EAS Journal of Nutrition and Food Sciences
Abbreviated Key Title: EAS J Nutr Food Sci
ISSN: 2663-1873 (Print) & ISSN: 2663-7308 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-6 | Issue-3 | May-Jun; 2024 | DOI:10.36349/easjnfs.2024.v06i03.002

Original Research Article

Evaluation of the Microbiological Quality and the Expiration Date of Seasoning Food Broths Based on the Edible Mushroom *Psathyrella tuberculata* and Local Ingredients in Côte d'Ivoire

Farman Ouattara Ahmed[1,2*], Kouamé N'zebo Désiré[3], Koné-Boko Tadiogo Naty Amine[1], Dadié Thomas[1], Anin Atchibri Louise[2]

[1]Laboratoire de Microbiologie et de Biotechnologie des aliments, Département des Sciences et Technologies des Aliments, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire
[2]Laboratoire de Nutrition et de Sécurité alimentaire, Unité de Formation et de Recherche des Sciences et Technologies des Aliments, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire
[3]Laboratoire de Biotechnologie, Agriculture et valorisation des Ressources Biologiques, Unité de Formation et de Recherche de BIOSCIENCES, Université Félix Houphouët Boigny de Cocody, Abidjan, Côte d'Ivoire

Article History
Received: 28.03.2024
Accepted: 04.05.2024
Published: 24.05.2024

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: The objective of this work was to evaluate the microbiological quality and to determine the Expiry Date of three formulations of seasoning food broths based on the edible mushroom, *Psathyrella tuberculata*. The pH, titratable acidity, and humidity were determined using the AOAC method. Mesophilic aerobic germs, total coliforms, sulphite-reducing Clostridium, *Escherichia coli*, yeasts, moulds, staphylococci, and *Salmonella spp*. were quantified using standard microbiological methods. The Expiry Date of the formulated seasoning food broths powders was determined using an ageing test and predictive microbiology. The results obtained indicated that the ingredients and seasoning broths had a pH of less than 7 with a moisture content of between 8 and 11% for the ingredients and 9 and 10% for the seasoning food broths. Titratable acidity ranged from 0,01 to 0,6 for the ingredients and from 0,5 to 0,54 for the seasoning broths. The results showed that total coliforms, *Escherichia coli* strains, sulphite-reducing anaerobes and *Salmonella spp* were absent in all samples. However, the loads of mesophilic anaerobes and fungal flora detected from day one to day forty-five were within Codex Alimentarius standards. The Expiry Date (ED) for B90, B70 and B50 seasoning food broths. Were 56,77 days, 64,98 days and 72,6 days respectively. The powders analysed were of satisfactory microbiological quality. The Expiry Date (ED) obtained are those of three seasoning food broth formulations without preservative food additives.

Keyword: Edible Mushroom *Psathyrella Tuberculata*, Expiry Date, Ingredients, Microbiological Quality, Seasoning Food Broths.

INTRODUCTION

Seasoning food stocks or seasoning cubes are preparations obtained from plant-based substances with or without the addition of flavor enhancers, flavoring substances, spices and any other foodstuff intended to improve the palatability and authorized food additives (ASN, 2017). These are industrialized food products that are becoming essential in African cuisines. According to Pivot (2002), these industrial broths are present in the public space of West African cities. In addition to the culinary side, they are enriched with essential micronutrients such as iron and zinc, thus providing bioactive substances that are very useful for the good health of consumer populations (Bowley, 2005; OMS, 2006). However, certain food additives such as sodium chloride and monosodium glutamate, often used at high levels because of their preservative and flavor enhancing properties, have harmful effects on the health of consumers (Studios, 2018). On the other hand, certain food plants such as wild edible mushrooms, spices and their natural extracts endowed with antimicrobial, antioxidant, antiseptic, analgesic and anti-inflammatory

***Corresponding Author:** Farman Ouattara Ahmed
Laboratoire de Microbiologie et de Biotechnologie des aliments, Département des Sciences et Technologies des Aliments, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire

properties (Mohammedi, 2006) are used both for their nutritional quality and for their therapeutic properties.

In Côte d'Ivoire, the seasoning food broths or seasoning food cubes produced are not sufficiently documented and the instructions for use are not always followed. With the aim of improving the health of populations consuming these broths, seasoning food broths have been formulated without food additives from wild edible mushroom (*Psathyrella tuberculata*), cassava starch and spices such as powders onion, garlic, parsley which are important sources of vitamins, fiber, protein and minerals such as potassium, calcium, magnesium, iron and zinc. However, these ingredients can be contaminated with microorganisms. According to Ibtissem and Lilia (2017), several spoilage microorganisms and pathogens such as yeasts, molds, *Clostridium perfringens*, *Escherichia coli*, *Salmonella* spp, *Listeria monocytogenes* and *Staphylococcus aureus* have been found in spices and herbal mixtures. Spices. Dehydrated seasoning broths should be formulated from ingredients obtained in accordance with the Code of Hygienic Practice for Dehydrated Fruits and Vegetables, including Edible Fungi as recommended by the Codex Alimentarius Commission (CAC /RCP 5-1971). They should comply with any microbiological criteria established in accordance with the Principles for the Application of Microbiological Criteria for Foods (CAC/GL 21-1997). Compliance with all standards involving good hygiene practices in the food chain during production, primary processing and packaging can help to preserve dehydrated food products over a long period of time **(**ASN, 2017). In addition, it is recommended to indicate a Date of Minimum Durability (DMD) on the label of grocery products, canned goods and frozen products to protect the health of consumers.

