EFFECT OF ULTRAVIOLET-A AND ULTRAVIOLET-C LIGHT ON THE CONCENTRATION OF VITAMIN D_2 AND MECHANICAL PROPERTIES OF OYSTER MUSHROOMS DURING GROWTH

TINDIBALE EDWARD

A Thesis Submitted to the Graduate School in Partial Fulfilment for the Requirements of the Award of Master of Science Degree in Physics of Egerton University

EGERTON UNIVERSITY

JULY, 2015

DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted in part or whole for an award in any institution.

Signed: ……. Date: …….

Tindibale Edward

SM13/3075/11

RECOMMENDATION

This thesis has been submitted for examination with our approval as supervisors for examination according to Egerton University regulations.

Signed: ……. Date: ……..

Dr. M. S. K. Kirui

Department: Physics

Egerton University

Signed: ……. Date: ……..

Prof. J. O. Omolo

Department: Chemistry

Egerton University

COPYRIGHT

DEDICATION

This thesis is dedicated to the most important people in my life: my father Mr. Benson Lukorito, my mother Mrs. Carolyne Lukorito and my siblings.

ACKNOWLEDGEMENT

I thank the Almighty God for the care and protection He gave me throughout the research period. My sincere gratitude goes to Egerton University for having granted me a chance to pursue my postgraduate studies. I am really thankful and grateful to my supervisors Dr. M. S. K. Kirui and Professor J. O. Omolo for giving me excellent guidance and encouragement. Their enthusiastic attitude, knowledge, and commitment for the advancement of science in the field of material science, drove me to explore innovative knowledge in this field. Their intellectual coherence facilitated completion of this work. I really appreciate their inspiring discussions and critical reviews made, in writing of this thesis. My sincere gratitude goes to Eng. P. M. Odhiambo and Mr. R. G. Ngumbu in Physics Laboratory, Egerton University for excellent technical assistance given to me during the laboratory experiments. I wish to express my thanks to Prof. N. I. Wagara and Mr. N. Muchiri for support towards mushroom growth. I owe my heartfelt gratitude to my father (Mr. Benson Lukorito) and mother (Mrs. Lukorito) for a rousing my scientific curiosity during childhood, and their endless support and encouragement given to me throughout my life. I am indebted to them for life and will never be able to compensate. I wish to thank my colleagues for their support and friendship that made me remain focused during my studies.

ABSTRACT

The sun emits ultraviolet radiation in the form of ultraviolet-A (UV-A), ultraviolet-B (UV-B), and ultraviolet-C (UV-C) bands. Ultraviolet light has the potential to boost vitamin D_2 production in mushrooms which are the only non-animal food source of vitamin D for humans. The ergosterol in mushrooms, a component of fungal cell membranes which serves the same function as cholesterol in animal cells, can be converted into vitamin D_2 by exposure to controlled UV light. However, mushrooms grow in dark locations that receive inadequate sunlight to facilitate conversion of ergosterol to vitamin D_2. The mechanical properties of the mushrooms produced after irradiation during growth is a very important factor to consider to meet consumers' needs. This study investigated the effects of UV-A and UV-C light on both the concentration of vitamin D_2 in oyster mushrooms during growth and the mechanical properties oyster mushrooms. Experiments were carried out with exposure of the mushrooms with UV-A (365nm) and UV-C (254nm) light during growth. The exposure time ranged from 10 minutes to 60 minutes at intervals of 10 minutes and irradiation was done for three days. Vitamin D_2 concentration for UV-A and UV-C band was determined. The absorbance of vitamin D_2 for UV-A light was 0.18 - 0.49 for 10 - 60 minutes of irradiation while for UV-C light the vitamin D_2 content was 0.38 - 0.81 for 10 – 60 minutes of irradiation. On the other hand, the mechanical properties were investigated. The average storage modulus for UV-A and UV-C ranged from 7.614MPa and 6.850MPa for 10 minutes to 5.269MPa and 5.046MPa for 60 minutes exposure time respectively. The average loss modulus for UV-A and UV-C ranged from 1.779MPa to 0.5792MPa for 10 minutes exposure to 2.449MPa to 2.065MPa for 60 minutes exposure time respectively and the average loss factor for UV-A and UV-C ranged from 0.2771MPa and 0.1157MPa for 10 minutes to 0.3893MPa and 0.4450MPa for 60 minutes exposure time respectively. There was a significant difference between the storage modulus, loss modulus and loss factor of the irradiated samples by both UV bands with reference to the control sample, $p < 0.05$. UV-C light irradiated samples had higher loss modulus and loss factor but low storage modulus as temperature increased from 35 - 100^0C with respect to the control sample while UV-A light irradiated samples had lower loss modulus, low loss factor and higher storage modulus than UV-C irradiated samples. This study showed that oyster mushrooms with a well-defined content of vitamin D_2 can be obtained with mechanical properties that do not affect the quality of the mushrooms.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

CA	Controlled atmosphere
CHF	Congestive Heart Failure
DMA	Dynamic Mechanical Analyzer
DNA	Deoxyribonucleic Acid
DM	Dry matter
DV	Dry Value
IU	International Units
LVDT	Linear Variable Differential Transformer
MF	Magnetic Field
PUV	Pulsed Ultraviolet
TPA	Texture Profile Analysis
TA	Thermal Analysis
UV	Ultraviolet
UV-A	Ultraviolet –A
UV-B	Ultraviolet –B
UV-C	Ultraviolet -C

CHAPTER ONE

INTRODUCTION

1.1 Background information

Ultraviolet (UV) light is electromagnetic radiation with a wavelength shorter than that of visible light, but longer than X-rays, in the range 100 nm to 400 nm, (Hockberger, 2002). UV light is measured as wavelengths of light in units of nanometers (nm) and its intensity is measured in microwatts/centimeter square ($\mu W/cm^2$). Ultraviolet light below 290nm is blocked by the ozone layer, and would cause much damage to living organisms if it penetrated the atmosphere, (Betsy *et al*., 1997). What remains of ultraviolet in sunlight after atmospheric filtering is responsible for the formation of vitamin D peak production occurring between 295 and 297 nm in all organisms that make this vitamin including humans. Figure 1 shows ultraviolet radiation in electromagnetic spectrum (Bintsis, *et al*., 2000)

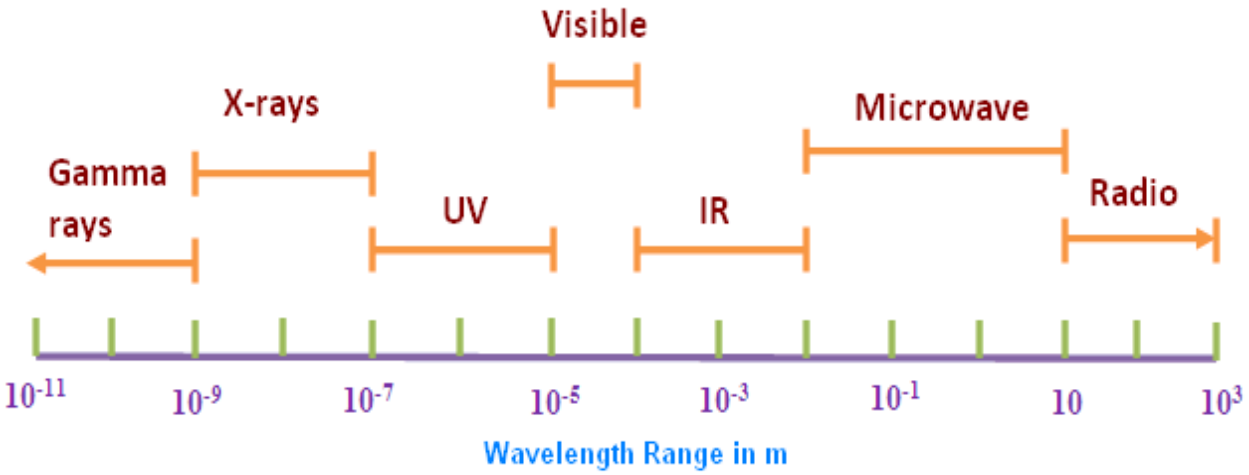


Figure 1: Ultraviolet radiation in electromagnetic spectrum.

The invisible ultraviolet spectrum makes up one specific portion of sunlight. This unique portion accounts for three percent of all solar radiation reaching the earth (Sereana and Wand, 2000). The sun emits ultraviolet radiation in the form of ultraviolet-A (UV-A: 315-400nm), ultraviolet-B (UV-B: 280-315nm), ultraviolet-C (UV-C: 200-280nm) bands and vacuum UV: 100-200nm (Meulemans, 1986). The UV-B light band provides the light that converts a precursor into Vitamin D_3 in the skin (Alexander *et al*., 2006). Over exposure to UV-B radiation leads to direct deoxyribonucleic acid (DNA) damage and sunburn (Matsumu and Ananthaswamy, 2004).

Vitamin D is a fat soluble vitamin required by the body to regulate calcium and phosphorus in the human body and in mineralization of bones (Chung *et al*., 2009). Furthermore, it is clear that receptors for vitamin D are present in a wide variety of cells, and meaning this vitamin has biological effects that extend far beyond control of mineral metabolism (Ovesen *et al*., 2003). Vitamin D consists of two different compounds, vitamin D_2 from ergosterol and vitamin D_3 from animal products or the action of sunlight on a cholesterol-like precursor, 7-dehydrocholesterol, which is in the skin (Lips, 2006). Ingested vitamin D_2 and endogenously produced D_3 are converted to the biologically active form, 1, 25-dihydroxyvitamin D (1, 25$(OH)_2$D) (calcitriol) in the human body (Lorraine *et al*., 2011).

Daily "doses" of sunshine can improve Vitamin D intake and hence this vitamin has been referred to as the sunshine vitamin. Light hitting the skin from the sun's rays stimulates the production of this vitamin and hormone. But lifestyles in modern times have led to limited exposure to the sun and for this reason, it is extremely important to have a diet high in vitamin D_2 or take a vitamin D_2 supplement. Decreased exposure to sunlight (Westerdahl *et al*., 2000) has reduced the synthesis of vitamin D_2 attributable to exposure to UV radiation. Therefore, vitamin D synthesis in the skin is not very reliable. This is a fact that is even more pronounced in the elderly (Kim and Moon, 2000). Studies have also shown that some wild mushrooms have naturally occurring levels of vitamin D_2 in the range of 2.91-58.7 μg/100g fresh weight (Teichmann *et al*., 2007). Vitamin D_2 content of mushrooms can also be enhanced through the use of UV light irradiation (Jasinghe *et al*., 2007; Ko *et al*., 2008).

Vitamin D deficiency is an ever increasing problem in human nutrition and health. Research has suggested that it affects much more than the classic diseases of rickets in children and osteomalacia in adults resulting from inadequate bone mineralization (NIH, 2008). Vitamin D deficiency has been linked to diseases such as cardiovascular disease (Wang *et al*., 2008) and cancer (Lappe *et al*., 2007). Other diseases with links to vitamin D deficiency include hypertension, stroke, diabetes, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, periodontal disease, mental illness, propensity to fall and chronic pain (Cannell *et al*., 2008).

Mushrooms have been harvested for thousands of years for food and medicines (Roupas, *et al*., 2012). Of the estimated 1.5 million species of fungi, about 10,000 produce the fruiting bodies which are referred to as mushrooms. While commercial harvesting of wild mushrooms continues

today, most of the world's supply comes from commercial mushroom growers. The Chinese first cultivated shiitake (*Lentinus edodes*) mushrooms around 1100 AD, with domestication efforts beginning centuries earlier. White button mushrooms (*Agaricus species*), most familiar to Americans and Europeans, were first domesticated in France in 1650 (Royse, 1997).

Commercial production began in the United States in the 1880s. *Agaricus* is the leading mushroom crop worldwide. Oyster mushrooms (*Pleurotus species*) were more recently domesticated, and now rank second in world production. Shiitake mushrooms, which are very popular in Asian cultures, rank third. Many other edible mushrooms, such as straw and wood ear mushrooms, are gaining in popularity. Roughly 300 mushroom species are edible, but only 30 have been domesticated and 10 are grown commercially which include: *Lentinula edodes* (Shiitake), *Agaricus bisporus* (White button), *Pleurotus species* (Oyster), *Auricularia species* (Wood ear), *Volvariella volvacea* (Straw), *Flammulina velutipes* (Enokitake) *Tremella fuciformis* (jelly fungus), *Hypsizygus marmoreus* (Bunashimeji), *Pholiota nameko* and *Grifola frondosa* (Maitaki) (Chang, 1996).

The cultivation of oyster mushrooms has spread out rapidly worldwide, as a natural result of their remarkable biological features and the versatile technology available. In general, two main trends can be identified, first with respect to private commercial enterprises operating primarily on a large or small scale for accumulation of capital, and involving intensive cultivation tending to be highly mechanized and secondly rural production for satisfying regional needs, which is performed through rustic cultivation methods. Developing countries have benefitted by the latter trend, in terms of its local contribution to food production, rural development, and sustainable agriculture. The protein content of oyster mushrooms can be considered as their main nutritional attribute. Average values in the range of 10.5-30.4%, on a dry weight basis, have been reported (Martinez, 1998).

People are often reliant on dietary intake of vitamin D to satisfy their requirements, however foods naturally rich in vitamin D are few in number and in many cases not widely consumed. However, even in those foods considered the richest sources of vitamin D, the levels are highly variable. For example, the content of vitamin D in fish can vary significantly both between and within species and according to whether they are wild or farmed; the vitamin D content of farmed salmon has been shown to be only approximately 25% that of wild salmon (Lu *et al*., 2007).

The number and variety of vitamin D fortified foods available on the market differs significantly between countries but most commonly includes milk, breakfast cereals and margarines (Lehtonen *et al*., 2008). Furthermore, the small number of foods available is likely to have limited impact on population dietary intake. For example, certain population groups are low consumers of milk, one of the more commonly fortified foods (Park *et al*., 2001). Also consumption of large quantities of milk may lead to increased need for vitamin D as milk may block vitamin D receptors due to the bovine albumin protein which is a molecular mimic of vitamin D. Immune reaction against the milk protein, may result in an autoimmune reaction against the vitamin D receptor, making it difficult for the vitamin D hormone to bind (Parez Maceda *et al*., 1991).

Studies have shown browning of white button mushrooms with UV treatment (Koyyalamudi *et al*., 2009; Teichmann *et al*., 2007). UV-B treatment has been done on harvested mushrooms while still fresh. The postharvest treatment increased the levels of vitamin D_2 in the mushrooms from 2.77 μg/g to 36.7 μg/g, 68.6 μg/g, and 106.4 μg/g for pileus, middle, and gill parts, respectively (Ko *et al*., 2008). This study focused on oyster mushrooms as a source of vitamin D_2 as a result of UV-A and UV-C light irradiation while putting in consideration the shelf life of this mushrooms rich in vitamin D_2 by studying their mechanical properties with respect to UV-A and UV-C light irradiation.

There are a limited number of natural dietary sources of vitamin D leading to a real need for alternatives to improve dietary intake (De Borst, *et al*., 2011). Enhancement of foods with vitamin D is a possible mode for ensuring increased consumption and thus improved vitamin D status (Lorraine *et al*., 2011). Mushrooms are the only non-animal-based food containing vitamin D_2 and ergesterol hence are the only natural vitamin D_2 sources for vegetarians (Mattila *et al*., 2000). Among them, the two most popular mushrooms, among consumers, in the world are the shiitake mushroom (*Lentinus edodes*) and the button mushroom (*Agaricus bisporus*). Mushroom quality is of great significance when it comes to its consumption and shelf life (Gonzalez *et al*., 2000). Factors contributing to quality include stiffness and texture. Since not all harvested mushrooms can be consumed at once, they need to be of good quality so that they can be stored for later consumption.

1.2 Statement of the problem

In modern times, some people spend more time in houses, offices, cars and shopping malls hence have limited exposure to sunlight which is a rich source of vitamin D. Foods rich in vitamin D are scarce and in many cases not widely consumed. This has led to low or lack of dietary intake of vitamin D. The richest sources of vitamin D include fish liver oils, oily fish, egg yolk, and wild mushrooms. Reliance on fish, milk and eggs is likely to have limited impact on the population dietary intake, since certain population groups are low consumers of milk and some of these foods are expensive and scarce hence novel methods for producing a wider variety of vitamin D rich foods are needed.

Mushrooms are rich in vitamin D_2 if exposed to ultraviolet light. There's low exposure of cultivated mushrooms to ultraviolet light since they are unable to convert sunlight into energy for photosynthesis. This limits conversion of ergosterol in mushrooms into vitamin D_2 (ergocalciferol). Mushroom tissue comprehensive stiffness is an important factor contributing to mushrooms quality. The short shelf life of mushrooms is an obstacle to the distribution and marketing of the fresh product. Thus, need for mushrooms rich in D_2 and prolonging post-harvest storage is a challenge.

1.3 Objectives

1.3.1 General objective

To investigate the effect of controlled UV-A and UV-C light on both the concentration of vitamin D_2 and mechanical properties of oyster mushrooms during growth.

1.3.2 Specific objectives

1. To determine the change in concentration of vitamin D_2 in oyster mushrooms that have been exposed and those that have not been exposed to UV-A and UV-C light during growth.
2. To determine the impact of exposure time of UV-A and UV-C light on the concentration of vitamin D_2 in oyster mushrooms during growth.
3. To determine the change in storage modulus, loss modulus and loss factor of oyster mushroom as a result of exposing them to UV-A and UV-C light at various time intervals and those that have not been exposed to UV-A and UV-C light during growth.

1.4 Hypotheses (H_0)

1. There is no significant difference in the levels of vitamin D_2 concentration in oyster mushrooms that have been exposed to UV-A and UV-C light.
2. Different exposure time of UV-A and UV-C light on oyster mushrooms during growth has no significant impact on the concentration of vitamin D_2.
3. There is significant no difference in modulus of elasticity in oyster mushrooms that have been exposed to UV-A and UV-C light during growth and those that have not been exposed

1.5 Justification of the study

Vitamin D deficiency is becoming a challenge that needs to be addressed. This study aims at addressing this problem by production of edible mushrooms that are rich in vitamin D_2. A lot of expenses are incurred in buying foods that have been enriched or fortified by vitamin D. Similarly some of the available vitamin D supplements are not enough for the population dietary needs and part of the population does not consume them. Mushrooms on the other hand are cheap and not scarce compared to fish liver oils and oily fish which are good sources of vitamin D. Enriching food supplements with vitamin D_2 is more expensive and time consuming compared to irradiation of mushrooms with UV-A and UV-C light during growth. Similarly more expertise are needed in vitamin D food fortification process compared to those who are needed in enhancement of vitamin D_2 in mushrooms.

CHAPTER TWO

LITERATURE REVIEW

2.1 Vitamin D and its significance

Vitamin D was discovered in 1920, culminating the long search for a way to cure rickets, a painful childhood bone disease (Raphael, *et al*., 2013). Within a decade, the fortification of foods with vitamin D was under way, but solving the problem of rickets was only the beginning of research into vitamin D. Results suggest that vitamin D may have an important role in many aspects of human health, from bone fractures to prostate cancer, cardiovascular disease, neuromuscular problems, and diabetes. Scientists have defined vitamins as organic (carbon containing) chemicals that must be obtained from dietary sources because they are not produced by the body's tissues. Vitamins play a crucial role in the body's metabolism, but only tiny amounts are needed to play that role. Vitamin D breaks the other rules for vitamins because it's produced in the human body, it's absent from all natural foods except fish and egg yolks, and even when it's obtained from foods, it must be transformed by the body before it can do any good (Beard, *et al*., 2011). Vitamin D deficiencies were rare when most men rolled up their sleeves to work in sunny fields, but as work shifted from farms to offices that changed. A number of factors can play a role, such as limited exposure to sunlight especially for the older adults, those in residential care or household and for people who wear concealing cloths for religious or cultural purpose. The same is true for people with very dark skin since melanin in dark skin affects UV penetration hence need for more UV exposure to make vitamin D (Jablonski and Chaplin, 2002). Similarly vitamin D deficiency is common among people hospitalized for a long time, people with a disability or chronic diseases, night shift and in-door workers such as factory workers who have limited incidental UV exposure throughout the day and breast fed babies or mother with low vitamin D.

2.1.1 Vitamin D

Vitamin D which refers to several forms of this vitamin (either vitamin D_2 or D_3) is one of the least understood and most underutilized therapeutic interventions available. Despite overwhelming research concerning the use of vitamin D, it continues to be largely neglected as a powerful clinical tool. Vitamin D_2 (ergocalciferol) is the synthetic form of vitamin D that can be formed from the plant steroid called ergosterol by UV irradiation. Vitamin D_3 (cholecalciferol) is obtained from animal products or the action of sunlight on a cholesterol-like precursor, 7-dehydrocholesterol, that is in the skin (Holick *et al*., 2007).

