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A Novel Thickening Agent Made from a Mixture of Cassava and Corn Flour for the Production of Edible Mushroom Growing Media

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Abstract

According to the FAO, sub-Saharan Africa population's diet has a real deficit in animal protein for several reasons, including low animal production, rapid population growth, economic factors, etc. To compensate for this deficiency, the population turn to consuming mushrooms harvested seasonally, which are unfortunately becoming scarce due to deforestation. This has led to a shift towards mushroom cultivation, which necessarily requires the use of agar as a reference solidifying agent for the culture medium. However, agar is an imported product that is not accessible to all social classes. Hence, the aim of this research was to compare agar with three other solidifying agents: A1, A2, and A3, composed respectively of 1:1; 1:2, and 2:1 (cassava flour:corn flour, pretreated in an oven at 157.5 ±3.54 °C). When preparing the culture media with these three solidifiers, the best gel was obtained from solidifying agent A3, with which the fungal species *Pleurotus oestratus* was inoculated. On the third day, the mycelia had invaded all nine tubes of the agar medium, almost reaching the mother culture at the same time (three days) as the reference medium prepared with agar as the solidifying agent (PDA). To ensure the viability of our culture medium prepared with A3, the seed stock and seed led to the production of sporophores, proving that solidifying agent A3 can effectively replace agar, especially in developing countries.

Keywords: solidifier; culture medium; cassava flour; corn flour; mushrooms.

INTRODUCTION

Food insecurity in sub-Saharan Africa is a crucial problem affecting more than hundreds millions of people. According to the FAO (2024), more than 280 million people suffer from chronic hunger. This makes sub-Saharan Africa the region with the highest prevalence of undernourishment in the world and with high levels of child malnutrition. Previous studies have shown that several factors, such as lack of infrastructure, limited resources, armed conflict, poor access to primary health care services, and population growth, are at the root of malnutrition (FANZO, 2012; Regnier et al., 2025). Human nutrition must contain a good level of essential amino acids, which are the basis of protein food quality to combat food insecurity. In fact, according to Stryer *et al.* (1981), everyone should have them in their diet. Animal proteins are generally considered to be of high quality due to their composition of essential and digestible amino acids (Lovedeep *et al.*, 2022). Although their consumption should increase with population growth (Shah, 2021), unfortunately this trend is decreasing in low-income countries due to difficult socioeconomic conditions (Muleya, 2022), lack of promotion of livestock farming, and armed conflicts.

In an attempt to find an alternative to animal proteins, mushrooms are being consumed, as they are more nutritionally beneficial (Oei, 1993) because, in addition to protein, they contain other compounds such as minerals, vitamins, and antioxidants that are beneficial to the well-being of populations (Regnier *et al.*, 2025) in sub-Saharan Africa. These mushrooms are generally harvested seasonally in forests with high humidity. Unfortunately, global warming (Thomas and Vazquez, 2022) and deforestation (Kadhila-Muandingi et al., 2012) are significantly reducing mushroom availability in forest. Scientists have carried out studies to domesticate mushrooms using mainly agricultural wastes (Akter et al., 2022, Bertin, 2021; Gwanama et al., 2011). However, this approach requires the use of solidified culture medium by a specifically, agar, which is generally unaffordable for all social classes, especially in sub-Saharan Africa.

Agar is a sulfated sugar polymer composed mainly of D-galactose, 3,6-anhydro-1-galactose, and gluconic acid. It provides a solid pure culture medium (Prescott et al., 2003).

To our knowledge, no other solidifying agent have been used to replace agar (Chang and Quimo, 1989), as evidenced in the mycology collection at the Catholic University of Louvain, which lists 44 culture media using only agar as a solidifying agent (MUCL, 1998). The aim of this research is therefore to study another locally produced solidifying agent that is accessible to all as a substitute for agar.

MATERIAL AND METHODS

MATERIAL

Cassava and corn flours, as well as sweet potatoes, were purchased at the Ngaba market and rice (pudding) was purchased at Kingabwa market (along the Congo River), all in Kinshasa. *Pleurotus ostreatus* seeds were obtained from the mycology laboratory, and agar was obtained from the microbiology laboratory of the Life Sciences Department, Faculty of Science and Technology, University of Kinshasa. Auricularia and Schyzophillum were harvested from our plot in the commune of Ngaliema/Kinshasa.

