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Formulation of a sporophore-based supplementary food: the case of the sporophore of the edible fungal species *Pleurotus ostreatus* (Jacq. ex. Fries) Kummer (1871) grown on banana (Musa spp) leaves

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A B S T R A C T

The aim of this study is to develop a suitable treatment for the fruiting substrate based on banana leaves to produce sporophores of *Pleurotus ostreatus*, and formulate some high-quality supplementary food rich in energy and proteins. The plant material consisted of maize (*Zea mays* L), rice (*Oryza sativa*) and groundnuts (*Arachis hypogaea*) and the fungal material used in this work consists of fungal strain 11113 of *Pleurotus ostreatus*. The substrate plant material used consisted of banana leaves, and these leaves were enriched with sawdust, wheat bran and spent grain. Our food supplement was obtained by mixing sporophore powder, rice flour, corn flour and peanuts in different proportions. Proximate analyses were carried out to assess the nutritional profile of the supplementary feed and organoleptic characteristics were assessed. The findings showed that the mycelium was whiter, rhizomorphic and very dense. The appearance of fruiting bodies was observed on day 7 of fruit induction and the harvesting of sporophores from the first flush took place on day 10 in the control treatment. The supplementary feed is rich in protein (20.5%), fats (9.6%) and carbohydrates (52.8%). Further trials need to be carried out on this substrate, while modifying the proportions of ingredients, in order to improve yields.

Keywords: Sporophore; Formulation; Banana leaves; *Pleurotus ostreatus*; Kinshasa.

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1. Introduction

Malnutrition affects children under five, mainly during the complementary feeding period and it is the cause of at least 50% of child deaths worldwide precisely in developing countries such as the Democratic Republic of the Congo (DRC) (Kandala et al., 2011; Akilimali et al., 2022). DRC is affected by this scourge of malnutrition, give a worrying nutritional situation for children under five, i.e. 43% of children are affected by chronic malnutrition while 8% of children suffer from acute malnutrition (Lezama and Oyewale, 2018; Cush et al., 2019; Zawadi, 2023). This child malnutrition already appears around the age of 6 months, a period that corresponds to the introduction of complementary foods (Udoh et al., 2016; White et al., 2017; Harrison et al., 2023). To prevent infant malnutrition, which mainly affects children in developing countries, WHO and UNICEF insist on several points, including strict breastfeeding from 0 to 6 months and a balanced food intake during weaning (Govender et al., 2022). Moreover, malnutrition is characterized by a deficiency in the protein and energy required for a child's development (Govender et al., 2022; Ajmal, et al., 2022).

The introduction of liquid or semi-liquid food supplements in the infant's diet is essential to supplement the intake of breast milk in order to meet their growing nutritional needs beyond 6 months, and to avoid nutritional deficiencies and slowed growth during this weaning period (Dillon, 1989, Abeshu et al., 2016, Aimal et al., 2022, Harrison et al., 2023). After the age of 6 months, breast milk becomes qualitatively and quantitatively insufficient for infants

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whose nutritional needs increase (Agbangnan et al., 2014, Balogun et al., 2015). However, in developing countries, many mothers give their children, from 6 to 60 months, porridges that are most often prepared with local cereal flours (maize, millet, sorghum, etc.) (Marcel et al., 2022). These porridges fill the child's stomach and temporarily suppress the appetite, but they are not very nourishing, as they are low percentages of proteins, vitamins and minerals. Their energy density is less than 60 kcal per 100 ml, i.e. less than milk. The result is malnutrition, especially so-called protein-energy malnutrition (Macrel et al., 2022; Cheickna et al., 2023).

In Africa, over two thirds of the population rely on forest products, either in the form of subsistence derived from a wide range of Non-Timber Forest Products (NTFPs), including edible mushrooms (Degreef et al., 2016, Batubenga et al., 2021). However, the flora of DRC contains a variety of species of high nutritional value, many of which have been used for thousands of years for different uses (Hoare, 2007). Indeed, it has been reported that mushrooms make a significant contribution to the subsistence of African populations, especially the sub-Saharan part, thanks to their considerable nutritional value (Bharucha & Pretty, 2010; Degreef et al., 2016, Fernandes et al., 2021). Edible mushrooms contain reasonable amounts of protein, carbohydrates, fibers (non-digestible complex carbohydrates), minerals, and vitamins, while being low in calories, fats, cholesterol, and sodium (Loria-Kohen et al., 2014, Tounkara et al, 2017, Raman et al., 2021).