Vitamin D_3 (cholecalciferol) is required by the body to properly absorb calcium via the intestine. Vitamin D_3 is not the final active form in the Vitamin D series, but it is a key step (Melissa**,** 2002). UV-B doesn't directly 'make' Vitamin D_3 instead it converts 7-dehydrocholesterol to previtamin D_3 as shown in figure 1, which is then thermally isomerized to Vitamin D_3, (Holick, 2004). Vitamin D_2 (ergocalciferol) is obtained from plant sources. Vitamin D_2 and D_3 follow the same pathway for activation. After being created in the skin or consumed in the diet, the liver hydroxylates at the 25^{th} carbon to 25-hydroxyvitamin D (25(OH) D). The kidney as well as other tissue, converts vitamin D_2 and D_3 to its most active metabolite 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), also known as calcitriol (Holick *et al*., 2007).

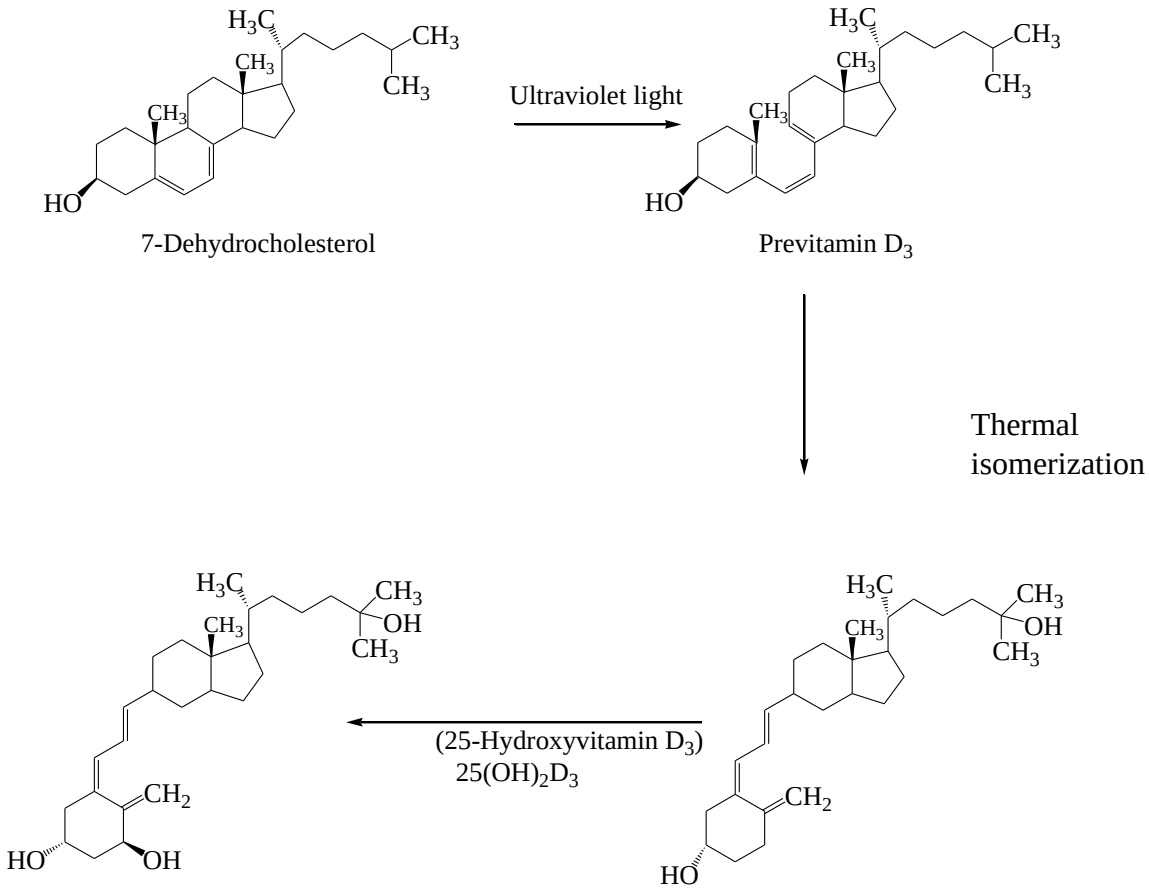


Figure 2: Conversion of 7-Dehyrocholesterol to vitamin D3 in the skin in the presence of ultraviolet light.

Vitamin D_2 and D_3 can be further classified into vitamin D_4 (22,23 dihydroergocalciferol); vitamin D_5 (sitosterol or 24-ethylcholecalciferol) and vitamin D_6 (stigmasterol) according to their side

chain structures (Napoli *et al*., 1979). Vitamins D_2 and D_3 have very similar structures except that vitamin D_2 has one more double bond and a methyl group compared with vitamin D_3. Vitamin D along with the vitamins A, E and K is categorized into the group of "fat soluble" vitamins. Overdosing of vitamin D is potentially toxic in view of its hypercalcemic effect (Adams and Lee, 1997). However there are no reported cases of vitamin D overdose (Marriott, 1997), on the contrary, there are concerns about the inadequate dietary intake of this vitamin.

2.1.2 Significance of vitamin D

Vitamin D reduces mortality, (Zittermann *et al*., 2009) thus low blood levels of vitamin D are associated with increased mortality. However, both an excess and a deficiency in vitamin D appear to cause abnormal functioning and premature aging (Tuohimaa, 2009). Vitamin D deficiency causes osteomalacia, a bone-thinning disorder that occurs exclusively in adults, and characterized by proximal muscle weakness and bone fragility (Cranney *et al*., 2007). Moderate to high doses may reduce cardiovascular disease risk but are of questionable clinical significance, (Wang *et al*., 2010). Low levels of vitamin D are associated with multiple sclerosis, tuberculosis and cancers however taking supplements does not appear to help people with prostate cancer (Ascherio *et al*., 2010; Buttigliero *et al*., 2011; Nnoaham and Clarke, 2008).

Vitamin D has been reported to have effects on immune function (Beard *et al*., 2011). It has been postulated to play a role in influenza with lack of vitamin D synthesis during the cold seasons as one explanation for high rates of influenza infection during cold seasons (Cannell *et al*., 2006). For viral infections, other implicated factors include low relative humidity produced by indoor heating and cold temperatures that favour virus spread (Bruce *et al*., 2010).Vitamin D further influences maturation, differentiation and migration of antigen present in cells (May *et al*., 2004).

There are a number of malignancies associated with insufficient solar UV-B radiation and suggestions that these could be reduced significantly by increased UV-B exposure or supplementary vitamin D consumption (Grant, 2002). Vitamin D deficiency has been shown to be associated with several types of cancers such as breast (Berube *et al*. 2004), prostrate (Kamradt *et al*., 2003), and colon cancers (Tangpricha *et al*., 2001). It is now well established that apart from having an important role in calcium homeostasis and skeleton maintenance, the active analogs of vitamin D act as growth regulators on hyperproliferative cells including cancer cells.

Congestive heart failure (CHF) has been found to correlate with serum vitamin D concentrations (Zittermann *et al*. 2009), and therefore it has been suggested that vitamin D deficiency may be a contributing factor in the pathogenesis of CHF in adults. In addition, vitamin D deficiency has been found to contribute to the heart failure in infants (Carlton-Conway *et al*. 2004). Moreover, there are a number of observations of cardiovascular diseases, which are associated with vitamin D insufficiency that have been reported in the literature (Norman *et al*. 2002). All these findings suggest that vitamin D plays a favourable role in the prevention of heart diseases.

Obesity is a global public health crisis (WHO, 1997), and it is recognized as a major public health problem of global significance (Gill *et al*. 1999). There is evidence reported in the literature that vitamin D deficiency has been linked with obesity (Kamycheva *et al*., 2002). In addition, people who are obese are likely to be deficient in vitamin D because of decreased bioavailability of vitamin D due to its deposition in the adipose tissue (Wortsman *et al*., 2000).

In addition to the above described major chronic diseases, vitamin D deficiency has been found to be associated with arthritis (McAlindon and Felson, 1996), hypertension (Pfeifer *et al*. 2001) psoriasis (Kira *et al*., 2003). Moreover, vitamin D has been suggested for therapeutic applications in the treatment of several diseases including hyper-proliferative diseases, secondary hyperparathyroidism, post-transplant survival, and various malignancies (Mehta and Mehta, 2002).

2.1.3 Sunlight as a source of vitamin D

Human exposure to solar ultraviolet radiation has important public health implications. Evidence of harm associated with overexposure to UV has been demonstrated in many studies. Skin cancer and malignant melanoma are among the most severe health effects, but a series of other health effects have been identified. Reports in the literature provides a quantification of the global disease burden associated with UV (MacKie, 2000). The information presented forms a knowledge base for the prevention of adverse effects of UV exposure that is achievable with known and accessible interventions. UV prevention focuses on protecting the skin and other organs from UV radiation. On the other hand, a moderate degree of UV exposure is necessary for the production of vitamin D which is essential for bone health. Additionally, evidence emerges that low vitamin D levels are likely to be associated with other chronic diseases. Thus, public health

policy on ultraviolet radiation needs to aim at preventing the disease burden associated both with excessive and with insufficient UV exposure.

Living organisms on earth have evolved over millions of years as the planet and its atmosphere have changed. Selection pressures related to ultraviolet radiation have likely been instrumental in the development of different skin pigmentation in humans, as they have migrated from areas of high ambient ultraviolet radiation to areas of lower ambient ultraviolet radiation, (Jablonski, and Chaplin, 2002). The contrasting requirements of protection from excessive ultraviolet radiation and receiving sufficient sunlight to promote the production of vitamin D by the skin have meant that those inhabiting low latitudes, with high ultraviolet radiation intensity, have darker skin pigmentation for protection from the deleterious effects of ultraviolet radiation, while those in higher latitudes have developed fair skin to maximize vitamin D production from much lower ambient ultraviolet radiation.

There has been more rapid human migration out of the areas in which man evolved, to all other parts of the world. No longer is our skin pigmentation necessarily suited to the environment in which we live. While dark-skinned populations at low latitudes have very low levels of melanoma and cancers of the skin, migration of these people to areas of high latitude has seen an increase in the incidence of rickets and osteomalacia (Shaw, and Pal, 2002). Fair skinned populations who have migrated to low latitudes have experienced a rapid rise in the incidence of melanoma and non-melanoma skin cancers. In addition, behavioural and cultural changes in the twentieth century have meant that many of people are now exposed to more, or less, ultraviolet radiation than ever before.

Meanwhile, industrialized society has produced chlorofluorocarbons (CFCs) that react chemically with the stratospheric ozone that has shielded earth from most of the harmful wavelengths of ultraviolet radiation (Ha-Duong, *et al*., 2003). The resulting loss of stratospheric ozone has been associated with increasing levels of some types of ultraviolet radiation reaching the earth's surface. It is difficult to assess changes in ultraviolet radiation due to stratospheric ozone depletion, using ground based measurements, due to ultraviolet radiation changes associated with fluctuations in cloud cover and increase in lower atmospheric pollution. Increases in ambient ultraviolet radiation will be associated with increased adverse health effects due to excessive ultraviolet radiation exposure in the absence of behavioural changes and efforts at sun protection. The net health gain or loss from higher levels of ambient ultraviolet radiation will thus depend on

the interaction of increased ambient ultraviolet radiation levels, skin pigmentation of those exposed and behavioural changes influencing personal exposure.

Almost everyone has some exposure to ultraviolet radiation on a daily basis. It is an exposure that cannot be entirely avoided and, anyway, to strive for zero exposure would create a huge burden of skeletal disease from vitamin D deficiency. However, evaluation of the burden of disease created by excess exposure to ultraviolet radiation is very important since avoidance of excess exposure is a relatively simple public health message. The effect of solar radiation on human health depends on the amount and type of radiation impinging on the body. This in turn depends on, firstly, the concentration of atmospheric ozone that is available to absorb ultraviolet radiation, particularly UV-B. Next, the amount and spectral structure of radiation reaching the body is dependent on the angle at which the sun's rays pass through the atmosphere at low latitudes (closer to the equator) there is more intense solar ultraviolet radiation with a greater proportion of shorter wavelengths, related to the low angle of incidence of the incoming radiation (Sayre, 1998).

Increasing altitude increases ultraviolet radiation intensity by decreasing the air mass through which solar irradiation must pass. Similarly, time of day and season as well as presence of clouds, dust, haze and various organic compounds can alter the intensity of incident solar radiation. Variations in cloud cover usually reduce ground level ultraviolet radiation, although this effect is highly variable, depending on the characteristics of the cloud itself. Indeed, cloud cover can result in increased ground level of ultraviolet radiation if both direct sunlight and light scattered from clouds, reach the earth's surface (Madronich, 1998).

Naturally, humans obtain vitamin D through cutaneous synthesis in the presence of ultraviolet B (UV-B) from sunlight and as well as from the diet. UV-B (UV-B; wave length 290 – 315 nm) represents approximately 1.5 % of the total solar spectrum (Hollosy, 2002). The precursor of vitamin D_3, 7-dehydrocholesterol found in the adipose tissues of the body can be converted to vitamin D_3 in the skin, and this process is supported by sunlight (Feldman *et al*., 1996). The cutaneous production of vitamin D under exposure to sunlight depends on number of factors such as latitude, season, exposure to direct sunlight, skin colour, and age (Engelsen, *et al*., 2005).

Most of the higher energy X-rays, gamma rays, and cosmic rays never make it through the atmosphere due to their absorption by ozone layer, leaving only UV, visible, and infra-red (IR) rays. Ozone absorption even takes care of the highest energy UV radiation, blocking radiation

below 290nm. The portion of the sunlight-spectrum that reaches the earth's surface is limited (Fligge, 2000) as shown in Figure 2 below.

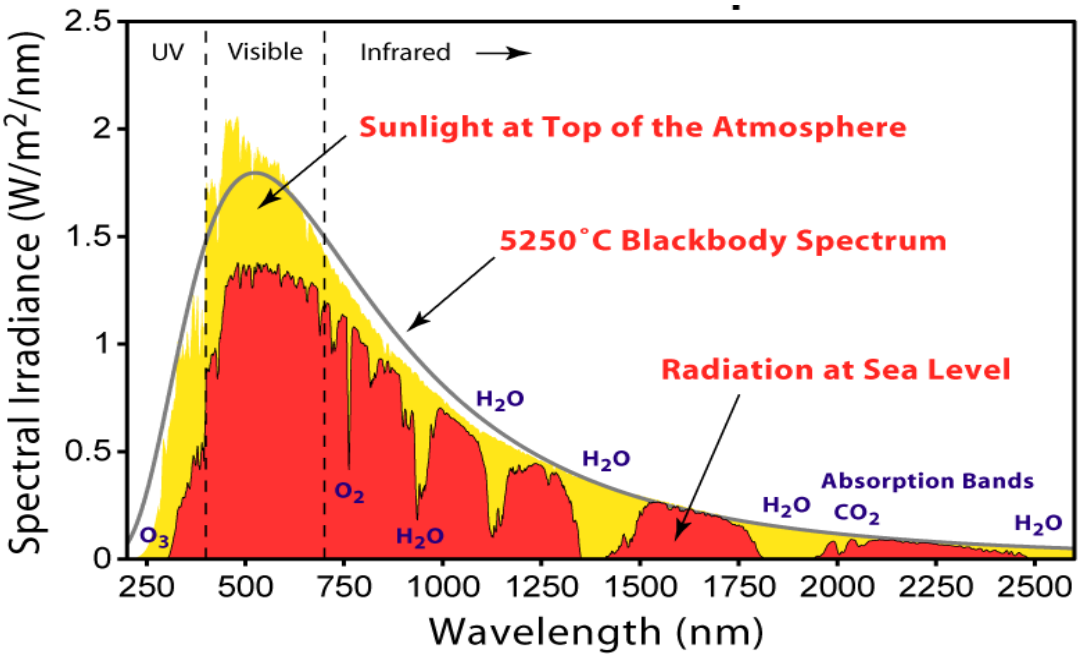


Figure 3: Solar radiation spectrum (Fligge, 2000)

Sunscreens suppress cutaneous vitamin D synthesis (Matsuoka *et al*., 1987). Age-related decline in skin thickness may contribute to the age-related decline in 25(OH) D (Need *et al*., 1993). Environmental factors such as latitude, season, and time of the day influence the cutaneous production of vitamin D (Holick, 1995). The prevalence of vitamin D deficiency is higher in people with darker skins than people with white skins (Shanna *et al*., 2002). Moreover, people with dark skins need to spend up to six times longer in the sun to obtain the same amount of vitamin D as a white person since the increased skin pigment can greatly reduce the penetration of ultraviolet radiation into the skin (Clemens *et al*., 1982). However, "Sun bingeing" may cause skin cancers (Wharton and Bishop, 2003).

In addition, excessive exposure to ultraviolet radiation, produces undesirable inactive by-products of pre-vitamin D, such as tachysterol and lumisterol by photo-isomerization (Havinga, 1973). The evidence reviewed in this section suggest that there are a number of factors involved in cutaneous production of vitamin D under exposure to sunlight, and therefore the adequate exposure is not easily defined. On the other hand, still there are pro and counter arguments on the

risks and benefits of sunlight among the scientific community, which keeps the question unreciprocated.

2.1.4 Dietary sources of Vitamin D

Vitamin D_3 may be obtained in limited amounts from animal food products such as butter, margarine, milk and milk products, liver and other meats, and eggs. Oily fish (including mackerel, sardines, salmon and trout) and fish liver oils provide more substantial amounts of vitamin D but are eaten only by a minority of people. Vitamin D rich dietary sources are tabulated in Table 1 (Holick, 2013).

Table 1: Vitamin D levels in ten different types of foods.

FOOD	**Concentration of vitamin D [IU per100g]**
Sardine	1150-1570
Cod liver Oil	1200
Tuna	250
Mushrooms	60-90
Egg Yolk	25
Mackerel	820-1100
Herring	320-480
Salmon	150-550
Pork	45
Beef	9-42

There are many factors that can influence vitamin D levels and particularly endogenous synthesis. They include skin pigmentation, latitude, avoidance of sun exposure, increased age, weight, low dietary intake of vitamin D rich foods and supplements and malabsorption disorders. Dark pigmented skin needs far more UV-B exposure time to produce the same amount of vitamin D than lightly pigmented skin, because melanin is a highly effective natural sunscreen and interferes with the production of vitamin D (Chen, *et al*., 2007).

At high latitudes there is less available UV-B year-round. In particular, at latitudes above 35°N there is not enough UV-B for cutaneous synthesis for some months of the year, due to the

zenith angle of the sun (Raphael *et al*., 2013). It is important to note that a sunscreen can reduce cutaneous synthesis up to 95% (Holick *et al*., 2007). The skin loses the ability to synthesize vitamin D with age, particularly in adults older than 70 years (Holick, 2004). Higher adiposity has been associated with lower vitamin D levels which may result from adipose tissue being used as a storage site for vitamin D (Blum *et al*., 2008), or may be associated with reduced sun exposure in obese individuals.

