

Proximate composition and mycological characterization of peanut butter sold in retail markets of Abidjan (Côte d'Ivoire)

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ABSTRACT

Objective: The aim of this work was to contribute to the food safety of Ivorian consumers by investigating the proximate composition and the toxic fungal contamination of peanut butters offered for retail sale on the different markets of Abidjan.

Methodology and results: Peanut butter samples (45) were collected from the main markets of the 9 communes of Abidjan District and their physicochemical and mycological characteristics were determined. Statistical analyses were performed on the data obtained. Mean proximate composition was as follow: moisture (1.03 – 4.50 %), pH (6.25 – 6.72), titratable acidity (9.18 – 18.48 meq/100 g), ash (5 – 5.5 %), crude fibre (5 – 6.78 %), protein (21 – 30 %), lipids (41 – 50 %), carbohydrate (15 – 26 %) and energy (560 – 640 kcal/100 g). The total fungi isolated ranged from 10⁴ to 10⁶ CFU/g. Eight (8) genera of fungi were isolated: *Mucor*, *Alternaria*, *Helminthosporium*, *Geotrichum*, *Fusarium*, *Cladosporium*, *Penicillium* and *Aspergillus*. The predominant fungi belonged to *Aspergillus* genus (20.22 – 51.65 %) followed by *Helminthosporium* (0 – 47.44 %) and *Penicillium* (0 – 41.54%). The mycotoxigenic fungi were isolated with a frequency of 14.81, 13.95, 9.1 and 21.62 % for *Aspergillus versicolor*, *Aspergillus ochraceus*, *Aspergillus flavus* and *Aspergillus parasiticus*, respectively.

Conclusions and application of findings: Peanut butter sold in retail markets in Abidjan District are nutritive and could meet the dietary needs of the population. However, the presence of mycotoxigenic fungi represents a public health problem. Therefore, good manufacturing processing and good hygiene practices would help to minimize fungal contamination in order to obtain good sanitary peanut butters.

Key words: Peanut butter, physicochemical characteristics, fungal contamination, retail markets.

INTRODUCTION

Groundnut or peanut (*Arachis hypogaea* Linn) is a plant which belongs to the family of *Fabaceae* (Eke-Ejiofor *et al.*, 2012). Botanically, groundnut is a legume although it is widely identified as a nut and has similar nutrient profile with tree nuts (Ros, 2010). This annual plant is generally distributed in the tropical, sub-tropical and warm

temperate areas and represents the second most important legume in the world based on total production after soybean (Pattee & Young, 1982; Redden *et al.*, 2005). The average world production of groundnut pods amounted to about 35.88 million tons/year from 24.4 million hectare and the total production in sub-Saharan Africa

was 8.2 million tons/year from 9.5 million hectare (USDA, 2012). The main producing countries are China, India, Nigeria, United States, Indonesia and Sudan. Peanut constitutes a major annual oilseed crop and a good source of protein containing high lysine content, which makes it a good complement for cereal (Okaka, 2005). The proximate biochemical composition of mature groundnut seeds is per 100 g edible portion: moisture (6.5 g), protein (25.8 g), lipids (49.2 g), carbohydrate (16.1 g), dietary fibre (8.5 g), calcium (92 mg), magnesium (168 mg), phosphorus (376 mg) and iron (4.6 mg) (USDA, 2010). However, peanut contains some anti-nutritional factors such as phytic acid, condensed tannins, trypsin and amylase inhibitor, that may limit its usage and nutritional value (Njintang et al., 2001). Moreover, groundnuts are liable to fungal contamination during handling, storage and transportation, exposing them to the risk of contamination with aflatoxin (Polixeni & Panagiota, 2008; Mutegi et al., 2012). Indeed, groundnuts can be contaminated with aflatoxin during pre- and post-harvest processing and the risk of contamination increases along the marketing chain due to poor handling practices (Kladpan et al., 2004; Kaaya et al., 2006). The main aflatoxin producing fungi in groundnuts are *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*, which mostly infect groundnuts as a complex (CAST, 1998; Varga et al., 2012). *Aspergillus flavus* grows in groundnuts when the moisture content exceeds 9 % and has optimum growth conditions of between 25 and