The present study aims to evaluate the microbiological quality and the Expiry Date (ED) of seasoning food broths formulated with the edible mushroom (*Psathyrella tuberculata*) and ingredients composed of onion (*Allium cepa*), garlic (*Allium sativa*), parsley (*Petroselinum crispum*) and cassava (*Manihot esculentus*) produced in Côte d'Ivoire.

MATERIAL AND METHODS

Material

The plant material consisted of *Psathyrella tuberculata*, a species of domesticated edible wild mushroom (made cultivable) in Hermankono (Garo) in the Lôh Djiboua region in southern Côte d'Ivoire, vegetables (garlic cloves, bulbs of onion, parsley leaves) and cassava tuberous roots. The mushrooms and cassava roots were collected after cultivation in Hermankono, while the vegetables were purchased at the Forum market in Adjamé (Abidjan).

METHODS

Preparation of Ingredient Powders

The edible parts (carpophore and stem) of the mushroom were sorted and washed three times with healthy tap water then spread out and dried on a drying rack placed on a table in the sun until obtaining crispy dry mushrooms. The dried mushrooms, once wrapped in sterile food plastics, were transported to the laboratory and then dried again in a steamroom (Memmert UN 260) oven at 45°C for 48 hours. Garlic cloves (*Allium sativa*) of the purple autumn variety (sprint) from the Middle East and onion bulbs (*Allium cepa*) of the purple variety of Galmi from Niger, were cleaned, washed with healthy tap water and cut by hand with a knife into very thin slices for better drying. The thin slices were placed widely spaced on aluminum foil in the steamroom (Memmert UN 260) oven at 55°C for 72 hours. Parsley leaves (*Petroselinum crispum*) of the curly type, were detached, washed three times with 5% bleach and then rinsed three times with healthy tap water. They were then placed very far apart on trays in the steamroom (Memmert UN 260) oven at 45°C for 72 hours. The cassava tuberous roots (*Manihot esculentus*) were peeled, washed three times with healthy tap water then crushed, rinsed three times with healthy tap water and ground in a previously cleaned traditional grinder. The ground material obtained is then put in a 35 litre container containing tap healthy water and then the mixture is filtered with an aluminium sieve of 450 µm mesh. For starch extraction, the collected whitish water was allowed to stand for at least 5 hours until the starch sedimented in the bottom of the vessel. The wet starch recovered was dried in the steamroom (Memmert UN 260) oven at 45°C for 72 hours. Each dried product was ground using a blender and each ground material obtained was sieved using a 250 µm mesh stainless steel sieve. Mushroom powder (MP), garlic powder (GP), onion powder (OP), parsley powder (PP) and starch powder (SP) were immediately packed each in a plastic sterile food.

Formulation and Preparation of Seasoning Food Broths

The B90, B70 and B50 seasoning broth powders were developed with an Excel-assisted formulation system, using a calculation system based on the matrix formulation method (Loba *et al*., 2019). Using the nutritional values from the list of ingredients (PC, PAI, PO, PP and PA) and the trial-and-error method described by Olusayo *et al*., (2013), the nutritional requirements to be met were obtained with a deviation of ±10%. The equation system used was as follows:

$$a11X1 + a12X2 + \cdots + a1nX3 = b1$$
$$a21X1 + a22X2 + \cdots + a2nX3 = b2$$
$$an1X1 + an2X2 + \ldots + anmXn = b3 \quad (1)$$

With
a = the nutrient content (protein, fibre, potassium, sodium, calcium, magnesium and polyphenol).
X = the proportions of ingredients to be mixed.
b = the nutritional requirements to be met.
The various nutrient values (in g/100g of powders: protein, fibre, potassium, sodium, calcium, magnesium and polyphenol) were automatically calculated using formulas developed with Excel software.

The seasoning broths formulated were presented in Table 1 with the different proportions of ingredients and the codes (B90, B70 and B50) identifying these seasoning broths. Code B90 designates seasoning broth formulated with 90% *Psathyrella tuberculata* mushroom powder, 05% onion powder, 02% garlic powder, 02% parsley powder, 01% cassava starch powder. B90 codes; B70 and B50 designate food broths of seasonings composed respectively of 90%; 70% and 50% *Psathyrella tuberculata*.

Table 1: Different seasoning food broths based on *Psathyrella tuberculata*

Ingredients (%)	B90	B70	B50
Mushroom powder (MP)	90	70	50
onion powder (OP)	05	15	25
garlic powder (GP)	02	07	12
Poudre de persil (PP)	02	07	12
starch powder (SP)	01	01	01
Water (ml)	10	10	10

B90: Dehydrated seasoning food broth with 90% of the base ingredient, B70: Dehydrated seasoning food broth with 70% of the base ingredient, B50: Dehydrated seasoning food broth with 50% of the base ingredient.

Analysis of Physico-Chemical Parameters
PH Measurement

The pH was determined by the AOAC method (1990). A quantity of 5 g of sample of the different powders was mixed in 50 ml of distilled water and centrifuged at 4200 rpm for 10 min with a centrifuge (Sigma). The pH reading was taken directly in the filtrate after calibration of the pH meter (Sension TM^{+}).

Measurement of Titratable Acidity

The titratable acidity was determined by the AOAC method (1990). A quantity of 5 g of sample of the different powders was mixed in 50 ml of distilled water and centrifuged at 4200 rpm for 10 min with a centrifuge (Sigma). The measurement of the titratable acidity was made in 10 ml of the filtrate and titrated with a solution of NaOH (0,1N) until a persistent pink color after the prior addition of 2 to 3 drops of phenolphthalein.

