EFFECT OF PLASTICIZER CONCENTRATION ON THE SENSORY, MECHANICAL AND BARRIER PROPERTIES OF CASSAVA STARCH-MUSHROOM (*PLEUROTUS PULMONARIUS)* EDIBLE FILMS

***Ojo Mofoluwaso Olufunmilola[1], Igbabul Bibiana Dooshima[2] Alkali Shian Joseph[2]**
*Corresponding author
1 Department of Food Science and Technology, Federal University of Technology Minna Niger State Nigeria
2 Department of Food Science and Technology, Unversity of Agriculture, Makurdi. Benue State Nigeria

ABSTRACT: *Edible films were produced from 6g blends of cassava starch (CS) and mushroom from cassava starch and mushroom (Pleurotus pulmonarius) composite flours were prepared using cassava starch flour (CS) and mushroom (MF) ratios of 100:00, 90:10, 80:20, 70:30 and 60:40 CS: MF with glycerol as a plasticizer in varying levels of 0%, 2%, 4%, 6% and 8% using the casting method and the mechanical, barrier and sensory properties were studied. Films were formed in all the suspensions in this study and the addition of plasticizer to these film-forming solutions helped to overcome the brittleness and fragile nature of the unplasticized cassava starch mushroom (Pleurotus pulmonarius) films. Results of the mechanical properties of the CSMF films ranged from 1.27 to 10.11Mpa, 5.09 to 21.71mm and 19.15 to 24.53 MPa respectively. Elongation at break (EAB) increased with glycerol concentration up to 4% and decreased at higher concentrations. Water vapour permeability (WVP) and film solubility (FS) ranged from 6.03 to 9.98 g mm m^{-2} d^{-1}kPa and 15.76 to 39.79% respectively. There was a general Increase in WVP and FS with the increase in glycerol content and lower mushroom flour inclusion. Film thickness (FT) ranged from 0.15 to 0.44mm. FT decreases with an increase in glycerol concentration. The sensory attributes indicated that the increase in MF substitution resulted in increased opacity and improved flavour with higher acceptability at lower substitution while at higher concentrations (6 and 8%) of glycerol lower sensory scores were observed. However, CSMF 90:10% at 2% glycerol was the most acceptable. Glycerol behaved like a typical plasticizer. Edible films had substantial barrier properties and mechanical strength to withstand stress during handling. Cassava starch and mushroom (P. pulmonarius) based edible films could be used in food packaging and agricultural industries*

KEYWORDS: edible films, cassava starch, mushroom (*p.pulmonarius*) glycerol, sensory, mechanical and barrier properties.

INTRODUCTION

Edible films and coatings are any non-rigid, thin material used for wrapping or coating food materials and drugs to extend the shelf life of the product which may be consumed together or separated before consumption (Erkmen and Barazzi, 2018). Edible films act as a barrier between food components and the environment, thereby enhancing the

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mechanical properties of some fragile products thus reducing mechanical damage during transportation, processing and storage (Hans and Gennadios, 2005; Skurty *et al*., 2010). Edible films material should not alter the appearance, smell, and taste of the product therefore, film material should be as thin as possible acquiring adequate mechanical properties to protect food material. Edible films and coatings invented now are specially produced to increase their functionalities by incorporating natural or chemical antimicrobial agents, antioxidants, enzymes or functional ingredients such as probiotics and nutraceuticals (Embuscado and Huber, 2009). Edible films can enhance the nutritional value of foods by carrying basic nutrients and/or nutraceuticals in their matrix (Joblin, 2004). Films formations require the use of at least one component capable of producing a structural matrix with sufficient cohesiveness and additives that improve the functionality and efficiency of the films (Donhowe and Fennema, 1994). In the recent past, proteins, polysaccharides and lipids have been utilized as base materials in edible film production (Bourtroom, 2009), polysaccharides such as starches, cellulose derivatives and plant gums have been extensively studied as edible films and coatings for food packaging and preservation. Starch solutions and gels have been used in several studies as base film cast in food packaging (Liu and Han, 2005; Lourdin*et al*., 1997; Zhang and Han, 2006). Plasticizers used are polyhydric alcohols such as glycerol and sorbitol.

Cassava (*Manihot esculenta*) is a major source of starch which is widely cultivated as an annual crop in tropical and subtropical regions. According to FAO (2014). Africa accounts for over 54% of the world's cassava with Nigeria at the foremost globally producing above 54.8 million metric tonnes (MT) annually. Starch is a polysaccharide consisting of amylose and amylopectins fractions made of glucose moieties linked by glycosidic bonds. Cassava starch has been extensively used to produce films (Souza *et al*., 2012; Belibi*et al* 2014; Muller *et al*.,2011; Praseptiangga *et al*., 2017) and was described as odourless, isotropic, tasteless, non-toxic, colourless and biologically degradable. Cassava starch is largely considered material for edible films production due to its high digestibility, low production cost, high degree of decomposition in the natural environment, and nutritional benefits.