30°C, and water activity of 0.99 with a minimum of 0.83, while production of aflatoxin occurs optimally at 25°C and water activity of 0.99 (Ribeiro et al., 2006). According to IARC (2002), aflatoxin produced by *Aspergillus sp.*, has immunosuppressive effects and epidemiological studies have shown a positive correlation between aflatoxin intake and the incidence of liver cancer. Peanuts and its derivatives are often classified as street food which satisfies essential need of the urban population by being affordable and available (Donkor et al., 2009). Peanut seeds are eaten raw, boiled or roasted, made into butter or paste and are used for thickening soups (Campos-Mondragón et al., 2009). Peanut butter is made by grinding dry roasted groundnuts into a paste (Mutegi et al., 2009). Peanuts are also used as major ingredients in the formulation of weaning food with other cereals such as sorghum, corn, and millets because of their high protein and omega 6 fatty acid contents (Iro et al., 1995). In Côte d'Ivoire, the use of marketed peanut butter in the confection of sauce is very popular among the urban population. Generally, peanut butter is produced by traditional methods characterized by unknown hygienic conditions. To our knowledge, there is no scientific data on physicochemical and microbiological quality of marketed peanut butters. Therefore, the aim of this work is to assess the physicochemical properties and the level of toxic fungal contamination of peanut butters sold in retail markets.

MATERIAL AND METHODS

Sample collection: Peanut butters were collected for 8 days (June 2012) in the main markets of the 9 township of Abidjan (Côte d'Ivoire): Abobo, Adjamé, Attécoubé, Cocody, Koumassi, Marcory, Port-Bouet, Treichville, Yopougon identified as AB, AD, AT, C, K, M, P, T and Y. A total of 45 samples used for analysis were obtained as follow: peanut butters were randomly and aseptically collected from different sellers in each market and immediately transported in icebox (4°C) to the laboratory. The collected peanut butters were then aseptically mixed together in a dough trough to constitute five (5) samples of 25 g each per township. The samples obtained were stored at 4°C until further analysis.

Physicochemical analysis: Proximate analysis was carried out using the AOAC (2000) standard methods. The moisture content was determined by the difference of weight before and after drying the sample in an oven (MEMMERT, Germany) at 105°C until constant weight. Ash fraction was determined by the incineration of dried sample (5 g) in a muffle furnace (PYROLABO, France) at 550°C for 12 h. The percentage residue weight was expressed as ash content. pH and titratable acidity were determined as follow: 10 g of peanut butter sample was homogenized with 100 mL of distilled water and then filtered. The pH value was recorded after the electrode of pH-meter (HANNA, Spain) was

immersed into the filtered solution. The filtered solution was titrated to the end point with sodium hydroxide solution 0.1 N and phenolphthalein as indicator. For crude fibre, 2 g of sample were weighed into separate 500 mL round bottom flasks and 100 mL of 0.25 M sulphuric acid solution was added. The mixture obtained was boiled under reflux for 30 min. Thereafter, 100 mL of 0.3 M sodium hydroxide solution was added and the mixture were boiled again under reflux for 30 min and filtered under suction. The insoluble residue was washed with hot water and dried to a constant weight in an oven (MEMMERT, Germany) at 100°C for 2 h, cooled in a desiccator and weighed. The weighed sample was then incinerated, and weighed for the determination of crude fibre content. Proteins were determined through the Kjeldhal method and the lipid content was determined by Soxhlet extraction using hexane as solvent. Total carbohydrate was determined by the formula:

$$100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ fat} + \% \text{ ash}).$$

The calorific value (energy) was calculated as follow: (protein x 4) + (carbohydrate x 4) + (lipid x 9).

The results of ash, fibre, protein, lipid and carbohydrate contents were expressed on dry weight basis.

