPPH (*r* values)

r value	*Pleurotus djamor* R22			*Pleurotus citrinopileatus*		
	FRAP	TEAC	DPPH	FRAP	TEAC	DPPH
Total Polyphenols	0.942	0.804	0.969	0.754	0.894	0.914
Total Flavonoid content	0.973	0.970	0.960	0.828	0.899	0.952
Lycopene	0.930	0.967	0.923	0.915	0.828	0.843
B-carotene	0.964	0.936	0.974	0.941	0.891	0.959

Pearson correlation coefficient: Values tested with 1-way ANOVA test

Significance at $p< 0.05$

4.2.8 DPPH Radical Scavenging properties (RSA)

DPPH radical scavenging properties showed a concentration dependency (Fig. 4). Percentage inhibition increases with sample concentration with the highest percentage inhibition recorded at 20mg/mL for all the four growth levels (Fig. 4 and 5). Radical scavenging was concentration dependent. Radical Scavenging Ability were significantly different between the primordials of the basidiocaps (EYFB, YFB) and the mature basidiocaps MFB for both species (*P<0.05*) with the primordials comparing positively with BHT L-AA, and α-Tocopherol. Although spawn mycelia phase and the fruiting phase showed a reduced capacity, they were not significantly different (*P<0.05*). Besides, spawn mycelium can be an alternative source of important bioactive molecules to substitute mature fruiting bodies, which tend to have reduced values (*P<0.05*) in *P. djamor* R22.

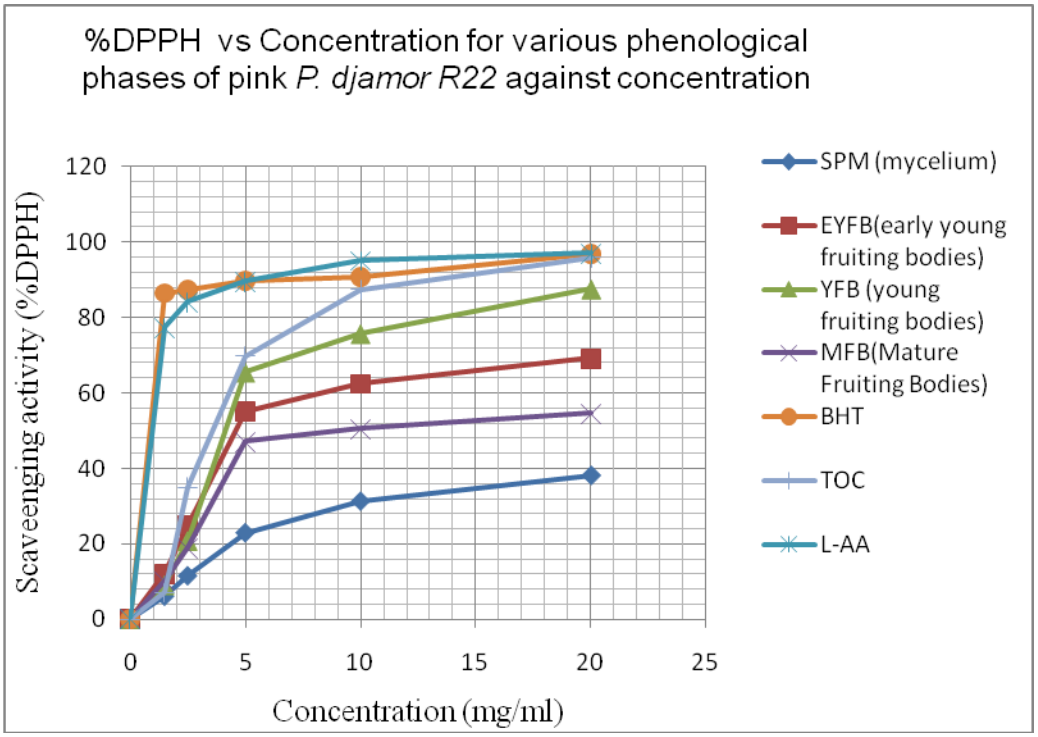

Figure 4: DPPH radical scavenging activity of various phenological states of *Pleurotus djamor* R22. L-ascorbic acid (L-AA), α-Tocopherol (TOC), Butylated hydroxytoluene (BHT)

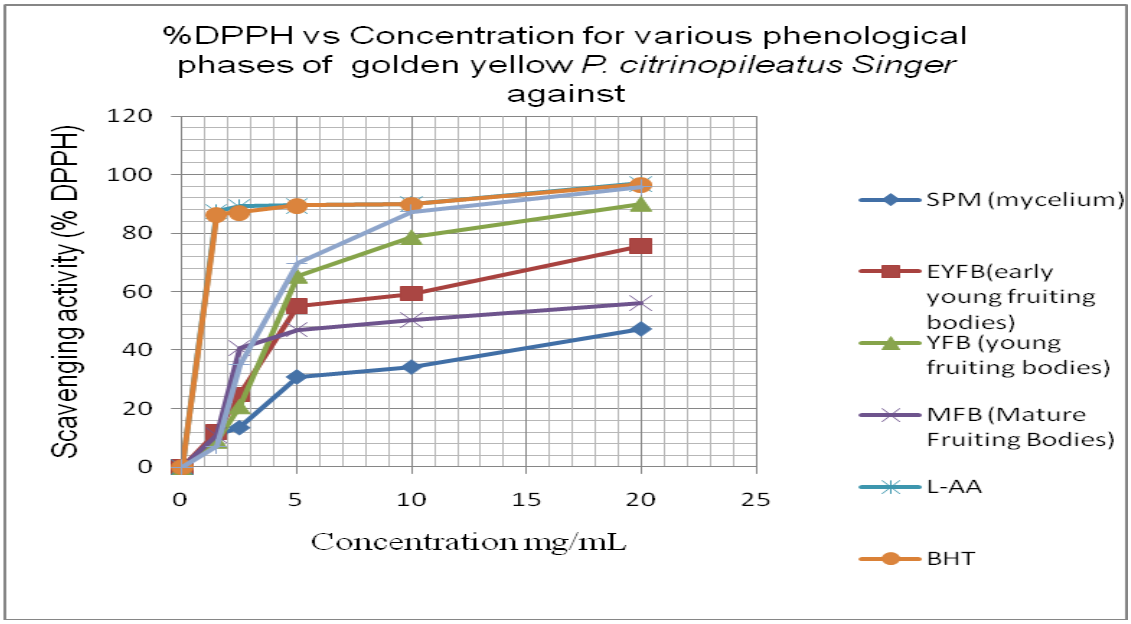

Figure 5: DPPH radical scavenging activity of ethanol extracts of various phenological states of *Pleurotus citrinopileatus* Singer. L-ascorbic acid (L-AA), α-Tocopherol (TOC), Butylated hydroxytoluene (BHT)

4.2.9. Metabolomic profiling

4.2.9.1 Distribution of compounds across phenology

Profiling of compounds across phenology in *P. djamor* (GA1, GA2, GA3, and GA4) shows the presence several compounds with different accurate mass and percentage confidence. Analysis of spawn mycelium (GA1) captured 11 compounds, early young basidiocaps (GA2) 13 compounds, young basidiocaps (GA3) 14 compounds, while mature fruiting bodies gave 16, the highest compounds (GA4). Twenty three (23) compounds with significant hits were positively identified (Table 9)

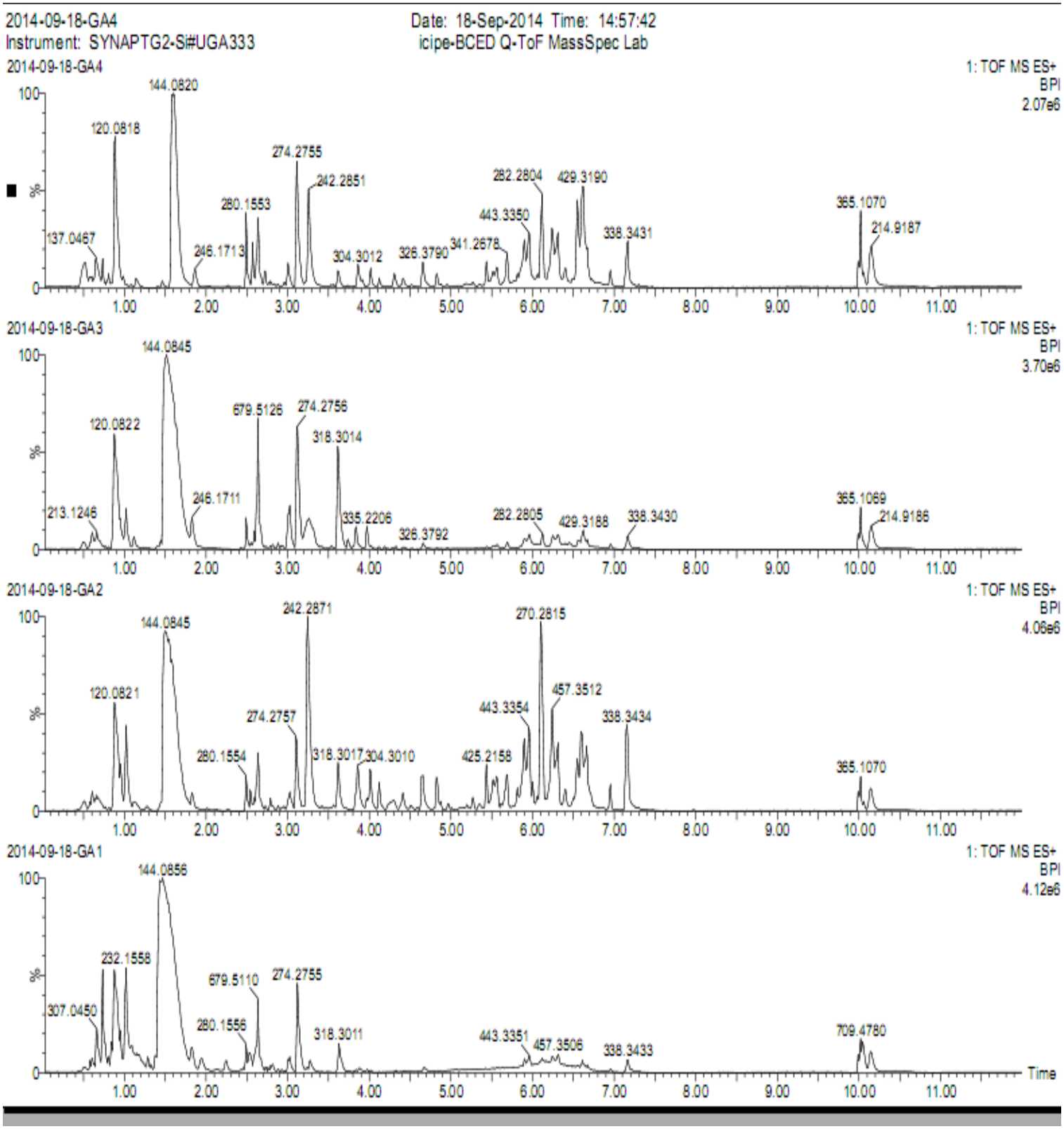

Figure 6: Accurate mass of compounds across four phonologies in *P. djamor R22*

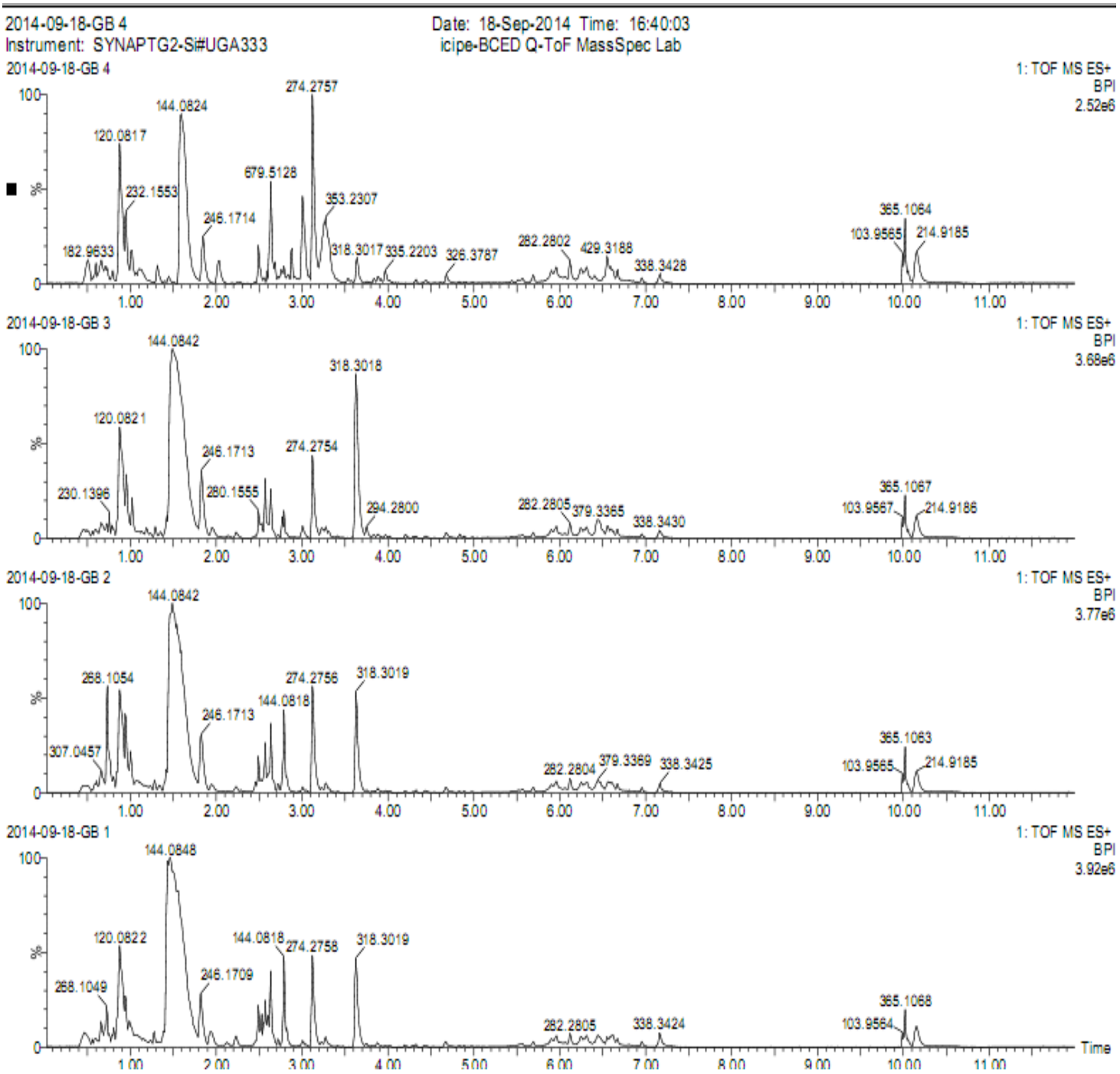

Figure 7: Accurate mass of compounds across four phonologies in *P. citrinopileatus*