METHODS

Flours pretreatment

The two flours were heated separately at 157±3.54°C in an oven for 50-60 minutes. Meanwhile, 50 g of sweet potato were peeled, washed with tap water and then with distilled water, before cutting it into small pieces.

Preparation of Gel

In a 500 mL backer was placed, 50 g of chopped potatoes and 250 mL of distilled water It was heated on a hot plate while, adjusting the volume of water until a juice was obtained. Then, 15 g of solidifier (A1, A2, or A3, as appropriate) was gradually added to the juice on the hot plate while stirring with a spoon, followed by addition of 5 g of dextrose until a gel was obtained. The gel was divided into 9 test tubes (3-5 mL). The tubes containing the gels were then plugged with cotton wool, covered with aluminum foil, and sterilized at 1 atm in an autoclave for 15 minutes. The tubes were cooled to ambient temperature in an inclined position to ensure a good seeding surface, and the solidification was evaluated several hours later.

Inoculation and obtention of starter for mushroom culture

After solidification of the medium (A3 called Ms Med), it was inoculated with the *Pleurotus oestratus* specie on the 5th day, and 3 days after inoculation, the mycelium invaded the medium in all 9 tubes without contamination.

Preparation of the reference culture medium (PDA)

The control gel medium was prepared using the same procedure, taking 50 g of sweet potato, 5 g of agar, and 5 g of dextrose. The resulting gel was divided into 14 tubes containing 3 to 5 mL

Obtaining the starter culture of the reference medium (PDA)

The starter culture was obtained after inoculating the *Pleurotus Ostreatus* species and waiting for mycelium to appear in the tubes.

Seedbed preparation on unhusked rice

The seedbed was prepared by taking 792 g of unhusked rice, washed with tap water to avoid impurities and dust, then soaked in water for 24 hours and boiled on a hot plate until it was well cooked. After cooling, the water was drained off, 16.16 g of slaked lime was added and mixed. The mixture was distributed into jars of 500 mL to 1/3 of their capacity. The jars were covered with cotton wool and topped with aluminum foil, then sterilized at 1 atm for 15 minutes in an autoclave**.**

Preparation of the fruiting substrate for two media and obtaining the seed for two media.

The sawdust from a sawmill of Kindele area in Mont-Ngafula municipality/Kinshasa, was used as fruiting substrate for two media seed. After sieving, 1,800 g sawdust were taken and mixed with 23 % wheat bran, 2% sugar, 2% slaked lime and 3 liters of tap water. The mixture obtained was divided in two parts in jars: 6 for the A3 medium (cassava-corn flour) and 6 jars B medium (PDA). The inside of each jar lid was covered with cotton wool and topped with aluminum foil, then sterilized at 1 atm in an autoclave for approximately 15 minutes. The different media were seeded in the inoculation box with the semi-white.

Final substrate preparation and sporophores production

a. Final substrate preparation

The final substrate (20,000 g) was obtained by mixing 15,000 g of sawdust, previously fermented for 4 days, with 5,000 g of fermented and drained banana leaves. To this mixture was added, 15% wheat bran (3,000 g) and 2% slaked lime (400 g). Then, 800 g of this mixture was packed into small transparent plastic bags, pasteurized for 2 hours in a barrel heated to approximately 100°C, and then repasteurized 12 hours later to prevent contamination. After cooling, the bags were seeded in the inoculation box and kept in the incubation box at 25±1°C until white spots appeared.

b. Sporophores production of the two media

The fruiting substrate were prepared by weighing 20 kg of sawdust about 18 bales (800 g each) and placing it in

the inoculation box (9 for each medium) with only rice seeds and kept in cabinets for incubation at ±27°C.

RESULTS AND DISCUSSION

RESULTS

Gel preparation by using three different solidifiers

Conditions of preparation of the solidifiers are shown in Table 1. The gelatinization time decreases in the following order A1, A2 and A3. The solidifier A1 did not solidified whereas it took 72 hours for A2 and 48 hours for A3 to solidify. Only A3 didn't disolidified therefore it was used in subsequent experiment

Table 1. Gel obtention from three solidifiers.

