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Mycobiota of cocoa: From farm to chocolate

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ABSTRACT

The present work was carried out to study the mycobiota of cocoa beans from farm to chocolate. Four hundred and ninety-four samples were analyzed at various stages of cocoa processing: (i) primary stage at the farm (fermentation, drying, and storage), (ii) secondary stage at processing (testa, nibs, liquor, butter, cake and powder) and (iii) the final chocolate product (dark, milk, white and powdered) collected from retail outlets. Direct plating or dilution plating on Dichloran 18% Glycerol agar were used for cocoa beans and processed product analyses, respectively. Fungi were isolated and identified using different keys of identification. The largest numbers and diversity of fungi were observed in the samples collected at the farm, especially during drying and storage. The species with the highest occurrence among samples were: *Absidia corymbifera*, *Aspergillus* sp. *nov.*, *A. flavus*, *Penicillium paneum* and yeasts. A total of 1132 potentially toxigenic fungi were isolated from the following species or species groups: *A. flavus*, *Aspergillus parasiticus*, *Aspergillus nomius*, *Aspergillus niger* group, *Aspergillus carbonarius* and *Aspergillus ochraceus* group. The highest percentage of toxigenic fungi was found at the drying and storage stages. The industrial processing reduced the fungal contamination in all fractions and no fungi were found in the final chocolate products. The knowledge of which fungi are dominant at each processing stage of cocoa provides important data about their ecology. This understanding leads to a reduction in fungal spoilage and mycotoxin production in this product.

1. Introduction

Chocolate quality may be influenced by a variety of environmental, agronomic and technological factors to which the cocoa beans are subjected from the opening of the fruit until the end of industrial processing. Among these factors, it is known that the microorganisms present in the fermentation play an essential role in the development of the sensorial characteristics of chocolate (Rohan, 1964; Schwan and Wheals, 2004; Afoakwa et al., 2008). The intensity of microbial proliferation and the presence of one or another group of microorganisms in the fermentation and drying steps are crucial for the development of particular characteristics in cocoa beans leading to a final product of good quality (Schwan and Wheals, 2004).

In general the processing of cocoa starts at the farm, where the primary processing is carried out. Following the opening of freshly harvested pods, the beans are placed in wooden boxes, allowing natural fermentation for about 6 days. After fermentation, beans are transferred to sun drying platforms where a gradual reduction in moisture content occurs. When moisture content reaches values sufficiently low (6–7%), beans are transferred to storage rooms, then later bagged and marketed. At the manufacturing stage or secondary processing, the dried fermented cocoa beans are roasted and grinded and then follow two distinctive processing lines giving as final product powdered cocoa or chocolate (Wood, 1985).

Research has shown a steady and marked presence of fungi during the fermentation process and during the drying and storage steps (Ribeiro et al., 1986; Ardhana and Fleet, 2003; Mounjouenpou et al., 2008; Sanchez-Hervas et al., 2008). When present, filamentous fungi occur with higher intensity in the last days of fermentation and are usually related to the formation of off flavors and deterioration (Schwan and Wheals, 2004).

Besides the deteriorative potential and consequent influence on sensorial quality of cocoa and chocolate, the presence of fungi in food is also a public health issue due to the possibility of mycotoxin formation. *Aspergillus*, *Penicillium* and *Fusarium* are the main genera capable of producing toxic secondary metabolites, many of which

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have mutagenic, teratogenic and carcinogenic effects on humans and animals (IARC, 1993). In a previous study, Copetti et al. (2010) when evaluating whole cocoa beans found the presence of ochratoxin A at all stages of cocoa processing on the farm, with the major incidence during drying (<0.01–5.54 μg/kg) and storage (<0.01–4.64 μg/kg). In that study, the capability for production of ochratoxin A by the isolates of potentially ochratoxigenic species was evaluated, finding out that all *Aspergillus carbonarius* and *Aspergillus ochraceus* group isolates were ochratoxin A producers whereas only about 5% of *Aspergillus niger* presented such ability.

