



Evaluation of the Microbiological and Physico-Chemical Quality of Soybean Flour (*Glycine max* L. Merrill) as a Food Supplement for Infants Sold in Daloa

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Authors' contributions

This work was carried out in collaboration among all authors. Author KKA Athanase designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OTST and KKC managed the analyses of the study. Authors CI and Konate Ibrahim managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study is to assess the presence of harmful microorganisms and to characterize some physicochemical parameters in the soya flour sold in Daloa. To carry out the work, sixty (60) samples of soybean flour were collected by purchase in PMI (20), supermarkets (20) and in certain markets (20) made up of grains that will be transformed into flour according to the defined conditions. by ourselves. His samples will be transferred to the microbiology laboratory for analysis. A count to assess the microbiological quality was carried out. The assay of some chemical parameters and the determination of some physical parameters were performed. The different pH

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values obtained are all alkaline. Microbiological analysis revealed compliance of average microbial loads of fungi (yeasts and molds) below 10^3 CFU / g and aerobic mesophilic bacteria below 10^5 CFU / g. On the other hand, the average microbial loads of total coliforms do not comply with the defined microbiological criteria. Furthermore, with regard to the potentially pathogenic germs in *Bacillus cereus* occurrences, there is no conformity of the average loads of the three types of flour. The defined criterion being 10 CFU / g. As for *E. coli* and *S. aureus*, only F1 flour complies with microbiological criteria. There is a presence of *Salmonella* in 60% of the samples of the F1 flour. Good practices should be observed in the processing of grains into flour in order to avoid possible contamination of the flour.

Keywords: Soybean; supplement; infant; flour; fillers.

1. INTRODUCTION

Food security is one of the world's priorities for humans. In recent decades, the growth of foodborne illnesses in the world and in particular in Côte d'Ivoire has often been linked to the presence of microorganisms or their toxins in food [1]. Over 40% of children in developing countries are chronically malnourished. Making quality, locally produced infant flours available would help tackle one of the main causes of this malnutrition [2]. Infant flour formulas have been consumed by millions of infants for many years. They represent the vast majority of breastmilk substitutes commonly used in the world. These flour preparations consist of milk substitutes and cereal-based preparations such as soy [3]. However, flours can occasionally be contaminated with pathogens during the processing of soybeans [4]. Also the hygienic practices during harvesting, processing and marketing implemented could be at the origin of an increase in the number of emerging pathogens. In addition, the dangers that represent a risk for the safety of soybean meal can occur from the harvest of the raw material, through to consumption, including processing [4]. Among these are Enterobacteriaceae and Enterococci. Some of these microorganisms are emerging opportunistic pathogens that take on particular characters and have remarkable properties of resistance to desiccation and osmotic stress, allowing them a longer survival in contaminated flour [5]. A correlation between infection caused by certain Enterobacteriaceae and their presence in flour 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 [5]. For investigated outbreaks, death rates ranging from 20 to more 80% in some cases have been reported [6]. But the subjects at high risk,

susceptible to contracting an infection from the consumption of soy flour are newborns, in particular, premature babies. low birth weight infants or immunocompromised infants and infants born to HIV / AIDS positive mothers [4]. In the Ivory Coast and particularly in Daloa, there are no data on the sanitary and microbiological quality of the soya flour sold and particularly those relating to the presence of pathogenic germs. Also, there is no surveillance giving statistics of diseases resulting from the consumption of flour in general and soy flour in particular contaminated by microorganisms. The main objective of this study is to assess the presence of harmful microorganisms and to characterize some physicochemical parameters in soy flour.

2. MATERIALS AND METHODS

2.1 Sampling

A total of 60 soybean flour samples consisting of locally produced flours sold in PMI (F1), flour which was powdered by ourselves using grains (F2) and flour industrially manufactured sold in supermarkets (F3) in Daloa. These samples were transported aseptically to the laboratory to perform the various analyzes.

2.2 Methodology

2.2.1 Soybean meal production

The grains purchased on our respective markets were transformed into flour before the physicochemical and microbiological tests were carried out. The production of flour requires several unit operations. These are sorting, roasting, pulping, crushing, sieving, blending and packaging. After the operation, the flour obtained is packaged in sterile stomascher paper for further work (Fig. 1).

