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Effect of Packaging on Microbiological Quality of *Azolla Filiculoides* and *Moringa Oleifera* Flour

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Abstract

Since many foods consumed by human are from animal, the risk of germs transmission through the consumption of products from animals like pig and poultry nourished with contaminated flours remains high. This study aims firstly to access the hygienic quality of two flours Moringa oleifera and Azolla filiculoides and then to select best stocking conditions for their conservation. Those flours were produced and stored in three packing in three different environments for microbiological and phytochemical analyses. On the whole, 36 samples of each flour were followed and analyzed during two months time, according to standardized methods ISO and AFNOR to research anaerobes sulphito-reducers (ASR), coliforms thermotolerants, Escherichia coli, yeasts, moulds and Staphylococcus aureus germs presence. The results showed that storing in plastic bag and tin in refrigerated and non-ventilated environments are suitable for conservation during two months without anaerobes sulphito-reducers (ASR) and Staphylococcus aureus presence.

Keywords: *Moringa oleifera, Azolla filiculoides, flour, microbiological quality.*

Introduction

The food sanitary safety is a major concern of the authorities and food industry. If legal provisions exist for the products used for human consumption, it remains less effective with animal's feeds. According to Soltner (1994) [1], the cost related to the animals feeds account for 70% of the total cost of production. From then on, the reduction of the food costs becomes a significant concern for the small stockbreeders of developing countries. The use of nonconventional local feed in the diet of monogastric reasonably seems to be an alternative to conventional commercial feed [2]. Several work reported the use of *Azolla Spp* [3], [4], [5];and of *Moringa oleifera* [6], [7], [8], [9] in animal feeds. Azolla is used as food supplement for variety of animals including pigs, rabbits, chickens, ducks and fish [10]. Seultrope reported that Azolla is harvested in large quantities and utilized as fodder for cattle and pigs. It was also found that broilers feed with Azolla resulted in growth and body weight values similar to those resulting from the use of maize-soya bean meal. Das et *al* (1994) [11] found that digested Azolla slurry remaining after biogas production was suitable as fish pond fertilizer. *Moringa oleifera* Lam., a member of the family Moringaceae, is a fast-growing plant widely available in the tropics and subtropics with great economic value importance for the food and medical industry [12]. The seeds are a rich in oil and protein source and can be used for the purification of water. The roots are a source of spices. The leaves are rich in carotene, iron and ascorbic acid [13], [14]. It has been reported that leaves and green fresh pods are used as vegetables, and are rich in carotene and ascorbic acid which can be used as livestock feed. Twigs are reported to be very palatable to ruminants and to have a good level of crude protein [15].

Moreover, drying reduces the microbial growth in the flour because relative humidity of the leaves becomes too low to permit proliferation of micro- organisms. However, the levels of microorganisms in flour depend mainly on the drying and the quality of the packaging conditions. However, the mode of production and preservation of the flours of *Moringa oleifera* and *Azolla filiculoides* flours leaves did not always guarantee the harmlessness of this food complement. These flours are highly perishable because of their high moisture and protein content. They also provide a breeding space for the growth of microorganisms.

The aim of this work is to evaluate the microbiological quality of these two flours preserved in three packages (plastic bag, jute bag and can) and stored in three

environments (refrigerated, ventilated, unventilated) at various dates.

Material and Methods

Samples collection

The fresh leaves of *Moringa oleifera* were collected in the town of Abomey-Calavi at Zogbadjè while *Azolla filiculoides* were reaped in a pond near the Laboratory at the Wetlands of the University of Abomey-Calavi. They were collected and used to produce flours.

Flours production

Moringa oleifera and *Azolla filiculoides* leaves were processed into flours following method developed by [16] slightly modified at washing level of the leaves. The leaves of *Moringa oleifera* were sundried for 3 days (figure 1) while those of *Azolla filiculoides* for 4 days (figure 2).

Experiment

Each flour produced was packaged in three types of packaging: plastic bags, jute bags, cans. These packaging material containing flours were then stored in three different environments: the refrigerator (4°C), a ventilated room and in an unventilated room.

Chemical Analyses

Dry matter (DM), crude protein (CP) and total ash contents were determined according to the method of AOAC (1990). Organic matter (OM) was calculated by subtracting the total ash from DM.