The bioavailability of vitamin D_2 from UV treated mushrooms (*A. bisporus*) has been investigated (Stephensen *et al*., 2012). The study was conducted using a group of healthy male (n = 14) and female (n = 24) adults 20–59years of age recruited from the University of California. Subjects participating in the study had high baseline serum 25(OH)D levels which ranged from 60 to 100 nmol/L, concentrations that are consistent with significant sun exposure by the participants. Participants were randomized to one of four groups:(Group1) negative control group administered non-UV treated mushrooms plus a placebo capsule;(Group 2) UVB treated mushrooms containing a target value of 10µg (400 IU) vitamin D2 per serving plus placebo capsule; (Group3) UV-B treated mushrooms containing a target value of 25µg (1000 IU) vitamin D_2 per serving plus placebo capsule;(Group4) non-UV treated mushrooms plus 25µg (1000IU) of vitamin D2 supplement capsules. The authors reported that consumption of UVB-treated mushrooms produced statistically significant increases in serum 25(OH)D_2 concentrations on weeks 3 and 6 relative to that observed in subjects consuming the control non-UV-treated mushroom preparations where vitamin D_2 levels remained low (<6 nmol/l) throughout the study. On week 6, changes in serum 25(OH)D concentrations in Groups 1through 4were 1.22 ±1.65,13.8 ±2.42,12.7 ±1.22, and 32.8 ±1.26nmol/l respectively. The authors concluded that vitamin D_2 from UV-treated mushrooms is absorbed and converted to 25(OH)D_2 with an efficiency similar to that seen for vitamin D2 from supplements. In a single blind, placebo controlled, parallel group study (Urbain *et al*., 2011), the bioavailability of vitamin D2 from UVB treated button mushrooms was evaluated in healthy adults deficient in vitamin D. Twenty-six healthy male (n = 9) and female (n = 17) Caucasian subjects (aged<45years and BMI of18.5– 26 kg/m^2) were recruited from the employees of the University Medical Center Freiburg (Germany) for participation in the study. All subjects included in the study displayed low (650nmol/l) serum 25(OH)D levels and normal (2.2-2.7mmol/l) serum calcium levels. Subjects were not permitted to frequent tanning salons, and were instructed not to consume vitamin D supplements or fish oil capsules, and consumption of fish was

limited to no more than once per week. Subjects recruited to the study were randomized to one of three treatment groups: (1) vitamin D mushroom group (n = 8), vitamin D supplement group (n = 9), or placebo control group (n = 9). For production of the vitamin D mushrooms, whole button mushrooms (*A. bisporus*) were treated with UVB light at a wavelength of 304nm for 25min at ambient temperature (21^0C) (1.5J/cm 2) resulting in vitamin D concentrations of 491µg/100 g (19,460IU/100 g) fresh mushrooms. Subjects randomized to the vitamin D mushroom group consumed an experimental soup containing 28,000 IU of vitamin D_2 once weekly (daily equivalent of4000 IU)provided by inclusion of vitamin D mushrooms in the soup. A placebo orange juice also was consumed by these individuals. The vitamin D supplement group consumed a standard mushroom soup and were administered 28,000 IU of vitamin D_2 provided by the addition of vitamin D_2 supplement drops to orange juice. Subjects in the placebo group consumed 60IUofvitamin D_2 from a conventional mushroom soup and a control orange juice. The authors reported that the hypercalcemia (serum calcium >2.7mmol/l) was the main safety endpoint and that serum calcium remained within the reference range at all-time points. The authors also stated that ‘‘No physical symptoms were reported during the study.’’ The authors concluded that the ‘‘bioavailability of vitamin D_2 from vitamin D_2-enhanced button mushrooms via UV-B irradiation was effective in improving vitamin D status in young healthy adults.’’ The authors also concluded that the capacity of vitamin D2 from UV-B treated vitamin D mushrooms to increase circulating levels of 25(OH)D was equivalent to that observed in subjects consuming the vitamin D_2 supplemented orange juice. The bioavailability of vitamin D_2 from the consumption of wild Chanterelle mushrooms (*Cantharellus tubaeformis*) has been reported (Outila *et al*., 1999). Bioavailability was evaluated by monitoring changes in serum 25(OH)D using a competitive protein binding assay at weeks 1.5 and 3 of treatment. The authors reported that individuals randomized to the vitamin D_2 treatment groups displayed time dependent increases in serum 25(OH)D levels relative to levels observed in subjects consuming the control diets. No differences in serum intact parathyroid hormone or urinary calcium were noted among the groups at week 3.Strontium absorption, which is an indicator or predictor of calcium absorption, also was not different between the groups at week 3. However, strontium levels were significantly reduced relative to baseline in the subjects not consuming vitamin D, an effect not observed in the supplemental groups. Finally, a case report also was identified in the literature in which the diagnosis of vitamin D deficiency in a vegetarian patient was self-treated by the individual through

the exclusive consumption of fresh mushrooms exposed to UV light (Ozzard *et al*.,2008). The vitamin D mushrooms were produced by the individual who purchased a UV-B bulb from a local hardware store and exposed 200 g of button mushrooms (*A. bisporus*) daily from a distance of approximately 15 cm. Mushrooms were stir-fried before consumption, and consumed on a daily basis for 3 months. Biochemical values obtained at baseline and after 3months of mushroom consumption demonstrated normalization of vitamin D status in the individual however mushrooms used in the bioavailability studies had been exposed to UV-B light during postharvest.

2.2 Ergosterol in mushrooms and its conversion to vitamin D_2

The history of research on ergosterol in fungi starts from the 1970's and a number of studies have been carried out in this field. Ergosterol content in cultivated mushrooms varies according to the composition of the cultivation media (Trigos, 1997). Furthermore, ergosterol content also varied among the different mushroom species (Mau *et al*., 1998). Ergosterol is the most abundant sterol found in mushrooms (Mattila *et al*., 2002), and its content has been shown to be higher in cultivated mushrooms (6.02 – 6.79 mg/g dry matter) than in wild mushrooms (2.96 – 4.89 mg/g dry matter). The effect of light source of UV has been found to be an important factor on the conversion of ergosterol under UV irradiation (Jasinghe and Perera, 2006).

Conversion of ergosterol in mushrooms to vitamin D_2 under UV irradiation was observed Mau *et al*., (1998) and it was reported that the conversion was higher under UV-B compared with UV-C. It was also reported that vitamin D_2 in *A. bisporus* (fresh mushrooms) and *A. bitorquis* (high temperature mushrooms) both irradiated with UV-C at 12^{o}C for 2 hours increased from 2.20 and 4.01 µg/g of dry weight to 7.30 and 5.32 µg/g respectively. However, the effect of UV-A, which represents approximately 6.3 % of the incoming solar radiation (Hollosy, 2002), on the conversion of ergosterol in mushrooms to vitamin D_2 during growth has not been reported in the literature. Even though Mau *et al*. (1998) has reported the effect of UV-B and UV-C on the conversion of ergosterol in mushrooms to vitamin D_2, the maximum value of vitamin D_2 obtained was only 12.28 µg/g. In their study, there was no mention of the determination of mechanical properties of mushrooms having been analysed as after UV irradiation. In their study also UV irradiation was during postharvest.

Mushrooms can produce considerable vitamin D_2 levels when exposed to UV-B light through the conversion of natural ergosterol, the production rate depends on the irradiation dose and temperature (Ko, *et al*., 2008). The vitamin D_2 content in these irradiated mushrooms appears

relatively stable when refrigerated (Roberts *et al*., 2008) and the retention of vitamin D_2 in mushrooms following cooking and storage has been reported as high, at 86% and greater (Mattila *et al*., 1999). Even after 6 years of storage, dried mushrooms have appeared to retain much of their vitamin D_2 content (Rangel *et al*., 2002). Shiitake mushroom has been valued as a food that promotes longevity and has attracted public attention as a health food effective in preventing various diseases. As a functional food, shiitake contains all eight essential amino acids as well as a good blend of vitamins and minerals including vitamins A, B, C, and D. Vitamin D_2 has been used successfully to treat rickets and other diseases related to vitamin D deficiency.

Much work has been done on drying of mushrooms of various kinds. (Gothandapani *et al*., 1997 and Martinez-Soto *et al*., 2001) have reported on the influence of drying conditions on the quality of oyster mushrooms. The distribution of ergosterol in different parts of the mushroom vary significantly and the moisture content is critical in terms of conversion of ergosterol to vitamin D. The optimum moisture content for conversion was 70% on a wet weight basis. In a drying operation the moisture content of the mushroom for irradiation is important if the aim is to optimize the conversion of ergosterol to vitamin D. Thus, in a continuous drying operation, for example, the product should be subjected to UV irradiation before the moisture content of the product drops below 70% on a wet weight basis (Perera, *et al*., 2003). To avoid cases of continuous drying operation for the purpose of conversion of ergesterol to vitamin D_2 before moisture drops below 70% on wet weight basis while in this study, investigation on the conversion of ergesterol to vitamin D_2 was carried out during growth.

Previous studies (Koyyalamudi *et al*., 2009) have shown that *A. bisporus* mushrooms contain an abundance of ergosterol, which on exposure to UV irradiation is converted to vitamin D_2. A study (Feeney 2006) showed that while vitamin D_2 could be increased with postharvest exposure of *A. bisporus* (white button mushroom) to UV-C light to a level of over 800% of the daily value (DV) in an 84g serving, the exposure time to achieve such levels was 5 minutes. Another study (Ryan *et al*., 2011) compared the compositional changes in *A. bisporus* mushrooms exposed to sunlight with those occurring after commercial ultraviolet (UV) light processing. Following UV-B exposure, there were no significant changes in vitamin C, folate, vitamins B_6, vitamin B_5, riboflavin, amino acids, fatty acids and ergosterol. Sunlight exposure resulted in a 26% loss of riboflavin, evidence of folate oxidation, and unexplained increases in ergosterol (9.5%). It was concluded that compositional effects of UV-B light are limited to changes in vitamin D and

show no detrimental changes relative to natural sunlight exposure and, therefore, provide important information relevant to the suitability and safety of UV-B light technology for vitamin D enhanced mushrooms.

Vitamin D_2 content in shiitake mushroom by a combination of UV-B irradiation and hot air drying has been reported to increase (Lee *et al*., 2002). A study on pulsed UV light (PUV) (Robert *et al*., 2012) was investigated as a means to rapidly increase vitamin D_2 content in fresh button mushrooms (*A. bisporus*). Vitamin D_2 was found to increase following 3 pulses. Following 12 pulses, D_2 began to approach a maximum concentration of 27 μg/g. The D_2 produced with 3 pulses decreased from 11.9 to 9.05 μg/g after 3 days of storage; however, D_2 levels remained nearly constant after this point throughout an 11-day shelf life study.

Similarly in another study (Ko *et al*., 2008), fresh sliced Shiitake mushrooms and white button mushrooms were exposed to UV-B and the concentration of vitamin D_2 was investigated. After the exposure to UV-B, the concentration of vitamin D_2 was increased from 2.77 μg/g to 36.7, 68.6, and 106.4 μg/g for pileus, middle, and gill parts, respectively. A research on *Portabella* mushrooms which investigated the effects of UV-B treatment of *Portabella* mushrooms at three intensities (0.5, 0.75 and 1.0mW/cm^2), three doses (0.5, 1.0 and 1.5J/cm^2) and two post-harvest times (1 and 4 days) on D_2 formation and degradation during storage (Roberts *et al*., 2008). The concentration of vitamin D_2 of about 10μg was present after four days of storage. Mushrooms are highly valued with respect to their flavour and are reputed to have medicinal value. Ergosterol undergoes photolysis when exposed to UV light to yield a variety of photo-irradiation products, the principal ones being pre-vitamin D_2, tachysterol and lumisterol. The pre-vitamin D_2 undergoes spontaneous thermal rearrangement to vitamin D_2 (Jones *et al*., 1985). Even though the conversion of ergosterol, in mushrooms, to vitamin D_2 is not a new idea there are data on the irradiation of mushrooms in the literature. Mau *et al*., (1998) reported that UV-B was more effective than UV-C in conversion of ergosterol to vitamin D_2 However, only limited information is reported in the literature about vitamin D_2 concentration in oyster mushrooms when exposed to UV-A and UV-C light during growth and thereafter mechanical properties analysed.

2.3 Shelf life of mushrooms

The short shelf life of mushrooms due to postharvest changes, such as browning, cap opening, stipe elongation, cap diameter increase, weight loss and texture changes, to their high respiration rate and lack of physical protection to avoid water loss or microbial attack

(Akram and Kwon, 2010) is an obstacle to the distribution and marketing of the fresh product. The effect of gamma and electron beam irradiation on the physcochemical and nutritional properties of mushrooms has been carried out with the objective of increasing the shelf life of mushrooms (Beaulieu *et al*., 2002). Thus, prolonging postharvest storage while preserving their quality would benefit the mushroom industry as well as consumers. There has been extensive research on finding the most appropriate technology for mushrooms preservation. Gamma, electron-beam and UV irradiation have been shown to be potential tools in extending the postharvest shelf life of fresh mushrooms. Studies evaluating the effects of ionizing radiation are available mainly in cultivated species such as *Agaricus bisporus*, *Lentinus edodes* and *Pleurotus oestreatus*. Mushrooms global economic value is now staggering, and the reason for the rise in consumption is a combination of their value as food (Kalac, 2009) and their medicinal or nutraceutical properties (Ferreira *et al*., 2009; Ferreira *et al*., 2010).

Wild mushrooms are collected for consumption being a good source of digestible proteins, carbohydrates, fibres and vitamins (Kalac, 2009; Heleno *et al*., 2011; Ouzouni *et al*., 2009). Dry matter content is usually about 100g/Kg. Structural polysachrises and proteins comprise the main components of dry matter, while the lipid content is low. Chitin, glycogen, mannitol and trehalose are typical carbohydrates constituents. The proportion of essential amino acids is nutritionally favourable, while the content of n-3 fatty acid is negligible (Kalac, 2009). Furthermore macrofungi have a history of traditional use in oriental therapies and modern clinical practices continue to (for example: phenolic compounds, polyketides, terpenes, steroids and polysachaccharides) with immunomodulatory, cardiovascular, liver protective, antifibrotic, antiinflammatory, antidiabetic, antiviral, antimicrobial activities and antitumor properties (Ferreira *et al*, 2010; Poucheret, *et al*, 2006).

A study on prolonging shelf life of mushrooms by UV irradiation indicated that mushrooms perishability can be reduced by UV irradiation after harvest (Akihiro, 2013). Attempts have been made to develop a technique or method that will extend the shelf life of different varieties of fruits and vegetables (Acedo, 1997) but to date, few or none at all has been done specifically for mushroom. It was reported that irradiation when used alone can cause the development of undesirable sensory and chemical changes in some food items (Thakur and Singh, 1995). However, these changes can be prevented if irradiation is used in combination with other methods, such as heating, cryogenic temperature or vacuum packaging. In literature, it was reported that

irradiation of mulberry leaves with UV reduced the feeding responses of *Bombyx mori* silkworm larvae (Mochida, 1995). An organic extract analysis of UV-irradiated leaves showed to have induced the production of moracins C and N which act as feeding deterrents. Furthermore, it was reported, that UV-B radiation induced a rapid increase in intracellular carotenoids (Goes *et al*., 1994).

Food irradiation may be considered as a second big break through after pasteurization (Kalyani and Manjula, 2013). It was argued that acceptance of a new food technology is not simply related to the characteristics of the process itself, but the needs, beliefs and attitudes of individual food consumers and the nature of economic, political and social environment in which food choices take place (Henson, 1995). Although this innovative technology has already been recognized worldwide, industrial application of this technology is still very limited. Henceforth, in the present undertaking, an attempt was made to utilize this novel technology in farm product. Specifically, an attempt was made to use ultraviolet (UV) radiation irradiation on mushroom in an attempt to extend its shelf life after harvest and in this study the focus is on how the UV radiation affects mechanical properties of the mushroom during growth.

Whereas mushroom growers are faced with a challenge of how to maintain the freshness of the produce before it gets to the market or how to extend the shelf life until it gets to the consumer, apparently there are limited solutions to this challenge reported in the literature. Studies have been conducted relevant to post harvest problems, however, results are varied and diversified. Maybe, this diversity could be attributed to the highly diverse varieties of farm products so that, a method best suited for one product, may not necessarily be effective to other farm harvest.

The mushrooms of the *Pleurotus* genus are more delicate and sensitive than the *Agaricus* genus and they start deteriorating immediately within one day after the harvest. Once deteriorated, these fruiting bodies can cause severe gastrointestinal discomfort. Under ideal climatic conditions, shelf life of these mushrooms is about 10 days, their quality being affected predominantly by storage temperature. The shelf life can be reduced from 9 days at 2 °C to 3 days at 18 °C (Lukasse and Polderdijk, 2003). Therefore, cooling the fresh mushrooms can be an alternative regarding their distribution and sale, thus increasing their shelf life (Villaescusa and Gil, 2003). For long periods of conservation, the traditionally used method for *Pleurotus* genus mushrooms is the convective drying at 45-65 ºC (Arora *et al*., 2003).

Dehydration is a classical method of food conservation, based on the principle that the reduction of the water activity of the product must be conducted until defined levels that guarantee the microbiological and physicochemical stability (Lewicki and Jakubczyk, 2004). The dehydrated mushrooms can be rehydrated by water immersion before the consumption. The rehydration characteristics of dried products are used as a quality parameter and indicate if physical and chemical changes occurred during the drying process due to process conditions, pre-treatments and sample composition. Rehydration capacity of *Agaricus bisporus* fruiting bodies was studied after hot-air dehydration, where in one case microwave was used at the end of the dehydration process and in another case no microwave was used. The power of the applied microwaves was measured through the temperature of the food center. Rehydration capacity was better using hot-air dehydration without the use of microwaves. This work dealt with the evaluation of a conservation process for *Pleurotus ostreatus* fruiting bodies and did not study the mechanical properties of the mushroom and the mushrooms under investigation had not been exposed to ultraviolet light during growth (Funebo and Ohlsson (1998).

Prolonging postharvest storage, while preserving their quality, would benefit the mushroom industry as well as consumers. Extended shelf life is a key factor for making any food commodity more profitable and commercially available for long periods of time at the best possible quality. The producer will benefit from the longer shelf-life to develop the market over greater distances. Gamma irradiation (Beaulieu *et al*., 2002) and electron beam irradiation (Koorapati *et al*., 2004) have been shown to be potential tools in extending the postharvest shelf life of fresh mushrooms. The highly perishable nature of mushrooms remains a problem for the progress of this industry (Gautam *et al*., 1998). In fact, fresh mushrooms can only be stored for a few days until they lose freshness and quality. There are many methods to extend the shelf-life of mushrooms. They include modified atmosphere packaging (MAP) (Roy *et al*., 1995), controlled atmosphere (CA) storage (Lopez-Briones *et al*., 1992), coating (Nussinovitch and Kampf, 1993), refrigeration (Mau *et al*., 1993), cultivating with $CaCl_2$ solution (Miklus and Beelman, 1996) and using sorbitol (Roy *et al*., 1995). Although CA storage is effective to lower respiration rate and increases shelf-life of fruits and vegetables, it is not appropriate for mushrooms, which have extremely high respiration rates (Roy *et al*., 1995). Also, authors found that MAP could have a damaging effect, causing anaerobic respiration as well as potential growth of anaerobic pathogens (Varoquaux *et al*., 1999).

A potentially attractive alternative is exposure to ionizing radiation, and previous papers have suggested this method is highly effective in inhibiting physical changes associated with postharvest deterioration and maintaining a fresh product appearance (Kader, 1986). Food processing by employing radiation is well established as a physical, non-thermal mode of food preservation (cold pasteurization) that processes foods at or nearly at ambient temperature. Irradiation of food products causes minimal modification in the flavour, colour, nutrients, taste, and other quality attributes of food. However, the levels of modification in flavour, colour nutrients and taste might vary depending on the basic raw material used, irradiation dose delivered, and on the type of radiation source employed (gamma, X-ray, UV, electron beam) (Mexis *et al*., 2009).

Gamma radiations are short wavelength, high energy photons, and have deep penetrating power. Gamma rays come from spontaneous disintegration of radioactive nuclides (Cobalt 60 or Cesium 137) as their energy source. During irradiation, the radioactive nuclides are pulled out of storage (water pool) into a chamber with concrete walls that keep any gamma rays from escaping (Yaqvob *et al*., 2013). Gamma irradiation alone and in combination with refrigeration has been shown to prolong shelf life through reducing moisture loss and improving colour and appearance (Ajlouni *et al*., 1993). Doses of *γ*-irradiation inhibited cap opening, stalk elongation and browning and reduced the level of microbial contamination of *Agaricus bisporus*.

Electron-beam irradiation levels above 0.5 kGy reduced total plate counts, yeast and mould, and psychotropic counts to below detectable levels and prevented microbial- induced browning. Although, colour was preserved by irradiation as evidenced by the higher values, they showed that the irradiation at 1 kGy was most effective in extending shelf-life of mushroom slices (Koorapati *et al*., 2004). The onset of fruit body softening, splitting and browning compared with non-irradiated controls and test samples subjected to lower or higher irradiation doses significantly delayed (by 6–9 days) as a result of ^{60}Co γ-irradiation dose of 1.2 kGy (Xiong *et al*., 2009). Irradiation with 1.2 and 1.6 kGy also had a positive effect on other indicators of mushroom tissue senescence, resulting in smaller decreases in soluble protein levels and more protracted increases in proteinase activity.

Furthermore, γ-irradiation extended the storage life (Roy *et al*., 2000) and *Pleurotus pulmonarius* (Xia *et al*., 2005) without adversely affecting key nutritional components. Radiation treatments also delayed cap opening and browning, and lowered the rates of decomposition and

weight loss, in the straw mushroom, *Volvariella volvacea* (Liu *et al*., 2003; Ye *et al*., 2000). Fan (2005) found that irradiation increased the phenolic content and antioxidant capacity of both tissue types of all vegetables at day 4 and day 8. The results suggest that irradiation increased nutritional quality of leafy vegetables, but some adverse visual quality changes were encountered. In addition, it was concluded that irradiation treatments of carrot and kale juice improve the microbiological safety with maintaining or even enhancing the antioxidative activity (Song *et al*., 2006).

