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Evaluation of Some Microbiological and Physicochemical Parameters of Peanut Pastes Collected in Some Public Markets in the City of Daloa, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Peanuts, grown on most continents, are processed and consumed in several forms including peanut paste. The latter is subject to contamination of all kinds including pathogenic microorganisms. The general objective of this study is to evaluate the microbiological quality and

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some physicochemical parameters of peanut pastes sold in Daloa. To carry out this study, seventy (70) peanut paste samples were collected. The physicochemical parameters, namely pH, humidity level, dry matter level, titratable acidity, sugar level and ash level, were evaluated using referenced methods. The microbiological parameters were also evaluated taking into account the standards in force. This involves counting and researching spoilage germs and pathogenic germs. The CFU/g load for mesophilic aerobic germs varies from 38.103±1671.35 to 50.103±2378.45CFU/g. For the fungal flora, the loads vary from 1.103± 0.00 to 4.103±2249.89 CFU/g. No *Bacillus cereus* species were found in the samples analyzed. Regarding the search for *Escherichia coli*, the charges vary from 20.103±12 to 46.103±12. For *Staphylococus aureus*, the loads vary from 19.103±14 to 37.103±64. The presence of Salmonella is noted in all the samples from the different markets in this study. As for the phyisco-chemical parameters, the pH values vary from one sample to another with a low average pH of 6.1 ± 0.01 from the Orly market and a high pH of 6.40 ± 0.00 from the Orly market. of the large market. The titratable acidity values are 0.5± 0.00 at the Orly market and 0.93± 0.6 at the highest value at the Lobia market. The humidity level is between 0.94±0.42 and 3.33±3.21. The ash rate values are between 3.33± 0.58 and 8.8± 0.26. The sugar level is between 1.00±0.00 and 2.33±0.58. The presence of pathogenic microorganisms in peanut pastes is a major public health problem.

Keywords: Peanut paste; pathogens; E. coli; B. cereus S. aureus.

1. INTRODUCTION

The peanut (Arachishypogaea), also called peanut or ground pistachio [1], is the twelfth largest crop production in the world. It is cultivated on all continents, in approximately 120 countries over a total area of 24.6 million hectares for a production of 38.2 million tonnes per year [2]. The largest peanut producers are located on the Asian, African and American continents. Thus China, India, Nigeria and the United States occupy the first four places globally [2]. In much of sub-Saharan Africa, peanuts are an important crop for both domestic consumption and marketing [3]. In Ivory Coast, it is produced mainly in the Northern and Central regions [4]. Ivory Coast is the 17th largest producer in the world with production estimated at 88,000 tonnes per year [2]. Peanut exports represent only 6% of global production due to local consumption of the majority. Peanut is an oilseed protein whose seeds contain approximately 45-50% lipids, 25-30% proteins and 5-12% carbohydrates. It is also an important source of sugars, vitamin E and A [5]. Its oil is of better nutritional quality compared to other oilseeds such as soya [6]. Known for its involvement in medicine, peanut is used in the diagnosis of pimples and asthma attacks and also used in traditional African and Indian medicine [7]. The peanut produced around the world is mainly transformed into several derivatives which are used in the composition of food products. Peanut flour and butter are used in the food industry for the manufacture of biscuits and other derived products. Also the inshell peanut is a staple food in some African countries [8]. Peanuts are particularly important for infant health due to their high content of many nutrients essential for growth such as proteins, fats and calcium. Peanuts are consumed in shelled, unshelled, paste and oil form. It is used in the preparation of numerous dishes [9,10]. Nutritionally, peanut paste is rich in fat with improved digestibility of certain nutrients. Despite its multiple nutritional, economic and health actions, peanut paste can occasionally be contaminated by pathogens during the processing of peanut grains [11]. Also the hygiene practices implemented during harvesting, processing and marketing could be the cause of an increase in the number of emerging pathogens. Furthermore, the dangers that represent a risk to the safety of peanut paste can occur from the harvest of the raw material, to consumption, including processing [11]. Among these are enterobacteria, molds and enterococci. Some of these microorganisms are emerging opportunistic pathogens which have particular characteristics and possess remarkable properties of resistance to desiccation and osmotic stress, allowing them longer survival in contaminated peanut paste [12,13]. This situation therefore leads the consumer to be very vigilant regarding the product which could constitute a real danger for the health of populations and the environment. In Ivory Coast and particularly in Daloa, there is no data on the health quality of peanut pastes sold on public markets and particularly those relating to the presence of pathogenic germs. There is also no monitoring providing statistics on illnesses resulting from the consumption of peanut grains in general and