Oyster mushrooms (*Pleurotus spp*) are edible and constitutes the third group of mushroom that is a good source of protein and fibre. Their nutritive value varies according to the genetic structure of species, physical, chemical and substrate composition of growing medium with the time of harvest. Edible mushrooms contain the most commonly occurring non-essential amino acids needed for human nutrition and all the essential amino acids as well as lysine which is deficient in most cereals is the most abundant amino acid in mushrooms (Sadler, 2003). In recent times, there have been increased interests in the utilization of mushrooms for human nutrition, medicinal uses, bioremediation and bioconversion of lignocellulose wastes and recycling of agricultural residues (Okeke *et al*., 2003).

Plasticizers are integrated into edible coatings and films and are widely used in polymer industries as additives (Białecka-Florjanczyk and Florjanczyk, 2007). Hydrophilic compounds such as glycerol, glycol, sorbitol and glucose are commonly used as

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plasticizers in hydrophilic formulations and recently, disaccharides such as sucrose and monosaccharides (e.g., fructose, and mannose) have been investigated (Zhang and Han, 2005 and Sothornvit and Krotcha, 2005). Plasticizers type and concentration affect the film properties (Siepmann *et al*., 1997; McGinity, 1997). However, the most efficient plasticizers will generally have a similar structure to the polymers they plasticize (Cao *et al*., 2009). Plasticizers affect both barrier and mechanical properties by modifying the film structure, chain mobility and diffusion coefficient of permeants by interfering with the polymeric chains to increase their flexibility, workability, or distensibility. Plasticizer has an affinity for water molecules around it thereby reducing intermolecular interactions of the main constituents (Ke and Sun, 2001). Polyols such as sorbitol, glycerol and polyethene glycol are the major plasticizers used have employed.At the end of the study, it is expected that the developed cassava starch and mushroom (*P. pulmonarius*) biodegradable films will fill the gap of the rising consumer needs for safe, healthy and environmentally friendly packaging films.

MATERIALS AND METHODS

Sample Collection

Cassava cultivar (TMS 30572) and mushroom (*P. pulmonarius*) fruiting bodies were obtained from Benue State Agricultural Development Authority and Federal Institute of Industrial Research Oshodi (FIIRO) Lagos Nigeria respectively.

Sample Preparation

Cassava Starch extraction: Starch was extracted using the wet method as described by (Benesi *et a*l., 2004) with slight modification. Two kilograms (2kg) of fresh tubers were peeled, washed, chopped into approximately 1cm cube and crushed to produce a pulp which was then suspended in ten times its volume of deionised water and stirred for 5 min. This was sieved using double-layered cheesecloth to remove the fibrous material leaving the starch solution. The filtrate was allowed to stand for 2 hours and the water was decanted. The mixture was stirred again for 5 min and filtration was repeated until it was considerably free of fibre deposit. The starch from the filtrate was allowed to settle, the sediment was removed and dried in the oven (PEK medical) at 40^{o}C for 72 hours, milled into powder, sieved and packaged.

Preparation of mushroom flour

Freshly harvested mushroom (*P. pulmonarius*) fruiting bodies were cleaned, sorted, sliced and dried in the oven at 50^{o}C for 24hrs, milled with the use of attrition mill (Model R175A) into flour. The flour was sieved, properly packaged in a transparent polyethene bag and stored.

Production of edible films

Cassava starch (CS) and mushroom (*P. pulmonarius*) flour (MF) were mixed at ratios 100:0, 90:10, 80:20, 70:30, 60:40 w/w CS: MF using a household Kenwood mixer maintained at speed No. 5 for 20min to achieve homogenous blending. The cast method described by (Araujo –Farro *et al*., 2010) was adopted for the preparation of the cassava

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starch and mushroom (CSMF) based edible films. Preliminary studies in the laboratory showed that cassava starch and mushroom (*P. pulmonarius*) flour blends at a concentration less than 6g and at glycerol concentration above 10% is not sufficient to obtain a strong supporting matrix as the resulting films were soft, sticky and peeling was difficult. Therefore, 6g was selected as the optimum concentration of the flour samples of cassava starch mushroom in the formation of the edible films using glycerol concentration at 0, 2, 4, 6 and 8%.

Film solutions were prepared by mixing 6g of each blend with glycerol at 0, 2, 4, 6, and 8% levels respectively using distilled water. The suspensions were stirred for 1h on a magnetic stirrer (250 rpm) at room temperature to obtain the film-forming mixtures. Each film-forming suspension was heated in a water bath at 80^{o}C for 15min with continuous stirring using a glass rod to obtain the film-forming solution. Further heating continued for 15min, cooled to 50^{o}C and cast unto flat levelled non-stick trays to set. Once set they are dried in an oven (PEK Medical, USA) with air circulation at 40°C for 48h. The dry films sheets were carefully peeled off the trays, packaged in polythene pouches and stored in dry shelves.

Film conditioning

The films were stored at controlled conditioned of 25^{o}C and 53% relative humidity in desiccators over diluted sulphuric acid for 48h prior to use

Cassava Starch and Mushroom (*P. pulmonarius*) Films Characterization

Mechanical properties

The mechanical properties (tensile strength, elongation at break and Elastic modulus) were determined using a Tensometer (Berlin Germany). The method described by Thomazine *et al*. (2005) was adopted. Samples were cut into small rectangles (25 × 100 mm) and fixed in the grips probe. The initial grip separation distance was fixed at 50 mm and the moving rate set was 0.9 mm/s. The tensile strength (force at the break/initial cross-sectional area) and elongation at break ($\Delta l/l_o$) directly from the stress-strain curves and the elastic modulus was calculated as the slope of the initial linear portion of this curve.

