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APPLICATION OF MAIZE STARCH-PEANUT SHELL NANOCOMPOSITE PACKAGING ON MUSHROOM UNDER VARYING MOISTURE, THICKNESS, AND COLD STORAGE

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ABSTRACT

In this research, a maize starch-peanut shell nanocomposite film was applied to package mushroom under varying moisture, thickness, and cold temperature conditions. The film was developed by congealing 18 g starch, 0.38 g peanut nanoparticles, 16 g glycerol in 300 ml distilled water at 70ºC, and its thermal, mechanical, barrier and microstructural behaviors were determined using standard methods. The film was applied to package oyster mushroom by varying the thickness (5– 10 mm) and moisture content (77.18 –91.14 %, wb) of the product, and thereafter storing it under 4– 8 ºC cold temperature condition. The results revealed ~ 5% weight fraction degradation at ≤ 310 ºC with endothermic peaks occurring at 250 ºC and 400 ºC, which corresponds to phase transition points where the film was thermally stable. The deformation pattern of the film at atomic level mimics a natural plastic material, with a heterogeneous particle size distribution across the film matrix. The permeability coefficients were 0.68×10^{-10}, 2.10×10^{-10} and 14.0×10^{-10} cm^3 (STP) $cm/cm^2 s cm$ Hg for nitrogen, oxygen, and carbon-dioxide, gases, respectively. Also, the microbial load of the packaged product significantly decreased with an increase pH, moisture, and temperature ($p< 0.05$). Thus, the film can be suitable for mushroom packaging.

Keywords: Peanut nanoparticles, Maize starch, Nanocomposite film, Mushroom, Packaging

INTRODUCTION

Mushrooms are a type of fungus that normally grows above ground, on soil, or on its food supply. They are fleshy, spore-bearing fruiting bodies and are well-known for their incredible health advantages. They are a great addition to diet because they are loaded with vitamins and minerals and provide many different recipes taste. Also, they are a low-calorie food that packs a nutritional punch and has been recognized as an important diet recipe due to its rich composition of vitamins, minerals, and antioxidants. The benefits are better attained when they are consumed fresh without transformation into other forms. Thus, the need to preserve the products in its natural state has resulted in the quest for modern techniques **(Farhoodi, 2015; Jafarzadeh *et al.*, 2019)** to maximize the economic and nutritional values.

Many preservation methods have been reported for fresh mushrooms. This includes drying, freezing, tincturing, pickling, and so on **(Donglu *et al.*, 2016; Manikantan *et al.*, 2021; Nasiri *et al.*, 2022)**. Drying involves the removal of moisture to prevent its deterioration and to allow the product to be stored and used for a longer period than having to use them when fresh. But this can cause off-flavor, color, and nutritional quality decline due to heating **(Nasiri *et al.*, 2022).** The addition of lemon juice solution can help extend the shelf-life and prevent color degradation, but this can cause chemical interference by activating enzymes that may cause quality decline in the food. Freezing is another option of preservation which is often used in conjunction with blanching, steaming, or frying **(Fadeyibi, 2022)**. The effect of the freezer on the product has however made this approach less attractive due to the chilling injury it can cause **(Alabi *et al.*, 2022)**. The extracts and tinctures are used to preserve the beneficial constituents in mushrooms. Many of the mushrooms that are tinctured contain polysaccharides, beta-glucans, triterpenes, phenols, sterols, statins, enzymes, and other indole compounds that are beneficial to the consumers **(Donglu *et al.*, 2016; Gholami *et al.*, 2017; Wang *et al.*, 2021)**. But this method of preservation is destructive since the products are transformed into forms to distort their natural physical state during processing. Pickling involves keeping the product in a container or jar followed by adding some preservative such as salt or glucose to prevent deterioration and extend its shelf-life. This also can cause product quality interference and affect its texture thereby promoting off-flavor and color degradation in the product **(Gholami *et al.*, 2017)**.

