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Microbiological Analysis, Minerals, Nutritional Values and Anti-Nutrients of *Tacca leontopetaloïdes* before and after Storage

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Abstract The different species of Taccas are very spread in the Sambirano region, especially the *Tacca leontopetaloides*. Bush populations use "Kabija" starch as a substitute food. Analysis of minerals present as Ca, K, Mg, Na, P were determined. The results of the analysis indicate that the levels in mg / 100mg are 10.19, 45.93, 7.80, 1, 54, 11.99 for samples fresh (FMF) and 33.93, 128.64; 22,90; 4.50; 31,50 for the dry state (FMS) before storage. On the other hand, the mineral values decrease after 6 months of storage, the levels in mg / 100g, either, 5.04; 20.01; 2.99; 0.22; 4.22 for FMF and 13.22; 62; 9.89; 1.56; 13.55 for (FMS). In starch, nutritional values ash, lipids, proteins and carbohydrates are measured in g / 100g rates either, 0.30; 0.14; 0.09; 91.02 for (FMF) and 0.80; 0.15; 0.12; 87.07 for (FMS) before storage; water is present with 7.5% fresh (FMF) and 5.3% dry (FMS). After 6 months of storage, nutrient levels decrease by 0.1 g / 100 g; 0.08 / 100g; 0.05 / 100g; 40 / 100g for (FMF) and 0.5 / 100g; 0.08 / 100g; 0.07 / 100g; 38.89 for (FMS); water also decreased with 4% for (FMF) and 3% for (FMS). But, it is necessary to do the study on anti-nutrients like Polyphenols, Oxalate and Saponins. The levels in starch should be lower than 2.01 mg / 100 g for Phenols, 140 mg / 100 g Oxalates and 60 mg / 100 g Saponins for (FMF) and 3.6 mg / 100 g; 270.8mg / 100g; 23mg / 100g for (FMS). Anti-nutrients levels also decrease after storage. First and foremost, quality control and microbiological analysis before and after storage are very important for food safety.

Keywords *Tacca leontopetaloïdes*, Physico, Chemical Analysis, Microbiological Study

1. Introduction

The part of the Sambirano is in the northern part of Madagascar, Ambanja district in the DIANA region, with rainy climates favorable to the development of *Tacca leontopetaloides*. Previously, the population of the region is limited to harvesting "Kabija" which pushes the wild in the forest. At present, the population begins to cultivate "Kabija" in association with rice on slash. One to several leaves come from the center of the plant with petioles (leaf stems) from 17 to 150cm long. The leaves are large and deeply divided, 30 to 70cm long and up to 12cm wide. The upper side of the leaves has depressed veins while the lower surface is shiny with yellow fat veins. The flowers, in violet greenish clusters, are carried by the upper stems. The plant is usually dormant for part of the year.

Later, the new leaves come from the round of the underground tubers. The tubers are hard as potatoes, with a brown skin and a white interior. The new leaves of "Kabija" develop from the end of October onwards during the rainy season. At the end of 4 months, "Kabija" is harvested even if the stem does not die. One foot of "Kabija" has at least 2 kinds of tubers, one small and the other protruding. The large tuber is hung to produce the starch. The small tubercle ensures the harvest of the following season. Thus, the number of protruding tubers corresponds to the number of seasons of "Kabija". In addition, the tubers of "Kabija" remain dormant for almost 4 months in the soil. It is sold to the market in the form of starch. The starch of "Kabija" or *Tacca leontopetaloïdes* constitutes the main feeding of the populations of the bush in the northern part of Madagascar, in substitution of the rice during the periods of welding and rain. It is sold to the market in the form of starch. Our study is based on the two types of starch: fresh material starch (FMF) and dry matter starch (FMS).

Tacca is a genus of plants of the family Taccaceae according to the classical classification, or of the Dioscoreaceae according to the phylogenetic classification. It comprises about 30 species of monocotyledonous plants. *Tacca leontopetaloides* is a plant of the forests of Madagascar [3, 7, 21, 30, 40].

Our survey is based to the two types of starches: starch of the cool matter (FMF) and the starch of the dry matter

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(FMS).

It is why the main objective of the present work is of the physico-chemical analysis of the two samples and follow-up the microbiological analysis. This survey is divided in three parties. First, bibliographic survey. The second is dedicated to the methods of consistent analysis of results of the physico-chemical parameters before and after the conservation and the survey of microbiological. Third, the discussion and finally the conclusion.