Determination of Moisture Content

The moisture content was determined according to the AOAC method (1990). A quantity of 5 g of sample of the different powders was weighed and then dried in the steamroom (Memmert UN 260) oven for 24 hours at 105°C. After drying, the sample was cooled in a desiccator for 1 hour, then weighed and the moisture content was determined according to formula 1:

$$\text{Humidity in (\%)} = \frac{(\text{Initial weight} - \text{final weight})}{\text{Initial weight}}\ x\ 100 \quad (2)$$

Microbiological Analysis
Collection, Transport and Storage Conditions of Samples

The samples (ingredient powders and seasoning broths) were removed aseptically using sterile spatulas and individually wrapped in sterile stomacher bags. A total of 17 samples (5 samples of ingredient powders and 12 samples of seasoning food broth powders) were taken and sent to the microbiology laboratory. The seasoning broth powders were stored under aseptic conditions at room temperature to determine a use-by date.

Germs Sought and Microbiological Criteria

The main germs sought are mesophilic aerobic germs, total coliforms, sulphite-reducing anaerobic bacteria (Clostridium sulphite-reducing), *Escherichia coli*, yeasts and moulds, *Staphylococcus* and *Salmonella spp*. The microbiological criteria applicable to spice powders and seasoning broths given in the Codex Alimentarius standards (ASN, 2017) were used to interpret the microbiological quality of the powders.

Germ Enumeration

The count of the germs studied was carried out from the stock suspension and decimal dilutions as described by the French association for standardization (AFNOR, 2004).

The enumeration of mesophilic aerobic germs was carried out on Plate Count Agar (PCA) (Fluka

BioChemica 70152) according to the standard ISO 4833 (2003). A volume of 1 ml of the stock suspension and decimal dilutions were deposited in empty sterile Petri dishes, then approximately 15 ml of sterilized supercooled Pate Count Agar were poured into them, then homogenized and left to cool. A double layer of about 5 ml of white agar was then poured. Cultures were incubated at 30°C for 72 hours. Only Petri dishes with colonies between 30 and 300 were retained. For the enumeration of total coliforms, it was carried out on Violet Red Bile Lactose agar (VRBL, Merck 10660, Merck, Darmstadt, Germany) according to standard NF-V-08-015 (2009). Thus, a volume of 1 ml of the stock suspension and decimal dilutions were deposited in empty sterile Petri dishes, then approximately 15 ml of the sterilized supercooled VRBL agar were poured into them, then homogenized and left to cool. A double layer of approximately 5 ml of the same agar was then poured. Cultures were incubated at 30°C for 24 and 48 hours. Petri dishes with pink-red, round and lenticular colonies between 15 and 150 were selected. As for sulphite-reducing anaerobic (SRA) bacteria, their enumeration was carried out on supercooled TSN (Tryptone-Sulphite-Neomycin) agar, according to standard NF-V-08-061(2004). Indeed, a volume of 1 ml of the stock suspension and decimal dilutions were inoculated deeply into tubes containing 20 ml of sterile TSN agar. The tubes were incubated at 46° C. for 48 hours to count the sulphite-reducing Clostridium forming large black colonies in depth. The tubes where the colonies were between 15 to 150 were retained.

As regards the enumeration of *Escherichia coli* on the Rapid E. coli 2 medium, it was carried out according to standard NF ISO 16649-2 (2001). For this, a volume of 0,1 ml of the stock suspension and decimal dilutions were inoculated by plating on the surface in sterile Petri dishes pre-poured with Rapid E. coli 2 agar. The cultures were incubated at 44° C for 24 hours. Petri dishes with purple colonies with colonies between 15 and 150 were retained. The enumeration of fungal flora (yeasts and moulds) on Oxytetracycline-Glucose (OGA) agar was carried out according to ISO 21527-2 (2008) standard. Indeed, a volume of 0.1 ml of the stock suspension and decimal dilutions were spread on the surface of approximately 15 ml of sterile OGA agar precast in sterile Petri dishes and incubated at 30°C for 72 hours. The Petri dishes selected are those with translucent and ovoid colonies for yeasts and whitish and fluffy for moulds with colonies between 15 and 150. The enumeration of *Staphylococcus aureus* was determined on Baird Parker agar according to the ISO 6888-1 (2004) standard. A volume of 0.1 ml of the stock suspension and decimal dilutions were spread on the surface of approximately 15 ml of Baird-Parker agar containing an emulsion of egg yolk and tellurite (Oxoid) precast in petri dishes. Sterile and incubated at 37°C for 24 hours. The Petri dishes selected are those with black colonies with a whitish transparent halo with colonies between 15 and 150. The search for *Salmonella spp* in the samples was carried out according to the ISO 6579 (2002) standard following the stages of pre-enrichment, enrichment, isolation and identification. The stock suspensions containing the samples were first incubated at 37°C for 24 hours for the pre-enrichment step, then 0.1 ml of the pre-enriched suspension was added to a tube containing 10 ml of Rappaport Vassiliadis (RV) broth incubated at 37°C for 24 hours, followed by subculture of a loop of enriched medium by streaking on *Salmonella-Shigella* (SS) agar incubated at 37°C for 24 hours. Colorless, transparent colonies with or without a black center were then identified using biochemical tests.