Packaging is yet another option of preserving the mushroom especially when smart packaging materials are used in conjunction with either the freezing or pickling approach. The material can contain the product and help to monitor and control the exchange of gases and vapor within the packaging system **(Fang *et al.*, 2021; Gholami *et al.*, 2017; Sami *et al.*, 2020; Wang *et al.*, 2021)**. This will work effectively in the presence of the nanoparticles, which are normally incorporated into the film matrix to ensure that the physical stability and other attributes of the packaged product are maintained over an extended storage period. Typically, **Zhang *et al.*(2019)** produced a nanocomposite film for the packaging of white mushrooms by incorporating silica nanoparticles into a mixture of glucomannan and carrageenan protein sources. Other material nanofillers, such as Zinc oxide **(Nasiri *et al.*, 2021)**, sustainable plant sources **(Donglu *et al.*, 2016)** have been incorporated with either a starch or a protein source to produce packaging materials for mushroom preservation. However, the available films are only able to contain the products and extend their shelf-life for few days, usually less than 30 days, after which deterioration will set in. Thus, they are not reliable for a long-term packaging of the product since they are unable to monitor the physical changes in them, such as thickness and moisture content variabilities. Hence, there is a need to develop a film that can control these indices during the preservation of the fresh mushroom storage beyond its normal storage life. The objective of this research was to develop a maize starch-peanut nanocomposite film for application in oyster mushroom packaging under varying moisture, thickness, and cold temperature conditions.

MATERIALS AND METHODS

Preparation of maize starch

A 3 kg of dried white maize was purchased at Malete market, Malete, Kwara State, Nigeria. The city of Malete is located on Latitude 8º 42′ 34.74″ N and Longitude 4º 27′ 52.272″ E. The maize was processed to extract starch by soaking it in hot water for 5 h, and thereafter ground into paste before it was filtered using a muslin sack. The product was decantated to separate the water and the starch before drying using an air circulated oven dryer (Model- Air flow direction: vertical and horizontal, 2kW, 50- 250 deg. C, China) for 10 h. The dried starch was subjected to particle size analysis using the mechanical sieve shaker (Model- Φ 200 mm, freq. 221 t/min) to obtain the fine particles of the starch with an approximate particle size of 15.3μm. The fine starch was stored in a sealed polyethylene bag for subsequent experiment.

Production and characterization of peanut shell nanoparticles

A 3 kg of peanut shell was sourced from a peanut oil processing plant in Bida, Niger State, Nigeria. The city of Bida is on Latitude 9º 4′ 56.352″ N and Longitude 6 º 0′ 0.108″ E. The shells were milled using the attrition mill to form a powder of varying particle sizes. The mechanical sieve shaker was used to separate the particles of the powder into various categories of sizes. The particle size of the fine materials was determined using a dispersive light scattering equipment (Zetaziser vr 7.0). The film was developed by congealing 18 g starch, 0.38 g peanut nanoparticles, 16 g glycerol in 300 ml distilled water at 70ºC to form a thermoplastic solution. This was based on previous studies and preliminary investigation **(Farhoodi, 2015; Huang *et al*., 2015).** The solution was poured into a prepared plastic mold (400 × 300 mm size) and placed in an air circulated oven dryer at 60ºC and 75% RH to dry for 24 h. The film was carefully peeled off from the mold and its thickness measured as 26.23 μm using a plastic gauge before it was sealed using a polyethylene bag to avoid hydration before the packaging experiment.

Characterization of the film

Titration was used to measure the permeability of the nanocomposite film to the following gases: oxygen, carbon dioxide, nitrogen, hydrogen, and methane **(Hadassah and Sehgal, 2006)**, while the Karl Fischer method was used to determine the water-vapor permeability**(Kviesitis, 1971)**. In the titration procedure, a sample of film was placed in a specially made lens mold and exposed to carbon-oxide gas. The concentration of the gas emitted across the film was then measured **(Hadassah and Sehgal, 2006)**. The released gas was collected by dissolving in ethanol, and the solvent's titration was used to determine the carbon-oxide permeability. For the oxygen, nitrogen, hydrogen, and methane gases, the experiment was repeated three times, and the three average gas measurement readings were recorded as the permeabilities of the nanocomposite film. The Karl Fischer method involves dissolving a sample of the film in alcohol to create a solution, which is then titrated against sulfur dioxide assisted buffer. **(Schöffski and Strohm, 2000)**. Analytical measurements of the amount of moisture absorbed by the film sample were made after the sulfur dioxide in the titration cell was oxidized with iodine solution **(Schöffski and Strohm, 2000)**. The experiment was carried out three times, and the water-vapor permeability of the nanocomposite film was determined by averaging the moisture content measured in each instance. Thermogravimetric analyzer (TGA 55- TA Instrument) in conjunction with a Differential Thermal Analyzer (DTA) were used to determine the thermal property of the nanocomposite film **(Fadeyibi *et al.*, 2017; Shanks, 2010)**. This process involved heating the film continuously and gradually to 600ºC in 2ºC increments while maintaining a regulated nitrogen environment. The consequent change in weight was measured. This led to weight depreciation owing to temperature exposure. The glass transition, crystallization, melting, and sublimation phase transitions that took place during the TGA examination were described by the DTA. The experiment was repeated three times, and the average weight loss, impact temperature, and transformation energy were noted as the thermal properties. Scanning electron microscopic examination (SEM, electron microscope model Joel JSM-7600F) was used to analyze the nanocomposite film's microstructural characteristics **(Nikov *et al.*, 2020)**. The analysis was carried out by employing an Energy-Dispersive x-ray Spectroscopy (EDS) connected to the SEM to analyze the film sample's elemental composition.

