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Physicochemical characteristics of the formulated culture media using locally available root crops

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Key words: Physicochemical, Formulated culture media, Natural and synthetic culture media, Root crops, Tube, Taro, Cassava, Dextraose agar

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Abstract

The study aimed to develop fungal culture media in dehydrated form utilizing selected locally available root crops such as cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), ube (*Dioscorea alata* L.), taro (*Colocasia esculenta*) and potato (*Solanum tuberosum*). Specifically, the objectives of the study was to determine the physicochemical characteristics of the formulated culture media such as the color, clarity, gel strength, ash, moisture, crude protein, crude fat and total carbohydrate contents. From the formulated combination, 39 grams of the formulated culture medium was suspended in 1000 ml of distilled water. The agar powder acted as a gelling agent for the medium. The resulting solution was boiled until all constituents were dissolved. It was autoclaved for 15 minutes at 121°C. The pH was adjusted based on the following requirement of the fungi: *Saccharomyces cerevisiae* 4-6, *Aspergillus niger* 5.5 and *Rhizopus stolonifer* 7-8. The media was dispensed into sterile Petri dish, taking care to distribute equally at approximately 20-25ml per petri dish. Based on the findings of the study: (1) the formulated culture media possessed the necessary physicochemical characteristics of culture media for the culture of fungi; and (2) Cassava, sweet potato, ube, taro and potato with dextrose and agar powder showed comparable effects on the growth of fungi under the study. For future researches and studies, the following may be considered: (1) the formulated fungal culture media utilizing local rootcrops are recommended for the cultivation of *Saccharomyces cerevisiae, Aspergillus niger* and *Rhizopus niger;* and (2) a study may be conducted on the shelf-life of the formulated fungal culture media.

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Introduction

Culture media play a pivotal role in any microbiology laboratory. They are widely employed for isolation, identification and sensitivity testing of different pathogenic microorganisms. Most of the laboratories usually prepare their own media for routine diagnostics as well as research purposes (Basu *et al.*, 2005). Without high-quality media, the possibility of achieving accurate, reproducible and repeatable microbiological test results is reduced. A microbiological culture medium is a substance that encourages the growth, support, and survival of microorganisms.

Culture media contains nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals, and gelling agents (for solid media). Culture media has been used by microbiologists since the nineteenth century. Even with the increased use of rapid methods the majority of techniques found in the pharmaceutical quality control laboratory require growth media. For the assessment of culture media, no one definitive standard exists.

Media containing high carbohydrate source, nitrogen source are required for the growth of fungi at pH range of 5 to 6, and a temperature range from 15 to 37˚C. There are two general types of fungal culture media: natural and synthetic. Natural media are composed of natural substrates, such as herbaceous or woody stems, seeds, leaves, corn meal, wheat germ, and oatmeal etc. Natural media are usually easy to prepare but they have the disadvantage of their unknown composition. Some examples include corn meal agar, potato dextrose agar, V-8 juice agar, and dung agar. Synthetic media, on the other hand, contain ingredients of known composition. These types of media can be duplicated with precision each time they are made and contain defined amounts of carbohydrates, nitrogen, and vitamin sources. Czapek- Dox medium, glucose-asparagine and Neurosporacrassa minimal medium fall in this category.

One of the standard approaches to the laboratory diagnosis of fungal infections is the cultivation of the causative fungus and its subsequent identification. For any fungus to be cultivated for any purpose, it is necessary to provide the appropriate biochemical and biophysical environments. The biochemical or nutritional environment is made available as culture medium (ASM, 2019).

However, these culture media are not readily available and expensive and thus their usage in small diagnostic laboratories has undoubtedly decreased. With this situation at hand, the protocols of proper disease diagnosis which involves the isolation and identification of the etiologic agent in a disease has been commonly disregarded.