Determination of the Expiry Date of Dehydrated Seasoning Food Broths

The Expired Date was determined using the methodology developed by Bokomena (2021) based on the ageing test and predictive microbiology on dehydrated seasoning food broths. The determination of the expiry date was made by four (04) series of analyzes on three (03) samples of the dehydrated seasoning food broth, with a regular interval of fifteen (15) days. The evolution of each germ of alteration was represented on a table, then the latter interpreted by clouds of points. The linear regression lines of each germ were then represented and the one with the greatest slope among the two lines was considered. The equation of this significant line must then be found in order to deduce the date on which the reference criterion is reached. The risk α is fixed as equal to 5%.

With y be the number of colonies forming units per gram of sample of the significant spoilage germ, and x the time. The linear regression line determined on Excel has the equation:

$Y=a\,x + b.$ (3)

At t = x,

Y = reference criterion, which makes it possible to calculate t.

The margin α=5% must be taken into account.

$ED = t \pm \alpha$ (4)

With

ED: Expiry Date

t: Time when the number of spoilage germs is equal to the reference microbiological criterion

α: risk taken for margin of error

The Colonies Forming Unit (CFU) load of mesophilic aerobic germs and fungal flora counted in the dehydrated seasoning food broths over the period of 45 days in a fifteen-day interval made it possible to draw the curves of evolution of the aerobic mesophilic germs and fungal flora. Thus, the trend curves were determined with their different equation of the form [y=a x + b] and their coefficient of determination [R^2] marked on the graph. For the maximum threshold values given by the Codex Alimentarius standards (ASN, 2017), M = Y = 10^5 for AMG and M = Y = 10^3 for FF, we can determine the

value of x which indicates the number of days on which the product is fit for consumption.

Statistical Analyzes

Analysis of variance (ANOVA) was performed with Statistica version 7.1 software to study the degree of difference between variables. In the event of a significant difference between the parameters studied, the classification of the means (homogeneous groups) was carried out with Duncan's test. The significance level (α) is 0,05. Statistical differences with a probability value less than 0.05 were considered significant. When the probability is greater than 0,05, the statistical differences are not significant.

RESULTS

Physico-Chemical Characteristics of Ingredient Powders and Formulated Seasoning Food Broths

The results of the physico-chemical analyzes of the ingredient powders and the formulated seasoning food broths are presented in Tables 2 and 3 respectively. Table 2 shows that the pH is lower in onion powder (4,36 ± 0,01) but higher in mushroom powder (6,5 ± 0,00). There is a significant difference at the 5% threshold at the level of the pH of the various ingredients. Regarding the moisture content, it is relatively higher at the level of onion powder (OP) (10,83 ± 0,09). There is no significant difference on the one hand between the moisture content of onion powder (OP) and that of starch powder (SP) and on the other hand between those of garlic powders (GP), mushroom powder (MP) and parsley powder (PP) on the other hand there is a significant difference between those of onion powder, starch powder and those of garlic powder, mushroom powder and parsley powder. Titratable acidity is higher in parsley powder with a value of 0,6±0,00 compared to other ingredient powders ($P \leq 0,05$). After formulation, Table 3 shows that the highest moisture content determined comes from the seasoning broth (B70) with a content of 9,92 ± 0,05 compared to the other formulated seasoning broths ($P \leq 0,05$). Furthermore, the highest pH was determined in B90 broth and the titratable acidity in B50 broth ($P \leq 0,05$).

Table 2: Physico-chemical characteristics of ingredients

Parameters	Ingredients				
	SP	GP	OP	MP	PP
pH	6,24±0,01^{b}	5,92±0,01^{c}	4,36±0,01^{e}	6,5±0,00^{a}	5,82±0,00^{d}
Humidity (%)	10,60±0,05^{a}	8,60±0,86^{b}	10,83±0,09^{a}	8,29±0,05^{b}	8,62±0,11^{b}
Titratable acidity (%)	0,0147±0,00^{e}	0,54±0,00^{b}	0,44±0,00^{c}	0,363±0,01^{d}	0,583±0,00^{a}

Values are means ± standard deviations of three measurements (n = 3). On the same line, the numbers followed by the same letter are not significantly different at the 5% level. SP: Starch powder from cassava tubers, GP: Powder from dried garlic cloves, OP: Powder from dried onion bulbs, MP: Powder from dried *Psathyrella tuberculata* fungus, PP: Powder from leaves of dried parsley.

Table 3: Physico-chemical characteristics of formulated seasoning food broths

Parameters	Formulated Seasoning Food Broths		
	B90	B70	B50
pH	6,39±0,00^{a}	6,2±0,01^{b}	5,96±0,00^{c}
Humidity (%)	9,27±0,29^{b}	9,91±0,05^{a}	9,33±0,39^{b}
Titratable acidity (%)	0,49±0,02^{b}	0,50±0,00^{b}	0,54±0,00^{a}

Values are means; ± the standard deviations of three measurements (n = 3). On the same line, the numbers followed by the same letter are not significantly different at the 5% level. B90: Seasoning broth formulated with 90% of the dried *Psathyrella tuberculata* mushroom, B70: Seasoning broth formulated with 70% of the dried *Psathyrella tuberculata* mushroom, B50: Seasoning broth formulated with 50% of the mushroom *Psathyrella tuberculata* dried.