Currently, data on mycobiota occurrence during the different periods of the primary processing of cocoa under natural conditions and on fungal contamination in the products of secondary processing are limited. Therefore, the aim of this research was to evaluate the mycobiota of cocoa during the processing steps and in the final chocolate product, correlating the results with the levels of water activity (a_w) in the products.

2. Methods

2.1. Samples

A total of 494 samples of cocoa, corresponding to the stages of primary processing on grower farms (226 samples), secondary processing at the manufacturing plants (168 samples) and chocolate sold on the market in the form of powder and bars (100 samples) were evaluated.

The 226 samples of integral cocoa beans (beans and pulp) (not less than 200 g each sample), from the primary processing stage, were collected in the cocoa producing region of Ilheus-Bahia (Brazil). The cocoa samples came from three major farms and represented the different stages of production: 25 samples before fermentation, 51 samples with different times of fermentation (1–6 days), 85 samples collected at different times of sun drying on movable roof platforms (1–14 days) and 65 samples during the storage period (up to one year).

One hundred and sixty-eight samples from secondary processing were evaluated at the different stages of cocoa processing. The cocoa samples were collected as follows: 19 testa (shell) samples, 29 of nibs, 25 of liquor, 25 of cocoa butter, 26 of cake and 44 of powder. These samples came from three Brazilian manufacturers located in the same region of the farms.

The 100 samples of chocolate were acquired from the market, corresponding to dark (25), milk (25) and white (25) chocolate in bars; and in powder (25).

2.2. Analysis of water activity

The water activity of samples was directly determined in triplicate in an Aqualab, Series 3TE instrument (Decagon, USA), at 25 ± 0.1 °C. Each sample was placed in a disposable cup, sealed in the analytical chamber until vapor equilibrium was reached, and had the dewpoint detected by infrared.

2.3. Mycobiota of cocoa beans

The cocoa beans were superficially disinfected by immersion in a solution of sodium hypochlorite 0.4% for 2 min to remove the surface contaminants. Then 11 beans were placed on 3 Petri plates containing agar Dichloran Glycerol 18% with Chloramphenicol (DG18) (pH 5.6), with a total of 33 beans analyzed per sample. The plates were incubated at 25 °C for 7 days and the results expressed as a percentage of beans infected internally, according to Pitt and Hocking's (2009) methodology.

For the samples of industrially processed cocoa, the technique of dilution plating was applied. Under aseptic conditions, 25 g of each sample were weighed and 225 mL of sterile peptone water 0.1% were added. Then, aliquots of the serial dilutions were prepared and inoculated on plates containing DG18. The plates were incubated at 25 °C for 7 days and the results expressed in colony forming units per gram of sample (CFU/g), according to Pitt and Hocking's (2009) methodology.

After incubation, the plates were examined and all the fungal species were first isolated on Petri plates containing Czapek Yeast Extract Agar (CYA) to be later identified by specific protocols for each genus.

2.4. Identification of fungi

The isolated *Aspergillus* sp. and *Penicillium* sp. were grown in Czapek yeast autolysate (CYA) agar and Malt extract agar (MEA). The genus *Penicillium* was identified according to Pitt (2000) and Samson et al. (2002), and identification of species in the genera *Aspergillus* and *Eurotium* was performed according to Klich and Pitt (1988), Samson et al. (2004) and Frisvad et al. (2004). The other isolates were identified according to descriptions of Pitt and Hocking (2009) and Samson et al. (2002), supplemented with other sources when necessary.

3. Results

3.1. Fungi and water activity during the primary processing of cocoa

Table 1 shows the values of a_w found during the primary processing of cocoa beans at the farm. The a_w values showed a large decrease between the stages, with the biggest variation observed during the sun drying. The frequency of occurrence and the level of infection by each fungal species isolated during fermentation, drying and storage of cocoa are presented in Table 1. A considerable difference in fungal presence, both qualitative and quantitative, was observed between the processing stages.

