

## Full Length Research Paper

## Nutritional value of a dietary supplement of *Moringa oleifera* and *Pleurotus ostreatus*

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Received 29 November, 2016; Accepted 10 April, 2017

*Moringa oleifera* and *Pleurotus ostreatus* are widely used as food or food supplements. They are demonstrated to have many beneficial effects on nutritional status and human health. The aim of the present study was to evaluate the nutritional value of *M. oleifera* and *P. ostreatus* mixture in specific proportions. The mushroom species was cultivated at the Mushroom Biotechnology Laboratory and *M. oleifera* at the botanical garden of the University Cheikh Anta Diop de Dakar, Senegal. The compost of *P. ostreatus* culture was corn and straw peanut. Mixtures of different proportion powders of *M. oleifera* and *P. ostreatus* were used for investigations. Results showed that the product contain 35.08% crude proteins, 14.28% carbohydrates, 22.71% fat, 20.96% fibers and 6.98% of total ash. The energy was 401.79 kcal for 100 g of dry matter. Among minerals, potassium (1566.83 mg/100 g) are the most abundant mineral element followed by phosphorus (318.55 mg/100 g), calcium (284.33 mg/100 g) and magnesium (253.14 mg/100 g); the less abundant was copper with 0.53 mg/100 g. This study shows that the used proportion of *M. oleifera* leaves and *P. ostreatus* powders mixture could be a good source of minerals, proteins and carbohydrates.

**Key words:** *Moringa oleifera*, *Pleurotus ostreatus*, dietary supplement.

### INTRODUCTION

Many people living in developing countries all over the world suffer of hunger -12.9% of the population is underfed. Sub-Saharan Africa is the region with the

highest prevalence of hunger. About 25% of the populations suffering from chronic malnutrition live in sub-Saharan Africa (FAO, 2015). Every one child on six in

developing countries, so like 100 millions of children, is suffering of insufficient weight (WHO, 2010). In the world, 66 millions of children go to school with an empty belly and of which 23 million live in Africa (WFP, 2012). According to World Food Program (WFP, 2012), reported that annually 3.2 billions of dollars for feeding 66 millions of children who go to school. However, according to current's provisions, by 2020, Africa will have the most increasing percentage of death because of non-communicable diseases. Among the risk's factors, bad feeding and a poor consumption of fruits and vegetables could be the cause of 1.7 million of death (WHO, 2010). In order to fight hunger and diseases, many organisms and governments, encourage the use of vegetal species as minerals and proteins sources (FAO, 2015). In some developing countries like Senegal and Cameroon, governments encourage the use of naturals and bio-products against under-feeding which cause many infantile mortality and morbidity (UNICEF, 2007; Shetty, 2010). Many studies have been shown the health and nutritional interest of edible mushrooms (Zhang *et al.*, 2016; Alam *et al.*, 2008; Pornariya and Kanok, 2009). Mushrooms and some plants provide proteins, carbohydrates, minerals, fibers, vitamins, minerals and fatty acids (Khatun *et al.*, 2012; Okwulehie *et al.*, 2014; Barros *et al.*, 2007). They have therapeutic properties and many of them have been used in medicine all over the world (Badalyan, 2014). Researchers have shown that *P. ostreatus* has antitumor effects, antioxidant properties, antihyperlipidemic effects, antidiabetic effects, (Zhang *et al.*, 2016; Abrams *et al.*, 2011; Alam *et al.*, 2008; Elmastas *et al.*, 2007; Jayakumar *et al.*, 2007; Jayakumar *et al.*, 2006). Also, the plant *M. oleifera* has multiple therapeutic effects (Farooq *et al.*, 2007; Ferreira *et al.*, 2008; Sholapur and Patil, 2013). High malnutrition rates are common in the pastoral regions in different parts of the world. In order to solve this complex problem, simple alternative solutions that contribute considerably to immediate food self-sufficiency are required. The objective of the present study was to determine the nutritional value of *Moringa oleifera* and *Pleurotus ostreatus* mixture in specific proportions.

## MATERIALS AND METHODS

### Plant material

The mushroom *P. ostreatus* was cultivated and harvested at the Biotechnology Laboratory of mushrooms at the University Cheikh Anta Diop de Dakar (UCAD), Senegal. They have obtained on a substrate from straw peanut and rice bran; the mycelium was

inoculated on corn seeds. Fresh leaves of *M. oleifera* were collected from the Botanical garden of UCAD, identified and authenticated by a taxonomist from the Laboratory of Botany and Plants systematic, UCAD.