2. Material and Methods

Thus, the number of protuberant tubers corresponds to the number of seasons of" Kabija ". Besides, the tubers of "Kabija" remain nearly sleeping 4 months in soil [32, 48].

2.1. Production of the Starch

2.1.1. Production of the Starch of Fresh Material (FMF) According to the Traditional Method

To extract fresh starch (FMF), the fresh tubers were grated using a stainless steel rape with a mesh size of 0.09 mm^2. The grafts are subjected to a treatment of 3 repetitive dips of 6 hours in tap water for a total duration of 18 hours. Each soak is done with 15 liters of water per 1 kg of grating. After each hour, the settling water is discarded, another quantity of water added for kneading followed by a rest. Then tap water is added for a final mixing followed immediately by pressing and decanting. The starch is collected after the supernatant water, which has become clear after 60 minutes, has been poured. The starch is dried in the sun on an impregnable polyethylene [7-10].

2.1.2. Production of Dry Matter Starch (FMS)

Dry matter starch (FMS) is obtained after three successive soakings of the flour (500 μm) of *Tacc*a *leontopetaloides* for 3 hours. The dilution cycles are 15 liters of water per 700 g of tuber flour. The differences between the two soakings are the rest time after kneading (6 hours for FMF and 3 hours for FMS) and settling time after pressing (1 hour for FMF and 1.50 hours for FMS) [7-10].

2.2. Analyses of the Minerals

The starch is gobbled in an oven to mitten to 550°C to get a white ash that contains the minerals.

The mineral contents Ca, Mg, K and Na are determined by atomic absorption spectrophotometry. After humidification, 5 to 25 ml of concentrated hydrochloric acid is added. The suspension is then boiled, and then filtered. The phosphorus content is determined by colorimetry or by spectrophotometry at 560 nm [9, 15].

2.3. Analysis of the Nutritional Value

- The lipids: the sample is treated by the hexane. Five grams of sample are introduced in cartridges to extraction during six hours. The extract taken is placed in a drying oven at 75°C during one hour until obtaining of a constant mass.
- The raw ashes: the sample of 5 g appropriated is placed in an oven to mitten adjusted to 550°C. The white ashes are weighed after cooling [7, 8, 12].
- The proteins: the raw protein has been determined by the Kjedahl method [7, 10, 35].
- The glucides are measured out by spectrophotometry to 490 nm. The method of Fischer and Stein is applied and she uses the DNS to 540 nm to value the soluble sugars [7-12].

2.4. Dosage of Factors Anti-Nutrients and Toxins in the Flour of "Kabija"

In this method, the last soaking and mixing are used, because they contain the toxic substances and tannins anti-nutrients.

Determination of total phenolic content was based on reaction with Folin Ciocalteu reagent. The blue coloration obtained has a maximum absorption at 725 nm. The tannins were determined using the spectrophotometric method using acidified vanillin and tannic acid as standard (λmax = 500 nm). Total oxalates: The total oxalic acids were evaluated by the potassium permanganate solution titration method (AOAC, 1970). The determination of the saponin content was made using the afrosimetric method based on the formation of stable foams by Koziol saponins [9, 10, 20].

2.5. Microbiological Analysis after 2 Months of Storage of the Starches of "Kabija"

The microbiological quality of the starch of "Kabija" is the subject of an interesting survey after the manufacture and the storage, because the starch is very sensitive to mildew because of the humidity during the drying. The micro-organisms in the starch of "Kabija" preexist in the raw matter before his transformation, but can be brought also accidentally in the starch. The present micro-organisms are harmful micro-organisms constituted by pathogenic germs, of the micro-organisms of change and contamination [2-5].

a) Studied samples

Dry matter starch (FMS)

b) Types of the studied micro-organisms

- flora aerobe total mésophile to 30°C
- Total Coliforms to 30°C
- Escherchia coli - glucuronidase (+) to 37°C
- *Staphylococci coagulases* (+) to 37°C
- Bacteria sulfitoreducteurs to 37°C
- Yeasts and mildews to 25C
- *Salmonella* (3)

c) Mother solution

One takes 10g of "Kabija" and add 90g of water peptone stamped. The whole is ground during 60s in the STOMACHER. After the solution mother is sudden a set of

decimal dilutions

d) Method of calculation

- **sowing in depth** [2-5]

$$N = \frac{\sum a}{V\,(n_1 + 0{,}1\,n2)d} \quad \text{ou} \quad N = \frac{\sum a}{V - n.d}$$