Table 9: Identity of compounds based on accurate mass and empirical formula

CE	COMPOUND NAME
1 HIGH ENERGY	SPERMIDINE
166	DL Phenylalanine
232	N-[(2S)-2-Ammoniopropanoyl]-L-alanyl-L-alaninamide
250	Ethyl N-([1,2,4]triazolo[1,5-a]pyrimidin-2-ylcarbonyl)glycinate
268 WITHOUT N	2-(3,4-Dimethoxyphenyl)chromenium
268, 136	M/Z 136 ADENINE
268	6R,10bS)-10b-methyl-6-phenyl-1,2,3,5,6,10b-hexahydropyrrolo[2,1-a]isoquinoline
268 b	Adenosine
268H	Adenosine
296	5-Acetoxy-2-phenyl-1-benzofuran-3-carboxylic acid
307 WITHOUT N	2,2'-(3,7-Dioxo-5,7-dihydro-1H,3H-furo[3,4-f][2]benzofuran-1,5-diyl)diacetic acid
331n	2-Isopropyl-5-methylphenyl biphenyl-4-carboxylate
331	N-[4-(dimethylamino)phenyl]-3,4,5-trimethoxybenzamide
RT 1.03	Ethyl N-([1,2,4]triazolo[1,5-a]pyrimidin-2-ylcarbonyl)glycinate
RT 1.943 WITHOUT N	2-Benzyl 3-methyl 4-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-1-(methoxymethoxy)-2,3-naphthalenedicarboxylate
RT 2.25 LOW ENERGY WITH N	Entecavir monohydrate
RT 2.25 LOWHIGH ENERGY WITH N	N,N-dimethyl-adenine
RT 2.49A	NO HIT
RT 2.49B	4-Methyl-2-oxo-2H-chromen-7-yl hexopyranosyl-(1->6)hexopyranosyl-(1->6)hexopyranoside
RT 2.53	7-Isopropyl-6-methoxy-1,2-dimethylphenanthrene
RT 2.63 HIGH ENERGY	NO HIT
RT 2.63	NO HIT
RT 3.12	N-[3-(Dimethylamino)propyl]-3-{[3-(dimethylamino)propyl]amino}-2-methylpropanamide
RT 3.021 HIGH	NO HIT

ENERGY MZ 369 WITHOUT N	
RT 3.021 HIGH ENERGY	NO HIT
RT 3.267	Phytosphingosine

4.3.1 Optimization of mushroom based yoghurt product

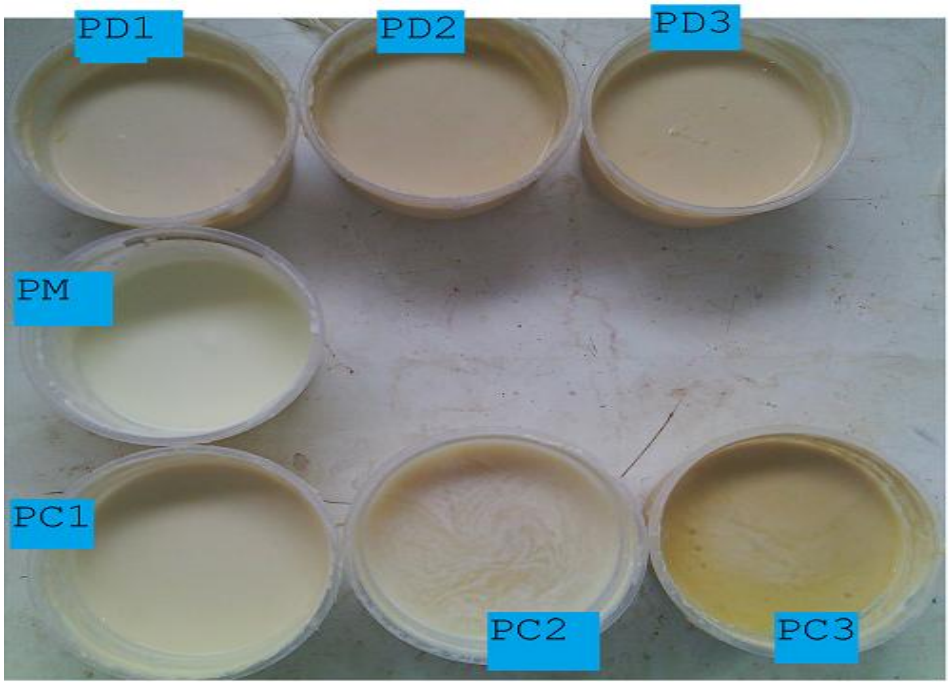

Figure 8: Pictorial representation of mushroom based yoghurt product

PD1 (1.5% *P. djamor*); PD2 (3.0% *P. djamor*); PD3 (5.0% *P. djamor*); PC1 (1.5% *P. citrinopileatus*), PC2 (3.0% *P. citrinopileatus*), PC3 (5.0% *P. citrinopileatus*); PM (conventional yoghurt)

Different formulations were prepared but only three per species managed to set. These were the PD1 (containing 1.5% *P. djamor*); PD2 (3.0% *P. djamor*); PD3 (5.0% *P. djamor*); PC1 (1.5% *P. citrinopileatus*), PC2 (3.0% *P. citrinopileatus*), PC3 (5.0% *P. citrinopileatus*); and PM (conventional yoghurt). Formulation with 7% mushroom did not set after 24hours of incubation and was not included for further analysis. Untrained panelist of 17 individuals was constituted and used to evaluate sensory attributes results shown (table 15). Four products namely; PD1, PC1, PC2, and PD2 showed comparable attributes to conventional yoghurt.

4.3.2 Physicochemical characteristics

4.3.2.1 Setting time and pH

The product pH changed with incubation duration. pH was used to determine duration of fermentation (setting time). Increased total solids (corresponding with increased percentage of mushroom added) extended the setting duration significantly ($p<0.05$). The lowest concentration (1.5%) recorded the fastest decrease in pH. Product with replaced skim milk did not set and therefore was not included in organolepsis.

Table 10: Changes in pH of the mushroom based yoghurt formulations during fermentation

PRODUCT	pH (HOURS)					
	1 hour	2 hours	4 hours	6 hours	8 hours	22 hours
PC1	5.21 ± 0.02^{a}	4.67 ± 0.02^{b}	4.61 ± 0.02^{b}	4.527 ± 0.025^{b}	4.426 ± 0.015^{c}	4.256 ± 0.025^{b}
PC2	4.957 ± 0.035^{a}	4.717 ± 0.060^{a}	4.69 ± 0.046^{a}	4.627 ± 0.021^{a}	4.52 ± 0.04^{a}	4.13 ± 0.079^{c}
PC3	6.137 ± 0.093^{a}	5.711 ± 0.045^{a}	5.673 ± 0.057^{a}	4.916 ± 0.068^{b}	4.823 ± 0.100^{b}	4.323 ± 0.191^{c}
PC4	5.827 ± 0.230^{b}	5.68 ± 0.113^{b}	5.53 ± 0.139^{b}	5.16 ± 0.259^{b}	4.820 ± 0.132^{b}	4.72 ± 0.191^{a}
PD1	5.25 ± 0.615^{c}	4.98 ± 0.435^{c}	4.727 ± 0.307^{c}	4.593 ± 0.146^{b}	4.55 ± 0.122^{b}	4.373 ± 0.011^{c}
PD2	6.093 ± 0.221^{a}	5.573 ± 0.139^{b}	5.287 ± 0.246^{b}	4.75 ± 0.123^{c}	4.57 ± 0.04^{d}	4.263 ± 0.179^{e}
PD3	5.857 ± 0.207^{b}	5.68 ± 0.236^{b}	5.51 ± 0.332^{b}	5.207 ± 0.240^{b}	4.797 ± 0.161^{c}	4.59 ± 0.098^{d}
PD4	6.02 ± 0.350^{c}	5.747 ± 0.074^{d}	5.5 ± 0.19^{d}	5.21 ± 0.111^{d}	5.001 ± 0.061^{d}	4.883 ± 0.154^{d}
PCD	6.047 ± 0.237^{a}	4.81 ± 0.095^{b}	4.423 ± 0.060^{c}	4.223 ± 0.047^{e}	Nd	Nd

Values expressed as mean±SD,

Value with same superscript in the same row denotes they are not significantly different at $p< 0.05$

4.3.2.2 Viscosity

Viscosity of the yoghurt was significantly different after fermentation for all formulations. Concentration of the initial mushroom added during preparation affected viscosity. Product that had skim milk replaced with mushroom powder did not set and thus less viscous. Low percentage (1.5%) mushroom for both (PC1 and PD1) were not significantly different from the plain yoghurt (PM) before fermentation ($p<0.05$). all products after fermentation showed significantly different viscosity.

Table 11: changes in viscosity of the mushroom based yoghurt formulations before and after fermentation

changes in viscosity (Centipoise)		
Product	Before fermentation	After fermentation
PC1	1481.384±122.168[a]	9630.365±78.27101[b]
PC2	1695.802±201.280[a]	10461.74±82.5885[d]
PC3	1926.017±170.482[b]	7478.119±223.0845[c]
PD1	1579.278±107.381[a]	10788.78±307.6952[a]
PD2	1817.290±165.540[b]	9381.73±154.3593[b]
PD3	1993.718±108.4262[b]	7735.937±120.7968[e]
PM	1571.982±102.791[a]	6602.246±88.45173[c]

Values expressed as mean±SD, *n* =3

Value with different superscript denotes they are significantly different at $p< 0.05$

4.3.2.4 Moisture content and total solids

Moisture content decreased while total solids increased with increasing mushroom concentration. Increased solids had significant effect ($p<0.05$) on other physicochemical properties like pH, TTA, and acceptability profiles (Table 12).

Table 12: Moisture and total solid content of the mushroom based yoghurt formulations

Moisture and Solid content of mushroom based yoghurt		
Product	Moisture content (%)	Total solids (%)
PC1	80.356±0.172[c]	19.644±0.172[a]
PC2	80.316±0.197[c]	19.684±0.197[a]
PC3	79.011±0.285[a]	20.989±0.285[b]
PD1	80.495±0.911[a]	19.505±0.911[a]
PD2	78.683±0.665[c]	21.317±0.665[b]
PD3	76.669±0.330[e]	23.331±0.330[d]
PM	81.808±0.270[c]	18.192±0.270[a]

Values expressed as mean±SD, *n* =3

Value with same superscript in the same column denotes they are not significantly different at $p< 0.05$

4.3.1.5 Total titratable acidity (%TTA)

Percentage TTA increased significantly during fermentation in all formulations (Table 13). There was insignificance difference between the various formulations of the same species before and after fermentation.

Table 13: Total titratable acidity of the mushroom based yoghurt formulations

content of mushroom based yoghurt after fermentation		
Product	TTA before fermentation	TTA after fermentation
PC1	$0.2^{\cdot} \pm 0.05^{c}$	0.459 ± 0.02^{a}
PC2	0.3 ± 0.01^{c}	0.396 ± 0.123^{a}
PC3	0.4 ± 0.01^{c}	0.468 ± 0.01^{a}
PD1	0.1 ± 0^{c}	0.333 ± 0.031^{b}
PD2	0.1 ± 0.01^{c}	0.369 ± 0.001^{b}
PD3	0.2 ± 0.05^{c}	0.468 ± 0.021^{a}
PM	0.1 ± 0^{c}	0.27 ± 0.081^{d}

Values expressed as mean±SD,

Value with same superscript in the column denotes they are not significantly different at $p< 0.05$

4.3.1.6 Colour after fermentation

The colour of products evaluated showed a hue range for yellow (90^{o}), positive a* indicated red to purple range while positive b* indicated yellow hue (table 14). These colour range correspond with the initial colour of the raw material used. There was insignificance different between the various formulations ($p<0.05$) (Table 14).

Table 14: Colour content of the mushroom based yoghurt formulations

Colour content of mushroom based yoghurt			
	L*	a*	b*
PC1	89.2 ± 0.3^{a}	1.1 ± 0.02^{c}	13.3 ± 0.2^{a}
PC2	83.1 ± 0.1^{a}	1.2 ± 0.1^{c}	18.3 ± 0.4^{b}
PC3	83.9 ± 0.2^{a}	0.1 ± 0.05^{c}	18.9 ± 0.5^{b}

PD1	86.4±0.1[a]	0.2±0.05[c]	14.6±0.2[a]
PD2	88.5±0.3[a]	0.7±0.01[c]	12.9±0.1[a]
PD3	83.2±0.3[a]	1.6±0.03[c]	18.4±0.4[b]

Values expressed as mean±SD, *n* =3

Value with same superscript in the same column (Hue and chroma) denotes that they are not significantly different at $p< 0.05$

4.3.2. Organoleptic analysis Basic sensory evaluation and hedonic testing

Acceptability of unflavoured mushroom formulated yoghurt showed comparative profiles with pure yoghurt (Table 15). Formulation with low concentration of mushroom formulation exhibited better acceptability that was comparable to pure unflavoured yoghurt. However, overall acceptability of 1.5% and 3% did not differ significantly with the control.

Table 15: acceptability profiles of mushroom based yoghurt formulations

Organoleptic study						
Batches	Taste	Flavour	Thickness	Taste	Colour	Overall acceptability
PC1	4.01[a]	4.77[c]	6.69[d]	2.54[a]	3.11[d]	4.17[a]
PC2	4.65[b]	5.84[e]	7.01[d]	5.32[c]	3.59[d]	4.35[a]
PC3	5.29[c]	7.06[b]	7.94[e]	5.27[c]	3.01[d]	6.03[b]
PD1	3.96[a]	4.91[c]	7.05[d]	2.63[a]	3.78[d]	4.08[a]
PD2	4.77[b]	5.04[c]	5.39[a]	4.21[b]	3.37[d]	4.51[a]
PD3	5.98[c]	6.78[b]	9.80[c]	5.74[c]	3.21[d]	5.87[b]
PM	3.03[e]	2.12[a]	3.26[b]	2.51[a]	3.78[d]	3.96[a]

Values expressed as mean±SD, *n* =17

Value with same superscript in the same column denotes that they are not significantly different at $p< 0.05$.

4.4.1 Colour development and relative strength

Table 16 and 17, shows colour changes during the extraction processes for both species. The intensity (chroma) L* value (measure of colour brightness), while the hue angle (measure of colour) and the intensity of its content. However, the difference in brightness and intensity

between different duration were not significantly (p>0.05). The hue and chroma values of dye bathes from both species were calculated from the L* a* b* values.