### *M. oleifera* and *P. ostreatus* powder mixture preparation

Carpophores of *P. ostreatus* were washed with distilled water and then heated at 50°C for 10 min (Manzi *et al.*, 2004). They were drained and dried at 40°C till a dry texture was obtained which was then molded in a sterilized at 170°C for 30 min local molding machine in order to obtain a fine powder. The leaves of *M. oleifera* were washed thoroughly two times with distilled water, and put inside the bag containing 1% of sodium hypochlorite for 5 min as describe by Bénissan *et al.* (2012). They were washed again with distilled water and dry on tissue paper in a clean room sheltered from the sun during 7 days. The dry leaves were then reduced to get powder for further exploration by an artisanal machine after sterilization at 170°C during 30 min (Rutala *et al.*, 2008). According to the recommendation of FAO (2015), WHO (2007) and Goyens (2009), one mixture of two powders (*M. oleifera* and *P. ostreatus*) was prepared in function of the recommended daily intake under the aseptic conditions. These studies notified that when people take 30 g of *M. oleifera* and 15 g of *P. ostreatus*, more than 50% of daily recommended intakes of total proteins, calcium, potassium, magnesium, phosphore, iron, manganese, copper, zinc are covered by these proportions. For that reason, a mixture with these two species according to these recommendations was made.

All metallic serving utensils were sterilized with local autoclave at 170°C for 30 min, plates in autoclave at 121°C for 20 min, the others that didn't support high temperature were sterilized at UV for 30 min.

### Method of analysis

#### Chemical composition analysis

Chemical composition of sample (*M. oleifera* and *P. ostreatus* mixture powder in 2:1 proportions) was determined for moisture, crude protein, total fat, minerals (sodium, calcium, magnesium, phosphorus, potassium, iron, copper, zinc, manganese). Chemical analysis of samples was conducted at the "Laboratoire National d'Analyses et de Contrôle" in Dakar (Senegal), at Institute of Medical Research and Medicinal Plants studies MINRESI and "Centre Pasteur du Cameroun". The determination of crude proteins was made by mineralization process in the flasks, the distillation by machine VELP® *Scientifica* UDK 127 device, and titration relatively with the Kjeldahl method according to the AOAC (2002). Total lipids were determined by the method of Soxhlet through the solubilization of lipids in an organic solvent, hexane. The samples weighed in Whatman paper and placed in extraction inners of the balloons ramp heating Bistabil BRAND 6 positions and heated for 12 h and then weighed (AOAC, 2002). Total ash was determined by the weight difference by incinerate the samples in the oven electronic Heraeus (T 5042, Germany) at 550°C for 24 h carbohydrates by the difference between dry matter and (crude proteins+ total ash + fats+ total fibers). Energy was calculated by

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using carbohydrates, fats and crude proteins contents (Italian law, 1993). Minerals as calcium, magnesium, potassium, sodium, copper, iron, manganese, zinc, phosphorus are determined by flame atomic absorption spectrometry after mineralization of parallel samples in microwave mineralization (Milestone model START-D, Italy) with power of 800 W. The dry matter was determined by the method of drying according to the AOAC directions (AOAC, 2002). Samples of known weight were placed at 105°C for 48 h. The water content was calculated by the difference of dry matter obtained before and after drying.

### Microbiological analysis

Sanitary quality of samples (*M. oleifera* powder, *P. ostreatus* raw powder, and mixture powders of *M. oleifera* and *P. ostreatus* cooked) were analyzed by identifying microorganisms including thermoresistants coliforms, *Escherichia coli*, *Salmonella* and Fungi commonly used as indicators of sanitary quality of water and foods (NF V08-060, 2009; NF ISO 16649-2, 2001; NF V08-061, 2009; NF ISO 21527-2, 2008). The detection of *Salmonella* was investigated according to the standard method of NF EN ISO 6579 (2002). A portion of each 25 g of mixture product was placed in 225 ml of buffered peptone water for pre-enrichment. The homogenized solution was incubated at 37°C for 24 h. Then, 0.1 ml of the solution was added in a tube containing 10 ml of Rappaport Vassiliadis broth (Biolife, Italy) incubated at 42°C for 24 h. The selective isolation was realized on medium Hektoen agar (Biolife, Italy). After researching thermoresistants coliforms, *Escherichia coli* were searched at 44°C for 24 h. For each tube of 10 ml of *EPSI* (*Eau Peptonée Sans Indole*), one colony was plated (NF ISO 16649-2, 2001). According to the standard method, Sulfite-reducer Anaerobe in mixture product was carried out by conventional methods NF V08-061(2009) based on the use of Tryptone Sulfite Néomycine medium (Biolife, Italy).