Microbiological Quality of Ingredient Powders and Formulated Seasoning Food Broths

The microbiological quality of the powders of the ingredients and of the formulated seasoning food broths was evaluated and mentioned respectively in Table 4 and 5. Table 4 shows that the load of mesophilic aerobic germs varied from $4,65.10^{1}$±0,41 to $3,9.10^{2}$±8,16 CFU/g. This load is higher in the mushroom powder ($3,9.10^{2}$±8,16) with $P \leq 0,05$, likewise the fungal load which varies from 10 to 60 CFU/g is higher in the mushroom powder. However, there is a total absence of other germs. The results of the microbiological quality of the edible mushroom seasoning food broths are recorded in Table 5. The microbiological quality was evaluated over a period of 45 days on four (04) occasions with 15-day intervals. This table shows that the load of mesophilic aerobic germs varied from $4,65.10^{1}$±0,41 to $3,9.10^{2}$±8,16 CFU/g on day 1, from $7,18.10^{2}$±8,49 to $9,05.10^{2}$±4, 08 CFU/g on day 15, from $1,84.10^{3}$±1,63.10 to $2,31.10^{3}$±2,44 CFU/g on day 30 and from $4,3.10^{3}$±0 to $5,6.10^{3}$±8,1.10 CFU/g on day 45 on the other hand, the fungal load varied from 10 to 60 UFC/g on day 1, from 50±0 to 90±8,16 CFU/g on day 15, from $1,1.10^{2}$±0 to $2,1.10^{2}$±0 on day 30 and from $2,9.10^{2}$±8,16 to $5,2.10^{2}$±8.16 at day 45. The load of mesophilic aerobic

germs is significantly different at the p<0,05 threshold between the different seasoning food broths, on the other hand that of the fungal flora is not significantly different at the p<0,05 threshold between all the different food broths of seasoning. On the other hand, total coliforms, strains of *Escherichia coli*, sulphite-reducing anaerobic bacteria and *salmonella spp* were not detected in all the different powders during this study. It should also be noted the absence of pathogenic germs both in the ingredients and in the formulated broths.

Table 4: Microbial profile of ingredients

Parameters (CFU/g)	Ingredients				
	SP	GP	OP	MP	PP
MAG	$1,09.10^{2}\pm1,63^{b}$	$4,65.10^{1}\pm0,41^{e}$	$6,65.10^{1}\pm2,04^{d}$	$3,9.10^{2}\pm8,16^{a}$	$80,45.10^{1}\pm2,04^{c}$
Total coliforms	0	0	0	0	0
E. coli	0	0	0	0	0
Yeasts and moulds	10 ± 0^{a}	0	0	60 ± 0^{a}	30 ± 0^{a}
SRA	0	0	0	0	0
S. aureus	0	0	0	0	0
Salmonella spp	Absence/25g	Absence/25g	Absence/25g	Absence/25g	Absence/25g

Values are means ± standard deviations of three measurements (n = 3). On the same line, the numbers followed by the same letter are not significantly different at the 5% level. MAG: mesophilic aerobic germs, *E. coli*: *Escherichia coli*, SRA: sulphite-reducing anaerobic, *S. aureus*: *Staphylococcus aureus*, SP: Starch powder from cassava tubers, GP: Powder from dried garlic cloves, OP: Powder from dried onion bulbs, MP: Powder from the dried *Psathyrella tuberculata* mushroom, PP: Powder from dried parsley leaves.

Table 5: Microbial profile of formulated seasoning food broths

Days	**Formulated Seasoning Food Broths**	**Parameters (CFU/g)**						
		MAG	***Total coliforms.***	***E. coli***	**Yeasts and moulds**	**SRA**	***S. aureus***	***Salmonella spp***
1	B90	$3,8.10^{2}\pm1,63.10^{a}$	0	0	30 ± 0^{a}	0	0	Absence/25g
	B70	$3,3.10^{2}\pm1,63.10^{b}$	0	0	20 ± 0^{a}	0	0	Absence/25g
	B50	$2,45.10^{2}\pm2,04.10^{c}$	0	0	20 ± 0^{a}	0	0	Absence/25g
15	B90	$9,05.10^{2}\pm4,08^{a}$	0	0	$90\pm8,16^{a}$	0	0	Absence/25g
	B70	$8,25.10^{2}\pm4,08^{b}$	0	0	$55\pm4,08^{b}$	0	0	Absence/25g
	B50	$7,18.10^{2}\pm8,49^{c}$	0	0	50 ± 0^{b}	0	0	Absence/25g
30	B90	$2,31.10^{3}\pm2,44.10^{a}$	0	0	$2,1.10^{2}\pm0^{a}$	0	0	Absence/25g
	B70	$2,13.10^{3}\pm1,63.10^{b}$	0	0	$1,4.10^{2}\pm0^{a}$	0	0	Absence/25g
	B50	$1,84.10^{3}\pm1,63.10^{c}$	0	0	$1,1.10^{2}\pm0^{a}$	0	0	Absence/25g
45	B90	$5,6.10^{3}\pm8,1.10^{a}$	0	0	$5,2.10^{2}\pm8,16^{a}$	0	0	Absence/25g
	B70	$4,85.10^{3}\pm4,08.10^{b}$	0	0	$3,45.10^{2}\pm1,22.10^{b}$	0	0	Absence/25g
	B50	$4,3.10^{3}\pm0^{c}$	0	0	$2,9.10^{2}\pm8,16^{c}$	0	0	Absence/25g

Values are means ± standard deviations of three measurements (n = 3). On the same line, the numbers followed by the same letter are not significantly different at the 5% level. MAG: mesophilic aerobic germs, *E. coli*: *Escherichia coli*, SRA: sulphite-reducing anaerobic, *S. aureus*: *Staphylococcus aureus*, B90: Seasoning food broth formulated with 90% of the dried *Psathyrella tuberculata* mushroom, B70: Seasoning food broth formulated with 70% of the dried *Psathyrella tuberculata* mushroom, B50: Seasoning food broth formulated with 50% of the mushroom *Psathyrella tuberculata* dried.