3.1.1. Fermentation

During the six days of fermentation the fungi most commonly isolated were: yeasts, *Monascus ruber*, *Penicillium paneum*, *Geotrichum candidum* and *Absidia corymbifera* (Table 1). In this period, the a_w of cocoa beans was in the range of 0.99–0.98 (Table 1). In general, filamentous fungi were found at low levels during this stage.

3.1.2. Drying

At the end of the fermentation the beans were transferred to the sun drying platforms, although some farms also adopt drying in artificial dryers. In this work the samples correspond to beans collected exclusively at sun drying, which usually takes 7–14 days or more, depending on weather conditions. The fungi most frequently isolated during drying were: *Abs. corymbifera*, *P. paneum*, yeasts, *Aspergillus* sp. *nov.* (related to *A. tamarii*), *A. flavus*, *A. parasiticus*, *A. candidus*, *A. niger* and *Eurotium chevalieri* (Table 1). The new species may be the same as *A. cacao* (*nomen nudum*), mentioned by Raper and Fennell (1965), but this species has never been validly described.

During this period the a_w values declined from about 0.99 found on the first day to around 0.49 at the end of the period (Table 1). This huge variation was also reflected in the diversity of the fungi isolated. At the beginning of drying, the beans showed a higher a_w, so there was a predominance of fungi found at the end of fermentation (*Abs. corymbifera*, *P. paneum* and yeasts). With a reduction in a_w the mycobiota started to change to fungal species typically

Table 1
Frequency of occurrence, mean and variation in the level of infection of cocoa beans by fungi during fermentation, sun drying and storage.

Processing stage (number of samples)	Fermentation (51)			Sun Drying (85)			Storage (65)		
Mean a_w (range)	0.99 (0.98–0.99)			0.81 (0.49–0.99)			0.65 (0.40–0.85)		
Fungi	FO (%)	AI (%)	RI (%)	FO (%)	AI (%)	RI (%)	FO (%)	AI (%)	RI (%)
Absidia corymbifera	15.69	4.94	ND – 97	73.17	26.19	ND – 100	69.23	31.83	ND – 100
Aspergillus candidus	1.96	0.06	ND – 3	20.00	2.19	ND – 27	18.46	1.43	ND – 21
A. carbonarius	1.96	0.06	ND – 3	5.88	1.48	ND – 45	9.23	2.40	ND – 66
A. clavatus	0	ND	ND	1.18	0.04	ND – 3	0	ND	ND
A. flavus	3.92	0.16	ND – 6	37.64	11.30	ND – 100	32.31	7.74	ND – 100
A. fumigatus	3.92	0.65	ND – 12	11.76	1.69	ND – 66	1.54	0.09	ND – 6
A. niger group	3.92	0.24	ND – 9	16.47	2.65	ND – 60	26.15	4.15	ND – 51
A. ochraceus group	0	ND	ND – 0	5.88	0.28	ND – 9	3.08	0.09	ND – 3
A. parasiticus	1.96	0.10	ND – 6	24.39	5.65	ND – 78	13.85	2.22	ND – 48
A. penicillioides	0	ND	ND	0	ND	ND	15.39	2.20	ND – 60
Aspergillus sp. *nov.*	3.92	0.24	ND – 9	48.24	18.25	ND – 100	41.54	14.20	ND – 100
A. sydowii	3.92	0.06	ND – 3	2.35	0.35	ND – 24	18.46	2.58	ND – 42
A. ustus	ND	ND	ND	0	ND	ND	1.54	0.05	ND – 3
A. versicolor	1.96	0.06	ND – 3	0	ND	ND	6.15	1.71	ND – 57
Ascomycetes	0	0.12	ND – 3	1.18	0.04	ND – 6	0	ND	ND
Cladosporium sp.	0	ND	ND	0	ND	ND	1.54	0.05	ND – 3
Dematiaceous hyphomycetes	0	0.06	ND – 3	4.70	3.09	ND – 100	6.15	0.28	ND – 9
Emericella nidulans	0	ND	ND	0	ND	ND	3.08	0.18	ND – 9
Eurotium amstelodami	5.88	0.84	ND – 27	8.23	0.51	ND – 10	35.38	12.32	ND – 100
E. chevalieri	0	ND	ND	12.94	1.13	ND – 27	21.54	2.97	ND – 66
E. rubrum	0	ND	ND	9.41	0.92	ND – 18	29.23	7.37	ND – 100
Eupenicillium sp.	0	ND	ND	0	ND	ND	1.54	0.51	ND – 33
Fusarium solani	0	ND	ND	1.18	0.04	ND – 3	0	ND	ND
Geotrichum candidum	19.61	9.14	ND – 100	10.59	5.10	ND – 100	6.15	0.78	ND – 21
Monascus ruber	25.49	3.69	ND – 33	4.70	0.56	ND – 24	10.77	1,66	ND – 60
Mucor sp.	5.88	0.53	ND -21	1.18	0.11	ND – 9	3.08	0.23	ND – 9
Neosartorya fischeri	0	ND	ND	1.18	0.11	ND – 6	1.54	0.09	ND – 6
Paecilomyces variotii	3.92	0.24	ND – 12	4.70	0.41	ND – 21	10.77	0.69	ND – 12
Penicillium citrinum	0	ND	ND	8.23	2.96	ND – 39	7.69	1.75	ND – 51
P. fellutanum	0	ND	ND	0	ND	ND	1.54	0.51	ND – 33
P. paneum	23.53	2.69	ND – 27	58.82	17.31	ND – 100	16.92	3.37	ND – 66
Rhizopus sp.	1.96	0.16	ND – 6	9.41	1.06	ND – 33	10.77	0.74	ND – 12
Syncephalastrum sp.	1.96	0.06	ND – 3	0	ND	ND	15.38	2.82	ND – 42
Wallemia sebi	0	ND	ND	0	ND	ND	1.54	0.32	ND – 21
Yeasts	41.18	28.12	ND – 100	48.23	20.33	ND – 100	9.23	3.42	ND – 69