As a consequence, there is unnecessary and inadequate administration of medications that would possibly result to the development of resistance by these microorganisms. Analyzing such circumstances, simple culture media in dehydrated form with low cost using abundant naturally occurring resources such sweet potato (*Ipomoea batatas*), cassava, (*Manihot esculenta*), ube (*Dioscorea alata* L.), taro (*Colocasia esculenta*), and potato (*Solanum tuberosum*) shall be formulated. Generally, the study aimed to develop fungal culture media in dehydrated form utilizing selected locally available root crops such as cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), ube (*Dioscorea alata* L.), taro (*Colocasia esculenta*), and potato (*Solanum tuberosum*). Specifically, the objectives of the study was to determine the physicochemical characteristics of the formulated culture media such as the color, clarity, gel strength, ash, moisture, crude protein, crude fat and total carbohydrate contents.

Materials and methods

Research Design

The study aimed to develop fungal culture media in dehydrated form utilizing commonly available rootcrops such as cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), ube (*Dioscorea alata* L.), taro (*Colocasia esculenta*), and potato (*Solanum tuberosum*).

Materials

The five rootcrops used in the study were Cassava (*Manihot esculenta*), Sweet potato (*Ipomea batatas*),

Ube (*Dioscorea alata* L.), Taro (*Colocasia esculenta),* and Potato (*Solanum tuberosum*) which were procured from local markets. They were processed into powder using a knife, pan, stove, hot air oven and sieve. The components of the culture media were the powdered root crop, dextrose and agar powder (food grade) mixed with distilled water. A weighing scale was used to measure the proper amount of each component of the formulated culture media (Table 1). The formulated culture media prepared was sterilized through an autoclave. Test fungi (*Saccharomyces cerevisiae*, *Aspergillus niger* and *Rhizopus stolonifer*), sterile inoculating needle, alcohol lamp, sterile petri dishes, and incubator were utilized in the cultivation of the fungi. A digital Vernier caliper, digital single lens reflex (DLSR) camera and a calculator were employed in data gathering. Commercial Potato Dextrose Agar was used as control.

Formulation of the Culture Media

There were five rootcrops used in the study namely: cassava (*Manihot esculenta*), sweet potato (*Ipomea batatas*), ube (*Dioscorea alata* L.), taro (*Colocasia esculenta),* and potato (*Solanum tuberosum*). Each rootcrop was peeled, washed, grated and oven-dried at 80°C. It was then pulverized and sieved. The root crop powder was combined with extrose powder, and agar powder (food grade) following the formulation in Table 1.

Preparation of the Formulated Culture Media

Using the formulations in Table 1, 39 grams of the formulated culture medium was suspended in 1000 ml of distilled water. The agar powder acted as a gelling agent for the medium.

Table 1. Comparison of the formulated culture media per liter of distilled water.

Level (%)	Root Crop Powder (g)	Dextrose (g)	Agar (g)
75	18.0	6.0	15.0
85	20.4	3.6	15.0
95	22.8	1.2	15.0

The resulting solution was boiled until all constituents were dissolved. It was autoclaved for 15 minutes at 121°C. The pH was adjusted based on the following requirement of the fungi: *Saccharomyces cerevisiae* 4-6, *Aspergillus niger* 5.5 and *Rhizopus stolonifer* 7-8. The media was dispensed into sterile Petri dish, taking care to distribute equally at approximately 20-25ml per petri dish.

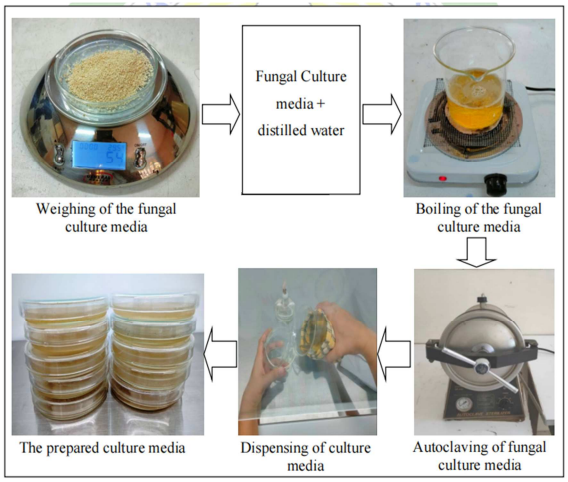

Fig. 1. Preparation of Formulated Culture Media.