Expiry Date (ED) of Dehydrated Seasoning Food Broths

Mesophilic aerobic germs and fungal flora constituted the main germs to be monitored four (04) times over an interval of fifteen (15) days (Day 01 to Day 45). The evolution of mesophilic aerobic germs and fungal flora from day 01 to day 45 are represented respectively by figures 1 and 2.

Figure 1 shows the evolution of mesophilic aerobic germs in the different broths. Thus, the microbiological life of B90 broth is equal to 59,75 days and its safe consumption margin α = 5% is equivalent to 2,98 days, therefore the expiry date of B90 broth has been set at 56, 77 days. As for the microbiological shelf life of B70 broth, it is equal to 68.40 days with the safe margin of consumption α = 5% equivalent to 3,42 days, therefore the expiry date of B70 broth was 64, 98 days. Finally, the microbiological lifespan of B50 broth is equal to 76,42 days and its safe consumption margin α = 5% is equivalent to 3,82 days, so the expiry date for B50 broth will be set at 72,6 days.

Figure 2 shows the evolution of the fungal flora in the different broths. Thus, the microbiological lifespan

of B90 broth is equal to 7,45 days with the safe consumption margin α = 5% being equivalent to 0,37 days, which makes it possible to determine the expiry date of B90 broth as being 7,08 days. For broth B70, its microbiological lifespan is equal to 10,61 days but its safe consumption margin α = 5% being 0,53 days, thus its expiry date is 10,08 days. Finally, the microbiological lifespan of broth B50 being 12,64 days, its safety margin of consumption α = 5% also of 0,63 days, consequently its expiry date is 12,01 days. The comparison of the evolution of the two germs leads us to choose that of the MAG as a reference since the growth of the fungal flora is much lower than that of the mesophilic aerobic germs. Finally, the use-by date of the B90, B70 and B50 seasoning food broths is 56,77 days, 64,98 days and 72,6 days respectively.