The gamma irradiation had effects on whiteness, weight loss, electrolyte leakage, total soluble solid, vitamin D and phenol content. During the storage, decreases of the mushroom whiteness were observed for all mushrooms, control and treated. Whiteness of pileus and stipe is often used as important index of visible quality, since rapid discoloration occurs after harvest. Most of the researchers agree that irradiated mushrooms retain their original skin colour for longer periods or darken less rapidly than unirradiated mushrooms (Thomas, 1988). Weight loss is one of the physiological parameters used as quality indicator in fruits. Weight losses in controls and irradiated samples increased in parallel during the experimental storage period. The weight loss was because of evaporation of water from the fruit surface as a result of respiration and transpiration. Electrolyte leakage rate of mushrooms were lowered as significantly by application of 0.5, 1 and 1.5 kGy compared with control in storage times. Electrolyte leakage is an index of the semipermeable properties of cell membranes, and a reduction in membrane integrity resulting from lipid peroxidation increases membrane leakage and enhances cell senescence (Hildebrand, 1989). Decreased rates of membrane lipid peroxidation and membrane leakage observed following treatment of *V.* V*olvacea* and *L. edodes* fruit bodies with ^{60}Co irradiation (Ye *et al*., 2000) and calcium chloride (Li *et al*., 2000)), respectively, were accompanied by marked prolongation of postharvest mushroom freshness. Fan and Sokorai (2002) found that irradiation increased electrolyte leakage in fresh cut iceberg lettuce.

There was a decrease in the total soluble solid with 1, 1.5 and 2 kGy as well as control. Total soluble solid was earlier reported to be the major respiration substrate in *A. bisporus* during postharvest storage and steady decreases in the soluble solids concentration were previously reported in fruit bodies stored at cold-temperatures (Tseng and Mau, 1999). Researchers showed that irradiation increases phenolic content in vegetables (Fan and Sokorai, 2002), which may in turn influence appearance, flavour and nutritive values and irradiation also significantly enhanced

total phenols content, especially between days 1 and 4, whereas vitamin C was lowered. Gamma radiation causes a notable damage on vitamin C of *A. bisporus* but the changes which occurred during eighth day of storage, irradiated mushrooms were similar to those of the non-irradiated at temperature 4°C. The possible reason for accelerated decrease of ascorbic acid in irradiated and non-irradiated samples might be enhanced respiration resulting in increased enzymatic activity causing rapid degradation of ascorbic acid; irradiation also could not control the loss of ascorbic acid.

2.4 Mechanical properties of mushrooms

The mechanical properties mainly result from the structure, physical state and rheology. These properties are needed for process design, estimating other properties, characterizing foods, and quality determination. Texture is one of the important factors to evaluate quality of mushroom. Undesirably, the stability of texture can be only maintained for a very short period of storage, it is usually changed quickly after harvest (Nichol, 1985). Stiffness, toughness, brittleness and pliability are considerable characteristics during the analysis of fruit body texture. Improving mushroom quality and texture as well have been preceded by several methods. Gormley and MacCanna (1967) assessed the texture by measuring the force required to shred a bulk sample. Gormley (1969) indirectly measured the changes through dry matter content, from the report; the texture may be changed due to the changes of cellular materials and moisture loss. In addition, a record on the softening of fruit body during storage (Szczesniak and Kahn, 1971) was valuable contribution to develop the analysed texture method. Beelman *et al*., (1987) based on the changes of firmness and toughness to analyse the texture of *A. bisporus* fruit bodies.

McGarry and Burton (1994) developed method to measure tissue compressive stiffness where they were able to evaluate the changes of button mushroom texture in different sizes and stages. Another method to analyse the texture properties basing on the changes of tenderness, pliability, toughness and brittleness of post harvested and cooked mushroom has been reported (Kojo *et al*., 2004). Previous researches revealed some relations between textural characteristics and constituents of fruit bodies like hyphae density which was proved to be one of criteria that determined the stiffness in *A. bisporus* (McGarry and Burton, 1994). In further detail, when the hyphae pack more closely, the air space in mushroom fruit body will decrease so textural properties are also affected. Another determined stiffness criterion was cell turgidity which was reflected by water content in mushroom (Nichol, 1985; Beelman *et al*., 1987).

Dynamic mechanical analysis (DMA 983) can be used to gain insight into the factors affecting food quality through simulation of processing conditions. There have been, however, relatively few studies on the dynamic mechanical properties of food, although a comparative DMA study on starches of wheat has been reported (Roulet *et al*., 1988). Experiments using DMA were performed on two food products, commercial white bread and dried pasta. The DMA storage and loss moduli obtained provided valuable information about the softness and water keeping properties of bread, as well as the cooking characteristics of pasta.

A research on the influence of temperature of freeze drying process on the mechanical properties of dried mushroom (Kramkowski *et al*., 2001) indicated that high value of stress in the fresh mushrooms undergoes high reduction in effect of freeze drying process and the freeze dried material preserve approximately constant stress value in a wide strain range. The products preserved by the method feature with relatively low quality deterioration (smell, taste, colour, chemical composition) when compared with the fresh product. Such behaviour follows from the fact that the material is dried at low temperature, under significantly reduced pressure in a drying chamber, assuring sterility of the process (Krokida *et al.,* 2000). The dried product is usually directed to the storage houses, what demands for appropriate durability and mechanical resistance. Therefore, the improvement of freeze drying process is strictly connected with the recognition of the relations between the process parameters and mechanical properties of resulted dried material. However, only limited information is reported in the literature about mechanical properties of mushrooms especially oyster that have been exposed to UV-C and UV-A light during growth.

A study on anisotropy of mechanical properties of mushroom (A. *bisporus)* was carried out by (Jerzy *et al*., 2013). Strength tests, hysteresis tests and creep tests of mushrooms compressed between two parallel plates were carried out however the mushroom sample used had not been exposed to UV-A and UV-C during growth. A study on colour and some physical properties of frozen mushroom (*Agaricus bisporus*) dipped in different antioxidant solutions was carried out by Buket *et al*., (2011). The research exhibited the large effect of antioxidant solutions on the final quality of frozen mushrooms which has short life for fresh consumption. The research did not investigate the mechanical properties and the mushrooms used had not been irradiated by UV light either during growth or after harvest. Mushroom consumption has increased remarkably because of their desirable aroma and taste and high nutritional content (Vizhanyo and Jozsef, 2000).

Colour, fresh and clean appearance and uniform closed buttons also have high importance for mushroom quality and consumer preferences (Gonzalez *et al*., 2000).

Mushrooms are soft textured and highly perishable, beginning to deteriorate shortly after harvest (Walde, 2006). Because of their short shelf life under normal ambient conditions of temperature and humidity, their preservation is of most importance. Dehydration, canning, freezing, among others, have been found to be suitable for their preservation (Bernas, 2006). Dehydration is one of the important preservation methods employed for storage of mushroom and dehydrated mushrooms are valuable ingredients in a variety of sauces and soups. As mushrooms are very sensitive to temperature, choosing the right drying method can be the key for a successful operation (Giri and Prasad , 2007). The preservation of aroma is essential for accessing quality of processed food products, and in particular for the case of mushrooms, which are very much used for culinary preparations because of their unique aroma. Freeze-drying, being a low temperature process, causes less deterioration in the aroma compounds of food products. In this process water is eliminated by sublimation from a frozen state, and the temperature of the product remains very low during the operation (Kompany and Rene, 1995). Texture is the result of complex interactions among food components. This property of fruits and vegetables is affected by traits such as cellular organelles and biochemical constituents, water content, and cell wall composition. Thus, any external factor affecting these traits can modify texture and can, therefore, lead to changes in final product quality.

The changes in texture occurring during the processing of plant materials or certain physiological events have been related to tissue and cell microstructural changes (Marsilio, 2000). In a sensory point of view this property is generally defined as the overall feeling that a food gives in the mouth and is therefore comprised of properties that can be evaluated by touch (Sams, 1999). Raquel and Maria (2011) in their study on influence of freeze-drying treatment on the texture of mushrooms and onions indicated that texture is composed of several textural properties which involve mechanical (hardness, chewiness, and viscosity), geometrical (particle size and shape) and chemical (moisture and fat content) characteristics. The texture parameter, together with appearance and flavour, are the organoleptic quality attributes which determine the acceptability of food by the consumer. Hence, there has been a great interest in the development of methods to predict and control the texture of plant-based foods, particularly in relation to processing treatments. Instrumental texture profile analysis (TPA) is one of the methods to determine the

texture by simulating or imitating the repeated biting or chewing of a food. The textural properties: hardness, springiness, cohesiveness, and chewiness were then determined and adhesiveness was practically zero in the fresh mushrooms and zero in the freeze-dried ones. Hardness decreases very much with this treatment, either in the cap or in the stalk. Chewiness is another textural property that varies quite much with freeze-drying, contrarily to cohesiveness, which practically does not change. Springiness also decreases with drying, although not in a much accentuated way. When the two parts of the mushroom are compared, it is observed that the cap is much harder, almost two times harder, has slightly lower cohesiveness and springiness and a little higher chewiness. However, the mushrooms used were button mushrooms and they had not been exposed to ultraviolet light during growth. In addition, the textural properties studied did not involve viscoelastic properties which were the main focus in this research where oyster mushrooms were used.

Various parameters related to the thermal characteristics of *A. bisporus* mushroom have been investigated (Guizani, *et al*., 2013). The glass transition of *A. bisporus* containing un-freezable water was studied to determine the stability of dry mushroom during its storage, whereas freezing point versus solids content was studied to determine the stability for the frozen *A. bisporus*. It was not possible to trace glass transition in samples containing low water, which indicated the structural complexity of mushroom in terms of thermal behaviour. However, samples containing higher water content exhibited glass transition. Maximal freeze concentrated solids was found as 0.782 g/g sample with the characteristic temperature of end point of freezing being 30^0C. The un-freezable water (reactive water) in mushrooms was observed as 0.218 g/g sample. However these mushrooms used had not been irradiated by UV-C and UV-A light during growth and no investigation were done with respect to modulus of elasticity of the mushrooms and the vitamin D_2 content of the mushrooms as a result of UV irradiation during growth.

Mushrooms are an important source of nutrients and of a variety of secondary metabolites, including various phenolic compounds, known for their excellent antioxidant activities (Mau *et al*., 2002). Various antioxidant compounds are used widely in different food products that help in providing protection to oxidative damages by free-radical molecules (Kumari *et al*., 2011). Along with its consumption as fresh, *A. bisporus* can be preserved in the dried, frozen and freeze dried forms. The major objective in drying is the reduction of moisture content to a certain level depending on the type of food, which allows safe storage and preservation (Seiiedlou *et al.*, 2010).

However, drying methods play an important role in production of the dried vegetables and the bioactive compounds, their antioxidant capacity might be lost during drying process (Hung and Duy, 2012). The bioactive compounds of mushroom and their antioxidant capacity might as well be lost during the preservation. Moisture sorption isotherms represent the equilibrium relationship between water activity and moisture content of foods at constant pressure and temperature. A sound knowledge of the relationship between moisture content and equilibrium relative humidity is essential in the formulation of foods and in their storage stability (Tsami *et al*., 1999).

Thermal analysis determines different phases and states of foods as a function of water content and temperature (Rahman, 2006). The state diagram, a plot of thermal characteristic temperatures as a function of solids content, is used to clearly visualize food's characteristics (Rahman, 2009). It has been reported in the literature that food can be considered very stable at its glassy state. Glass transitions of pure components are more commonly reported in the literature, than the real foods, which are more complex multi-components mixtures. Molecular mobility of glassy materials is significantly reduced below its glass transition. This in turn delays various deteriorative changes such as texture loss, enzymatic spoilage and flavour loss in foods during storage (Mitchell, 1998). It has been also suggested in previous studies that the concept of glass transition should be added along with the existing concept of water activity to get a better understanding about the factors governing the stability of foods (Rahman *et al*., 2005 and Sablani *et al*., 2007). The glass transition temperature values of dates (Guizani *et al.*, 2010), tuna fish (Rahman *et al.*, 2003), king fish muscle (Sablani *et al*., 2007), strawberry (Roos, 1987), garlic (Rahman *et al*., 2005) and grapefruit (Fabra *et al*., 2009) were presented in the literature. The glass transition of fresh mushroom has been shown to change as a function of cooling rate (Haiying *et al*., 2007). The partial glass transition temperatures of mushroom samples decreased when the cooling rate increased from 4.0 to 10.0^0C/min, but when the cooling rates increased from 1.0 to 4.0^0C/min, the trends were not significant (Guizani, *et al*., 2013). However, the storage modulus, loss modulus and loss factor of oyster mushrooms subjected to UV-C and UV-A irradiation are scarce in the literature.

A study on air drying kinetics and quality characteristics of oyster mushroom (*Pleurotus ostreatus*) influenced by osmotic dehydration (Kulwinder *et al*., 2014) indicated that mushroom is one of a non-conventional source of food production, requiring negligible area and being indoor activity and free from natural calamities. It has a great potential for the production of protein rich

quality food and for recycling of cellulose agro-residues and other wastes. It is reported that drying is a comparatively cheap and the easiest mean to increase the shelf life of high moisture products. On the other hand, mushrooms are very sensitive to temperature, therefore choosing a proper method of drying is a very important decision. The osmotic process is a method of partial removal of water from product by immersing it in a hypertonic solution. The process is facilitated by the osmotic pressure difference between the food material (hypotonic medium) and concentrated osmotic solution (hypertonic medium). As osmotic dehydration does not give a product of low moisture content considered for shelf stable therefore osmosed products are further dried up to desired moisture content, in association with other methods of food preservation including freezing, vacuum dehydration and oven or freeze drying (Torringa *et al*., 2001).

The combination of osmotic dehydration with hot air drying, also known as 'osmo-convective drying' has been proposed by many researchers (Kumar *et al*., 2009). The commodities dried under uncontrolled conditions yield poor quality in terms of texture, rehydration, colour and flavour owing to uneven drying and sometimes longer exposure to high temperature. Therefore, osmo-convective drying must be applied in order to improve the rehydration characteristics, texture, and colour of dehydrated products to a great extent. The dry weight, nutrition-elements and organoleptic tests, and storage life of such products are also increased.

For quality evaluation, similar drying experiments were conducted under same drying conditions. Drying was terminated when moisture content reached 7% or below on dry basis. The samples were then allowed to come to room temperature, packed and stored for quality analysis. The dried sample was analysed for its quality by estimating the colour, rehydration ratio and sensory characteristics. Osmotic treatment can prolong pigment retention and minimize the browning associated with natural oxidative processes, thus making products more attractive to consumers. However the impact of air drying kinetic on the storage modulus, loss modulus and loss factor of the mushrooms were not explained. Furthermore, the concentration of vitamin D_2 with respect to the osmotic dehydration was also not explained (Kulwinder *et al*., 2014).

The magnetic treatment effects on mushroom spawn growth and yield have been studied (Jamil *et al*., 2011). The spawn of mushroom were exposed to full-wave rectified sinusoidal magnetic field (MF). The spawn were grown under controlled magnetic field treatment laboratory conditions. The magnetic field treatment resulted in significant increase in the growth and yield of mushroom. The selected spawn were exposed to a controlled MF and sowing was done in the

vegetable seed laboratory. The pre-sowing MF treatments were administered using an electromagnet consisting of two pairs of cylindrical coils, each formed of 4 000 turns of 0.42 mm enamelled copper wire. The MF treatment was applied according to the method of (Iqbal *et al*., 2012). However with respect to MF treatment, there was no analysis of mechanical properties of the mushrooms subjected to the treatment.

A part from mechanical properties of mushrooms having an influence on their quality, they also affect the mushrooms in terms of the suitability of the mushroom for automated harvesting. A study on influences of compost and casing layer depths on the mechanical properties of mushrooms was carried out (Noble *et al*., 1997) and the depths of the casing and compost had significant influences on the mechanical properties of mushrooms in terms of firmness and suitability of the crop for robotic harvesting. Previous measurements showed that the torque required to fracture the stipe (stem) at the point of connection to the bed was, on average, less than the torque required to detach the cap (pileus) from the stipe (Hiller, 1994). However, in some mushrooms, detachment of the cap, splitting of the stipe or cup slippage occurred at a lower torque than that required to remove mushrooms from the bed (Reed and Tillett, 1994), and therefore resulted in low value, damaged or bruised mushrooms.

Previous robotic harvesting tests showed that mushrooms grown in polythene bags were more firmly attached to the bed and prone to slippage failure than mushrooms grown in shallower containers (Noble *et al*., 1997). Anecdotal evidence also indicates that mushrooms grown with a deeper layer of compost have a firmer texture. The energy supplied to test samples to achieve a set displacement (firmness) increased with compost depth but decreased with increasing casing depth. The most firm (requiring the greatest energy) mushrooms were those grown on deep (260 mm) compost and covered in shallow (25 mm) casing; the least firm mushrooms were grown on shallow compost and deep casing. The plastic deformation of test samples increased with casing depth and with deep casing, was greater for mushrooms grown in shallow compost than for those grown in deep compost. The second flush produced mushrooms which were firmer but more prone to plastic deformation than the first flush.

A shallower compost depth significantly reduced the detachment torque and increased the proportion of mushrooms which were successfully detached at the base, however, this treatment also produced less firm mushrooms which were also more prone to stipe splitting than mushrooms grown in deeper compost, but had no influence on the susceptibility of the mushrooms to bruising.

The mushrooms grown on deep compost required considerable energy to harvest and made an audible 'click' when detached, while mushrooms grown on shallow compost required much less energy and were occasionally detached unintentionally by a touch. The reduction in yield per unit of cropping area would also make the shallow compost treatment uneconomic. Shallow casing increased the proportion of mushrooms that were successfully detached at the base and resulted in mushrooms that were firmer, less likely to deform plastically, but that showed greater discolouration resulting from a standard mechanical treatment. The torque (62 Nmm) necessary to detach mushrooms of 40-45 mm diameter was less than that required in the present experiment in most mushrooms, twisting the cap did not result in cap detachment from the top of the stipe (Hiller, 1994). However, in the present experiment, such cap detachment and stipe splitting occurred for 16-50% of the mushrooms tested, depending on the compost and casing depth. This indicates that a twist action alone would result in unacceptable losses using a robotic harvester thus a twist combined with a bending action is therefore necessary (Noble *et al*., 1997). Compost and casing depths have major influences on the mechanical properties of mushrooms and may enable the desired properties for robotic harvesting to be achieved.

However, biological mechanism of how compost and casing depths can affect mushroom structure were not explained and there's limited information in the literature on how the mechanical properties of mushrooms are affected by UV light irradiation. The influence of casing on the mushroom biomechanical properties is unlikely to directly involve nutritional factors as casing is a non-nutritional material, although a greater casing depth increases the distance between the nutritional source and the fruit-bodies.

2.5 Viscoelasticity moduli and loss factor theory

Viscoelastic materials simultaneously exhibit a combination of elastic and viscous behaviour. While all substances are viscoelastic to some degree, this behaviour is especially prominent in polymers. There's however limited information in the literature concerning the viscoelastic behaviour of mushrooms that have been UV irradiated especially during growth. The region are shown in figure 3 as: 1- glassy region, 2- glass transition region, 3- rubbery plateau region, 4- rubbery flow region and 5- liquid flow region (Sperling, 2006).

Various techniques have been used to study mechanical properties of food. They include Instron 5566 stress testing machine and the dynamic mechanical analysis using the Dynamic Mechanical Analyzer, (DMA 983). In this study DMA 2980 was used to monitor the mechanical

properties of mushrooms. The processing and handling of mushroom products can significantly affect the material's texture, flavour, and appearance. Historically, the methods used to evaluate and predict these properties have been somewhat arbitrary and non-quantitative. The use of analytical instrument techniques such as thermal analysis provides a more quantitative, reproducible way for characterizing food products. Dynamic Mechanical Analysis (DMA), for example, can provide information about the mechanical properties of food and how they are affected by various processing conditions (Roulet *et al*., 1988).

