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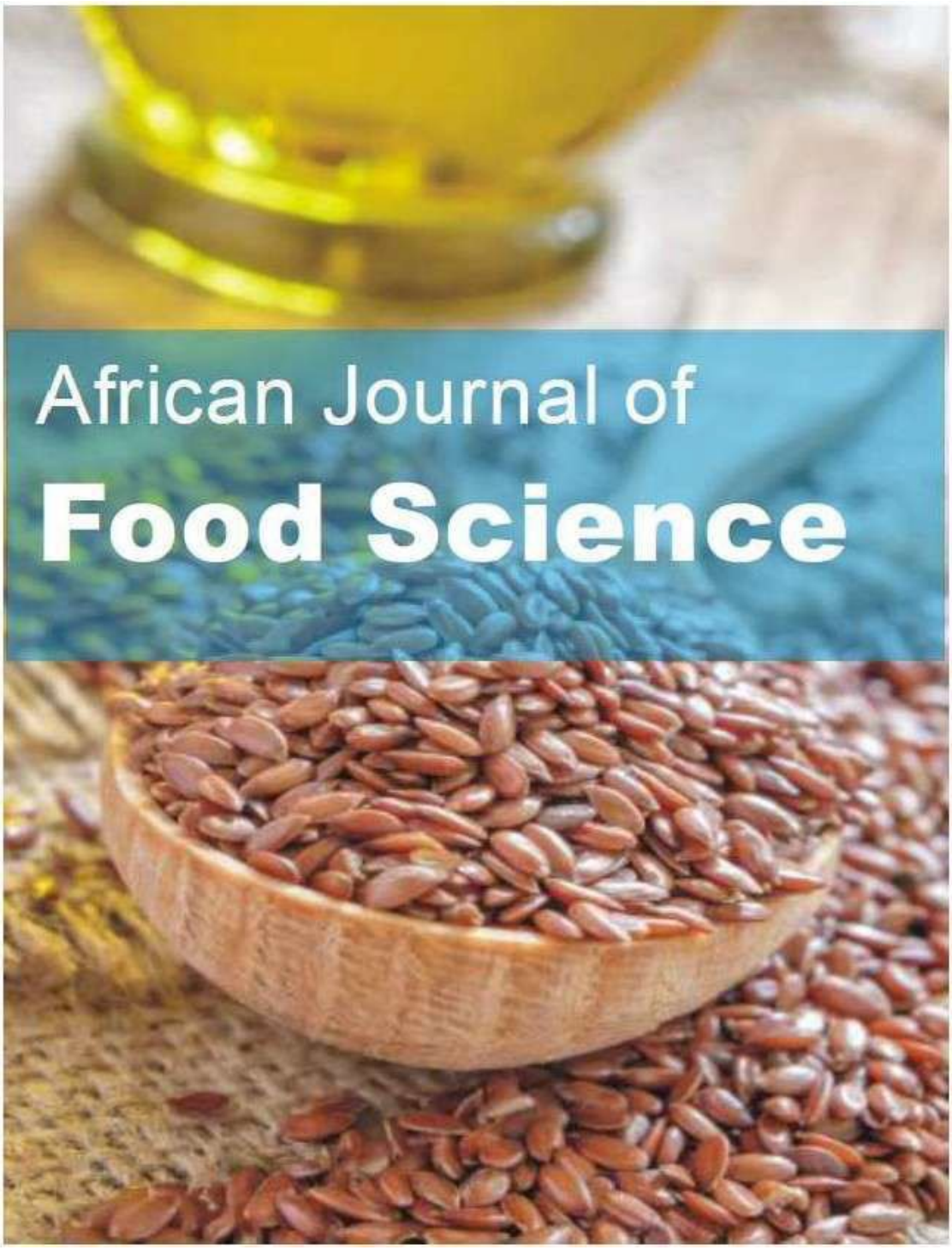

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Table of Content

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African Journal of Food Science

Full Length Research Paper

Isolation and characterization of fungal strains from the seeds of Bambara groundnut (*Vigna subterranea* (L.) Verdcourt) produced in Burkina Faso

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Bambara groundnut is the main food legume after cowpea, especially in rural areas in Burkina Faso. Due to inappropriate production and storage conditions, the seeds are susceptible to contamination by several fungi. Therefore, the present study was conducted to isolate and identify the postharvest fungi associated with Bambara groundnut seeds produced in Burkina Faso. To do this, a total of 99 seed samples were collected in the three agro ecological zones of Burkina Faso. The sanitary analysis of the seeds was carried out using the blotting paper method with slight modifications. The isolation and purification of the isolates was performed on Potato Dextrose Agar medium while their identification was done through macroscopic and microscopic phenotypical characterization using different culture media (Malt Extract Agar and Czapeck Dox Agar) and different identification keys. A total of 421 fungal strains were isolated and the predominant genera were *Aspergillus* belonging to section *flavi* (66.84%), *nigri* (59.04%), and *Macrophomina* (26.49%). This study shows that Bambara groundnut seeds produced in Burkina Faso are contaminated by several fungal strains and that seed infection rates by these fungi differ according to the agro-ecological zones. Post-harvest and storage techniques need to be improved to limit crop losses.

Key words: Agro-ecological zone, Bambara groundnut, seeds, fungal strains, macroscopic and microscopic characteristics, Burkina Faso.

INTRODUCTION

Bambara groundnut is the second most important food legume after cowpeas for numerous populations, especially during the dry season in Burkina Faso (Ouoba et al., 2016). In order to have their crops available throughout the year, rural farmers use several traditional storage techniques. Unfortunately, these technics do not

provide the expected protection to the seeds that are damaged due to several agents including insects and fungi (Ouoba et al., 2016; Kpatinvoh et al., 2017). Fungal contamination of food commodities can cause considerable economic losses through the reduction of their organoleptic and nutritional qualities. According to the Food and Agriculture Organization of the United Nations (FAO), about one quarter of the world's production is annually lost due to uncontrolled fungal growth, representing an economic loss of 5-10% (FAO, 2010). These fungi make food products dangerous because of the production of toxic metabolites such as mycotoxins which are detrimental to human and animal health (Pereira et al., 2013; Olagunju et al., 2018; Okayo et al., 2020). The identification of post-harvest fungi implicated in food spoilage and the production of mycotoxins is an essential step in achieving food security.

During its production, Bambara groundnut can be contaminated by toxinogenic fungi present in the soil (Olagunju et al., 2018), which may lead to accumulation of mycotoxins in the seeds. Studies conducted in South Africa revealed that the seeds were contaminated with fungi and mycotoxin (aflatoxins) at concentrations ranging from 0.01 to 0.1 ppm (Shabangu, 2009; Olagunju et al., 2018). In Burkina Faso, the research on Bambara groundnut has mainly consisted in making agronomic characterizations (Ouedraogo et al., 2008), assessing in a real environment the best technical options to increase its productivity and the pathogens contaminating the vegetative system (Ouoba et al., 2019). However, data on fungal and mycotoxinogenic contamination of the seeds and the risks associated with them remain very limited. The contamination, the development of fungi and the production of mycotoxins can vary depending on the environmental conditions such as temperature, humidity etc. and the storage conditions (Njoroge et al., 2019; Baddi et al., 2021). Burkina Faso is divided isn three agro-climatic zones with different environmental conditions and traditional storage practices that could impact the mycoflora of stored seeds. Therefore, this study was conducted to isolate and characterize the fungal strains associated with the seeds of Bambara addressed two important questions: (i) what are the main fungal strains contaminating the seeds of Bambara groundnut in Burkina Faso? (ii) How do agro-ecological conditions affect the distribution and the infection rates of the fungi?