FO = Frequency of occurrence % (number of samples that contained a fungal species/total of samples evaluated, %); AI = Average of infection (sum of infection level/total number of samples); RI = Range of Infection % (Range of infected beans in a sample, %); ND = Not Detected.

isolated from intermediate moisture foods, such as the genus *Aspergillus* and xerophilic fungi such as *A. candidus* and *E. chevalieri*, especially after some of the volatile acetic acid had evaporated.

In the course of drying, an increased occurrence of *Aspergillus* section *Flavi* and *Aspergillus* section *Nigri* was also observed, which are important groups of potentially aflatoxin and ochratoxin producers, respectively. The prevalence of beans infected with *A. flavus* reached 100% in some samples. This aflatoxigenic species was isolated from 11.3% of drying samples. The values found for *Aspergillus parasiticus* were lower. Regarding the potentially ochratoxigenic fungi, *A. niger* was the most prevalent species, followed by *A. carbonarius* and the group of *A. ochraceus* at a lower number (Table 1). At one stage of the drying process, these species were exposed to moisture and temperature that may allow mycotoxin production.

3.1.3. Storage

In stored samples, there was an increase in the number of xerophilic species of *Eurotium* such as *E. amstelodami*, *E. chevalieri* and *E. rubrum*. *Aspergillus penicillioides* was primarily isolated at this stage, reaching up to 100% of infection in some samples. The water activity decreased from 0.85 to 0.40 (Table 1). A small increase in the diversity of the species *Aspergillus* and *Penicillium* was noticed. The number of yeasts and *G. candidum* suffered a drastic reduction, while *Abs. corymbifera* persisted.

3.2. Fungal contamination and water activity during industrial processing and in the final product

The number of fungi isolated from samples after industrial processing was low ($<10–10^3$ CFU/g, data not shown), mostly corresponding to xerophilic species. Table 2 presents the isolated species, as well as the number of samples in which they were present.

The cocoa testa was the industrial product with the highest fungal contamination, basically by the same species previously isolated from stored beans. *Abs. corymbifera*, *E. amstelodami* and *A. flavus* were the predominant species.

Despite the heat treatment to which the samples were submitted, 55% of the nib samples analyzed showed the presence of some fungal species, following a pattern of species similar to that found in the shell.