### Data analysis

All experimental results are the averages of two parallel measurements (means  $\pm$  SD). Quantitative data were expressed as means and standard deviation (SD) of at least 2 measurements. Each experimental set was compared with one way analysis of variance (ANOVA) procedure using Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., Chicago, IL, USA). Duncan's new multiple range test was used to determine the differences of means. P values <0.05 were regard as significant.

## RESULTS

The macronutrients levels are presented in Table 1. The highest content of carbohydrates was  $25.13 \pm 0.20$  mg for *P. ostreatus*,  $36.81 \pm 0.21$  mg was the highest value of crude proteins in *M. oleifera* powder. Raw *P. ostreatus* had the highest content in fibers. The most abundant macronutrients in the mixture were crude proteins followed by fats, fibers and carbohydrates.

As can be seen in the Table 2, *M. oleifera* had the highest content of calcium, magnesium, manganese and sodium. Others minerals (iron, potassium, zinc, copper and phosphorus) were abundant in raw *P. ostreatus*. The most abundant mineral in the mixture was potassium followed by phosphorus, calcium and magnesium.

As can be concluded from data present in Table 3, almost elements contents except sodium could cover minimum 20% of DRI for children (1 to 8 years). For some nutrients, the levels were more than 100% of DRI.

Some minerals covered a minimum of 20% DRI while others contained more than 100% of children's DRI within the age group 9-18 years (Table 4). This is valid for adults who had more than 100% of crude proteins, iron and manganese (Table 5).

As can be concluded from data present in tables, almost elements contents could cover more than 20% of DRI of all groups. For some nutrients (proteins, iron, manganese), we have noticed more than 100% of DRI.

According to referenced standards, we didn't find any pathogens in the product (Table 6).

## DISCUSSION

The dried leaves of *M. oleifera* and *P. ostreatus* are rich in macronutrients and minerals. According to Breene (1990), Çokuner and Özdemir (2000), Bénissan et al. (2012), proteins content in 100 g dried matter of *P. ostreatus* ranged from 19 to 39 g; for *M. oleifera* average amounts 35 g. In this study, the protein content is in conformity with Shin et al. (2007). Watanabe et al. (1994) found that the carbohydrates value of *P. ostreatus* as 47.9 g in 100 g dry matter which is higher than this study, however, Alam et al. (2008) found that a value as 37.8 g which is also not compatible with result 16.70 g in 100 g dry matter. This difference might be due to the difference of environment factors. The results are similar those of Moyo et al. (2011) who worked on nutritional characterization of *M. oleifera* leaves. The most abundant mineral in both species is potassium 1566.8 mg followed by phosphorus 318.5 mg and calcium 284.3 mg and the least content was copper with 0.53 mg.

According to Daily Reference Intakes in the Essential Guide of Nutrients Requirements (EGNR) (2006), sodium contents is low and the great amount of potassium suggest the utilization of this mixture in anti-hypertensive diet because of the fact, potassium from fruits and vegetables can lower blood pressure. These results and observations are similar those of Manzi et al. (1999). Calcium was observed to be higher compared with other plant sources (Nkafamiya et al., 2010). It plays a key role in bone health. In fact, more than 99% of total body calcium found in the teeth and bones. Calcium deficiency can result from inadequate intake or poor intestinal absorption and can cause osteopenia, osteoporosis, and an increased risk of fractures (EGNR, 2006). Interestingly too, iron, commonly deficient in many plant-based diets, which is a necessary component of hemoglobin and myoglobin for oxygen transport and cellular processes of growth and division (EGNR, 2006), an essential trace element for normal functioning of the central nervous