Therefore, they can play an important role in the formulation of the supplement food (Pérez-Moreno et al., 2021). Yet, the formulation of a high-quality, energy-, protein- and mineral-rich supplementary food based on mushroom powder (*Pleurotus ostreatus*) is possible (Tolera & Abera, 2017, Dhillon et al., 2023). In such circumstances, it would be advisable to develop a supplementary food based on local, energy-balanced, and protein-rich products. It is necessary to note that the incorporation of local resources like peanuts should be cautious in order to avoid allergies to consumers precisely children though its high content of fats. Yet, there is growing evidence that early introduction of peanut products, in controlled amounts, can reduce the risk of peanut allergies in some children. However, this approach should be taken with caution and should be guided by healthcare professionals, particularly in malnourished populations where children's immune systems may be compromised. While peanuts are a nutritious ingredient, the risk of allergic reactions necessitates careful handling, consideration of alternative ingredients, and clear communication with consumers.

The aim of this study is to develop a suitable treatment for the fruiting substrate based on banana leaves to produce sporophores of *Pleurotus ostreatus*, and formulate high-quality supplementary food rich in energy, protein and minerals. The relevance of this study is to fight against malnutrition and famine in Kinshasa, and in DRC by providing a protein-rich, energy-rich supplementary food.

2. Materials and Methods

2.1. Study area

This study was carried out at the Laboratoire de Myciculture, Life Sciences Mention, Faculty of Sciences and Technologies, University of Kinshasa, Kinshasa, DRC where fungi were cultivated and at the Laboratoire de Nutrition at Institut Supérieur des Techniques Médicales de Kinshasa, Kinshasa in DRC where proximate analyses were carried out.

2.2. Material

The plant material consisted of maize (*Zea mays* L), rice (*Oryza sativa*) and groundnuts (*Arachis hypogaea*), were purchased at the Gambela market in Kasa-vubu municipality, Kinshasa, DRC. The fungal material used in this work consists of fungal strain 11113 of *Pleurotus ostreatus*, which was obtained from the Laboratory of Systematic Mycology and Myciculture, Mention of Life Sciences, Faculty of Sciences and Technologies, University of Kinshasa, Kinshasa, DRC.

2.2.3. Substrate preparation

The culture substrate is defined as the material on which the mycelium grows i.e. the support on which the mycelium will grow until it produces sporophores (Oei, 2005). Mycelium growth is determined by the internal conditions of the substrate and the climate around it. Its composition will determine its selectivity, i.e. its suitability for the needs of the cultivated fungus and its unsuitability for competing organisms (Ibeghouchene and Slimani, 2020). The substrate plant material used in this work consists of banana leaves. These leaves were enriched with sawdust, wheat bran and spent grain.

2.2.3.1. Banana leaves (Musa spp)

The dried banana leaves used in this study were harvested from the garden of a residential plot in the Tchad district, Mont-Ngafula municipality, Kinshasa.

2.2.3.2. Additives

a) Sawdust

Sawdust is an essential growing medium for mushroom fruiting in the laboratory, as it provides the nutrients and structure necessary for mycelium development and sporophore emergence (the mushrooms themselves). The sawdust used as an additive to the fruiting substrate used in this study was purchased from a carpenter's workshop at the Intendance de l'Université de Kinshasa, Lemba municipality in Kinshasa.

b) Wheat bran

Wheat bran comprises the outer tissues of the wheat kernel, including the pericarp, testa, hyaline layer, and aleurone layer. It is produced after grinding the grains and sieving to remove the endosperm and embryo layers. It contains a high level of nitrogenous matter and starch along with cellulose. It is also rich in iron and phosphorus. Wheat bran is a common ingredient in mushroom growing substrates because it provides essential nutrients and fiber to the mycelium, promoting sporophore development and ensuring abundant fruiting. Its role is to improve the structure of the substrate, retain moisture, and provide a source of carbon and other nutrients necessary for mushroom growth, making it an excellent supplement for optimizing laboratory crop yields.

c) Slaked lime

Slaked lime has no direct importance in mushroom fruiting, as it is not a component of the substrates or culture media used in laboratories for commonly cultivated mushrooms. However, it is sometimes used as a substitute for peat in certain mushroom growing substrates, which alters the composition of the nutrient medium and influences fruiting through its effect on pH. Its role is not related to fruiting itself, but rather to preparing the substrate to allow mycelium growth. This additive acts as a buffer and it helps stabilize the pH of the mixture at near-neutral values, and facilitates the early stages of

mycelial invasion. It is obtained after the complete reaction of quicklime with water.

d) Spent grain

These are residues from the brewing of cereals, generally used for animal feed. They are mainly produced by breweries and distilleries manufacturing alcohols and bioethanol, and correspond to all the non-soluble elements remaining after fermentation and transformation of grain starch into alcohol.