Preparation of the Potato Dextrose Agar

Thirty-nine (39) grams of Potato Dextrose Agar was suspended in 1000 ml distilled water then boiled to dissolve the medium completely. The media was autoclaved for 15 minutes at 1210C and cooled to 45 - 500C. The pH was adjusted then mixed well before dispensing on sterile plates.

Physicochemical Characterization

Important tests were carried out, i.e. (1) Visual Test for Color; (2) Visual Test for Clarity, and (3) Gel Strength.

Visual Test for Color

The color of the prepared culture media was determined using colorimetric chart.

Visual Test for Clarity

The clarity of the media should be examined for optical artifacts.

Gel Strength

The gel strength should not be over-hard or over-soft, but firm and usable

Data Gathered

Physicochemical characteristics

These were the color, clarity, gel strength, moisture, ash, protein, sugar, and fat contents of the formulated culture media.

Data Analysis

All data were expressed as mean + standard error of the mean (SEM). The gathered data were analyzed using standard deviation and Analysis of Variance (ANOVA). The Tukeys honestly significant difference (HSD) test and Post hoc test were used in the determination of the significance of the difference between means.

Results and discussion

Physicochemical Characteristics of the Formulated Culture Media

The physicochemical characteristics of the formulated culture media are presented in Table 2. Results showed that in 100 grams, the formulated culture media like cassava dextrose agar contained 6.18g ash, 5.11g moisture, 0.69g crude protein, 0.16g crude fat, and 87.86g total carbohydrate. Likewise, sweet potato dextrose agar contained 6.86g ash, 5.33g/100g moisture, 1.84g crude protein, 0.16g crude fat, and 85.81g total carbohydrate; ube dextrose agar contained 7.22g ash, 6.03g moisture, 3.04g crude protein, 0.11g crude fat, and 83.60g total carbohydrate; taro dextrose agar contained 7.40g ash, 4.47g moisture, 1.94g crude protein, 0.10g crude fat, and 86.09g total carbohydrate and potato dextrose agar contained 5.38g ash, 6.24g moisture, 0.14g crude protein, 0.04g crude fat, and 88.20g total carbohydrate.

Table 2. Physicochemical characteristics of the formulated culture media (g/100g)

Culture media	Cassava dextrose agar	Sweet potato dextrose agar	Ube dextrose agar	Taro dextrose agar	Potato dextrose agar
Chemical characteristics					
Ash	6.18	6.86	7.22	7.40	5.38
Moisture	5.11	5.33	6.03	4.47	6.24
Crude Protein	0.69	1.84	3.04	1.94	0.14
Crude fat	0.16	0.16	0.11	0.10	0.04
Total Carbohy drate	87.86	85.81	83.60	86.09	88.20
Physical Characteristics					
Color	Light yellow	Light yellow	Lavander blush	Ivory	Ivory
Clarity	Semi Transparent	Semi Transparent	Semi Transparent	Semi Transparent	Semi Transparent
Gel strength	Firm	Firm	Firm	Firm	Firm

Culture media contains the nutrients needed to sustain a microbe. Culture media can vary in many ingredients allowing the media to select for or against microbes (Boundless, 2021). Media generally contain a source of carbon, nitrogen and vitamins.

Glucose (dextrose) is the most widely utilizable carbon source, and hence is the most commonly used in growth media. Fructose and mannose are the next most commonly utilized sugars by fungi and are found in media from natural sources. Sucrose (table sugar) may be used in some media. Nitrogen sources include peptone, yeast extract, malt extract, amino acids, ammonium and nitrate compounds (Zimbro, 2009). Media containing high carbohydrate source, nitrogen source are required for the growth of fungi at pH range of 5 to 6, and a temperature range from 15 to 37˚C. There are two general types of fungal culture media: natural and synthetic. Natural media are composed of natural substrates, such as herbaceous or woody stems, seeds, leaves, corn meal, wheat germ, and oatmeal etc. Natural media are usually easy to prepare but they have the disadvantage of their unknown composition. Some examples include corn meal agar, potato dextrose agar, V-8 juice agar, and dung agar. Synthetic media, on the other hand, contain ingredients of known composition. These types of media can be duplicated with precision each time they are made and contain defined amounts of carbohydrates, nitrogen, and vitamin sources (Basu *et al*., 2015).