The liquor fraction and butter showed low contamination and fungi were not detected in the cake samples. On the other hand, fungi were detected in approximately one third of the cocoa powder samples analyzed in this study.

No fungi were isolated from the 75 samples of chocolate bars evaluated. Of the 25 samples of powdered chocolate analyzed, only one showed fungal contamination (10^4 CFU/g) and the species isolated were: *E. amstelodami*, *E. rubrum*, *A. niger*, *A. flavus*, *Paecilomyces variotii*, *Penicillium citrinum* and *Aspergillus* sp. *nov.*

Table 2
Fungal contamination in cocoa's secondary processing products from industry.

Product (a/b)	Shell (19/19)	Nibs 16/29)	Liquor (4/25)	Butter (1/25)	Cake (0/26)	Powder (11/44)
Abs. corymbifera	14/19	7/29	–	–	–	1/44
A. candidus	–	1/29	–	–	–	1/44
A. flavus	11/19	8/29	1/25	–	–	3/44
A. niger	8/19	8/29	–	–	–	2/44
Aspergillus sp. *nov*	7/19	2/29	–	–	–	1/44
A. sydowii	1/19	1/29	–	–	–	2/44
A. terreus	3/19	–	–	–	–	1/44
A. versicolor	2/19	2/29	–	–	–	–
Cladosporium sp.	–	1/29	–	–	–	–
E. amstelodami	12/19	7/21	–	1/25	–	4/44
E. chevalieri	10/19	11/21	–	–	–	–
E. rubrum	7/19	5/21	–	–	–	–
Em. Nidulans	5/19	7/29	–	–	–	–
Yeast	3/19	–	3/25			–
Paecilomyces sp.	8/19	–	–	–	–	2/44
P. citrinum	3/19	–	–	–	–	2/44
P. paneum	1/19	–	–	–	–	–
P. variabile	–	–	–	–	–	1/44

a/b = number of samples with fungi/total number of samples evaluated.

Table 3 shows the a_w found in the different products. The a_w values of industrial and commercial samples ranged between 0.18 and 0.62.

4. Discussion

4.1. Fungi and water activity during the primary processing of cocoa

This study confirmed the relationship between the availability of water in the food and the ability of microorganisms to grow.

4.1.1. Fermentation

According to Schwan and Wheals (2004) cocoa pulp inside a not injured cocoa pod is microbiologically sterile but is subsequently contaminated with microorganisms when the fruit is opened. These microorganisms mainly come from the pod surface, workers' hands and knives, insects, baskets used to transport the beans and pulp and contribute to the subsequent spontaneous fermentation process (Schwan and Wheals, 2004) The role of filamentous fungi in the microbial succession of cocoa fermentation is not totally known. It is believed that an extensive fungal development at the end of fermentation causes increased deterioration in the consecutive phase of drying (Gilmour and Lindblom, 2008). It is known that these microorganisms can cause hydrolysis of the pulp, produce acids, off flavors and alter the taste of the cocoa beans (Schwan and Wheals, 2004). Besides this, some fungal species can also produce mycotoxins in cocoa (Petithuguenin, 2002; Gilmour and Lindblom, 2008; Copetti et al., 2010, 2011).

Considering some physiological aspects of the more prevalent fungi isolated at fermentation, Pitt and Hocking (2009) remark that *G. candidum*, a yeast-like fungus, grows well under microaerophilic conditions and high a_w and *M. ruber* and *P. paneum* are also adapted to environments of low oxygen pressure and show good growth under these fermentation conditions. *P. paneum* is a species closely related to *Penicillium roqueforti*, which has an optimum pH for growth between 4.0 and 5.0, and tolerates high levels of CO_2 (Taniwaki et al., 2001, 2010), as well as organic acids commonly found in the fermentation of cocoa (Vivier et al., 1992). Both species were also present in the mycobiota of other foods fermented by lactic or acetic acid bacteria (O'Brien et al., 2006; Storm et al., 2008).