MATERIALS AND METHODS

Collection sites and seeds sampling

Samples of Bambara groundnut (seeds or pods) were collected in 2020 from 47 sites throughout the three agro-ecological zones (Sahelian, Sudano-Sahelian and Sudanian) of Burkina Faso (Figure 1). Sample locations were randomly selected in each of the three agro-ecological zones from a list initially drawn up according to accessibility, the level of Bambara groundnut production and the need to cover the study area. Accordingly, 9 sites were considered in the Sahelian zone, 25 sites in the Sudano-Sahelian zone, and 13 sites in the Sudanian zone. Seed samples (1,000 to 2,000 g) were randomly collected from farmers in each agro-ecological zone (25, 51 and 23 from the Sahelian, Sudano-Sahelian and Sudanian zones, respectively) making a total of 99 samples. Each sample was placed in a sterile plastic bag and transported immediately to the laboratory. The samples were further divided into two equal parts; the first portion was used for the study while the other was stored (refrigeration) for further analyses.

Seeds health testing

The standard blotter method described by Mathur and Kongsdal (2003) was used with slight modifications, to detect fungi growth from seeds in the presence of humidity. Two hundred (200) untreated seeds from each sample were placed on moistened blotters in Petri dishes at the rate of 10 seeds per dish and incubated for 7 days at 20-25°C under alternating cycles of 12 h near Ultraviolet Light (NUV) and 12 h darkness. Then, the individual seeds were examined for the presence of fungi under a stereo-microscope. A preliminary identification of each fungus developed on the seeds was made by examining the mycelium and/or conidia under a compound microscope and the different strains present on each seed were recorded. Then, the infection rate of each fungus and the percentage of infected samples were computed using the following formulae (Marasas et al., 1988):

$$\text{Infection rate (\%)} = \frac{\text{Number of seeds infected with a fungal strain}}{\text{Total number of seeds}} \times 100 \quad (1)$$

$$\text{Percentage of infected sample} = \frac{\text{Number of samples contaminated with a fungal strain}}{\text{Total number of samples}} \times 100 \quad (2)$$

Isolation and purification of the developed fungal strains

Each visible mycelial growth on the seeds was isolated by collecting a fragment of the mycelium using a sterilized needle that was placed in the center of a Petri dish containing PDA medium for growth. During the collection, precautions were taken to avoid the contact with other neighbouring mycelia from the same seed; then, successive subcultures were performed on PDA medium in order to

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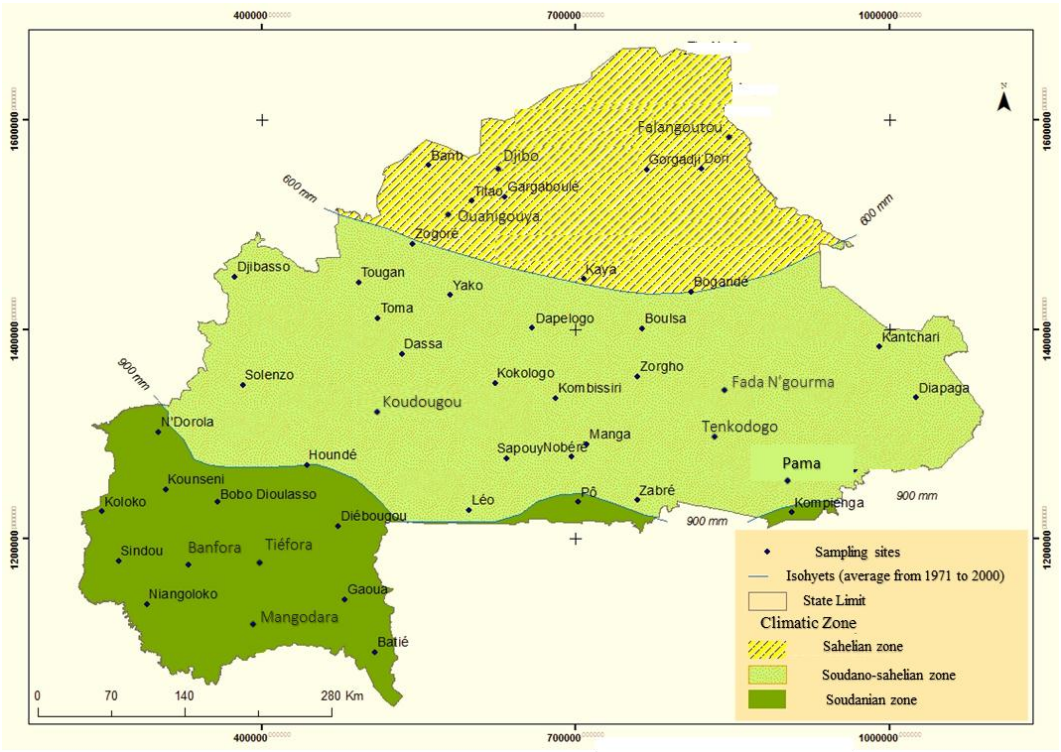


Figure 1. Sites of collection of the Bambara groundnut seeds samples.

purify the isolated fungus. The subculturing was carried out by placing a fragment of the mycelium in the center of a new Petri dish using a sterilized loop. The purified strains obtained were kept on PDA at 4°C.

Identification

Three culture media (potato dextrose agar, malt extract agar and czapeck dox agar) were used to identify the purified strains based on their phenotypical and cultural characteristics. Furthermore, the fragments of the mycelium stained with methylene blue were observed under a microscope. The identification keys used were those described by Samson et al. (1996), Pitt and Hocking (1997), Botton et al. (1999) and Mathur and kongsdal (2003).

Data analyses

Seed-borne fungi of Bambara groundnut and infection rates were determined using equations 1 and 2. The distribution of fungal strains on the samples and between agro-ecological zones was compared by the Analyses of Variance (ANOVA); it was done with the Duncan's Multiple Range (DMR) test at the significance level of $p < 0.05$ using Statistical Analysis System, version 8.

RESULTS

Mycoflora of Bambara groundnut seeds

A total of 421 fungal strains belonging to five genera were detected from the seeds collected in the three agro-ecological zones: *Aspergillus*, *Macrophomina*, *Rhizopus*, *Cladosporium* and *Penicillium* (Table 1). The health testing of the seeds revealed that all of the tested seed samples were infected by at least two fungal strains. The mycoflora can be split into three groups based on the percentage of infected samples: (i) *Aspergillus* belonging to section *flavi* and section *nigri* infected all samples with high infection rates (3.50-100% and 9.00-99%, respectively); (ii) *Macrophomina* and *Rhizopus* were isolated from 92.92 and 91.91% of the samples with infection rates varying from 0.50 to 85% and 1 to 99.50% respectively; (iii) *Cladosporium* and *Penicillium* contaminated 32.32 and 8.08% of the samples with infection rates of 0.5-7.00 and 0.50-54.50%, respectively.

Distribution of fungal strains in the three agro-ecological zones

The results show that all fungal isolates were detected in all of the three agro-ecological zones. *Aspergillus* of the sections *flavi* and *nigri* contained all of the samples regardless of the agro-ecological zone. *Macrophomina phaseolina* and *Rhizopus* spp. were detected in more than 80% of the samples in the three agro-ecological zones (Table 2). *Cladoporium* spp. and *Penicillium* spp. were isolated from less than 40% of the samples. The statistical analysis (ANOVA at 5% level) revealed a positive effect of the agro-ecological zone on the infection

Table 1. Fungal isolates and infection rates of Bambara groundnut seeds.

Fungal isolate	Infected sample (%)	Infection rate (%)
Aspergillus section *flavi*	100.00	3.50-100.00
Aspergillus section *nigri*	100.00	9.00-99.00
Macrophomina	92.92	0.50-85.00
Rhizopus	91.91	1.00-99.50
Cladosporium	32.32	0.5-7.00
Penicillium	8.08	0.5-7.50

Table 2. Distribution of post-harvest fungi of Bambara groundnut in the three agro-ecological zones of Burkina Faso.