Table 16: Changes in Chroma of the extracts during extraction process

	Chroma of colour during extraction (hours)			
	1hr	3hrs	6hrs	8hrs
EP1	24.38524±2.105[b]	25.41515±2.382[b]	24.993±1.941[a]	26.81679±1.001[b]
EP2	29.12336±0.628[b]	30.39112±1.010[b]	32.28452±2.603[b]	26.84865±0.9[b]
EP3	30.64719±0.923[b]	28.68449±2.640[b]	34.12228±0.441[b]	29.06303±2.074[b]
ED1	29.98083±1.107[b]	30.01666±1.850[b]	31.78459±0.172[b]	29.31552±0.193[b]
ED2	29.30836±1.864[b]	28.89221±3.014[b]	31.23348±1.559[b]	28.70679±0.485[b]
ED3	30.89741±0.911[b]	28.39243±1.068[b]	33.25568±0.863[b]	27.89928±2.619[b]

Values expressed as mean±SD; Value with same superscript in the column denotes they are not significantly different at $p< 0.05$

(EP1, EP2, and EP3 various designs for *P. citrinopileatus*) ED1, ED2, ED3 represents various designs for *P. djamor*)

Table 17: Hue angles of the extracts during extraction process

Hue of colour during extraction (hours)				
	1	3	6	8
EP1	7.06673±0.984[a]	7.460566±0.051[a]	7.125016±0.088[a]	-5.34918±0.427[a]
EP2	56.91015±1.156[b]	58.45425±2.038[b]	56.75293±1.968[b]	55.77724±1.103[b]
EP3	56.30993±2.031[b]	57.52881±1.824[b]	60.50504±3.622[b]	53.95813±1.950[b]
ED1	48.92194±2.604[b]	51.22095±1.039[b]	52.15664±1.091[b]	48.04184±1.006[b]
ED2	47.76576±1.089[b]	51.46407±1.716[b]	48.24459±0.035[b]	50.65442±3.721[b]
ED3	55.94991±1.472[b]	51.43474±0.905[b]	55.7843±2.001[b]	50.08925±0.845[b]

Values expressed as mean±SD,

Value with same superscript in the column denotes they are not significantly different at $p< 0.05$

4.4.2 Fastness of fibres without mordants

Fibres without both alum and copper sulphate mordants were evaluated for their ability to retain dyes without effects of these mordants. The table below shows high fastness in

unmordanted fabrics. Fabrics show poor characteristics of retaining natural dyes without molecules that helps it from fading the dye.

4.4.3 Fastness of mordanted fibres

Alum, copper sulphate, and their mixture (1:1; w: w) were evaluated for their mordanting properties on both silk and cotton polymer materials. Different shades of colour hues were obtained as shown in (Fig.9, 10, 11, 12). Copper sulphate exhibited a blue hue unlike Alum as shown (fig. 9). Fabric polymers without mordants (fig. 11) exhibited significant colour fastness unlike fabrics that were mordanted (fig. 10, 12). There was no significant difference between the two species considering their colour hues.

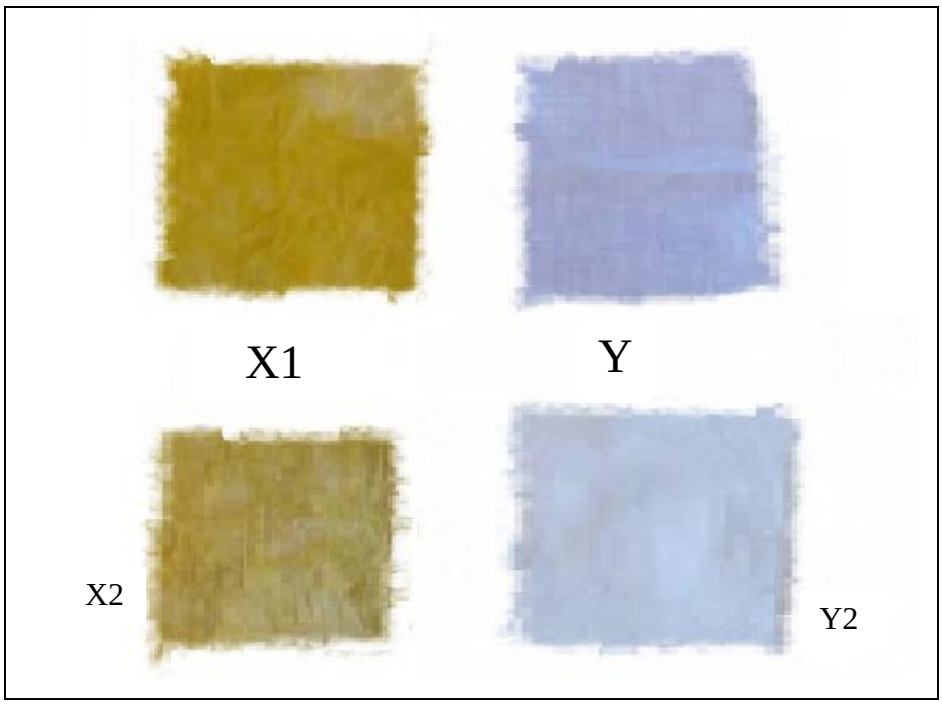

Figure 9: pictorial presentation of the effect of mordant and dye extract on the fabric
Gray scale values of stained fabric (X1=4; Y=5; X2=2; Y2=3)
X-Alum mordant (X1 *Pleurotus citrinopileatus*, X2 *P. djamor*); Y – Copper sulfate (Y1 *P. citrinopileatus* Y2 *P. djamor*)

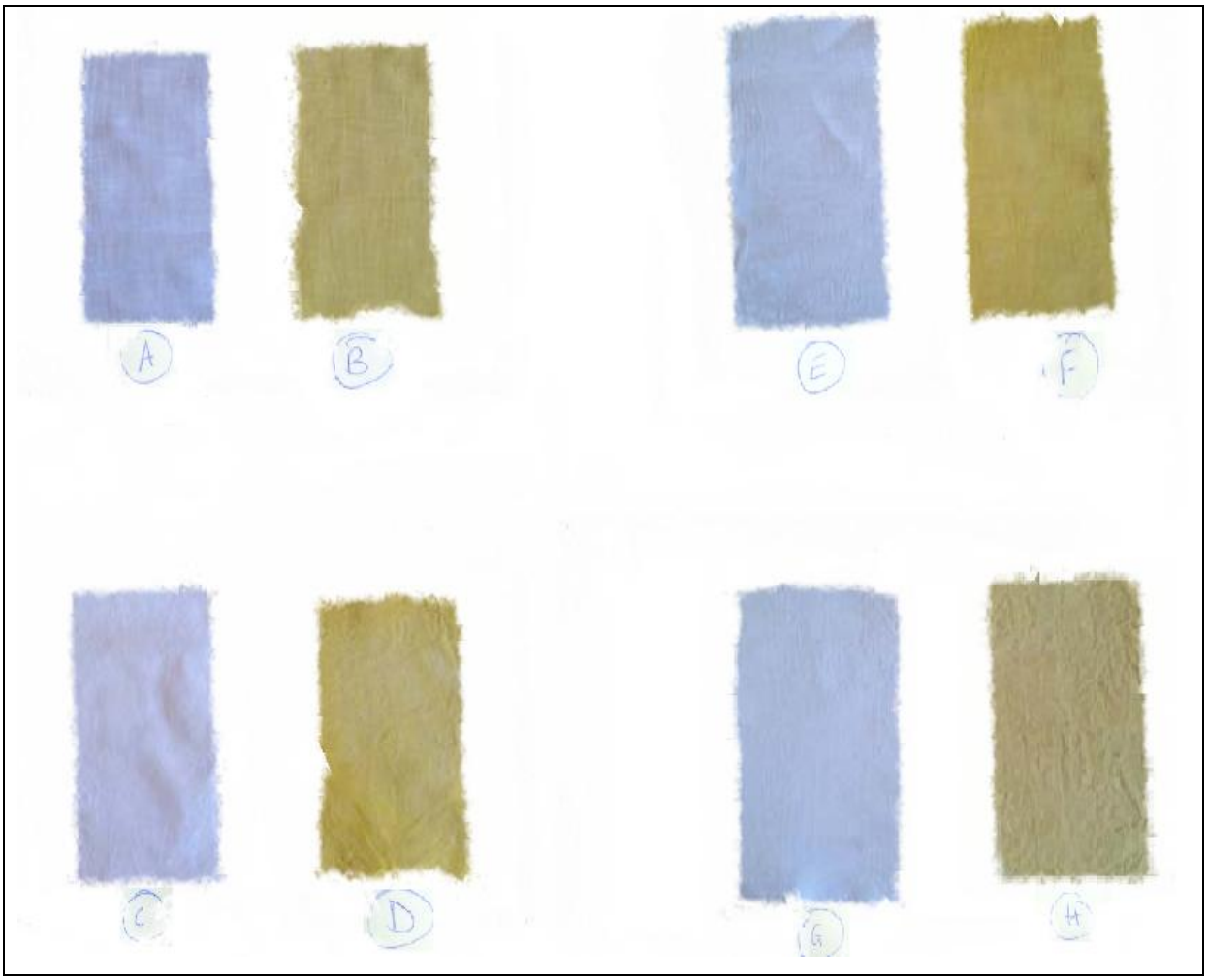

Figure 10: pictorial presentation of mordanted cotton dye with mushroom extract
Gray scale values of stained fabric (A=5, C=3; B=3, D=2) (E=5, G=3, F=4/3, H=2)
B/D-Alum mordant, A/C-Copper sulfate mordant (*Pleurotus citrinopileatus*);
E/G-Copper sulfate, F/H-Alum mordant (*P. djamor*)

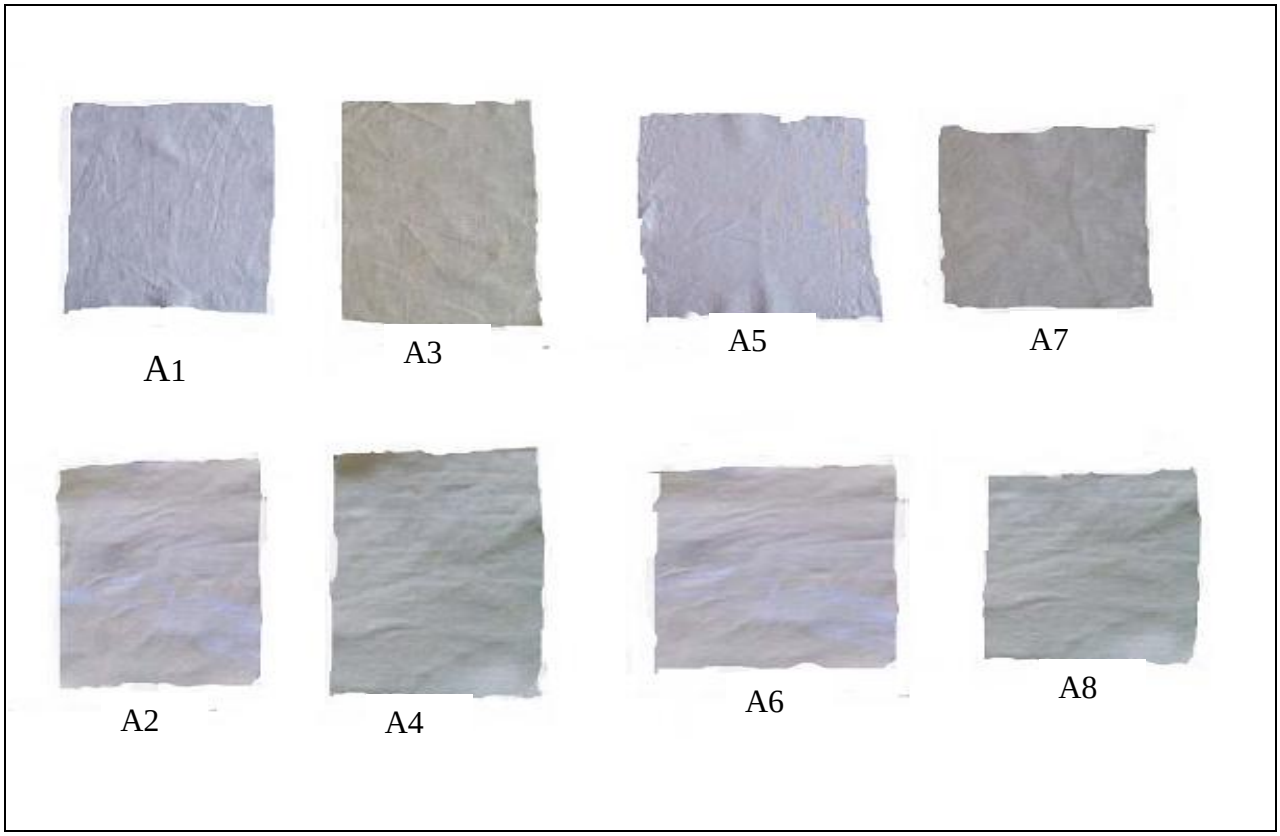

Figure 11: pictorial presentation of fastness in unmordanted fabrics dye with mushroom extract

Gray scale values of stained fabric (cotton, A1=2, A2=1; A3=2, A4=1) (silk, A5=2, A6=1, A7=2, A8=2)

A1, A2, A3, A4 (*Pleurotus citrinopileatus*);

A5, A6, A7, A8 (*P. djamor*)

