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Formulation and Characterisation of a Mushroom-based Pate Enriched with Macrotermes Subhyalinus

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Abstract
The aim of this work was to produce and characterise termite-enriched mushroom-based pâté. To achieve this, the following methodology was adopted. Six formulas were generated by incrementing the additions of edible mushrooms and termites, named P-100, P-80, P-60, P-40, P-20, and P-CO. Various parameters were then evaluated, including pH, water-holding capacity, oil-holding capacity, foaming capacity, emulsifying capacity, thiobarbituric index and nutritional parameters. The results showed that water retention capacity ranged from 27.98 to 80.98%, and pH values from 5.55 to 6.06. Foaming capacities ranged from 1.12 to 2.43, while emulsifying capacity and emulsion stability values ranged from 48.05 to 39.74% and 97.29% to 80.64% respectively. After cooking the pâtés, physico-chemical analyses revealed that protein content ranged from 0.8 to 8.8%, fat content from 8.87 to 29.16% and dry matter from 64.53% to 32.25%. The TBA index fluctuated between 0.73 mg MDA/kg and 0.14 mg MDA/kg. The textural analyses carried out on the different pâtés varied between 29.17 N and 7.05 N. The hedonic test carried out on the different pâtés led to the conclusion that all the pâtés were appreciated by the panelists, with the emphasis on the p-60 formulation. This study has shown that it is possible to formulate termite-based pâtés. This new product would significantly increase the protein content, which would lead to better feeding.

Keywords: Termites, Fungi, Hedonic Test, Nutritional Values.

Introduction

Meat is a costly protein source and considering Cameroon as a developing country not everybody is able to afford meat nor its products and this is a major problem. It was once believed that all charcuterie products must at all times be made from meat. However, there have been attempts to produce some charcuterie products like pâté from fruits, vegetables and even fungi. Globalization is affecting food production and consumption chains worldwide. Consumers all over the world are increasingly getting a wider variety of food products of higher quality and of lower prices [10]. In addition to increase in demand arising from the population growth, increase in demand for protein particularly animal-derived proteins is generally noted, and given by increase in socio-economic changes such as rising incomes, increased urbanization and aging population whereby the contribution of protein to healthy aging is increasingly recognized. However, all proteins are not the same, they vary in terms of nutritional profile, digestibility, bioavailability, consumer's acceptance etc. [1]. In Africa, there exist different alternate protein sources apart from animal protein which are also very nutritious and also have some health benefits. Examples of these protein sources include; soy beans, edible insects etc. some these listed protein sources are mostly considered unconventional. Spreadable liver pâtés are very popular food products [3]. These are pastes normally produced with pork liver, precooked pork backfat, water, sodium caseinate and small quantities of other additives (Nangmou et al., 2016) in which animal sources may vary depending on the country of production. In this work, we replaced meat with mushrooms because of its nutraceutical properties which are absent in meat. Pâtés are regarded as food products with high animal fat content, ranging from 35% to 50% (Nangmou et al., 2016), and since the consumers' demands for healthier foods are intensifying, some alternatives have been proposed to make these products healthier, particularly those aiming at suppressing hard fat amounts like the above stated references. In this work pork back fat is being replaced with termites, given that termites are very high in MUFA and PUFA's and a source of protein as well. Recently, nutritional directives encourage the consumption of increased dosages of n-3 PUFA and MUFA, in order to reduce the risk of cardiovascular diseases, stroke, heart failure, and atrial fibrillation in a significant manner (Martínez-Carrera et al., 2002). We set ourselves the objective to produce pâté from mushroom enriched with termites as a source of lipid and additional protein.

Materials and Method

For this part of our work, we will be looking at the biological materials, reagents and equipment used for the fabrication of pâté from mushroom enriched with insects.

Materials

Biological Materials

This study was carried out in the process engineering department, hall of food technology laboratory and GETA (Genie Enzymatique et Technologie Alimentiare) of the National Advance School of Agro-industrial Science of the university of Ngaoundere in cameroon. The materials used will be listed below.

