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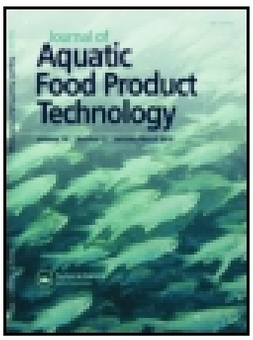

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Potential Utilization of Dagaa (*Rastrineobola argentea*) Protein Hydolysate in Formulation of Media to Support Microbial Growth and Production of Bacterial Secondary Metabolites

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ABSTRACT

Dagaa fish hydrolysate (DFH) and dagaa protein hydrolysate (DPH) were prepared from dagaa using Alcalase enzyme. Proximate, mineral analysis, and potential use of DFH and DPH in microbial media were evaluated. Dagaa media formulations were used to culture bacteria commonly used for amylase production (*Bacillus subtilis* and *Rhodobacter capsulatus*) and that used as a bio-pesticide (*Metarhizium anisopliae*). Whole dagaa had the highest protein (5.2 ± 0.1 mg/ml), ash (13.5 ± 0.4%) and fat (2.36 ± 0.2%) content, while DPH had the least protein (2.6 ± 0.0 mg/ml) content. Mineral content was significantly different ($p < 0.05$) between dagaa and its hydrolysates. Dagaa and LB had the highest Sodium (22.7 ± 1.1 mg/ml) and calcium (0.3 ± 0.0 mg/ml), content respectively. Sustenance of amylase production in DPH-starch and standard media by both microorganisms was significantly different ($p < 0.05$). Sporulation rate of *M. anisopliae* in experimental media was not significant ($p > 0.05$).

KEYWORDS

Dagaa; hydrolysates; culture media; proximate analysis and mineral analysis

Introduction

Lake sardine or silver cyprinid, locally known as dagaa (*Rastrineobola argentea*), accounts for the second largest fish catch in Lake Victoria, Kenya (Salehe et al. 2012). It is relatively cheap and available compared to Nile perch (*Lates niloticus*) (Owaga et al. 2010). Despite the large harvest of dagaa, most of the catches are lost due to endogenous enzyme spoilage as well as lack of good drying methods during the rainy season (Nyeko 2008). The post-harvest losses can be managed by exploitation of the high nutritive content of dagaa through preparation of dagaa fish protein hydrolysate (Ogonda et al. 2014). Previous studies have indicated that the species is rich in protein and fats (Bille and Shemkai 2006; Ogonda et al. 2014).

The use of fish materials as a source of nutrients for microbes was reported as early as 1949 (Tarr and Deas 1949). Fish hydrolysate has been incorporated into culture media mostly as a source of nitrogen (Dufosse et al. 2001; Petrova et al. 2021). Microorganisms grown on media that have fish peptone as a nitrogen source have better, if not equal, microbial growth titers than those grown in standard media (Ovissipour et al. 2009). Fish protein hydrolysate from hake filleting waste appears to be of sufficient nutritional value to support the growth of bacteria and archaea (Martone et al. 2005). Fish peptones from tuna, cod, red salmon, and unspecified fish were compared to one made with casein using a new method based on Gompertz modeling of microbial growth (Dufosse et al. 2001; Sathivel et al. 2003). Cumulative results obtained from six species of bacteria, yeasts, and fungi showed that in most cases the fish peptones were very effective as a source of nitrogenous compounds in microbial culturing (Dufosse et al. 2001). Fish peptones have also been used as a source of protein in the growth of infectious microorganisms such

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as *Staphylococcus aureus* (Fallah et al. 2015). The use of fish peptones has also helped to curb the growing problem of pollution from fish waste, as well as giving value in addition to fish waste.

The assessment of the nutritional value of the fish is necessary in order to determine if specific fish hydrolysates are suitable for use as a source of nitrogen for microbes. This is done by proximate and mineral component analysis (Cui and Wootton 1988). Nutritional demands of microbes grown on culture media are unique to the microorganism being cultured, but culture media have macronutrients such as carbohydrates, lipids, and proteins, as well as micronutrients (minerals and vitamins) (Oberhardt et al. 2015; Prentice 2005). The nitrogen/protein source is usually the most expensive component in preparation of culture medium (Aspmo et al. 2005; Martone et al. 2005). Macronutrients are typically required in large amounts to maintain cell structure and metabolism of microorganisms, while micronutrients are required in small amounts for enzyme function and maintenance of protein structure (Prentice 2005).