Spent grain is an ideal substrate for mushroom fruiting in the laboratory due to its richness in essential nutrients, such as sugars and starch, and its biodegradable organic matter content. After pasteurization or sterilization to eliminate contaminants, spent grain provides everything necessary for mycelium growth. In addition, fungal metabolism breaks down the cell walls of the grains, releasing more nutrients and promoting better fruiting.

2.3. Preparing the final substrate, obtaining the production culture and harvesting sporophores

2.3.1. Preparing the final substrate

The final substrate is the support on which the mycelium will grow until it produces sporophores (Oei, 2005). Indeed, mycelium grows best when the substrate is relatively compacted in the culture bags and there is not too much free space between the particles (Yang & Qin, 2023).

To achieve this, banana leaves were cut into small pieces using a machete, then placed in a bag and soaked in tap water for 48 hours before being drained for 24 hours. The substrate additives - sawdust, wheat bran, slaked lime and spent grain - were not soaked, but used dry.

We then proceeded to prepare three treatments, with the exception of the control treatment. The proportions of the different treatments prepared are given in Table 1.

The table shows that four types of treatments were prepared. To achieve this, we weighed 98% dry banana leaves enriched with 2% slaked lime for the control treatment (T0); 68% banana leaves, 20% sawdust, 10% wheat bran, and 2% slaked lime for treatment T1; 68% dry banana leaves, 20% sawdust, 10% spent grain, and 2% slaked lime for treatment T2; 73% dry banana leaves, 20% sawdust, 5% wheat bran, and 2% slaked lime for treatment T3.

After mixing, the substrates obtained were placed in heat-resistant bags measuring 29.4 cm high by 19.2 cm wide, which we doubled to increase their resistance to the heat of sterilization. The bags were filled with 500 g of substrate and closed with a foam plug, which was wedged into a plastic ring about 2.5 cm in diameter and 2.5 cm high at the neck of the bag, thus forming the bundles.

2.3.2. Moisture content

The moisture is calculated using the following formula (Eq. (1)):

$$\text{Moisture (\%)} = \frac{Mf - Ms}{Mf} \times 100 \quad (1)$$

Where: Mf: Fresh matter (in grams); Ms: Dry matter (in grams).

2.3.3. Heat treatment

After bagging, the sachets were placed in the autoclave for sterilization at 120°C for one hour. After sterilization, all bags were cooled to 30°C before spawning.

2.3.4. Spawning and incubation

After the substrates had cooled, spawning was carried out under aseptic conditions, in an inoculation box next to the flame of an alcohol lamp used to sterilize the sampling material within a radius of around 15 cm from the flame, using the seed (*Pleurotus ostreatus*) on sawdust at a rate of two tablespoons per bundle.

The spiked bundles were then sealed with a foam stopper caught in a plastic ring. After spawning, the bundles were placed in a ventilated cabinet for incubation at room temperature. Incubation took place in the dark until the substrate was completely invaded by mycelium.

2.3.5. Fruit induction and harvesting

This stage involved transferring the mycelial bundles to the mushroom house in the Biology Experimental Garden (Faculty of Sciences and Technologies), precisely in the fruiting shed. The hut is made of mats and its floor is lined with pieces of fired brick. - The hut provides the following conditions: high moisture, adequate light and moderate temperature. In the garden, before the bundles were placed in the mushroom house, and after the caps had been removed, the bags containing the mycelial blocks were incised with a new razor blade. The incisions were in the shape of a 2 cm-long sign of the cross. In the mushroom house, the mycelial bundles were placed on shelves. The hut was watered daily with tap water to maintain its moisture.

In fact, just after the fruiting bodies appeared on the substrate, we waited until they developed into mature sporophores, i.e. the moment when the cap began to become slightly concave. For harvesting, we detached the sporophores from the substrate in such a way as not to damage it. The yield was calculated using the following formula (Eq. (2)):

$$\text{Yield (\%)} = \frac{\textbf{\textit{Weight of fresh sporophores}}}{\textbf{\textit{Weight of watered substrate}}} \times 100 \quad (2)$$

2.4. Mushroom drying

After harvesting, the sporophores produced were oven-dried for 5 days at 45°C and were ground using a sieve (mesh size 500-60 000 µm) stainless steel screen. The sporophore powder obtained was added to the various cereal flours given below to formulate a complementary feed.

2.5. Corn flour

The corn flour was obtained from corn kernels. The kernels were manually sorted to remove panicle and cob debris, washed, oven-dried at 45°C for 30 minutes, then roasted in an electrotech household oven at 100°C for 45 minutes, then ground and sieved using a sieve (mesh size 500-60 000 µm) stainless steel screen.

2.6. Rice flour

Rice grains were sorted by hand, washed, dried in an oven at 45°C for 30 minutes, then ground and sieved using a stainless steel screen (mesh size 500-60 000 µm).

Table 1. Proportions of ingredients in the final substrate.