N: Numbers of colonies
∑a: The sum of the Ufc in two dilutions
V: Volume of seeded inoculum
n1: number of first dilution boxes
d: dilution factor corresponds to the 1st dilution
n2: number of second dilution boxes
n: number of box

- **sowing in surfaces for the Staphylococci coagulase (+)** [2-4, 45]

$$N = \frac{\sum a}{V\,.1.1.F}$$

$$a = \frac{b^c}{A^c} + C^c - \frac{b^{nc}}{A^{nc}}\,.\,C^{nc}$$

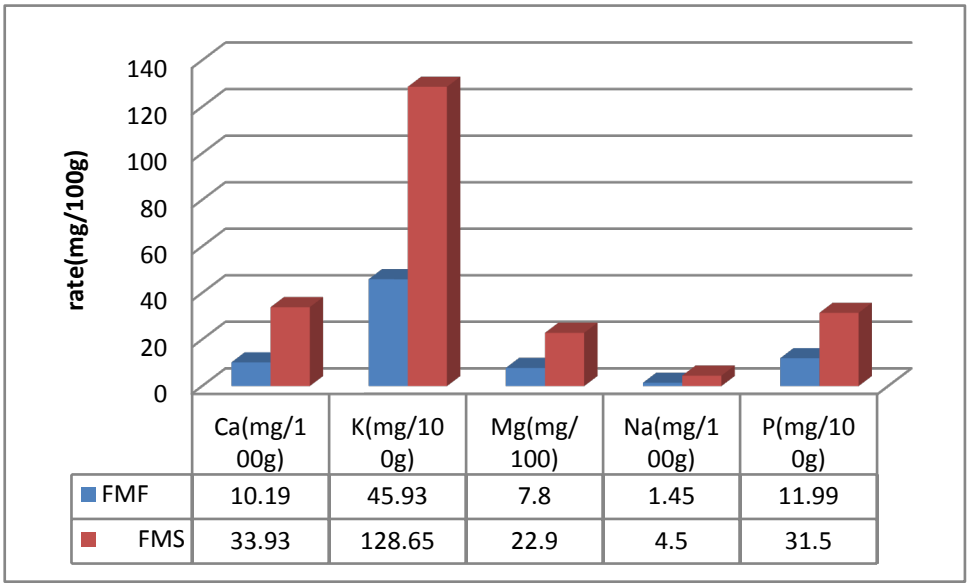
A^c: number of characteristic colonies planted out
A^{nc}: number of colonies non feature planted out
b^c: number of colonies of feature of presumed positive Staphylococci
b^{nc}: number colonies non feature of presumed positive Staphylococci
C^c: number of characteristic colonies of positive Staphylococci presumed for it limps
C^{nc}: does Sum of the columns of Staphylococci to positive coagulase identified in two limp.
F: rate of dilution to the 1st dilution
V: volume spread on every limps

3. Results and Discussions

3.1. Dosage of the Minerals of "Kabija" before the Conservation

The Figure and the Table show the rates of the major minerals in the starch of "Kabija" in the cool state (FMF) and to the dry state (FMS).

Mineral	FMF	FMS
Ca (mg/100g)	10,19	33,93
K (mg/100g)	45,93	128,64
Mg (mg/100g)	7,80	22,90
Na (mg/100g)	1,54	4,50
P (mg/100g)	11,99	31,50