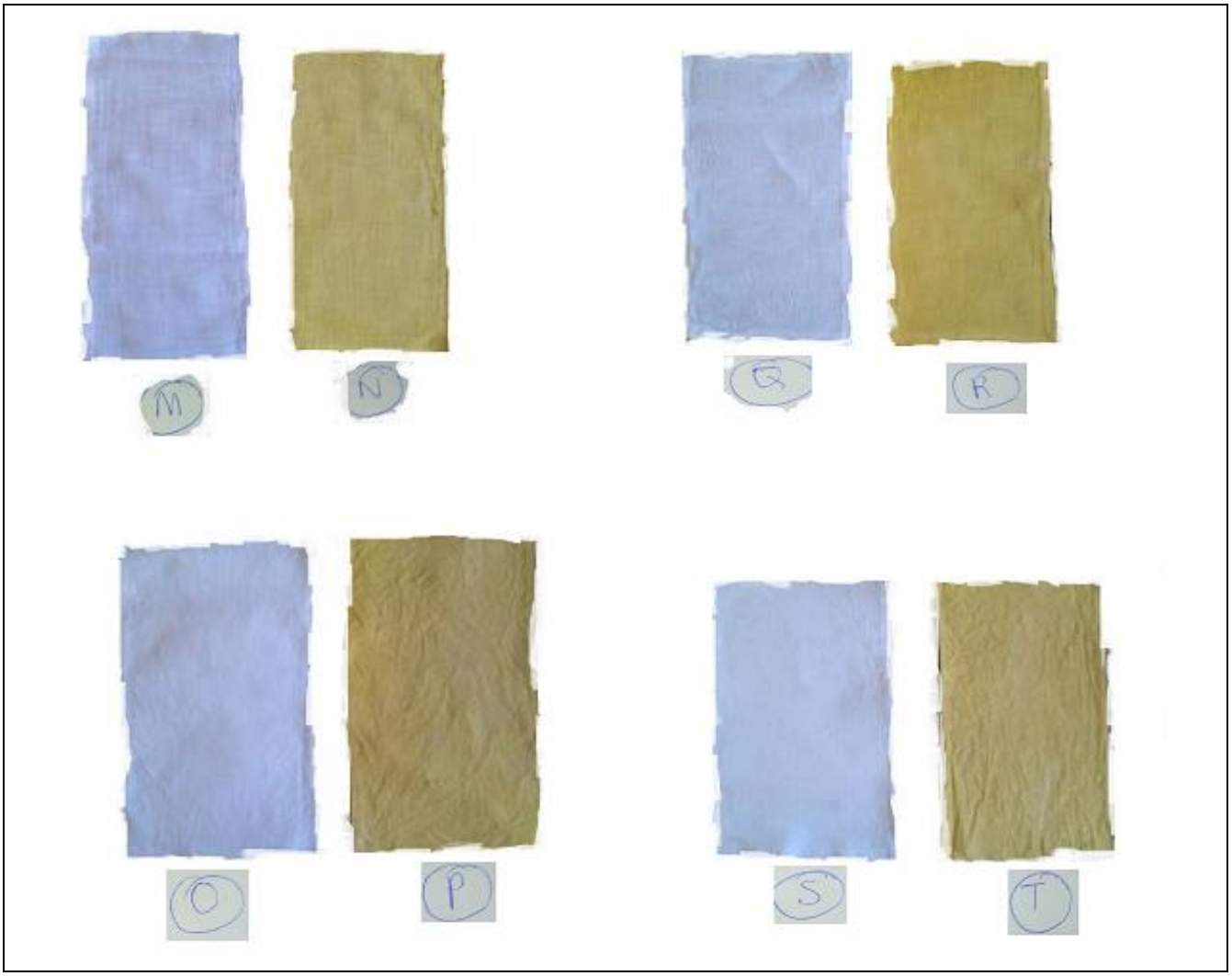

Figure 12: pictorial presentation of mordanted silk dye with mushroom extract
Gray scale values of stained fabric (M=5, O=3; N=3, P=2) (Q=5, S=3, R=4/3, T=2)
N/P-Alum mordant, M/O-Copper sulfate mordant (*Pleurotus citrinopileatus*);
Q/S-Copper sulfate, R/T-Alum mordant (*P. djamor*)

4.5.1 Percentage absorbance as a measure of degradation

The measure of decolouration increased with increasing concentration of SMS for the concentration of Levafix blue CA (LVBCA) for both *Pleurotus citrinopileatus* and *P.*

djamor. Three treatments (50gms of *P. djamor* spent substrate in 100mg LVBCA, 25gm of *P. citrinopileatus* spent substrate in 200mg LVBCA, and 100gm of *P. citrinopileatus* spent substrate in 50mg LVBCA reported decolouration that were not significant from their respective preceding treatment ($p > 0.05$) from . In table 19, 100gms of SMS reported the highest percentage decolouration of 50mg Levafix at 88.606±0.114. The lowest degradation was reported in 10gm of SMS for 200mg LVBCA. However, the lowest concentration of SMS (10gms) reported the best decolouration (degradation)

Table 19: Percentage absorbance of Levafix blue CA treated with SMS as a measure of degradation

	Pleurotus djamor LBCA				*Pleurotus citrinopileatus* LBCA			
SMS	25 mg	50 mg	100 mg	200 mg	25 mg	50 mg	100 mg	200 mg
0 gm								
10 gm	37.300±0.022^{a}	18.656±0.016^{f}	16.912±0.073^{f}	3.950±0.001^{p}	50.908±0.058^{h}	27.057±0.016^{t}	19.805±0.046^{f}	9.833±0.040^{m}
25 gm	66.018±0.136^{b}	59.291±0.016^{e}	53.756±0.056^{h}	10.841±0.003^{m}	64.491±0.188^{b}	43.315±0.075^{k}	30.681±0.028^{t}	30.972±0.036^{t}
50 gm	75.153±0.05^{c}	68.956±0.016^{b}	69.919±0.032^{b}	33.638±0.021^{t}	85.491±0.054^{d}	83.637±0.016^{d}	71.383±0.013^{g}	38.971±0.033^{a}
100 gm	88.015±0.046^{d}	85.342±0.043^{d}	74.289±0.033^{g}	43.498±0.052^{k}	86.441±0.037^{d}	88.606±0.114^{d}	72.806±0.03^{g}	40.847±0.049^{a}

Values expressed as mean±SD, $n = 3$

Value with same superscript denotes they are not significantly different at $p < 0.05$

4.5.2 Comparison of degradation treatments

4.4.2.1 Spent substrate of *P. djamor*

Increasing the concentration of spent mushroom substrate at a constant concentration of levafix blue CA leads to increased discolouration of the dye. However, this phenomenon was did not exhibit linear relationship (fig. 13)

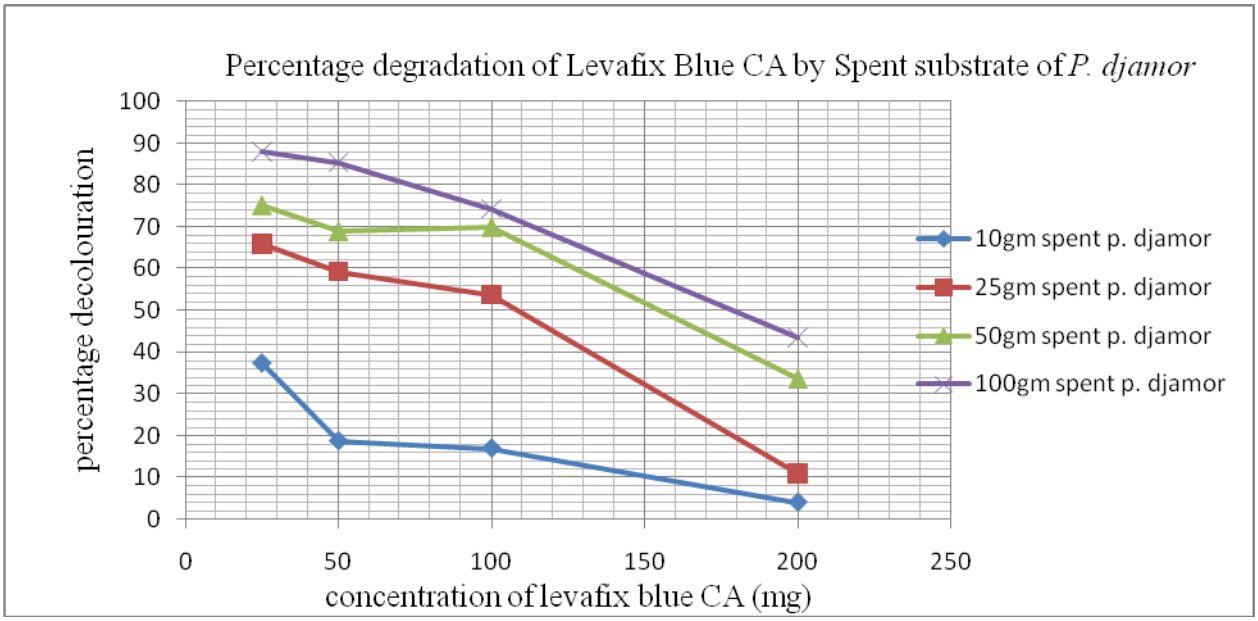

Figure 13: Comparison of spent substrate from *P. djamor* concentration in biodegradation of Levafix

4.4.2.2 Spent substrate of *P. citrinopileatus*

Unlike *P. djamor*, treatment by 50gms and 100gms of spent substrate did not show any significance different ($p>0.05$). However, similarity in increased concentration of spent mushroom substrate at a constant concentration of levafix blue CA leading to increased discolouration of the dye was noted. This study did show a linear relationship as well (Fig.14).

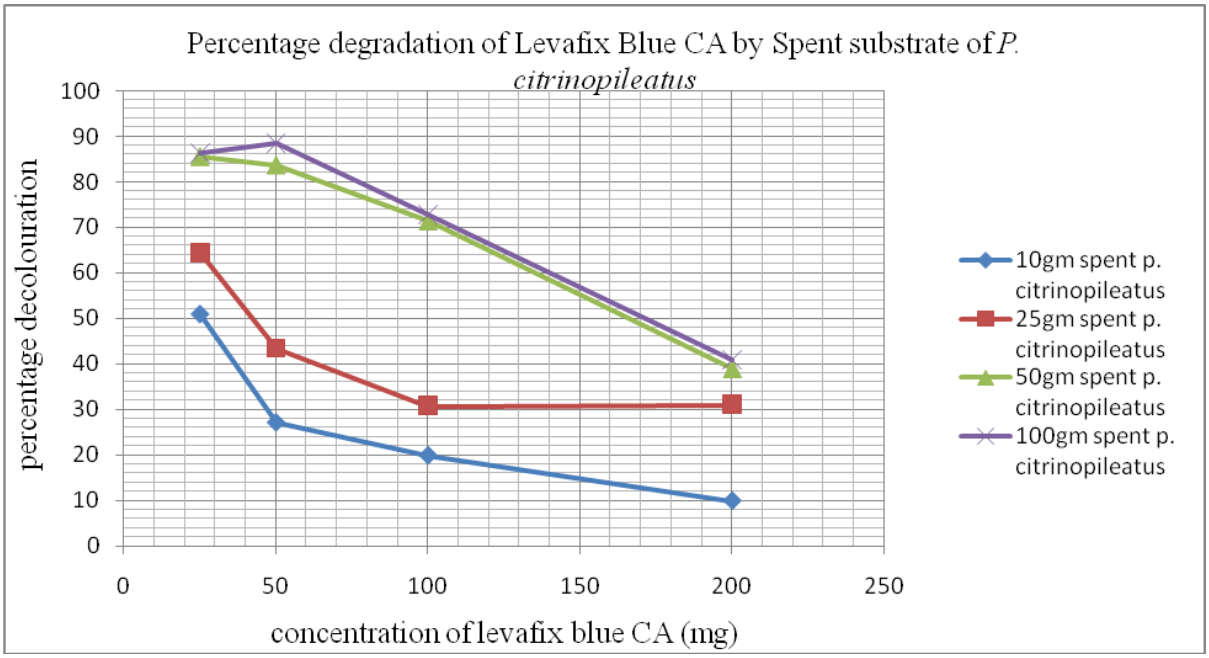

Figure 14: comparison of spent substrate from *P. djamor* concentration in biodegradation of Levafix

4.4.2.2 Comparison of biodegradation potential by spent substrate of *P. citrinopileatus* and *P. djamor*

Biodegradation percentages for treatments in both species exhibited comparative trend with insignificant values between then ($p>0.05$) with the exception of 50gms treatment of *P. djamor* which showed a significant degradation potential than a similar concentration of *P. citrinopileatus* (fig.15)

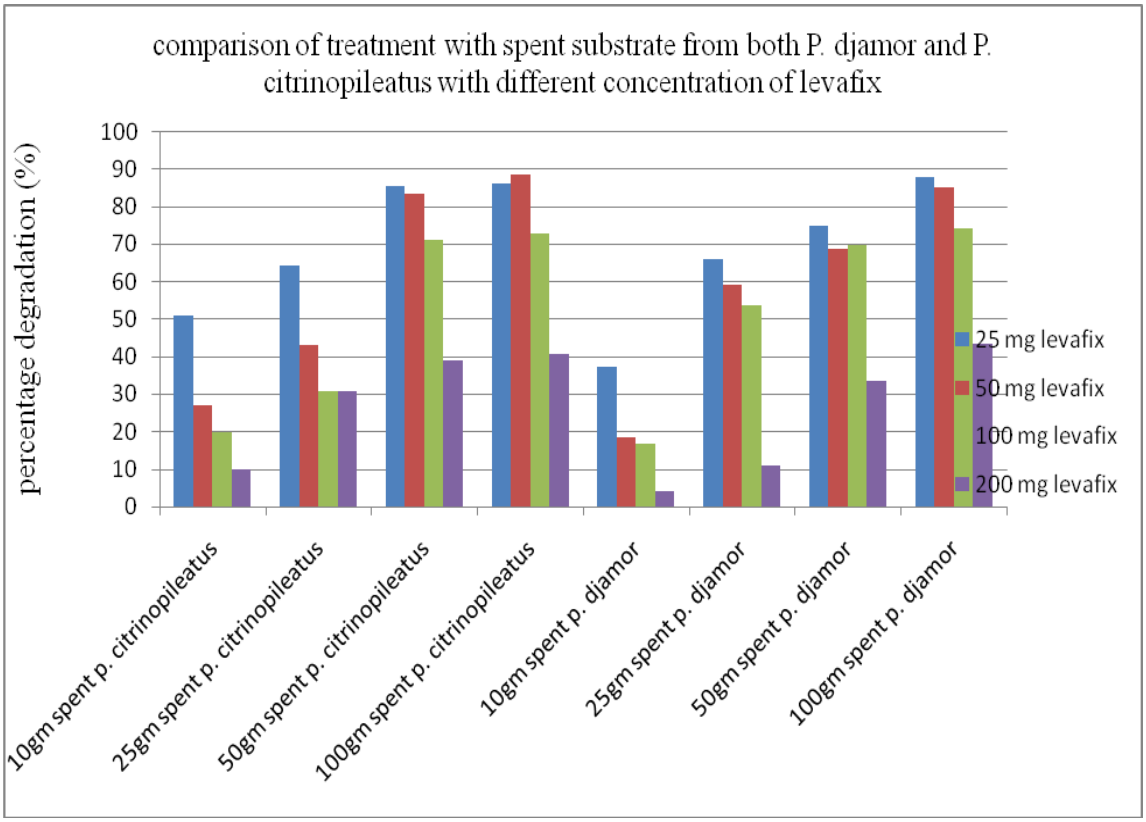

Figure 15: comparison of similar treatment of Levafix by spent substrate from both *P. djamor* and *P. citrinopileatus*.

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Substrate Suitability

The highest biological efficiency was observed on bean straw substrate when growing *P. citrinopileatus*. These findings agree with those of Musieba *et al.* (2012) who reported similar results for the specie. Previous study by Kimenju *et al*., (2009) on substrate suitability for *P. ostreatus* found comparable results on pin head formation and biological efficiency. These species are characterised by rapid colonization of substrates (Table 1) with earnest emergence of basidiocaps spawned at 7.5%. Previous studies have shown that spawning rate determines the spawn run, the pinhead formation, and formation of primordia with 2.5 to 10% as the preferred range (Yang *et al.,* 2013). In our study, both species colonizes various organic residues as their sole substrates (without supplementation) with good biological efficiency, which justifies their good conversion potential as discussed below.