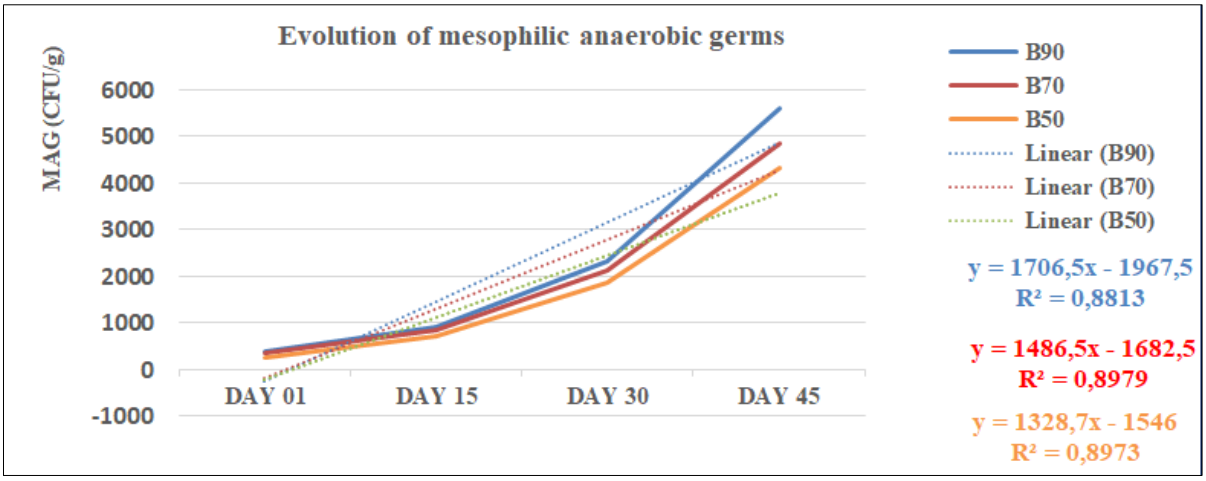

Fig. 1: Evolution of MAGs from day 01 to day 45

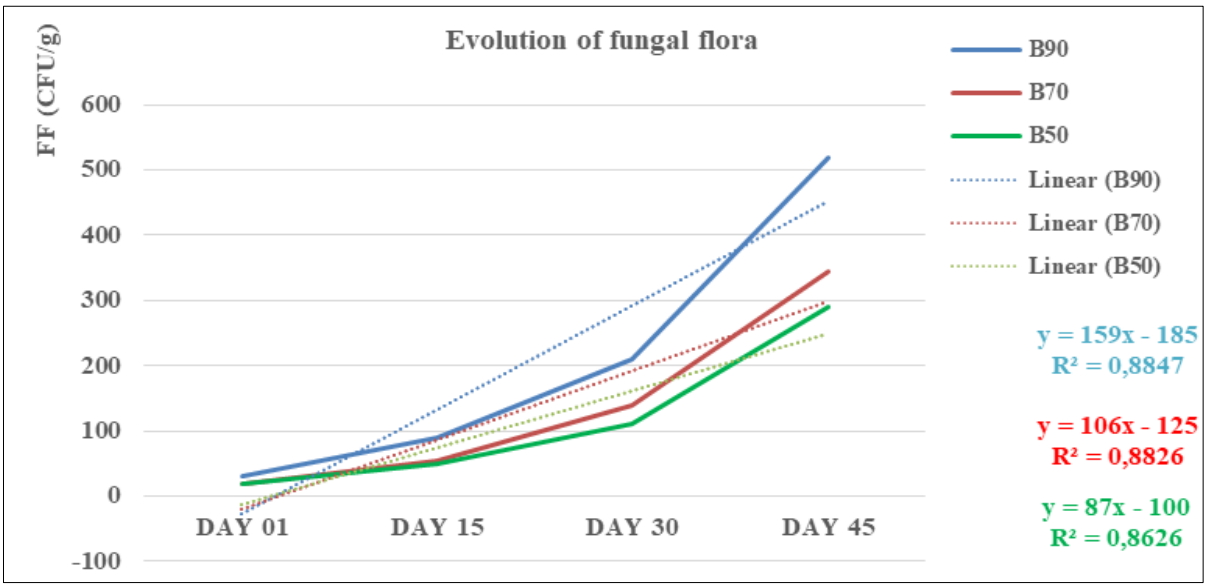

Fig. 2: Evolution of fungal flora from day 01 to day 45

DISCUSSION

The powders of the ingredients and the formulated seasoning food broths have a relatively low pH compared to the pH determined in the moringa-based seasoning broth from Bokomena (2021) and all have a pH below 7, which shows that these different powders are acidic. According to Aryee *et al.*, (2006), food acidity is a factor in good food preservation. In addition to these factors favourable to the good conservation of the powders, these powders must have moisture contents lower than the limit value (10%) recommended by the WHO and the FAO (2019) for dried vegetables and condiments.

In fact, water content is an important factor in maintaining food quality, as increased moisture facilitates the growth of microbes and ultimately destroys the organoleptic and marketable quality of foods.

Concerning the loads of mesophilic aerobic bacteria (MAP) and fungal flora (FF) found in the powders of ingredients and seasoning food broths, this study showed that the powder of the edible mushroom,

Psathyrella tuberculata and that of the seasoning food broth B90 contained the highest loads respectively than the other powders of ingredients and seasoning broths. However, the loads of GAM and FF found in all the powders were below those tolerated by Codex Alimentarius standards (ASN, 2017). The non-detection of *Staphylococcus aureus* strains in all the powders could be explained by the heat treatment of 55°C applied to these matrices during oven drying. In addition, the non-detection of *Staphylococcus aureus* strains in garlic powder and seasoning food broths could also be explained by the antimicrobial activity of the latter on these strains. This deduction is in agreement with the results obtained by Okombe and Nzuzi (2019) indicating that the aqueous or methanolic extract of garlic has bactericidal power on strains of *Staphylococcus aureus* and several bacteria.

The non-detection of faecal contamination indicators (total coliforms, faecal coliforms (*Escherichia coli*), and sulphite-reducing anaerobic bacteria and frequently sought-after pathogenic microorganisms (*Staphylococcus aureus* and *Salmonella spp*) shows that the correct Hygiene and manufacturing practices were respected from sampling to conservation, including the production of the various ingredient powders and seasoning food broths.

The microbiological quality of all these powders is therefore considered satisfactory according to the microbiological criteria applicable to spice powders given by the Codex Alimentarius standards (ASN, 2017). However, acceptable levels of contamination could lead to a loss of organoleptic and/or commercial characteristics over a long shelf life for these powders.

A dehydrated foodstuff of satisfactory microbiological quality can be kept for a long time. However, the manufacturer or consumer needs to know how long it will keep, which is why it is necessary to determine the Expired Date (ED) of these seasoning food broths.

According to Belaidouni (2015), the most important criterion for determining the Expiry Date is the microbiological criterion. For this reason, an ageing test of dehydrated seasoning food broths kept under normal conditions of storage and presentation for sale, in other words at room temperature, was carried out over a period of fifteen (15) days on four (04) occasions to better assess the microbiological parameters.

The Expired Date was determined by studying the evolution of the main spoilage germs present, in particular mesophilic anaerobic germs (MAG) and fungal flora (FF). Indeed, the work of Belaidouni (2015) indicates that the higher the spoilage germ load, the more easily the food product is spoilable. Figures 1 and 2 show that the growth of fungal flora is much lower than that of GAM. This result explains why the study of the evolution of GAMs is taken as the reference study for determining the Expired Date. The methodology adopted is in line with the work carried out by Rakotoniaina (2005) indicating that the study of total flora is the best method for assessing the microbiological quality of foods. Thus, the ageing test and predictive microbiology used in the methodology of Bokomena (2021) on the evolution of total flora made it possible to determine the Expired Date of seasoning food broths.

The Expired Dates obtained for B90, B70 and B50 seasoning for broths were 56,77 days, 64,98 days and 72,6 days respectively. These Expired Dates are lower than those obtained by Bokomena (2021) for moringa-based seasoning food broth, which is 143 days. These results could be explained by the presence of a food additive in the moringa-based seasoning broth. No preservative additives were added to the B90, B70 and B50 formulations, but salt was added to the moringa-based seasoning food broth formulated by Bokomena (2021).

CONCLUSION

This study showed that all the powders analysed had a pH of less than 7, and were therefore acidic, with a moisture content favourable to their preservation, and of satisfactory microbiological quality. The Expired Date (ED) for B50, B70 and B90 seasoning broths, determined over a period of 45 days with an interval of 15 days on 4 occasions, are 56,77 days, 64,98 days and 72,6 days respectively. However, the results obtained for these Expired Dates are acceptable for dehydrated products formulated without the addition of preservative food additives.

Acknowledgements

The authors would like to thank the managers of the central laboratory of the University Nangui Abrogoua and the microbiology laboratory of Yopougon technical school in Abidjan for access to the technical facilities.