peanut paste in particular contaminated by microorganisms. Indeed, microorganisms can cause more or less serious disorders in humans through or without food. It is also with the aim of overcoming the problems caused by these microorganisms that this scientific investigation was initiated. The general objective of this study is to evaluate the presence of harmful microorganisms and to characterize some physicochemical parameters in peanut pastes sold in Daloa.

2. MATERIALS AND METHODS

2.1 Sampling

The biological material used to carry out this study consists of peanut pastes sold in the public markets of the city of Daloa. All samples were collected by purchase. A total of fifteen (70) peanut paste samples were collected to carry out the work. Ten samples of peanut paste were taken per market. These are the Abattoir Market, Grand Marché, Tazibouo Market, Kennedy Market, Soleil Market, Lobia Market and Orly Market. They were transported in a cooler containing ice to the laboratory to carry out physicochemical and microbiological analyses.

2.1.1 Physicochemical analyzes

2.1.1.1 Determination of dry matter

The dry matter is determined on a 10 g sample of peanut paste by drying in an oven at a temperature of 105°C until a constant weight is obtained [14].

2.1.1.2 Determination of ashes

The ash rate was determined by the gravimetric method on 10 g of sample introduced into a crucible of known weight, then incinerated in a muffle furnace at 550°C for 24 hours. After cooling, the crucible containing the ashes was weighed. The ash rate is obtained according to the following formula [15]:

$$\text{Ashes (\%)} = \frac{m_2 - m_0}{m_1} \times 100$$

Where,

m 0: mass of the empty crucible (in grams)
m 1: test portion (in grams)
m 2: mass of the crucible containing the ashes (in grams)

2.1.1.3 Dosage of titratable acidity

The titratable acidity rate is determined according to the method proposed by AOAC [15]. Ten (10) g of sample are ground in a porcelain mortar then mixed with 30 ml of distilled water. Ten (10) ml of the suspension obtained after this mixture are taken and one to two drops of phenophthalein are added to it. The dosage is carried out immediately after preparation of the suspension with a 0.1N sodium hydroxide solution until it turns pale pink. Two tests are carried out and the average of the tests is considered. The titratable acidity rate is determined by the following expression:

$$\text{Acid levels} = \frac{\text{Vol(NaOH)} \times \text{N(NaOH)} \times 0{,}09}{\text{Test portion}} \times 100$$

Where,

Vol (NaOH) = Volume of NaOH (in ml) used for the dosage
N (NaOH) = Normality of the NaOH solution
Test portion = 10 ml

2.1.1.4 Determination of pH

the pH of a solution is the decimal cologarithm of the hydrogen ion activity of the solution; it is expressed in pH units. In this study, PH was determined according to the potentiometric method using the electrode of a PH meter (WTW PH 302) [15].

2.1.1.5 Determination of humidity level

Moisture content is generally determined using a thermogravimetric approach, i.e. loss through drying. At this level, the sample is heated and the weight loss due to moisture evaporation is recorded. The drying oven combined with a balance and desiccation are among the most common techniques used to analyze humidity levels. The moisture content of the peanut paste was determined by drying in an isothermal oven at 105 ± 5 °C to constant mass [15] which was expressed as a mass percentage. A glass crucible was dried in an oven at 105±5 for 15 min then cooled in a desiccator for 45 min and weighed (m0). The crucible containing 10 g of paste was weighed again (m1) and dried in an oven at 105 ± 5 °C until a constant mass was obtained (24 ± 1 h). After cooling for 45 min in a desiccator, the crucible containing the dried sample was weighed (m2). The measurements were carried out in duplicate. The water content

or humidity level of the dough was calculated using the formula below

$$T_{H_2O} = \frac{m_1 - m_2}{m_1 - m_0} \times 100$$

Where,

TH2O = Humidity rate,
m0 = empty mass of the glass crucible,
m1 = total mass of the sample + the glass crucible,
m2 = total mass of the dry sample + the glass crucible.