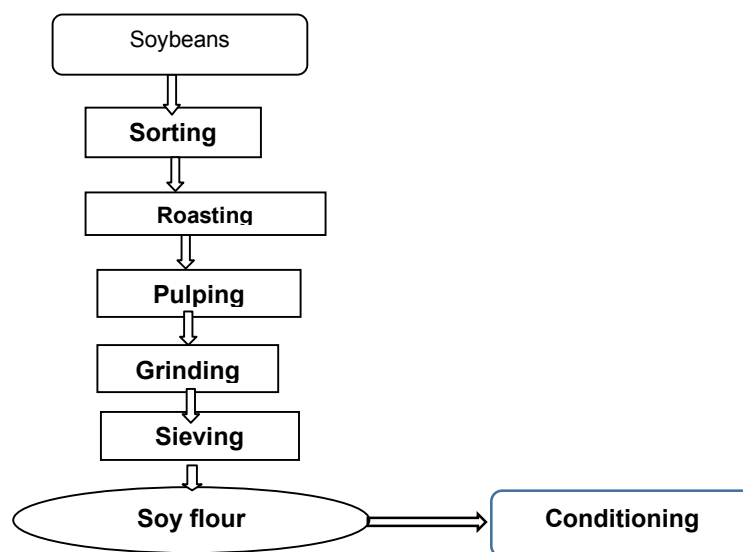


Fig. 1. Diagram flour soy grain processing

2.1.2 Determination and determination of physicochemical parameters

The determination of dry matter, ash, pH, moisture content and titratable acidity assay were performed according to the method proposed by [7].

2.1.3 Microbiological analyzes

Twenty-five grams of soy flour is aseptically weighed into sterile stomascher paper. A volume of 225 ml of buffered peptone water (Biorad group, Paris, France) is added to it. The resulting mixture was homogenized for one minute. This mixture constitutes the stock suspension which will be left to stand for 30 minutes at laboratory temperature in order to allow revivification of the microorganisms. From this initial suspension, a series of decimal dilutions is then carried out [8].

2.1.3.1 Enumeration of germs

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 is well homogenized. This technique concerns Sabouraud media with chloramphenicol, PCA and VRBL. Another quantity of 0.1 ml is placed in a Petri dish containing 20 ml of agar previously prepared and poured. Then the 0.1 mL is spread on the surface of the agar using a sterile spreader. The seeded dishes are left on the bench to solidify the agar. The boxes thus solidified are incubated at 25 ° C / 7 days for the enumeration of yeasts

and molds [9], at 30 ° C / 24 H for total coliforms [10,11], at 30 ° C / 72 H for aerobic mesophilic organisms [12], at 37 ° C / 48 H, at 45 ° C / 24 H for the detection and enumeration of *E. coli* [13] at 37 ° C / 24 to 48 hours for the detection and enumeration of *Staphylococcus aureus* [14] and at 30 ° C / 24 H for the detection and enumeration of *Bacillus cereus* [15].

2.1.3.2 Research of salmonella

The detection of *Salmonella* was carried out in 4 stages according to ISO standard 6579. These are pre-enrichment, enrichment, isolation and finally reading and identification.

3. RESULTS AND DISCUSSION

3.1 Monitoring of Physicochemical Parameters

The physicochemical parameters vary from one type of flour to another. Thus, at the level of titratable acidity, the values are 0.73 ± 0.13 , 0.63 ± 0.01 and 0.85 ± 0.21 respectively for the flours F1, F2, and F3. The pH of all three types of flour are alkaline and humidity levels are low. The dry matter evolves from 94.53 ± 3.29 for the F1 flour to 96.78 ± 0.90 for the F2 flour and to 94.21 ± 0.55 for the F3 flour. The ash content varies from 5.39 ± 0.79 , 5.12 ± 1.26 , and 3.93 ± 1.58 respectively for the flours F1, F2 and F3. The sugar levels are relatively low and are 8.89 ± 2.20 for F1, 6.8 ± 0.26 for F2 and 8.67 ± 3.88 for F3 (Table 1).