Performance evaluation of the nanocomposite film

A 3000 g of freshly harvested oyster mushroom was purchased from a local farm located at Idofian, Kwara State, Nigeria. The produce was washed in distilled water to remove dirt and other foreign materials before it was divided to three portions. The samples were placed inside three separate desiccators containing 0.1 molar salt solutions (sodium chloride, calcium chloride and potassium chloride) to vary the moisture content in range of 77.18 –91.14 % (wb). The thickness of the product was varied by using a cutting tool, which was first disinfected with 95% alcohol, to slice the mushroom on a clean table into different thicknesses of 5, 7 and 10 mm. 100 g of each of the samples was wrapped using the nanocomposite film and sterilized before being placed on Petri dishes. After treatment, the samples were kept in the refrigerator under 4, 6 and 8ºC cold temperatures for 45 days. A total of 27 main samples were thus obtained at the end of storage, as shown in Table 1, and the microbial loads were determined using the methods reported by **Fadeyibi *et al*. (2017)** and **Sunmonu *et al.* (2020)**. The experiment was replicated three times, and the average value and standard deviation were recorded as the microbial loads of the packaged oyster mushroom.

Table 1 Samples of packaged oyster mushroom

s/n	Moisture (%, wb)	Thickness (mm)	Temp (ºC)	s/n	Moisture (%, wb)	Thickness (mm)	Temp (ºC)
1	77.18	5	4	15	91.14	7	6
2	84.22	5	4	16	77.18	10	6
3	91.14	5	4	17	84.22	10	6
4	77.18	7	4	18	91.14	10	6
5	84.22	7	4	19	77.18	5	8
6	91.14	7	4	20	84.22	5	8
7	77.18	10	4	21	91.14	5	8
8	84.22	10	4	22	77.18	7	8
9	91.14	10	4	23	84.22	7	8
10	77.18	5	6	24	91.14	7	8
11	84.22	5	6	25	77.18	10	8
12	91.14	5	6	26	84.22	10	8
13	77.18	7	6	27	91.14	10	8
14	84.22	7	6				

Optimization of film process variables

The response of each treatment (thickness, temperature, and moisture content) to the microbial loads was analyzed to determine the best film formulation, using a Box-Behnken Design Methodology in Design Expert (ver. 10), as shown in Table 2. Also, the F-value and p-value were determined for each variable and their interaction to ascertain whether the optimization model was significant or not.

Table 2 Treatment levels of variables used in film development.

Optimization variables	Unit	Range			Constraints
		-1	0	+1	
Thickness	μm	5	7	10	-1≤+1
Temperature	deg C	4	6	8	-1≤+1
Moisture content	%, wb	77.18	84.22	91.14	-1≤+1

Statistical analysis

The results of the barrier, mechanical, thermal, and structural characterizations of the nanocomposite film were illustrated graphically. The data were analyzed in triplicate, and the average values and standard deviations were determined, to reduce experimental error. The effects of the moisture content, thickness and cold temperature on the microbial load obtained from the packaging using the nanocomposite film packaging (**Fadeyibi, 2022**) were determined using ANOVA at 5% level of significance (**Yisa *et al.*, 2018**). A 3-level factorial design was used to obtain the best combination of the process conditions for a minimum microbial load on the packaged mushroom samples by maximizing the moisture content, thickness, and temperature conditions.