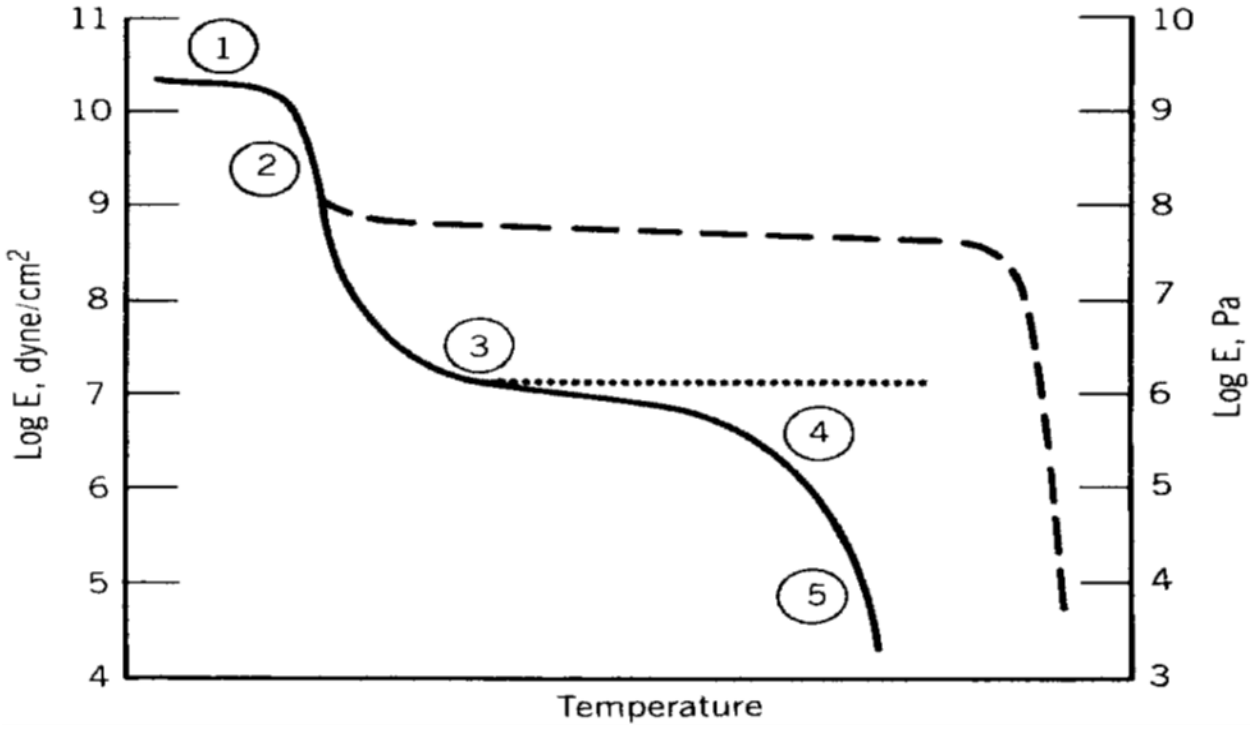


Figure 4: Five regions of viscoelastic behaviour for a linear, amorphous polymer. Also illustrated are effects of crystallinity (dashed line) and cross-linking (dotted line). (Sperling, 2006)

In DMA, the sample is clamped between the ends of two parallel arms which are mounted on low force flexure pivots allowing motion only in the horizontal plane. The distance between the arms is adjustable by means of a precision mechanical slide to accommodate a wide range of sample lengths (from 1 mm up to 65 mm). In addition, a variety of clamping configurations is available to accommodate different material types. An electromagnetic motor attached to one arm drives the arm/sample to a strain (amplitude) selected by the operator. As the arm/sample system is displaced,

the sample undergoes flexural deformation. A linear variable differential transformer (LVDT) mounted on the driven arm measures the sample's response to the applied stress and uses that information to calculate the modulus and damping properties of the material. The rate of deformation (frequency) can be selected by the operator from a wide range (0.001 to 10 Hertz). A frequency of 1 hertz is often used to provide the best compromise between sensitivity and time of analysis. DMA can be simply described as applying an oscillating force to a sample and analysing the material's response to that force. From this, one calculates properties like the tendency to flow from the phase lag and the stiffness (modulus) from the sample recovery. These properties are often described as the ability to lose energy as heat (damping) and the ability to recover from deformation (elasticity). When subjected to stress, a material will exhibit deformation or strain. The slope of stress against strain curve gives the relationship of stress to strain and is a measure of the material's stiffness. The modulus is dependent on the temperature and the applied stress.

For an isotropic perfect elastic material subjected to sinusoidal varying tensile strain ε at a frequency below that required to induced resonance vibrations, the deformation hence the strain occur in-phase with the stress (Carter, 1990).

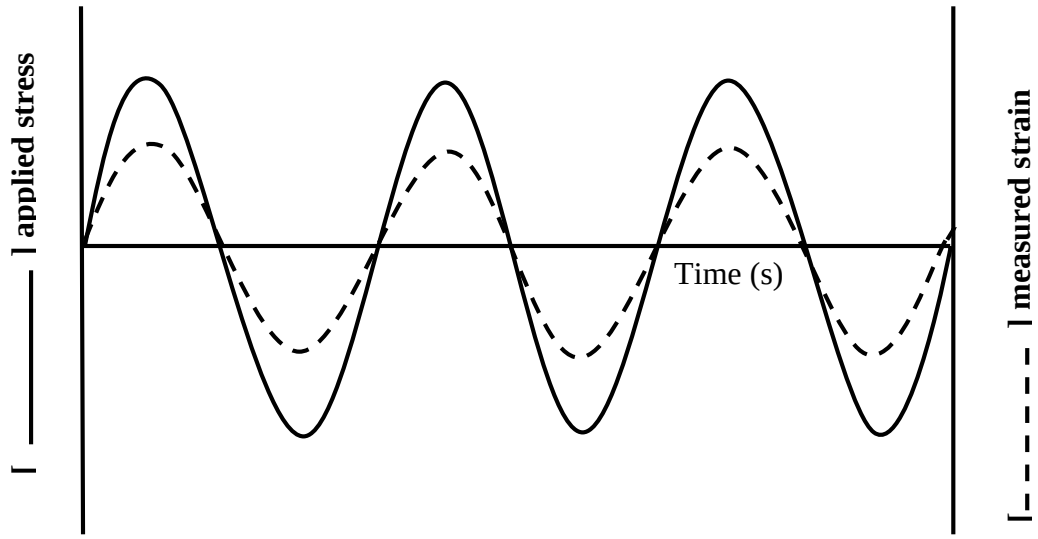


Figure 5: Response of an elastic material (stress in-phase with strain)

For linear viscoelastic behaviour, under steady state conditions, the stress σ sustained by the sample is also sinusoidal; but the stress is out of phase say by an angle δ, (Bryant and Roger 1991).

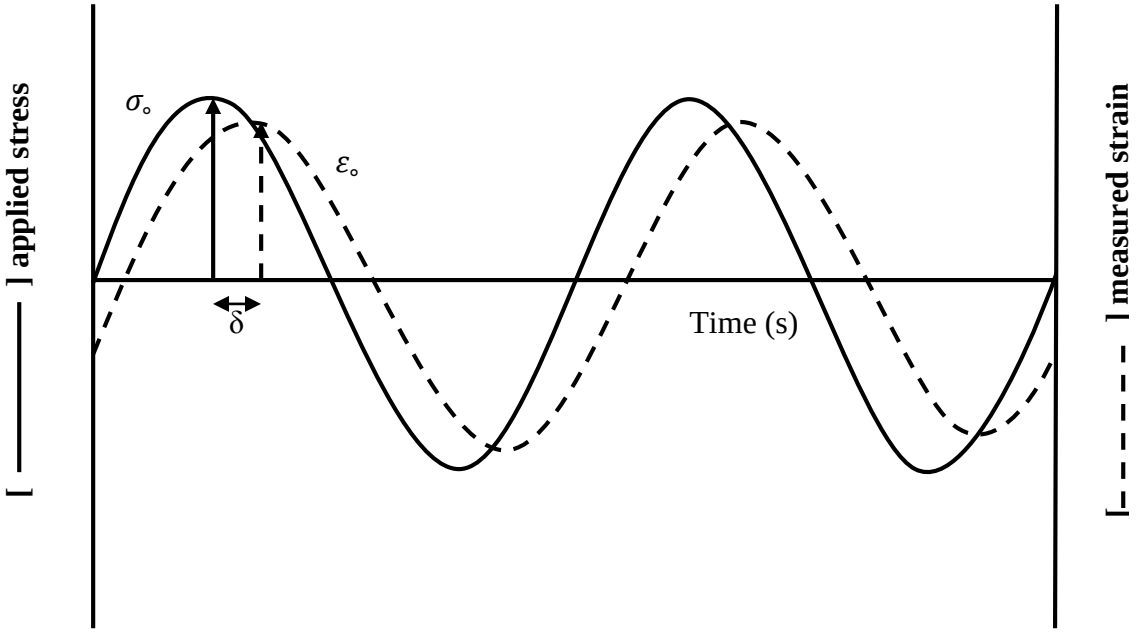


Figure 6: Response of a viscoelastic material (stress out of phase with strain)

Therefore

$$\varepsilon = \varepsilon_\circ \, cos\omega t \qquad 1$$

$$\sigma = \sigma_\circ \cos(\omega t + \delta).$$

$$= \sigma_\circ cos\delta cos\omega t - \delta_\circ sin\delta sin\omega t \qquad 2$$

Where **ω** is the angular frequency, t is the time and $\delta_\circ$ and $\varepsilon_\circ$ are the amplitudes of stress σ and strain ε respectively.

From equation 2 the stress is resolved into two components:

a) $\sigma_\circ cos\delta$ and it's in-phase with strain,

b) $\delta_\circ sin\delta$, which is 90◦ out of phase with the strain

Therefore the material behaves partly as a solid and partly as a viscous liquid, and the stress-strain relationship is written as; $\sigma = \varepsilon_\circ \, E' cos\omega t - \varepsilon_\circ \, E'' sin\omega t$ 3

Where the component moduli are given by,

$$E' = \frac{\sigma_\circ}{\varepsilon_\circ} cos\delta \text{ and } E'' = \frac{\sigma_\circ}{\varepsilon_\circ} \, sin\delta \qquad 4$$

Equation 3 and 4 suggest that tensile modulus can be specified in complex form E*. This further implies that the strain and the stress cycles are represented by the real parts of

$\varepsilon^* = \varepsilon_\circ expi\omega t$ and $\sigma^* = \sigma_\circ expi(\omega t + \delta)$ respectively.

$$\text{Then } E^* = \frac{\sigma^*}{\varepsilon^*}$$

$$E^* = \frac{\sigma_\circ}{\varepsilon_\circ} expi\delta$$

$$= \frac{\sigma_\circ}{\varepsilon_\circ} (cos\delta + isin\delta)$$

$$= E' + iE'' \qquad 5$$

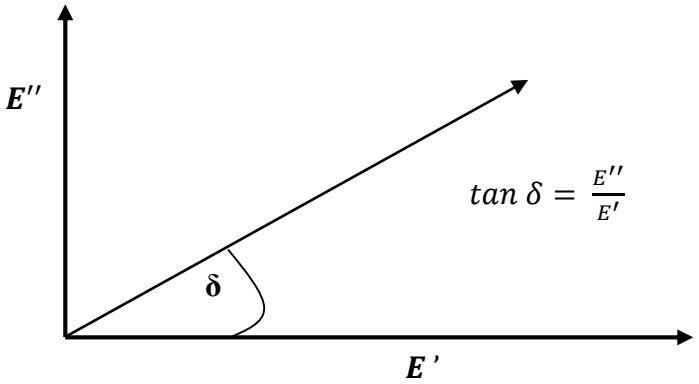


Figure 7: Storage E' and loss Moduli E" and tan δ

The real part of the modulus E'which is in phase with the strain is termed as the storage modulus since it is proportional to the peak and the energy stored. It represents the elastic behavior of the material. The imaginary part of the modulus E'' is out phase with the strain, is proportional to the net energy dissipated per cycle and is known as the loss modulus. It represents the viscous part of the material response. The ratio $\frac{E''}{E'}$ = tan δ is called as the loss factor or damping factor. It's a dimensional less quantity and is the rate of energy lost per cycle to the energy stored and hence recovered per cycle. This loss factor gives information about the samples viscous or elastic behavior. E' E'' and tan δ depends on the test frequency and also on the temperature, and each is used to characterize dynamic mechanical properties either at a given frequency or temperature or, preferably over a range of these variables. Viscoelastic properties of a material changes as it degrades. The changes of these properties have been used to infer the influence of temperature of freeze drying process on the mechanical properties of dried mushroom after harvest (Kramkowski *et al*., 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Growing of oyster mushrooms

Wheat grains were prepared for grain spawn by being boiled, drained, filled in containers and sterilized. The substrate was then prepared from wheat straw and was pasteurized by hot water immersion to kill contaminants. The wheat grains and straw were cellulosic substrates that formed the media for growth of the mushroom. The pasteurized substrate was then spawned after ensuring that the substrate has cooled down to 30°C. The spawn was mixed with the substrate when filling the perforated bags of dimensions 15cm by 20cm by 30cm. The bags were thirteen and were labelled B1 - B13. Spawn run followed where the mycelium was grown through the substrate. The bags once spawned were placed in a mushroom house. The mycelium colonized the substrate in two to three weeks and started to form small fruiting bodies. Near darkness, controlled temperature and humidity conditions were provided. Humidity was maintained between 80-100% by spraying water several times per day and the temperature was maintained between 15 - 25°C.

3.2 Exposure of mushrooms to UV light during growth

UV-C light (254nm) and UV-A light (365nm) irradiation begun once the mushrooms cap started opening from the stem. An 8W Ultraviolet fluorescent lamp made by UVITEC (model LF-204.LS) was used. The lamp irradiated at the ranges (254 nm) and (365nm) with a switch that shifted between the two ranges and the measured intensity was 3.5 W/m^2 for 365nm and 0.0327W/m^2 for 254nm. Bag labelled B1, the control, was not exposed to UV-C and UV-A light. Six bags labelled, B2 to B7 were exposed to UV-C light while another set of six bags labelled B8 to B13, were exposed to UV-A light. Beginning with the lowest exposure time of 10 minutes for bags B2 and B8, and subsequent 10 minutes increment for the next bag up to 60 minutes for the highest exposed bags (B7 and B13) was done, for UV-C and UV-A respectively. This irradiation procedure was repeated for three days. Once the caps were fully opened and separated from the stem, the mushrooms were ready for harvesting. Harvesting was done by holding the mushrooms by their stalks and breaking them off carefully from the substrate. Samples were picked from each bag, freeze dried and ground into a powder. The powder was then used for quantification of vitamin D_2 using UV spectrophotometry.

3.3 UV spectrophotometry analysis

The samples for spectrophotometric analysis were prepared by the method previously described by Perera *et al*, (2003) where 0.5g of B1 mushroom sample powder was weighed into 250 ml round bottom flasks and mixed with 4 ml of sodium ascorbate solution (17.5 g of sodium ascorbate in 100 ml of 1 M NaOH), 50 ml of ethanol and 10 ml of 50% potassium hydroxide. The mixture was saponified under reflux at 80^0C for one hour then it was immediately cooled to room temperature and transferred into a separating funnel. The mixture was first extracted with 15 ml de-ionized water, followed by 15 ml ethanol and then with a three-stage n-pentane extraction of volumes 50, 50 and 20 ml, respectively. The pooled organic layers were washed three times with 50 ml of 3% KOH in 5% ethanol and then finally with deionized water until neutralized. The organic layer was transferred into a round bottom flask rotary and was evaporated to dryness at 40^0C and immediately re-dissolved in 5 ml ethanol. The sample was passed through a 0.45 μm non-pyrogenic filter. This procedure was repeated using samples of B2 - B13. UV spectroscopy, which is based on measurement of the intrinsic absorption of calciferols, plays a very modest role in quantification of vitamin D_2. In this study, spectrophotometric determination of vitamin D_2 were then determined by method previously described by Saad, (1978) where calciferol reacts with 11N hydrochloric acid in the presence of symmetrical tetrachloroethane to develop a greenish yellow colour with maximum absorption at 440-460nm. Aliquot of 2ml of the prepared samples of B1 - B13 were evaporated to dryness on a boiling water bath. Then 1ml of 11 N hydrochloric acid and 1ml of symmetrical tetrachloroethane were added and the tube was warmed for 10 minutes on the water bath with occasional shaking. After cooling the volume was completed to 7ml with acetone and the absorbance was measured using a spectrophotometer by putting a reference blank solution (the solution of the sample that had not been irradiated during growth) in a cuvette and placed in the spectrophotometer. The absorbance of the reference blank was determined at 450nm. The blank was removed and the cuvette containing sample solution for B2 was put in the spectrophotometer and the absorbance was determined at 450nm. This procedure was repeated for samples in B3 – B13.

3.4 Dynamic mechanical analysis (DMA)

The harvested samples were tested for their storage and loss factor using DMA-2980 instrument. DMA 2980 analytical instrument is used to test the physical properties of material. A compressional clamp of diameter 12.5mm which handles samples of thickness 3.00mm was used.

The samples for experimental studies were cut into cylindrical shapes of diameter 12.50mm and thickness 3.00mm. Each of the sample was placed on the compressional clamp at a time and subjected to changes in stress induced by an oscillating force. The amplitude and the phase of the displacement in the sample in response to applied oscillating force over a range of temperature were measured. Measurement of loss and storage moduli and the loss factor was obtained directly from the DMA.

The storage modulus gives the amount of energy the sample stores, the loss modulus gives the amount of energy dissipated by the sample when a sinusoidal force is applied. The loss factor is also called the damping factor and it is measured as an angle to indicate the lag between the stress and strain giving information about the samples elastic nature. Calibration of the DMA using standard samples preceded any measurement to ensure reliability of the machine output data.

The storage moduli, loss moduli and loss factors of the samples that had been exposed to UV-A and UV-C light during growth and those had not been exposed were recorded as graphs of storage modulus, loss modulus and loss factor against temperature and experiment was again repeated after 10 days of storage at a temperature of 10°C.

3.5 Statistical analysis

The results were statistically analysed by analysis of variance (ANOVA). The evaluation of equality of means was carried out by the one-way analysis of variance using the F distribution to assess significance. The data were expressed as means ± SD (standard deviation). The test results were considered significant only after reaching $p < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Change in Concentration of Vitamin D_2 in Oyster Mushrooms exposed to 254nm and 365nm UV-light During Growth

The exposure of UV-C light during growth for 60 minutes resulted in absorbance of 0.81 compared to 0.49 for UV-A light during growth (Table 2). High values of absorbance implies high concentration of vitamin D_2. The difference between the UV-C and UV-A absorbance values can be ascribed to a higher efficiency of vitamin D_2 conversion at exposure to UV-C during the growth than UV-A light. The increase in the absorbance after repeated exposure may be explained by a carryover of ergosterol formed in the small mushrooms left growing for the next day. The absorbance was much lower in mushrooms exposed for 10 minutes under both UV light bands.

Table 2: Absorbance values of solutions of samples irradiated at different times by UVA and UV-C light during growth.

Time of irradiation (minutes)	UV-A absorbance	UV-C absorbance
10	0.18	0.38
20	0.22	0.45
30	0.31	0.53
40	0.49	0.65
50	0.49	0.81
60	0.49	0.81

The samples of the mushrooms that were grown without exposure to UV-C and UV-A light were found to have lowest absorbance values indicating that there was very little Vitamin D_2 present. The conversion of ergosterol to vitamin D2 under UV-C and UV-A light bands were shown to be significantly different ($p < 0.05$). The absorbance of vitamin D_2 under both UV light bands increase gradually as time of exposure increases up to 50 minute for UV-C and remains

constant up to 60 minutes of irradiation. In the case of UV-A irradiation, the absorbance of vitamin D_2 increases up to 40 minutes and remains constant at 60 minutes of irradiation. The increase in the absorbance of vitamin D_2 was as a result of conversion of ergosterol in the mushroom to vitamin D_2. The absorbance of vitamin D_2 for the untreated samples ranged between (0.14 ± 0.02). A study on comparison of UV-A and UV-C for vitamin D_2 producing capacity in commercial mushroom species has been reported (Teichmann, *et al*., 2007). For the mushrooms studied, vitamin D_2 formation follows the order UV-C > UV-A after 2 hours of post-harvest exposure with UV-A light not result in any significant changes in vitamin D_2 concentrations from 0 to 2 hours. In this study, there was significant change in vitamin D_2 for both UV-A and UV-C samples with respect to the control sample for 1 hour. Therefore, the most probable reason for differences obtained in vitamin D_2 production after exposure to UV-A light between this study and that of (Teichmann, *et al*., 2007) could be explained in part by different methodology for vitamin D_2 quantification in mushroom samples, different exposure handlings, different exposure time (during growth). Furthermore it has been reported that temperature, moisture and the part of the mushrooms tissues (gills or caps) exposed to UV irradiation play a role for vitamin D_2 yield (Perera, *et al*., 2003); (Jasinghe and Perera, 2006).

Change in concentration of vitamin D_2 under both UV- bands increased with respect to time of exposure. The vitamin D_2 level of the biologically active treated mushrooms increased substantially on both wavelengths. In the case of UV-C treatments, even the shortest time period (10 minutes) was enough to cause twice as high vitamin D_2 level in the mushrooms as in control. UV-A irradiation did not cause as intensive change in vitamin D_2 concentration as experienced in case of UV-C radiation.

4.2 Effect of duration of irradiation on the conversion of ergosterol to vitamin D_2 in oyster mushrooms during growth.

The yields of vitamin D_2 concentration, after the irradiation of the mushrooms for 40 minutes were shown to be significantly higher. The vitamin D_2 concentration obtained by irradiation of longer durations by UV-C band were higher than the observed change in concentration of vitamin D_2 with respect to UV-A light irradiation. Figure 7 shows comparison of absorbance of vitamin D_2 for oyster mushrooms irradiated by UV-A and UV-C light during growth and untreated mushrooms at a different times of exposure. The conversion was almost completed within 40 minutes.