Solidifiers	A1	A2	A3
gelatinization time (minutes)	25	20	15
solidification time (hour)	-	72	48
disolidification time (hour)	-	>72	Never

Legend: A1: cassava and corn flours (1:1); **A2:** cassava and corn flours (1:2); **A3:** cassava and corn flours (2:1)

It was observed that A1 and A2 media were not solidified only A3 named Ms Med and served to inoculate *Pleurotus oestratus.* The Figures 1 and 2 show the Ms Med stater obtained at 155 and 160 °C respectively and invaded after inoculation with *Pleurotus oestratus*.

Figure 1. Ms Med stater treated at 155°C.

Figure 2. Ms Med stater treated at 160°C.

The two gel media preparation

Parameters in the preparation of Ms Med medium compare to the reference medium are shown in Table 2.

Table 2. The two gel media preparation in six test tubes for each one.

Parameters	Ms Med medium (cassava-corn flour)	Reference medium (PDA)
Solidification time (days)	2	1
Duration before inoculation (days)	4	3
Duration of mycelium invasion (days)	3	2
Completion duration (month)	3	1 ½

The results in Table 2 show that Ms med medium solidified slightly later (2 days) than reference medium (reference). It was also noted that the time before inoculation of Ms Med medium was long (4 days) compared to that of the reference medium, which was 3 days. Similarly, the invasion time for Ms Med medium was considered to be as long as that for reference medium. Finally, we observed a slow depletion of Ms Med medium (3 months) compared to reference medium (1½ months).

This delayed solidification of Ms Med medium can be explained by a slow loss of water from this medium compared to reference medium. Indeed, reference medium contains agar as a solidifier, which is a pure gelling agent optimized to form a solid network, and its gelation and solidification are rapid due to the good network it forms. Ms Med medium, on the other hand, contains a mixture of cassava and corn flour as a solidifier. The latter is composed of starch and many other impurities that can disrupt the network, leading to a less clear gel that is slower to form. Starch forms gels through gelatinization and then retrogradation, but these gels are generally less rigid, more sensitive to water and temperature, and often classified as weak gels (Chamorro et al., 2025). As for the slow invasion of Ms Med medium, it has been reported that mycelium does not grow well in a medium with a high water content (Lahouar et al., 2016; Han and Nout, 2001). Thus, as Ms Med medium slowly loses its water content, this would explain the slow invasion, as it takes time for the medium to become solid.

The two media observation

Table 3. Visual observation of the two gel media used.

No	Culture medium	Visual appearance	Effect
01	Ms Med medium (cassava-corn flour)	Less clear	Not easy to observe mycelium
02	Reference medium (PDA)	Clear	Easy to observe mycelium

The results in Table 3 show that the two culture media do not have the same visual appearance. Indeed, the Ms Med medium was brownish, while the reference medium was yellowish (Figure 3). The brown color of Ms Med medium could be explained by the Mayer reaction undergone by the cassava flour. The Figure 3 shows the observation invasion of the two staters (Ms Med [left] and reference [right]).

Figure 3. Ms Med and reference invaded

Appearance, invasion, and speed of mycelium in both media

The table 4 gives the mycelium growth in the two culture media.

Table 4. Mycelium growth in the two culture media.

Culture medium	Appearance after inoculation (hours)	Invasion after apparition (hours)	Invasion speed
Ms Med medium (cassava-corn flour)	24	72	Faster
Reference medium (PDA)	24	72	Fast

As can be seen in Table 4, the evolution of the mycelium in both media was the same in terms of appearance after inoculation and subsequent invasion. However, it was found that the rate of mycelium invasion was faster in Ms Med medium than in reference medium. This difference between the two media could be explained by the chemical composition of the different solidifiers used. It was found that agar is composed of agarose and agaropectin (Lin et al., 2022), which are sugar polymers, while the cassava-corn flour mixture is composed of starch (amylose and amylopectin), which are sugars, but also contains proteins, fibers, and possibly lipids (Chamorro et al., 2025; Ubalua et al., 2014). In addition, this rapid invasion in Ms Med medium is thought to be due to the activity of water in this medium. This can be explained by the late solidification of Ms Med medium (2 days), which means that even though the medium had solidified, there was still some residual moisture favorable to mycelium invasion. This corroborates the work of Pardo *et al.* (2005), who found that mycelium needs water for invasion. Zhang *et al.* (2023) showed that carbon and organic nitrogen promote rapid mycelial growth. Therefore, the rapid growth of mycelium in Ms Med medium is likely due to the presence of carbon (the main source of energy) and nitrogen from the starch and proteins in the solidifying agent used. Contrary to previous studies (Boulfelfel *et al.*, 2022, Neelam *et al.*, 2013, and Hoa and Wang, 2015), which showed that reference medium (PDA) was the favorable culture medium that improved both mycelial extension and density levels of *Pleurotus ostreatus*, this study proved that the medium used (cassava-corn flour) was even more favorable than PDA in terms of colonization and mycelial growth and, above all, more economical for developing countries.