RESULTS AND DISCUSSION

Barrier and microstructural characteristics of the nanocomposite film

The results of the permeability of the nanocomposite film are shown in Fig. 1. In accordance with the current literature, packaging of perishable food items, such as oyster mushrooms, typically results in the production of gases such as oxygen, hydrogen, nitrogen, water vapour, carbon dioxide, or methane (**Ghosh and Singh, 2022; Kim *et al*., 2014; Zhao *et al*., 2022**). Because of the potential for structural imbalance, the shelf life may be impacted. It is therefore necessary to regulate and keep check on the gaseous concentration in order to prevent gas accumulation. The results shown in Fig. 1 show that the nanocomposite film is a great material that can manage the gas concentration (**Mohammadpour and Naghib, 2021**) by enabling additional hydrogen (50×10^{-10} cm^3 (STP) cm/cm^2scm Hg %) and carbon dioxide (14×10^{-10} cm^3 (STP) cm/cm^2scm Hg) gases, and lesser nitrogen (0.68×10^{-10} cm^3 (STP) cm/cm^2scm Hg) and oxygen (2.1×10^{-10} cm^3 (STP) cm/cm^2scm Hg) gasses to pass through. The packaged oyster mushrooms may benefit from a stable microsphere with a high carbon dioxide and low oxygen levels. Due to the limited oxygen gas supply in the packaging system, the product will therefore undergo anaerobic respiration; as a result, the carbon dioxide level will be increased to lengthen the product's shelf life. This supports the findings of **Qin et al. (2021),** who increased the shelf-life of a corn cellulose by using graphene oxide nanosheets, and **Zhang et al. (2020),** who increased the water vapour transmission rate of a film by adding lignin to polyvinyl acetate. In other similar works, **Raja and Xavier (2021)** functionalized a silane nanoparticle on nano clay to improve the maintaining quality of the film during packing, and **Fadeyibi and Osunde (2021)** added a zinc nanoparticle to cassava starch. Considering the foregoing, it follows that during food packaging, the nanocomposite film can regulate the gaseous concentration within the microsphere.

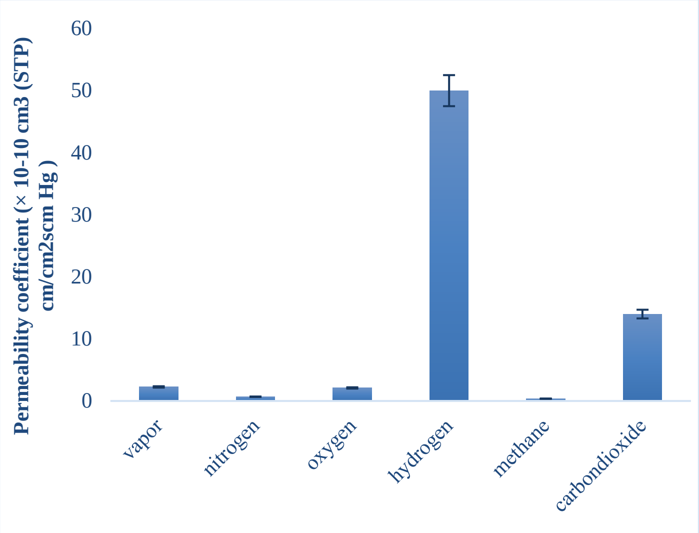

Figure 1 Permeability of the nanocomposite film

Figure 2 displays SEM images of the nanocomposite film. The arrows point to areas of shape and size anomalies due to the emergence of voids within the matrix, demonstrating the presence of heterogeneous particles in the film. According to the Mie theorem, the size distribution falls within a narrow band length of 500–700 nm (**Goh *et al.*, 2014; Hao *et al.*, 2020**), which translates to a particle size range of 10.55–11.77 nm. The existence of the molecules of starch and glycerol, which are dispersed throughout the film matrix to form the pores necessary to balance the gases in the microsphere, meant that the size of the nanoparticles was obviously less than the matrix size of the film. Related investigations that support this conclusion include the microstructural characterizations of the AIN/Y nanocomposite film **(Li et al., 2018),** MoO3-TiO2 nanocomposite film **(Li et al., 2001),** and ZnO-graphene oxide thin film nanocomposites film **(Trinh et al., 2021).** Also, **Nikov et al. (2020)** reported a comparable outcome in their study on the microstructural characterisation of nanocomposites made by laser ablation in a magnetic field. In line with the film under study, the physicochemical and microstructural characteristics of a polyvinyl alcohol mixture with a clay nanoparticle likewise showed no evident aggregation within the film matrix.