Agro-ecological zones	Infection rates (%)					
	Aspergillus* section *flavi	***Aspergillus* section *nigri***	***Macrophomina phaseolina***	***Rhizopus* spp**	***Cladosporium* spp**	***Penicillium* spp**
Sahelian zone	57.96 b	49.34 b	21.68 b	38.60 a	0.36 a	0.18 a
Sudano-sahelian zone	62.48 ab	56.98 a	25.48 b	30.72 a	0.72 a	0.19 a
Sudanian zone	66.84 a	59.97 a	33.98 a	30.217 a	0.3913 a	0.19 a
Average	62.35	55.74	26.49	32.59	0.55	0.19
Agro-ecological zones	**Infected samples (%)**					
	Aspergillus* section *flavi	***Aspergillus* section *nigri***	***Macrophomina phaseolina***	***Rhizopus* spp**	***Cladosporium* spp**	***Penicillium* spp**
Sahelian zone	100.00	100.00	88.00	80.00	36.00	16.00
Sudano-sahelian zone	100.00	100.00	94.11	96.07	37.25	3.92
Sudanian zone	100.00	100.00	95.65	95.65	17.39	8.69

rates of several fungi (Table 2). Three strains including *Aspergillus* belonging to section *flavi* and section *nigri* and *M. phaseolina* presented significant differences in relation to their distribution in the three agro-ecological zones. Indeed, the mean infection rate of *Aspergillus* belonging to section *flavi* was significantly higher in the Sudanian zone (66.84%) than the Sahelian zone (57.96%); regarding the distribution of *Aspergillus* belonging to section *nigri*, the mean infection rate obtained in the Sahelian zone (49.34%) was significantly lower than that of the Sudanian (59.97%) and the Sudano-sahelian (56.98%) zones. As for *M. phaseolina*, its mean infection rate was significantly higher in the Sudanian zone (33.98%) compared to the other zones (25.48 and 21.68% for Sudano-sahelian and sahelian zones respectively). For a given fungus, infection rates with different letters are significantly different.

Characteristics of the isolated strains

The fungal isolates were identified based on the determination of macroscopic (cultural) and microscopic (morphological) characteristics. The macroscopic identification was performed through the observation of the fungal colonies on specific media. The color, form, size, appearance and apical growth of each fungal strain were determined. The microscopic identification was mainly based on the determination of the morphological characteristics of the mycelium (presence/absence of septa, color, differentiation etc.) and spores (shape, color, wall texture etc.). The following fungal strains were identified: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Aspergillus* sp., *Macrophomina phaseolina*, *Rhizopus* sp., *Cladosporium* sp., *Penicillium notatum*, *Penicillium* sp. (Table 3).

DISCUSSION

This study presents the contamination of Bambara groundnut seeds produced in Burkina Faso based on the determination of phenotypical characteristics. The post-harvest mycoflora contains species of the genus *Aspergillus*, *Macrophomina*, *Rhizopus*, *Cladosporium* and *Penicillium*. These fungi are very common in the soil and air through their spores (Abdoullahi et al., 2019) and frequently isolated from poorly stored dried food. Strains of the genus *Aspergillus* were isolated from all of the seed samples in the three agro-climatic zones with very high infection rates (66.84% for section *flavi* and 59.04% for section *nigri*). However, these values were higher in

Table 3. Macroscopic and microscopic characteristics of the isolated strains.

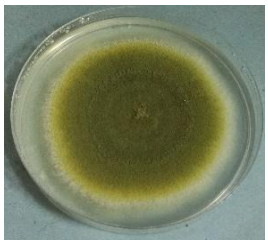
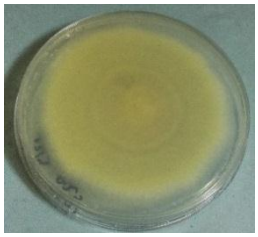
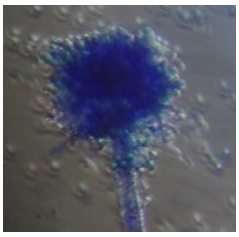
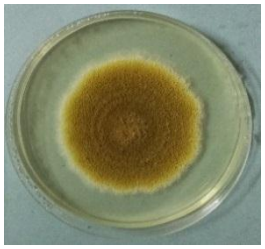
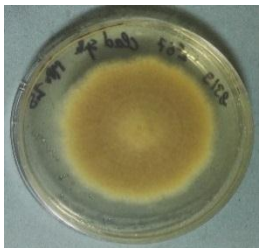
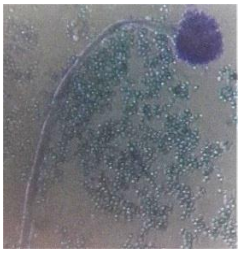
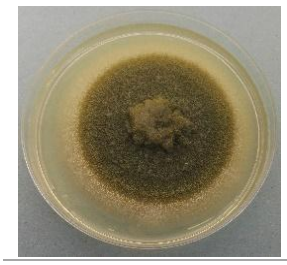
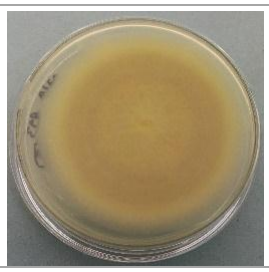
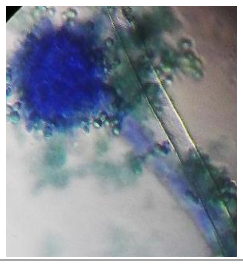
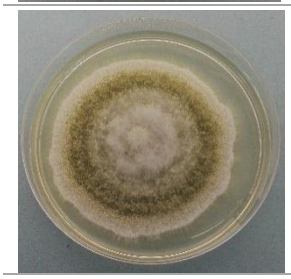
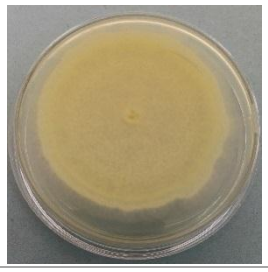
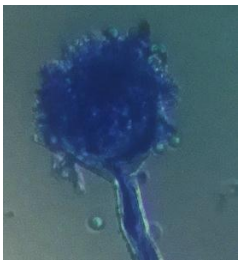
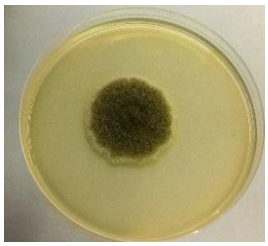
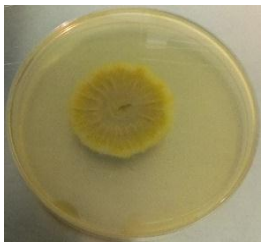
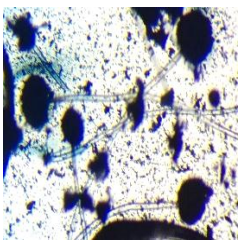
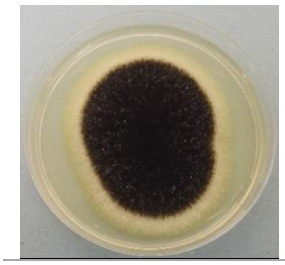
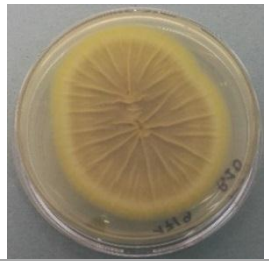
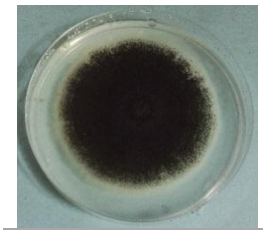
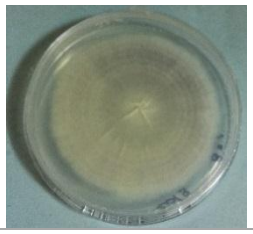
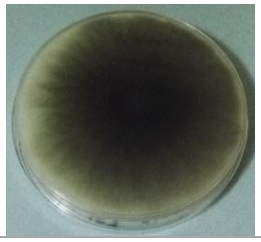
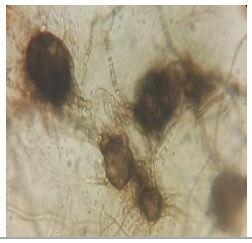
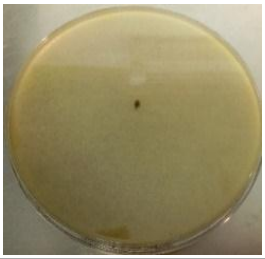
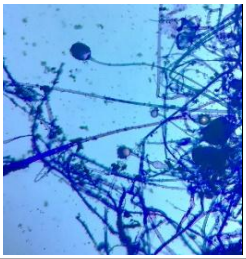
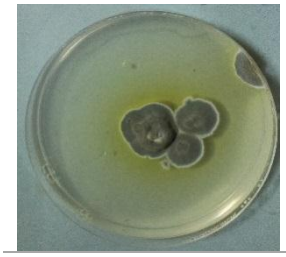
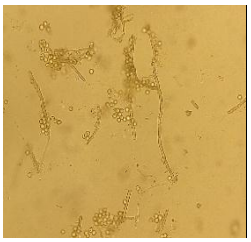
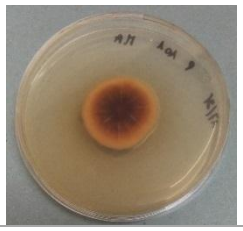
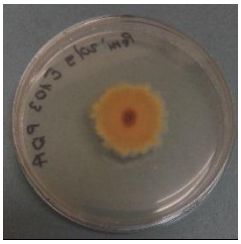
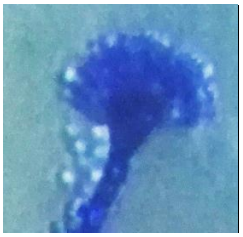
Macroscopic characteristics		Microscopic characteristics	Fungal strains
Recto	Verso		
			*Aspergillus flavus*
			*Aspergillus flavus*
			*Aspergillus parasiticus*
			*Aspergillus fumigatus*
			*Aspergillus* sp
			*Aspergillus niger*