Oyster Mushrooms (Pleurotus ostreatus)

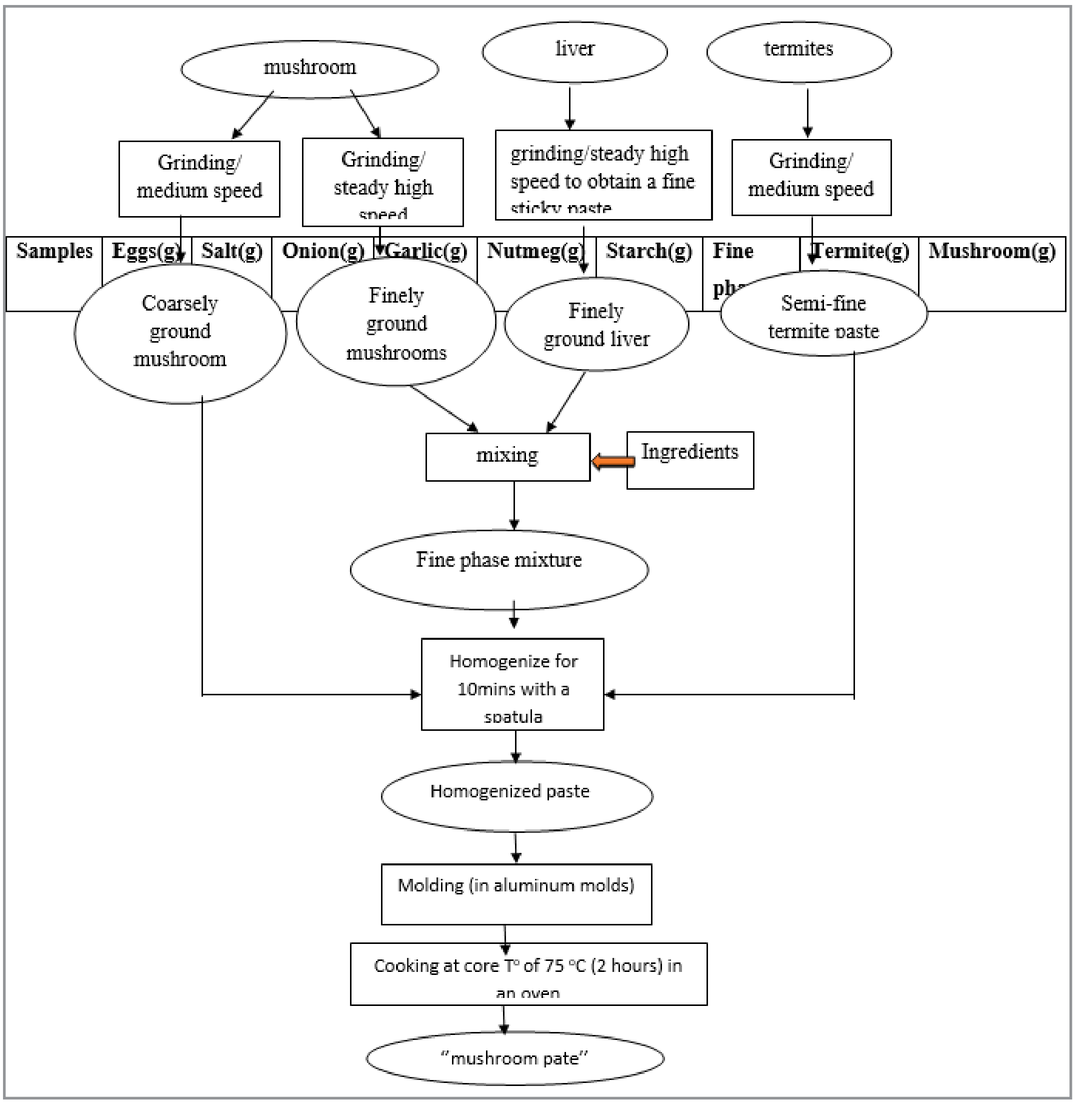
These oyster mushrooms were grown on corn cobs as a substrate and bought from a mushroom farmer. The liver used during this work was the liver from beef. The termites used during this work were gotten from the market.

Method

The diagram below shows a plan out of how the production would adhere.