5.1.2 Substrate colonization, degree of mycelium density, and formation of primordia

Previous study by Narain *et al.,* (2008), depicts that development of primordia and mycelia growth largely depends on substrates. Bean straw reported the shortest period of substrate colonization and improved degree of mycelium density in both species. This agrees with previous reports that showed bean straw as the best substrate for oyster mushrooms. Similarly, Yang *et al* (2013) found that oyster mushrooms on bean straw substrates have longer stipe length. In comparison, the emergence of 1st primordia in this residue was delayed in *P. citrinopileatus* than in *P. djamor*. The rate of mycelium growth in wheat straw and sugarcane baggase were significantly different in *P. citrinopileatus* but similar in *P. djamor*. These organic substrates showed a uniform degree of mycelium density on the substrates for both species. This trend was also replicated in the duration it take for substrate colonization in both species ($p>0.05$). The only noted difference in their growth parameters is on primordia formation with sugarcane baggase having early basidiocaps ($p<0.05$) unlike previous studies (Musieba *et al.,* 2012) that reported the same on bean straw. However, the degree of mycelia growth of *P. citrinopileatus* in rice straw was statistically different than *P. djamor*. This study shows that sawdust has poor growth characteristics for both species, which is not different from previous attempts (Mandeel *et al.,* 2009). Banana leaves and maize stovers showed

moderate growth features. The best substrates with superior degree of mycelium surface density, easy substrate colonization and early emergence of primordia were bean straw, rice straw, wheat straw, and sugarcane baggase in that order for both specie.

5.1.3 Effects of substrate on pining per flush, MBD and MBW

Determination of pinning duration across substrates is an important factor in selection of appropriate substrates. Scientist argues that the earlier the pining duration the better because mushroom colonization is likely to acquire contamination from the surrounding over a lengthy incubation periods (Singh and Kanaujia, 2009). Wheat straw, bean straw, and sugarcane baggase reported a shorter duration that was similar but statistically different from other substrates during the 1st flush in *P. citrinopileatus*. However, during the 2nd flush in the same specie wheat straw reported the shortest duration with bean straw, rice straw, banana leaves, and sugarcane baggase not different statistically ($p>0.05$). For *P. djamor* only maize stovers and sawdust reported significantly different pinning duration from other substrates during the first flush. The 2nd flush showed a different variation with bean straw and wheat straw recording shorter intervals, a similar trend was recently reported (Musieba *et al.*, 2012). MBD could be attributed to market quality of mushroom; previous studies grouped this parameter in several subsets as an indicator for market quality (Onyango *et al.*, 2011). Our study reports mean sizes of basidiocaps per substrates to compare substrate suitability for both species. Interestingly, wheat straw in both species which had reported lower numbers of pin head recorded statistically significant sizes (134.67±55) than the bean straw which has superior growth parameters. Sugarcane baggase (122.5 in *P. citrinopileatus*) and rice straw in *P. citrinopileatus* (100.5) also exhibited superior market quality in terms of basidiocap sizes than other substrates. The spawning rate of 7.5% could have compromised the optimal sizes. This is because increased number of pin heads signifies competition for nitrogen from substrate whose rate of depletion increases with maturity as previously reported (Yang *et al.*, 2013). *Pleurotus djamor* exhibited varied MBD across substrate with wheat straw and bean straw statistically similar ($p>0.05$). Selection of either bean straw, wheat straw, or sugarcane baggase substrates could perform best for this specie. Harvest per flush varied between substrates for both species (Table 3). Although sugarcane baggase recorded the highest MBW during the 1st flush, its value in the 2nd flush declined drastically when compared with other substrates in *P. citrinopileatus*. However, for *P. djamor* bean straw recorded the highest MBW during the 1st flush followed by sugarcane baggase and rice straw. The 2nd flush of rice

straw reported the highest MBW that was not statistically significant from that reported by bean straw. Based on fresh harvest per flush for both species for both species *P. citrinopileatus* provides a better MBW than *P. djamor* for the six substrates, which suggests the need for supplementation (Wang, 2010). Wheat straw is the currently preferred substrate across East Africa, recent studies recommended bean straw (Musieba, *et al*., 2012), our study recommends Rice straw and sugarcane baggase. This study justifies the need to diversify on substrate selection with rice straw, maize stovers, and sugarcane baggase as potential candidates.

5.1.4 Effects of substrates on Mean Basidiocap Number (MBN), Biological Efficiency (BE) and Pin head abortion

Our study demonstrates that bean straw exhibits a better substrate conversion efficiency indicated by the highest number of basidiocaps. This shows that ligninocellulosic enzymes easily degrade these residues to release nutrient for the basidiomycetes (Sanchez, 2010). Rice straw reported the second best conversion, but these two reported high pin head abortion. In fact bean straw reported the highest statistical value than all other six substrates. This could denote that although these substrates have high MBN the rate of abortion may be heightened by increased competition for nutrient; which could be solved by lowering the spawning rate to ensure optimal growth based on substrate nutritional content (Narain et al., 2008). Wheat straw reported fewer MBN but had a statistical higher BE compared to bean straw in *P. citrinopileatus*. The general trend observed is that an increase in MBN leads to more abortive heads which could be attributed to growth stressors like diminished nutrients and competition for nitrogen from the substrate (Yang *et al.,* 2013). This could be solved by lowering spawning rate (Dahmardeh *et al*., 2010).

Comparatively *P. citrinopileatus* exhibited higher MBN than *P. djamor* ($p<0.05$) in all six substrates, a similar trend of increased MBN lead to increased abortive heads. Interestingly, BE of *P. djamor* were not statistically different in Bean straw and rice straw, as was the case for sugarcane baggase, maize stovers, and wheat straw. This shows that although the frequencies of pin head abortion were significantly different between these substrates it did not affect production significantly. In this context pin head abortion can be used as a guide to supplementation practices and selection of spawning rate for any substrate to improve on maturity of most formed pin heads. Supplementation will improve long term nutritional availability and translate to increased MBN and BE (Wang, 2010; Yang et al., 2013). This

justifies that *P. citrinopileatus* and *P. djamor* like other oyster mushrooms have better conversion efficiencies and the ability to degrade organic lignocelluloses materials into edible biomass (Mamiro and Mamiro, 2011). Both species exhibited high mycelia colonization rates characterised by efficient yield of sporosphores, emergence of primordials, and basidiocaps

5.2.0 Phytochemicals, Total Antioxidants, Polyphenols, Flavonoids, and Carotenoids across Phenology

5.2.1 Relative abundance of phytochemicals

Many previous studies documents bioactivity of lyophilized, fresh and dried mushrooms samples without contextualizing the distribution of bioactive molecules across phenological and physiological phases (Barros *et al.,* 2007b; Reis *et al.,* 2011). We designed and developed spawn mycelium (part was included for analysis) and part of it used for substrate inoculation to obtain two primordials phases and one mature fruiting body phase. These were used for phytochemical abundance analysis and several in vitro assays to assess their distribution.

This study documents essential health promoting bioactive molecules across phenological phases of two unique basidiomycetes. Primordial phases of both species are rich in phytochemicals like flavones, phenols, phytosterols, and terpenoids with average quantities of saponins and tannins (figure 2 & 3). These findings are in agreement with those from previous studies on phytochemical on various Kenyan edible mushrooms (Wandati et al., 2013). Previous studies on chemical composition of various maturity stages [Barros et al., 2007a, b) revealed that immature spores have high content of bioactive molecules, which agrees with our finding in these unique species. However, in the present study, phytochemicals like cardiac glycosides, anthraquinones and tannins were not detected. These finding would provide leads to mycologists interested in biotechnological processes of solid states fermentation to tap these molecules at an appropriate phenological phase for either pharmaceutical or nutraceutical functions. Most of these bioactive molecules in other crops have been documented structurally and pharmacologically as containing pharmacological properties like anti-diabetic, anti-inflammatory (Lee *et al.,* 2012) and anticancer (Roslin and, Anupam 2011). This highlights the need of scientists to begin bioprospecting for alternative health promoting molecules from the primordials of basidiocaps.

5.2.2 Total Phenolic Content

Phenolic content was expressed as Gallic acid equivalent. Phenolics are derived from renewable natural resources, they are characterized as non-enzymatic compounds whose potential in antioxidant and other health promoting properties like anti-inflammatory have drawn immense scientific attention (Chang 2006). Our findings show that these compounds had a phenological variation (Table 1). Primordial phases of both *P. citrinopileatus* and *P. djamor* R22 recorded the highest values for the polyphenols. Previous studies recommended immature spores (Barros, *et al.,* 2007b), unlike Soares *et al.,* (2009) who suggested both young and mature because the phenol content differences were minor. For *P. djamor* R22 there was no significant difference in TPC levels in mycelium and mature fruiting body in *P. djamor* R22 (p=0.192). This suggests that spawn mycelium of *P. djamor* R22 could as well be used as a potential source of polyphenols instead of obtaining mature fruiting body. Perhaps, a better approach would involve using fermentation procedure to concentrate these molecules at mycelia phase. Our study collaborates previous finding by others who used button mushrooms (Soares *et al.,* 2009) that reported variation in chemical and bioactive compounds with mature spores presenting lower antimicrobial and bioactive activities as opposed to immature spores. Our study used whole mushroom unlike (Oboh and Shodehinde, 2009), which majored on different parts of mushroom like pileu and stipes. Primordial stages provided a better source for bioactive molecules. High phenolic acid contents in the primordial phases contribute partially to their high antioxidant properties. Polyphenol content increases with the growing of the basidiomycete, so that young coloured fruiting bodies have lower values but this increase up to a certain level before beginning to decline as the mushroom ages.

5.2.3 Total Flavonoid content (TFC)

There was significant difference in the total flavonoid content in the four phonological levels ($p<0.05$) for both *P. citrinopileatus* and *P. djamor* R22 (Table 1). In both species, primordial reported the highest flavonoid values (Table 1).This observation agrees with that of Barros and others (Barros *et al.,* 2007b). Total flavonoid contents of *P. citrinopileatus* recorded in all the growth levels were significantly different ($p<0.05$). This denotes that as the mushroom grows the contents of flavonoids increases to a certain level before declining sharply. Similarly, other studies have shown that bioactive molecules in *Agaricus* mushrooms changes with maturity so that the harvesting stage determines their quantities (Soares et al., 2009). Besides, Barros *et al.,* (2007b) demonstrated that immature *Lactarius piperatus* have high

contents of phenol compound than their mature parts. The existing explanation for possible low values in the mature stages is the possibility of involvement of these molecules in defensive processes against aging stressors (Ferreira *et al.,* 2009). However, Sultana *et al.,* (2009) demonstrates that secondary metabolites vary depending on the solvent used for their extraction. Ethanol extracts may not give a complete profile hence our values could not be effectively compared with other studies that used non-food grade solvents like methanol and ethyl acetate.

5.2.4 Total carotenoids, β- carotene, and Lycopene

Pigment molecules like β- carotene and Lycopene have been reported previously in edible fungi and their potential roles in antioxidant properties studied (Robaszkiewicz *et al.,* 2012). Our work showed varied concentrations of these molecules across the four phenological states with primordials drawing attention. Although the total carotenoid was significantly high their corresponding β- carotene and Lycopene were very low (Table 2). Both spawn mycelia phases reported very little values, which shows they are deprived of these essential phytochemicals. However, 1st and 2nd primordials phases of both species exhibited the highest contents of β- carotene and Lycopene, which warrants interest. Barros et al., (2007c) and Robaszkiewicz *et al.,* (2012) reported low quantities of these bioactive molecules in their respective studies, which agree with our values in the mature fruiting bodies because their studies were not phonologically designed. However, increased values in our study could be attributed to the species pigmentation (golden yellow and pink oyster). These molecules have pharmacological value because supplementation of diet with *β*-carotene has shown increased plasma antioxidant properties amongst the aged women (Meydani *et al.,* 1994), while Lycopene has protective value in preventing oxidative modification of lymphocyte DNA (Meydani et al., 1994, Robaszkiewicz et al., 2012). Pharmacological benefits of these molecules highlight the need to identify new renewable sources with potent molecules that can be targeted for pharmaceutical or neutraceuticals health benefits. Primordial phases of both *P. djamor* R22 and *P. citrinopileatus* offer a better outlet of these health promoting molecules.

5.2.5 2, 2'–azinobis (3-ethylbenzothiazoline-6-suslfonic acid (ABTS assay)

Differential potential of phenological states of two basidiomycete species to inhibit lipid peroxidation was determined using $ABTS^{+}$ assay with Trolox used in the calibration of standard curve. Primordials showed a better activity to vitamin E analogue (Table 3).

Generally, all samples were concentration dependent. The potency of primordials in the decolouration of ABTS radicals could be attributed to high phenolic and flavones contents (Table 1). Edible fungi have been shown to possess analogous defensive systems comparable to those found in other plants to help fight biotic and abiotic factors (Vaz *et al.,* 2011). These features have been exploited for pharmaceutical and nutraceutical properties (Meydani et al., 1994; Robaszkiewicz *et al.,* 2012, Vaz, *et al.,* 2011). Our study strengthens the importance of targeted phenological phase to maximize on these potentials. These two species are unique because they not only occur in different coloration but have varied distribution of bioactive molecules across their developmental phases. This warrants mycologists and other scientists in product development to have a clear perspective on the importance of getting these essential molecules in right quantities.

5.2.6 Reducing Power assay

Several in vitro assays have been validated in the determination of antioxidant properties of compounds. These methods scavenge or quench free radicals, inhibit peroxidation of lipids or chelates for metal ions (Robaszkiewicz et al., 2012). Reduction power of these samples was determined with BHA and BHT as positive controls. Our finding revels that reducing power varies along the phenological state, with primordials exhibited significant potential to reduce potassium ferricyanide than both the spawn mycelia and the fruiting phase ($p<0.05$). Reduction power in basidiomycetes can be attributed to potent antioxidant property in the primordial phases of basidiocaps. These *in vitro* assays justified the existing differential distribution of antioxidant properties along phenological states of both *P. citrinopileatus* and *P. djamor* R22.

5.2.7 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) Antioxidant activity

Radical scavenging properties of these extracts were concentration dependent (fig.4 & 5). Primordial phases of *P. djamor* R22 and *P. citrinopileatus* exhibited high antioxidants properties compared with the mature fruiting bodies ($p<0.05$). The RSA were significantly different between the young basidiocaps (EYFB, YFB) and the mature basidiocaps MFB for both species (*P<0.05*). These finding highlights the potential of primordials as potent sources of bioactive molecules as antioxidants. These finding corroborates previous studies showing a similar trend in Kenyan mushrooms of 0.58 mg/mL to 4.58 mg/mL (Wandati *et al.,* 2013) and from other regions like Brazil, with 0.76 to 17mg/mL (Barros *et al.,* 2007b]. Besides, this shows that *P. djamor* R22 has a better ability to scavenge for free radicals than *P.*

citrinopileatus at both EYFB and YFB ($p<0.05$). However, other than maturity, these properties could also be speculated to depend on a number of other factors such as mushrooms parts utilized, the species, and the substrates used in their cultivation (Oboh and Shodehinde 2009). Any discrepancy in these values may be attributed to other factors like substrates, medium used, which possibly may affect the quantity of phytochemicals extracted (Sultana *et al.*, 2009). Generally, our study depicts that spawn mycelium can be an alternative source of important bioactive molecules to substitute mature fruiting bodies, which tend to have reduced values (*P<0.05*) in *P. djamor* R22.