Table 3. Contd.

			*Aspergillus niger*
			*Macrophomina phaseolina*
			*Rhizopus* sp
			*Cladosporium* sp
			*Penicillium notatum*
			*Penicillium* sp

the Sudanian zone than in the Sahelian and Sudano-Sahelian zones. In fact, drying has always been a challenge for farmers. In some areas like the Sudanian zone of Burkina Faso where rainy season can last up to 6 months, frequent rain events can make the weather humid and the sky almost cloudy most of the time. As the sun is the main source of energy used for drying, insufficient light intensity in this area can last the drying time of the pods. As mentioned by Olagunju et al. (2018), insufficiently dried seeds are prone to mould, especially

Aspergillus, and to rot during storage. In general, *Aspergillus* has a wide geographical distribution, but is more often associated with warm climate regions (Hedayati et al., 2007; Perrone et al., 2020). The optimal growing temperature for most *Aspergillus* species is between 25 and 40°C. For this reason, they grow very well in the so-called 'dry' food products like Bambara groundnut.

The predominance of genus *Aspergillus* in the mycoflora of Bambara groundnut seeds has been reported by previous studies (Shabangu, 2009). Our results are similar to those of Olagunju et al. (2018) who isolated the genus predominantly from Bambara groundnut seeds produced in South Africa, with infection rates of 52.5%. The high infection rates obtained from our study could be explained by the common presence of spores of *Aspergillus* in the air, soil and materials used to remove the pods. Hocking (2006) has also reported that fungi of the genus *Aspergillus* easily colonize food crops when storage conditions are not adequate; that could also explain their high presence. In addition, several studies conducted using other food samples including olives (Roussos et al., 2006), stored wheat (Belkacem-Hanfi et al., 2014), coffee bean (Djossou et al., 2015), dried fishes (Abdoullahi et al., 2019), groundnut kernels (Okayo et al., 2020), wheat and sorghum silages (del Palacio and Pan, 2020) and cassava (Ono et al., 2021) have revealed the presence of strains of *Aspergillus*. Besides the presence of these strains, aflatoxins B1, B2, G1, G2 and ochratoxins A were detected in the samples.

Species of the genus *Penicillium* are very common in soils, organic substances and food commodities. These fungi proliferate mainly during storage (Kpatinvoh et al., 2017). *Penicillium* was detected in all the agro-climatic zones with low infection rates. Its presence in Bambara groundnut seeds could be the result of inadequate storage conditions. Its low infection rates could be explained by the significant growth of fungi such as *Aspergillus* sections *flavi* and *nigri*, *Rhizopus* sp. and *M. phaseolina* which rapidly invade the seeds inhibiting the growth of other fungi. Indeed, these invasive fungi are able to colonize the environment in a very short period of time, considerably reducing the space necessary for the growth of other fungal species. Studies have highlighted the presence of *Penicillium* species in the seeds of Bambara groundnut (Olagunju et al., 2018) as well as in cowpea seeds during the storage (Kpatinvoh et al., 2017). *Penicillium* strains are responsible for food spoilage. In addition, several mycotoxins are produced by a variety of *Penicillium* species during food storage including cyclopiazonic acid (*P. chrysogenum*), penicillic acid (*P. cyclopium*), patulin or clavacin (*P. expansum*, *P. griseofulvum*), citrinin (*P. expansum*) and ochratoxin A (*P. verrucosum*) (Pitt, 2000)

Macrophomina conidia are common in soils (Iqbal et al., 2010). Since Bambara groundnut seeds develop underground, the fungus could colonize the pods, leading to infection. Its average infection rate was significantly higher in the Sudanian zone than in the Sahelian and Sudano-sahelian zones. This could be explained by the relatively high rainfall and humidity in this zone compared to the other zones. It can cause seeds to rot during storage, destruction of seedlings during emergence and complete wilting of the plant (Zida et al., 2008; Ouoba et al., 2017). Under high temperatures (30-35°C) and low soil humidity (below 60%), this fungus can cause substantial yield losses in crops such as soybean and sorghum (Kaur et al., 2012).

Fungi of the genus *Rhizopus* were detected in all three climatic zones with approximately equal average infection rates. Since *Rhizopus* is a common air- and soil-borne fungus, it can infect Bambara groundnut seeds during harvest and post-harvest operations. It can rapidly colonize decaying plants and fruits and cause soft rot in some crops with high humidity content such as sweet potato (Pang et al., 2021).

Fungi of the genus *Cladosporium* were also found in all three climatic zones at relatively low infection rates. These low values could be explained by the important competitive development of fungi such as *Aspergillus* section *flavi* and *nigri*, *Rhizopus* spp. and *M. phaseolina*. The genus includes common saprophytic, phytopathogenic and human pathogenic species. With their small conidia, usually formed in branched chains, they are well adapted to easily spread in large numbers over long distances. Some of the species are responsible for food spoilage while others can cause allergy or even plant or animal disease with sometimes significant environmental impact (Bensch et al., 2012). Studies have shown *Cladosporium* to be pathogenic through the appearance of lesions on inoculated leaves of *Vicia faba* (faba bean) (El-Dawy et al., 2021). The existence of the fungus in pods can cause capillary spread of the inner tissue, resulting in the formation of white felted spots extending into the pod cavity (El-Dawy et al., 2021).

