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Comparative Study of Some Physico-Chemical, Microbiological Parameters and Origins of Faecal Contamination of Three Types of Soybean Flour Sold in the City of Daloa (Côte d'Ivoire)

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A B S T R A C T

The aim of this study is to compare the physicochemical and microbiological parameters of soya flour, but also to determine the origin of faecal contamination of the latter. To carry out the work, three types of flour consisting of 60 soybean samples were used. In addition, the method proposed by AOAC, (1995) made it possible to determine the physicochemical parameters such as dry matter, ash, pH, moisture content and titratable acidity dosage. The microbiological analyzes enabled the enumerations of yeasts and molds, fecal coliforms, aerobic mesophilic germs, Bacillus cereus, detection and enumeration of *E. coli*, *Staphylococcus aureus* and *faecal streptococci*. The determination of the origins of faecal contamination was carried out according to the work of Borrego and Romero (1982). The results show that there is no significant difference between the different types of flour at P> 0.05 at the 5% level for the different physicochemical parameters. However, at the level of microbiological parameters, a significant difference is noted between the F2 flour and the two other types, namely F1 and F3, at the level of *Mesophilic Aerobic Germs*, *E. coli* and *Staphylococcus aureus*. There is also a significant difference between the F3 flour and the two other types of flour namely the F 1 and the F2 at the level of Bacillus cereus. Furthermore, a significant difference is also noted between F1 flour and the two other types of flour, namely F2 and F3 in faecal streptococci. In addition, we see the source of contamination of F1 is of mixed origin, predominantly human, while F2 and F3 flour, the source of contamination is strictly human. Key words: comparison, origin, feces, streptococci, coliforms.

Keywords

Flour, plant, agronomic, nutritional and economic

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Introduction

Soybean (*Glycine max* [L.] Merr.) Is a legume cultivated for its seeds which are particularly rich in food proteins (Kouamé *et al.*, 2007) offering many advantages whether on the plant, agronomic, nutritional and economic. It is used in human and animal food. Very rich in nutrients, soy is taken as a food of choice. Due to its nutritional qualities, soy can completely replace meat or fish (Hubert, 2006). In addition, flour made from soybeans is a very recognized enrichment. It can easily be incorporated into other food flours to enrich them with nutrients and thus strengthen cereals and other flour products (Hubert, 2006). Flours can be contaminated with pathogens during the processing of soybeans (FAO / WHO, 2006).

Also the hygiene practices from harvest through processing and marketing observed could promote an increase in the number of emerging pathogens. On the other hand, flour formulas for infants have been consumed by millions of infants for many years. They represent the vast majority of breast milk substitutes commonly used in the world in general and in Côte d'Ivoire in particular.

These preparations consist of special foods, milk substitutes and cereal-based preparations such as soy flour (Codex Alimentarius Commission, 2013). PPNs that meet current microbiological standards are not sterile products.

They can occasionally be contaminated with pathogens (FAO / WHO, 2006) such as emerging opportunistic pathogenic Enterobacteriaceae. (FAO / WHO, 2006). A correlation between infections caused by certain microorganisms and their presence in PNPs has been clearly established. Bacteria of the genus Cronobacter spp. have been implicated in several severe neonatal epidemics such as meningitis, sepsis and gastroenteritis in children (FAO / WHO, 2007). The objective of this study is to compare the physicochemical and microbiological parameters of soybean meal, but also to determine the origin of faecal contamination.

Materials and Methods

Sampling

Around sixty (60) samples of soybean flour composed of flour sold in PMI (children's health center) (F1), grains bought at the market and transformed into flour under aseptic conditions in the laboratory (F2) and flour from industrial units sold in supermarkets (F3) in Daloa.

All these samples were transported in a cooler containing carboglaces to the laboratory for microbiological and physicochemical analyzes.