5.2.8 Correlation between TPC, TFC, β-Carotene, Lycopene and DPPH, TEAC, FRAP

There is a strong positive (*r* value >0.8) and significant correlation ($p<0.001$) between TPC, TFC content, β-Carotene, Lycopene and various in vitro antioxidant assays, DPPH, ABTS, and FRAP for *P. djamor* R22 and *P. citrinopileatus* Singer (Tables 4). It is therefore feasible to allude that flavonoids, polyphenols, β-Carotene, and Lycopene may play a significant role in conferring total antioxidant properties of the extracts. Study by Froufe et al., (2009) reported that flavonoids and polyphenols could have additive effects on antioxidant. Given that our samples were uniquely coloured, unlike most previous findings that did not use such species, which could explain existence of β-Carotene, and Lycopene. However, other studies did not find any significant correlation between flavonoids and RSA (Soobrattee et al., 2005; 2006, Gan *et al.*, 2013). Fractions from the two primordial phases depict a much lower concentration of total antioxidant properties, which suggest the possibility of combined effects of all intact bioactive molecules determined in high total antioxidative potential. The significance of this study is attributed to high phenolic and flavonoid compounds in primordial stages of basidiocaps (Table 2). Interestingly, spawn mycelia could also be a potential target for these value added properties. Barros (2007c) reported that phenolic compound and other secondary metabolites like carotenoids, tocopherols, and ascorbic acids contributed to radical scavenging activity. However, if these metabolites are to be validated for additional functions, their targeted extraction with column chromatography, purification, determination of structure and alignment of data in database will shed more light for future pharmacological application.

5.2.9 Total antioxidant properties of Primordial fractions

Having shown superior properties on DPPH, FRAP, TEAC/ABTS, TFC and TPC, fractions from *P. djamor* R22 and *P. citrinopileatus* Singer primordials were eluted and tested for *in*

vitro DPPH and TEAC assays. Generally all the fractions reported lower antioxidant values than their initial extracts ($p<0.05$) (Fig.6). This could suggest a synergistic potentiated effect when fractions act together. However, given that this is the first ever phenological study on basidiomycetes, these finding would lead scientists in natural products to bioprospect for potential application of targeted active biomolecules for pharmaceutical and nutraceutical application.

5.2.10 Metabolomic profiling (LC-MS)

Distribution of compounds across the four phenological phases for both species was different. For instance *P. djamor* reported 54 hits of compounds while *P. citrinopileatus* reported hits of compounds. This is the first study to profile and identify bioactive molecules in mushroom. Previous studies by Barros et al., (2007) identified compounds from edible mushroom based on commercial standards. No existing literature describes profiling of bioactive compounds using untargeted approach like LCQToFMS. Our study identified amino acid derivative compounds that have never been previously reported in literature. These compounds could be incriminated to confer health benefits associated to mushrooms. Compounds with significant hits were derivatives of various amino acids. A synergistic activity of these compounds could have potentiated the total antioxidant properties.

5.3.1 Mushroom in value added yoghurt

Intake of daily product like yoghurt has been proved to improve lactose maldigestion by lowering bad cholesterol (Alvarez-Leon *et al.,* 2006; Cueva and Aryana, 2008). Among the health benefits yoghurt supplies significant quantities of vitamins, quality proteins, potassium, calcium, and phosphorous (Karagul, et al., 2004). Addition of different substances or additives with additional health benefits potentiates on these benefits (Dureja et al., 2003; Sanodiya et al., 2009). Mushroom contains numerous health promoting benefits like therapeutic properties, minerals, vitamins, that can be transferred to other food items to leverage on their benefits (Ferraira, 2009). Mushrooms have been used as vegetable, development of alternative medicines for numerous ailments and culinary in high end hotels (Chang 2006). However, no literature exists to show attempts of fortifying daily products like yoghurt with mushroom. Addition of mushroom to daily product to improve quality of low fat products like yoghurt is an area of interesting research considering the value addition being brought on board. Many herbs and plant based fruits and vegetables have been

attempted in value addition potential for yoghurt with acceptable profiles (Coisson, et al., 2005; Husseina et al., 2011). This study used different concentration (1.5%, 3%, 5%, and 7.5%) of mushroom powder for the development of yoghurt. The products had different physicochemical and organoleptic characteristics.

5.3.2 Physicochemical properties of mushroom yoghurt

Acidity of yoghurt is one of the important attributes in determining acceptability and fermentation process of yoghurt (Routray and Mishra, 2011; Tamine and Robinsin, 2007). Addition of mushroom at different concentration had a significant effect on this physicochemical property of yoghurt. Low concentrations (1.5% and 3.0%, 5.0% mushroom) took a significant duration to ferment than the plain yoghurt, while the high concentration (7.5%) did not set. The maximum rate of yoghurt acidification increased significantly ($p<0.05$) with increasing concentration of mushroom powder from both species. This could be ascribed to high phenol and organic acid components in the mushroom which has been reported to impart buffering capacity (Zibadi & Watson, 2004). Espírito et al., (2012) and Varghese and Mishra (2008) demonstrates that buffering capacity is proportional to total solids which leads to extended fermentation time. Their findings are comparable to our finding. Previous studies in reported an extended setting duration for peanut and cashew nut yoghurt without milk (Isanga and Zhang, 2008, Aroyeun, 2004). Addition of total solids in yoghurt has been shown to increase the time it take for proteins to get in contact with lactic acid formed by the bacteria hence a slow decline in pH and lengthy fermentation process (Tamine and Robinsin, 2007). Considering that 7.5% did not contain skim milk, fermentation could not be finalised because coagulation did not take place since the protein content could not sustain the process. Lucey and Singh (1998) showed that coagulation of protein leads to development of the process. This could suggest that increasing total solids had an impact on the activity of lactic bacteria fermentation. The increase in viscosity could be attributed to the formation of a three dimensional gel matrix by the curdling process when milk protein associates with lactic acid released by bacteria during fermentation process (Lucey and Singh 1998). Low concentration (1.5% and 3%) had comparable attributes with plain yoghurt despite the increase in viscosity. Highly mushroom concentrated yoghurt exhibited poor viscosity, pH and setting duration. Previous studies found that addition of fruits to yoghurt exhibits poor texture, weak body and increased syneresis (Celik and Bakirci, 2003). This shows that formulations that do not affect activity of Lactic acid bacillus could be used to

develop yoghurt products with value added properties. This study did not address changes on physicochemical properties over storage. It is likely that some attributes would change with the storage and this may change the product acceptability (Narayana and Gupta, 2013). According to Al-Kadamany et al., (2003) and Lee et al., (1990) during storage, activity of lactic acid diminishes protein content and consequently lowering the pH, which may affect palatability of the product hence the need to assess these changes over weeks.

5.3.3 Acceptability of yoghurt fortified with mushroom

Popularity of yoghurt and any most daily food component largely depends on sensory attributes (Karagul et al., 2004; Tamine and Robinsin, 2007). Different preparation of probiotic yoghurt fortified with mushroom had different sensory properties. Very low concentrations (1.5% and 3%) were comparable to plain yoghurt in overall acceptability unlike 5% and 7.5% which recorded poor scores. These findings are comparable with previous studies that fortified yoghurt with cashew nut, peanut and soy (Aroyeun, 2004; Osundahunsi, *et al*, 2007; Kumar and Mishra, 2004). However, properties of very low concentrated mushroom could have been improved by flavouring mushroom yoghurt with either vanilla or straw berry because flavouring improves sensory attributes and overall acceptability (Adegoke et al., 2013). Addition of flavours and other components increases consumer options which affect marketability and retention of consumer interest (Isanga and Zhang, 2008). Our study aimed at testing the effects of mushroom on colour, taste, flavour and overall organoleptic attributes which were comparable with unflavoured mushroom. Since 7.5% product did not set, it was less viscous and very thin making it tasteless to the eye. Low concentrations scored better in colour than plain yoghurt despite the increase in viscosity. Other studies also reported a similar trend when yoghurt was fortified with fruits like Mango and papaya (Kumar and Mishra, 2003; 2004). Perhaps the best approach would involve designing experiment to ascertain the maximum concentration of mushroom that is fermentable and attain their organoleptic properties with vanilla and strawberry fortification before commercialisation.

5.4.1 Colour development and fastness on polymer fabrics

Extraction of dye bath depends on the extraction conditions with duration playing a critical role. Evaluation of colour development with time helped identify optimal stage when most of the dye molecules have been obtained. Based on colour parameters L^* a^* and b^* lightness

increased until it flattened after 8hrs which was selected as the optimal duration. The bath was used for dyeing purposes. During extraction, the concentration increased with time until it flattened off suggesting no more extraction. Although this depends on other factors like temperature, PH, and darkness, duration is the most studied condition because other parameters are used to reduce extraction period.

Colour fastness is the ability of polymer material to resist changes in colour characteristics upon dyeing (Patricia, 1982). Pure polymer fabrics were evaluated for their colour fastness based on lightness and washing (Mahangade et al., 2009) with aqueous extracts of *Pleurotus citrinopileatus* and *P. djamor*. Both species exhibited varied shades of hues ranging from fairly good to good (3-4). The most notable observation is the absence of colour staining for both dye baths which suggests retention of colour molecules within the polymer materials. Lack of colour staining has been a success of most previous studies experimenting application of natural dyes on various mordanted textile materials (Kumaresan et al., 2011a, b). Other studies have reported similar trends in natural dyes obtained from plants like Bixa and Pomegranate tested on fabric materials (Das *et al.,* 2007; Adeel *et al*., 2009). A fairly fastness for washing is reported for both dye baths of coloured basidiomycetes. Cotton fastness ranged from fair to fairly good (2-3) for both species unlike silk which reported fairly good to good with washed fastness (3-4). This suggests that silk unlike cotton polymer have superior dyeing properties (fastness) with both extracts. Studies show that cotton is the most challenging polymer material for use with natural dye because of poor fastness with most dyes from nature (Adeel *et al.,* 2009). Washing fastness did report a significance difference for both species (3 -4). Changes occasioned by washing were not significant in silk for both species; however cotton polymer reported a significant variation with dye bath from *Pleurotus citrinopileatus.* Mushroom dyes especially from wild non-edible species like Boletus sp., Macrolepiota sp., Auricularia spp have been investigated and tested for their colour fastness with comparable findings obtained (Mibei *et al.,* 2009). Coloured edible mushrooms are hereby reported for the first time on their potential for non-food application especially in dyeing of polymer fabric materials. Most of these studies reported varied colour shades that depended on the type of mordant used (Anitha and Prasad 2007). Mordanting helps in leveraging colour properties in the polymer material. In comparison with polymer fabrics without mordants it can be concluded that mordants played a critical role in colour enhancement and retention.

5.4.2 Effects of mordanting

Mordants are salts associated with enhancement of fabric colour (Bhattacharya, and Shah, 2006). They make it possible to produce different hues with the same colour bath. Two mordants (alum and copper sulphate) were evaluated for their enhancement of dyeing properties for both dye bathes from *Pleurotus citrinopileatus* and *P. djamor*. Our finding agrees with previous suggestions that each mordant imparts a different hue from the same bath of dye (Anitha and Prasad 2007). Silk gave conspicuously diverse hues with fairly good colour fast in both species. These hues are more diverse in *P. djamor* which suggest a potentiated effort in these basidiomycetes to produce coloured molecules. Different mordant imparts different hue irrespective of dye bath source (Kumaresan et al., 2007). However, hues in cotton polymer were fair and fairly good in *P. citrinopileatus.* Mordanting could have played a significant role in preventing colour staining because the unmordanted fabric exhibited colour staining especially on the cotton material. Cotton has been reported to stain poor to fairly good with most natural dyes (Adeel *et al.,* 2009). Copper sulphate gave a diverse hue from those exhibited by alum. This is comparable to previous findings (Kongkachulchay et al., 2002). In comparison, the mixture of copper sulphate and alum was excellent when compared to alum alone but not as diverse as copper sulphate alone. Several studies evaluating various ratio mixtures of mordants and their effect on dyeing properties found to be significant than the individual mordants (Kumaresan et al., 2011). A combination of several mordants optimized at specific ratios gives a diverse range of hues that would appeal to consumers (Kumaresan et al., 2007). The effect of mordant is inseparable from natural dyes especially when using cotton polymer material because they have poor fastness characteristics (Samanta, and Agarwal, 2009). However, silk and other materials like wool have reported excellent fastness with other natural dyes (Das et al., 2007; Kongkachulchay, et al., 2002). The scope of this study is to give a baseline on the viability of using coloured edible basidiomycetes as a source of dye for polymer material

5.5.1 Decolouration of levafix blue CA by Spent Mushroom Substrate

Changes in the intensity of colour measured as optical density were noted in the various treatment procedures. For an increase in the concentration of SMS the decolouration percentage increased significantly. This study agrees with previous studies that utilized spent substrate mushroom from species like P. oestratus in remediating environmental pollutants (Papinutti and Forchiassin, 2010; Singh et al., 2010). Other studies demonstrates application of SMS in various bioremediation studies, for instance Ahlawat *et al.,* (2010) ascribes that

fungicides can be remediated from environment using spent substrates, while Chiu *et al* (2009) reports on its application in decontaminating soils with spilled petroleum products. Gonzalez et al., (2012) describes the enzymatic potential in SMS for bioremediation application. Our study did not document the specific enzymes involved in the remediation and descolouration, a gap that need to be addressed to identify the specific enzymes with the potential to breakdown the chromophore in Levafix blue CA. Levafix is a reactive dye with high performance efficiency making it preferred in industrial applications (Dye.com, 2013). However, its removal and treatment procedure utilizing chemical and physical methods are not only expensive and unsustainable but also inefficient (Papinutti and Forchiassin, 2010). Spent substrate is a sustainable alternative that would not only alleviate on environmental contamination but also ensure effective utilization of simple knowledge.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

The main purpose of substrate suitability study is to determine organic residues that give optimal growth performances of high biological efficiency, marketable basidiocaps and low pin head abortion over a given number of flushes with a given spawning rate. This study showed that bean straw, sugarcane baggase, wheat straw, wheat straw, and rice straw gives better growth performances for both *P. citrinopileatus* and *P. djamor* than maize stovers, banana fibres, and sawdust residues. Although bean straw had the highest mean basidiocaps during the first flush compared to other substrates, its Biological Efficiency did not differ significantly to that of rice straw in both species. Therefore, mean basidiocap number did not affect Biological efficiency. Comparative studies with different spawning rates could have better explained the association between mean basidiocap and Biological efficiency. Sawdust residue reported the poorest survival*; P. citrinopileatus* did not report any growth unlike *P. djamor* that reported poor response with this residue. Studies on supplementation of poor substrates could have leveraged on their productivity, however supplementation incurs additional costs. Spawning rate at 7.5% could have potentiated formation of abortive heads; a lower spawning rate could have maximised survival and fecundity of these basidiomycetes in the substrates. Nevertheless, our study compared seven substrates between *P. citrinopileatus* and *P. djamor* with the former reporting superior growth parameters than the latter. This discrepancy could be attributed to adaptability to climatic conditions because *P. citrinopileatus* is an indigenous while *P. djamor* is an exotic strain. A better explanation could be arrived at by designing comparative studies between indigenous species (e.g. *P. citrinopileatus* and another indigenous spp) another set to include two or more exotics spp (*P. djamor* However, Biological Efficiency between the two species for the individual six substrates did not differ significantly which suggests a good opportunity to domesticate and commercialise both species with the selected optimal organic residues.