Flow Diagram for the Fine Phase Production

The figure below shows the flow diagram for the production of the mushroom pâté after raw materials have been sorted and washed



Pâté formulations

Table 1: Formulations for Various Pâté Samples

P-100	50	12	6	3.6	4	20	100	200	0
p-80	50	12	6	3.6	4	20	100	160	40
p-60	50	12	6	3.6	4	20	100	120	80
p-40	50	12	6	3.6	4	20	100	80	120
p-20	50	12	6	3.6	4	20	100	40	160
p-control	50	12	6	3.6	4	20	100	0	200

The table below shows the various formulations made with different percentages of mushroom and termite incorporation.

Cooking of "Mushroom Pâté"

After obtaining the desired formulations, the same quantity of the homogenized pastes was filled into metallic molds. Packaged samples were introduced in an oven at a temperature of 45-50oC for 3hours. The pâtés were cooked until the core temperature of the product got to 75oC. At the end of the cooking, the samples were removed and cooled down at room temperature for textural, physical, and sensory analysis. The main steps for the ''mushroom pâté" production were; trimming/sorting of mushroom, liver and termites, chopping/mixing, formulation of the desired pastes, molding, cooking, cooling packaging and storage.

Sorting and Trimming/Washing

At this level the mushrooms were trimmed, termites were sorted and frozen given that they were conserved in the freezer and the liver was denerved to carefully remove any redness or glands and they are chopped into regular slices to ease grinding and later on they are washed.

Salting

The mushrooms, termites and liver, were salted in two different methods, the liver and mushrooms were salted by immerging them in a brine solution with 40g and 100g of salt respectively, and the termites were salted dry with 84g of salt for 24-48hrs before production.

Chopping and Mixing/ Cutting

The next day the (day of production) the mushroom was divided into two parts the first part wa finely chopped and the second part was coarsely chopped. The chopped mushrooms were transferred into different two different metallic bowls and the cutter was cleaned. In the mixer, the liver was ground in to a very fine texture. The ground liver was transferred and placed into another metallic bowl and the mixer was cleaned. Finally, the termites were ground into a medium texture, then removed and placed into a metallic bowl as well. The liver and finely chopped mushrooms were mixed together with ingredients to form the fine phase.

Formulation of Pastes

Being the most delicate step of the preparation process, it consists of mixing the ground ingredients and additives in different proportions. Then, knead this mixture so as to obtain more or less homogeneous and seasoned batter. The mixture is made in a plastic or aluminum container using a wooden spatula or electric blender. During mixing, there is possible reduction of particle size (if an electric blender is used.

Molding

Different baking molds are used but the molds which was used was an aluminum bowl.it is necessary to determine the shape which allows an optimal slice weight. The portion of pâté recommended per person is 40g

• Cooking: the pâté is prepared using a dry method of cooking in an oven for 2hours at a temperature of 75°C.

• Cooling: The baked pâtés were transferred into a plastic bag and sealed with a sealing machine and placed in a fridge

Physico-chemical Analysis Carried out on Uncooked Pâté

The characterization of physico-chemical properties of the raw pâté and their different formulations are shown through these analyses.

Determination of pH

10g of our sample was placed in 100ml of distilled water and mixed for 5mins.The pH meter electrode is immersed in a beaker containing the mixed sample. The pH value is read on the bulletin board.

Foaming Capacity and Stability

The method us dis the method by Srinivas and Rao Narasinga (1986). 3g of the sample is added to 100ml of distilled water. The mixture is whipped using a supermix 50mill(france) for 5min and introduced into a graduated cylinder of 250ml ,the volume of foam is read for 30seconds. Foaming stability is how stable the foam can be after a given time.it is measured after 0min, 20min, 40min,60min,120.

Emulsifying Capacity and Stability of Emulsion

0.4g of the sample is mixed with 20ml of water in 250ml, 20ml of refined palm oil is added to the mixture which is vortexed tube and then the solution is homogenized with a vortex for 60 then centrifuged at1500rpm for 15min. For the emulsion stability, the emulsion formed is heated at 85oC for 15mins in a water bath to measure its stability. After heating, it is brought to room temperature (25oC) then centrifuge 1500 rpm for 5min and the volume of emulsion formed is noted.