Several fish species, such as catfish, *Plotosus lineatus*, and *Arius maculate*, have been investigated for the presence of minerals. Some of the mineral elements identified include calcium (Ca), magnesium (Mg), iron (Fe), sodium (Na), and zinc (Zn) (Manikandarajan et al. 2014; Tenyang et al. 2014). Proximate analysis of proteins and fats in various fish species has indicated that fish are very rich in these two micronutrients but lack carbohydrates (Mustapha et al. 2014). Fish compares favorably to eggs, milk, and meat in the nutritional value of its protein (Olomu 1995).

This study is therefore aimed at carrying out proximate analysis of macronutrients and mineral analysis of dagaa and its derivatives in order to determine the best hydrolysate to incorporate in microbial culture media.

Materials and methods

Preparation of dagaa fish hydrolysate (DFH) and dagaa protein hydrolysate (DPH)

The dagaa used in the study were bought from Nairobi City Market, Kenya. Fish hydrolysate was prepared by use of a commercial exogenous protease Alcalase® 2.4 L as described by Seniman et al. (2014). Alcalase is a serine protease enzyme from Bacillus licheniformis. The DFH was prepared using the protocol described by Ogonda et al. (2017). The optimum working temperature of the enzyme was 56°C at a minimum stirring speed using a Stuart stirrer (UK) at a pH of 7 and predetermined enzyme substrate ratio of 2% (v/w) for 6 h to achieve hydrolysis of dagaa (Ogonda et al. 2017). The DFH contained both proteins and fats after enzymatic hydrolysis, and therefore it was mixed with hexane (Sigma Aldrich, St. Louis, MO, USA) to extract lipids in order to yield dagaa protein hydrolysate, which was devoid of fats and only contained proteins. Hexane oil extraction for DFH entailed mixing of DFH with hexane solvent, decanting, and extraction of the DPH devoid of fats by use of a micropipette (Bonilla-Méndez and Hoyos-Concha 2018). DPH was then transferred into 15 ml falcon tubes and spun in a refrigerated centrifuge (Hanil Science Industrial, Korea) at 12000 rpm for 20 minutes at 4 °C. The DPH was stored at −20 °C for later use as an alternative source of protein to peptone and tryptone containing media.

Preparation of standard Sabouraud's dextrose (SD) media and modified Horikoshi media

Sabouraud's dextrose medium was prepared according to Saigal et al. (2011). To 1 L of distilled water, 10 g of mycological peptone and 40 g of dextrose (Sigma Aldrich) were dissolved. In cases where Sabouraud's dextrose agar was required, 15 g of agar (Sigma Aldrich) was added. The mixture was then autoclaved at 121°C, 15 psi for 15 minutes, then cooled, and was ready for use. The media with agar was poured into Petri dishes before it solidified.

Modified Horikoshi medium was prepared according to Horikoshi (1971), with a few modifications that included the use of 10 g starch (Sigma Aldrich), 5 g peptone (Sigma Aldrich), 5 g yeast extract, 1 g

potassium dihydrogen phosphate (KH2SO4) (Sigma Aldrich), 0.2 g magnesium sulphate (MgSO4) (Sigma Aldrich), and 15 g of agar (Sigma Aldrich) in 1 L of distilled water.

Preparation of formulated dagaa protein hydrolysate-starch media (DPH-starch)

The DPH-starch medium was prepared by mixing 10 g of starch, 5 g yeast extract, 300 ml of DPH (15.84 g/ml of protein, equivalent protein content as standard media determined by biuret protein concentration analysis), 1 g of KH_2SO_4 and 0.2 g of $MgSO_4$ in 1 L of water. When preparing the agar plates for the DPH-starch media, 15 g of agar was added per liter of media. The mixture was autoclaved at 121°C, 15 psi for 15 minutes, then cooled, and was ready for use.

Preparation of formulated dagaa protein hydrolysate-dextrose (DPH-dextrose) for fungal growth

To prepare the DPH-dextrose media, DPH protein content had to be equivalent to that of standard media. Therefore, the formulated DPH-dextrose media for fungi were made up of 300 ml of DPH and 40 g of dextrose/L.

Proximate analysis

In this study, proximate analyses of moisture content, ash minerals, proteins, and lipids content were performed in triplicate.