Microbiological Analyses

The yeasts, moulds, thermotolerant coliforms, *E. coli*, *Staphylococcus aureus*, sulphite-reducing anaerobes were determined as followed: 10 g sample were diluted in sterile (90 ml) peptone water and then homogenized. Appropriate decimal dilutions were prepared for bacterial analysis according to ISO methods. Total Mesophilic Aerobic Bacteria were enumerated with Plate Count Agar (PCA) after incubation at 30 ˚C for 72 h. Yeasts and moulds were enumerated on Oxytetracycline Glucose Agar (OGA) after incubation at 25˚C for 5 days. Total and Thermotolerant Coliforms and *Escherichia coli* were counted in Brilliance *E. coli* Agar (Coliform Selective Medium)) after incubation at 37 °C for 48 h. *Staphylococcus aureus* was determined by the spread plate method using Baird-Parker agar with egg yolk tellurite emulsion (Oxoid Ltd.). The plates were incubated at 37 °C for 48h (Lancette et Tatini, 1992). Sulphito-reducer Anaerobes were enumerated in Bacto Sulfite Agar (BSA) after incubation at 37 ˚C for 48 h.

Identifications were made during the two months using the method of Scotch (Samson *et al*., 1995) [28] for the moulds investigation.

Results

Manufacture

Drying under sun led us to get crumbly leaves as show the figures 1 and 2 respectively for *Moringa oleifera* and *Azolla filiculoides* flours.

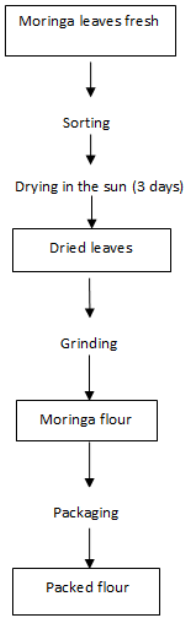

Figure 1: Technological diagram of production of *M. oleifera* flour

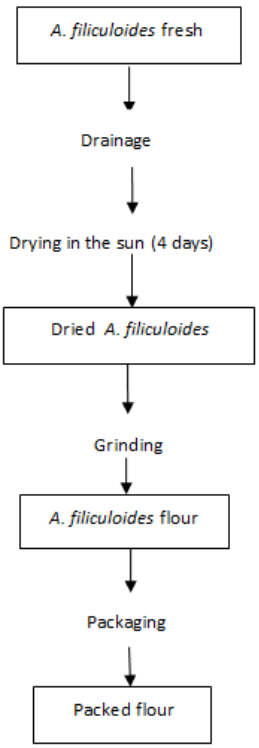

Figure 2: Technological diagram of production of *A. filiculoides* flour

Chemical analysis

The results related to the chemical analyses of the two flours are presented in table 1. The dry matters and the crude protein content were similar, with mean values of 94.19% and 27.70%; 94.98% and 27% (w.b) respectively for *Moringa oleifera* and *Azolla filiculoides* flours.

Table 1: Chemical composition (% DM basis)

Attributes	*M.oleifera*	*A.filiculoides*
Dry matter	94,19±0,7	94,98±0,5
Total ash	14,02±0,9	15,64 ±1,0
Organic matter	85,99±1,0	84,36±0,9
Moisture	5,81±0,1	5,02 ±0,5
Crude protein	27,70±1,4	27,00±1,6

Microbiological characteristics

Microbiological quality with the production of the flours is given in table 2.

Table 2: Microbiological quality of Moringa and Azolla flours the production day

Parameters (log_{10} cfu/g)	*M. oleifera* flour	*A.filiculoides* flour	Safety standard
TMA	4,25	3,44	5
Yeasts	1,25	<1	4
Moulds	1,43	<1	4
Total coliforms	<1	<1	3
Thermotolerant coliforms	<1	<1	3
E. coli	<1	<1	2
S .aureus	<1	<1	3
SRA	<1	<1	3

TMA: Total Mesophilic Aerobic

SRA: Sulphito- reducer Anaerobes

From figures 3 and 4, the trend of yeasts and moulds counts during the conservation of Moringa flour is shown. The load of yeasts on the leaves of *Moringa oleifera* flour in the jute bag becomes higher than in the other packing in the refrigered environment. The same tendency is observed in the non-ventilated and ventilated environment. We notice that the jute bag supports more the development of the moulds within the flour of *Moringa oleifera*. This growth reaches the standard in ventilated environment and remains in on this side and tends towards this one respectively in the environments refrigerated and non-ventilated. By comparing the results of the three environments, we notice that the ventilated environment supports the more the development of yeasts. Values obtained in packing for each environment are below the standard (4 log_{10} cfu/g). The ventilated environment was the medium where the evolution in the jute bag reached the desired suitable standard; therefore it supports more the development of the moulds.