Color Determination

To confirm the colors of each product observed with the naked eyes, the CIE method was used. The method consists in taking images of each sample in a wooden box having a bulb reflecting white light with a digital camera and the image obtained analyzed using Image J version 1.4.3.67 to identify and calculate the values corresponding to each type of color. The values of L*, C*, h (lightness/darkness, Chroma and hue respectively) were identified according to the approach of McGuire (1992). The results obtained were expressed as an average of three trials

Textural Analysis

The texture evaluation was performed using Stable Micro System's TAXT2 texture analyzer (Survey, Great Britain). This device allows one to perform compression tests in the center of a slice 25 mm thick pâté using a flat end 3.5 cm in diameter (method A A C C,74-09, 1987). Its interest is to characterize the product with the help of an instrumental measurement and to permit it follow the example for the evolution of the flexibility of the crumb in time. The measurement criterion used is the maximum compressive force for deformation by 40%. This criterion allows good discrimination pâtés and corresponds to ratings close to those made by the consumer.

Determination of the Thiobarbituric Acid (TBA)
The TBA index was determined according to the procedure described by Veron, Witte, Gray, Krause, Miltone and Bailey (1970). 10g of samples are crushed and homogenized for 15min. in 25ml of extractive solution (20% of trichloroacetic acid in 2M phosphoric acid at 4oC). the stuffing is transferred to a 100ml flask with 20ml of distilled water. The overall volume of the sample is reduced to 100ml with distilled water and the whole is homogenized by agitation. 25ml are filtered through Watman No. 1 filter paper. Then, 25ml of the filtrate is transferred to a test tube (15x200mm) containing 2.5ml of TBA (0.005m in distilled water). The tubes are closed and the solutions mixed with a vortex. The tubes are then kept in the dark at room temperature (20 ° C) for 15 hours. The color obtained after this time is measured at the UV / Visible Spectrophotometer (Pharmacia Biotech Ultrospec 4000, England) at 530NM. The value of the TBA is obtained by multiplying the absorbance by 5.2 (Veron et al., 1970). This is expressed in mg of Malononic aldehyde per kg of fresh pâté sample.

Determination of the Water Retention Capacity
The water retention capacity (CRE) of different pâté was determined by the method of Hamm, (1960) modified by Zayas and Lin (1988). A 0.5 g sample of raw pâté was placed between two sheets of Whatman No. 4 filter paper and pressed between two flat glass plates for 20 min. Under a mass of 1Kg. The area of the filter paper soaked with the juice from the squeezed raw pâté as well as the area occupied by the pâté (without juice) were measured.

Determination of the Total Ash Content
The total ash is the calcination residue at 550 ° C of the organic materials. The principle consists of incineration at 550 ° C in a muffle furnace of a test sample of the drying material at 105 ° C, to constant weight and the weighing of the residue obtained according to AOAC standard (1990).

Protein Content
The method used for the protein content is the kjeldahl method AFNOR, 1984

Determination of Total Lipid Content
The total lipid content was determined by the hot extraction method at Soxhlet described by Bourely (1982).

Determination of Water Content and Dry Matter
Dry mass or total dry residue is all compounds that do not volatilize under the drying conditions defined by the method used. The determination of the dry matter was carried out by AFNOR method 1982).

Sensory Analysis on "Mushroom Pâté"
Sensory evaluation is a scientific discipline used to evoke measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Sidel and Stone, 1993). Sensory analysis methods; difference tests, descriptive analysis and consumer acceptance testing can be used (Lawless and Heymann, 1998).

Results and Discussion

Physicochemical Composition of Elaborated Pâtés
The different incorporations of termite in the pâté as the lipid phase and protein source affected their physicochemical properties. the table 2 below shows the physicochemical analysis carried out on uncooked pâté. physicochemical composition of all sets of uncooked pâté samples; P-100: 100% termite incorporation; P-80: 80% termite incorporation P-60:60% termite incorporation, P-40:40% termite incorporation, P-20:20% termite incorporation, P-C0: control sample.