Determination of dry weight content

Crucibles were cleaned and dried in an oven (Memmert, Germany), cooled, and weighed on a weighing balance (Mettler Toledo, Switzerland). The dry dagaa was homogenized using a homogenizer (Sumeet Research and Holdings Company, India), placed into the dried crucibles, and weighed in triplicate. These were investigated for dry weight content using air oven method according to AOAC method 950.46 (AOAC 1995). Crucibles with the dry dagaa sample were oven-dried at 105 °C overnight. Samples were left in a desiccator (Thermo Fisher Scientific, Waltham, MA, USA) for 30 minutes to cool before reweighing. The same procedure was used to determine the dry weight content of the following samples: DFH, DPH, and Sabouraud's dextrose broth (SDB).

Dry weight was estimated using the formula:

$$\%\text{Dry weight} = \left(\frac{\text{The weight of dried sample}}{\text{The original wet weight}}\right) * 100 \quad \text{(i)}$$

Determination of ash content

Ash content was determined according to AOAC method 950.153 (AOAC 1995). A muffle furnace (Baird and Tatlock, England) was preheated at 550 °C. Dried dagaa, DFH, and DPH samples from moisture determination were then labelled and left to burn in the furnace for 6 hours. The ash was cooled in a desiccator before weighing.

Ash content was determined according to the formula:

$$\%\text{Ash weight} = \left(\frac{\text{The weight of ash}}{\text{The original wet weight}}\right) * 100 \quad \text{(ii)}$$

Determination of crude lipid content

Total crude lipid content was determined using the modified Dyer and Bligh method of lipid extraction (Bligh and Dyer 1959; Iverson et al. 2001). In this method, 10 g of ground dagaa was weighed in triplicate in falcon tubes. To this, 10 ml of distilled water, 20 ml of chloroform (Sigma Aldrich) and 40 ml of methanol (Sigma Aldrich) was added and homogenized for 2 minutes. Another 20 ml of chloroform was added and homogenized for a further 30 seconds. To the homogenate, 20 ml distilled water was added and homogenized for an additional 30 seconds. The resultant homogenate was then centrifuged for 10 min at 16000 x g. The organic phase containing chloroform was pipetted off to a glass centrifuge tube. The weight of the tubes was determined before and after addition of 1 ml chloroform. The glass tubes with the chloroform were placed in the oven at 70 °C. After evaporation of the chloroform, the tubes were cooled to room temperature and reweighed. The same protocol was applied for pre-prepared DFH and DPH samples. Lipid content was determined using the formula:

$$\frac{\text{Lipid}}{\text{100g sample}} = \frac{\text{Lipid in tube} * (\text{volume of chloroform in total})}{\text{Amount of sample weighed in(g)} * \text{ml chloroform evapourated}} \quad \text{(iii)}$$

Mineral analysis

Mineral elemental concentrations were determined using atomic absorption spectrophotometry (ASS) (Shimadzu, Japan) according to AOAC (1975) and flame photometry (Sherwood Scientific, UK) according to Masamba et al. (2015). The mineral elements were investigated for dagaa, DFH, and DPH and included: Ca, Na, Mg, Fe, Zn, potassium (K), nickel (Ni), and copper (Cu), which have been found in most fish from previous studies (Fawole et al. 2007; Younis et al. 2011). Mg, Zn, Fe, Ni, and Cu were analyzed using AAS, while Na, K, and Ca were analyzed using flame photometry (Overman and Davis 1947).

Preparation of samples for mineral analysis

Dagaa, DFH, and DPH samples were prepared in triplicate. Each sample was then dissolved in 5 ml nitric acid (Sigma Aldrich) and made up to 20 ml with distilled water. The mixture was then heated on a hot plate (Stuart) until a third of the total volume was achieved. The solution was filtered with Whatman filter paper 1 (Cole-Parmer, Vernon Hills, IL, USA) and analyzed for minerals using AAS as described by Steiner-Asiedu et al. (1991) and Atta et al. (1997) and flame photometry for minerals mentioned. Using AAS, the wavelength, sample dilution, and the concentration of standards varied for the different minerals as shown in Table 1. Standards of each mineral were prepared in varying concentrations and then read against a blank (mixture of distilled water and concentrated nitric acid). The standard concentrations were used to prepare standard curves for extrapolating the concentration of different minerals in dagaa, DFH and DPH.