The ventilated environment supports the growth of yeasts for *Azolla filiculoides* flour. The number of moulds increased in all the environments on the fifteenth day of conservation. The ventilated environment supports the development of these moulds. At the end of two months of conservation, this microbial load reaches the standard only in the ventilated environment

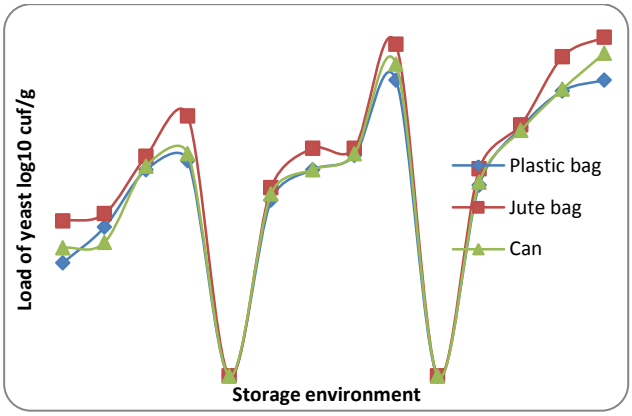

Figure 3: Yeasts evolution in *Moringa oleifera* flour on refrigerated (R), non-ventilated (NV) and ventilated (V) environment at 15, 30, 45, 60 days

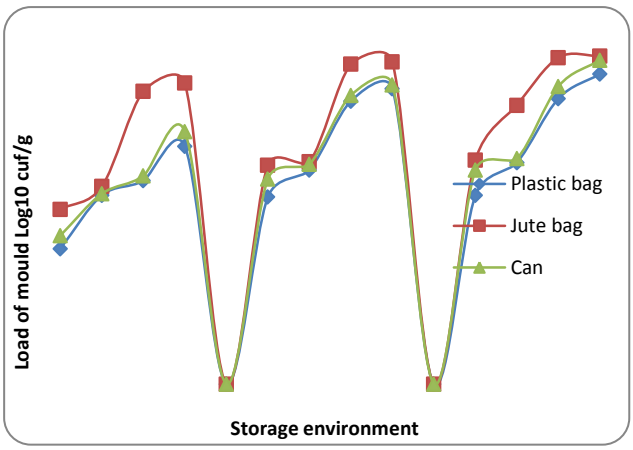

Figure 4 : Moulds evolution in *Moringa oleifera* flour on refrigerated (R), non-ventilated (NV) and ventilated (V) environment at 15, 30, 45, 60 days

For Azolla flour, the jute bag remained the most favourable for the development of yeasts in each environment (figure 5). This growth accelerated more in ventilated environment and exceeded the standard (4 log_{10} cfu/g) at the end of 60 days.

The jute bag remains the packaging (figure 6) the most favourable for the development of the moulds in all the environments. At the end of two months' time, the load

in this last packaging reached the standard envisaged in ventilated environment.

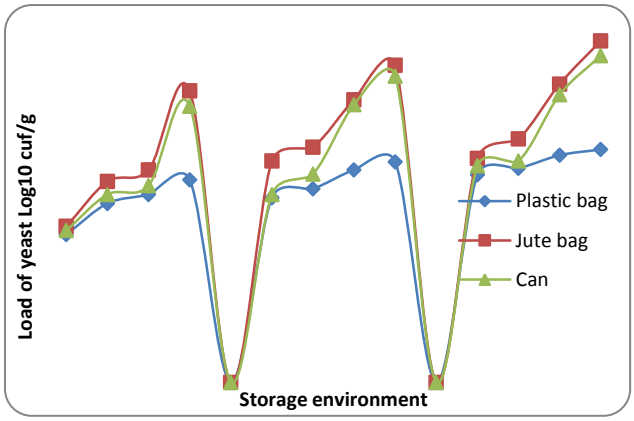

Figure 5: Yeasts evolution in *Azolla filiculoides* flour on refrigerated (R), non-ventilated (NV) and ventilated environment at 15, 30, 45, 60 days

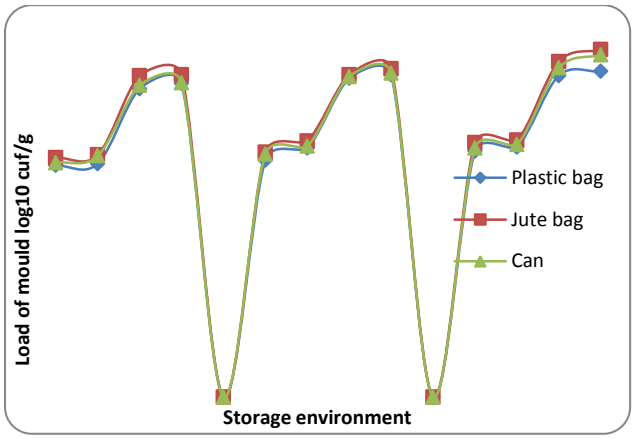

Figure 6 : Moulds evolution in *Azolla filiculoides* flour on refrigerated (R), non-ventilated (NV) and ventilated environment at 15, 30, 45, 60 days

Discussion

The average content of crude protein present in the *M. oleifera* flour was 27.7%. This value is very close to that reported by Oduro *et al.,*(2008) [19], Ayssiwede *et al.*,(2011) [7], Etalem *et al.*,(2013),[20]. Lower to the figure reported 30.3% by Moyo and Masika (2011) [30]. The crude protein of Azolla flour was 27%. It is higher than that of Alalade *et al.*., 2006[3] which used the species pinnata. Vanhove and Lopez (1982) [21] noted that the content of crude proteins of Azolla can vary from 13 to 34.5 %. Sanginga and Vanhove (1989) [22] allotted the variations of the nutritional composition of Azolla to the diversity of the stocks of Azolla, in the environmental conditions such as the temperature, the light intensity and of the nutritive elements of the ground which affect consequently, their morphology of growth and the composition.