Mycological analysis: The isolation of fungi was carried out according to the agar dilution method as described by Pitt *et al.* (1992). Ten (10) gram from each peanut butter sample, were homogenized with

90 mL of buffer peptone water (AES Laboratory, France) and serial decimal dilutions (10^{-1} to 10^{-4}) were performed. Fungal species were isolated on the semi selective Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Biokar Diagnostics, France). The medium was poured into sterile Petri dish and 0.1 mL of each sample suspension was spread-plated onto the DRBC agar in triplicate. The plates were incubated for 5 to 7 days at 25°C. Fungal isolates were sub-cultured on Malt Extract and Czapek Yeast medium agars (Oxoid, UK) and incubated for 5 to 7 days at 25°C for purification. Fungi were identified by using taxonomic schemes based on microscopic observation and culture appearance including colonies colours, texture, reverse colour, hyphae arrangement, conidia shape and nature of spores (Singh *et al.*, 1991). For the differentiation between *Aspergillus parasiticus* and *Aspergillus flavus* colonies, AFPA agar (Oxoid, UK) supplemented with chloramphenicol, was used. The total fungal count for each plate was expressed as colony-forming units per gram of sample (CFU/g). Each genus or specie identified was then expressed as percentage (%) of the total isolated fungi.

Statistical analysis: All the analyses were performed in triplicate and the data were analyzed using EXCELL and STATISTICA 7.1 (StatSoft). Differences between means were evaluated by Duncan's test. A significance difference was established at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Physicochemical properties: The physicochemical composition of peanut butter samples tested is shown in Table 1. All the parameters generally showed significant difference ($p < 0.05$) except for ash, fibre and protein contents of peanut butter samples. The lowest value of moisture content was 1.23 ± 0.20 % for the township C while the highest value was 4.30 ± 0.20 % for the township AB. These values are lower than that (7.48 %) of raw peanut seeds (Ayoola & Adeyeye, 2010) and this fact could be explained by the decrease of moisture content during the roasting which is an important step of peanut butter processing (Campos-Mondragón *et al.*, 2009). pH values of the marketed peanut butters ranged from 6.25 to 6.72 and these values are comparable to those (6.3 - 6.5) reported by FDA (2007). These values were also positively correlated with the titratable acidity ranged from 9.18 to 18.48 meq/100 g (Table 1). The mean value for ash (5.5 %) found in this study was higher than that (3.4 %) of raw peanut seeds indicated by Eke-Ejiofor *et al.*

(2012). Indeed, Ayoola and Adeyeye (2010) stated that roasted groundnut, used for peanut butter manufacturing, was more advantageous in mineral content than the raw ones. The average crude fibre content in this results (5 %), indicate an ability of peanut butter to maintain a normal intestinal tract because diet low in crude fibre may cause constipation and colon diseases. In view to their protein content (21 - 30 %), the studied peanut butters could be considered as a valuable source of protein in improving the nutrition status of humans (FAO/WHO, 2007). In addition, the lipid content of the marketed peanut butters falls within the range (41 - 48 %) reported by Makeri *et al.* (2011) for peanut paste prepared with two Nigerian cultivars of *Arachis hypogea*. This result suggested that peanut it is an interesting oleaginous crop for which the implementation should result in the economic well-being of rural people. Lipids also play a nutritionally role in providing essential fatty acids for humans. The relatively highest values of energy (560 - 640

kcal/100 g) of peanut butter samples analyzed in this study may be attributed to the highest values of their protein and fat contents. Moreover, these energy levels could cover the recommended energy for an adult, which is estimated to 800 kcal per day (FAO, 1973).

Fungal contamination: The fungal count of peanut butters, collected from markets, is given in Table 2. The mycoflora was mainly represented by eight genera: *Mucor*, *Alternaria*, *Helminthosporium*, *Geotrichum*, *Fusarium*, *Cladosporium*, *Penicillium* and *Aspergillus* (Table 2). The predominant fungi belonged to *Aspergillus* genus (20.22 – 51.65 %) followed by *Helminthosporium* (0 – 47.44 %) and *Penicillium* (0 – 41.54%). In addition, *Aspergillus* strains were isolated from all the samples analyzed whatever the commune. The peanut butter samples collected from Port-Bouet were more contaminated with *Helminthosporium* (44.44 ± 3.00 %) and *Geotrichum* (25.95 ± 2.00 %) while those collected from Treichville were more contaminated with *Alternaria* (31.11 ± 2.50 %) and *Fusarium* (26.66 ± 1.00 %). The highest occurrence of *Mucor* (30.23 ± 2.50 %) was noted for the samples collected from Cocody. The total fungi count enumerated ranged from 10^4 to 10^6 CFU/g with the highest value ($120 \pm 5.00 \times 10^4$ CFU/g) for the samples collected from Attécoubé. Other studies made in Kenya, Benin and Mali, have also revealed the occurrence of *Aspergillus*, *Fusarium* and *Penicillium* in peanut butters and other peanut products (Adjou et al., 2012; Keita et al., 2013; Ndong'u et al., 2013). The presence of *Aspergillus* sp. implies a risk of mycotoxin production and represents a health risk for the consumers (Sultan & Magan, 2010). According to Pittet (1998), the mycotoxins produced by *Aspergillus* sp. include aflatoxins and ochratoxin A (OTA). Mycotoxins have attracted worldwide attention due to the significant losses associated with their impact on human and animal health, and consequent national economic implications (Bhat & Vashanti, 1999). Mycotoxins can be acutely or chronically toxic or both