Barrier Properties

Water vapour permeability

The method of Bertruzzi *et al.* (2003) was adopted for the determination of water vapour permeability. The conditioned films were sealed on the mouth of glass dishes containing distilled water with silicon adhesive to give a good seal. The glass dishes were placed in desiccators at 25^{o}C and 52% humidity maintained respectively using a saturated sulphuric acid solution in thermostatically controlled incubators. The water vapour transferred through the films was determined by measuring the weight change periodically until a constant weight was reached within 6 h. Weight loss was plotted over time to obtain a moisture transmission curve. The permeabilities were calculated

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from the slopes of the linear regression of weight loss versus time. Water vapour permeability was calculated from the following equation:

$$WVP = CX/A\Delta P \quad (1)$$

Where X= Film thickness (m), A is the area of the exposed film (m^2),
ΔP = Water vapour differential across the film and
C =The slope of a plot of weight change of the dish versus time.
Slopes were calculated by linear regression

Film solubility

Film solubility (FS) in water is defined as the content of dry matter solubilized after 24 h of immersion. A modification of the method described by Jangchud and Chinnan (1999) was used to measure film solubility. Film pieces of 20mm x 20mm were dried in a vacuum oven for 24h and then weighed to the nearest 0.0001g for the initial dry mass. Films were immersed into 20mls of distilled water in a 50ml screw cap bottle containing 0.01g/100ml sodium benzoate. The tubes were capped and placed in a water bath in a shaking water bath for 24h at 25^{o}C. The remaining solution and film paper were poured into filter paper, rinsed with 10ml of distilled water and dried at 70^{o}C in a vacuum oven for 24h to determine the dry mass of the film. The total soluble matter was calculated from the initial mass and the final dry mass using the following equation:

$$\%\ \text{Film solubility} = \frac{\text{Film mass before test} - \text{Film mass after test}}{\text{film mass before the test}} \quad (2)$$

Film thickness

Film thickness was measured with a handheld digital micrometre (Mitutoyo, Japan) having a sensitivity of 0.001mm. Ten thickness measurements were taken randomly at different points on each cassava starch mushroom edible film and the mean values were calculated.

Sensory evaluation

The method described by Ihekoronye and Ngoddy (1985) was adopted using a twenty-member panel for sensory evaluation of cassava starch mushroom (*P. pulmonarius*) films. All panel members were trained by the open discussion method prior to sensory tests. Edible film samples from the various blends were provided in coded white plastic plates. The order of presentation of samples to the panel was randomised. Each sensory attribute was rated on a 7-point Hedonic scale and evaluated for transparency (extremely transparent to opaque), flavour (extremely pleasant to nauseating), mouthfeel (extremely chewable to putty-like), appearance (extremely appealing to extremely unappealing) and general acceptability (highly acceptable to total reject). Crackers and cold water were provided to panellists to rinse in between tastes to minimise the residual effects of the films in the mouth.

Statistical analysis

The data obtained from this study were subjected to one-way analysis of variance (ANOVA) conducted on each of the variables and the least significant difference (LSD) test at the significant level of $P<0.05$ was performed using SPSS software (version 20, SAS, Inc., New York, NY) for Windows to compare the difference between treatment means. Results were expressed as the mean± standard deviation of triplicate determination.

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RESULTS AND DISCUSSION

Mechanical Properties of Cassava Starch Mushroom (*P. pulmonarius*) Films as influenced by glycerol concentration

In this experiment, edible films from cassava starch and mushroom flour without plasticizer were observed to be relatively brittle and broke easily and this made handling difficult when cutting to obtain the appropriate dimension for mechanical properties analysis. As a result, the mechanical properties for the edible films at 0% glycerol were not carried out. According to Garcia *et al*. (2002), unplasticized matrices are often brittle and rigid once dried and due to strong interactions between polymer chains that favours crystal development which may results in defects or cracks on the films when peeled off. This may also account for the rigidity of cassava mushroom (*P. pulmonarius*) edible films without glycerol (plasticizer) and subsequent cracking when cutting hence the difficulty in handling. Similar effects were observed in the unplasticised films from mungbeans and soy-protein (Wittaya, 2015) and edible films from chia seed mucilage (Dick *et al*., 2015).