2.1.1.5 Sugar level

The sugar level is measured using a refractometer. A quantity of 10 g of the sample was taken using a balance then 90 mL of distilled water was added. The solution obtained is put in a centrifuge at 4000 rpm. After 12 min, the tubes are removed from the centrifuge. Approximately 0.1 mL of the supernatant is taken and then introduced into the refractometer for observation in daylight.

2.1.2 Microbiological analysis

2.1.2.1 Preparation of culture media

The different media used to carry out the work were prepared according to the manufacturer's recommendations mentioned on the different boxes. These are the Eau Tamponnée broths used in the enrichment and pre-enrichment phases and the Rappaport broth from Vassiliadis used for selective enrichment for the search for *Salmonella*. There are also agars such as Plate Count Agar (PCA) used for the enumeration of mesophilic aerobic germs, Sabouraud Chloramphenicol agar used for the enumeration of yeasts and molds, Baird Parker agar used for research and enumeration of *Staphylococcus aureus*, Rapid *E. coli 2* agar used for the detection and enumeration of *Escherichia coli*, Hektoen agar is used for the isolation of Salmonella and Mossel agar used for the detection and enumeration of *Bacillus cereus.*

2.1.2.2 Preparation of the stock suspension and decimal dilutions

Twenty-five grams of the peanut paste are weighed aseptically using a balance in an Erlenmeyer flask. A volume of 225 ml of buffered peptone water is added. The mixture was homogenized for 2 min to obtain the mother suspension corresponding to the 100 dilution. This solution is left to stand for 30 min at laboratory temperature, in order to dissolve and allow revivification of the microorganisms. From this suspension, a series of decimal dilutions is then carried out. Decimal dilution consists of reducing the density of the peanut paste in microorganisms, first to 1/10 then to 1/100 and so on until reducing the microbial concentration of the mother suspension to the factor of 10^n . A quantity of 1 mL of stock suspension is taken and then introduced into the first test tube containing nine (9) mL of sterile tryptone salt broth previously prepared. Then another quantity of 1 mL is taken again from the first tube and introduced into a second tube also containing 9 mL of sterile tryptone salt broth. This operation continues until the desired dilution is obtained [16].

2.1.2.3 Seeding and incubation

One milliliter of each dilution obtained is introduced into the Petri dishes. A quantity of 20 mL of previously prepared medium is poured into the Petri dish. The whole thing is well homogenized. The inoculated plates are left on the bench for the agar to solidify. The plates thus solidified are incubated at 25 °C for 7 days for the counts of yeasts and molds on Sabouraud with Chloramphenicol, at 30 °C for 72 hours for the mesophilic aerobic germs on Plate count Agar. A quantity of 0.1 mL of each decimal dilution concerned is placed in a Petri dish containing 20 mL of agar previously prepared and poured. Then the 0.1 mL are spread on the surface of the agar using a sterile spreader. The inoculated plates are incubated at 45°C for 24 hours for the detection and enumeration of *E. coli* on Rapid *E. coli* 2 medium and at 37°C for 24 to 48 hours for the detection and enumeration of *Staphylococcus aureus* on Baird Parker and at 30°C for 24 hours for the research and enumeration of Bacillus cereus spores after treatment at 80°C/10 min on Mossel medium.