Phenological documentation of bioactive molecules in mushroom is an important perspective. It seeks to guide entrepreneurs interested in developing mushroom products to target physiological states with optimal health promoting molecules. The viability of this application depends on duration of spawning and formation of first pin heads. However, economic viability of this design ought to be ascertained through an effective investigation of cost-benefit analysis and its practicality. As the study unravels, primordial and early basidiocaps offers significant polyphenols, flavonoids, and total antioxidant properties than both spawn mycelium and mature fruiting bodies. However, most products in the market are

developed from mature fruiting bodies. This suggests a significant deficiency in the health properties of those products. Essentially, perceived health molecules in mushrooms is what lures consumers to buy mushroom products. Our study justifies the need for a paradigm shift in considering primordial and early basidiocaps as the appropriate sources of mushroom products. Interestingly, spawn mycelium could be an alternative source for concentrated bioactive molecules. Perhaps the best approach for harvesting these health molecules from spawn mycelium is through biotechnological approaches like solid state or liquid state fermentation. New studies that culture molecules in bioreactors should compare between solid state and liquid state fermentation to ascertain the most appropriate process for harvesting these molecules.

Value addition is an emerging aspect of improving health and creating industrial opportunities. Medicinal properties of mushroom justify its use in ethnomedicinal from ancient times. However little has been done to assess the possibility of transferring bioactive health promoting molecules and ascertaining their value added potential in daily products like yoghurt. Therefore, this study is the baseline from which future aspects could be considered. Low concentration of mushroom powder exhibited comparable scores with plain yoghurt in overall acceptability despite the increased viscosity. Perhaps an increase in milk quantity could have lowered the viscosity. Colour attributes were palatable and this justifies the need to consider coloured basidiomycetes as a potential fortifying agent. However, one challenge of these products is the lengthy duration to end fermentation process. A notable observation is that substitution of skim milk with mushroom powder could not complete setting process. This justifies the need to include conventional components of yoghurt processing when fortifying. There is high likelihood that yoghurt fortified with mushroom has improved flavonoids, polyphenols, antioxidant properties and other health benefits in specific mushroom used. The best approach to ascertain these properties is to use a more specialised protocol like LCMS and GCMS to identify and quantify these bioactive molecules in the product compared with conventional yoghurt. This study did not ascertain the appropriate shelf for the products. Shelf life is also an important attributes which should have been monitored over several weeks. Changes in physicochemical attributes correlate with the duration of storage, storage condition, and the overall acceptability. Such changes need to be ascertained prior to further developments and commercialisation.

Basidiomycetes can be an alternative source of dye for dyeing polymer materials. Mordanting gives a diverse range of hues on the same dye bath which makes the process irreproducible because such hues depend on the type of mordant used. Although colour fastness based on

their washing and lighting characteristics did not differ significantly in silk for both species of basidiomycetes, cotton reported significant hue shades in *P. citrinopileatus*. However, this study did not verify the individual dye particle retained in the fabric through a specialised RT-HPLC/MS protocol. Such a specialised protocol may help identify specific dyeing molecules attributed to coloration and help in the identification of targeted extraction to optimize dye extraction process. This study unravelled an interesting perspective to colour development along phenological phases. Primordial were reported as the best source for optimal flavonoids, polyphenols, carotenoids and β-carotene molecules which are strongly correlated to colouration. As the basidiomycetes develop mature fruiting bodies tend to have reduced quantities of these bioactive molecules. This suggests the need to consider early basidiocaps as the optimal source for dyeing molecules. The main challenge may be addressed by cost benefit-analysis, which ought to leverage on the potential of using primordial as opposed to mature fruiting bodies. Besides, lack of information databases of basidiomycetes that yields dye bathes and their extraction, concentration, and application protocols is the main challenge.

Activity of a consortium of microorganisms' previously reported in spent mushroom substrate have the potential to degrade and discolour levafix blue CA. this demonstrate the potential application of these waste in remediating the environment from synthetic reactive dyes and dye wastes. This study did not identify the kinetics potentiating discoloration and degradation of the levafix blue CA dyes. This would have enable identification of optimal mycoremediation conditions. Besides, identification and characterization of the specific microorganisms involved in bioremediation would have given leads to their commercial utilization.

REFERENCES

Adeel S, Ali S, Bhatti IA and Zsila F. (2009). Dyeing of cotton fabric using Pomegranate (Punica Granatum) aqueous extract. Asian Journal of Chemistry; 21(5): 3493-3499

Adegoke GO, Musenbi S, Wachira S, Muigai C, Muigai F, Ngumba E, Karanja P, Onyango C, Adoko, GM (2013). Production of Probiotic Yoghurt Flavored with the Spice, Aframomum danielli, Strawberry and Vanilla, *Food and Public Health*, 3(2): 92-96

Adosinda, M., M. Martins, C. Ferreira, I. M. Santos, M. J. Queiroz and N. Lima, (2001). Biodegradation of bioaccessible textile azo dyes by Phanerochaete chrysosporium,‖ J. Biotechnol.,vol 89, , pp 91-98.

Ahlawat OP, Gupta P, Kumar S, Sharma DK, Ahlawat K (2010) Bioremediation of fungicides by spent mushroom substrate and its associated microflora. Indian J Microbiol 50:390–395

Akula R, Ravishankar GA. (2011). Influence of abiotic stress signals on secondary metabolites in plants, *Plant Signal Behav,*; 6(11): 1720–1731. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3329344/

Álvarez-León EE, Román-Viñas B, Serra-Majem L (2006). Dairy products and health: a review of the epidemiological evidence. Brit. J. Nutr. 96: 94-99.

Al-Kadamany E, Khattar M, Haddad T, Toufeili I (2003). Estimation of shelf-life of concentrated yogurt by monitoring selected microbiological and physicochemical changes during storage. LWT - Food Sci. Technol. 36: 407-414.

Anitha K., & Prasad SN. (2007). Developing multiple natural dyes from flower parts of Gulmohur. Current Science; 92(12):1681-1682.

AOAC (Association of Official Analytical Chemists). (1996). Official Methods of Analysis., Washington. D.C.

Aroyeun, S. O. (2004). "Optimization of the utilization of cashew apple in yoghurt production," Nutrit Food Sci, vol. 34, pp. 17-19,.

Asamudo NU, Dada AS, Ezeronye OU (2005). Bioremememediation of textile effluent using Phanerochaete chrysosporium. Afr. J. Biotechnol. 4(13):1548-1553.

Barros L, Baptista P, Estevinho LM, Ferreira I. (2007a). Effect of fruiting body maturity stage on chemical composition and antimicrobial activity of *lactariu* ssp. Mushrooms. *J. Agric. Food Chem.*; 55(21): 8766-8771. http://dx.doi.org/10.1021/jf071435+

Barros L, Baptista P, Ferreira ICFR. (2007b). Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food. Chem. Toxicol.*; 45(9): 1731–1737. http://dx.doi.org/10.1016/j.fct.2007.03.006

Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira ICFR. (2008). Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food. Chem. Toxicol.*; 46(8): 2742-2747. http://dx.doi.org/10.1016/j.fct.2008.04.030

Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P. (2007c). Total Phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food. Chem.,*; 103(2): 413- 419. http://dx.doi.org/10.1016/j.foodchem.2006.07.038

Bhattacharya, S.R and Shah, A.K (2006). Metal ion effect on dyeing wool fabric with Catechin. Colouration Technology, 116(1), 10 – 20.

Brand-Williams W, Cuvelier M, Berset C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Sci. Technol.*; 28(1): 25–30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5

Cai LY, Shi FX, Gao X. (2011). Preliminary phytochemical analysis of Acanthopanantrifoliatus(L.) Merr. Journal of Medicinal Plants Research. ;5(17):4059–4064

Celik S and Bakirci I (2003) Some properties of yoghurt produced by adding mulberry pekmez (concentrated juice). International Journal of Dairy Technology 56 26–29

Cheung LM, Cheung PCK, Oei VEC. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *J. Food Chem.*; 81(2): 249-255. http://dx.doi.org/10.1016/S0308-8146(02)00419-3

Chiu SW, Ching ML, Fong KL, Moore D (1998) Spent oyster mushroom substrate performs better than many mushroom mycelia in removing the biocide pentachlorophenol. Mycol Res 102:1553–1562

Chiu SW, Gao T, Chan CSS, Ho CKM (2009) Removal of spilled petroleum in industrial soils by spent compost of mushroom Pleurotus pulmonarius. Chemosphere 75:837–842

Coisson JD, Travaglia F, Piana G, Capasso M and Arlorio M (2005). Euterpeoleracea juice as a functional pigment for yogurt. Food Research International 38 893–897.

Collins, P. (1991). Annatto legislation in Europe. In: *Proceedings of 1st International symposium on Annatto; Campinas, Brazil; 17-21 June 1991*; edited by Carvalho, P.R.N. and Yabiku, H.Y. Campinas, Brazil: Instituto de Tecnología de Alimentos

Collins P. (2000). Colouring our foods in the last and next millennium. Int. J Food Sci. Technol, , 35, 5-22

Coulibaly, AY, Hashim R, Sulaiman SF, Suleiman O, Ang LZP, Ooi KL. (2014). Bioprospecting medicinal plants for antioxidant components, *Asian Pacific Journal of Tropical Biomedicine*,; 4(9): 740 - 746

Cueva O, Aryana KJ (2008). Quality attributes of a heart healthy yogurt. LWT - Food Sci. Technol. 41:537-544

Das D, Maulik SR and Bhattacharya SC. (2007). Dyeing of wool and silk with Bixa orellana. Indian Journal of Fibre and Textile Research; 32: 366-372

Delgado-Vargas, F, Jimenez, A. R, & Paredes-López O. (2000). Natural Pigments: Carotenoids, Anthocyanins, and Betalains — Characteristics, Biosynthesis, Processing, and Stability, *Critical Reviews in Food Science and Nutrition*, 40(3):173–289.

de Carvalho LMJ, Gomes PB, Godoy RLO, Pacheco S, do Monte PHF, de Carvalho JLV, Nutti MR, Neves ACL, Vieira ACRA. Ramos SRR. (2012). Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (Cucurbita moschata Duch): A preliminary study. *Food Res Int* 47,; 337–340. http://dx.doi.org/10.1016/j.foodres.2011.07.040

Dureja H, Kaushik D., Kumar V. (2003) Developments in nutraceuticals, Indian Journal of Pharmacology; 35: 363-372

Espíndola, LHS, Espindola, FS, Freitas, GR, Brandeburgo, MAM. (2007). Biodegradation of Red 40 Dye by the Mushroom Pleurotus Sp Florida; *Biosci. J Uberlândia*, v. 23, n. 3, p. 90-93

Espírito S.A.P; Perego P. Converti A. Oliveira M.N. (2012). Influence of milk type and addition of passion fruit peel powder on fermentation kinetics, texture profile and bacterial viability in probiotic yoghurts, *LWT - Food Science and Technology* 47; 393- 399

Faraco, V.; Pezzella, C.; Miele, A.; Giardina, P. & Sannia, G, (2009). Bioremediation of coloured industrial wastewaters by the white-rot fungi Phanerochaete chrysosporium and Pleurotus ostreatus and their enzymes Biodegradation, 20: 209-220

Ferreira ICFR, Barros L, Abreu RMV. (2009). Antioxidants in wild mushrooms. *Curr. Med. Chem.*; 16(12): 1543-1560. http://dx.doi.org/10.2174/092986709787909587

Ferreira ICFR, Vaz JA, Vasconcelos MA, Martins A. (2010). Compounds from wild mushrooms with antitumor potential. *Anticancer Agents Med Chem.*; 10(5): 424-436. http://dx.doi.org/10.2174/1871520611009050424

Froufe HJC, Abreu RMV, Ferreira Erkurt, E. A., U. N. Ali and H. Kumbur, (2007). Decolourization of synthetic dyes by white rot fungi, Process Biochem., vol 42, , pp 1429-1435

Gan CH, Nurul AB, Asmah R. (2013). Antioxidant analysis of different types of edible mushrooms (*Agaricus bisporous* and *Agaricus brasiliensis*), *Int. Food Res. J.* 20(3): 1095-1102. Accessed from http://www.ifrj.upm.edu.my/20%20%2803%29%202013/8%20IFRJ%2020%20%2803%29%202013%20Asmah%20%28306%29.pdf

Gao JF, Wang JH, Wu XL, Zhang QA, Peng YZ (2011) Protonated spent mushroom substrate as a potential low-cost biosorbent for the removal of Reactive Red 15 from aqueous solutions. Fresen Environ Bull 20:51–62

Gautam R., Bassi A.S., Yanful E.K. (2006). A review of biodegradation of synthetic plastic and foams. *Applied Biochemistry and Biotechnology* 141: 85-109

Gonzalez Matute R, Figlas D, Mockel G, Curvetto N (2012) Degradation of metsulfuron methyl by Agaricus blazei Murrill spent compost enzymes. Biorem J 16:31–37

Guinot, P., Annick, G., Giles, V., Alain, F and Claude, A. (2008). Primary flavonoids in Marigold Dye: Extraction, Structure and Involvement in the Dyeing process. Phytochem. Anal (19), 46 – 51.

Hari, R.K., T.R. Patel and A.M. Martin, (1994). An overview of pigment production in biological systems: functions, biosynthesis and applications in food industry. Food Rev. Intern., 10: 49-70.

Harborne JB. (1973). Phytochemical methods. A Guide to modern techniques of plant analysis. London: Chapman and Hall;.

Hernandez-Luna CE, Gutierrez-Soto G, Salcedo-Martinez SM (2007). Screening for decolorizing basidiomycetes in Mexico. World Journal of Microbiology and Biotechnology 24, 465-473

Hirasawa M, Shouji N, Neta T, Fukushima K, Takada K. (1999). Three kinds of antibacterial substances from Lentinus edodes (Berk.) Sing. (Shiitake, an edible

mushroom). *Int. J. Antimicrob. Agent.*; 11(2): 151-157. http://dx.doi.org/10.1016/S0924-8579(98)00084-3

Husseina, MM Fatma AM Hassanb, HH Abdel Daymc, A Salamac, AK Enabb, Asmaa A, Abd El-Galila (2011). Utilization of some plant polysaccharides for improving yoghurt consistency. Annals of Agricultural Science 56(2) 97–103

Hutkins R.W., (2006) " Microbiology and Technology of Fermented Foods," UK, Oxford, Blackwell Publ Ltd, pp. 104-134.