Microbiological analysis technique

The various microbiological analyzes were carried out taking into account the following standards. These are the NF EN ISO 6887-1, 2017 standards which were used for the preparation of the initial suspension and the decimal dilutions, ISO 21527-1, 2008 for the enumerations of yeasts and molds at 25 ° C / 7 days, NF V08-060 and NF ISO 4832, 2006 at 30 ° C / 24 H for fecal coliforms, NF V 08-051, 1999 at 30 ° C / 72 H for aerobic mesophilic bacteria, ISO 4832, 2006 at 45 ° C / 24 hours for the detection and enumeration of *E. coli*, NF ISO 16649-2, 2001 at 37 ° C / 24 to 48 hours for the detection and enumeration of *Staphylococcus aureus*, ISO 7932, 2004 at 30 ° C / 24 H for the detection and enumeration of *Bacillus cereus* and NF / ISO 16310: 2011 at 44 ° C / 8h for faecal streptococci.

Expression of microbiological results

For the calculation of the average microbial loads after enumeration, the following mathematical formula was used.

$$N = \frac{\Sigma Ci}{(N_1 + 0,1N_2)d.V}$$

N: number of colony in CFU / ml;

ΣCi: sum of the characteristic colonies counted on all the dishes selected;

N1: number of dishes retained at the first dilution;

N2: number of dishes retained at the second dilution;

d: dilution rate corresponding to the first dilution;

V: volume of the inoculum collected.

Determination of physicochemical parameters

The determination of physicochemical parameters such as dry matter, ash, pH, moisture content and titratable acidity assay were performed by the method proposed by AOAC, (1995)

Determination of the origin of faecal contamination

It is based on the criteria defined by Borrego and Romero (1982).By taking into account the fecal coliform / faecal streptococci ratio, the origins of product contamination can be determined (Table 1).

Statistical analyzes

Analysis of variance (ANOVA I) and Tukey's test were the statistical tools used for data analysis. This analysis was performed using STATISTICA 7.1 software (Statsoft, France).In the event of significant differences between the parameters studied, the classification of the means was made according to the Newmann-Keuls test. The significance level is 0.05.Statistical differences with a probability value less than 0.05 (P <0.05) are considered significant. When the probability is greater than 0.05 (P> 0.05) the statistical differences are not significant. If there was a significant difference (p <0.05) between the means, Tukey's test was performed to determine the different classes of homogeneity.

Results and Discussion

Comparative study of the physico-physical parameters of the different types of flour

Analysis of the table shows that there is no statistically significant difference between the different types of flour at P> 0.05 at the 5% level for the different parameters (Table 2).

Comparative study of the microbiological parameters analyzed

Analysis of the results shows that there is no statistically significant difference between the different types of flour at P> 0.05 at the 5% level for fecal coliforms and yeasts and molds. Statistical differences with a probability value less than 0.05 (P <0.05) are considered significant so there is a significant difference between F 2 flour and the other two types namely F 1 flour and flourF3 at the level of Mesophilic Aerobic Germs, *E. coli* and *Staphylococcus aureus*. There is also a significant difference between the flour F3 and the two other types of flour namely the flour F 1 and the flour F2 at the level of *Bacillus cereus*. Furthermore, a significant difference is also noted between F1 flour and the two other types of flour, namely F2 flour and F3 flour in faecal streptococci (Table 3).

Origin of faecal germs

The ratios of the mean microbial loads of fecal coliforms and fecal streptococci (CF / SF) of the different types of soybean meal studied were 0.81;4.12 and 4.15 respectively for the flours F1, F2 and F3.These reports would indicate that these faecal flora are all of various origins. In addition, we note that for F1 flour that 0.7 <R <1 hence the source of contamination is of mixed predominantly human origin, while the F2 and F3 flours, R> 4 hence the source of contamination is strictly human (Table 4).