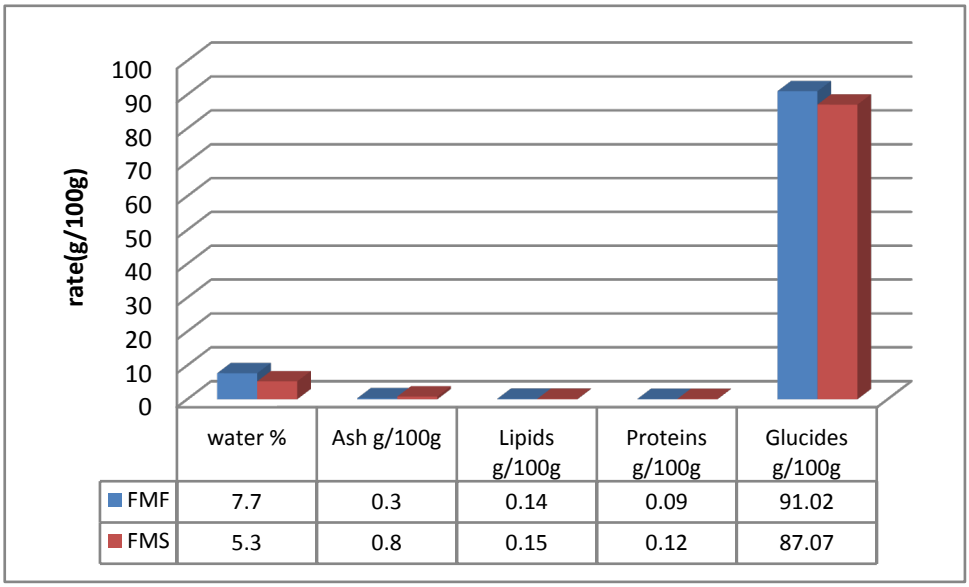


The rates of the minerals are very different in the two samples. The one of the Ca is distinctly more elevated in the FMS that in the FMF. The rate of the 5 elements is more important in FMS that in FMF.

3.2. Nutritional Value of the Tuber of "Kabija" before the Conservation

The picture and the face indicate the rate of the big organic compound in the dry starch (FMS) and cool (FMF).

Parameters	FMF	FMS
Water %	7,7	5,3
Ash g/100g	0,30	0,80
Lipids g/100g	0,14	0,15
Proteins g/100g	0,090	0,12
Glucides g/100g	91,02	87,07



The weak content in water of each of these samples dehydrated constitutes a factor limiting the proliferation of the micro-organisms, food agents of determination of the constituent. She permits conservation easy of flour and the starches of "Kabija" thus.

The content in ash of a food is indicatory of his global content in mineral salts. The starches of the dry matter (FMS) are distinctly richer in ashes. The weak content in ashes of the starches would be due to the washing by the water of soaking.

The content in lipids: the contents in lipids of "Kabija" show particularly weak values. The FMF has a content of 0,14 g g/100 and the FMS a content of 0,15 g g/100. These results show that *Tacca leontopetaloïdes* doesn't constitute a good source lipidique.

The content in proteins of FMF is more or less equal to the one of FMS. Difference doesn't exist practically between the contents in proteins of the 2 samples. Be sides this content in protein is in any case weak.

The rate of sugars: in FMF of "Kabija", he is equal to 91,02 g g/100, while in the FMS he is of 87,07 g g/100. The sugars of the tubers of "Kabija" are essentially composed of starch. Indeed, the digestible starch and the soluble sugars constitute the available sugars. The starches of "Kabija" prove to be of good sources of digestible starch.

3.3. Tenor in Some Factors Anti-Nutrients

The tuber of "Kabija" contains toxic compounds for the man and livestock.

The table shows some of these anti-nutritional factors

Parameters	FMF	FMS
Polyphenols (mg/100g)	2,01	3,6
Oxalate (mg/100g)	140,2	270,8
Saponins (mg/100g)	60,19	23