Rudrappa (2021) reported that cassava has nearly twice the calories than that of potatoes and perhaps one of the highest value calorie foods for any tropical starch-rich tubes and roots. 100 g root provides 160 calories. Their calorie value mainly comes from sucrose which accounts for more than 69% of the total sugars.

Amylose (16-17%) is another major source of complex carbohydrates. Cassava is very low in fats and protein than in cereals and pulses. Nonetheless, it has more protein than that of other tropical food sources like yam, potato, plantains, etc. Based on the USDA National Data Base, the nutrition value per 100 gram of raw cassava root are the following: Energy- 160Kcal, Carbohydrates- 38.06g, Protein- 1.36g, Total Fat- 0.28g, Cholesterol- 0mg, Dietary Fiber- 1.8g, Vitamins like: Folates- 27µg, Niacin- 0.854mg, Pyridoxine- 0.088mg, Riboflavin- 0.048mg, Thiamin- 0.087mg, Vitamin A- 13 IU, Vitamin C- 20.6mg, Vitamin E- 0.19mg, Vitamin K- 1.9 µg; electrolytes like Sodium- 14mg and Potassium- 271mg; and minerals like Calcium- 16mg, Iron- 0.27mg, Magnesium- 21mg, Manganese- 0.383mg, Phosphorus- 27mg, and Zinc- 0.34mg. However, Waisundara (2017) added that cassava roots and leaves are deficient in the sulphur containing mino acids, methionine and cysteine, and some nutrients are not optimally distributed within the rest of the plant's physiology.

The aqueous and ethanolic extracts of raw cassava tuber contain alkaloids, flavonoids, tannins, reducing sugars and anthocyanosides, but do not contain cardiac glycosides, anthraquinone, phlobatinnins and saponins. Vitamins A, C and E and minerals, namely, calcium, magnesium, phosphorus, iron, sodium, and chloride ions were identified in the raw and boiled cassava tubers and raw leaves and their levels were significantly reduced by boiling (Ebuehi *et al.*, 2018).

Cassava is basically composed of starch. Cassava starch is composed of two components: amylose and amylopectin. An amylose is a long straight chain of polymer of anhydroglucose units. An amylopectin is a branched chain compound, also of anhydroglucose units (Bartlebay Research, 2013). Chalapathi *et al.* (2010) concluded that the binding capacity of *Manihot esculenta* starch would be many times greater than that of industrial starch. They had sufficient hardness to withstand the wear and tear during the handling of the tablets for the various evaluation studies. The physical appearance of the tablets was same for both formulated as well as industrial starch.

Sweet potatoes are becoming a research focus in recent years due to their unique nutritional and functional properties. Bioactive carbohydrates, proteins, lipids, carotenoids, anthocyanins, conjugated phenolic acids, and minerals represent versatile nutrients in different parts (tubers, leaves, stems, and stalks) of sweet potato. The unique composition of sweet potato contributes to their various health benefits, such as antioxidative, hepatoprotective, anti-inflammatory, antitumor, antidiabetic, antimicrobial, antiobesity, antiaging effects (Wang *et al.*, 2016). According to Ware (2019), a 124 gram serving of mashed sweet potato contains: Energy (calories)- 108, Protein- 2g, Fat- 3g, Carbohydrate- 18.7g, Fiber- 2.48g, Iron- 0.7mg, Calcium- 40.8mg, Magnesium- 19.8mg, Phosphorus- 50.8mg, Potassium- 259mg, Sodium- 306mg; Selenium 0.9mcg, Vitamin C- 12.8mg, Folate- 7344mg, Choline- 14.4mg, Vitamin A- 823mcg, Bcarotene- 9470mcg, Vitamin K- 5.1mcg, and cholesterol- 1.24mg.