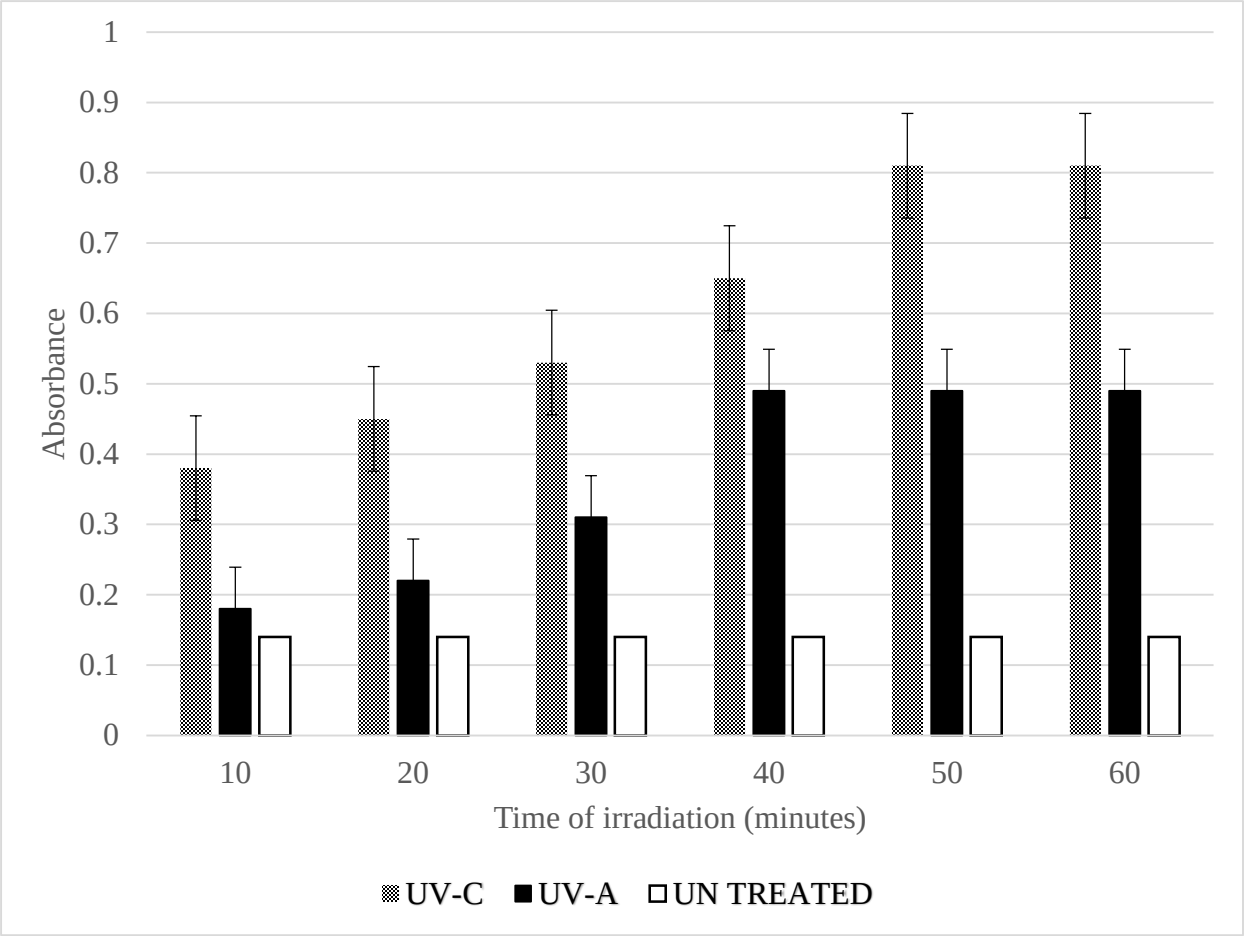


Figure 8: Comparison of absorbance of vitamin D_2 for oyster mushrooms irradiated by UV-A and UV-C light during growth and untreated mushrooms at different time of exposure.

Prolonged irradiation does not increase vitamin D_2. Previtamin D intermediates also absorb UV radiation, producing tachysterol and lumisterol by photoisomerization (Havinga, 1973; Webb *et al*., 1989), and prolonged irradiation produces irreversible ‘‘over-irradiation products’’ by dimerization and ring cleavage (Braun *et al*., 1991). After a certain irradiation time (50 minutes) neither UV-A nor UV-C light enhanced the vitamin D_2 content. Jasinghe and Perera (2006) experienced the same decline of vitamin D_2 level during their studies of UV treatments on post-harvest mushrooms.

4.3 Determination of the change in storage modulus, loss mosulus and loss factor of oyster mushroom as a result of exposing them to UV-C and UV-A light during growth.

The storage modulus for control, UV-A and UV-C irradiated samples at different temperatures was determined over the temperature range of 25 - 100^{0}C of which 35 -100^{0}C was chosen for analysis. There was a drop in storage modulus after irradiation of the samples by both UV-A and UV-C light during growth. The samples irradiated by UV-C had a higher drop compared to those treated by UV-A light as indicated in the table 3. This indicates that irradiation of samples with UV-C light lowers storage modulus of the mushrooms. The samples under UV-C and UV-A exhibited similar viscoelastic behaviour. The regions included the glassy region in which the samples were hard, springy or rock like.

Table 3: The average storage modulus (E′) and its percentage drop at different exposure times for UV-A and UV-C light from 35 -100^{0}C.

Samples irradiation time (minutes)	Average E′ (MPa)		Percentage (%) drop in E′	
	UV-A	UV-C	UV-A	UV-C
0	8.987	8.987	-	-
10	7.614	6.850	15	23
20	6.367	5.760	29	36
30	5.874	5.540	35	38
40	5.506	5.250	39	42
50	5.269	5.046	41	44
60	5.269	5.046	41	44

In this region the bending of the bonds was occurring and temperature range was 35 - 85^{0}C. Glass transition region then followed in which the samples softened and thus became less hard as storage modulus decreased and tan δ peaks, temperature ranging from 80 - 95^{0}C. The samples then started undergoing slippage of main chain (rubbery plateau region) and the temperature ranged from 95 - 100^{0}C. Samples that were irradiated for longer durations (40, 50 and 60 minutes) registered lower values of storage modulus especially for UV-C irradiated samples compared to those that were irradiated for short time intervals (10, 20 and 30 minutes).

The low storage modulus for both UV-C and UV-A irradiated samples as temperature increases can be attributed to low levels of ergosterol which was subjected to photolysis and yielded photo irradiation products, the principal ones being vitamin D_2, tachysterol and lumisterol when the mushrooms were irradiated by UV-C and UV-A light during growth. Samples under UV-C irradiation had lower storage modulus as temperature increased because they had higher levels of vitamin D_2 concentrations and this indicated that most of the ergosterol in this samples had undergone photolysis during irradiation. This means ergosterol presence in the samples increases the storage modulus since it's a component of the mushrooms cell membrane.

Tables 4 below shows the loss modulus for both the control andUV-A irradiated samples. Loss modulus of the mushrooms represents the energy lost as heat and is a measure of vibrational energy that has been converted during vibration and that cannot be recovered.

Table 4: Loss modulus (MPa) for both control and UV-A-irradiated oyster mushroom samples at different temperatures (^{0}C).

	Loss Modulus (MPa)					
Temperature (^{0}C)	**Control Sample**	**UV-A 10 min**	**UV-A 20 min**	**UV-A 30 min**	**UV-A 40 min**	**UV-A 50 and 60 min**
35	1.40	1.20	0.90	0.72	0.60	0.59
40	1.40	1.23	0.90	0.72	0.60	0.59
45	1.40	1.25	0.90	0.72	0.60	0.59
50	1.45	1.28	0.90	0.72	0.60	0.59
55	1.47	1.30	0.90	0.72	0.60	0.59
60	1.50	1.35	0.95	0.72	0.60	0.59
65	1.60	1.45	1.10	0.75	0.60	0.59
70	1.70	1.50	1.25	0.80	0.64	0.59
75	1.85	2.00	1.40	1.10	0.90	0.59
80	3.40	2.70	2.50	1.60	1.15	0.68
85	4.10	3.00	2.60	1.70	1.40	0.53
90	3.60	2.70	2.00	1.50	1.30	0.53
95	2.75	1.65	1.36	1.00	0.78	0.53
100	2.50	1.60	1.25	0.90	0.70	0.53

There was a significant difference in the loss modulus between the irradiated samples and control samples, $p < 0.05$. As temperature increased from 35 - 100^{0}C, the E″ decreased with highest decrease of 26% for UV-A 10 minutes, 37% for UV-A 20 minutes, 58% for UV-A 30 minutes, 66% at for UV-A 40 minutes and 87% at for UV-A 50 and 60 minutes at 85^{0}C.

Tables 5 shows the loss modulus for control, UVC irradiated samples. The loss modulus for this samples was higher than that of the control sample as temperature increased from 35^{0}C to 75^{0}C and it was lower than that of the control from 85^{0}C to 100^{0}C. This indicates that UV-C samples have a higher loss modulus than UV-A samples. This high loss modulus can be ascribed to presence other irradiation products apart from vitamin D2, which are tachysterol and lumosterol which increases the energy lost per unit circle.

Table 5: Loss Modulus (MPa) for both control and UV-C irradiated samples against temperature (^{0}C)

	Loss Modulus (MPa)					
Temperature	**CONTROL**	**UV-C 10 min**	**UV-C 20 min**	**UV-C 30 min**	**UV-C 40 min**	**UV-C 50,60 min**
35	1.4	1.72	1.59	1.57	1.55	1.54
40	1.4	1.72	1.59	1.57	1.55	1.54
45	1.4	1.72	1.62	1.57	1.55	1.54
50	1.45	1.68	1.65	1.56	1.55	1.54
55	1.47	1.81	1.73	1.61	1.55	1.56
60	1.50	1.92	1.88	1.73	1.68	1.62
65	1.60	1.98	1.94	1.85	1.80	1.75
70	1.70	2.85	2.78	2.70	2.67	2.61
75	1.85	3.50	3.35	2.90	2.92	2.80
80	3.40	3.61	3.41	2.84	2.81	2.71
85	4.10	3.40	3.11	2.79	2.75	2.58
90	3.60	3.12	2.71	2.63	2.59	2.47
95	2.75	2.71	2.59	2.51	2.46	2.40
100	2.50	2.46	2.41	2.37	2.35	2.25

Tables 6 shows tan δ (loss factor) for both control and UV-A irradiated samples. Loss factor of mushrooms sample is the measure of the energy lost in terms of the recoverable energy and represents mechanical damping or internal friction in viscoelastic system. There was a significant difference in loss factor between the control and irradiated samples, $p < 0.05$. It was noted that the loss factor for UVA samples was lower than that of the control samples. The low loss factor of UV-A irradiated samples indicated that the samples had an elastic strain component.

Table 6: Tan δ for both control and UV-A-irradiated oyster mushroom samples at different temperatures (^{0}C)

	Tan δ					
Temperature (°C)	**Control**	**UV-A 10 min**	**UV-A 20 min**	**UV-A 30 min**	**UV-A 40min**	**UV-A 50 and 60 min**
35	0.13	0.12	0.12	0.09	0.09	0.08
40	0.13	0.12	0.12	0.09	0.09	0.08
45	0.13	0.13	0.12	0.09	0.09	0.09
50	0.13	0.14	0.12	0.09	0.09	0.09
55	0.14	0.14	0.13	0.11	0.09	0.09
60	0.15	0.15	0.13	0.11	0.09	0.09
65	0.16	0.17	0.15	0.12	0.09	0.10
70	0.17	0.18	0.16	0.12	0.11	0.11
75	0.19	0.25	0.17	0.17	0.16	0.11
80	0.27	0.43	0.35	0.25	0.21	0.13
85	0.48	0.49	0.43	0.30	0.25	0.11
90	0.69	0.55	0.47	0.37	0.32	0.14
95	0.67	0.51	0.45	0.34	0.29	0.20
100	0.60	0.50	0.42	0.32	0.27	0.20

Tables 7 shows tan δ (loss factor) for both control and UV-C irradiated samples. The samples irradiated by UV-C light during growth had high loss factor than those irradiated by UV-A light. The high values of loss factor in UV-C light treated samples were higher than those of the control

sample and UV-A sample. This indicated that the UV-C mushrooms samples had a non-elastic strain component.

Table 7: Tan δ for both control and UV-C irradiated oyster mushroom samples at different temperatures (^{0}C)

	Tan δ					
Temperature (^{o}C)	**Control sample**	**UV-C 10 min**	**UV-C 20 min**	**UV-C 30 min**	**UV-C 40 min**	**UV-C 50;60 min**
35	0.13	0.19	0.20	0.22	0.24	0.24
40	0.13	0.20	0.22	0.22	0.25	0.25
45	0.13	0.21	0.23	0.24	0.25	0.26
50	0.13	0.21	0.24	0.25	0.25	0.26
55	0.14	0.23	0.27	0.26	0.26	0.26
60	0.15	0.26	0.30	0.29	0.29	0.29
65	0.16	0.27	0.32	0.32	0.32	0.32
70	0.17	0.39	0.50	0.48	0.50	0.50
75	0.19	0.49	0.6	0.53	0.55	0.55
80	0.27	0.53	0.63	0.54	0.55	0.56
85	0.48	0.57	0.6	0.55	0.58	0.63
90	0.69	0.69	0.63	0.62	0.68	0.68
95	0.67	0.61	0.65	0.68	0.70	0.73
100	0.60	0.60	0.62	0.67	0.69	0.70

Irradiation of samples for 60 minutes had no further change on the storage modulus, loss modulus, and loss factor of the samples as samples under this time duration recorded similar values as those under 50 minutes of irradiation. A study on anisotropy of mechanical properties of mushrooms (*Agaricus bisporus)* (Jerzy *et al*., 2013) indicated that the value of the apparent elasticity modulus (E) was treated as a modulus which determined relation between the calculated value of stress and relative strain was accepted as a measure of the material resistance to the compression load. The mushrooms were selected on account of the shape, size and the term of cropping. First group consisted of species of 25-35 mm dimensions, in the second group of the same crop, mushrooms dimensions were 35-45 mm. Group 3 was composed of mushrooms of 35-45 mm dimensions, but

they came from a later crop.A test machine Instron 5566 with a tensometric head 2525-806 of a scope up to 1kN (precision 0.25%) was applied. Compression tests were carried out in a parallel direction (axial) to the stem of a mushroom and in the perpendicular directions (radial). The elasticity modulus, was not determined over a range of temperature and it was established to be higher with respect to axial loading (0.54 MPa) than that obtained in radial loading (0.27MPa). However, the findings in the present study indicated higher mechanical properties and they were based on storage modulus, loss modulus and loss factor of the mushroom that had been exposed to UV light during growth and the temperature range (35 - 100°C) was factored in the mechanical property analysis. After 10 day of storage, at a temperature of 10°C, there was no significant difference in loss modulus, storage modulus and loss factor of the treated samples with respect data collected 10 day earlier. The control samples on the other had a decrease in storage modulus for both UV-A and UV-C irradiation samples after 10 day of storage as indicated in table 8. This indicate that mushroom irradiation helps in maintaining their mechanical properties for a longer period hence preserving quality

Table 8: The average storage modulus (E′) at different exposure times for UV-A and UV-C light from 35 -100^{0}C after 10 days of storage.

Samples irradiation time (minutes)	**Average E′ (MPa)**	
	UV-A	**UV-C**
0	8.033	8.033
10	7.512	6.647
20	6.221	5.543
30	5.703	5.349
40	5.414	5.098
50	5.109	4.975
60	5.012	4.837

It should be noted that wild mushrooms are not exposed to UVC light as this spectrum of light is completely absorbed by ozone in the upper atmosphere, however UVC light has along-history of safe use in the production of purified and semi-purified food grade sources of vitamin D_2 that are synthesized from ergosterol (Simon *et al*., 2013). Since wild sun exposed mushrooms

have a long history of safe consumption in the food supply, a comparison of the effects of sunlight on mushroom composition with those occurring in mushrooms processed using UV light was conducted and no compositional changes of nutritional or toxicological significance were observed (Simon *et al*., 2011). The effects of electromagnetic radiation on foods have been a subject of extensive research. Light within the ultraviolet wavelengths of the electromagnetic spectrum represent a form of non-ionizing radiation, which have limited effects on biological molecules, and the corresponding capacity of the technology to impart significant unintended effects on foods is low (Simon *et al*., 2013). Studies evaluating the bioavailability of vitamin D mushrooms in humans and animals have been reported and it been confirmed that vitamin D in mushrooms is bioavailable and mushrooms are a nutritionally relevant food source of vitamin D (Urbain, *et al*., 2011).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Exposing mushrooms to UV light during growth causes measurable increases in the vitamin D_2 content and as a result, mushrooms can provide appreciable amounts of vitamin D_2 to the diet. Irradiation of mushrooms with UV-A and UV-C light during growth leads to increase in concentration of vitamin D_2 with UV-C light recording a higher concentration than UV-A light. The concentration of vitamin D_2 will vary depending on the type of light and duration of exposure. Therefore artificial UV irradiation can convert the natural ergosterol content of mushrooms into vitamin D_2 not only in case of post-harvest mushrooms, but during growth as well

The variation of exposure time of irradiation also has an impact on the concentration of vitamin D_2 where by concentration increased with time of irradiation up to 40 minutes for UV-A and 50 minutes for UV-C light bands.

Irradiation of mushrooms during growth with UV-A and UV-C light leads to a decrease in storage modulus with increase in temperature with UV-C irradiated samples having a higher decrease than UV-A irradiated samples. Loss modulus and loss factor decrease with respect to control sample for UV-A light irradiation. For UV-C irradiated samples, the loss modulus and loss factor increase with respect to control sample. UV-C light had a greater impact on the mechanical properties of oyster mushrooms compared to UV-A light. This changes in mechanical properties did not affect the quality of the mushroom and shelf life was improved.

5.2 Recommendations

i. Storage modulus, loss modulus and loss factor for irradiated oyster mushrooms be studied using different substrates in growth mechanisms so as to identify the relationship between the substrate and mechanical properties of mushrooms.

ii. The storage modulus, loss modulus and loss factor for UV irradiated oyster mushrooms be studied below room temperatures of 30 °C using Nitrogen in DMA 2980.This is to take into account the extreme winter weather conditions in some parts of the world.

iii. Test trials to be carried out so as to study the bioavailability of vitamin D_2 from irradiated oyster mushrooms during growth and establish the effectiveness in improving vitamin D status.

iv. The very high concentrations of vitamin D_2, loss modulus and loss factor after exposure to UV-C irradiation makes it worth continuing investigations with different wavelengths of UV light and irradiation intensities.

v. Investigation using clinical studies of therapeutic potential of vitamin D_2 from irradiated oyster mushrooms and its applications could be useful for dieticians, nutritionists, and other relevant health professionals for formulation of therapeutic diets. The literature reviewed it this thesis, clearly indicates that many common diseases (heart diseases, obesity, diabetes, cancers, hypertension, and arthritis) are associated with vitamin D deficiency. In addition, vitamin D is now being used in therapeutic applications in the treatment of several diseases including hyperproliferative diseases, post-transplant survival and various malignancies.

REFERENCES

Acedo, A. L. (1997) Improving the quality and shelf life of vegetable and fruits by evaporative cooling storage. 19th Scientific Meeting. *National Academy of Science and Technology*. Westin Plaza, Pasay City, Metro Manila.

Adams J and Lee G. (1997) Gains in bone mineral density with resolution of vitamin D intoxication. *Annals of Internal Medicine*, **127**: 203-206.

Ajlouni S., Beelman R and Thompson B (1993) Influence of gamma irradiation on quality characteristics, sugar content, and respiration rate of mushrooms during postharvest storage in food flavors, ingredients and composition; charalambous, C., Ed.; *Elsevier Publishing Cooperation.: Amsterdam, The Netherlands*: 103-121.

Akihiro N. O (2013) The Efficacy of Ultraviolet (UV) Radiation to Protract the Shelf Life of *P. florida*. *Advanced Biomedical research,* **4** (3): 34-38.

Akram K and Kwon J. H. (2010). Food Irradiation for Mushrooms: A Review. *Journal of Korean Society for Applied Biological Chemistry*, **53**: 257-265.

Alexander B.J., Richard S.L., Robert, M.D and Cheryl, B. (2006) Vitamin D: A clinical perspective. Metametrix clinical laboratory department of science and education.

Arora, S., Shivhare, U.S., Ahmed, J., Raghavan, G.S.V. (2003), Drying kinetics of *Agaricus bisporus* and *Pleurotus florida* mushrooms. *American. Society of Agricultural. Engineering*, **46**: 721-724.

Ascherio A., Munger K.L and Simon, K.C. (2010) Vitamin D and multiple sclerosis. *Lancet Neurology*, **9** (6): 599–612.

Beard J.A., Bearden A and Striker R. (2011). Vitamin D and the anti-viral state. *Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology*, **50** (3): 194–200.

Beelman R.B., Okereke A and Guthrie B. (1987). Evaluation of textural changes related to Post-harvest quality and shelf life of fresh mushroom. Development in crop science cultivating edible fungi, *Elsevire, Amsterdam*, **10**: 251-258.