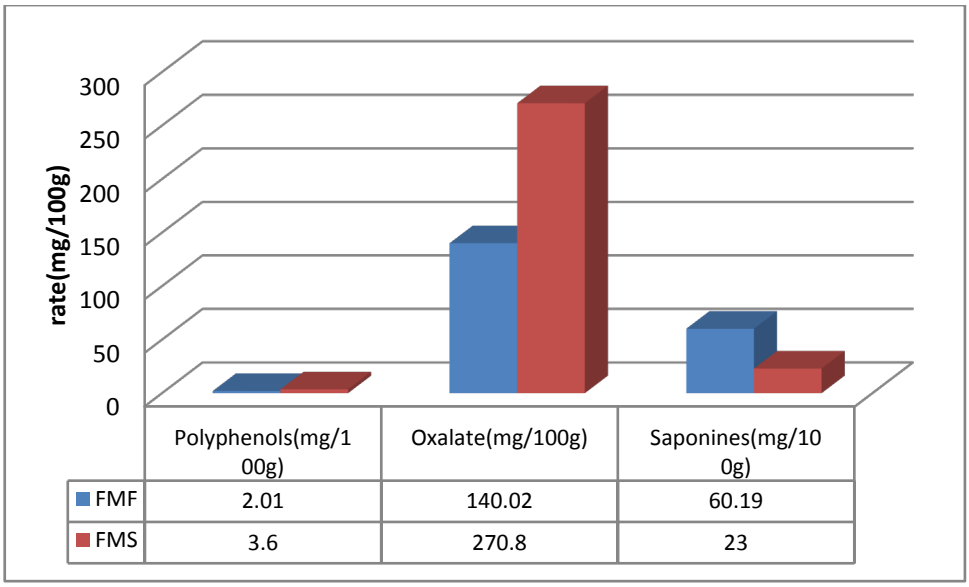


Figure of rate of this factor anti-nutrients

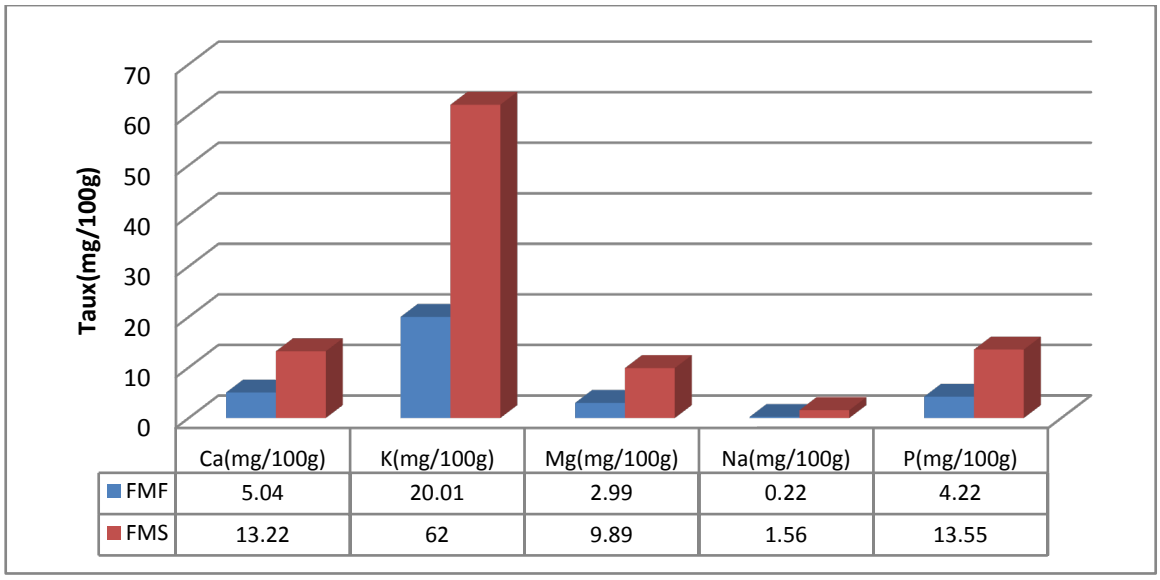
- Difference of the rates of these factors doesn't exist between FMF and FMS. Otherwise, he is shown that concentrations in phenol of 2,7 g mg/100 don't produce any negative effects when these products are consumed by the animals. Soaking is one of the ways to detoxify the tuber.
- Figure shows a clean difference on the other hand between the content in oxalate of FMF (140,2 g mg/100) and the one of the FMS (270,8 g mg/100). However, at the rate of 0,78%, the oxalates binds to the calcium and other minerals as the iron of which they reduce the bioavailability. Therefore, the potential nutritional risk of consumption of *Tacca leontopetaloides* dims, especially as one knows that the raphides responsible for the irritation of the mouth, the throat and the bitterness is in weak percentage (5 to 20%) in relation to the shapes in bows. Be sides, when one does a correct cooking before all consumption, the totality of the oxalate is destroyed [9, 10, 40, 45].
- The Saponins is precursors natural of the corticosteroids. The content in Saponins of the starches is of 60,19 g mg/100 (0,060%) at the FMF and 23 g mg/100 (0,023%) at the FMS.

In fact, the Saponins is innocuous to 1%, they are irritating to 5%. TO a rate 1,5%, their effects are reversible. Otherwise, ingested daily to the quantity of 15 mg, the Saponins is not assimilated but are endowed of a power cholesterol-lowering when they set to cholesterol and the biliary salts. A reduction of the intestinal absorption of cholesterol results from it. Thus, the Saponins have an interest at a time deleterious and beneficial [9, 10, 37, 44].