Appearance, spread, and mycelium speed in semi-white production

The evolution of the mother spawn (Ms Med and reference) is shown in Table 5.

Table 5. Mother spawn A et B evolution on unhusked rice.

Medium	Bottle number	Appearance duration (hours)	Invasion duration (hours)	Invasion speed (cm/day)
Ms Med	3	12	48	1,5
Reference	3	12	60	1

The observations in Table 5 show that for the same substrate (unhusked rice), mother spown of Ms Med had a faster invasion rate than that of reference medium, at 1.5 and 1 cm per day, respectively. Similarly the mother spawn of Ms Med had a shorter invasion time than that of the reference, showing that Ms Med mother spawn adapted more easily and grew faster than the reference. This could be explained by the nutrients that Ms Med mother spawn had originally consumed (Hoa *et al.*, 2015).

The Figure 4 shows the mother spawn of the reference and Ms Med media.

Figure 4. Reference and Ms Med mother spawn.

Seed production

Table 6 shows the results of seed production from Ms Med and reference mother spawn

Table 6. Seed production on sawdust.

Spawn	Bottles	Begining Invasion	Completion invasion
Ms Med	6	48 h after	3 weeks
reference	6	48 h	2 weeks

As seen in Table 6, the two spawns of Ms Med and reference had the same invasion time in the substrate used (sawdust).

The Figure 5 shows the Ms Med seed production.

Figure 5. Ms Med seed production

Sporophores Production

Table 7 shows the results of the production of sporophores Ms Med and reference, each consisting of 5 bundles.

Table 7. Production of Ms Med and reference sporophores in each of the 5 fruiting bundles.

Flesh (days)	Bundle weight (g)	Ms Med sporophores weight (g)	reference sporophore weight (g)
1	800	30	-
2	800	35	30
3	800	53	5
4	800	78	50
5	800	57	-
Total	**-**	**253**	**85**

The results in Table 7 show that, with an average temperature of 30°C over five days, Ms Med seed produced more sporophores than reference seed, namely 253 g compared to 85 g for reference sporophore. Given that we had the same species of mushroom (*Pleurotus oestratus*), this difference in production would be due to the amount of nutrients contained in each of these seeds. Therefore, Ms Med seed would have a higher nitrogen content than the reference, which promoted its overproduction (Hoa and Wang, 2015). The Figures 6 and 7 show the mushroom produced from Ms Med and reference seeds.

Figure 6. Mushroom from Ms Med seed.

Figure 7. Mushroom from reference seed.

CONCLUSION

This research focused on producing a culture medium solidified by a solidifying agent other than agar, which is more widely used and less affordable, especially in developing countries such as the Democratic Republic of Congo. By preparing two culture media, Ms Med and reference, the first solidified with a mixture of cassava flour and corn (2:1) and the second with agar, it was shown that Ms Med medium solidified later (2 days) than the reference (1 day) and had a longer depletion time (3 months) than the reference (1½ months). These results show that the solidifier used in Ms Med enriched the medium with nutrients (carbon and nitrogen), which promotes mycelial growth, sporophore multiplication (Table 5), and therefore high yield in despite stressful conditions (30°C). In addition, Ms Med medium with late solidification retained a certain amount of moisture favorable for mycelial growth and had a long depletion period, which is an advantage for its preservation and prolonged use. Given that the solidifier used in Ms Med medium can be obtained with local and inexpensive materials while giving the best results as well as the medium using agar, we believe that it can replace the later in the cultivation of edible mushrooms in developing countries.

Competing Interests: The authors declare that there are no competing interests.

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