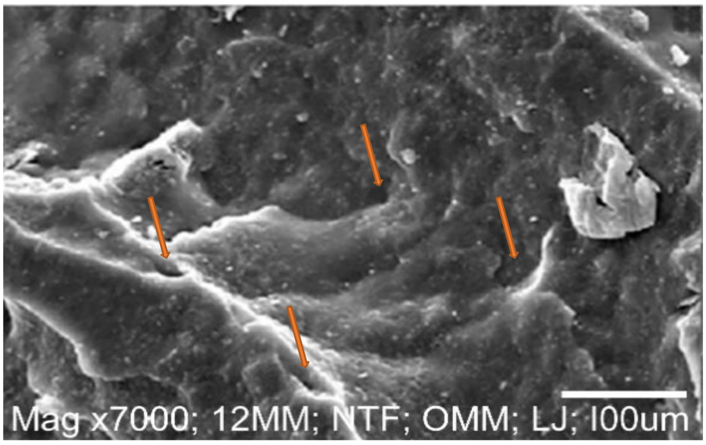

Figure 2 SEM micrographs of the nanocomposite film showing appearance of voids

Thermal behavior of the nanocomposite film

The TGA/DTA results of the nanocomposite film is shown in Fig. 3. The weight lost increases with an increase in the temperature from 0- 600°C. The results revealed ~ 5% weight fraction degradation at ≤ 310 °C with endothermic peaks occurring at 250 °C and 400 °C, which corresponds to phase transition points where the film was thermally stable. The phase transition of the film increases with temperature, and the heat of decomposition was 4.85 J/g, according to the DTA data on the curve. In order to degrade just 1g of the film sample within the glass transition or threshold temperature, 4.85 J of heat energy had to be released. Related studies that support low weight degradation of film below the glass transition temperature include **Manikandan et al. (2019)** on the thermal stability of a green film based on the blend of poly (vinyl alcohol) and Eleusine coracana composite film, and **Thomas et al. (2021)** on thermal stability of blend of natural rubber and nano cellular whisker composite film. The film may not be acceptable for packaging above this critical temperature since a greater portion of its weight was lost at higher temperature.

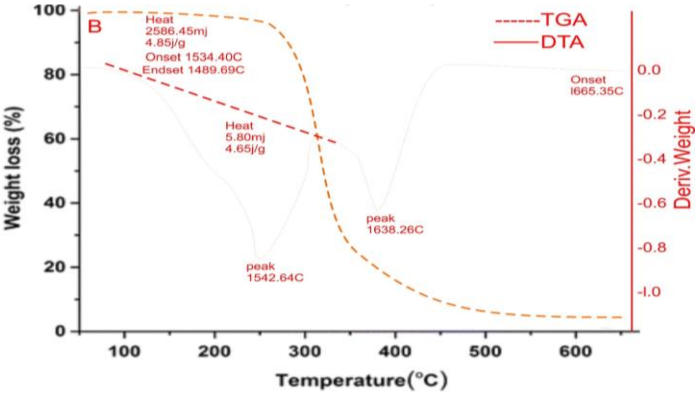

Figure 3 Effect of temperature on weight degradation of the nanocomposite film

Effect of process variables on the microbial load of packaged oyster mushroom

The effects of moisture, thickness, and cold temperature on the microbial loads of the packaged oyster mushroom using the nanocomposite film are shown in Table 3. The microbial load of the packaged product significantly decreased with an increase pH, moisture, and temperature (p< 0.05). The effect of moisture was significant while that of thickness and temperature were not significant on the number of bacteria and fungi loads in the packaged product as shown in Table 4. The interaction and quadratic effects of the variables on the film performance were also significant (p< 0.05) except that of the temperature.