Final substrate	Ingredients	Proportion (g)	Proportion (%)	Moisture content (%)
T_0	Banana leaves	3000	98	76
	Slaked lime	61	2	
T_1	Banana leaves	3000	68	60
	Sawdust	882	20	
	Wheat bran	441	10	
	Slaked lime	88	2	
T_2	Banana leaves	3000	68	71
	Sawdust	882	20	
	Spent grain	441	10	
	Slaked lime	88	2	
T_3	Banana leaves	3000	73	71
	Sawdust	821	20	
	Wheat bran	205	5	
	Slaked lime	82	2	

T1: First treatment; T2: Second treatment; T3: Third treatment; T0: Control treatment

2.7. Peanut flour

Peanut seeds were manually sorted, roasted, sieved (mesh size 500-60 000 µm) and ground.

2.8. Complementary feed formulation

Our food supplement was obtained by mixing sporophore powder, rice flour, corn flour and peanuts in the following proportions: 50g of sporophore powder, 15g of corn flour, 15g of rice flour and 20g of peanut powder. These ingredients were mixed by hand until a homogeneous mixture was obtained.

2.9. Proximate analyses

Proximate analyses to assess the nutritional profile of the supplementary feed were carried out to determine moisture, crude proteins, crude fibers, total lipids, total ash, total carbohydrates and energy value (Bongo et al., 2019, Elawati et al., 2022, Oyenike et al., 2022, Zakuani et al., 2023). Crude protein was calculated using the conversion factor of (N × 4.38). The carbohydrate content was calculated by difference of major components while the energy content (E) was calculated using the Atwater factor (Sanni et al., 2020) as follows (Eq. (3)):

$$E = (\text{glucides} \times 4) + (\text{lipids} \times 9) + (\text{proteins} \times 4) \quad (3)$$

All analyses were performed in triplicate as recommended in the literature These analyses were carried out in the biochemistry laboratory of the nutrition-dietetics section of the Institut Supérieur des Techniques Médicales de Kinshasa (ISTM), Kinshasa, DRC.

2.10. Organoleptic characteristics

A survey form was used to record respondents' assessments for the organoleptic characteristics of our supplementary food (porridges) (Zakuani et al., 2023), which we compared with 2 other commercial slurries that we used as controls. The survey-participation technique was used, and interviewees were randomly selected in the Life Sciences Mention.

The sensory assessment of the porridge (sporophore-based supplementary food) was conducted following the methodology described by Nilugin et al., (2015), Eke-Ejiofor et al. (2023), Zakuani et al., (2023), utilizing a questionnaire on 7 days of storage. Sporophore-based supplementary food were used for the evaluation and labeled with random numbers and this food was compared to two commercial porridges precisely Protivap and Cerelac which were used as controls. A total of 40 trained semi-panelists, who regularly consume these slurries, were randomly selected to participate in the study. The panel was constituted of which 25 women and 15 men, aged between 20 and 35, selected from the students and staff of Life Sciences Mention, Faculty of Sciences and Technologies, University of Kinshasa located in DRC. To uphold ethical standards, appropriate protocols were implemented to protect the rights and privacy of all participants throughout the study. The panelists evaluated the color and flavor of the samples, using a 3-point hedonic scale ranging from 3= like extremely to 1= dislike extremely. Water was used for rinsing the mouth between each tasting to avoid after taste. It should be noted that the semi-panelists were regular consumers of supplementary food and who were neither sick nor allergic to any of the raw materials used for the production of this porridge. The samples were coded and presented in identical containers.

2.11. Statistical analyses

Descriptive statistics (means and standard deviation) were used to different treatments and biochemical analyses. In order to

compare different treatments, Friedman test was used considering our data where each semi-panelist constituted a repetition with a p-value <0.05 as being significant. The data were processed with SPSS 23.0 software.

3. Results and Discussion

3.1. Sporophore production

3.1.1. Mycelial growth on the final substrate

The characteristics of the mycelium revealed that it was white in color, rhizomorphic in appearance and very dense. Mold growth was observed on 20% of bales in the control treatment, and no mold growth was observed in the first, second and third treatments.

Fig. 1 shows the mycelial invasion time of *Pleurotus ostreatus* on fruiting substrates from different treatments.

The onset of mycelial invasion was perceptible on day 3 of incubation on all bundles for all treatments. We observed total invasion of bundles by mycelium on day 18 for the first treatment (T_1) and on day 20 for the control treatment (T_0), the second treatment (T_2) and the third treatment (T_3).