Beaulieu M., D'Aprano G and Lacroix, M. (2002). Effect of dose rate of gamma irradiation on biochemical quality and browning of mushrooms *Agaricus bisporus*. *Radiation Physics and Chemistry*, **63**: 311–315.

Berube S., Diorio C., Verhoek-Oftedahl W and Brisson J (2004). Vitamin D, calcium, and mammographic breast densities. *Cancer Epidemiol Biomarkers and Prevention*, **13**: 1466-72.

Bernas E., Jaworska G and Kmiecik W. (2006) Storage and processing of edible mushrooms, *ACTA Scientiarum Polonorum - Technologia Alimentaria*, **5** (2):5-23.

Betsy M., Sutherland Hardy K., Meltz M and Glickman R. (1997) Ultraviolet Radiation Hazards to Humans, Medical Physics Publishing, Madison.

Bintsis T., Litopoulou-Tzanetaki E. and Robinson R. (2000) Existing and potential applications of ultraviolet light in the food industry – A critical review. *Journal of the Science of Food and Agriculture* **80**: 637–645.

Buket E., Erdogan K., and Hikmet O (2011) Colour and some physical properties of frozen mushroom *Health Nutrition,* **2** (1): 6-12

Buttigliero C., Monagheddu C., Petroni P., Saini A., Dogliotti L., Ciccone G and Berruti A. (2011) Prognostic role of vitamin D status and efficacy of vitamin D supplementation in cancer Patients: A systematic review. *The oncologist* **16** (9): 1215–1227.

Blum M., Dolnikowski G., Seyoum E., Harris S.S., Booth S.L., Peterson J., Saltzman E and Dawson Hughes .B. (2008) Vitamin D (3) in fat tissue. *Endocrine* **33***:* 90-94.

Braun M. M., Fub W and Kompa K. L. (1991) Improved photosynthesis of previtamin D by wavelength of 280-300 nm. *Journal of Photochemistry and Photobiology A: Chemisty*, **61**: 15-26.

Bruce D., Ooi J. H., Yu S and Cantorna M. T. (2010) Vitamin D and host resistance to infection. Putting the cart in front of the horse. *Experimental biology and medicine (Maywood, N.J.)* **235** (8): 921–927.

Bryrant W. R and Rogers C. B. (1991) Physical methods of chemistry. Volume 3,2nd edition. Wiley- Interscience publication. New York.

Carlton-Conway D., Tulloh R., Wood L and Kanabar D (2004) Vitamin D deficiency and cardiac failure in infancy. *Journal of the Royal Society of Medicine*, **97**: 238-239.

Carter, R. E (1990) Rheology of food, pharmaceutical and biological materials with general rheology. Elselvier science publisher ltd. New York.

Cannell J.J., Hollis B.W., Zasloff M and Heaney R.P. (2008) Diagnosis and treatment of vitamin D deficiency. *Expert opinion on pharmacotherapy*. **9** (1):107-118.

Cannell J. J., Vieth R., Umhau J. C., Holick M. F., Grant W. B., Madronich S., Garland C. F and Giovannucci, E. (2006) Epidemic influenza and vitamin D. *Epidemiology and Infection,* **134** (6): 1129–1140.

Chang, S.T. (1996) Mushroom research and development - equality and mutual benefit. *Mushroom Biology*, **2**: 1-10.

Chen T.C, Chimeh F, Lu Z, Mathieu J, Person K.S, Zhang A, Kohn N, Martinello S, Berkowitz R, Holick M.F (2007) Factors that influence the cutaneous synthesis and dietary sources of vitamin D. *Archives of Biochemistry and Biophysics,* **460**(2):213–217.

Chung M., Balk, E.M., Brendel M., Lau J., Lee J., Lichtenstein A., Patel K., Raman G., Tatsioni A., Terasawa T and Trikalinos, T.A. (2009) Vitamin D and calcium: A systematic review of health outcomes. *Evidence report/technology assessment,* (**183**): 1–420.

Clemens T.L, Henderson S.L, Adams J.S, and Holick MF (1982). Increase skin pigment reduces the capacity of skin to synthesize vitamin D_3. *Lancet* **319**: 74-76.

Cranney A., Horsley T., O'Donnell S., Weiler H., Puil L., Ooi D., Atkinson S., Ward L., Moher D., Hanley D., Fang M., Yazdi F., Garritty C., Sampson M., Barrowman N., Tsertsvadze A and Mamaladze V. (2007) Effectiveness and safety of vitamin D in relation to bone health. *Evidence report/technology assessment,* (**158**): 1-235.

De Borst M.H, De Boer R.A, Stolk R.P, Slaets J.P and Wolffenbuttel B.H (2011) Vitamin D deficiency: universal risk factor for multifactorial diseases? *Current Drug Targets* 12: 97-106.

Engelsen O, Brustad M, Aksnes L and Lund E (2005) Daily duration of vitamin D synthesis in human skin with relation to latitude, total ozone, altitude, ground cover, aerosols and cloud thickness. *Photochemistry and Photobiology*, 81(**6**):1287–1290.

Fabra, M. J., Talens, P., Moraga, G. and Martinez-Navarrete, N. (2009). Sorption isotherm and state diagram of grapefruit as a tool to improve product processing and stability. *Journal of Food Engineering* **93** (1): 52–58.

Fan X and Sokorai B (2002) Sensorial and chemical quality of gamma irradiated fresh-cut iceberg lettuce in modified atmosphere packages. Journal of Food Prot. 65: 1760–1765.

Fan X (2005) Antioxidant capacity of fresh-cut vegetables exposed to ionizing radiation. *Journal of Science Food Agriculture*. **85**: 995–1000.

Feeney M.J. (2006). Optimizing Vitamin D_2 in Mushrooms; Pilot study to expose mushrooms to ultraviolet light. *Mushroom News* **54** (5): 2-24

Ferreira, I. C. F. R., Barros, L., andm Abreu, R. M. V. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chemistry*, **16**: 1543-1560.

Ferreira, I. C. F. R., Vaz, J. A., Vasconcelos, M. H., and Martins, A. (2010). Compounds from wild mushrooms with antitumor potential. *Anti-cancer Agents in Medicinal Chemistry*, **10**: 424-436.

Feldman D., Malloy P.J and Gross C (1996) Vitamin D: metabolism and action. In: Marcus R., Feldman D., Kelsey J., eds. Osteoporosis. San Diego: Academic Press: 205-235.

Fligge M, Solanki SK (2000) The solar spectral irradiance since 1700. Geophys Res Lett 27:2157–2160.

Funebo, T and Ohlsson, T. (1998), Microwave-assisted Air Dehydration of Apple and Mushroom. *Journal of Food Engineering*, **38**: 353-367.

Gautam S., Sharma A and Thomas P (1998) Gamma irradiation effect on shelf-life, texture, polyphenol Oxidase and microflora of mushrooms (*Agaricus bisporus*). *International Journal of Food Science Nutrition* **49**: 5-10.

Gill T.P, Antipatis V.J and James W.P.T (1999) The global epidemic of obesity. *Asia Pacific Journal of Clinical Nutrition*, **8**:75.

Giri K and Prasad S (2007): Drying kinetics and rehydration characteristics of microwave-vacuum and convective hot-air dried mushrooms, *Journal of Food Engineering*. **78**: 512-521.

Goes, J. I., Handa, N., Taguchi, S. and Hama, T. (1994). Effect of UV-B radiation on a fatty acid composition of the marine phytoplankton Tetraselmis sp. - Relationship to cellular pigments. *Marine Ecology - Progress Series*. **114**: 259-274.

Gonzalez E., Gimenez M., Olarte C., Sanz S and Simon A (2000). Effect of packaging conditions on the growth of microorganisms and the quality characteristics of fresh mushrooms (A. *bisporus*) stored at inadequate temperatures. *Journal of Applied Microbiology* **89**: 624-632.

Gormley T.R. and MacCanna C. (1967) Prepackaging and shelf life of mushrooms: *Irish Journal of Agricultural Research* **6**: 255-265.

Gormley T.R. (1969) Texture studies on mushrooms: *Journal of food technology* **4**:161-169

Gothandapani L., Parvathi K and Kennedy J (1997) Evaluation of different methods of drying on the quality of oyster mushrooms: *Journal of Drying Technology* **15**: 6–8.

Guizani, N., Rahman, M. S., Klibi, M., Al-Rawahi, A. and Bornaz, S (2013) Thermal characteristics of *Agaricus bisporus* mushroom: freezing point, glass transition, and maximal-freeze-concentration condition. *International Food Research Journal* **20** (4): 1945-1952.

Guizani, N., Al-Saidi, G. S., Rahman, M. S., Bornaz, S. and Al-Alawi, A. A. (2010). State diagram of dates: glass transition, freezing curve and maximal-freeze-concentration condition. *Journal of Food Engineering* **99** (1): 92-97.

Grant W.B (2002) An ecologic study of dietary and solar ultraviolet-B links to breast carcinoma mortality rates. *Cancer* **94**: 272-281.

Ha-Duong, M., Mégie, G., and Hauglustaine, D. (2003) A Pro-Active Stratospheric Ozone Protection Scenario, *Global Environmental Change-Human and Policy Dimensions*, **13**(1): 43-49.

Haiying, W., Shaozhi, Z. and Guangming, C. (2007). Experimental study on the freezing characteristics of four kinds of vegetables. *LWT-Food science and Technology* **40**: 1112-1116.

Havinga E (1973) Vitamin D example and challenge. *Experientia* **29**: 1181-1193.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., Santos-Buelga, B., and Ferreira, I. C. F. R. (2011). Targeted metabolites analysis in wild *Boletus* species. *LWT*, **44**: 1343-1348.

Henson, S. (1995). Demand -side constraints on the introduction of new food technologies – the case of food irradiation. *Food Policy*.**20**: 111-127.

Hildebrand D.F (1989) Lipoxygenase. *Plant Physiology*. **76**: 249– 253.

Hiller S. 1994. Some properties of mushrooms. *International Agrophysics* 8: 635-642.

Hockberger P. E. (2002) A history of ultraviolet photobiology for humans, animals and microorganisms. *Photochemistry and Photobiology.* **76** (6):561–579.

Holick M.F (1995) Environmental factors that influence the cutaneous production of vitamin D. *American Journal of Clinical Nutrition* **61**(S):638S-645S.

Holick M.F (2004) Vitamin D: Importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *American Journal of Clinical Nutrition* **79***:*362-71.

Holick M.F (2007) Vitamin D deficiency. *New England Journal of Medicine* **357**: 266-81.

Holick M.F, Chen T.C, Lu Z, Sauter E. (2007) Vitamin D and skin physiology: a D-lightful story. *Journal of Bone Mineral Research*; **22**(2):28-33.

Holick MF (2013) Vitamin D: physiology, dietary sources, and requirements. In: Allen, LH, Prentice A, Caballero B (edn), Encyclopedia of Human Nutrition, (3rd edn), vol. 4, Academic Press, Oxford, UK.

Hollosy F (2002) Effect of ultraviolet radiation on plant cells. *Micron* **33**: 179-197.

Hung, P. V. and Duy, T. L. (2012). Effects of drying methods on bioactive compounds of vegetables and correlation between bioactive compounds and their antioxidants. *International Food Research Journal* **19** (1): 327-332.

Iqbal M., Haq Z., Jamil Y., and Ahmad M .R (2012). Effect of pre-sowing magnetic treatment on properties of pea. *International Agrophysics*, **26**:25-31.

Jablonski, N.G. and Chaplin, G (2002) The evolution of human skin coloration. *Journal of Human Evolution.* 39 (1): 57-106

Jamil Y., Zia ul Haq., Iqbal M., Perveen. T and Amin. N (2011) The effect of magnetic field (MF) produced by a home-made electromagnet for the treatment of spawn on its growth and yield. *International Agrophysics,* **26**: 375-380

Jasinghe V. J and Perera C. O. (2006). Ultraviolet irradiation: The generator of vitamin D_2 in edible mushrooms. *Food Chemistry,* **95**: 638–643.

Jasinghe V.J., Perera C.O and Sablani, S.S. (2007) Kinetics of the conversion of ergosterol in edible mushrooms. *Journal of Food Engineering,* **79** (3): 864-869.

Jerzy B., Gabriel C and Paskalis G. (2013). Anisotropy of mechanical properties of mushrooms (*Agaricus bisporus)*. *Polish Society of Agricultural*: Engineering ISSN 1429-7264.

Jones G., Seamark D. A., Trafford D. J. H and Makin H. L. J. (1985) Vitamin D: Cholecalciferol, ergocalciferol, and hydroxylated metabolites. In A. P. Deleenheer, W. E. Lambert and M. G.M. Ruyter. Modern chromatographic analysis of the vitamins, chromatographic science series, **30**: 73–127. New York, Basel: Marcel Dekker Inc.

Kader A. A (1986) Potential applications of ionizing radiation. *Journal American Food Technology*. **40**: 117–121.

Kalac, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, **113**: 9-16.

Kalyani. B and Manjula. K. Irradiation of Mushrooms – An Overview (2013) International *Journal of Science and Research International Journal of Science and Research*, **2**: (12)

Kamradt J., Rafi L., Mitschele T., Meineke V., Gartner B.C., Wolfgan T., Holick M.F and Reichrath J (2003) Analysis of the vitamin D system in cutaneous malignancies. *Recent Results Cancer Research*, **164**: 259-269.

Kamycheva E., Joakimsem R.M and Jorde R (2002) Intakes of calcium and vitamin D predict Body Mass Index in the population of northern Norway. *Journal of Nutrition* **132**:102-106.

Kim J. H. and Moon S. J. (2000) Time spent outdoors and seasonal variation in serum concentrations of 25-hydroxyvitamin D in Korean women. *International. Journal of Food Science Nutrition* **51** (6): 439–451.

Kira M., Kobayashi T and Yoshikawa K (2003) Vitamin D and skin. *J Dermatol* **30**:429-437.

Ko, J.A., Lee B.H., Lee S and Park H.J (2008) Effect of UV-B exposure on the concentration of vitamin D_2 in sliced shiitake mushroom (*Lentinus edodes*) and white button mushroom (*Agaricus bisporus). Journal of Agricultural Food Chemistry,* **56**: 3671–3674.

Kojo A., Suzuki Ayuho., Suzuki Akira., Cheong J.C., Nishizawa M. and Watanabe, T. (2004) Changes in shape, colour and texture of fruiting bodies of *Hypsizygus marmoreus* during post-harvest storage: 119-124

Koorapati, A., Foley, D., Pilling, R., and Prakash, A. (2004). Electron-beam irradiation preserves the quality of white button mushrooms (*Agaricus bisporus*) slices. *Journal of Food Science*, **6**: 25–29.

Kompany E and Rene A (1995): The Freeze-drying conditions for improved aroma retention in cultivated mushrooms (*Agaricus bisporus*), *LWT-Food Science and Technology Journal Elsevier*, **28**: 238-240.

Koyyalamudi S.R., Jeong S.C., Song C.H., Cho K.Y and Pang G. (2009) Vitamin D_2 formation and bioavailability from *Agaricus bisporus* button mushrooms treated with ultraviolet irradiation. *Journal of Agriculture. Food Chemistry,* **57** (8): 3351-3355.

Kramkowski R., Banasik K. and Gawlik P. (2001) The influence of temperature of freeze-drying process on the mechanical properties of dried mushrooms, *Journal of polish agricultural universities* **4** (2)

Kulwinder Kaur, Satish Kumar, and Md. Shafiq Alam (2014). Air drying kinetics and quality characteristics of oyster mushroom (*Pleurotus ostreatus*) influenced by osmotic dehydration. *Journal of Agricultural Engineering International*: **16**(3): 214－222.

Kumar V., Kumar G and Sharma P. D., (2009). Effect of osmo-convective drying on quality of Litchi. *Journal of Agricultural Engineering*, **46**(4): 31-35.

Kumari, D., Reddy, M. S. and Upadhyay, R. C. (2011). Nutritional composition and antioxidant activities of 18 different wild *Cantharellus* mushrooms of northwestern Himalayas. *Food Science and Technology International* **17** (6): 557-567.

Krokida M. K., Maroulis Z. B., Saravacos G. D., 2000, The effect of the method of drying on the colour of dehydrated products. *International. J. Food Science. Technology*. **35**: 53-59.

Lappe J.M., Travers Gustafson D., Davies K.M., Recker R.R and Heaney R.P. (2007) Vitamin D and calcium supplementation reduces cancer risk: Results of a randomized trial. *American. Journal of Clinical Nutrition*, **85** (6):1586-1591.

Lee J. S., Kim S. J., Ahn R. M., Choi H. S., Choi H. R., Yoon S. K., Hong W. S., Whang H. S., Kwon D. J and Kim Y. J. (2002) The effect of UV-B irradiation and hot-air drying on the vitamin D_2 content of shiitake mushroom (*Lentinus edodes*). *Korean J. Soc. Food Cookery Science,* **18** (2): 173–178.

Lehtonen Veromaa M., Mottonen T., Leino A., Heinonen O.J., Rautava E and Viikari J. (2008) Prospective study on food fortification with vitamin D among adolescent females in Finland: Minor effects. *British Journal of Nutrition,* **100**: 418-423.

Lewicki, P.P and Jakubczyk, E. (2004), Effect of hot air temperature on mechanical properties of dried apples. *Journal of Food Engineering*, **64**: 307-314.

Li J.Y., Huang W.N., Cai L.X and Hu W.J (2000) Effect of postharvest calcium treatments on physiological and biochemical changes in shiitake (*Lentinus edodes*). *Fujian Journal of Agricultural Science*. **15**: 43–47.

Lips P. (2006) Vitamin D physiology. *Progress in Biophysics and Molecular Biology-science direct,* **92** (1): 4–8.

Liu C., Xu H.Q., Wang H and Cai J (2003) Fresh keeping of mushroom by irradiation. *Acta Agriculture Nucleatae Sinica.* **17**: 363–366.

Lorraine B., Louise O., Magdalena S., Michael J. G and Anne P. (2011) The potential role of vitamin D enhanced foods in improving vitamin D status. *Nutrients*, **3** (12): 1023-1041.

Lopez-Briones G.L., Varoguaux P., Chambroy Y., Bouquant J., Bureau G and Pascat B (1992) Strorage of common mushroom under controlled atmospheres. *International Journal of Food Science Technology*. **27**: 493–505.

Lu Z., Chen T., Zhang A., Persons K., Kohn N., Berkowitz R., Martinello S and Holick, M. (2007) An evaluation of the vitamin D_3 content in fish: Is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? *Journal of Steroid Biochemistry Molecular Biochemistry,* **103**: 642-644.

Lukasse, L.J.S and Polderdijk, J.J. (2003), Predictive modelling of post-harvest quality evolution in perishables, applied to mushrooms. *Journal Food Engineering*, **59**: 191-198.

MacKie, R.M. (2000) Effects of Ultraviolet Radiation on Human Health. *Radiation Protection Dosimetry*. **91** (1-3): 15-18.

Madronich, S (1998). Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Journal of Photochemistry and Photobiology,* **46** (1-3): 5-19

Martinez-Soto G., Ocana-Camacho R and Paredes-Lopez (2001) Effect of pretreatment and drying on the quality of oyster mushrooms (*Pleurotus ostreatus*). *Journal of Drying Technology*, **19** (3–4): 661– 672.

Marsilio V., Lanza B., Campestre C and De Angelis M. (2000) Oven-dried table olives: textural properties as related to pectic composition, *Journal of Science Food Agriculture*. **80**:271-276.

Marriott, B.M. (1997) Vitamin D supplementation: a word of caution. *Ann International Med* **127**: 231-233.

Martinez C. D (1998). Oyster mushrooms. *McGraw-Hill Yearbook of Science and Technology 1999*. Ed.: M. D. Licker. McGraw-Hill, Inc., New York: 242-245.

Matsuoka L., Ide L., Wortsman J., Maclaughlin J., and Holick M. (1987) Sunscreens suppress cutaneous vitamin D_3 synthesis. *Journal of Clinical Endocrinol Metabolism* **64**: 1165-68.

Matsumu Y and Ananthaswamy, H. (2004) Toxic effects of ultraviolet radiation on the skin. *Toxicology and Applied Pharmacology* **195** (3):298–308.

Mattila P., Ronkainen R., Lehikoinen K and Piironen, V. (1999) Effect of household cooking on the vitamin D content in fish, eggs, and wild mushroom. *Journal of Food Composition and Analysis*, **12** :153-160.

Mattila P., Suonpaa K and Piironen V. (2000) Properties of edible mushrooms. *Nutrition*, **16** (7/8): 694–696.

Mattila P. H., Lampi A. M., Ronkainen R., Toivo J and Piironen V. (2002) Sterol and vitamin D_2 contents in some wild and cultivated mushrooms. *Food Chemistry*, **76:** 293–298.

Mau J. L., Chen P. R and Yang J. H (1998) Ultraviolet irradiation increased vitamin D2 content in edible mushrooms. *Journal of Agricultural Food Chemistry* **46**: 5269-5272.