The mechanical properties of the edible films prepared from cassava starch mushroom (*P. pulmonarius*) edible films are shown in Table 2. The TS ranged from 1.27Mpa to 10.11Mpa at CS: MF 60:40 and100:00 respectively.100% cassava starch films had higher values than the composite films. The tensile strength (TS) expresses the maximum stress developed in a material during the tensile test. It offers a measurement of integrity as well as heavy-duty use of films (Perez *et al*., 2012). Edible films as food packaging function to protect food during handling, transportation and marketing. Therefore, high TS is required for edible films, though this depends on the intended application of the films (Chinma*et al*., 2012; Llker *et a*l., 2012; Pital and Rakshit, 2011). Decrease in TS as the concentration of the glycerol and mushroom (*P. pulmonarius*) flour (MF) incorporation increased in the films was observed could be explained by the loss of intermolecular interactions among cassava starch molecules and mushroom (*P. pulmonarius*) flour (Warkoyo *et al*., 2014; Sobral *et al*., 2001) observed that the increase in plasticizer concentrations increased the moisture content of the film because of its high hygroscopic nature, which also contributes to the reduction of the forces between the adjacent macromolecules. In addition, Liu *et al*. (2013) reported that increasing the glycerol concentration in the manufacture of starch-chitosan films tend to lower TS values. The TS observed in this study are lower than that reported by Chinma *et al.* (2012) on edible films from cassava starch-soy protein isolate edible films but higher than that reported by Castro de Cruz (2012) on edible films from cassava starch – glycerol blends incorporated with nutraceuticals. The phenomenon of a decrease in TS with an increase in plasticizer concentration has been reported by several other researchers (Saremnezhad *et al*., 2011; Arrieta *et al*., 2013; Wiset*et al*., 2014). On the basis of high TS requirements, edible films at 2% glycerol at various compositions may be applicable.

In this study, elongation at break (EAB) values varied between 5.09 to 21.71mm at CSMF 60:40, 8% glycerol and 80:20, 4% glycerol respectively. There was an increase

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in the EAB values up to 4% glycerol in the films and a decrease was observed as the glycerol content increases. The elastic modulus (EM) for the edible films varied between 19.15 to 24.53Mpa at CSMF 60:40, 6% glycerol and 100:00, 2% glycerol. There was an observed decrease in the elastic modulus (EM) as the mushroom substitution increases. Elongation at break (EAB) describes the nature of the film's plasticity the ability of the films to extend before breaking. Plasticity or extensibility is generally required for a film to retain its integrity when applied to food products (Galus and Lenart, 2013). Concentration, type of base material, and solvent used in the manufacture of films are factors determining EAB. An increase in EAB as the concentration of the glycerol increased to 4% followed by a decrease at higher glycerol concentration may be due to the addition of glycerol which promotes the interactions among cassava starch, mushroom (*P. pulmonarius*) and glycerol through hydrogen bonding in films (Oses *et al*., 2009), and a reduction in the strength of intermolecular forces that improves mobility between molecular chains leading to an increase in elongation may have occurred up to 4% concentration. The film breakage leading to low EAB values at above 4% glycerol did not occur as a result of the film brittleness but as the concentration of the glycerol increased the films were more ductile resulting in easy breakage (Katili *et al*., 2013). In addition, the mushroom (*P. pulmonarius*) flour may have caused a rearrangement in the film's network, inability to form a cohesive and continuous matrix thus decreasing the film resistance to elongation and therefore reduced stretch-ability of the mushroom included edible films. A similar result was observed by Peroval *et al*. (2004). In relation to thickness, a more stretchable matrix was formed in thicker films, possibly as a result of these films having a better organization of the cassava starch – mushroom network chains and a greater cross-sectional area which permits greater extension under stress than the thinner films. Thinner films reach a limit in their ability to stretch quicker than thicker films. (Jansson and Thuvander, 2002) found that starch film strength and strain showed a strong dependence on film thickness.

Elastic modulus (EM) is a fundamental measure of the film stiffness, the higher the EM, the higher the stiffness of a material. Higher stiffness of edible films has been reported to be advantageous (Mali *et al*., 2002; Chinma *et al*., 2012). High EM has been correlated with high peak viscosity (Ojo *et al*., 2017; Chinma *et al*., 2012). Higher viscosity value has been reported in our earlier studies on cassava starch mushroom (*P. pulmonarius*) flour blends (Ojo *et al*., 2017), higher peak viscosity was observed in 100% cassava starch than in the composite blends therefore high EM values at higher concentration of cassava starch at different glycerol concentration may be attributed to the high peak viscosity. Furthermore, a decrease in EM as glycerol concentration increases may be attributed to plasticising effect of glycerol due to decrease in starch-mushroom flour interactions and increase in the mobility of the chain allowing the films to be less resistant and more elastic.

Barrier Properties of Cassava Starch Mushroom (*P. pulmonarius*) Edible Films

The results of the water vapour permeability (WVP) are as shown in Table 3. In this study, water vapour permeability (WVP) ranged from 5.73 to 9.98g mm m^{-2}day kPa at CS:MF 90:10%, 2% glycerol CS:MF 70:30%, 8% glycerol respectively while the film

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solubility (FS) values ranged from15.76% to 39.79% at CSMF 60:40, 0% glycerol and CSMF 100:00, 8% glycerol respectively. Thin and flexible films were obtained from plasticised cassava starch mushroom (*P. pulmonarius*)films with film thickness (FT) ranging from 0.15mm at CSMF 70:30 and 80:20 at 8% glycerol to 0.44mm at CSMF 90:10 and 60:40 at 0% glycerol concentration. This result implies that there was a significant ($p<0.05$) difference in the barrier properties of the samples.