The characteristics of the mycelium, notably its white color and rhizomorphic appearance, enabled us to identify the presence or absence of molds. In the control treatment, 20% of bales were contaminated by green mold, whereas no mold was observed in the first, second and third treatments. Total invasion of bales by mycelium on day 18 for the first treatment (T_1) and on day 20 for the control treatment (T_0), the second treatment (T_2) and the third treatment (T_3). Fig. 2 presents the steps of mycelial invasion on the substrate.

3.1.2. Fruit induction and sporophore harvesting

After mycelium had completely invaded the substrate and primordia had appeared on all bundles of all treatments, all bundles were transferred to the mushroom house for sporophore development. The appearance of fruiting bodies was observed on day 7 of fruit induction and the harvesting of sporophores from the

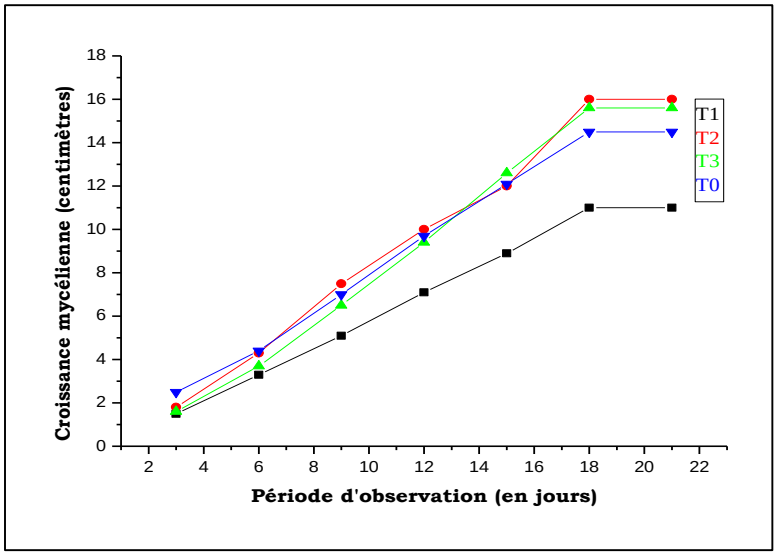

Fig. 1. Mycelium growth rate in different treatments. T0: Control treatment; T1: First treatment; T2: Second treatment; T3: Third treatment.

Fig. 2. Steps of evolution of the sporophore

first flush took place on day 10 in the control treatment. While the appearance of fruiting bodies was observed on day 2 of fruit induction and the harvesting of sporophores from the first flush on day 5 (First treatment).

Fruiting buds appeared on the 2nd day after fruit induction, and sporophores were harvested on the 10th day on bundles from the second treatment; on bundles from the third treatment, fruiting buds appeared on the 2nd day after induction, and harvesting took place on the 8th day.

The yields obtained are given in Table 2. It was observed that the average sporophore yields were 14.2 ± 3.1% for T_1, 16.0 ± 4.8% for T_2, 12.4 ± 2.6% for T_3 and 7.2 ± 2.6 for T_0. Different treatments showed an important yield than the control.

The average sporophore yields obtained in T_2 and T_1 (16.0±4.8% and 14.2±3.1% respectively) were much higher than those obtained in T_3 and T_0 (12.4±2.6% and 7.2±2.6% respectively). This difference in sporophore yield is explained by the difference in additive proportions. Boudouma (2009) and Sztupecki et al. (2023) reported that wheat bran is composed of 14.5% nitrogenous matter, 10.6% cellulose, 28.4% hemicellulose and 20.8% starch. This composition of wheat bran is in addition to that of the base substrate, which contains lignin and cellulose among other compounds. The same applies to sawdust and spent grain. Kurtzman et al. (1982) and Dissasa (2022) have shown that carbon sources suitable for mycelial growth include starch, glucose, fructose, maltose, mannose, sucrose, pectin, cellulose and lignin.

Comparison of the yields obtained in this study with the literature shows that, the yields obtained in this study are lower than the theoretical yield of 20 % recommended by Oei (1993) for a substrate to be considered suitable for the production of an edible fungal species after the harvest of 3 yeasts. On the other hand, the results obtained (26.4%) with the species *Pleurotus florida*, after the harvesting of three yeasts by Mata (1999), using water hyacinth are very much higher than those (16.0± 4.8%; 14.2±3.1%; 12.42±2.6% and 7.2±2.6%) obtained in this study, as well as those obtained (34.1%) by Nzuzi (2017) with a substrate based on banana pseudostems enriched with soy flour.