Bacteria at the production day reveals that the various flours do not contain any of the following germs: total coliforms, thermotolerant coliforms, *Staphylococcus aureus*, sulfito-reducers anaerobe. These results are in conformity with those of Tété-Benissan *et al.* (2012) [23] in Togo.

The presence of the moulds in the products is inherent to the quality of the raw materials (*M. oleifera*, *A. filiculoides*), and with healthiness related to the chain of transformation or conditioning of the latter [24]. Their presence in great number in the bag of jute all along the 60 days of conservation is related to the matter and the manner of manufacture of this latter. Indeed this bag is made out of plastic, permeable to oxygen. As Beaulieu (2007) [25] affirmed plastic packaging is permeable with oxygen. The jute bags belonging to plastic packing having pores are permeable with oxygen. The moulds are usually aerobic germs [26] and this justifies their great number.

Their weak growth in the refrigerated environment can be justified by the reduction of oxygen and the low temperature. The growth observed in the non-ventilated environment can be related to the reduction of the ambient air circulation through this medium.

The load of moulds detected 15 days after the preservation, in the flour of M. oleifera preserved out of plastic bag, jute bag and can are respectively 2.30 log10 cfu/g ; 2.73 log10 cfu/g and 2.49 log10 cfu /g in the ventilated environment. The results are consistent with those obtained by Bidossessi *et al.* (2013) [24] which found 2.77log10cfu after 7 days of conditioning. The studies conducted in Togo on the microbiological composition of the powder of *M. oleifera* by Tété-Benissan *et al.* (2012) [23] also revealed the presence of moulds in this latter.

The results obtained prove the production day of the flours which highlight, the absence of *E coli*, *S. aureus*, the anaerobes sulphito-reducters, the thermotolerant coliforms. The appearance of *E coli* 30 days after, in the flour of *M. oleifera* preserved in plastic bag and in can on the non-ventilated environment could be due to the contamination of this environment by these germs and because the conditions are favourable for them in these two packings, they developed there. The *E coli* are being optional anaerobic germs [27].

The trends observed for *E coli* count in *A. filiculoides* flours preserved on plastic bag and can in the non-ventilated environment need to be further explored by later studies. Indeed, these micro-organisms which appear in the product after 30 days of conservation (2.30 $\log_{10}$ cfu/g for jute bag, 2.60 $\log_{10}$ cfu/g for the can) disappear completely after 60 days in these two packings. This absence could be explained by the evolution of the moulds which inhibit them in the flour; it is with to say a competition between these two germs. There are some modification about internal factors (aw, pH) and external factors like T° and environment condition (relative humidity)

The total flora in the flours the day of the production could be explained on the one hand by the season and

the site of harvest of for the plants, and on the other hand by the not hygienic handling of the materials during drying and grinding. The presence of the aerobic flora mesophilic results generally in a not very hygienic handling, manufacture in an unsatisfactory environment. The vegetable matters being taken in dry season and in an environment not protected, dust can be the source of the contamination by the total flora. The drier and the mill used during the production can also be sources of contamination by these micro-organisms. But let us announce that the found quantities are in-on this side standard.

Yeast multiplication is lasting all the time of the conservation. This multiplication is certainly inherent to their commensality for the crop products and animal in general [26]. The results for the flour of *M. oleifera* are in conformity with the results obtained by Bidossessi *et al.* (2013) [24] and Tété-Bénissan *et al.* (2012) [23].

Conclusion

The flour of *M. oleifera* and *A. filiculoides*, constitute interesting food for animals feeding. Over two months, we noted that the jute bag has permitted the fast development of yeasts and moulds due to the environment in which it is found. The plastic bag and can slightly allowed the growth of *E. coli* in a quantity generally below the normal standard. The ventilated environment was the environment in which the yeasts and the moulds generally exceeded the standard. Importantly, the non-ventilated environment is the environment in which *E. coli* developed. In the environment refrigerated, the values of the germs that pushed remained in-on this side standard during the two months of conservation.

Taking into account the results obtained, we can conclude that the three environments are favourable for the conservation of the flours. It is enough to exploit packaging. In the refrigerated environment, the flours were preserved in three packing for two months without microbial deterioration. For the ventilated environment, the plastic bag and the can are adapted for the preservation. With regard to the non-ventilated environment, the plastic and jute bags are suitable.

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