Extracellular amylase enzyme production by Bacillus subtilis and Rhodobacter capsulatus

The potential of DPH to maintain the growth of microbes that produce extracellular enzymes was tested. Two organisms, B. subtilis and R. capsulatus, that produce the extracellular enzyme amylase were grown on standard modified Horikoshi agar media and formulated DPH-starch agar media at 37°C for 16 hrs overnight in a metabolic incubator without agitation (Gerhardt, Germany). The ability of the microbes to produce extracellular amylase was noted by the presence and size of a halo in the middle of an iodine-stained starch containing petri dish. The microbes were then grown in corresponding broth media, and enzyme production was determined by dinitrosalicylic acid (DNS) assay. Each of the 200 µl of bacteria (overnight culture) was inoculated into separate 250 ml Erlenmeyer flasks with 100 ml of the two different media and separately grown at 37°C and 120 rpm in a metabolic

Table 1. Preparation of samples of dagaa, DFH, and DPH for the mineral analysis of magnesium, zinc, iron, nickel, and copper using atomic absorption spectrometry (ASS) and sodium, potassium, and calcium flame photometry (FP).

Mineral	Technique	Wavelength (nm)	Standard concentration (ppm)	Sample Dilution factor
Magnesium (Mg)	Atomic absorption spectrometry (AAS)	285.2	0.2, 0.4, 0.6, 0.8, 1, 2	Dagaa (5000X), DFH, DPH (10X)
Zinc (Zn)	AAS	213.5	1, 2, 3, 4, 5	Dagaa (50X), DFH, DPH (0X)
Iron (Fe)	AAS	248.2	1, 2, 3, 4, 5	Dagaa (5X), DFH, DPH (0X)
Nickel (Ni)	AAS	232.1	0.5, 1, 2, 3, 4, 5	Dagaa, DFH, DPH (0X)
Copper (Cu)	AAS	324.7	0.25, 0.5, 1, 2	Dagaa, DFH, DPH (0X)
Sodium (Na)	Flame photometry (FP)	N/A	1, 2, 4, 6, 8, 10	Dagaa (5000X), DFH (14X), DPH (14X)
Potassium (K)	FP	N/A	1, 2, 4, 10	Dagaa (5000X), DFH (14X), DPH (14X)
Calcium (Ca)	FP	N/A	10, 20, 40, 60, 80, 100	Dagaa, DFH, DPH (0X)

shaker for intervals of 24, 48, and 72 hours. After each time interval, 10 ml of each sample was removed and centrifuged at 16000 x g for 20 minutes in a refrigerated centrifuge. The supernatant was kept, and the pellet was discarded. DNS reagent (Sigma Aldrich) and Rochelle's reagent {made using sodium potassium tartrate (Sigma Aldrich) were used for the DNS assay. The assay determines the rate of glucose formation during breakdown of starch catalyzed by the enzyme amylase (Mahlow et al. 2016; Miller 1959). A glucose standard curve using 10 mg/ml stock glucose solution was also prepared.

Sporulation rates of Metarhizium anisopliae

Sporulation rates of *M. anisopliae* were compared in both the standard media (Sabouraud's dextrose) and in the formulated media (DPH-dextrose). Sabouraud's dextrose agar and DPH-dextrose agar media were prepared, and 20 ml of each was then poured into separate Petri dishes. Previously cultured M. anisopliae (200 µl) was spotted on both plates and incubated at 25°C for 11 days in a metabolic incubator without agitation. Controls were not inoculated. On the 11th day of growth, the conidia were harvested under sterile conditions. The plates were flooded with 3 ml distilled water and swirled to suspend the spores in water. The conidia were washed and swirled in 0.2 ml of 0.05% Tween 80 for 20 minutes before making 3 X and 5 X dilutions. The mixtures of different conidia dilutions were filtered using a Whatman filter paper 1, and the concentration of conidia in each of the filtrates was determined on a haemocytometer (Precicolor HGB, Germany) under a light microscope at X 40 magnification.

The formula below was used to determine the concentration of M. anisopliae conidia:

$$\text{Conidia concentration}\left(\frac{\text{conidia}}{\text{ml}}\right) = \frac{(\text{total conidia count})}{\text{number of squares}} * 10\text{^}4 \quad \text{(iv)}$$

Statistical analysis

The results from proximate and mineral determinations were analyzed with one-way analysis of variance (ANOVA) using statistical software SPSS version 19 (SPSS Inc., Chicago, IL, USA). Treatment means were separated by Tukey's multiple comparison test at $\alpha = 0.05$. This was done in order to determine whether DFH or DPH had the best potential to support microbial growth compared to the standard media for the respective microbes. Media were compared based on their effect on the growth and biomass of individual microorganisms. All conclusions are based on experiments that were repeated in triplicate over time to ensure reproducibility of results.