3.4. Dosage of the Minerals of "Kabija" after 6 Months of Conservation

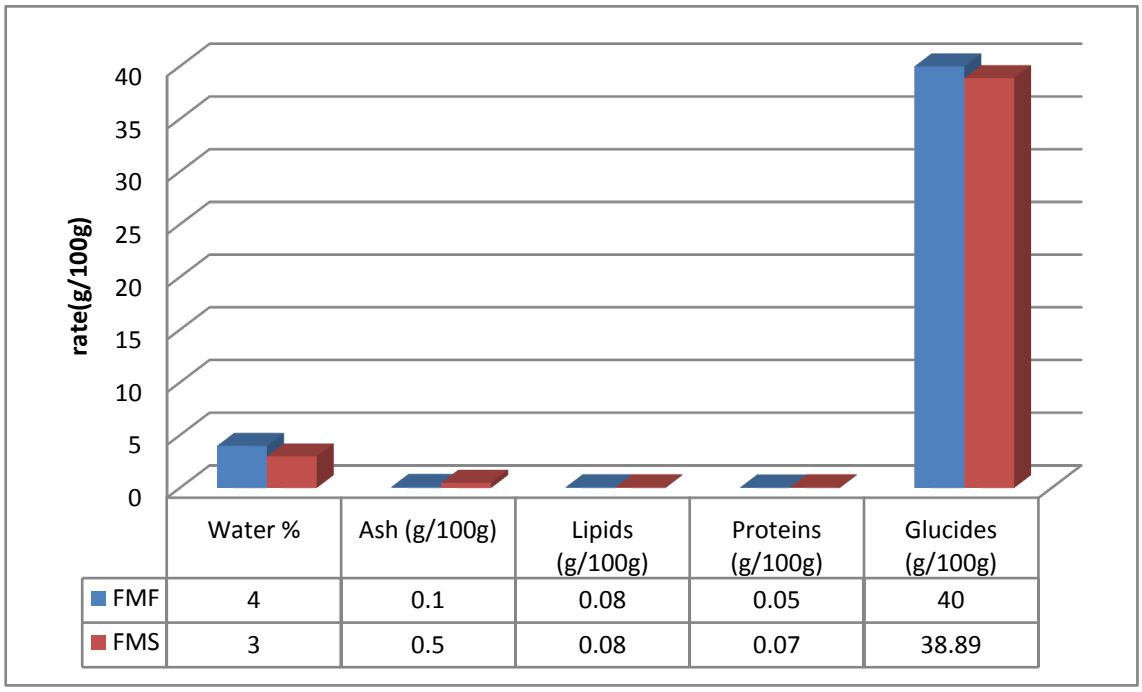
After 6 months of conservation the rates of the minerals decrease the 1/2. The volatile elements as the That, K, P, Mg loses more their values a lot in relation to the other minerals. Therefore the conservation influences the minerals in the "Kabija" to assure that the minerals are efficient during the consumption it is necessary to eat before six months of conservation.

Mineral	FMF	FMS
Ca (mg/100g)	5,04	13,22
K (mg/100g)	20,01	62
Mg (mg/100g)	2,99	9,89
Na (mg/100g)	0,22	1,56
P (mg/100g)	4,22	13,55



3.5. Nutritional Value of the Tuber of "Kabija" after 6 Months of Conservation

Parameters	FMF	FMS
Water %	4	3
Ash (g/100g)	0,1	0,5
Lipids (g/100g)	0,08	0,08
Proteins (g/100g)	0,05	0,07
Glucides (g/100g)	40	38,89



Thus, the rate of proteins decreases as the conservation continues. At the end of 6 months of conservation, "Kabija" loses 1/3 of his proteins.

The lipids are constituent elements of "Kabija". Their presence in "Kabija" is influenced by the length of conservation and the rate of lipids decreases with time.