Table 1. Physico-chemical parameters of the types of flour analyzed

Type of flour	Physico-chemical parameters					
	Titratable Acidity	pH	Humidity levels	Dry matter	Ash rate	Sugar levels
F1	0,73± 0,13	6,33 ± 0,09	5,48 ± 3,27	94,53 ± 3,29	5,39 ± 0,79	8,89 ± 2,20
F2	0,63 ± 0,01	6,33 ± 0,14	3,06 ± 0,71	96,78 ± 0,90	5,12 ± 1,26	6,8 ± 0,26
F3	0,85 ± 0,21	6,15 ± 0,32	5,93 ± 0,50	94,21 ± 0,55	3,93 ± 1,58	8,67 ± 3,88

F1: Flour sold in PMI (Health center)

F2: Grains transformed into flour during the work

F3: Flour processed industrially and sold in supermarkets

Table 2. Average microbial loads of potentially bacterial species Pathogens

Type of flour	<i>E. coli</i>	<i>S. aureus</i>	<i>B. cereus</i>
F1	10.10 ² ± 14	8.10 ³ ± 11	4.10 ² ± 88
F2	0 ± 0	0 ± 0	5.10 ² ± 108
F3	4.10 ⁴ ± 13	2.10 ⁴ ± 20	4.10 ³ ± 197
CM	10 CFU/g	10 ² CFU/g	10 CFU/g

CM: Microbiological criteria

F1: Flour sold in PMI (Health center)

F2: Grains transformed into flour during the work

F3: Flour processed industrially and sold in supermarkets

3.2 Monitoring of Microbiological Parameters

3.2.1 Potentially pathogenic bacterial species

The different types of soybean flour analyzed contain potentially pathogenic bacterial species, in particular *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*, with the exception of F2 flour, for which there is an absence of *Escherichia coli* and *Staphylococcus aureus*. The flour. The average loads of F1 and F3 flours are higher than the microbiological criteria prescribed by the reference standards. Furthermore, at the level of the F2 flour, only *B. cereus* has an average microbial load higher than microbiological criteria (Table 2).

3.2.2 Alteration and contamination flora

Different microbial flora has been found in the different types of soy flour sold in the streets of Daloa. These are alteration microflora and

certain flora suggestive of a deficit in good hygiene practices. These are fungal flora, total flora and total coliforms. All the samples are contaminated with these different microfloras. Apart from the average microbial loads of total coliforms which are above the expected microbiological quality standards, the other two floras, namely the fungal flora and the total flora, have average microbial loads in accordance with the microbiological criteria defined by the microbiological quality standards provided (Table 3).

3.2.3 Testing for *Salmonella* in different types of flour

It should be noted an absence of *Salmonella* in almost all of the samples analyzed in the F2 and F3 flours. On the other hand, there is presence of *Salmonella* in the samples of the F2 flour with twelve (12) positive samples, ie approximately 60% (Table 3).

Table 3. Average microbial loads of spoilage and contamination germs

Type of flour	Mesophilic aerobic germs	Total coliforms	Yeasts and molds
F1	2.10 ² ± 21	9.10 ² ± 35	332 ± 37
F2	10 ⁵ ± 60	7.10 ² ± 87	554± 61
F3	6.10 ² ± 11	5.10 ² ± 32	454 ± 55
CM	10 ⁵ CFU/g	10 CFU/g	10 ³ CFU/g

CM: Microbiological criteria

F1: Flour sold in PMI (Health center)

F2: Grains transformed into flour during the work

F3: Flour processed industrially and sold in supermarkets

Table 4. Search for the genus *Salmonella* in the types of soybean meal analyzed

Types of flour	Samples analyzed	Presence of <i>Salmonella</i>	% of positive samples	% of negative samples
F1	20	+	12 (60 %)	8 (40 %)
F2	20	-	0 (00 %)	20 (100 %)
F3	20	-	0 (00 %)	20 (100 %)
Total	60	-	12 (20,00 %)	48 (80 %)

F1: Flour sold in PMI (Health center)