As reported by McCabe (2019), purple yam (ube) is a starchy root vegetable that's a great source of carbohydates, potassium, and vitamin C. One cup (100 grams) of cooked ube provides the following: calories- 140, carbohydrates- 27 grams; protein- 1 gram; fat- 0.1 grams; fiber- 4 grams; sodium- 0.83% of the Daily Value (DV); potassium- 13.5% of the DV; calcium- 2% of the DV; iron- 4% of the DV; vitamin C- 40% of the DV; and Vitamin A- 4% of the DV. In addition, they are rich in powerful plant compounds and antioxidants, including anthocyanins, which give them their vibrant hue.

The Food and Agriculture organization (Onwueme, 1999) described that the main economic parts of the taro plant are the corms and cormels, as well as the leaves. The fresh corm has about two-thirds water and 13-29% carbohydrate. The composition of the carbohydrate fraction indicates that the predominant carbohydrate is starch. The starch itself is about four fifths amylopectin and one-fifth amylose. The amylopectin has 22 glucose units per molecule, while the amylose has 490 glucose units per molecule. The starch grains are small and therefore easily digestible.

This factor makes taro suitable as a specialty food for allergic infants and persons with alimentary disorders. However, the smallness of the starch grains makes taro less suitable as a source of industrial starch. The starch in the corm is more concentrated at the corm base than at the corm apex.

Taro contains about 7% protein on a dry weight basis. This is more than yam, cassava or sweet potato. The protein fraction is low in histidine, lysine, isoleucine, tryptophan, and methionine, but otherwise rich in all the other essential amino acids. The protein content of the corm is higher towards the corm's periphery than towards its center. This implies that care should be taken when peeling the corm; otherwise a disproportionate amount of the protein is lost in the peel. The proximate composition of the taro corm on a fresh weight basis are: moisture- 63-85%, carbohydrate (mostly starch)- 13-29%, protein- 1.4-3.0%, fat- 0.16-0.36%, crude fibre- 0.60-1.18%, ash- 0.60-1.3%, vitamin C- 7.9mg/100g, thiamine- 0.18mg/100g, riboflavin- 0.04mg/100g, and niacin- 0.9mg/100g.

Potatoes are rich in vitamins, minerals and antioxidants, which make them very healthy. Potatoes are an excellent source of many vitamins and minerals. One medium baked potato (6.1 ounces or 173 grams), including the skin, provides: calories: 161, fat: 0.2 grams, protein: 4.3 grams, carbs: 36.6 grams, fiber: 3.8 grams, vitamin C: 28% of the RDI, vitamin B6: 27% of the RDI, potassium: 26% of the RDI, manganese: 19% of the RDI, magnesium: 12% of the RDI, phosphorus: 12% of the RDI, niacin: 12% of the RDI, and folate: 12% of the RDI (Raman, 2018). Potatoes have only a trace of fat, and that tiny amount is split between saturated and polyunsaturated fat. They also have trace amounts of omega-3 fatty acids and omega-6 fatty acids. As a vegetable, they have no cholesterol (Lehman, 2020).

FAO (2017) as cited by Heuzé *et al.* (2018) described that potato is the world's fourth most important food crop after maize, wheat and rice with 381 million t (fresh weight) of tubers produced in 2014. Potato tubers are a source of high grade starch for industry. Potatoes have a wide range of industrial applications. They can be used in ethanol production, yield pulp for paper industry and they may also provide raw material to the chemical industry.

As reported by Heuzé *et al.* (2018), although potatoes are mainly considered an out most important source of starch, they contain high-quality protein in small amounts (2% fresh basis, i.e. 10% DM basis, which is comparable to cereal grains). Potatoes are an excellent source of lysine, but low contents of sulfur-containing amino acids limit their nutritive value. Martinez *et al.* (2019) added that among starches, potato starch is preferred because its paste is characterized by high clarity and neutral taste. This clarity is attributed to the high content of phosphate esters on the amylopectin chain. In addition, because of its granule size, purity, amylose and amylopectin chain lengths, the ability to exchange certain cations with corresponding effects on rheological behavior, and, to form thick viscoelastic gels upon heating and subsequent cooling, the potato starch is considered important and unique.