Physicochemical Analysis on Uncooked Pâté

Table 2: physicochemical Analysis Carried out on Uncooked Pâté

Paraeters	P-100	P-80	P-60	P-40	P-20	P-CO
pH	5.55 ±0.01^{a}	5.57±0.04^{bc}	5.58 ±0.03^{b}	5.59±0.05^{c}	5.67±0.06^{d}	6.06±0.07^{e}
Foaming capacity(%)	1.12±0.01^{a}	1.25±0.01^{a}	1.33±0.02^{ab}	2.02±0.05^{b}	2.20±0.07^{bc}	2.43±0.09^{c}
Emulsion capacity(%)	48.051±1.17^{c}	46.14±1.34^{bc}	45.45±1.23^{b}	42.30±1.46^{ab}	39.74±1.62^{a}	39.74±1.79^{a}
Emulsion stability(%)	97.29±3.21^{d}	91.67±2.86^{c}	88.57±2.57^{bc}	87.87±2.85^{b}	80.64±3.04^{a}	80.64±3.37^{a}

pH is a parameter which has a great influence on many properties of food. The results on figure 6 above shows that the pH of raw pâté is between 5.55 ±0.005c and 6.06±0.005c ($p<0.05$).; values corresponding to P-100 and P-CO respectively. The PH values reported on the table 6 above agrees with those reported in previous studies (AJ Martins, 2020). Those of the cooked pâté on table 5 varied from 6.17± 0.33a and 6.42±0.33e ($p<0.05$). of p-100 and P-CO respectively. The increase in pH in the raw pâté from 5.55 ±0.005c to 6.06±0.005c could be due to the termite incorporation as termites contain fatty acids and the control has no termite incorporation hence increases in basicity of the pâté. After baking, the pH values were found to have increased from 6.17±0.33a and 6.42±0.33e ($p<0.05$). of p-100 and P-CO respectively could be due to protein hydrolysis during cooking which released peptide and amino acids. The increase in pH after cooking has been reported by Manish et al.,2007.

The ability of food solutions to foam depends on the presence of flexible proteins as well as the presence of fat in food decreases the foaming capacity as shown on the figure above this is because fats have hydrophobic and hydrophilic ends as do proteins so fats compete with proteins for special alignment with gas bubbles. Considering the fact that termite have a higher lipid profile than proteins, figure 8 shows a progressive increase in foam capacity from 1.12% to 2.43%. values corresponding to P-100 and P-CO respectively. P-100 has more fat hence less foam, the drastic increase in the foaming capacity of PCO was due the absence of termite incorporation.

Emulsion capacity depends directly on proteins, since emulsion formation depends on the rapid adsorption, unfolding and reorientation of proteins at the oil-water interface. Thus this could justify the decrease from (48-39%). Values corresponding to P-100 and P-CO respectively, and given that termites are also high in protein and fat, p-100, the increase in emulsion in p-100 is justifiable. The progressive decrease in emulsion stability from 95% to72% could also be due to the pH of the protein solution. rheological properties of interfacial film and emulsion stability are influenced by the pH of the protein solution which affects protein solubility, net charge, and conformation of protein molecules at the oil-water interface (Das and Kinsella, 1990).

Water Retention Capacity

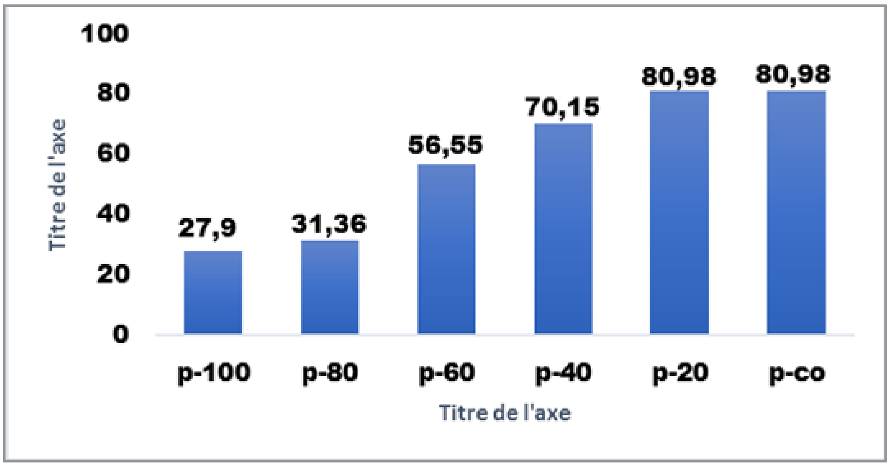


Figure 1: Water Retention Capacity

Table 3: the foaming Stability with Time.