**Table 1.** Macronutrients contents of the dietary supplement FMP16 (g/100 g DM).

Sample	Total ash	Fats	Crude proteins	Fibers	Carbohydrates	Total Sugar	<sup>a</sup> Energy
MO	8.77± 0.02	28.5±0.7	36.81±0.2	09.2±0.2	16.7±0.6	13±0.4	470±4
PO	5.71±0.04	27.37±0.54	28.97±0.06	12.83±0.33	25.13±0.20	12.86±0.61	463±4
MO+PO	6.98±0.33	22.71±0.05	35.08±0.160	20.96±1.425	14.28±1.310	11.78±0.145	402±4

<sup>a</sup>kcal/100 g DM. Values are means± standard deviation for two repetitions. FMP16: Code of product; DM, Dry matter; MO, Moringa oleifera leaves powder; PO, Pleurotus ostreatus raw powders; MO+PO, M. oleifera leaves powder + Pleurotus ostreatus cooked powder.

**Table 2.** Content of minerals in the prepared dietary supplement FMP16 (mg/100 g DM).

Sample	Iron	Calcium	Magnesium	Manganese	Copper	Potassium	Zinc	Sodium	Phosphorus
MO	20.96±0.01	583.3±8.05	306.19±0.40	5.18±0.00	0.48±0.01	1675.52±7.07	1.31±0.01	96.6	9.76±0.02
PO	24.3±0.07	21.22±0.63	86.35±0.09	0.74±0.00	0.66±0.00	2240.43±3.49	5.35±0.01	68.1	1693.57±3.41
PO+MO	19.66±0.07	284.33±1.40	253.14±0.45	3.70±0.02	0.53±0.01	1566.83±10.23	4.36±0.02	79.3	318.55±1.72

FMP16, Code of product; DM, Dry matter; MO, M. oleifera leaves powder; PO, P. ostreatus raw powders; MO+PO, M. oleifera leaves powder + P. ostreatus cooked powder. Values are means± standard deviation for two repetitions.

**Table 3.** Fulfillment of recommendation (Daily Reference Intakes) by the dietary supplement FMP16 (mg/100 g DM) for children (1-8 years)\*.

Elements	DRI*	Contents of FMP16 (/100 g)	Percentages of DRI covered by FMP16 (%)
Crude proteins	20	35.08	175
Carbohydrates	65	14.28	22
Fats	ND	22.71	ND
Fibers	19	20.96	110
Magnesium	80	253.14	316
Phosphorus	460	318.55	69
Copper	1.5	0.53	35
Iron	7	19.66	280
Manganese	1.2	3.70	300
Zinc	3	4.36	145
Potassium	3800	1566.83	41
Calcium	700	284.33	40
Sodium	1200	79.3	6

DRI, Daily reference intakes; crude proteins, carbohydrates, fats, fibers (g/day); magnesium, phosphorus, iron, manganese, zinc, potassium, copper, calcium, sodium (mg/day); ND, Not determined.\* Source: *EGNR*, Essential Guide to Nutrients Requirements (2006).

**Table 4.** Fulfillment of recommendation (Daily Reference Intakes) by the dietary supplement FMP16 (mg/100 g DM) for children (9 to 18 years)\*.

Elements	DRI*	Contents of FMP16 (/100 g DM)	Percentages of DRI covered by FMP16 (%)
Crude proteins	30	35.08	117
Carbohydrates	65	14.28	22
Fats	ND	22.71	ND
Fibers	38	20.96	55
Magnesium	410	253.14	60
Phosphorus	1250	318.55	25
Copper	0.7	0.53	75
Iron	15	19.66	130
Manganese	1.9	3.70	190
Zinc	5	4.36	87
Potassium	4500	1566.83	34
Calcium	800	284.33	35
Sodium	1200	79.3	6

DRI, Daily reference intakes; crude proteins, carbohydrates, fats, fibers (g/day); magnesium, phosphorus, iron, manganese, zinc, potassium, copper, calcium, sodium (mg/day) – ND, Not determined; \* Source: EGNR, Essential Guide to Nutrients Requirements (2006).

**Table 5.** Fulfillment of recommendation (Daily Reference Intakes) by the dietary supplement FMP16 (mg/100 g DM) for adults.