The presence of these fungi in the seeds of Bambara groundnut can negatively impact its agricultural production and consumers' health. Thus, the improvement of storage conditions to prevent seeds attacks by insects, rodents, fungi and their metabolites is necessary (Adetunji, 2007). The sorting and drying practices applied by farmers to have good quality seeds with low water content before storage should help reduce the risk of fungal infection of Bambara groundnut as suggested by Bankole and Adebanjo (2003). However, the high infection rates we obtained from the sun-dried seeds suggest the need for more research and investigations for adequate storage.

Conclusion

This study allows the determination of the major fungal strains associated with Bambara groundnut seeds in

Burkina Faso and also highlighted the influence of the three agro-ecological conditions on the distribution of several post-harvest fungi. It showed that Bambara groundnut seeds produced in Burkina Faso are contaminated by a wide range of fungi, including species incriminated in human and plant pathology. The analysis of the infection rates and frequency of occurrence of the fungi showed a clear predominance of strains of the genus *Aspergillus*. These fungi could be the cause of mycotoxin production in the seeds, which are involved in human and animals' diseases. Therefore, further studies should be conducted to test the mycotoxin production potential of these fungal isolates. In addition, a variety of potential pathogenic fungal species have been detected including *Rhizopus* sp. and *M. phaseolina*. The abundance and high frequency of these fungi in the samples can lead to a reduction in the nutritional value and the germinative capacity of the seeds. Inappropriate harvesting, transport, drying and storage techniques could explain the high presence of the fungal strains in the seeds. Therefore, initiatives to improve the storage techniques of Bambara groundnut seeds should be undertaken to limit their contamination and prevent post-harvest losses in order to contribute to food security.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Nutritional and physicochemical characterization of two products (jams and syrup) made from Antananarivo raketa fruits (*Opuntia ficus*-indica)

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The fruits of the prickly pear (raketa), like most fruits, are mainly made up of water, which limits their storage. The transformation of the fruits into jam or syrup allowed a longer preservation. As part of the valorization and conservation of food products in Madagascar, two products (jam and syrup) have been developed from raketa fruits. The present study focused on the nutritional characterization of raketa fruits and processed products, and the determination of some physicochemical parameters of processed products. The results of the analysis showed that the water content of raketa fruits was 87.76%, meaning a dry matter content of 12.24%. The carbohydrate content was 9.88% relative to the crude matter, the rate of reducing sugars was 25%. The other macronutrients were scarcely present, respectively 0.19% for lipids relative to crude matter and 1.31% for proteins. The energy value of fruits was 46.47 Kcal. These fruits had an almost neutral pH (6.35) and a titratable acidity of 1.0%. The processed products had a dry matter content of 65% for jam and 68% for syrup; the increase in these levels is due to the evaporation of water during cooking. For both products, carbohydrates were the most abundant macronutrients with levels around 65%, regarding reducing sugar contents, they were around 27.77%. Fat and proteins were almost negligible. The ash contents were 0.89% (jam) and 0.99% (syrup). The energy values were 256.51 Kcal for the jam and 266.41 Kcal for the syrup. The pH values were 3.86 (jam) and 4.36 (syrup).

Key words: *Opuntia ficus*-indica, prickly pear, syrup, jam, brix, Madagascar.

INTRODUCTION

Fruits are one of the most important crop productions (Nout et al., 2003). The production period of many tropical fruits lasts only a few months or a few weeks during which the producers do not always manage to sell their

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harvests. However, most fruits can only be kept for a few days after harvest, and about 22% of the production is lost (Bantayehu et al., 2019; Umar et al., 2015). Since fruits are easily perishable foodstuffs, effective means must be found to preserve them. The manufacture of drinks, syrup, jams, candied fruits or dried fruits helps to preserve fruit in order to enhance production surpluses and to avoid losses for producers (Rabemananjara, 2003).

According to Montagnac (1960), about fifteen fruits are endemic to Madagascar. According to its agro-climatic potential, Madagascar cultivated most fruit and vegetable species, both tropical and temperate, even if they are almost all introduced. According to Perrier de la Bathie (1921), the first Indonesian immigrants brought coconut, banana, jackfruit and large-fruited lemon; the Arabs (10^{th} and 13^{th} century) brought jujube, mango, lemon, lime, pomegranate, vines, and grapefruit in Madagascar; as for the prickly pear, *Opuntia ficus-indica*, known by the name "raketa », it was introduced in Madagascar by the first European navigators.

About raketa, the regions of the extreme south of Madagascar are the most productive, with an annual production of 1000 tons for the Androy region, but other regions also produce it such as Analamanga (Ramanampamonjy, 1998). The fruits, which are quite rich in sugars, can be preserved after processing and thus be consumed at a different period of their season. Raketa fruits are the staple food of the people of Androy during the lean season. Generally, the population harvested the fruit from December to May, which is why the study of this fruit and its preservation are of great interest in order to extend its availability throughout the year. From a nutritional point of view, the energy value for 100 g of fresh fruit is 50 Kcal, or 209 kJ (Cota-Sánchez, 2016). Raketa fruits are mainly composed of carbohydrates, in the form of sugars, such as glucose, galacturonic acid, glycosides and rhamnosides (Ginestra et al., 2009). The sugar content of fruits is influenced by environmental factors and cultural practices. Generally, fruits from dry areas are sweeter than those from wet or irrigated areas (Bourhia et al., 2020a). On the other hand, their protein and fat contents are very low, around 1% for both. In addition, the prickly pear is a source of fiber. They are also a source of micronutrients like vitamins especially vitamin C, provitamin A and vitamins of the group B; for minerals, there is a dominant quantity of magnesium (85 mg per 100 g), potassium, calcium and a non-negligible quantity of zinc, iron and copper. They also contained many interesting substances such as antioxidants, phenolic compounds and flavonoids (Cota-Sánchez, 2016).

Apart from their use as a food, these fruits are also used in the development of natural antioxidants, and for the manufacture of dyes (Rabemanantsoa, 2010; Bourhia et al., 2020b); in the preparation of alcoholic beverages from the sieved pulp (Espirad, 2002), in the pharmaceutical and cosmetic fields (Yahia and Saenz, 2011); they can also be stored in cans or frozen (Saenz, 2000; Yahia and Saenz, 2011). The fruits are very popular and give rise to several products, some of which are known and others are recently.

The fruits are rich in sugar with high acidity. They are characterized by their high water content, which allowed the rapid development of microorganisms, thus causing the deterioration of all the qualities, both organoleptic and nutritional. To solve this problem, two preservation techniques (jam and syrup) have been developed to further extend the shelf-life of the product. This solution also contributed to the development of the prickly pear processing sector and perfectly met the needs of the local population who needs it during difficult periods (in terms of nutrition). The objective of the present study was to perform a nutritional characterization of raketa fruits and processed products, and to determine some physicochemical parameters of processed products to assess their quality and their property to be preserved for a possible presentation to the consumer. The study joint the research framework of Laboratory of Biochemistry Applied to Food Sciences and Nutrition (LABASAN) (University of Antananarivo) which is interested in the preservation of fruits and vegetables.

MATERIALS AND METHODS

Sampling

The fruits were collected in the Analamanga region (Antananarivo, Madagascar) in March 2016. Sampling is an essential step, as the representativeness of the analysis results depended on it. The coefficient of variation or "CV" determines the homogeneity of the samples, which must not exceed 10% (AFNOR, 1987; Fermanian, 1991). Data processing was performed with XLSTAT 7.0. The fruits were subjected to the following processes before processing: sorting according to their weight, then washing and peeling.

Determination of nutritional compounds and physicochemical properties of fruits and processed products

Water and dry matter content

Moisture content was determined by oven drying method. This involved removing all of the free water from the sample by drying in an oven at 103°C until a constant weight was obtained (Bizot and Marti, 1991). Five grams of the sample were introduced into a capsule, previously tared, and dried at 103 ± 2°C for 24 h in an oven. Weighing preceded by cooling was carried out at regular time intervals (every hour) until the weight was constant, the amount of moisture was calculated from the resulting weight loss (AFNOR, 1993).