samples	t20	t40	t60	t120
p-100	97.61	96.42	95.23	92.23
p-80	98.23	98.76	96.76	95.06
p-60	95.19	95.18	93.38	90.18
p-40	89.24	86.23	86.23	86.23
p-20	97.53	92.32	90.56	90.56
p-co	95	92	92	92

The table above shows the stability of various pâtés with respect to time. The pâté with the most stable values was P-CO which had the same value from the t40-t120; corresponding to time at 40mins and time at 120minutes. The reduction of foam volume from t20 to t40 could have been due to restructuring of proteins and the stability from t40-t120 could have been due to the fact that the proteins have been totally restructured hence a stable foam volume.

Physicochemical Analysis Carried out on Cooked Pâté

Table 4: Physicochemical Analysis Carried out on Cooked Pâté

The values a–e Different letters (in the same row) mean that samples are statistically different (α = 0

Parameters	P-100	P-80	P-60	P-40	P-20	P-CO
pH	$6.17{\pm}0.33^{a}$	$6.22{\pm}0.41^{b}$	$6.32{\pm}0.33^{c}$	$6.29{\pm}0.33^{c}$	$6.36{\pm}0.33^{d}$	$6.42{\pm}0.33^{e}$
TBA(mg)	$0.72{\pm}0.11^{c}$	$0.63{\pm}0.15^{bc}$	$0.53{\pm}0.19^{bc}$	$0.51{\pm}0.14^{bc}$	$0.36{\pm}0.15^{bc}$	$0.14{\pm}0.04^{a}$
Texture	$29.17{\pm}0.07^{d}$	$15.79{\pm}0.05^{cd}$	$10.33{\pm}0.03^{bc}$	$7.05{\pm}0.04^{ab}$	$7.20{\pm}0.07^{ab}$	$7.05{\pm}0.06^{a}$
Moisture(%)	$35.46{\pm}1.14^{a}$	$51.21{\pm}1.13^{b}$	$56.37{\pm}1.21^{c}$	$58.86{\pm}1.25^{d}$	$64.76{\pm}1.18^{c}$	67.75±1.22cd
Dry matter(%)	$64.53{\pm}1.20^{d}$	$48.71{\pm}1.23^{c}$	$43.65{\pm}1.19b^{c}$	$41.15{\pm}1.13^{b}$	$35.14{\pm}1.17^{ab}$	$32.25{\pm}1.18^{a}$
Lipids(%)	$29.16{\pm}2.68^{d}$	$27.49{\pm}2.59^{cd}$	$25.31{\pm}2.61^{c}$	$20.47{\pm}2.62^{bc}$	$18.22{\pm}2.53^{b}$	$8.87{\pm}2.50^{a}$

Proteins(%)	8.8±1.29[e]	6.3±1.26[d]	5±0.90cd	2.9±0.76[c]	1.6±0.23[b]	0.8±1.06[a]

Dry matter measures nutrients upon their overall percentage in a formula after the moisture content has been removed. We observed that there is a significant difference p<0.05 of dry matter between the various Pâtés, with P-100 having the greater dry matter and P-CO having the lowest value. This is due to the difference in mushroom and termite incorporation as earlier stated in the moisture content, the water content of mushroom is very high this alone influenced the difference in dry matter as well as the moisture in termite is quite low, hence explaining why samples with higher termite incorporations have more dry matter than samples with less termite incorporation.

Lipids oxidation is a very serious problem faced by food industries nowadays since it leads to the reduction of the nutritional value, safety and shelf life of food. Thiobarbituric acid (TBA) is an intermediate product of oxidation of fatty acid compounds, generally use as an indicator of food oxidation (Issa, 2019). The data shows a progressive increase in the oxidation of lipid as the TBA composition increased as the incorporation of termite increases from (0.73±0.15a mg MDA/kg) of P-100 and (0.14±0.15c mg MDA/kg) of P-CO (of p>0.05). the icrease in the TBA index in P-100 was because of the high fat content which increases the substrate for oxidation and the low TBA index in P-CO was due the little or no fat present in the formulation hence low substrate available for oxidation. In contrast the TBA index according to (Issa, 2019) from two different sausage formulations increased with increase in the duration of conservation. F2B (0.58±0.03 mg MDA/kg), F1B (0.45±0.01 mg MDA/kg): the different quantities of formulations 2, and 1 respectively.