Permeability properties of the biomaterial films are significant as the films may be used in coating or packaging in order to protect products against water vapour or gases. Water can increase the rate of several reactions such as browning, lipid oxidation, vitamin degradation and enzyme activity, increase the rate of micro-organism growth and cause texture change, all of which are related to food shelf life and quality (Alves*et al*., 2007). One of the main functions of a food packaging is often to avoid or reduce moisture transfer between the food and the surrounding atmosphere, or between two components of a heterogeneous food product (Gontard*et al*., 1993). This function of packaging materials to minimize moisture transfer between the food and the surrounding environment is a crucial property for effective food packaging (Mali *et al*., 2004; Dias *et al*., 2010). Water vapour permeability (WVP) should be as low as possible in order to optimize the food package environment and potentially increase the shelf life of the food product (Hosseini *et al*., 2013). Cassava starch and mushroom (*P. pulmonarius*) edible films without glycerol had the lowest WVP values in all the samples, an increase in WVP as the glycerol concentration increases were also observed. These results could be related to the structural modifications of the starch-mushroom flour network produced by the plasticizer and to the hydrophilic nature of glycerol which supports the absorption and desorption of water molecules. A similar result was reported on hydrophilic films such as wheat gluten films (Gontard *et al*., 1993).

Plasticizer concentration and hydrophilic nature were found to be important factors in determining the moisture affinity of cassava starch films (Lagos *et al*., 2015; Mali *et al*.,2005) Factors that affect permeability also includes pores or cracks in the film permeating molecules which can penetrate through the film without any resistance. However, the use of a plasticizer like glycerol avoids or minimizes cracking of films during handling and storage and on the other hand, increases gas, water vapour and solute permeability of the films ((Lagos *et al*., 2015; Mali *et al*.,2005; Pavlath and Orts, 2009). With regards to synthetic polymers, the WVP obtained in this study was higher than that of low-density polyethylene (0.08gmm m^{-2}KPa) and high-density polyethylene (0.02 gmm m^{-2}KPa) but compare favourably with cellophane (7.27 gmm m^{-2}KPa). However, cassava starch mushroom (*P. pulmonarius*) films permeabilities were higher than cassava starch-based edible films (4.64 gmm m^{-2}KPa) as reported by Chinma *et al*. (2012) using cassava starch and soy protein concentrate and on the other hand lower than the values (23.28gmm m^{-2}KPa) reported by Riani *et al*. (2016).

Film solubility is advantageous in situations when the films will be consumed with a product that is heated prior to consumption and may also be an important factor that determines biodegradability of films when used as packaging material as high solubility

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would indicate lower water resistance (Hosseini *et al*., 2013). The 100% cassava starch film had a higher FS value than CS: MF composite films. It was also observed that high FS was influenced by the glycerol level and mushroom flour ratio, FS increased significantly ($P < 0.05$) with an increase in glycerol and decrease with an increase in MF ratio in the blends.

The increase in FS as glycerol concentration increases could be explained that at higher content of plasticizer, more molecules of plasticizer were untrapped in the cassava starch and mushroom (*P. pulmonarius*) flour cross-linked network and were able to escape into solution. While, lower content of plasticizer resulted in reduced plasticizer molecules untrapped in the crosslinked network and less able to escape into solution (Wittaya, 2013). Film solubility also decreased with the increased concentration of mushroom (*P. pulmonarius*) flour. Results obtained in this study were in agreement with the results of Bourtroom and Chinnan (2008).

Effect of Glycerol Content on the Sensory Properties of Cassava Starch Mushroom (*P. pulmonarius*) films

The results of the sensory properties of the edible films prepared from the cassava starch and mushroom (*P. pulmonarius*) flour is presented in Table 1.The sensory panellist rating for transparency ranged from 1.53 at CSMF 70:30, 8% glycerol and 60:40 at 6% glycerol to 6.85 (CSMF 100:00 at 2% glycerol). There was a significant difference ($p<0.05$) between the samples at lower mushroom substitution and at higher glycerol content. The 100% cassava starch at the different glycerol concentrations had the highest rating for transparency and there was an observed increase in opacity of the composites blends as MF increases.

All the composite films were slightly yellow – deep brown in appearance. As mushroom-cassava starch concentration of the film-forming solution increased, the colour of the films became more intensively brown. The appearance and general acceptability of biofilms is an important factor that is influenced by the colour. It determines to extent marketability, consumer acceptability and suitability for various applications (Srinivasa *et al*., 2003). High transparency value rating for 100% cassava starch in all the glycerol concentrations may be due to more preference for clear films (Sivarooban *et al*., 2008). The observed decrease in transparency of the composites blends is expected because the colour of the mushroom (*P. pulmonarius*) flour after drying was not as white as the cassava starch flour and further heating in the production of the edible films may have led to non-enzymatic browning due to the interaction of the protein amino acid and reducing sugars. This explains the least value of 1.53 at cassava mushroom (*P. pulmonarius*) 70:30 and 60:40 at 6 and 8% glycerol respectively.