Lipid content

The fat content was determined by extracting the lipids using a solvent. For six hours, the crude fat contained in 5 g of pulp was

extracted with hexane using a Soxhlet. The solvent was then removed *in vacuo* (AFNOR, 1993).

Protein content

The method used was the method of Kjeldahl which consists of assaying the nitrogen contained in the sample, making it possible to determine the total protein content using the conversion coefficient 6.25 (Godon and Loisel, 1991). The mineralization of the product led to the transformation of organic nitrogen into mineral nitrogen in the ammoniacal form $(NH_4)_2SO_4$. This reaction took place due to the oxidative action of H_2SO_4 boiling on organic matter in the presence of a catalyst, and the reduction of organic nitrogen to ammoniacal nitrogen. The latter was retained in the acid digestate in the form of sulfate. A quantity of 0.3 g of the sample was introduced into each flask and was added to 10 ml of concentrated H_2SO_4 and a catalyst such as $CuSO_4$, K_2SO_4, which is used to accelerate the mineralization process. The mineralisate, as well as the rinsing water from the flask, were transferred to the still tube for distillation. A 250 ml beaker containing 10ml of 4% boric acid and two drops of Tashiro (FLUKA, ref.36083-250ML) were placed below the distillate discharge pipe. The distillate collected in the mixture of boric acid and Tashiro's reagent was titrated with 0.1 N H_2SO_4 until the color changed into light purple. The volume of 0.1 N H_2SO_4 required for the assay was noted (AFNOR, 1993).

Ash content

In a muffle furnace of a known amount of sample, a quantity of 5 g of pulp was poured into a previously tared incineration pit and then incinerated at 550°C in a muffle furnace for five hours. After cooling, the crucible containing the ashes was weighed and the amount of ash was calculated (AFNOR, 1988).

Total carbohydrate content

The total carbohydrate content of the sample was deduced from the difference between the dry extract content and the sum of the protein, fat and crude ash contents (FAO, 1970; Adrian et al., 1995). The total carbohydrate content was obtained by subtracting from 100 g of dry matter the protein, fat and crude ash content.

GT% = 100% - (L% + P% + C%)

Where: GT% = total carbohydrate content, P% = protein content, L% = fat content, C% = crude ash content.

Reducing sugars content

The amount of reducing sugars needed to reduce an amount of copper dioxide in Fehling's liquor was determined using a solution of the sample juice. This reduction in Fehling's liquor was made visible by the boiling color change of the blue solution which turned into yellow in the presence of potassium ferrocyanide. A defecation of the fruit must be made before the dosage to avoid a false dosage of sugars. 10 g of the fresh sample were crushed, mixed with 50 ml of distilled water, and then stirred for 15 min. The mixture was then filtered with filter paper and the juice obtained was used for the determination of the reducing sugars. A quantity of 0.6 ml of CARREZ I (Roth, Ref. 9944.1) solution was added to this juice. After stirring, 0.6 ml of the CARREZ II (Roth, Ref. 9950.1) solution was poured into it, and the whole was stirred again. To obtain the defecated solution, filtration was performed. The defecated solution was dosed with 10 ml of Fehling's liquor. As soon as the yellow color appeared, the volume of the defecated solution to reduce Fehling's liquor was noted (Andrianoely, 2013).

Determination of total calorific values

The overall energy value is the energy released by the combustion of fat, carbohydrates and proteins in food. It was calculated according to the method of Greenfield and Southgate (1992), using the ATWATER coefficients (1 g of protein provides 4 Kcal, 1 g of fat provides 9 Kcal, 1 g of carbohydrate (glucose) provides 4 Kcal.).

Degree Brix measurement

The degree Brix (°Brix) of the pulp was determined before its transformation into jam and syrup, and the degree Brix of the transformed products was measured at the end of the cooking process. The measurement was done for each production batch to guarantee consumer safety. The fact that the products have reached sufficient dry matter content ensures their good shelf life. The degree Brix of the pulp was measured using an OPL Refractometer (°Brix from zero to 30). A drop of liquid was deposited on the glass of the Refractometer. The degree Brix was read directly at the scale, at the intersection of the scale and the boundary between the light fringe and the dark fringe. For processed products, the appropriate sugar level was determined using a ZEISS brand Refractometer (°Brix 50 to 90).

Pectin content

Pectins are precipitated by 90% alcohol. A precipitate forms after the action of alcohol with the pectins, and its size allows a good estimate of the pectin content. 5 ml of fruit juice were mixed with 10 ml of 90% alcohol. The whole was then stirred and then left to stand for 2 min. The formation of a gel (for fruits rich in pectins) and the formation of flakes with sediment (for fruits poor in pectins) can be observed (Barbara, 2008).

Determination of titratable acidity

Acidic titration is the titration of all the free and attached $H^=$ in acids. Each fruit is characterized by a varying degree of acidity. This acidity is measured by titration with NaOH. It is expressed in milliequivalent (mEq) per 100 g of pulp (Praden, 1985). The principle is based on titration with a sodium hydroxide solution in the presence of phenolphthalein as an appropriate indicator. 20 g of samples were crushed, mixed with 60 ml of distilled water, and then stirred for 15 min. The mixture was then filtered through filter paper and the resulting juice was used for the determination of the titratable acidity. 5 ml of the test sample were taken and added into a beaker, and four drops of phenolphthalein were added to it while stirring. Using a burette, the 0.1 N sodium hydroxide solutions were poured in until a persistent pink color was obtained.

Process applied to jam manufacturing

Table 1 shows the different ratios of pulp and sugar. After preparing the raketa fruits, the granulated sugar was added directly to the fruits in the pot and then put on the fire. The cooking time was approximately 10 min. Cooking was stopped after the jam has

Table 1. Proportion of sugar and pulp.

Number	1	2	3
Sugar level in g	100	60	50
Pulp weight in g	100	100	100

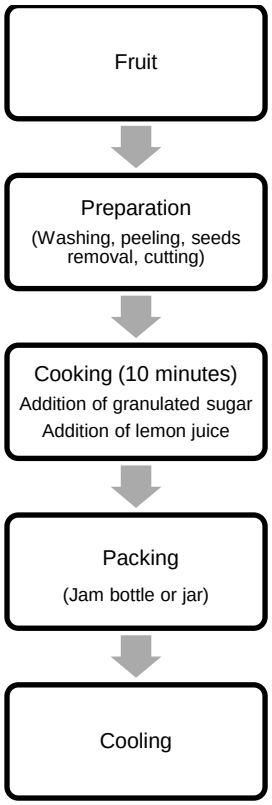

Figure 1. Production flow chart of Jam.

gelled in contact with a cold container. The sugar level was determined using a Refractometer. Acidification was necessary because the pH of 6.35 of the raketa fruit. For this, lemon juice with a pH between 2.2-2.4 was used (five drops) in order to reduce pH of the product at the level recommended by the standard (Codex Alimentarus, 2009) (pH between 2.8 and 3.9 for jam) for a better conservation. Moreover, the addition of acid at high temperature allowed the inversion of sucrose into fructose and glucose. The jam was put in hot jars; it was necessary to keep the heating under the cooking vessel during the filling in order to keep the jam always hot during packaging. Afterwards, the jam was quickly cooled. Indeed, the degradation of pectin continued if the temperature is maintained between 30 and 40 °C which could alter the taste and color of the product (Figure 1).