Regarding fat content, the incorporation of 100% termite lead to an increases in fat content (p>0.05), while the sample of P-CO showed a significant reduction (29.16% vs. 8.87%). From nutritional point of view, this reduction is a positive goal. This fact could be due the fact that termites have a greater lipid content compared to mushrooms. In the literature, the percentage of fat in pâté lies between 30-50%, in this case, the literature is slightly different from the results obtained.

The results shows a progressive decrease in the protein content from 8.8% to 0.2%(of p>0.05); values corresponding to p-100, and P-CO respectively, this is because termites are generally higher in proteins than mushrooms as seen in the results. Hence the greater the termite incorporation the greater the protein. In contrast the protein content of a meat based pâtés was slightly higher than that in this present report with protein content of 9% (J. Martins et al,2020).

Proximate compositions of pâté samples were significantly influenced by the termite and mushroom incorporation. The replacement of pork back fat by termites as a source of fat should have increased the moisture content of the pâtés but since mushroom has more water content, the figure above shows that the pâtés with higher mushroom incorporation and lesser termite incorporation was found to have greater values of moisture. In contrast, a previous study did not find differences in the proximate composition of pork pâté reformulated with partial and total fat replacement by olive oil (G. Aceites, 2016). In the present study, the moisture contents (67–34%) which doesn't totally agree with the results reported by other authors in pâté, who reported values of 51–52% in pâté reformulated with olive oil (G. Aceites2016), 50–53% in pâté reformulated with fish oil (Munekata et al, 2016 & Domínguez et al,2016,). P-100 had more moisture, this could be due to the presence of lipids in the pâté.

The textural parameter carried out was hardness. All pâtés were affected by the termite incorporation. The figure above shows that P-100 had a greater hardness value of (29.17±0.07d). This could be due to the increase in the protein content which lead to a greater gel formation from protein-water and protein-protein network. In contrast P-CO had the least hardness value of (7.05±0.07a) this could be due to the presence of fiber and water in mushrooms which disrupted the water-protein and protein-protein network, decreasing the gel strength of pâté. This result agrees with results of past research Lin et al,1998.

Table 5: Trichromatic Parameters

	p-100	p-80	p-60	p-40	p-20	p-co
L*	35.86±0.47[a]	37.99±0.47[b]	38.24±0.47[b]	38.74±0.47[b]	41.09±0.47[c]	57.66±0.47[d]
a*	5.65 ±0.07[e]	5.59±0.07[e]	5.37±0.07[d]	5.04±0.07[c]	4.68±0.07[b]	1.59±0.07[a]
b*	22.58±0.18[a]	24.29±0.18[c]	23.57±0.18[b]	23.89±0.18[bc]	25.513±0.18[d]	26.26±0.18[e]

The values a–e Different letters (in the same row) mean that samples are statistically different (α = 0.05).

Table 5 shows results of color parameter, color parameter L* which is for luminosity is shown to have progressively increased with decrease in termite incorporation. From (35.86±0.44347a) to (57.66±0.44347d). this is due to the fact that termites have a very thick brown coloration, which affects the brightness of pâté. As earlier stated the more the termites were incorporated, the lower the L* value. The color parameters (a*, b*) of the various pâtés tend according to the color space. The redness(a*) of pâtés increased upon increase in termite incorporation with highest value of (5.656±0.07e) corresponding to P-100, and the least value being (1.59233±0.07a) meanwhile the yellowishness (b*) of the pâtés increase with decrease in termite incorporation. This two parameters a*,b* respectively for redness and yellowishness both combine to give a pink coloration which is a characteristic of charcuterie products.

Conclusion and Perspectives

The aim of this work was to produce and characterize mushroom based pâté enriched with various incorporation of termite. To achieve this, we needed to know the nutritional value first of our raw materials: The results obtained after research on this showed that the nutritional values of our raw materials were up to the norms as UN accepts insects to be a source of protein for the future when the world's population increases enormously. After characterization of our final products, it showed that the termite incorporation had some influence on their physiochemical properties and the nutritional values have been improved.

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