Table 3 Effect of process variables on microbial loads of packaged mushroom

	Variables			Response (×10^5 cfu/g)	
s/n	Moisture (%, wb)	Thickness (mm)	Temp. (°C)	Bacteria count	Fungi count
1	77.18	5	4	3.60± 0.18	2.40± 0.12
2	84.22	5	4	4.52± 0.23	2.51± 0.13
3	91.14	5	4	5.67± 0.28	2.66± 0.13
4	77.18	7	4	4.11± 0.21	2.12± 0.11
5	84.22	7	4	5.74± 0.29	2.99± 0.15
6	91.14	7	4	4.33± 0.22	2.05± 0.10
7	77.18	10	4	6.54± 0.33	3.36± 0.17
8	84.22	10	4	5.89± 0.29	2.88± 0.14
9	91.14	10	4	5.55± 0.28	2.62± 0.13
10	77.18	5	6	2.55± 0.13	1.20± 0.06
11	84.22	5	6	2.01± 0.10	1.12± 0.06
12	91.14	5	6	2.3± 0.12	1.21± 0.06
13	77.18	7	6	3.11± 0.16	1.89± 0.09
14	84.22	7	6	2.99± 0.15	1.56± 0.08
15	91.14	7	6	3.26± 0.16	1.91± 0.10
16	77.18	10	6	4.38± 0.22	2.21± 0.11
17	84.22	10	6	4.47± 0.22	2.47± 0.12
18	91.14	10	6	3.78± 0.19	1.93± 0.10
19	77.18	5	8	1.99± 0.10	1.13± 0.06
20	84.22	5	8	2.00± 0.10	1.23± 0.06
21	91.14	5	8	1.83± 0.09	1.02± 0.05
22	77.18	7	8	2.55± 0.13	1.32± 0.07
23	84.22	7	8	2.89± 0.14	1.72± 0.09
24	91.14	7	8	3.13± 0.16	1.67± 0.08
25	77.18	10	8	4.11± 0.21	2.20± 0.11
26	84.22	10	8	4.66± 0.23	2.28± 0.11
27	91.14	10	8	5.01± 0.25	2.86± 0.14

Hence, over the course of 45 days of packaging, the nanocomposite film was successful in preventing the growth of microorganisms regardless of the sample wetness, thickness, or storage temperature. The effectiveness of starch combined with zinc nanocomposite film for tomatoes **(Fadeyibi *et al.*, 2017)**, slices of okra **(Fadeyibi *et al.*, 2019)**, and cucumber **(Fadeyibi *et al.*, 2020)** preservations has been demonstrated in related investigations. This indicates that the film has antibacterial properties, like other films of a similar nature, to limit the development of microbes while food is being preserved.

Table 4 Analysis of variance of microbial load as influence by the process variables

Parameters	Source	SS	df	MS	F-value	p-value
Bacterial count	A-Moisture	1.56×10^7	1	1.56×10^7	0.5593	0.4648^{ns}
	B-Thickness	1.79×10^9	1	1.79×10^9	63.95	$< 0.0001^*$
	C-Temp	1.67×10^9	1	1.67×10^9	59.93	$< 0.0001^*$
	AB	4.77×10^7	1	4.77×10^7	1.71	0.2086^{ns}
	AC	1683.14	1	1683.14	0.0001	0.9939^{ns}
	BC	1.13×10^8	1	1.13×10^8	4.05	0.0604^{ns}
	A²	1.18×10^7	1	1.18×10^7	0.4222	0.5245^{ns}
	B²	1.71×10^7	1	1.71×10^7	0.6111	0.4451^{ns}
	C²	4.99×10^8	1	4.99×10^8	17.88	0.0006^*
	Residual	4.75×10^8	17	2.79×10^7		
	Cor Total	4.79×10^9	26			
Fungi count	A-Moisture	10095.71	1	10095.71	0.0012	0.9732^{ns}
	B-Thickness	3.84×10^8	1	3.84×10^8	44.11	$< 0.0001^*$
	C-Temp	3.45×10^8	1	3.45×10^8	39.58	$< 0.0001^*$
	AB	2.77×10^6	1	2.77×10^6	0.3177	0.5804^{ns}
	AC	1.77×10^7	1	1.77×10^7	2.03	0.1723^{ns}
	BC	5.58×10^7	1	5.58×10^7	6.41	0.0215^*
	A²	5.68×10^6	1	5.68×10^6	0.6522	0.4305^{ns}
	B²	2.66×10^6	1	2.66×10^6	0.3051	0.5879^{ns}
	C²	1.19×10^8	1	1.19×10^8	13.71	0.0018^*
	Residual	1.48×10^8	17	8.71×10^6		
	Cor Total	1.12×10^9	26			