Results

Proximate analysis

Proximate analysis of dagaa, its hydrolysates, and two commercial media is given in Table 1. The unprocessed dagaa had the highest dry weight, protein, lipid, and ash content compared to DFH and DPH. The difference in these parameters between dagaa and its hydrolysates (DFH and DPH) was significant ($p < .05$). It is assumed that in the processing of hydrolysates, there is loss of macronutrients at every stage. As DPH is the most processed form of dagaa, it presented with the least macronutrient content. The ash content of dagaa was also significantly different ($p < .05$) from that of DFH and DPH. This is because dagaa contained more minerals than the DFH and DPH. The difference in ash content of DFH and DPH was not significant ($p > .05$). Protein content of dagaa was significantly different ($p < .05$) than that of DFH and DPH. The protein content of DFH and DPH was not significantly different ($p > .05$), indicating that there was little loss of protein during the removal of fat from DFH to yield DPH. The lipid content of dagaa was significantly different ($p < .05$) from that of DFH and DPH. The lipid content was significantly higher in DFH than in DPH. The DFH lipid content was significantly different from ($p < .05$) from DPH because DFH contains fat. Therefore, the richest sample out of the five in terms of macronutrient content was dagaa fish. Data on standard media Sabouraud's dextrose proximate analysis is as provided by Pere et al. (2017). It is assumed that the hydrolysis process leads to loss of nutrients.

Mineral analysis

Dagaa had the highest mineral content overall (Table 3). The mineral content in the dagaa hydrolysates was significantly less ($p < .05$) than in unprocessed dagaa due to losses associated with downstream processing. Na content was highest in the dagaa sample. The K content of dagaa was highest and lowest in DPH. Ca content was noted to be low in the two hydrolysates (DFH and DPH) and slightly higher in dagaa samples. Ni and Cu were not present in any of the samples, while Mg, Zn, and Fe were only present in dagaa at very low levels. In general, DFH and DPH samples had very low mineral content. Data on standard media Sabouraud's dextrose mineral analysis is as provided by Pere et al. (2017).

Rate of glucose formation on hydrolysis of starch by *B. subtilis* and *R. capsulatus* amylases in modified Horikoshi media and DPH-starch media

The rate of glucose formation on hydrolysis of starch by *B. subtilis* amylases was higher in DPH-starch media than in modified Horikoshi media. In the latter media, the rate of glucose formation increased, while in the former, the rate of glucose formation decreased over the three-day period as indicated in Figure 1. On the other hand, the rate of glucose formation upon starch hydrolysis by *R. capsulatus* amylase enzyme increased during the first 2 days in both media and then decreased on the third day (Figure 2). Generally, DPH-starch media showed higher glucose formation rates than modified Horikoshi media, as indicated in Figure 2. From the results, the rate of glucose formation due to the production of extracellular enzyme amylase by *B. subtilis* and *R. capsulatus* in modified Horikoshi and DPH-starch media were significantly different ($p < .05$).

Sporulation of *Metarhizium anisopliae* in SDB and DPH-dextrose media

SDB media had a slightly higher conidia concentration than DPH-dextrose media, as indicated in Table 4. Statistics revealed that sporulation rates of *M. anisopliae* in SDB and DPH-dextrose were not statistically different ($p > .05$).

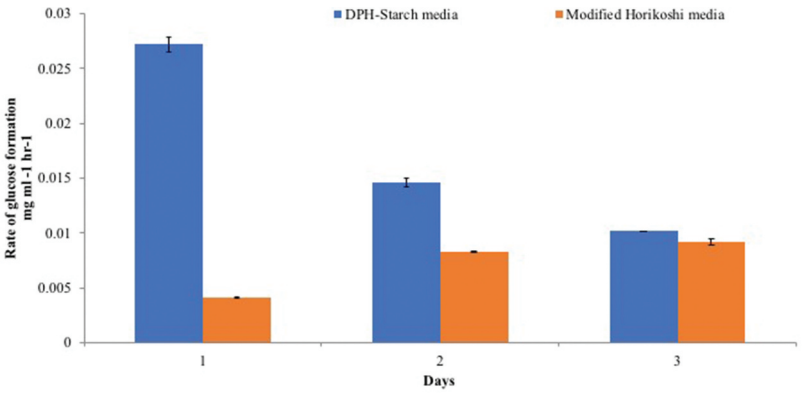

Figure 1. Rate of glucose formation due to breakdown of starch by *Bacillus subtilis* amylase enzyme over a 3-day period.