The physico-chemical characteristics of the three types of flour analyzed are variable. In addition, the pH of his samples are acidic and vary from 6.15 to 6.33. The measured titratable acidity content varies from 0.63 to 0.85 meq / 100g. The probabilities of the titratable acidity (p = 0.08> 0.05) and of the pH (p = 0.31> 0.05) determined, show that the three types of flour analyzed are not significantly different. This is explained by the fact that the soybeans processed into flour contain the same acidic compounds therefore the different manufactures did not have an impact on the acidity of these. This result is consistent with that of Soro *et al.,* (2013), who asserts that flours that have an acidic pH are better preserved against attack by microorganisms so these flours could be stored for a long time without risk of microbial spoilage. The dry matter content determined in this study varies from 94.21 to 96.78. This high content indicates a low humidity rate between 3.06 ± 0.71 and 5.93 ± 0.50 these humidity levels comply with the standard because dehydrated products must contain less than 15% humidity (Houphouët, 2016). Furthermore, there is no significant difference between the three types of flour analyzed. However, this low humidity is due to the heat accumulated in the grains. Indeed the grains have been previously roasted and spread, the heat will reduce the amount of water and the low humidity. Thus, these results could promote good conservation of flour for a long time without there being any risk of proliferation and microbial multiplication (WHO / FAO, 2007). According to Akubor, (2005), the determination of the water content is important, since it conditions the implementation of technological tests, such as bread making. Moisture levels are slightly elevated in F1 and F2 flours. These high humidity levels had already been notified in other works could be due to the fact that most millers use second-hand equipment whose performance is seriously reduced, which has the consequence of passing the product several times through grind in the mill if it is not properly hydrated. Probably the use of the right equipment would explain the low humidity levels in this study.

The hydrogen potentials (pH) of the different types of soy flour analyzed are acidic and vary between 5.62 and 6.11 for F3 flour; 6.24 and 6.57 for F 2; 6.18 and 6.32 for F 2 flour. These three types of flour are not significantly different in terms of pH. Moreover, these results are similar to those of cereal flours in other studies carried out by Wakil and Onilude (2009). It appears that the pH of flour can drop after one month of storage. This could be explained on the one hand by the continuity of the amylase activity of the amylase residues still active in the different types of flour, and on the other hand by the oxidation of fatty acids or be attributable to microbial enzymatic activities. (Saubade *et al.,* 2018). Moreover, according to previous work carried out by Soro *et al.,* (2013), flours which have an acidic pH are better preserved against attacks from certain undesirable microorganisms.

The observation of an increase in the ash content in the different types of flour is similar to the previous work reported by Leonel *et al.,*

(2005) for the ash content of Pachyrhizusahipa and those on cassava reported by Chotineeranat *et al.,* (2006). In addition, the ash content is the official means used to characterize the purity of flour (Ballogou *et al.,* 2018). According to GODON and Willm (1991), the determination of the ash offers the possibility of knowing the overall mineral content of the flours. The average microbial loads vary from one type of flour to another and from one parameter to another as well. Thus at the level of mesophilic aerobic germs, a significant difference is noted between the loads of the F2 and F3 flours and that of the F1 flour.

The average microbial load of F1 flour is low and this may be explained by its low moisture content. In fact, the soya beans have been roasted beforehand before transformation into flour. This would also explain the low load in aerobic mesophilic germs, but also in yeasts and molds. Moreover, in a recent study Tarhouni *et al.,* (2015) also showed that roasting grains before their transformation into flour considerably reduced the proliferation of certain germs. Concerning the fecal flora, there is no significant difference in the average microbial loads between the different types of flour at the level of fecal coliforms, on the other hand there is a significant difference between the F2 and F3 flours and that of the F1 flour which is less loaded. in germs at the level of faecal streptococci. However, the presence of germs of fecal origin in all types of flour could be explained by the non-compliance with good hygiene practices during the manufacture of these flours.

Table.1 Criteria for determining the origin of faecal contamination

Faecal coliforms / faecal streptococci ratio (R)	Source of contamination
R < 0,7	Strictly of animal origin
0,7 < R < 1	Mixed predominantly animal
1 < R < 2	Uncertain origin
2 < R < 4	Mixed predominantly human
R > 4	Strictly of human origin

Table.2 Comparative study of the different types of flour according to the physicochemical parameters

	Values of physicochemical parameters					
Types of flour	Titratable acidity	**pH**	Moisture rate **(g/100 g MF)**	Dry matter**(g/100 g DM)**	**Ash(g/100 g DM)**	Sugar levels
F 1	$0,73 \pm 0,13^{a}$	$6,33 \pm 0,09^{b}$	$5,48 \pm 3,27^{c}$	$94,53 \pm 3,29^{d}$	$5,39 \pm 0,79^{e}$	$8,89 \pm 2,20^{f}$
F 2	$0,85 \pm 0,21^{a}$	$6,15 \pm 0,32^{b}$	$5,93 \pm 0,50^{c}$	$94,21 \pm 0,55^{d}$	$3,93 \pm 1,58^{e}$	$8,67 \pm 3,88^{f}$
F 3	$0,63 \pm 0,01^{a}$	$6,33 \pm 0,14^{b}$	$3,06 \pm 0,71^{c}$	$96,78 \pm 0,90^{d}$	$5,12 \pm 1,26^{e}$	$6,84 \pm 0,26^{f}$