Elements	DRI*	Contents of FMP16 (/100 g DM)	Percentages of DRI covered by FMP16 (%)
Crude proteins	35	35.08	100
Carbohydrates	65	14.28	22
Fats	ND	22.71	ND
Fibers	38	20.96	55
Magnesium	420	253.14	60
Phosphorus	700	318.55	45
Copper	0.9	0.53	58
Iron	18	19.66	110
Manganese	2.3	3.70	160
Zinc	11	4.36	40
Potassium	4700	1566.83	33
Calcium	1200	284.33	23
Sodium	1500	79.3	5

DRI, Daily reference intakes; crude proteins, carbohydrates, fats, fibers (g/day); magnesium, phosphorus, iron, manganese, zinc, potassium, copper, calcium, sodium (mg/day) – ND, Not determined.\*Source: EGNR, Essential Guide to Nutrients Requirements (2006).

system and in the oxidation of carbohydrates, proteins and fats (Umar et al., 2007), was found in abundance in these two species. These results are comparable to data published by Moyo et al. (2011). Results from this study had higher levels of zinc 4.36 mg/100 g. These results corroborate studies by Barminas et al. (1998), who reported 25.5 mg/kg in dried *Moringa* leaves. Zinc has been shown to boost the immune system, is also required for cell reproduction and growth especially sperm cells (Brisibe et al., 2009). Raw *P. ostreatus* and *M. oleifera*

leaves were analyzed separately, then the mixture was done by mixing both, but in this case, *P. ostreatus* were cooked. According to Manzi et al. (2004), the effect of the cooking process is explained as a decrease of the nutrients contents of raw sample in the water, and consequently their concentration due to thermal degradation. The cooking process might affect the nutritional value by decreasing minimum 70% of his content. However, this decrease does not affect fibers which stay constant. Also, according to Barminas et al.

**Table 6.** Microbiological quality of the dietary supplement FMP16.

Strains	Results	References values (Unit)
Thermo-resistant coliforms	Absence	10/g-100/g <sup>a</sup>
<i>E. coli</i>	Absence	10/g-100/g <sup>b</sup>
Sulfito- reducer Anaerobe	Absence	<10/g <sup>c</sup>
<i>Salmonella</i>	Absence	Absence/25g <sup>d</sup>
Fungi	Absence	10/g-100/g <sup>e</sup>

a, NF V08-060 (2009); b, NF ISO 16649-2 (2001); c, NF V08-061(2009); d, NF EN ISO 6579 (2002); e, NF ISO 21527-2 (2008).

(1998) and Broin (2006), the mode of conservation and time between collection and analysis might influence the nutritional composition; it is worth mentioning that these analyses were completed 6 months after collection and transformation.

According to the daily recommendations in the EGNR (2006), by the Institute of Medicine of the National Academies, vitamins and minerals requirements (FAO, 2004), 100 g of this dietary supplement composed by *M. oleifera* and *P. ostreatus* might cover many recommended daily reference intakes of minerals and macronutrients. Percentages covered by each element are represented in Tables 3 to 5; showed that the dietary supplement is a good source of minerals and macronutrients more than many others plants which are used like food supplement (Bénissan et al., 2012; Moyo et al., 2011; Maiga et al., 2005).

## Conclusion

In conclusion, mushrooms such as *P. ostreatus* could be excellent food that can be used in alimentation for malnutrition problem in sub-Saharan Africa for their high contents in macronutrients and minerals. In this study confirmed the high nutritional quality of *M. oleifera* leaves which can be used to improve health and reduce malnutrition in the world. The data derived from nutritional value of this mixture of *M. oleifera* and *P. ostreatus* are clear indications that these species are rich in nutrients and had potential to be used as a food supplement and a promising dietary supplement that may overcome protein-energy malnutrition problem in the third world. For that reason, our dietary supplement composed of these two species could be a very good source of nutrients for reduce malnutrition rate.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

This work was supported by the AFIMEGQ project AF13FD0039. The authors gratefully acknowledge the Laboratory of Food Safety at the Biotechnology Centre of the University of Yaoundé I, Cameroon, the Laboratory of Mushrooms Biotechnology of the *Université Cheikh Anta Diop de Dakar*, Senegal, the *Centre Pasteur du Cameroun* and the Institute of Medical Research and Medicinal Plants, MINRESI, Cameroon. They are also grateful to the *Laboratoire de Biotechnologie de l'Institut de Technologie Alimentaire*, Dakar and all the students for their contribution in this work.

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