The cultivation of different local and exotic fungal species their yields as reported by some studies was much lower than this study. Kahambu (2019) who worked on two edible fungal species, *Pleurotus florida* and *Auricularia auricula* grown on *Saccharum officinarum* sugarcane bagasse (i.e. 5.4 ± 1.1% for *Auricularia auricula* and 0% for *Pleurotus florida*) ; Mokata (2020) on *Pleurotus sajor-caju* (1.5 ± 1.1% and 10.5 ± 1.9%); Nyamokombo (2019) on *Pleurotus florida* (Mont.) Singer and *Auricularia cornea* Ehrenb grown on male oil palm (*Elaeis guineensis* Jacq) inflorescences, respectively (4.1 ± 0.9% for substrate treatment made from male palm inflorescences and slaked lime; 6.0 ± 0.9% on male palm inflorescences, sawdust and slaked lime; 6.6±1.1 on male palm inflorescences, wheat bran and slaked lime; 6.0±0.7 on male palm inflorescences, sawdust, wheat bran, corn bran and slaked lime).
By improving the mass and texture of the substrate, it is possible to achieve or even exceed the 20% yield recommended by Oei (1993) for a substrate to be considered suitable for the production of an edible fungal species.

3.2. Determining the nutrient content of supplementary feeds

The nutritional content of our supplementary feed is presented in Table 3.

It was observed that the carbohydrate content (not including the fiber content) is 52.8%; the crude protein content is 20.5%; the lipid content is 9.1%; the crude fiber content is 8.8%; and the total ash content is 3.8%. This allows to say that the formulated supplementary feed better meets feed standards.

It should be noted that edible mushrooms are made up of dry matter and water, in varying proportions. To estimate the energy value of a feed, we need to know its dry matter content. A good supplementary feed must meet the necessary nutritional quality requirements to provide essential nutrients such as proteins, fats, fibers and carbohydrates in sufficient quantities. The findings clearly show that the supplementary feed is rich in protein (20.5%), fat (9.6%) and carbohydrates (52.8%) and the energy value was 375.1 kcal/100 g of fresh matter. The FAO/WHO standard for humidity or moisture content affecting food preservation indicates a moisture of 5%; this level coincides with this study. A moisture content in line with FAO/WHO recommendations. This will enable the supplementary food we have developed to last longer. The high moisture content of *Pleurotus ostreatus* also increases its significance as a functional food due to its ability to regulate metabolic processes, enhance waste removal, gut microbiome health and maintenance of homeostasis (Effliong et al. 2024). However, the low moisture content of the dry *Pleurotus ostreatus* signifies its less susceptibility to microbial infections and an increased shelf life span.

In terms of protein content, the supplementary feed has a protein content of 20.5%. This content also meets FAO/WHO standards, which stipulate that the protein content of flour intended for children should be between 15 and 22.6% (Tounkara et al, 2017). The proteins have the potential of replacing red and processed meat protein sources which contains heme iron, sulphur containing substances, mutagens that increases breast, colorectal cancer risks and other oxidative stress related diseases (Effliong et al., 2024).

The lipid content obtained, i.e. 9.6% of the supplementary feed, is well above FAO/WHO standards. This low content could be explained by the small proportion of peanut flour incorporated in the formulation of our supplementary feed, which is a lipid-rich oilseed. The same is true of the carbohydrate content, which stands at 52.8%, compared with the FAO/WHO standard of 65%. Carbohydrates are the source of energy that can be used by the body. Carbohydrates, proteins and fats are macronutrients required by the body for growth, provision of energy and maintenance of other body functions (Mutuku et al., 2022).

As for the energy value, that obtained in this work, 375 Kcal, is very close to the FAO/WHO standards of 400 Kcal (Tounkara et al., 2017). Effliong et al. (2024), reported that the energy value is a vital nutritional food component that helps to enhance the retaining and absorption of flavors leading to an increased food palatability.
Furthermore, the ash and fiber contents obtained are slightly higher than the FAO/WHO standards, i.e. 3.8% ash content versus 2.9% and 8.8% fiber content versus 5% (FAO/WHO standards, 2006). The ash content reflects the presence of various minerals which plays diverse roles in immune regulation, maintenance of homeostasis, disease prevention and maintenance of metabolic processes (Effliong et al., 2024). While fiber plays an important role in food substances and living organisms when consumed (Effiong et al., 2024). They help enhance food absorption, satisfaction, prevent constipation and serve as substrates for microorganisms. That is the reason why mushrooms should be a regular part of diet to prevent atherosclerosis and high cholesterol due to their high fiber content (Sultana et al., 2024).

Considering the antiglycemic effect of fungal polysaccharides, numerous studies have used fungal fiber to formulate functional foods. In addition to being satiating and nutritious, these products are an ideal complement to special diets such as diabetic and obesity diets (Singh et al., 2025). Seeing the richness of macronutrients that

Table 2. The sporophore yield of different treatments.