2.1.2.4 Testing for Salmonella

The search for Salmonella is carried out in 4 steps according to the standard [17]. However, during this work, 3 steps were carried out. This is Pre-enrichment which consists of diluting 25 g of sample to be analyzed in 225 mL of a diluent which is Buffered Peptone Water. The suspension obtained is left for approximately 30 min on the bench then incubated at 37°C/24

hours. Selective enrichment which consists of introducing 0.1 mL of suspension after 24 hours of incubation into 10 mL of sterile Vassiliadis Rappapport broth previously prepared and poured into a tube. The inoculated tubes are incubated at 41°C/18 to 24 hours. Isolation consists of inoculation by streak on the previously prepared Hektoen medium and poured onto a Petri dish at a rate of 20 mL is carried out using the Rappapport broth from Vassiliadis. The inoculated plates are incubated at 37°C/24 hours. This last step consists of Reading. Colonies with black centers are taken into account.

2.1.3 Expression of enumeration results

The following formula was used to calculate the average count of microorganisms in cfu/g [18].

$$N = \frac{\sum C}{V \times (n_1 + 0{,}1 \times n_2) \times d}$$

Where n1 is the number of plates from the very first dilution at which the colonies could be counted, n2 is the number of plates from the dilution which precedes the very first dilution at which the colonies could be counted, d is the rate of dilution of the first dilution at which the colonies could be counted. N is the average mushroom count in CFU/g or CFU/mL. ∑ C is the sum of colonies meeting the identification criteria on the boxes retained, V is the volume of the inoculum collected.

2.1.4 Statistical analysis

Excel software was used to calculate the different average loads. The results were subjected to analyzes of variance (ANOVA) carried out with Stastica 7.0 software in order to compare the means.

3. RESULTS

3.1 Monitoring of Physicochemical Parameters of Peanut Pastes

3.1.1 pH and titratable acidity

The determination of the pH of the different peanut paste samples made it possible to find the values which are recorded in the table below. They vary from one sample to another with a low average pH of 6.1 ± 0.01 from the Orly market and a high pH of 6.40 ± 0.00 from the large market. Likewise, the titratable acidity values are 0.5± 0.00 at the Orly market and 0.93± 0.6 at the highest value at the Lobia market (Table 1).

3.1.2 Humidity level

The average moisture content of peanut paste varies from one market to another. This rate is between 0.94±0.42 and 3.33±3.21. The highest humidity rate was 3.33 ± 3.21 came from the Lobia Market while the lowest rate came from the Abattoir Market with a value of 0.94 ± 0.42 (Table 1).

3.1.3 Ash rate and sugar rate

The different ash rate values vary from one sample to another. All values are between 3.33± 0.58 which is the lowest at the Orly market and 8.8± 0.26 the highest value at the Tazibouo market. The average sugar level of peanut paste varies from one market to another. This rate is between 1.00±0.00 value from the Orly market and 2.33±0.58 value from the Kennedy market (Table 1).

Table 1. Average physicochemical composition of peanut pastes from different markets

Origin of samples	Physico-chemical parameters analyzed					
	Titratable acidity	pH	Humidity level	Dry matter rate	Ash rate	Sugar level
1. Big Market	0,67± 0,06	6,42 ± 0,00	1,40±1,4	98,60±1,4	07,00±0,1	1.67±0.58
2. Orly Market	0,50± 0,00	6,11 ± 0,01	1,40±1,4	98.60±1,4	3,33±0,58	1,00±0.00
3.Slaughter house Market	0,83± 0,06	6,40 ± 0,02	0,94±0,42	99,06±0,41	5,87±0,81	1.67±0.58
4. Kennedy Market	0,63± 0,06	6,26± 0,01	2,47±1,55	97,53±1,55	6,67±0,58	2.33±0.58
5. Lobia Market	0,93± 0,06	6,33± 0,02	3,33±3,21	96,67±3,21	7,67±0,76	1.67±0.58
6. Tazibouo Market	0,53± 0,06	6,28 ± 0,03	1,20±0,6	98,80±0,6	8,80±0,26	1.67±0.58
7. Sun Market	0,69±0,18	6,30 ± 0,12	1,65±0,95	98,34±0,95	6,53±0,50	1.54±0.46