Potatoes contain a special type of starch known as resistant starch. This starch is not broken down and fully absorbed by the body. Instead, it reaches the large intestine where it becomes a source of nutrients for the beneficial bacteria in the gut. Resistant starch in potatoes is a source of nutrition for beneficial gut bacteria. They convert it to the short-chain fatty acid butyrate, which has been linked to reduced inflammation in the colon, improved colon defenses and a lower risk of colorectal cancer. Potatoes are rich in compounds like flavonoids, carotenoids and phenolic acids. These compounds act as antioxidants in the body by neutralizing potentially harmful molecules known as free radicals. When free radicals accumulate, they can increase the risk of chronic diseases like heart disease, diabetes and cancer (Raman, 2018).

The physical characteristics of the formulated fungal culture media are presented in Table 2. The color of the cassava dextrose agar as well as sweet potato dextrose agar is light yellow, ube dextrose agar is lavender blush, and taro dextrose agar as well as

potato dextrose agar is ivory. All of them are semi-transparent. However, there are visible artifacts in cassava, sweet potato and taro dextrose agar. The gel strength of all the culture media is firm.

Basu, *et al.* (2005) reported that quality of media directly affects the observations and inferences drawn from the cultural characteristics of microorganisms. Checking of different parameters of media such as growth supporting characteristics, physical characteristics, gel strength and batch contamination can help to assess their quality. The quality of the media depends directly upon the quality of the raw materials used for their preparation. Water is the most important raw material used for the preparation of culture media. The parameters to be checked are presence of copper ions, conductivity and pH.

Ideally there should be no copper ions present in water because it is inhibitory for the growth of microorganisms. The quality of petri dishes used for pouring of media is also an important factor. Sterilization of the media plays an important role in the quality of the media. Generally autoclaving is carried out for sterilizing the media. However, the time of autoclaving and the quantity of media sterilized should be closely regulated. The gross physical appearance of media often suggests the quality. Media prepared should be screened for physical characteristics such as excessive bubbles or pits, unequal filling of plates (uniform leveling), cracked medium in plate and freezing or crystallization. All the above mentioned characters can be checked visually by naked eye. However, for unequal filling of plates, thickness of medium can be checked at four points. These four points are the two ends of the two diameters of the plate, which are at right angles to each other. Thus all the four sides can be simultaneously checked. The 42 thickness at the four points is noted down and the mean thickness is determined and reported as mean thickness of the medium in the plate, which must be 4.0 ± 0.2 mm. The pH value of the medium is also one of the important physical characters, which must be checked. It can be measured while preparation of the medium before and after autoclaving by using the standard pH meter after proper calibration with standard buffers (Basu, *et al.,* 2005).

Conclusion and recommendations

The study aimed to develop fungal culture media in dehydrated form utilizing commonly available rootcrops such as cassava, (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), ube (*Dioscorea alata* L.), taro (*Colocasia esculenta*), and potato (*Solanum tuberosum*). Specifically, the objectives of the study were to determine the physicochemical characteristics of the formulated culture media such as the color, clarity, gel strength, ash, moisture, crude protein, crude fat and total carbohydrate contents. Based on the findings of the study, the following conclusions were derived: (1) the formulated culture media possessed the necessary physicochemical characteristics of culture media for the culture of fungi; and (2) Cassava, sweet potato, Ube; taro and potato with dextrose and agar powder showed comparable effects on the growth of fungi under the study. For future researches and studies, the following may be considered: (1) the formulated fungal culture media utilizing local root-crops are recommended for the cultivation of *Saccharomyces cerevisiae, Aspergillus niger* and *Rhizopus niger;* and (2) a study may be conducted on the shelf-life of the formulated fungal culture media.

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