depending on the nature of toxins and the dose consumed. In human, acute diseases include liver and kidney damage, attack on central nervous system (CNS), skin diseases and hormonal effects. Among the mycotoxins, aflatoxins produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* are natural carcinogenic compound causing mutation (Deng & Ma, 1998). The contamination level of the toxigenic flavi fungi is depicted by the Figure 1. The highest occurrence of these fungi were 14,81; 13,95; 9,1 and 21,62 % for *A. versicolor*, *A. ochraceus*, *A. flavus* and *A. parasiticus*, respectively (Figure 1). The observed fungi contamination of the studied peanut butters exposing the consumers to a potential risk of acquiring food borne disease. Indeed, food borne illnesses of microbial origin are a major international health problem associated to food safety in developing countries (WHO, 2002). The high susceptibility of peanuts contamination is mainly due to their nutritional content, useful to numerous fungi. If the hulls, which protect the seed against invasion by fungi, become damaged, the underlying cotyledons become susceptible to attack. This contamination, mainly due to the injury of the hulls, is favoured by insect attack, drought occurring at the end of the vegetative cycle and poor post-harvesting practices (Adjou et al., 2013). Contamination of street-vended food such as peanut butter has been attributed to exposure to polluted environment, poor sanitation and poor hygienic practices by the vendors (Mensah et al., 2002). There have been several suggested interventions to improve the hygiene of street foods such as peanut butter, which includes (1) education and training programs for vendors, (2) the improvement of vendors' equipment for preparation and storage, (3) the provision of adequate sanitation and the adoption of HACCP system in order to improve the efficiency of the surveillance system by detecting the hazards and focusing on the critical control points (WHO, 2002).

Table 1: Proximate composition of peanut butter samples sold in retail markets of Abidjan.

Parameters	Township investigated								
	AB (n=5)	AD (n=5)	AT (n=5)	C (n=5)	K (n=5)	M (n=5)	P (n=5)	T (n=5)	Y (n=5)
Moisture (%)	4.17 ^b ±0.20	3.20 ^a ±0.20	4.30 ^b ±0.20	1.23 ^d ±0.20	2.37 ^c ±0.30	2.87 ^a ±0.06	2.43 ^c ±0.11	1.53 ^d ±0.11	4.17 ^b ±0.25
Dry matter (%)	95.70 ^a ±0.20	96.80 ^b ±0.20	95.83 ^a ±0.20	98.77 ^d ±0.20	97.63 ^c ±0.30	97.13 ^c ±0.06	97.57 ^c ±0.11	98.47 ^d ±0.11	95.83 ^a ±0.25
pH	6.72 ^g ±0.00	6.67 ^a ±0.01	6.69 ^f ±0.00	6.66 ^a ±0.01	6.44 ^d ±0.00	6.40 ^c ±0.01	6.26 ^b ±0.01	6.58 ^e ±0.01	6.70 ^f ±0.01
Acidity (meq/100g)	17.33 ^a ±1.15	15.30 ^{ac} ±1.15	16.67 ^a ±1.15	15.33 ^{ac} ±1.15	12.31 ^b ±1.15	11.30 ^b ±1.15	10.33 ^b ±1.15	14.63 ^{bc} ±1.15	17.30 ^a ±1.15
Ash (%)*	5.67 ^a ±0.80	4.67 ^a ±0.60	4.87 ^a ±0.60	5.16 ^a ±1.00	5.50 ^a ±1.20	5.82 ^a ±1.16	5.61 ^a ±0.80	4.86 ^a ±1.00	5.47 ^a ±1.31
Fibre (%)*	5.47 ^a ±0.50	5.33 ^a ±0.60	5.00 ^a ±0.00	5.63 ^a ±1.14	5.15 ^a ±0.28	5.15 ^a ±0.28	5.00 ^a ±0.00	5.47 ^a ±0.50	5.31 ^a ±0.50
Fat (%)*	49.32 ^c ±0.34	47.37 ^{ab} ±0.52	49.03 ^{bc} ±0.49	46.12 ^a ±0.43	47.12 ^a ±0.78	47.28 ^{ab} ±0.86	42.58 ^d ±0.93	49.03 ^{bc} ±0.87	47.39 ^{ab} ±0.97
Protein (%)*	26.54 ^{ab} ±2.53	26.55 ^{ab} ±1.34	24.79 ^{ab} ±1.34	27.42 ^{ab} ±0.51	25.67 ^{ab} ±1.34	23.92 ^a ±1.34	23.33 ^a ±2.20	28.58 ^b ±2.20	25.96 ^{ab} ±2.20
Carbohydrate (%)*	17.29 ^c ±2.87	21.40 ^a ±1.87	21.55 ^a ±1.34	22.66 ^b ±1.45	19.84 ^e ±1.30	21.24 ^a ±2.21	23.92 ^a ±2.28	22.52 ^b ±3.08	18.95 ^d ±3.18
Energy (kcal/100g)	619.90 ^h ±24.66	617.85 ^g ±17.56	625.70 ^a ±15.15	609.97 ^c ±13.78	613.47 ^e ±14.27	613.06 ^d ±21.94	590.66 ^b ±22.89	625.92 ^a ±29.01	615.26 ^f ±30.24