F2: Grains transformed into flour during the work

F3: Flour processed industrially and sold in supermarkets

4. DISCUSSION

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. These results are appreciably similar to those of cereal flours in other studies [16]. It appears that the pH of flour can drop after one month of storage. This is either due to the continuity of the amylase activity of the amylase residues still active in the flours, or to the oxidation of fatty acids or to be attributable to microbial enzymatic activities [17]. Moreover, according to [18], 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 deterioration. The measured titratable acidity content varies from 0.63 ± 0.01 to 0.85 ± 0.21 meq / 100g, also defined as the acidity of the flour. This is explained by the fact that the soybeans processed into flour contain the same compounds (acid) therefore the different manufacture did not have an impact on the acidity of these. The dry matter content determined in this study varies from 94.21 ± 0.55 to 96.78 ± 0.90 . 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 [19]. This low humidity is due to the heat accumulated in the grains. The grains have been roasted and spread out beforehand, releasing the heat will reduce the amount of water, hence the low humidity. Such a result could promote good conservation of the flour for a reasonable period of time without risk of microbial proliferation [4]. Determining the water content is important, since it conditions the implementation of technological tests, such as breadmaking [20]. The dry matter content determined in this study is 94.53 ± 3.29 % for F1 flour, 94.21 ± 0.55 % for the F2 flour and $96.78 \pm 0.9\%$ for the F3 flour. These high levels indicate a low moisture content, as the

grains used for the production of the various flours had been previously dried. Lower moisture levels at 15.5% are recommended in order to keep the floury product for a reasonable period of time [6].

The different types of flour were contaminated with both *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*, potential pathogens with varying loads. Regardless of the different types of flour, the average microbial loadings of contaminants were well above prescribed standards. The average microbial loads of *E. coli* were $8.410^3 \pm 11$ CFU / g for artisanal flour 1 and $3.6.10^4 \pm 13$ CFU / g for industrial flour. The average microbial loads of the study are above those of recent work carried out by [20] on maize whose average microbial load values were between 0 and $3.3.10^1$ CFU / g. The massive presence of these potentially pathogenic germs in the F2 flours analyzed constitutes a danger to the health of the infant because they can lead to the production of toxins.

The average microbial loads of mesophilic aerobic germs are $1.4.10^2 \pm 21$ CFU / g, $10^5 \pm 60$ CFU / g and $6.1.10^2 \pm 11$ CFU / g, fungi (Yeasts and molds) are 332 ± 37 CFU / g, 554 ± 61 CFU / g and 454 ± 55 CFU / g respectively for F1 flour, F2 flour and F3 flour. These different loads are lower than the microbiological criteria for aerobic mesophilic germs and fungi of 10^5 CFU / g and 10^3 CFU / g respectively in infant flours [21]. These low loads could be explained by the very low moisture content in the various flours. Indeed, this moisture level is due to the prior drying of the soybeans. It should also be noted that the soy beans have been previously roasted before turning them into powder. The roasting / drying coupling would have allowed a significant reduction in the load of mesophilic aerobic bacteria and fungi. Recent work by [22] found average loads of yeasts and molds much lower, identical to the results of the study. Other authors such as [23] have also shown that drying and

roasting of grains reduced aerobic mesophilic germs in Tunisia.

The average microbial loads of total coliforms are respectively $9.6.10^2 \pm 35$ CFU / g, $6.6.10^2 \pm 87$ CFU / g, and $5.4.10^2$ CFU / g, for F1 flour, F2 and F3 flour. These loads are greater than the microbiological criteria which are set at 10 CFU / g. These microorganisms therefore constitute hygienic and sanitary indicators of the manufacturing processes and of the microbiological quality of the final product [4]. In this study, we note the presence of *Bacillus cereus* with average microbial loads of the various flours above the microbiological criteria set at 10^2 CFU / g. In general, all these contaminations reflect a lack of hygiene in food manufacturing [24]. *Salmonella* was detected in this study in F1 flour, the origin and manufacturing conditions of which are not known. These results could reflect the non-respect of good hygiene practices during the manufacture of flour [25].

5. CONCLUSION

The values of the physico-chemical parameters of the three types of flour analyzed are variable. The pH values of his samples of all three types of flour are acidic. The dry matter content determined in this study also varies from one type of flour to another and is high. This high content indicates low humidity. The evaluation of the microbiological parameters of the F2 and F3 flour, highlighted the absence of pathogenic microorganisms. While in F1 flour these pathogenic microorganisms have been detected. There are also levels of contamination from spoilage germs and potentially pathogens that have been detected. This worrying presence of microorganisms sometimes exceeds the threshold of the microbiological qualities of foods or food supplements intended for infants and young children.

The means at our disposal were limited, which did not allow us to make a larger and more representative sample. We would like the university and municipal authorities to get involved in this research which will be able to help our future mothers to improve the preparation of soy flour for their infants. This will also reduce the risk of contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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