The low level of filamentous fungi isolated during cocoa bean fermentation can be explained not only by the high population of bacteria and yeasts, which have competitive advantage because of their higher speed of multiplication and metabolites produced during their growth (i.e. alcohol and organic acids) but also because of the restrictive temperature, as this can rise above 45 °C after 48 h. However, in our study, one exception was the high prevalence of *Abs. corymbifera* in 8 samples. This species of zygomycetes is able to grow at temperatures of up to 54 °C (Pitt and Hocking, 2009).

A few studies have been carried out on the presence of fungi during cocoa fermentation. Ribeiro et al. (1986) studying filamentous fungi from cocoa fermentation in Brazil isolated the following species: *Aspergillus fumigatus*, *A. niger*, *Fusarium verticillioides*, *Fusarium oxysporum*, *Lasiodiplodia theobromae*, *Mucor racemosus*, *Mucor* sp., *P. variotii*, *P. citrinum*, *Penicillium implicatum*, *Penicillium spinulosum*, *Thielaviopsis ethaceticus*, *Trichoderma viride* and *Mycelia sterilia*. In our study we found some species described by those researchers; although others were not isolated. However, the taxonomy of *Penicillium* and *Aspergillus* has been significantly updated the last decade and so has the knowledge on the influence of acetic acid on the fungal mycobiota (Samson et al., 2010). In the Ribeiro et al. (1986) study the acetic acid tolerant *P. variotii* and *T. ethaceticus* were found, whereas we found other acetic acid tolerant species, such as *M. ruber*, *P. paneum* and *Byssochlamys nivea*. Likewise we found other species on cocoa with less acetic acid and lowered water activity. Another difference is the isolation methods and media used by Ribeiro et al. (1986), which differ from ours.

Mounjouenpou et al. (2008), reported the occurrence of filamentous fungi in an experiment where cocoa was processed under controlled conditions in Cameroon. Under conditions similar to those adopted on the farms evaluated in this study, the authors described the presence of *Aspergillus. versicolor*, *A. fumigatus*, *A. tamarii*, *Rhizopus nigricans*, *Fusarium* sp. and *A. niger* isolated at the end of the fermentation period. Some of the mentioned species were also isolated in our study, suggesting the existence of typical mycobiota present in cocoa, even from different continents.

Table 3
Water activity values of cocoa products from secondary processing at industry and chocolate from retail outlets.

Samples	Number of samples	Water activity (a_w)	
		Mean	Variation
Industry	**168**	**0.42**	**0.18–0.63**
Shell	19	0.48	0.32–0.63
Nibs	29	0.47	0.30–0.61
Liquor	25	0.38	0.18–0.62
Butter	25	0.49	0.42–0.55
Cake	26	0.32	0.24–0.48
Powder	44	0.39	0.19–0.59
Market	**100**	**0.44**	**0.21–0.58**
Powdered chocolate	25	0.51	0.43–0.58
Dark chocolate	25	0.41	0.21–0.51
Milk chocolate	25	0.40	0.34–0.54
White chocolate	25	0.44	0.35–0.55

The bold values in the table summarizes the data showed in details in the following lines.

4.1.2. Drying

The sun drying period has great importance for fungal development. At this stage there is a gradual reduction in water content of cocoa beans. With a reduction in a_w to below 0.88 the interruption of most bacteria and yeast growth occurs, although there is still enough water to sustain fungal multiplication (Beuchat, 1987).

In this work, the increase of *Aspergillus* section *Flavi* and *Aspergillus* section *Nigri* was shown in this phase of cocoa processing. These are important groups of potentially aflatoxin and ochratoxin producers, respectively. In studies carried out in Brazil,

Copetti et al. (2010, 2011) found a strong correlation between the presence of *A. carbonarius* and the level of ochratoxin A contamination in the samples, but only a weak correlation was found between the occurrence of potentially aflatoxigenic fungi and aflatoxin in the samples. Mounjouenpou et al. (2008) also reported higher OTA content in cocoa samples where *A. carbonarius* had been isolated, in Cameroon.