T	N.R.	N.S.	PS (g)	PFS			PT(g)	RDT%	Mean ± SD
				L1	L2	L3			
T_0	5	01	500g	25	14	10	49	9.8	7.2±2.6
		02		15	13	6	34	6.8	
		04		7	9	4	20	4	
		05		17	17	7	41	8.2	
T_1	7	01	500g	37	9	18	64	12.8	14.2 ±3.1
		02		41	15	5	61	12.2	
		03		42	27	9	78	15.6	
		04		44	8	5	57	11.4	
		05		55	10	38	103	20.6	
		06		43	8	14	65	13	
		07		38	12	19	69	13.8	
T_2	11	01	500g	54	30	16	100	20	16.2± 3.6
		02		85	30	19	134	26.8	
		03		30	20	12	62	12.2	
		04		40	19	13	72	14.4	
		05		27	25	12	64	12.6	
		06		50	34	22	106	21.2	
		07		40	25	18	83	16.6	
		08		30	18	18	66	13.2	
		09		30	20	12	62	12.4	
		10		41	20	15	76	15.2	
		11		27	22	19	68	13.6	
T_3	7	01	500g	25	6	11	42	8.4	12.42±2.6
		02		59	10	16	85	17	
		03		26	10	20	56	11.2	
		04		44	11	7	62	12.4	
		05		33	13	12	58	11.6	
		06		40	17	11	68	13.6	
		07		38	7	19	64	12.8	

T4: number of treatments; T0: control treatment; T1: first treatment; T2: second treatment; T3: third treatment; N.R.: number of replicates; N.S.: bag number, P.S.: substrate weight; PFS: fresh weight of sporophores; L1: first emergence; L2: second emergence; L3: third emergence; PT: total emergence weight.

Table 3. Nutrient composition of the supplementary feed expressed per 100g of fresh matter and dry matter.

Nutrients (%)	Fresh matter	Dry matter
Moisture	5±0.02	5±0.07
Crude proteins	20.5±0.1	21.6±0.2
Fats	9.1±0.3	9.6±0.5
Ashes	3.8±0.5	4±0.1
Crude fibers	8.8±0.9	9.3±0.5
Glucids	52.8	55.5±0.3
Energy (Kcal)	375.1 Kcal	394.8 Kcal

contain mushrooms, it should be noted as well that edible mushrooms are a great source of polysaccharides with a potential prebiotic effect because they contain indigestible polysaccharides such as glucans, chitin, hemicellulose, mannans, xylans and galactans (Navarro-Simarro et al., 2024).

Furthermore, mushrooms encompass a diverse array of bioactive constituents, which include phenolic acids, glycosides, volatile substances, alkaloids, flavonoids, organic acids, and a variety of biological catalysts such as amylases, cellulases, laccases, lipases, pectinases, proteases, phytases, and xylanases (Singh et al., 2025). And they display a large array of biological properties such as anti-inflammatory, antioxidant, anticancer, antidiabetic, antimicrobial and the healing capacity as well they enhance the gut microbiota (Singh et al., 2025). It should be noted that prebiotics are natural compounds that stimulate the growth of healthy intestinal microbiota, have gained considerable interest for their application in functional foods that can manipulate the composition of the microbiome and protect it from the proliferation of pathogenic bacteria (Navarro-Simarro et al., 2024).

3.3. Organoleptic characteristics

3.3.1. Color

The color appreciation of porridges according to color are shown in the Fig. 3. The figure shows that the majority of respondents (78.6%) stated that the supplementary feed had a very good color, compared with 21.4% of respondents who thought that the color of our feed was good. While 57.1% stated that porridge 1 has a very good color compared 64.3% who thought that porridge 2 has a very good color though 7.1% who thought that porridge 2 had a bad color. There was a statistical significance between the three porridges (Chi^2 = 57.100 for the supplementary food, Chi^2 = 20.100 for porridge 1 (Protivap) and Chi^2 = 56.800 for porridge 2 (Cerelac); df = 2 and p-value < 0.001 for the three formulas).

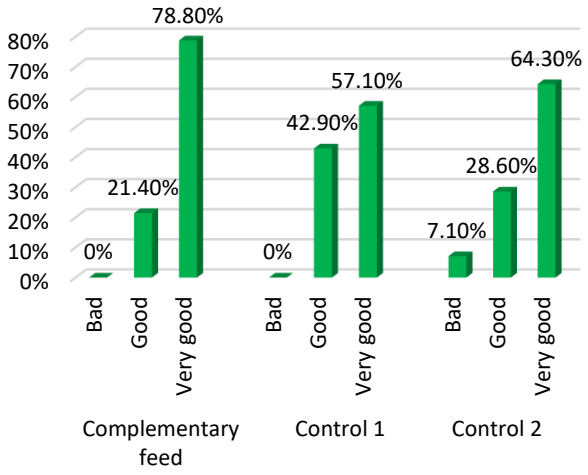

Fig. 3. Color appreciation.