Flavour ratings for the edible films ranged from 4.20 to 5.80 at CSMF 100:00 and 80:20 at 8% and 6% glycerol respectively. Generally, the composite blends had higher ratings than the 100% cassava starch edible films. There was no significant ($p\leq0.05$) difference in the flavour ratings at CSMF 70:30 and 60:40 in all the different glycerol concentrations. Composite blends had a higher flavour rating than the 100% cassava starch films. This may be attributed to the natural flavour found in mushrooms in

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addition to the flavour development which may have occurred during heating. It was observed that there was a significant ($p \leq 0.05$) difference amongst the films at cassava starch mushroom *(P. pulmonarius)* flour blends up to 80:20 and at the higher mushroom substitution of 70:30 and 60:40 there was no significant ($p \leq 0.05$) difference in the flavour of the films. Therefore, with respect to the flavour of the cassava starch mushroom *(P. pulmonarius)* edible films, the 70:30 is the optimal concentration further increase does not affect the flavour of the food.

Mouthfeel, which is used to determine the stickiness and chewability values of the film ranged from 2.60 to 6.40 both at 100% cassava starch, 8% and 2% glycerol. Films at 8% glycerol in all had the lowest rating. Stickiness and taste are important sensory attributes providing crucial information on the applicability of edible films and coating on food surfaces as protective layers (Kester and Fennema 1996). Generally, it is observed that the films produced at 8% glycerol had higher sticky surfaces in all the blends and therefore lower values than other composite blends. The stickiness could be attributed to phase separation and migration of glycerol onto the surface of the films. Phase separation of glycerol from the starch matrix at a higher glycerol level (27%) has been reported (Liu and Han, 2005), which may occur as a result of the increase in glycerol hence increase in water content in the film resulting in a decrease hydrogen bonding between cassava starch- mushroom (*P. pulmonarius*) at increased polyol concentration and polyoldiffusivity, and thus, phase separation may occur at higher concentration of glycerol (Godbillot *et al*., 2006).

The sensory scores for appearance ranged from 3.00 to 6.60, edible films from cassava starch mushroom (*P. pulmonarius*) blend at 60:40 and at 8% glycerol had the lowest and rated unappealing in appearance while the 100% cassava starch at 2% glycerol extremely appealing. Stickiness and taste are vital sensory attributes that provide essential information on the applicability of edible films and coating on food surfaces as protective layers (Kester and Fennema, 1986).

The increase in stickiness may be attributed to the high swelling power of cassava starch as reported by Srichuwong *et al*. (2005) and Chinma *et al*. (2012). Films stickiness has been reported to depend on the high water absorption capacity and swelling power of starch granules. Overall acceptability of edible films has been reported to be affected by the transparency, flexibility, brittleness and elasticity (Perez- Gallardo *et al*., 2012). As a qualitative observation, the surfaces of all plasticized films were smoother and more uniform than the unplasticized (control) film yielding a rough surface appearance. This may have contributed to the low overall acceptability of the unplasticized (0% glycerol) in all the various cassava starch mushroom blends. Overall acceptability ranged from 2.60 to 6.50 at CSMS 60:40, 8% glycerol and CSMS 90:10, 2% glycerol. The edible films from the 8% glycerol in all the various compositions were the least accepted.

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CONCLUSION

Cassava starch and mushroom (*P. pulmonarius*) films at 80:20 at 2% glycerol and 80:20 at 4% glycerol had higher values in terms of mechanical and barrier properties respectively. However, 90:10 at 2% glycerol films showed superiority in terms of sensory attributes. Edible films had substantial barrier properties and mechanical strength to withstand stress during handling. The film could be used in the food industry, pharmaceutical industry and agricultural industry.

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Table 1: Effect of Glycerol on the Sensory Properties of Cassava Starch and Mushroom (*P. pulmonarius*) Films