Process applied to syrup manufacturing

The amount of raketa pulp for making the syrup is 100 g, and the amount of sugar is 150 g. The fruits had undergone the various pretreatments, and then they had undergone an extraction by

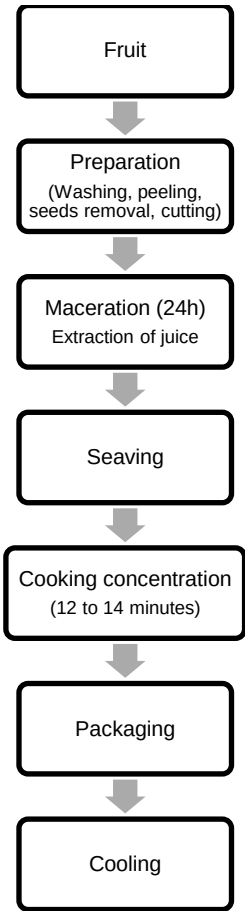

Figure 2. Production flow chart of syrup.

maceration which consists of letting the fruit cut into pieces rest in a solution of sugar for 24 hours. The difference in osmotic pressure caused the juice to exudate. The juice was then separated by sieving. The juice obtained passed through the process of cooking-concentration. This is the second manufacturing parameter to be taken into account, estimated from the amount of sugar used. The purpose was to remove as much water as possible from the juice so as to obtain concentrated syrup. Cooking was carried out immediately after sieving. The cooking time lasted about 12 to 15 min (2 to 5 min after boiling). For acidification, lemon juice (pH 2.2-2.4) has been added to decrease the pH of the product. Similar to the jam manufacturing, at the end of the cooking process, the amount of sugar and dry matter was determined using a Refractometer (Figure 2).

Microbial analysis

The microbiological quality reflects the safety and good hygiene practice during manufacture (AFNOR, 2002). The germs to be counted are the germs for fecal contamination test and the microorganisms that can spoil the products: *Staphylococcus aureus* Coagulase positive, *Escherichia coli*, *Bacillus cereus*, Yeasts, *Salmonella* sp. These analyzes were carried out in the microbiological laboratory of ACSQDA Madagascar. The sample analyzed was taken in accordance with aseptic precautions; all the

sampling materials were sterilized before and during the manipulation. In a sterile bottle, 25 g of the samples (jam and syrup) were suspended in 225 g of buffered peptone water.

The dilution was done according to the methods described in NF V08-010. A cascade dilution was carried out from the stock suspension. 1 ml of the suspension stock was introduced into a sterile tube, then 9 ml of distilled water was added, this is the 10^{-1} dilution. 1 ml of this mixture was then poured into another tube containing 9 ml of diluent which corresponds to the 10^{-2} dilution and so on until the final dilution.

Determination of S. aureus: Coagulase positive

The principle consists of determining the units of colony per gram of sample (cfu/g) after 48 h of incubation at 37°C. *S. aureus* were determined according to the standardized methods ISO 6888-1 and ISO 6888-2. The inoculum of each of the 3 successive dilutions 10^{-1}, 10^{-2} and 10^{-3} were inoculated on the surface in sterile Petri dishes containing beforehand approximately 15 ml of Baird Parker, in which 1 ml of Egg yolk with and 1 ml of Tellurite have been added. After 48 h, *S. aureus* were determined by the presence of Coagulase positive black colonies.

Enumeration of E. coli

The search for *E. coli* is very important from a health point of view. It is one of the germs indicative of fecal contamination in food. It is an enterobacterium isolated by ESCHERICH in 1881 which is a normal saprophyte from the intestinal tract of humans and animals. It can become pathogenic for humans under certain conditions. *E. coli* are among the causative agent of sepsis, diarrhea and also dysentery (Minor and Richard, 1998). The enumeration of all the characteristic colonies was made according to the ISO 16649-1 and ISO 16649-2. The TBX medium is selective for *E. coli* by the presence of dyes which inhibit the growth of all Gram-positive secondary flora. Among the Gram-negative bacteria, only *E. coli* produces blue colonies which are retained. Incubation was carried out at 37°C for 24 h.

Determination of B. cereus

The enumeration of *B. cereus* undertaken for this study was generally identical to the French standard XPV 08-058. The medium used was that proposed by Mossel et al. (1967). 1 ml of stock suspension 10^{-1} was inoculated on the surface of MOSSEL agar medium in which egg yolk had been previously added. Then the dishes were incubated at 37°C for 24 h.

Determination of yeast

The presence of yeast in food is commonly responsible of its deterioration. The methods described in NF V 08-059 were used in this study. The detection and enumeration of the yeasts were carried out on a SABOURAUD medium. 1ml of the inoculum corresponds to the 10^{-1} dilution of the stock suspension was inoculated into a Petri dish containing 15 ml of the medium. Seeding was done in depth and then incubated for 24 h at a temperature of 30°C. All the colonies formed were to be counted.

Determination of Salmonella sp

Enterobacteria isolated by Loeffler (1890), this bacterium is a dangerous pathogenic parasite of the intestines of humans and animals. The determination of Salmonella sp was carried out according to the methods NF EN ISO 6579. Rappaport-Vassiliadis selective medium for salmonella was used for enrichment before the culture. After enrichment, the suspension was plated using a selective medium for Salmonella. The genus Salmonella produces blue-green colonies after incubation at 37°C for 24 h.

Statistical analysis

All experimental treatments were done in triplicate. Data obtained from nutritional and physicochemical analysis were analyzed by Minitab 19.1 and differences between ranges of properties were determined using one way ANOVA at 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

After getting the results of degree brix of all jam products, Treatment 2 (consisting of 60 g of sugar) was the one that was used for the different analyzes. This T2 has degree brix which complies with degree brix standard recommended by Codex Alimentarius (2009). The results of the nutritional and physicochemical analysis of fruits and processed raketa products are recorded in the Table 2.

Nutritional composition

The water content of raketa fruits was high, around 87.76±0.61%. This result is comparable to those of Cota-Sánchez (2016) who found 87.5%. Salim et al. (2009) found a lower value (84.14%); this showed that these fruits are very rich in water. This very high water content of prickly pear fruit is a parameter which reflected the high perishability of this type of fruit and limited its suitability for storage at room temperature (Bouzoubaa et al., 2014). As for the transformation products, the water content was quite low varying around 35%, which allowed better preservation of the products. For jam, this value was close to the water content of fruit jams in general (31.23 to 33.36%) (Mohd Naeem et al., 2015). Moisture content of raketa fruit was significantly higher than derived product ($p<0.05$).

Raketa fruits contained trace amounts of fat (0.19%). Other studies have found similar values (ranging from 0.09 to 0.7%) (Cota-Sánchez, 2016; Salim et al., 2009). They are classified in the category of fruits very low in lipids such as grapes. The exclusive consumption of raketa fruit in the Southeast is inappropriate, as lipids are essential macronutrients in the human diet. In processed products, the fat contents were even significantly lower ($p<0.05$), varying between 0.01 and 0.03 g per 100 g of raw material. This low lipid content of the jam in this study is confirmed by Food Standards Australia New Zealand (FSANZ) (2018), the jams are generally low in fat. The

Table 2. Nutritionals and physicochemical characteristics of resultant Raketa products.

Parameter	Raketa pulp	Jam	Syrup
	Mean (SD)	Mean(SD)	Mean(SD)
Moisture (%)	87.76 ±0.61[a]	35.00±0.89[b]	32.42 ±1.24[b]
Dry matter (%)	12.24 ±0.62[a]	65.00±0.87[b]	67.58 ±1.26[b]
Lipid (%)	0.14 ±0.02[a]	0.03±0.03[b]	0.01 ±0.02[b]
Protein (%)	1.31 ±0.09[a]	0.20±0.02[b]	0.14±0.04[c]
Carbohydrate (%)	9.88 ±0.18[a]	63.91±1.21[b]	66.44±0.33[b]
Ash (%)	1.18 ±0.04[a]	0.86±0.03[b]	0.99 ±0.02[c]
Reducing sugar (%)	25 ±0.11[a]	27.77±0.11[b]	27.80 ±0.16[b]
Degree Brix	13.4±0.21[a]	65±2.03	68±2.25
Titratable acidity (mEq)	1.0 ±0.03[a]	1.5±0.01[b]	1.2 ±0.02[c]
pH	6.35 ±0.02[a]	3.89±0.06[b]	4.36 ±0.04[c]
Total calorific value (Kcal /100 g)	46.47 ±1.39[a]	256.51±1.84[b]	266.41±1.71[c]

Data are mean ± standard deviations of triplicate determinations. Means followed by the same letters in the same column are not significantly different from each other at 5% level of significance (p>0.05).

decrease may be due to the effect of cooking.