4.1.3. Storage

The xerophilic species that presented an increase in stored samples were reported as those responsible for considerable economic losses and common contaminants of stored grains, nuts, spices and cereal products (Samson et al., 2002; Pitt and Hocking, 2009). They have little importance regarding mycotoxin production according to Pitt and Hocking (2009), but long periods of storage of cocoa seeds should be avoided. Wood (1985) recommended that cocoa beans in tropical countries can have safe storage only for 2 or 3 months after which there is a problem of mold development. This happens because cocoa beans are hygroscopic and will absorb moisture under humid conditions until they reach equilibrium. In adverse conditions, such as low a_w, the fungal spores can remain viable for long periods, even without vegetative growth of hyphae. Storage under poor conditions, in which the cocoa is subjected to environments with high humidity, can provide suitable conditions for spore germination, fungal growth and decay, besides the possibility of toxin production. According to the same author, cocoa beans with a moisture content of 8% or more will turn moldy.

Research by Sanchez-Hervas et al. (2008) evaluated the mycobiota of 9 samples of cocoa stored from Sierra Leone, Equatorial Guinea and Ecuador and revealed the predominance of *Aspergillus* sections *Flavi* and *Nigri*. The mycobiota was similar to that found in our study, although the frequency of occurrence of the species was a little different, especially for xerophilic fungi.

4.2. Fungal contamination and water activity during industrial processing and in the final product

The low level of fungi in the final product is a result of industrial processing (roasting, grinding, pressing and alkalizing) which basically consists of technological processes that change the cocoa beans into products which actually taste like chocolate and at the same time reduces microbiological contaminants (Beckett, 2008). After pre-roasting of the beans, the testa is almost completely removed by winnowing (Minifie, 1999), which represents a significant reduction in the amount of contaminant microorganisms in the subsequent stages (ICMSF, 2005). The high temperature employed during the roasting and the procedures used to obtain nibs and liquor, coupled with the remaining moisture in the beans, should kill the microorganisms which can be present (Beckett, 2008). Treatments from 15 min to 2 h and 105–150 °C in this stage of chocolate production are capable of completely destroying the vegetative cells of microorganisms (ICMSF, 2005).

The low contamination found in the liquor and cake was expected due to thermal treatment/alkalization they were subjected to. In this process nibs, liquor, cake or cocoa powder are heated with alkali at temperatures of 85–115 °C, which produces a strong sterilizing effect due to the combined action of water, alkali and temperature (Minifie, 1999). According to this author, the cocoa powder's final microbiota is almost exclusively introduced during the subsequent stages of processing. The few isolated species were similar to those found in stored beans, suggesting a post-processing contamination. The a_w values of industrial and commercial samples are not conducive to microbial growth. There have been reports of development of xerophilic fungi such as *Bettsia alvei* (teleomorph of *Chrysosporium farinicola*), *Chrysosporium xerophilum* and *Neosartorya glabra* in deteriorated chocolate and chocolate confetti (ICMSF, 2005; Kinderlerer, 1997). Usually these episodes were due to inadequate storage problems arising from the formation of an environment with increased availability of water at the interface of the packaging and chocolate when it was stored in environments with high relative humidity (ICMSF, 2005).

5. Conclusion

The mycobiota encountered during the processing of cocoa beans were diverse and composed of at least 20 genera of filamentous fungi and yeasts. Two groups of fungi were predominant: those that tolerate the antifungal effect of acetic acid, and those that thrive when most of the acetic acid is evaporated. When there were still substantial amounts of acetic acid and carbon dioxide in the product, known acetic acid tolerant fungi, such as *P. paneum*, *P. variotii* and *Monascus* were present, but these species were less common on cocoa beans when the acetic acid disappeared and the water activity decreased. Regarding the presence of potentially toxigenic species and levels of water activity required for their multiplication and production of mycotoxins, the data found in this study suggest the drying stage as critical for mycotoxin production. The occurrence of xerophilic fungi increased in storage, thus they can be considered potential spoilers at this stage. With respect to the industrial processing, the water activity values found in processed products was rather low, not allowing for microbial growth.

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