Sample CS: MF	**Glycerol** (%)	**Transparency**	**Flavour**	**Mouthfeel**	**Appearance**	**Overall Acceptability**
100:00	0	$6.70^{ab}\pm0.17$	$4.40^{bc}\pm0.51$	$5.20^{b}\pm0.70$	$3.60^{e}\pm0.51$	$3.10^{e}\pm0.66$
	2	$6.85^{a}\pm0.10$	$4.60^{bc}\pm0.51$	$6.40^{a}\pm0.10$	$6.60^{a}\pm0.10$	$6.30^{a}\pm0.10$
	4	$6.30^{bc}\pm0.57$	$5.00^{ab}\pm0.10$	$4.40^{c}\pm1.24$	$5.60^{b}\pm0.90$	$6.00^{b}\pm0.24$
	6	$5.95^{c}\pm0.16$	$4.80^{a}\pm0.41$	$3.93^{c}\pm0.16$	$5.00^{c}\pm1.10$	$4.80^{c}\pm0.10$
	8	$5.85^{c}\pm0.83$	$4.20^{c}\pm1.20$	$2.60^{d}\pm0.50$	$4.20^{d}\pm0.77$	$3.60^{d}\pm0.13$
90:10	0	$5.40^{ab}\pm1.12$	$5.10^{ab}\pm0.65$	$5.00^{b}\pm0.93$	$3.60^{c}\pm0.51$	$4.25^{c}\pm0.59$
	2	$5.73^{a}\pm0.70$	$5.70^{a}\pm0.51$	$6.00^{a}\pm0.66$	$6.40^{a}\pm0.83$	$6.50^{a}\pm0.20$
	4	$4.93^{bc}\pm1.16$	$5.40^{a}\pm0.50$	$4.80^{b}\pm0.70$	$5.60^{b}\pm1.06$	$5.80^{b}\pm0.51$
	6	$4.47^{c}\pm0.92$	$5.20^{ab}\pm0.41$	$4.10^{c}\pm0.59$	$5.40^{b}\pm0.83$	$3.90^{d}\pm0.62$
	8	$3.87^{d}\pm0.74$	$4.80^{b}\pm1.01$	$3.00^{d}\pm0.82$	$3.73^{c}\pm0.70$	$2.75^{e}\pm0.55$
80:20	0	$4.25^{a}\pm1.40$	$5.20^{bc}\pm0.40$	$4.67^{b}\pm0.49$	$3.40^{c}\pm0.83$	$3.00^{d}\pm1.13$
	2	$4.35^{a}\pm1.30$	$5.60^{ab}\pm0.15$	$5.40^{a}\pm0.51$	$6.00^{a}\pm0.65$	$6.20^{a}\pm0.48$
	4	$3.85^{ab}\pm1.63$	$5.80^{a}\pm0.40$	$5.00^{ab}\pm0.30$	$5.13^{b}\pm0.51$	$5.60^{b}\pm0.57$
	6	$3.47^{bc}\pm0.74$	$5.20^{bc}\pm0.77$	$3.80^{c}\pm0.41$	$4.80^{b}\pm0.69$	$4.20^{c}\pm0.41$
	8	$2.60^{c}\pm0.83$	$4.80^{c}\pm1.01$	$3.40^{c}\pm0.50$	$3.60^{c}\pm0.63$	$3.33^{d}\pm0.49$
70:30	0	$4.40^{a}\pm0.91$	$5.00^{ab}\pm0.65$	$4.80^{b}\pm0.40$	$3.60^{cd}\pm1.55$	$2.80^{e}\pm1.01$
	2	$3.33^{b}\pm1.18$	$5.40^{a}\pm0.83$	$5.40^{a}\pm0.55$	$5.20^{a}\pm0.86$	$5.60^{a}\pm0.51$
	4	$2.80^{bc}\pm1.08$	$5.67^{a}\pm0.82$	$4.60^{b}\pm0.83$	$4.80^{ab}\pm1.06$	$4.40^{b}\pm0.83$
	6	$2.47^{c}\pm0.54$	$5.60^{a}\pm1.06$	$3.60^{c}\pm0.51$	$4.20^{bc}\pm0.39$	$3.60^{c}\pm0.54$
	8	$1.53^{d}\pm0.15$	$5.00^{ab}\pm1.13$	$3.20^{d}\pm0.50$	$3.47^{d}\pm0.74$	$3.33^{cd}\pm1.25$
60:40	0	$3.87^{a}\pm1.64$	$5.40^{ab}\pm0.84$	$5.20^{ab}\pm0.41$	$4.00^{bc}\pm2.07$	$3.00^{c}\pm1.13$
	2	$2.27^{b}\pm0.45$	$5.20^{ab}\pm0.77$	$5.60^{a}\pm0.42$	$5.00^{a}\pm0.64$	$5.20^{a}\pm0.41$
	4	$1.93^{b}\pm0.88$	$5.60^{a}\pm1.06$	$5.00^{b}\pm0.55$	$4.73^{ab}\pm0.88$	$4.93^{a}\pm0.70$
	6	$1.53^{b}\pm0.30$	$5.40^{ab}\pm0.50$	$3.60^{c}\pm0.32$	$3.73^{cd}\pm1.03$	$3.73^{b}\pm0.79$
	8	$1.67^{b}\pm0.49$	$5.20^{ab}\pm0.51$	$3.20^{c}\pm0.78$	$3.00^{d}\pm0.93$	$2.60^{c}\pm1.24$

Values with the same superscript within a column are not significantly ($p<0.05$)different
CS= Cassava starch flour, MF= mushroom flour

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Table 2: Effect of Glycerol on the Mechanical Properties of Cassava Starch and Mushroom (*P. Pulmonarius*) Films