REFERENCES

- AFNOR. (2004). Contrôle de la qualité des produits alimentaires. Association Française de Normalisation. Recueil des normes françaises. *Épices et Aromates*, 445.
- AOAC. (1990). Official methods of analysis. 15 th Ed. Association of Official Analytical Chemists, Arlington, VA, sec. 935-939.
- Aryee, F. N. A., Oduro, I., Ellis, W. O., & Afuakwa, J. J. (2006). The physicochemical properties of flour samples from the roots of 31 varieties of cassava. *Food control*, *17*(11), 916-922.
- ASN. (2017). Bouillons alimentaires d'assaisonnements-spécifications Projet de normes sénégalaises. Association Sénégalaise de Normalisation. NS 03-146. 13.

- Belaidouni, F. Z. (2015). La date de péremption des aliments, Mémoire pour l'obtention du diplôme du Master Biologie Option Science des aliments, Université Abou Bekr Belkaid - Tlemcen d'Algérie. 80.
- Bokomena, I. G. (2021). Étude de faisabilité technologique de la fabrication d'un bouillon alimentaire à base de Moringa (*Moringa oleifera*), mémoire de fin d'études pour l'obtention du diplôme d'ingéniorat, transformation et contrôle qualité des produits alimentaires, Université Athénée Saint Joseph Antsirabe (ASJA), 89.
- Bowley, A. (2005). Conference Report. *Nutriview* 1, 5-6.
- CAC/GL 21-1997. (1997). Principes et directives pour l'établissement et l'application de critères microbiologiques relatifs aux aliments. 6 p.
- CAC/RCP 5-1971. (1971). Code d'usages en matière d'hygiène pour les fruits et légumes déshydratés, y compris les champignons comestibles. 8 p.
- Codex, A. (2019). Programme mixte FAO/OMS sur les normes alimentaires, comité du Codex sur les épices et les herbes culinaires, 11.
- Ibtissem, C., & Lilia, R. (2017). Analyse microbiologique de quelques épices. Alimentation Humaine et Qualité des Produits, *Université Mouloud Hamméri de Tizi Ouzou d'Algérie*, 76.
- ISO 16649-2. (2001). Microbiologie des aliments Méthode horizontale pour le dénombrement des *Escherichia coli* β-glucuronidase positive. NF ISO 16649-2. Partie 2 : Technique de comptage des colonies à 44 °C sur milieu rapide d'E. coli 2.
- ISO 21527-2. (2008). Microbiologie des aliments, Méthode horizontale pour le dénombrement des levures et moisissures, Partie 2 : technique par comptage des colonies dans les produits à activité d'eau inférieure ou égale à 0,95. 9.
- ISO 4833. (2003). Microbiologie des aliments, Méthode horizontale pour le dénombrement des micro-organismes, Technique de comptage des colonies à 30 °C sur PCA. 19 p.
- ISO 6579. (2002). Microbiology of food and animal feeding stuffs, Horizontal method for the detection of *Salmonella spp*, Fourth Edition, 34.
- ISO 6888-1. (2004). Microbiologie des aliments, Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (*Staphylococcus aureus* et autres espèces), Partie 1 : Technique utilisant le milieu gélosé de Baird-Parker, Amendement 1 : Inclusion des données de fidélité, 18.
- Loba, S. E., Gbakayoro, J. B., Kouame, K. A., Grodji, G. A., & Brou, K. (2019). Formulations de Farines Composées Dont l'une à Base de Riz (Oryza sativa) et L'autre à Base de Maïs (Zea mays) Pour Enfants en âge de Sevrage. *European Scientific Journal*, *15*, 100-116.
- Mohammedi, Z. (2006). Études du pouvoir antimicrobien et antioxydant de quelques plantes de la région de Tlemcen. Produits naturels, *activité biologique*, 59.
- NF-V-08-015. (2009). Microbiologie des aliments, Méthode horizontale pour le dénombrement des coliformes totaux, Méthode par comptage des colonies obtenues à 37 °C. 6p.
- NF-V-08-057. (2004). Microbiologie des aliments. Méthode pour le dénombrement des staphylocoques à coagulase positive par comptage des colonies à 37 °C, Partie 1 : Technique avec confirmation des colonies.
- NF-V-08-061. (2009). Microbiologie des aliments - Dénombrement en anaérobiose des bactéries sulfito-réductrices par comptage des colonies.
- Okombe, E. V., & Nzuzi, M. V. (2019). Étude de l'activité antibactérienne (in vitro) des extraits aqueux et méthanoliques de l'ail (*Allium sativum L.*). *Journal of Applied Biosciences*, *141*, 14419 – 14425.
- OMS. (2006). Guidelines on Food Fortification with Micronutrients, Geneva. 342 p.
- Pivot, M. (2002). L'intégration locale des produits mondiaux-le bouillon cube au Sénégal ; Revue Maggi et la magie du bouillon Kub, Paris, Hoëbeke, 25.
- Rakotoniaina, H. H. (2005). Maîtrise des techniques d'analyses microbiologiques sur les produits charcutiers dans le cadre d'autocontrôle d'une société, rapport de stage technicien. Institut Supérieur Protestant Paul Minault, Antananarivo, 55.
- Studios. (2018). Bouillons de cube. Récupéré sur Santé actu : www.santeactu.com

Cite This Article: Farman Ouattara Ahmed, Kouamé N'zebo Désiré, Koné-Boko Tadiogo Naty Amine, Dadié Thomas, Anin Atchibri Louise (2024). Evaluation of the Microbiological Quality and the Expiration Date of Seasoning Food Broths Based on the Edible Mushroom *Psathyrella tuberculata* and Local Ingredients in Côte d'Ivoire. *EAS J Nutr Food Sci*, *6*(3), 77-85.