Data are represented as means ± SD (n=3). Mean with different letters in the same row are statistically different (p < 0.05) according to Duncan's test.

*: dry weight basis.

AB: Abobo, AD: Adjamé, AT: Attécoubé, C: Cocody, K: Koumassi, M: Marcory, P: Port-Bouet, T: Treichville, Y: Yopougon.

Table 2: Genera of fungi isolated from peanut butter samples sold in retail markets of Abidjan.

Fungi isolates (%)	Township investigated								
	AB (n=5)	AD (n=5)	AT (n=5)	C (n=5)	K (n=5)	M (n=5)	P (n=5)	T (n=5)	Y (n=5)
<i>Mucor</i>	6.00 ^a ±1.00	10.80 ^b ±0.80	7.70 ^c ±0.80	30.23 ^d ±2.50	0.00 ^g ±0.00	10.80 ^b ±0.80	7.41 ^c ±0.80	13.34 ^e ±0.80	9.10 ^f ±0.80
<i>Alternaria</i>	17.00 ^a ±1.00	21.05 ^b ±1.00	11.11 ^c ±0.80	0.00 ^g ±0.00	18.29 ^d ±1.00	0.00 ^g ±0.00	0.00 ^g ±0.00	31.11 ^e ±2.50	20.45 ^f ±1.00
<i>Helminthosporium</i>	6.00 ^a ±0.00	0.00 ^d ±0.00	13.67 ^b ±1.00	6.98 ^a ±1.00	0.00 ^d ±0.00	0.00 ^d ±0.00	44.44 ^c ±3.00	0.00 ^d ±0.00	7.95 ^a ±1.00
<i>Geotrichum</i>	0.00 ^g ±0.00	12.63 ^a ±1.00	15.38 ^b ±1.00	0.00 ^g ±0.00	0.00 ^g ±0.00	8.12 ^c ±1.00	25.93 ^d ±2.00	6.67 ^e ±1.00	3.41 ^f ±0.00
<i>Fusarium</i>	5.00 ^a ±0.00	0.00 ^d ±0.00	0.00 ^d ±0.00	0.00 ^d ±0.00	15.85 ^b ±1.00	0.00 ^d ±0.00	0.00 ^d ±0.00	26.66 ^c ±1.00	0.00 ^d ±0.00
<i>Cladosporium</i>	3.00 ^a ±0.00	0.00 ^d ±0.00	6.84 ^b ±1.00	0.00 ^d ±0.00	7.32 ^c ±1.00	0.00 ^d ±0.00	0.00 ^d ±0.00	0.00 ^d ±0.00	0.00 ^d ±0.00
<i>Penicillium</i>	28.00 ^a ±1.00	33.68 ^b ±2.50	18.80 ^c ±1.00	39.54 ^d ±2.00	31.71 ^b ±2.00	32.43 ^b ±2.00	0.00 ^f ±0.00	0.00 ^f ±0.00	27.27 ^e ±2.00
<i>Aspergillus</i>	35.00 ^a ±2.50	22.11 ^b ±1.00	26.50 ^c ±1.00	23.25 ^d ±1.00	26.83 ^e ±1.00	48.65 ^f ±3.00	22.22 ^g ±2.00	22.22 ^g ±2.00	31.82 ^h ±2.00
Total (CFU/g X 10⁴)	100 ^a ±5.00	95 ^b ±5.00	120 ^c ±5.00	43 ^d ±3.00	82 ^e ±3.00	37 ^f ±3.00	27 ^g ±2.00	45 ^h ±3.00	88 ⁱ ±3.00