3.2 Monitoring of Microbiological Parameters of Peanut Pastes

3.2.1 Flora of alteration and contamination

Different microbial flora were found in samples of peanut pastes sold in different markets. These are spoilage microflora and certain flora suggestive of a deficit in good hygiene practices. These are fungal flora and total flora. All samples from different markets are heavily contaminated by these different microflorae. The CFU/g load for mesophilic aerobic germs varies from 38.10^3±1671.35 to 50.10^3±2378.45CFU/g. For the fungal flora, the loads vary from 1.10^3± 0.00 to 4.10^3±2249.89 CFU/g. In addition, all the loads of these flora in CFU/g were all above the microbiological quality standards (Table 2).

3.2.2 Potentially pathogenic bacterial species

The peanut pastes analyzed contain potentially pathogenic bacterial species including *Escherichia coli* and *Staphylococcus aureus.* It should be noted at the same time, the presence of these two bacterial species in the majority of samples with often very high loads. These charges were also higher than the criteria prescribed by the reference standards. No *Bacillus cereus* species were found in the samples analyzed. For *Escherichia coli*, the loads vary from 20.10^3±12 to 46.10^3±12. For *Staphylococus aureus*, the loads vary from 19.10^3±14 to 37.10^3±64 (Table 2).

3.2.3 Testing for Salmonella in Bissap juices

It should be noted that Salmonella was present in the samples analyzed regardless of the peanut paste samples from the city's different markets. Furthermore, the frequencies of Salmonella presence vary from one market to another with 70 and 80% observed respectively at the Marché du Soleil and the Grand Marché (Table 3).

4. DISCUSSION

The physicochemical characteristics of the different peanut pastes analyzed vary. Furthermore, the pH of its samples is acidic and varies from 6.11 to 6.42. The measured titratable acidity content varies from 0.43 to 0.93meq/100g, also defining the acidity of peanut pastes. The values of titratable acidity and

Table 2. Average loads of samples from the different Daloa markets

Origin of samples	Yeasts and Molds	Aerobic Mesophilic Germs	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus cereus*
1.Big Market	1.10^3± 00^a	49.10^3±25^a	46.10^3±12^b	33.10^3±12^b	00±00^a
2. Orly Market	2.10^3±15^a	50.10^3±23^a	33.10^3±18^b	33.10^3±16^b	00±00^a
3.Slaughterhouse Market	3.10^3±64^b	44.10^3±60^a	28.10^3±57^a	19.10^3±14^a	00±00^a
4. Kennedy Market	1.10^3±64^b	38.10^3±16^a	20.10^3±12^a	22.10^3±12^a	00±00^a
5. Lobia Market	1.10^3±64^b	43.10^3±79^a	24.10^3±13^a	37.10^3±64^b	00±00^a
6. Tazibouo Market	3.10^3±77^b	47.10^3±28^a	40.10^3±64^b	23.10^3±10^a	00±00^a
7. Sun Market	4.10^3±22^b	48.10^3±12^a	30.10^3±77^a	34.10^3±64^b	00±00^a
Microbiological criteria	10^3CFU/g	10^6 CFU/g	10 CFU/g	10^3 CFU/g	10^3 CFU/g

Loads bearing the same letters in the same column are not significantly different at the 5% threshold (P<0.05)

Table 3. Search for the Salmonella genus in the peanut paste samples analyzed

Origin of samples	Total number of samples	Number of samples/Frequency (%)	
		Positifs	Négatifs
1.Big Market	10	8 (80 %)	2 (20 %)
2. Orly Market	10	6 (60 %)	4 (40 %)
3.Slaughterhouse Market	10	6 (60 %)	4 (40 %)
4. Kennedy Market	10	4 (40 %)	6 (60 %)
5. Lobia Market	10	4 (40 %)	6 (60 %)
6. Tazibouo Market	10	3 (30 %)	7 (70 %)
7. Sun Market	10	7 (70 %)	3 (30 %)
Total	70	38 (54,29 %)	32 (45,71 %)

determined pH show that the peanut pastes analyzed are not significantly different. This is explained by the fact that the peanut grains transformed into pastes contain the same compounds (acid) therefore the different manufacturing processes have not had an impact on the acidity of these. This result is consistent with that of Soro et al. [19], which states that doughs which have an acidic pH are better preserved against attacks by microorganisms so these doughs could be preserved for a long time without risk of microbial spoilage.