Mau J. L., Miklus M. B and Beelman R. B (1993) The shelf life of *Agaricus* mushrooms. In C. Charalambous (Ed.), The shelf life of foods and beverages, Amsterdam: Elsevier Publishing, Co: 255–288

Mau, J. L., Lin, H. C. and Song, S. F. (2002). Antioxidant properties of several specialty mushrooms. *Food Research International* **35**: 519–526.

May E., Asadullah K and Zugel U. (2004) Immunoregulation through 1,25-dihydroxyvitamin D_3 and its analogs. *Current Drug Targets Inflammation Allergy*, **3** (4): 377-393.

Melissa Kaplan (2002) MelissaKaplan's Herp. Calcium metabolism and metabolic bone disease. Care collection

Meulemans C.C.E. (1986) The basic principle of UV sterilization of water. In Ozone+ultraviolet water treatment, aquatec Amsterdam.

Mehta R.G and Mehta R.R (2002) Vitamin D and cancer. *J. Nutrition Biochemistry* **13**: 252-264.

Mexis S. F., Badeka A. V., Chouliara E., Riganakos K and Kontominas MG (2009) Effect of γ-irradiation on the physicochemical and sensory properties of raw unpeeled 7 almond kernels (*Prunus dulcis*). *Innovation Food Science Emergence Technology*. **10**: 87-92.

Miklus M.B and Beelman R.B (1996) $CaCl_2$ treated irradiation water applied to mushroom crops (*Agaricus bisporus*) increases Ca concentration and improves postharvest quality and shelf life. *Mycologia*. **88**: 403–409.

Mitchell, J. R. (1998) Water and food macromolecules. In Hill, S.E., Ledward, D.A. and. Mitchell, J. R. (Eds.). Functional Properties of Food Macromolecules, pp. 50–76. Gaithersburg: Aspen Publishers.

Mochida, M. (1995). The use of UV sterilization in the food industry. *Journal Food Hygienic Society Japan*. **36**: 549-551

McAlindon T and Felson D.T (1996) Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. *Annals of Internal Medicine*, **125**: 353-59.

McGarry, A and Burton K.S. (1994) Mechanical properties of the mushroom, *Agaricus bisporus Mycological Research*, **98** (2): 241-245.

Napoli J L, Fivizzani M A, Schnoes H K, & Deluca H F (1979) Synthesis of vitamin D_5: its biological activity relative to D_3 and D_2. *Archives of Biochemistry and Biophysics*, **197** (1):119-125.

Need A. G., Morris H. A., Horowitz M and Nordin C (1993) Effect of skin thickness, age, body fat, and sunlight on serum 25-hydroxyvitamin D. *American Journal of Clinical Nutrition,* **58**:882-85.

Nnoaham K.E and Clarke A. (2008) Low serum vitamin D levels and tuberculosis: A systematic review and meta-analysis. *International Journal of Epidemiology* **37** (1):113–119.

Nichol R. (1985) Post-harvest physiology and storage. The biology and technology of cultivated mushroom, John Wiley and Sons Ltd, 195-210.

NIH. (2004, 2008) Dietary supplement fact sheet: Vitamin D from http://ods.od.nih.gov/factsheets/vitamind.asp

Noble R,. Rama T., Miles S., Burton S and Stephens .T (1997) Influences of compost and casing layer depths on the mechanical properties of mushrooms, *Horticulture Research International. Ann. applied. Biology.* (1997), 131: 079-090.

Norman P., Moss I., Minder S., Gosling M and Powell J (2002) Maternal and postnatal vitamin D ingestion influences rat aortic structure, function and elastin content. *Cardiovascular Res* **55**:369-74.

Nussinovitch A and Kampf N (1993) Shelf life extension and conserved texture of alginate coated mushrooms (*Agaricus bisporus*). *Journal of Food Techenology*. **26**: 469–475.

Outila, T.A., Mattila, P.H., Piironen,V.I and Lamberg-Allardt,C.J.E (1999) Bioavailability of vitamin Dfrom wild edible mushrooms (Cantharellus tubaeformis) as measured with a human bioassay. *American Journal of Clinical Nutrition,* **69**: 95–98.

Ouzouni, P. K., Petridis, D., Koller, W.- D., and Riganakos, K. A. (2009). Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. *Food Chemistry*, **115**: 1575–1580.

Ovesen L., Brot C and Jakobsen, J. (2003) Food contents and biological activity of 25-hydroxyvitamin D: A vitamin D metabolite to be reckoned with? *Annals of Nutrition and Metabolism,* **47:**107–113.

Ozzard, A., Hear, G., Morrison, G and Hoskin M (2008) Vitamin D deficiency treated by consuming UVB-irradiated mushrooms. *British Journal of Haematology,* **58**:644–645.

Park Y.K., Barton C.N and Calvo, M.S. (2001) Dietary contributions to serum 25(OH) vitamin D levels [25(OH)D] differ in black and white adults in the united states: Results from NHANES III. *Journal of Bone and Mineral Research,***16**:281.

Perez Maceda B., Lopez Bote J. P., Langa C and Bernabeu C. (1991) Antibodies to dietary antigens in rheumatoid arthritis--possible molecular mimicry mechanism. *Clinica Chimica Acta journal,* **203** (2-3): 153-165.

Perera C., Jasinghe J., Ng' F. L and Mujumdar S. (2003) The effect of moisture content on the conversion of ergosterol to vitamin D in shiitake mushrooms. *Journal of Drying Technology,* **21**:1093–1101.

Pfeifer M., Begerow B., Minne H.W., Nachtigall D and Hansen C (2001) Effects of short-term vitamin D_3 and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *Journal of Clinical Endocrinol Metabolism,* **86**:1633-1637.

Poucheret, P., Fons, F., and Rapior, S. (2006). Biological and pharmacological activity of higher fungi: 20-Year retrospective analysis. *Mycologie*, **27**: 311-333.

Rahman, M. S., Kasapis, S., Guizani, N. and Al-Amri, O. (2003). State diagram of tuna meat: freezing curve and glass transition. *Journal of Food Engineering* **57** (4): 321–326.

Rahman, M. S., Sablani, S. S., Al-Habsi, N., Al-Maskri, S. S. and Al-Belushi, R. (2005). State diagram of freeze-dried garlic powder by differential scanning calorimetry and cooling curve methods. *Journal of Food Science* **70** (2): 135–141.

Rahman, M. S. (2006). State diagram of foods: Its potential use in food processing and product stability. *Trends in Food Science and Technology* **17**: 129–141.

Rahman, M. S. (2009). Food Stability beyond water activity and glass transition: Macro-micro region concept in the state diagram. *International Journal of Food Properties* **12** (4): 726-740.

Rangel Castro J.I., Staffas A and Danell, E. (2002) The ergocalciferol content of dried pigmented and albino *Cantharellus cibarius* fruit bodies. *Mycological Research,* **106**:70-73.

Raphael J. K, Zhiren Lu, Jaimee M. B, Jennifer E. W and Michael F. H (2013) Photobiology of vitamin D in mushrooms and its bioavailability in humans, *Dermatology-Endocrinology* 5(**1**) 165–176.

Raquel P. G. and Maria J. B. (2011). Influence of freeze-drying treatment on the texture of mushrooms and onions. *Croatian Journal of Food Science Technology* **3** (2) 26-31.

Reed J.N and Tillett R.D. (1994) Initial experiments in robotic mushroom harvesting. *Mechatronics* 4: 265-279

Robert B. Beelman., Michael D. Kalaras and Ryan J. Elias. (2012) Effects of postharvest pulsed UV light treatment of white button mushrooms (*Agaricus bisporus)* on vitamin D_2 contentand quality attributes. *Journal of Agricultural and Food Chemistry,* **59**(14): 7841-7853.

Roberts J.S., Teichert A and McHugh, T.H. (2008) Vitamin D_2 formation from post-harvest UV-B treatment of mushrooms (*Agaricus bisporus*) and retention during storage. *J. Agricultural and Food Chemistry,* **56**: 4541-4544.

Roos, Y. H. (1987) Effect of moisture on the thermal behavior of strawberries studies using differential scanning calorimetry. *Journal of Food Science* **52**: 146-149.

Roulet P., MacInnes W.M., Wuersch P., Sanchez R.M and Raemy A. (1988) A comparative study of the retrogradation kinetics of gelatinized wheat starch in gel and powder form using X-rays, differential scanning calorimetry, and dynamic mechanical analysis, Food hydrocolloids, **2** (5): 381-396.

Roupas P, Keogh J, Noakes M, Margetts C and Taylor P (2012) The role of edible mushrooms in health: evaluation of the evidence. *Journal of Functional Foods* 4: 687-709.

Roy S., Anantheswaran R.C and Beelman R.B (1995) Fresh mushroom quality as affected by modified atmosphere packaging. *Journal of Food Science*. **60**: 334–340.

Roy M.K, Chatterjee S.R and Bakukhandi D (2000) Gamma radiation in increasing productivity of *Agaricus bisporus* and Pleurotus sajor-caju and enhancing storage life of P. sajor-caju. *Journal Food Science Techenology.* **37**: 83–86.

Royse D. (1997) Specialty mushrooms and their cultivation. *Horticultural Reviews*, **19**: 59-97.

Ryan R. Simon, Katherine M. Phillips, Ronald L. Horst, and Ian C. Munro (2011) Vitamin D mushrooms: Comparison of the composition of button mushrooms (*Agaricus bisporus*) treated post-harvest with UVB light or sunlight, *Journal of Agricultural Food Chemistry* **59** (16): 8724–8732.

Saad, S.M. (1978) Spectrophotometric determination of vitamin D_2 by reaction of hydrochloric acid and tetrachloroethane. *Fresenius journal of analytic chemistry*, **293** (5): 416.

Sablani, S. S., Rahman, M. S., Al-Busaidi, S., Guizani, N., Al-Habsi, N., Al-Belushi, R. and Soussi, B. (2007). Thermal transitions of king fish whole muscle, fat and fat-free muscle by differential scanning calorimetry, *Thermochimica Acta* **462**: 56–63.

Sams E. (1999): Preharvest factors affecting postharvest texture, *Postharvest Biological Technology*. **15**: 249-254.

Sayre, R. (1998) Vitamin D versus Erythema: effects of solar angle and artificial sources. In: *Biological Effects of Light, Basel, Switzerland.*

Sereana H. D and Wand .B (2000) Ultraviolet radiation. The environment fact sheet series. Ohio State University.

Seiiedlou, S., Ghasemzadeh, H. R., Hamdami, N., Talati, F. and Moghaddam, M. (2010). Convective drying of apple: Mathematical modeling and determination of some quality parameters. *International Journal of Agriculture and Biology* **12** (2): 171–178.

Shanna N.O.D., Scanlon K.S., Cogswell M.E., Gillespie C., Hollis B.W., Looker A.C, Allen C., Doughertly C., Gunter E.W and Bowman B.A (2002) Hypovitaminosis D

Shaw, N. J and Pal, B. R. (2002) Vitamin D deficiency in UK Asian families: activating a new concern. *Archives of Disease in Childhood.* **86** (3): 147-149.

Simon R, Phillips K, Horst L and Munro C (2011) Vitamin D mushrooms: comparison of the composition ofbutton mushrooms (*Agaricus bisporus*) treated postharvest with UVB light orsunlight. *J.Agric.Food Chem.***59**:8724– 8732

Simon R, Borzelleca J, DeLuca H and Weaver M (2013) Safety assessment of the post-harvest treatment of button mushrooms (*Agaricus bisporus*) using ultraviolet light, *Food and Chemical Toxicology* **56**:278–289.

Song H.P., Kim D.H., Jo C., Lee C.H., Kim K.S and Byun M.W (2006) Effect of gamma irradiation on the microbiological quality and antioxidant activity of fresh vegetable juice. *Food Microbiology*. **23**: 372-378.

Sperling L.H. (2006). *Introduction to Physical Polymer Science.* John Wiley and sons, Inc. Publication. Canada.

Stephensen C.B., Zerofsky M., Burnett D.J, Lin Y.P., Hammock, B.D., Hall, L.M and McHugh, T (2012) Ergocalciferol from mushrooms or supplements consumed with a standard meal increases 25 hydroxyergocalciferol but decreases 25- hydroxycholecalciferol in the serum of healthy adults. *Journal of Nutrition*, **142** (7), 1246– 1252.

Szczesniak A.S. and Kahn E.L. (1971) Consumer awareness of attitudes to food texture: Adults. *J. Texture studies,* **2**: 280-295.

Tangpricha V., Flanagan J.N and Whitlatch LW (2001) 25-hydroxyvitamin D-1alpha-hydroxylase in normal and malignant colon tissue. *Lancet* **357**: 1673-74.

Teichmann A., Dutta P.C., Staffas A and Jagerstad, M. (2007) Sterol and vitamin D_2 concentrations in cultivated and wild grown mushrooms: Effects of UV irradiation. *LWT Food Science and Technology* **40** (5): 815-822.

Torringa, E., Esveld, I., Scheewe, R. D., Berg, and Bartels. P., (2001). Osmotic dehydration as a pre-treatment before combined microwave-hot-air drying of mushrooms. *Journal of Food Engineering*, 49: 185-191.

Thakur, B. R. and Singh, R. K. (1995). Combination processes in food irradiation. *Trends in Food Science Technology*. **6**: 7-11.

Thomas P (1988) Radiation preservation of foods of plant origin. Part VI. Mushrooms, tomatoes, minor fruits and vegetables, dried fruits and nuts. CRC *Food Science Nutrition*. 2**6**: 313-358.

Trigos A (1997) Ergosterol content in fruit bodies of *Pleurotus* is variable. *Micologia Neotropical-Aplicada* **10**: 93-96.

Tuohimaa, P (2009) Vitamin D and Aging. *The Journal of Steroid Biochemistry and Molecular Biology* **114** (1–2): 78–84.

Tsami, E., Krokida, M. K. and Drouzas, A. E. (1999). Effect of drying method on the sorption characteristics of model fruit powders. *Journal of Food Engineering* **38**: 381–392.

Tseng Y.H and Mau J.L (1999) Contents of sugars, free amino acids and free 50-nucleotides in mushrooms, *Agaricus bisporus*, during postharvest storage. *Journal of Science Food Agriculture*. **79**: 1519–1523.

Urbain, P., Singler, F., Ihorst, G., Biesalski, H.K and Bertz H (2011) Bioavailability of vitamin D2 from UV-B-irradiated button mushrooms in healthy adults deficient in serum 25-hydroxyvitamin D: a randomized controlled trial. *Eur. J. Clin. Nutr*. 65, 965–967.

Varoquaux P., Gouble B., Barron C and Yildiz F (1999) Respiratory parameters and sugar catabolism of mushrooms (*Agaricus bisporus* Lange.). *Postharvest Biological Technology*.**16**: 51-61.

Villaescusa, R and Gil, M.I. (2003), Quality improvement of *Pleurotus* mushrooms by modified atmosphere packaging and moisture absorbers. *Postharvest Biological. Technology,* **28**:169-179.

Vizhanyo T and Jozsef (2000). Enhancing colour difference in images of diseased mushrooms. *Computers and Electronics in Agriculture* **26**: 187–198.

Walde G., Velu V., Jyothirmayi T and Math R.G. (2006): Effects of pretreatments and drying methods on dehydration of mushroom, *Journal of Food Engineering*. **74**: 108-115.

Wang T.J., Pencina M.J., Booth S.L., Jacques P.F., Ingelsson E., Lanier K., Benjamin E.J., D'Agostino R.B., Wolf M and Vasan, R.S. (2008) Vitamin D deficiency and risk of cardiovascular disease. *Circulation* **117**: 503-511.

Wang L., Manson J.E., Song Y and Sesso H.D. (2010) Systematic review: Vitamin D and calcium supplementation in prevention of cardiovascular events. *Annals of internal medicine,* **152** (5): 315–323.

Webb A.R., DeCosta B.R., and Hollick M.F. (1989) Sunlight regulates the cutaneous production of vitamin D3 by causing photo-degradation. *Journal of Clinical Entomology and Metabolism,* **68**: 882-887.

Wharton B and Bishop N (2003). Rickets. *Lancet* **362**: 1389-400.

WHO, Obesity: preventing and managing the global epidemic. 1997: Geneva.

Westerdahl J., Ingvar C., Masback A and Olsson H. (2000). Sunscreen use and malignant melanoma. *International journal of cancer,* **87** (1): 145–150.

Wortsman J., Matsuoka L.Y., Chen T.C., Lu Z and Holick M.F (2000) Decreased bioavailability of vitamin D in obesity. *American Journal of Clinical Nutrition* **72**: 690-693.

Xia Z.L., Xiong X.Y and Jiang X.J (2005) The studies on *Pleurotus pulmonarius* by 60Co gamma irradiation. *Acta Laser Biological Sinica*, **4**: 60–64.

Xiong Q.L., Xing Z.T., Feng Z., Tan Q and Bian Y.B (2009) Effect of 60Co g-irradiation on postharvest quality and selected enzyme activities of *Pleurotus nebrodensis*. *Food Science Techenology,* **42**: 157–161.

Yaqvob M, Gholamali P, Farhood Z, Mahmood G and Vahid S, (2013) Improvement of shelf-life and postharvest quality of white button mushroom by 60Co γ-ray irradiation. *Plant Knowledge Journal Southern Cross Publishing Group*, **2**(1): 1-7

Ye H., Chen J.X., Yu R.C., Chen Q.L and Liu W (2000) The effect of irradiation on stored straw mushroom and the physiological mechanism. *Acta Agricultural Nucleatae Sinica*, **14**: 24–28.

Yokokawa H and Mitsuhashi T (1981) The sterol composition of mushrooms. Journal of *Photochemistry*, **20** (6): 1349-1351.

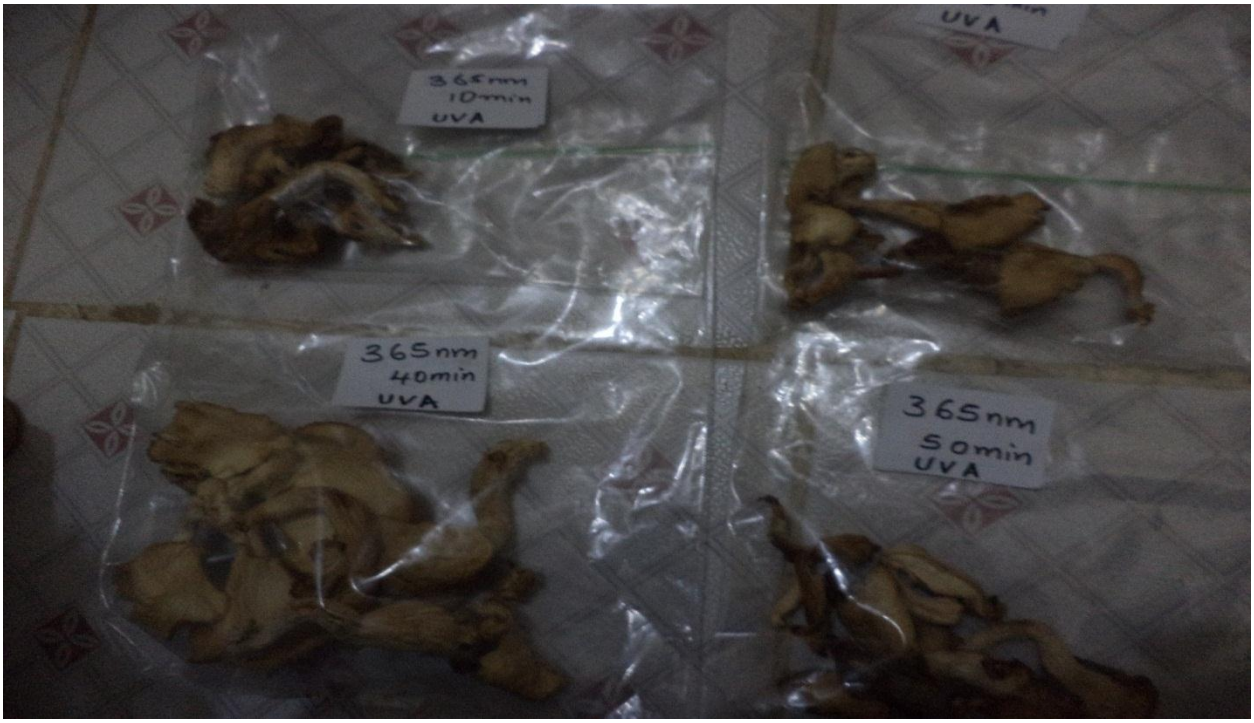
Zittermann A., Gummert J and Borgermann, J. (2009) Vitamin D deficiency and mortality. *Current opinion in clinical nutrition and metabolic care* **12** (6): 634–639.

APPENDICES

DMA (2980) Equipment.

Fig: Compression clamp

Harvested irradiated mushroom samples.



Dried oyster mushroom samples.