Data are represented as means ± SD (n=3). Mean with different letters in the same row are statistically different (p < 0.05) according to Duncan's test.

AB: Abobo, AD: Adjamé, AT: Attécoubé, C: Cocody, K: Koumassi, M: Marcory, P: Port-Bouet, T: Treichville, Y: Yopougon.

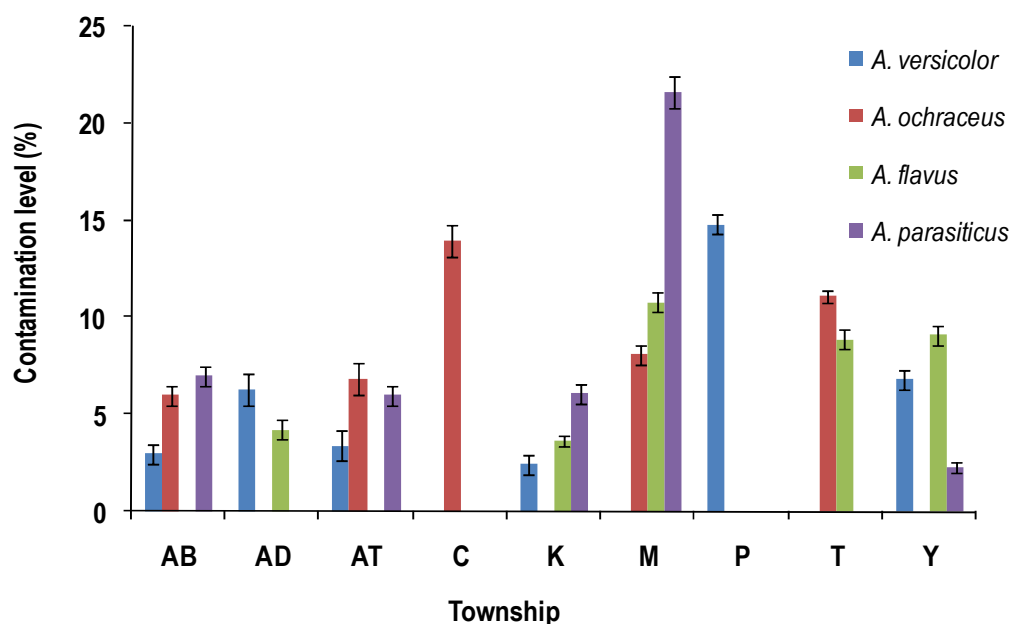


Figure 1: Level of mycotoxigenic *Aspergillus* species isolated from peanut butter samples sold in retail markets of Abidjan.

AB: Abobo, AD: Adjamé, AT: Attécoubé, C: Cocody, K: Koumassi, M: Marcory, P: Port-Bouet, T: Treichville, Y: Yopougon.

CONCLUSION

The studied peanut butters sold in retail markets of Abidjan are nutritive and could meet the dietary needs of the population when consumed with other foods. Indeed, these peanut butters constitute a valuable sources of protein, fat, crude fibre and minerals. However, the presence of toxigenic fungi as *Aspergillus flavus*, *Aspergillus ochraceus* and

Aspergillus parasiticus in these peanut products highlight a potential public health problem concerning the consumption by the consumers. Therefore, the need to educate both sellers and consumers on processing, food handling procedures and personal hygiene would help to minimize fungal contamination in order to obtain good sanitary peanut butters.

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