Sample CS:MF	Glycerol (%)	TS (MPa)	EAB (mm)	EM (MPa)
100:00	2	$10.11^{a}\pm0.26$	$15.35^{b}\pm0.06$	$24.53^{a}\pm0.06$
	4	$9.70^{b}\pm0.02$	$16.04^{a}\pm0.03$	$22.28^{b}\pm1.11$
	6	$7.90^{c}\pm0.01$	$10.31^{c}\pm0.01$	$22.69^{b}\pm0.98$
	8	$5.50^{d}\pm0.25$	$9.17^{d}\pm0.01$	$22.61^{b}\pm0.56$
90:10	2	$10.02^{a}\pm0.03$	$17.29^{b}\pm0.02$	$22.40^{ab}\pm1.75$
	4	$9.09^{b}\pm0.01$	$18.43^{a}\pm0.02$	$23.37^{a}\pm0.11$
	6	$7.09^{c}\pm0.01$	$11.52^{c}\pm0.00$	$23.04^{a}\pm0.24$
	8	$4.82^{d}\pm0.03$	$7.19^{d}\pm0.02$	$22.51^{ab}\pm0.75$
80:20	2	$9.42^{a}\pm0.03$	$17.01^{b}\pm0.02$	$23.96^{a}\pm0.34$
	4	$8.56^{b}\pm0.01$	$21.71^{a}\pm0.05$	$22.97^{bc}\pm0.61$
	6	$5.34^{c}\pm0.06$	$11.33^{c}\pm0.02$	$23.08^{b}\pm0.15$
	8	$4.56^{d}\pm0.07$	$7.25^{d}\pm0.01$	$22.39^{bc}\pm0.50$
70:30	2	$8.17^{a}\pm0.00$	$12.52^{b}\pm0.02$	$22.88^{a}\pm0.06$
	4	$7.64^{b}\pm0.02$	$17.2^{a}\pm0.01$	$19.71^{b}\pm1.11$
	6	$5.01^{c}\pm0.06$	$9.71^{c}\pm0.01$	$19.34^{c}\pm0.03$
	8	$2.56^{d}\pm0.07$	$5.58^{d}\pm0.01$	$19.94^{c}\pm0.70$
60:40	2	$7.14^{a}\pm0.13$	$10.37^{b}\pm0.07$	$21.10^{b}\pm0.12$
	4	$6.11^{b}\pm0.01$	$14.77^{a}\pm0.05$	$22.05^{a}\pm0.01$
	6	$3.47^{c}\pm0.03$	$8.81^{c}\pm0.05$	$19.15^{c}\pm0.02$
	8	$1.27^{d}\pm0.02$	$5.09^{d}\pm0.02$	$19.32^{c}\pm0.92$

Values with the same superscript within a column are ot significantly ($p<0.05$)different

CS= Cassava Starch,

MS= Mushroom (*P. pulmonarius*) Flour

EAB= Elongation at break

TS= Tensile strength

EM= elastic modulus

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Table 3: Effect of Glycerol on Barrier Properties of Cassava Starch and Mushroom (*P. Pulmonarius*) Films

Sample CS:MS	Glycerol (%)	WVP (g mm m^{-2}d^{-1}KPa^{-1})	FS (%)	FT (mm)
100:00	0	6.75^{e}±0.02	22.86^{e}±0.55	0.42^{a}±0.01
	2	7.75^{c}±0.04	30.25^{d}±0.28	0.40^{b}±0.02
	4	7.25^{d}±0.12	35.32^{c}±0.04	0.38^{c}±0.00
	6	8.82^{b}±0.09	37.62^{b}±0.23	0.16^{d}±0.01
	8	9.71^{a}±0.05	39.79^{a}±0.23	0.17^{d}±0.01
90:10	0	6.33^{d}±0.02	20.28^{e}± 0.64	0.44^{a}±0.01
	2	5.73^{e}±0.32	28.46^{d}± 0.07	0.37^{b}±0.03
	4	6.89c±0.01	31.97^{c}± 1.81	0.33^{c}±0.02
	6	7.94^{b}±0.05	37.67^{b}± 0.52	0.18^{d}±0.01
	8	9.14^{a}±0.01	39.50^{a}± 0.04	0.19^{d}±0.01
80:20	0	6.26^{e}±0.05	18.69^{e}±0.23	0.41^{a}±0.02
	2	6.74^{d}±0.02	25.31^{d}±0.03	0.38^{b}±0.01
	4	7.02^{c}±0.08	29.71^{c}±0.04	0.35^{c}±0.00
	6	8.34^{b}±0.02	36.73^{a}±0.13	0.16^{d}±0.01
	8	8.72^{a}±0.05	34.90^{b}±0.02	0.15^{d}±0.01
70:30	0	6.07^{d}±0.03	17.87^{e}±0.03	0.40^{a}±0.02
	2	7.06^{c}±0.06	22.51^{d}±0.47	0.29^{b}±0.01
	4	7.16^{c}±0.06	28.73^{c}±0.26	0.19^{c}±0.01
	6	8.51^{b}±0.06	34.12^{a}±0.11	0.19^{c}±0.00
	8	9.98^{a}±0.04	33.07^{ab}±0.05	0.15^{d}±0.01
60:40	0	6.03^{e}±0.09	15.76^{e}±0.01	0.44^{a}±0.01
	2	6.49^{d}±0.11	22.02^{d}±0.06	0.22^{b}±0.01
	4	7.30^{c}±0.07	27.43^{c}±0.09	0.21^{b}±0.01
	6	8.72^{b}±0.02	33.24^{a}±0.07	0.19^{c}±0.01
	8	9.85^{a}±0.06	32.48±0.06	0.16^{d}±0.00

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Values with the same superscript within a column are not significantly ($p<0.05$)differentCS= Cassava Starch, MS= Mushroom (*P. Pulmonarius*) Flour, WVP= Water vapour permeability FS=Film solubility, FT=Film thickness