All the means followed by the same letter in superscript on the same column are not significantly different at the probability threshold P <0.05.DM = dry matter MF = fresh matter F1: Flour purchased from the PMI (health center), F2: Flour obtained from grains purchased in public markets, F3: Flour sold in supermarkets

Table.3 Comparative study of the average microbial loads of the different types of flour

Types de farines	Germes Aérobies Mésophiles	Coliforms fécaux	Levures et Moisissures	*Escherichia coli*	*Staphylococcus aureus*	Streptocoques fécaux	*Bacillus cereus*
F1	$10^5 \pm 60^b$	$9{,}6.10^2 \pm 35^a$	332 ± 37^a	$9{,}5.10^2 \pm 14^b$	$8{,}410^3 \pm 11^b$	$11{,}5.10^3 \pm 147^b$	$3{,}9.10^2 \pm 88^a$
F2	$1{,}4.10^2 \pm 21^a$	$6{,}6.10^2 \pm 87^a$	554 ± 61^a	0 ± 0^a	0 ± 0^a	$1{,}6.10^3 \pm 115^a$	$5{,}0.10^2 \pm 108^a$
F3	$6{,}1.10^2 \pm 11^a$	$5{,}4.10^2 \pm 32^a$	454 ± 55^a	$3{,}6.10^4 \pm 13^b$	$2.10^4 \pm 20^b$	$1{,}3.10^3 \pm 96^a$	$3{,}5.10^3 \pm 197^b$

All the means followed by the same letter in superscript on the same column are not significantly different at the probability threshold P <0.05.DM = dry matter MF = fresh matter, F1: Flour purchased from the PMI (health center), F2: Flour obtained from grains purchased in public markets, F3: Flour sold in supermarkets

Table.4 Faecal coliforms / faecal streptococci ratio of the different types of soybean meal studied

Faecal coliforms / faecal streptococci ratio (R)	Values found	Source of contamination
F1	0,81	Mixed origin predominantly human
F2	4,12	Strictly of human origin
F3	4,15	Strictly of human origin

F1: Flour purchased from the PMI (health center), F2: Flour obtained from grains purchased in public markets, F3: Flour sold in supermarkets

With regard to potentially pathogenic germs, namely *E. coli* and *Staphylococcus aureus*, a significant difference is noted between the F2 flour and the other two flour, namely F1 and F3. Indeed we find a high microbial load in F1 and F2 flour. In recent studies, similar results have been found by Malete *et al.,* (2013), Sanou *et al.,* (2017) in infant flours produced in an artisanal way. Moreover, the presence of these germs reflects a health risk for the consumer. According to the work carried out by Mesa *et al.,* (2006) some strains of E. coli are pathogens causing diarrhea which can be fatal in warm-blooded humans and animals. These strains are usually found in environments where sanitary conditions are poor. Furthermore, the presence of potentially pathogenic germs such as *Bacillus cereus*, E. coli and *Staphylococcus aureus* is justified by the ubiquitous nature of the latter. These bacteria, which are very widespread in the environment and moreover saprophytic in humans and warm-blooded animals, are found in flour during processing. Also, the process for transforming these flours remains traditional. Thus, the production time is long and the inadequate hygienic conditions could constitute routes of contamination of these products. In addition, some authors such as Adjilea *et al.,* (2015) revealed in a recent study on the characterization of the traditional technology of production of flour from corn, that the milling would be a critical step, dependent on the sunshine and the level of healthiness of the immediate environment.

The comparative study carried out on the physicochemical and microbiological parameters revealed some significant differences on certain physical and microbiological parameters. The presence of potentially pathogenic germs in certain types of flour can cause serious illness, including death of infants. Therefore, the consumption of soy flour sold in markets, supermarkets and PMI would present risks for children and especially for infants. There are various sources of contamination of flour by faecal germs.

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