This low humidity level is due to the heat accumulated in the grains. In fact, the grains have been previously roasted and spread, when released, the heat will reduce the quantity of water, hence the low humidity level. Such a result could promote good conservation of peanut pastes for a reasonable period of time without risk of microbial proliferation. The water content of pasta is an important parameter which must be 15.5% [20] for the pasta to store properly. The samples taken from the different study sites present acceptable humidity (~15.5%). No sample has a value outside the given standards. According to Tchekessi et al. [21], the determination of the water content is important, since it conditions the implementation of technological tests, such as bread-making. The humidity levels of our study are respectively 1.65±0.95, 3.53±2.28 and 1.61±0.64 for the peanut pastes coming from the slaughterhouse market 2, the large market and Orly market. According to Tchekessi et al. [21] fresh peanut grains have a water content of 30 to 40% cannot be stored and would cause irreversible biological damage. Drying has the effect of quickly lowering the humidity level to around 15% then gradually to 8-10%.

The hydrogen potentials (pH) of the different peanut pastes analyzed are acidic and vary between 6.11 and 6.42. These results are significantly similar to those of cereal flours in other studies [21]. It appears that the pH of the paste may drop after one month of storage. This is due either to the continuity of the amylase activity of the amylase residues still active in the doughs, or to the oxidation of fatty acids or to be attributable to microbial enzymatic activities [22]. Furthermore, according to Soro et al. [19], doughs which have an acidic pH are better preserved against attacks by microorganisms.

The dry matter levels determined in this study are high and therefore indicate a low moisture content. In fact, the grains used for the production of the different pastes had been previously dried. Humidity levels below 15.5% are recommended in order to preserve the pasty product for a reasonable length of time [23]. The ash contents vary from 5.87±0.81 to 8.8±0.26. These very low levels could be explained by the dissolution of certain minerals in water [24]. Boli et al. [24] established that a high ash rate is most attributed to the mineral richness of the tuber. This is to say that the pastes studied are poor in minerals given their low ash content.

The average microbial loads of aerobic mesophilic germs are $44.8.10^3$ ± 36 CFU/g, 46.10^3± 38 CFU/g and $46.8.10^3$± 33 CFU/g. These different loads are lower than the microbiological criteria concerning aerobic mesophilic germs [25]. These low loads could be explained by the very low humidity level in the different doughs. In fact, this humidity level is due to the prior drying of the peanut grains. It should also be noted that the peanut grains were previously roasted before transforming them into paste. The roasting/drying combination would have allowed a significant reduction in the load of aerobic mesophilic germs. Saritha et al. [26] also showed that drying and roasting grains reduced aerobic mesophilic germs in Tunisia. Salmonella was detected in this study. These results could reflect non-compliance with good hygiene practices during pasta manufacturing.

The average microbial loads of mushrooms are higher than the microbiological criteria which are set at 103 CFU/g. These microorganisms therefore constitute hygienic and health indicators of the manufacturing processes and the microbiological quality of the final product [11]. Generally speaking, all these contaminations reflect a lack of hygiene in food manufacturing [27]. Peanut pastes were contaminated with both *Staphylococcus aureus* [28].

5. CONCLUSION

The evaluation of the microbiological parameters of the different peanut pastes highlighted the presence of pathogenic microorganisms. It should also be noted that the levels of contamination of spoilage germs and potentially pathogenic substances were detected. This worrying presence of microorganisms sometimes exceeds the threshold of the microbiological criteria for foods or food supplements intended for consumption. The physicochemical

parameters obtained indicate a better quality of peanut pastes which can be stored for a long time. However, the presence of its pathogenic germs is a serious public health problem.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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