3.3.2. Flavor

The results on the frequency of appreciation of porridges according to flavor are shown in Fig. 4.

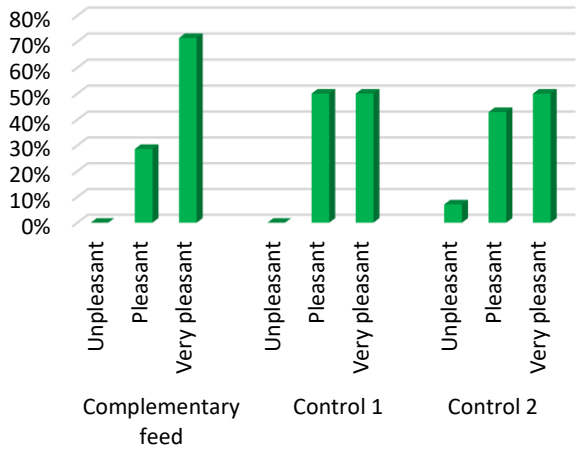

Fig. 4. Flavor appreciation.

The majority of respondents (71.4%) thought that the supplementary feed had a very pleasant taste, compared with 28.6% of respondents who stated that the taste of this supplementary feed was pleasant. 50% think that porridge 1 has a very pleasant flavor compared with 50% who think that porridge 1 has a pleasant flavor. 50% think that porridge 2 has a very pleasant flavor compared with 42.9% who think that porridge 2 has a pleasant flavor and 7.1% who think that porridge 2 has a bad flavor. The comparison of these three formulas considering the taste, the Friedman test, does not indicate a significant difference (Q= 1.458; df = 2; p-value = 0.516).

A comparison of the organoleptic characteristics of this supplementary feed with the commercial porridges (porridge 1 made with protivap and porridge 2 made with cerelac) revealed that the color of the supplementary feed was appreciated compared to the commercial ones used. The same observation applies to the taste, where the appreciation of the supplementary feed predominates over the porridges (71.4%). However, children are highly sensitive to taste, and their preferences strongly influence their willingness to consume a food product. A food that is nutritionally complete but unpalatable may lead to poor intake, reducing the intended nutritional benefits. Incorporating flavors that are familiar or pleasant to children can enhance acceptability and ensure consistent consumption. Additionally, Research suggests that early exposure to a variety of flavors can shape a child's food preferences later in life. Thus, providing a balanced yet enjoyable taste experience in a supplementary food product could not only improve immediate nutrition but also encourage healthier eating habits in the future.

Feyera (2025) reported that the commonly consumed supplementary feed consists of only cereals that do not contain adequate nutrients needed for proper body functioning and growth of older infants and young children, in many developing countries, and this is one of the great challenges. That's the reason, we were focused on choosing local resources which contain adequate nutrients to relieve this issue of malnutrition.

Mushrooms represent one of the world's greatest un-tapped resources of nutritious food. Cultivation of saprophytic edible mushrooms may be the only currently economical biotechnology for lignocellulose organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution. The consumption of mushrooms is now assuming greater importance in human diet worldwide (Okoro and Achuba, 2012). Thus, the necessity of cultivating and making supplementary food from

mushroom origin.

4. Conclusion

The main aim was to develop a suitable treatment for the fruiting substrate based on banana leaves to produce sporophores of *Pleurotus ostreatus* species, and to develop high-quality supplementary food rich in energy, protein and minerals based on the powder of an edible *Pleurotus ostreatus* mushroom species, with a view to improving the nutritional and economic health of the population of Kinshasa.

Pleurotus ostreatus strain adapted well to the different treatments of the fruiting substrate based on banana leaves, having colonized them well and produced sporophores. The latter remain interesting as substrates for the production of sporophores of the aforementioned fungal species, but require improvement through the addition of additives, which will make it possible to achieve the theoretical yield of 20% or more, according to which a substrate is considered suitable for the production of sporophores of an edible fungal species, as it has produced three yeasts in the three treatments.

The findings for the supplementary feed clearly show that, as prepared, the supplementary feed is rich in protein (20.5%), fat (9.6%) and carbohydrates (52.8%). These energy elements give it a total energy of 375 kcal per 100 g of fresh matter, and it meets the nutritional quality requirements for providing essential nutrients such as protein, fat, fiber and carbohydrates in sufficient quantities.

We recommend, however, that further trials be carried out on this substrate, while modifying the proportions of ingredients, in order to improve yields. We also recommend that further trials be carried out on this supplementary feed to improve lipid and carbohydrate content, to achieve the standard energy value (400 Kcal), mineral dosage and microbiological analysis to assess feed quality.

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Conflict of interest

The authors declare that there is no conflict of interest.

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