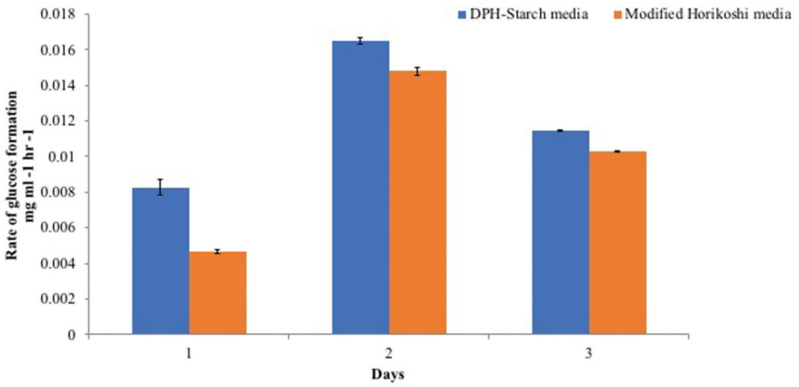

Figure 2. Rate of glucose formation due to breakdown of starch by *Rhodobacter capsulatus* amylase enzyme over a 3-day period.

Discussion

Dagaa protein hydrolysate contains lipids and is rich in proteins (Owaga et al. 2010; Ogonda et al. 2014). This makes it an excellent candidate for the fortification of food for all organisms including microbes. Post-harvest losses associated with dagaa are about 50%; this can be averted by processing DPH for use in microbiological media (Nyeko 2008).

In the current study, dagaa had more macro- and micronutrients than its hydrolysate derivatives (DFH and DPH). The downstream process of producing the hydrolysates from dagaa leads to loss of nutrients during sedimentation. Dagaa had higher ash content than DFH and DPH, indicating that they had slightly more inorganic matter than the latter (Table 2). DPH and DFH had the least ash content because they have very little inorganic matter. Most of it was lost during sedimentation as the dagaa was being processed into its constituent hydrolysates. Dagaa had the highest protein content, followed closely by DFH and DPH, respectively. The lower content of protein in the dagaa derivatives could be due to the loss of organic material by sedimentation during the hydrolysis process (Table 2). This nutritive loss after processing of fish by hydrolysis has also been noted in previous studies (Abraha et al. 2018). During the hydrolysis process, most of the protein content was lost as dagaa was converted to DFH by enzyme hydrolysis and as DFH was converted to DPH by the removal of fats using hexane through the process of sedimentation. DPH mostly contains soluble proteins, while DFH contains both soluble proteins and fats. This could also be attributed to a low degree of hydrolysis of dagaa leading to loss of protein content through the sludge produced during the hydrolysis process. This is noticeable when the proteins in dagaa decreased from 5.2 mg/ml to 4.2 mg/ml in DFH and

Table 2. Proximate analysis of dagaa, DFH, and DPH showing the moisture/dry weight, ash, protein, and lipid content.

Sample	Dry weight (%)	Ash content (%)	Protein content (mg/0.05 ml)	Lipid content (%)
Dagaa dwb	95.0 ± 0.0a	13.5 ± 0.4a	5.2 ± 0.1a	2.4 ± 0.2a
DFH wwb	9.7 ± 0.4aa	0.9 ± 0.0aa	4.3 ± 0.0a	1.0 ± 0.2a
DPH wwb	9.1 ± 0.3aa	0.7 ± 0.0aa	2.6 ± 0.0a	0.0 ± 0.0aa

Key: the abbreviation dwb indicates that the sample was analyzed on dry weight basis, while wwb indicates that the sample was analyzed on wet weight basis. Values are means ± standard deviation (SD) for triplicate analysis of samples mentioned. Values with a single letter indicate significant difference from the rest of the samples, while those with double letters indicates no significant difference to other samples according to one way analysis of variance (ANOVA).

Table 3. Mineral analysis of magnesium, zinc, iron, nickel, copper, sodium, potassium, and calcium content in dagaa, DFH, and DPH.