Raketa fruits were made up of 1.31±0.09% protein for this study. García et al. (2020) found a slightly higher protein (1.62%). The protein content of fruits is low. It decreased further after heat treatment. Most proteins break down during cooking. This explained the decrease in the protein content of jam and syrup. Mohd Naeem et al. (2015) also claimed the low protein content of fruit jams. This low amount of protein in the products is explained by the weakness of the protein potion of the ingredients used in the manufacture process.

The ash content of the pulp was 1.18±0.04%. This value is close to the result found by Cota-Sánchez (2016), Salim et al. (2009) and Garcia et al. (2020). The ash content of the syrup and the jam were respectively 0.99±0.02% and 0.86±0.03%. The value of the jam here is higher than that found by Mohd Naeem et al. (2015) for jams in general (0.12 to 0.2%). A significant decrease ($p<0.05$) of the amount of ash contents in all the finished products was noted after processing (approximately 0.25%).

Raketa fruits were rich in carbohydrates with a content of 9.88±0.18% of raw matter; they were the most abundant nutrient in these fruits. The total carbohydrate content in processed products was 7 times higher, around 65%. According to Mohd Naeem et al. (2015), jams generally contained a carbohydrate between 65.99 and 67.65%, which is similar to the results obtained in this study. The reducing sugar contents of the two processed products were very similar (around 27.80%), for the fruits it was around 25%. The amount increased significantly after transformation ($p<0.05$). The change of these levels is mainly due to the fact that during cooking, the water evaporated and the dry matter content increased.

The calorific value of the fruits of the prickly pear found in this study was 46.47 kcal per 100 g of fresh material. According to Santé Canada (2008), this value is average compared to other fruits such as avocado and strawberry, whose energy values are respectively 161 Kcal and 27 Kcal per 100 g of fresh material.

Jams are foods with a fairly high energy value, generally between 266 and 274 Kcal per 100 g of product (Mohd Naeem et al., 2015). The raketa jam produced for this work had a calorific value around 256.71 Kcal. Raketa syrup had a fairly high energy value of 266.41 Kcal ($p<0.05$). According to the French legislation of 1997, the calorific value of fruit syrup is between 220 and 350 Kcal. Caloric intake, whether for the fruit pulp of the prickly pear or its derived products, is largely provided by carbohydrates, as fats and proteins provide only a small amount of this energy.

Physicochemical properties

It is important to measure the degree Brix when processing fruit. The degree Brix level of the fruit should be adjusted, because the sugar concentration influenced the taste and texture of the products. The degree Brix designated the rate of soluble dry matter. Raketa Fruits had a Brix level of 13.4±0.21. Generally, the degree Brix of the fruits is between 4 and 15, it varied according to the maturity of the fruits and variety. The riper the fruit, the more the degree Brix increased (Monrose, 2009). Raketa fruits generally have a degree Brix ranging from 10 to 17 (Chougui et al., 2013; Cota-Sánchez, 2016; Garcia et al., 2020). The value found during this study was found within this range. The degrees Brix of the jam produced went from 65 and are consistent with the

Table 3. Microbial analysis (CFU/g).

Germ	*S. aureus*	*E. coli*	*B. cereus*	yeast	*Salmonella* sp.
Jam	$<10^2$	<10	$<10^3$	$<10^3$	ND
Syrup	$<10^2$	<10	$<10^3$	$<10^3$	ND
Standard (CFU/g)	$10^{2\ m}$	10	10^3	10^3	ND/25g
(DSAL, 2018) (CFU/g)	$10^{3\ M}$	10^2	10^5	10^4	

CFU=colony forming unit; ND = Non Determined; x<m = conform; m<x<M = poor; M<x = intolerable.

standard which must be greater than 65, according to Codex Alimentarius (2009). That of the syrup was 68; it complied with the standard established by CTA (1999) which must range between 65 and 70 °Brix. It emerged from these results that the soluble dry matter content of the processed products is 4 times higher than that of the pulp (13.4). Compared to the brix of raketa fruit, those of the two products were significantly different ($p<0.005$). This increase would be due, on the one hand, to the addition of sugar to the mixture and, on the other hand, to the evaporation of water during cooking, resulting in an increase in the sugar concentration in the mixture.

Raketa pulp was relatively low in acid, titratable acidity was 1.0 mEq. Indeed, the pH of prickly pear fruits was close to neutral and is relatively comparable with that of citrus fruits (Kelebek et al., 2008). After processing the fruit, the titrable acidity increased for each product, the addition of citric acid contained in the lemon therefore lowered the pH. Measuring the pH of processed products is essential to guarantee good gelation and consumer safety (Featherstone, 2016). The pH of the jam and syrup were 3.89±0.06 and 4.36 ±0.04 not exceeding 4.5 so the manufacture of jam and syrup made from raketa picked in Antananarivo can be exploited because of their pH which respected preservation standards (between 2.8 and 3.5 for jam; less than 4.5 for syrup) (Codex Alimentarius, 2009; Caetano et al., 2017; Dudez and Broutin, 2001; Martins et al., 2021). To develop a jam, certain basic rules must be respected, in particular the sugar level which must be between 63 and 70 °Brix, sufficient acidity corresponding to a pH not exceeding 4, a sufficient dry matter level at the beginning of the process, but especially the cooking time to avoid the degradation of pectin in order to ensure good gelation. Raketa jam had a sugar degree Brix around 65 which does not exceed the standard (Rahman et al., 2018; Anuar and Salleh, 2019; Kurniawati et al., 2019). Regarding the syrup, the sugar level was 68 °Brix. It is correct, in fact the standard indicated that the sugar level for the syrup must not be less than 65 °Brix and exceed 70 degree Brix (Martine, 1993).

Microbial analysis

Table 3 shows the results of microbial analysis of the two samples (Jam and Syrup). According to the reference (DSAL, 2018), “m” represents satisfactory concentrations of micro-organisms in the samples, and “M” represents unacceptable or intolerable concentrations of micro-organisms with insalubrious or damage conditions. Between the two parameters, the quality is poor. For each germ tested, the value obtained was below the limit established (bellow “m”), and *Salmonella* sp. was not found in the selected samples. The results affirm that the conditions of good hygiene practice and safety have been respected during the manufacturing process and the manipulation before and during the analysis. In terms of microbiology, the quality is acceptable for both products (jam and syrup). The low microbial levels may be the consequence of intense heat application during the jam and syrup manufacturing together with low pH and high sugar content (Alokun-Adesanya, 2019).

Conclusion

Although introduced long ago in Madagascar, raketa (prickly pear) received little interest in the past apart from the consumption of its fruit and use as fodder. Recent research has since demonstrated the various advantages and potential of its now promising transformation with high added value. At the end of this study, we were able to observe that raketa constitutes an important nutritional resource, especially for arid and semi-arid regions. Although it is an incomplete food: poor in proteins and lipids, its richness in carbohydrates, vitamins and water makes it an interesting remedy food. The syrup and jam made from raketa fruits are products with interesting nutritional, organoleptic and physicochemical characteristics which comply with manufacturing standards.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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