The optimum processing parameters for ideal packaging of the oyster mushroom is shown in Figure 4. The amounts of the bacteria (1500 cfu/g) and fungi (3500 cfu/g) loads were minimized at 91.14% moisture content, 8.3 mm thickness, and 7.8°C storage temperature which gives a desirability of 0.78. An accumulation of microorganisms in the food due its high moisture content was expected, but surprisingly, the product recorded lower number of the microbes at the cooling temperatures. Thus, the presence of the nanoparticles in the film matrix can create unfavorable environment for the growth and survival of the bacteria and fungi cells in the packaged mushroom to influence this observation.

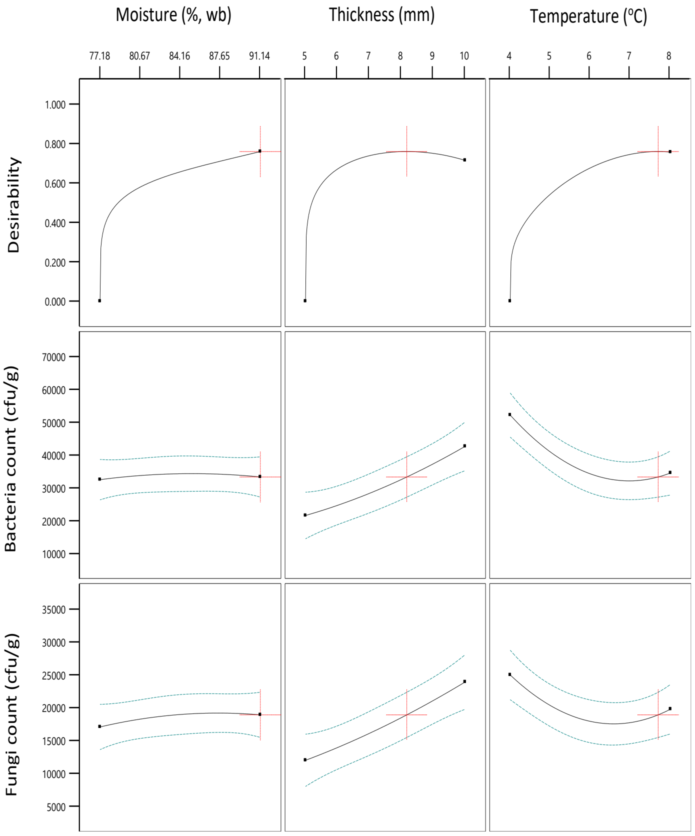

Figure 4 Optimum processing parameters for ideal packaging of mushroom

The nanocomposite-based packaging material is a similar material used for the packaging of volvariella volvacea with enhanced quality characteristics in terms of microbial growth inhibition **(Donglu *et al.*, 2019)**. **Gholami *et al.* (2017)** also corroborates this in their work on microbial growth inhibition and shelf-life extension of mushroom under cold storage using a smart packaging material. This inferred that the nanocomposite film can preserve the oyster mushroom at higher moisture, thickness, and cold storage.

CONCLUSIONS

The findings from this research revealed that ~ 5% weight fraction degradation at ≤ 310 °C with endothermic peaks occurred between 250 °C and 400 °C, which corresponds to phase transition points where the film was thermally stable. The deformation pattern of the film at atomic level mimics a natural plastic material, with a heterogeneous particle size distribution across the film matrix. The permeability coefficients were 0.68×10^{-10}, 2.10×10^{-10} and 14.0×10^{-10} cm^3 (STP) $cm/cm^2 s\,cm$ Hg for nitrogen, oxygen, and carbon-dioxide, gases, respectively. Also, the microbial load of the packaged product significantly decreased with an increase in the thickness, moisture, and temperature ($p< 0.05$). The interaction and quadratic effects of the variables on the film performance were also significant ($p< 0.05$). The optimum amounts of the bacteria (1500 cfu/g) and fungi (3500 cfu/g) loads occurred at 91.14% moisture content, 8.3 mm thickness, and 7.8°C storage temperature with a desirability index of 0.78. The optimal film can therefore be suitable for mushroom packaging.

Conflict of interest: The authors declare no conflict of interest.

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