	Concentration in mg/ml of specific mineral							
Sample	Mg	Zn	Fe	Ni	Cu	Na	K	Ca
Dagaa	0.5 ± 0a	0.0 ± 0a	0.0 ± 0a	0.0 ± 0aa	0.0 ± 0aa	22.7 ± 1a	4.9 ± 1a	0.0 ± 0a
DFH	0.0 ± 0aa	0.0 ± 0aa	0.0 ± 0aa	0.0 ± 0aa	0.0 ± 0aa	0.1 ± 0a	0.1 ± 0a	0.0 ± 0a
DPH	0.0 ± 0aa	0.0 ± 0aa	0.0 ± 0aa	0.0 ± 0aa	0.0 ± 0aa	0.1 ± 0a	0.0 ± 0a	0.0 ± 0a

Key: values indicate means ±standard deviations (SD) for triplicate analysis of samples mentioned. Values with single letter indicate significant difference from the rest of the samples, while those with double letters indicate no significant difference to other samples according to one way of variance (ANOVA).

Table 4. Sporulation rates of *M. anisopliae* in SDB media and DPH-dextrose media.

Microorganism	Media	Dilution factor	Conidia concentration (cell/ml)	Standard deviation	Standard error	Variance
Metarhizium anisopliae	SDB	10^3	$3.1*10^9$	1.8	1.0	3.2
	DPH-Dextrose	10^3	$2.9*10^9$	1.8	1.0	3.1
	SDB	10^5	$4.8*10^7$	2.5	1.4	6.2
	DPH-Dextrose	10^5	$4.7*10^7$	2.5	1.4	6.1

further reduced to 2.6 mg/ml in DPH (Table 2). DPH is comprised of only soluble proteins, and this could account for the reduced protein content.

Lipids are not a preferred source of carbon for most microbes due to the complex metabolic process compared to simple sugars and peptides or amino acids. DPH media, which is devoid of fats but rich in proteins with supplementation of minerals and some amino acids through the addition of yeast extract, provided a medium equivalent to standard microbial media such as SD and modified Horikoshi media for the growth of microbes. Minerals are micronutrients and are only required in small amounts (Prentice 2005). Due to the very low mineral content in DPH media, it is important to supplement DPH formulated media with minerals necessary for microbial growth (Table 3). Minerals are crucial in the growth and metabolism of these microbes. They function as cofactors (Nair 2008) in enzyme catalyzed reactions. Therefore, it is very important to supplement DPH with minerals when using it as a nitrogen source in formulated microbial media. This is of course dependent on the needs of the microbe being grown.

When the composition of DPH formulated media was adjusted in order to support the growth of *B. subtilis* and *R. capsulatus* in starch media, there was higher rate of glucose formation in DPH-starch media compared to modified Horikoshi media for both microorganisms (Figures 1 and 2). This is an indication that the amylase enzyme produced in DPH-starch media was higher than the amylase produced in modified Horikoshi media. Similar results were obtained from fish hydrolysates produced from fish wastes (viscera and chitinous material) tested as growth substrates for microbes that produce exo-enzymes: protease, lipase, chitinolytic, and ligninolytic enzymes (Rebah and Mileb 2013). The application of fish protein hydrolysates in exo-enzyme recovery from microbes saves on the cost of microbial enzyme production and helps to eliminate environmental pollution brought about by improper disposal of fish waste (Rebah and Mileb 2013).

The DPH-dextrose formulated media also sustained the sporulation of *M. anisopliae* species at the same rate as the standard Sabouraud's dextrose media (Table 4). In this case, sporulation rate

of *M. anisopliae* in DPH-dextrose formulated media was as good as in SDB standard media. Hence, DPH-dextrose can be substituted for Sabouraud's dextrose media in the growth of *M. anisopliae*.

Conclusion

DPH formulated media performed comparably well to commercial media (SD and modified Horikoshi media) in supporting microbial growth. The formulated media also supported the production of important metabolites in commercial microbes. DPH is potentially a good source for preparation of microbiological media with supplementation of missing micronutrients.

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Author's contribution

KP, EKM, BNM and VWW conceived and designed the experiments. KP performed all the experiments. EKM supervised the execution of the research. KP analyzed the data with guidance from BNM and VWW. KP and VWW processed the data and wrote the manuscript. All the authors revised and approved the manuscript.

Disclosure statement

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Recommendations

Further studies to access the chemical characterization of DPH should be done. This can help to determine the molecular weight of peptides in the hydrolysate as well as the content of amino acids and the degree of hydrolysis.

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