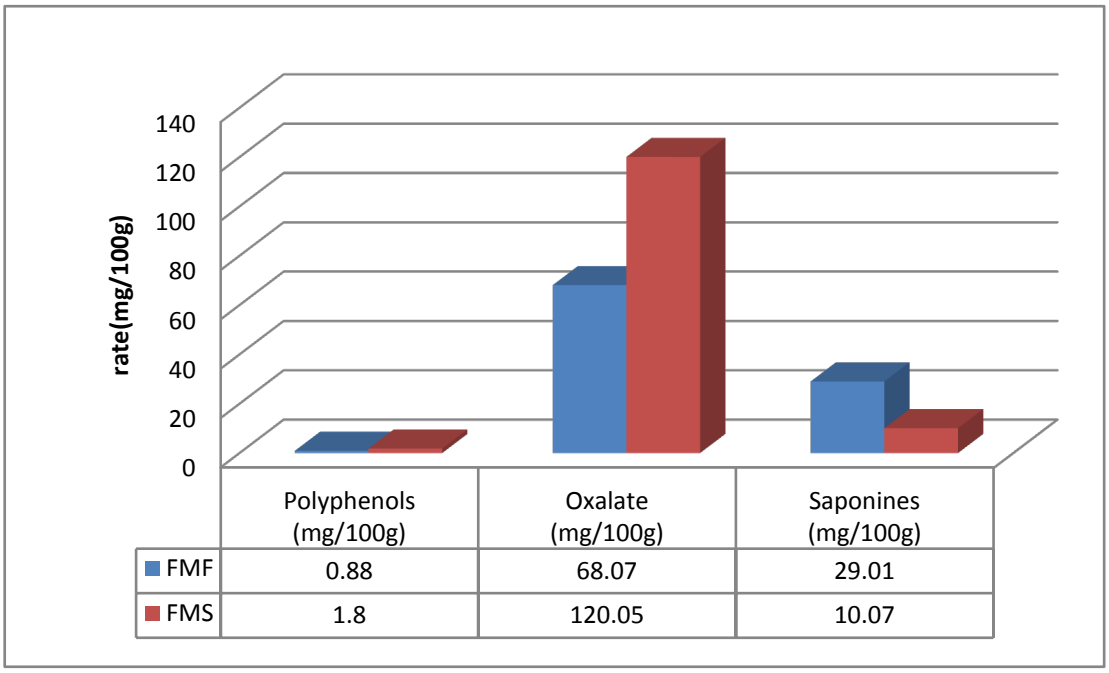
After 6 months of conservation, the rates of the composing lipid decrease of nearly 1 / 3.

The glucides are essential elements in the "Kabija", but after 6 months of conservation the rate loses 3/4 of initial rate nearly.

Concerning the presence of water in the starch of "Kabija", he is nearly decreased half of initial rate after 6 months, if the starch is kept well. But, if the case is contrary that wants to say kept badly, the activity of water increases and provoke the massive presence of mildews inside starch.

3.6. Content in Some Factors Anti-Nutrients after 6 Months of Conservation

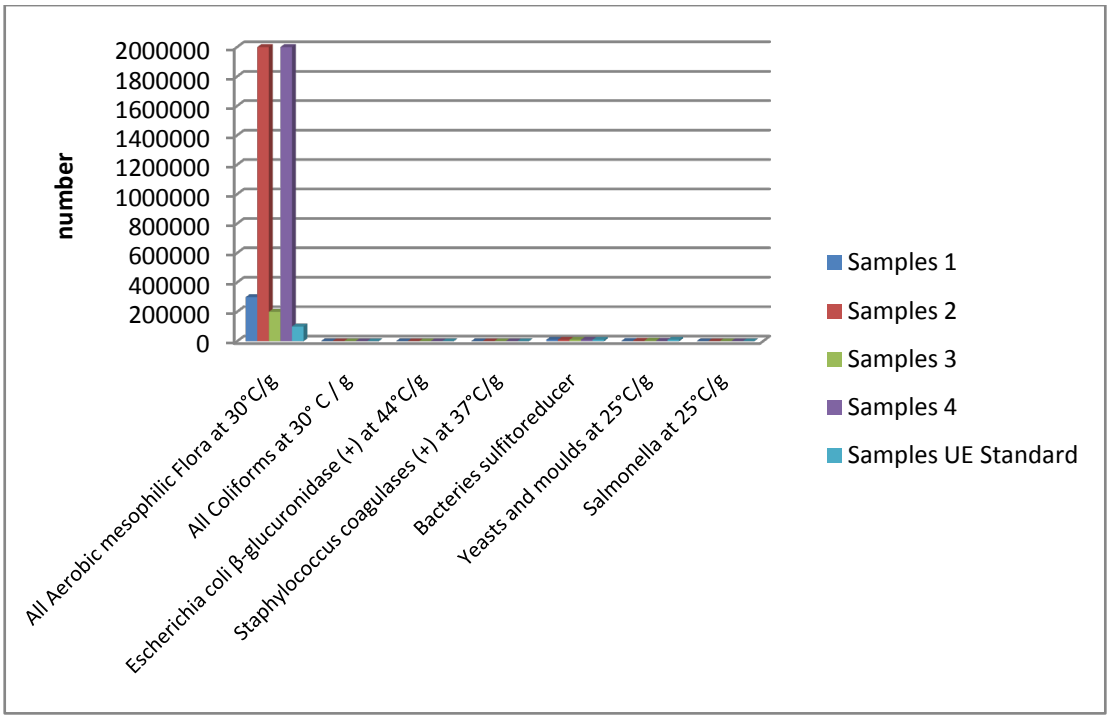
Parameters	FMF	FMS
Polyphenols (mg/100g)	0,88	1,8
Oxalate (mg/100g)	68,07	120,05
Saponins (mg/100g)	29,01	10,7