ICFR. (2009). A QCAR model for predicting antioxidant activity of wild mushrooms. *SAR. QSAR. Environ. Res.*; 20(5-6): 579–90. http://dx.doi.org/10.1080/10629360903408423

Isanga J, and Zhang G. (2008) "Screening of stabilizers for peanut milk based set yoghurt by assessment of whey separation, gel firmness and sensory quality of the yoghurt," Amer J. Food Technol, vol. 3, pp.127-133,.

Isanga J. and Zhang, G. (2008). "Screening of stabilizers for peanut milk based set yoghurt by assessment of whey separation, gel firmness and sensory quality of the yoghurt," Amer J. Food Technol, vol. 3, pp.127-133,

Isikhuemhen OS, Nerud F, Vilgalys R. (1999). Cultivation studies on wild and hybrid strains of *Pleurotus tuberregium* (Fr.) Singer on wheat straw substrate. *World J. Microbiol. Biotechnol.* 16(5): 431-435 http://dx.doi.org/10.1023/A:1008958319418

Jeong SC, Jeong YT, Yang BK, Islam R, Koyyalamudi SR, Pang G. et al. (2010). White button mushroom (*Agaricus bisporus*) lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats. *Nutr. Res.*; 30(1): 49-56. http://dx.doi.org/10.1016/j.nutres.2009.12.003

Karagul Y, Wilson C and White H (2004). Formulation and Processing of Yoghurt. Dairy Science 87 543-550

Kechi A., Chavan R.B., Moeckel R. (2013). Dye Yield, Color Strength and Dyeing Properties of Natural Dyes Extracted from Ethiopian Dye Plants; *Textiles and Light Industrial Science and Technology (TLIST)* (2)3, 137-145

Khalid A, Arshad M & Crowley DE (2008) decolourization of azo dyes by *Shewanella* spp. Under saline conditions, *Appl microbial Biotechnology* 79; 1053-1059.

Kongkachulchay, P; Shitangkoon, A and Chinwongamorn N. (2002). Studies on dyeing of silk yarn with lac dye. Effects of mordants and dyeing conditions. ScienceAsia, 28, 161 –166

Kumar P, Mishra HN (2003b). Effect of mango pulp and soymilk fortification on textural profile of set yoghurt made from buffalo milk. J. Texture Stud. 34(3): 249-269.

Kumar P, Mishra HN (2004). Mango soy fortified set yoghurt: effect of stabilizer addition on physico-chemical, sensory and textural properties. Food Chem. 87: 501-507.

Kumaresan, M., Palanisamy, P.N. and Kumar, P.E (2011a). "Application of eco-friendly natural dye obtained from flower of Spathodea campanulata using combination of mordants", European Journal of Scientific Research, Vol. 52(3);.306-312,

Kumaresan M., Palanisamy P.N. and Kumar P.E. (2011b). "Dyeing of cotton and silk fabrics using stem of Achras sapota extract: Effects of mordanting and dyeing process variables on colour yield and colour fastness properties", The Asian Journal of Experimental Chemistry, 6(1); 23-57

Law WM, Lau WN, Lo KL, Wai LM, Chiu SW (2003) Removal of biocide pentachlorophenol in water system by the spent mushroom compost of Pleurotus pulmonarius. Chemosphere 52:1531–1537

Lee J, Lim S, Kang S-M, Min S, Son K. (2012). Saponin inhibits hepatitis C virus propagation by up-regulating suppressor of cytokine signaling 2. *PLoS ONE.* ; 7(6): e39366. http://dx.doi.org/10.1371/journal.pone.0039366

Lee SY, Morr CV, Seo A (1990). Comparison of milk based and soymilk based yoghurt. J. Food Sci. 55(2): 532-536

Lucey J A and Singh H (1998) Formation and physical properties of acid milk gels: a review. Food Research International 30 529–542

Mahangade RR, Varadarajan PV, Verma JK and Bosco H. (2009). New Dyeing Techniques for Enhancing Color Strength and Fastness Properties of Cotton Fabric Dyed with Natural Dyes. Indian Journal of Fibre and Textile Research; 34:279-282.

Meydani, M. Martin, A. Ribaya-Mercado, J. D. Gong, J. Blumberg, J. B. and. Russell, R. M (1994). "β-carotene supplementation increases antioxidant capacity of plasma in older women," *J Nutr*,; 124(12): 2397–2403.

Mihalick J.E. and Donnelly K.M., Using metals to change the colors of natural dyes. J. Chem. Educ. 2006; 83(10): 1550-155

Mohamed IM, Mohamed MRM, Abdul BJ, Asarudeen A. (2011). Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms. *Pleurotus florida* and *Pleurotus eous*. *Int. Food. Res. J.*; 18: 579-582.

<http://www.scribd.com/doc/74839892/Determination-of-Total-Phenol-Flavonoid-and-Antioxidant-Activity>

Musieba F, Okoth S, Mibey RK, Wanjiku S, Moraa K. (2012). Suitability of selected local substrates for the growth of golden yellow *Pleurotus citrinopileatus* Singer: an indigenous Mushroom in Kenya. *Am. J. Food. Technol.,;* 9(3): 240- 244. http://dx.doi.org/10.3923/aj.2012.240.244

Narayana MNK and Gupta, VK (2013). Effect of total milk solid content adjusted by adding ultrafiltered milk retentate on quality of set mango yoghurt, *International Journal of Dairy Technology* 66(4) 570-575

Nijveldt RJ, Nood E, Hoorn DEC, Boelens PG, Norren K, Leeuwen PAM. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *Am. J. Clin Nutr.*; 74(4): 418-425. http://ajcn.nutrition.org/content/74/4/418.full

Oboh G, Shodehinde SA. (2009). Distribution of nutrients, polyphenols and antioxidant activities in the pilei and stipes of some commonly consumed edible mushrooms in Nigeria. *Bull. Chem. Soc. Ethiop.*; 23(3): 391-398. http://dx.doi.org/10.4314/bcse.v23i3.47663

Osundahunsi, O.F. et al. (2007). "Quality evaluation and acceptability of soy-yoghurt wit different colours and fruit flavours, " American J Food Technol, vol. 2, pp. 273-280,

Oyaizu, M. (1986). Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr*., 44, 307–315

Oyetayo VO. (2009). Free radical scavenging and antimicrobial properties of extracts of wild mushrooms. *Braz. J. Microbiol.*; 40(2): 380-386; accessed from<http://www.scielo.br/scielo.php?script=sci_pdf&pid=S1517-83822009000200031&lng=en&nrm=iso&tlng=en>

Papinutti L, Forchiassin F (2010) Adsorption and decolorization of dyes using solid residues from Pleurotus ostreatus mushroom production. Biotechnol Bioprocess Eng 15:1102–1109

Patricia, C.C (1982). The influence of mordant on the lightfastness of yellow natural dyes. Journal of the American Institute for Conservation (21), 43 – 58.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization

assay. Free Radic. *Biol. Med.*; 26: 1231–1237. http://dx.doi.org/10.1016/S0891-5849(98)00315-3

Reis FS, Heleno SA, Barros L, Sousa MJ, Martins A, Santos-Buelga C, *et al*. (2011). Toward the antioxidant and chemical characterization of mycorrhizal mushrooms from Northeast Portugal. *J. Food Sci.*; 76(6): 824-830, http://dx.doi.org/10.1111/j.1750-3841.2011.02251.x

Reis FS, Barros L, Martins A, Ferreira ICFR. (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an interspecies comparative study. *Food Chem. Toxicol.*; 50: 191-197. http://dx.doi.org/10.1016/j.fct.2011.10.056

Richard, MK., Tuigong R.D., & Makumba N.A.B. (2009). Fabric/Yarn dyeing using dyes extracted from locally available mushroom species. *International Conference on: Emerging Trends in Production, Processing and Utilization of Natural Fibres.* Held on: April 16th to 18th; Mayfair, Worli, Mumbai, India. Book of Papers (Vol 2)

Robaszkiewicz A, Bartosz, G. Ławrynowicz, M. Soszynski M. (2012). The Role of Polyphenols, *β*-Carotene, and Lycopene in the Antioxidative Action of the Extracts of Dried, Edible Mushrooms, *J Nutr Metab*. 173274, 9. http://dx.doi.org/10.1155/2010/173274

Roslin JT, Anupam B. (2011). Terpenoids as potential chemopreventive and therapeutic agents in liver cancer. *World. J. Hepatol.*; 3(9): 228-249. http://dx.doi.org/10.4254/wjh.v3.i9.228

Routray, W and Mishra, H.N. (2011) "Scientific and technical aspects of yoghurt aroma and taste; a review, " Compr Rev Food Sci Fd Safety, vol.10, pp. 208-210,

Samanta, A. K and Agarwal, P (2009). Application of natural dyes on textiles. Review Article. Indian Journal of Fibre and Textile Research. (34), 384 – 399

Sanodiya BS, Thakur GS, Baghel RK, Prasad GB, Bisen PS. (2009). Ganoderma lucidum: a potent pharmacological macrofungus. Curr pharm Biotechnolol, 10(8): 717-42

Sasek V, Cajthaml T (2005). Mycoremediation. Current state and perspectives. Int. J. Med. Mushrooms 7(3):360-361

Scalber A, Manach C, Morand C, Remesy C, Jimenez L. (2005). Dietary polyphenols and the prevention of disease. *Crit. Rev. Food Sci. Nutr.*; 45(5): 287–306. http://dx.doi.org/10.1080/1040869059096

Singh AD, Sabaratnam V, Abdullah N, Annuar MSM, Ramachandran KB (2010) Decolourisation of chemically different dyes by enzymes from spent compost of Pleurotus sajor-caju and their kinetics. Afr J Biotechnol 9:41–54

Singleton V L, Orthofer R, Lamuela-Ravento, RM. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods Enzymol.*; 299: 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1

Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. (2005). Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutat. Res.*; 579(1-2): 200–213. http://dx.doi.org/10.1016/j.mrfmmm.2005.03.023

Soobrattee MA, Bahorun T, Aruoma OI. (2006). Chemo preventive actions of polyphenolic compounds in cancer. *Biofactors.*; 27(1-4): 19–35. http://dx.doi.org/10.1002/biof.5520270103

Stamets P. (2000). Growing gourmet and medicinal mushrooms; 3rd ed. Berkeley- Toronto: Ten speed press;. p 153-167. Accessed from www.australasianmycology.com/pages/pdf/20/1/56.pdf

Stiles, D. (1991). Production and marketing of annatto in Kenya. *Proceedings of 1st International symposium on Annatto; Campinas, Brazil; 17-21 June 1991*; edited by Carvalho, P.R.N. and Yabiku, H.Y. Campinas, Brazil: Instituto de Tecnología de Alimentos Downham, A.,

Soares AA, de Souza CGM, Daniel FM, Ferrari GP, da Costa SMG, Peralta, RM. et al. (2009). Antioxidant activity and total phenolic content of *Agaricus brasiliensis* (Agaricus blazei Murril) in two stages of maturity. *Food Chem.*; 112(4): 775-781. http://dx.doi.org/10.1016/j.foodchem.2008.05.117

Sultana B, Anwar F, Ashraf M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules.*; 14(6): 2167-2180. http://dx.doi.org/10.3390/molecules14062167

Tamime A Y and Robinson R K (1999) Yoghurt: Science and Technology, 2nd edn. Cambridge: Woodhead Publishing Ltd

Tamine A.Y.and Robinsin, R.K. (2007). "Yoghurt Science and Technology," 3rd ed, Florida, Boca Raton,

Tamine AY, Robinson RK (2007). Yoghurt: Science and Technology. 3rd ed. Cambridge, Woodhead Publishing Limited p. 808

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.*; 39(1): 44-84. http://dx.doi.org/10.1016/j.biocel.2006.07.001

Varghese, K. S., & Mishra, H. N. (2008). Modelling of acidification kinetics and textural properties in dahi (Indian yogurt) made from buffalo milk using response surface methodology. International Journal of Dairy Technology, 61, 284-289

Vaz, J. A., Barros, L., Martins, A., Morais, J. S., Vasconcelos, M. H. and Ferreira, I. C. F. R. (2011). 'Phenolic profile of seventeen Portuguese wild mushrooms', *LWT-Food Science and Technology*; 44(1): 343-346

Velisek J., & Cejpek K. (2011). Pigments of higher fungi: A review. *Czech Journal Of Food Sciences*, 29,87-102.

Wandati TW, Kenji GM, Onguso JM. (2013). Phytochemicals in Edible Wild Mushrooms from Selected Areas in Kenya, *J. Food. Res.*; 2(3): 137-144. http://dx.doi.org/10.5539/jfr.v2n3p137

Wasser, SP. (2002). Review of Medicinal Mushrooms Advances: Good News from Old Allies, *HerbalGram - American Botanical Council;* 56:28-33

Zhou, Z.Y., & Liu J.K. (2010). Pigments of fungi (macromycetes). *Natural Products Report*, 27, 1531-1570.

Zibadi, S., & Watson, R. R. (2004). Passion fruit (Passiflora edulis): composition, efficacy and safety. Evidence Based Integrative Medicine, 1,183-187

APPENDICES

Appendix I: Sensory evaluation form

Sheet No 1 Date: ……..
Panelist age:…… Sex ……..

Test the provided samples either by tasting or visually, and using the 9-point hedonic scale provided, indicate the score of the stated attribute.

9-point hedonic scale

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

	SAMPLE CODES						
	PC1	PC2	PC3	PD1	PD2	PD3	P/D
1.APPEARANCE							
2.COLOR							
3.SMELL							
4.FLAVOR							
5.TASTE							
6.OVERALL APPEARANCE							

Comments: _____

_____

Appendix II: The Hunter's CIE (Commission Internationale de l'Eclairage) L* a* b* system colour space.

The Hunter L* (whiteness/darkness), a* (redness/greenness) and b* (yellowness/blueness) CIE (Commission Internationale de l'Eclairage) system colour space. Hue describes a visual sensation according to which an area appears to be similar to one or proportions of two of the perceived colours, red, yellow, green and blue. The hue angle is thus the actual colour. An angle of 0°C = red-purple hue, 90°C =yellow hue, 180°C= bluish green, 270°C=blue. Total colour change measures the changes in the three colour components: Lightness L*, red-green a* and yellow-blue b*. On a horizontal axis, positive 'a*' indicates a hue of red-purple, a negative 'a*' indicates bluish green, a positive 'b*' indicates yellow and negative 'b*' indicates blue.