After 6 months of conservation the rates of Polyphenols, Oxalates and Saponins decrease half. To assure the consumer in the danger to the anti-nutrients, one mean surer are to keep the starch before eating. Therefore, the conservation affects to the rates of the anti-nutrients.

3.7. Quality Microbiological of the Starch of "Kabija" after 6 Months of Conservation

Samples	01	02	03	04	UE standard
All Aerobic mesophilic Flora at 30°C/g	3.10^5	2.10^6	2.10^5	2.10^6	10^5
All Coliforms at 30°C / g	3.10^1	2.10^1	2.10^1	2.10^1	100
Escherichia coli β-glucuronidase (+) at 44°C/g	<10	<10	<10	<10	10
Staphylococcus coagulases (+) at 37°C/g	<100	<100	<100	<100	100
Bacteries sulfitoreducer at 37°C/g	10^4	10^4	10^4	10^4	10^4
Yeasts and moulds at 25°C/g	2.10^3	3.10^3	4.10^3	3.10^3	10^4
Salmonella at 25°C/g	Absence	Absence	Absence	Absence	Absence
Conclusion per sample	unsatisfied	unsatisfied	unsatisfied	unsatisfied	



Conclusion by non-satisfactory non satisfactory non satisfactory sample (m): criterias for dehydrated plant products.

The *Staphylococci* are the microorganisms that produce some neurotoxins. They come from the healthy or sick carriers during the manufacture. The rate is lower to 10 that want to say lower to the norm of criteria of the EU.

The yeasts and mildews are micro-organisms of change. They provoke the pigmentation, the formation of a viscous movie, the sparkling clearing in the products. Mildews entail the changes of the aspect, the texture, the taste, the odor and the deterioration of the quality organoleptic of the product by the production of indole and H_2S. It is very difficult to keep the starch of "Kabija" because of the humidity. He is necessary of good to dry it before the storage or the sale on the market.

The Coliforms are pathogenic bacteria as *Escherchia coli*. They are indicatory of fecal contamination. Is the rate of Coliforms superior to the norm of the EU whereas the one of *Escherchia coli* - *glucuronidase* (+) is lower to this norm. They are brought by wind and the dusts during the drying.

The *Staphylococci coagulases* (+) are micro-organisms few in the middle of production. Washing and the soaking decrease their rate during the manufacture. In this analysis, their rate is lower to 100, norms instituted by the EU.

Anaerobic sulphide-reducing bacteria are commensals of the intestine. They are also found in soil and reduce sulphites to sulphides. They are in highly resistant vegetative or sporulated forms.

The rate of bacteria sulfite-reducer is superior to the norm of the EU. The starch of "Kabija" is exposed directly to the display in the market. These bacteria are very abundant in the starch.

To be to the norm he agrees to respect the fashions of preparation, of the manipulation until the storage. To limit the microbial growth during the production, it is necessary to decrease the content of water non bound or of the free water and to use the H.A.C.C.P method during the preparation and the manufacture of products. So the drying can be considered like a method of conservation. Indeed, he prevents the proliferation of the micro-organisms before they are too numerous.

4. Conclusions

In the starch of "Kabija", the rate of proteins is weaker. However, the glucides, the calcium, potassium is more or less to a rate acceptable in the starch of "Kabija". But, after 6 months of conservation the rates of minerals and anti-nutrients decrease half. The preparation of "Kabija" is very sensitive to the microorganisms. Also, it is necessary to improve the technology of preparation while leaning on the HACCP methodology.

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