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Enteropathogens in cocoa pre-processing

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ABSTRACT

The European community lists cocoa among the products associated with major salmonellosis outbreaks in humans. Though cocoa products are not the only ingredients that may introduce *Salmonella* into chocolate, they have been implicated as the most prominent potential source of some outbreaks. The objective of this study was to investigate the presence of *Salmonella, Escherichia coli* and the level of total coliforms throughout the four different steps of cocoa pre-processing. The presence of *Salmonella* was detected in only one of the 119 samples analyzed – a sample of stored beans. Contamination by total coliforms and *E. coli* was highest during drying and storage, with percentages of up to 100% and 89% of positive samples. The environment, including the presence of vectors, intense handling and storage conditions appear to be the main critical points during pre-processing of cocoa contributing to contamination by these enteropathogens.

1. Introduction

The agro-industrial production of cocoa can be broken down into four basic steps: (1) cocoa growing on farms, which comprises the growing, harvesting and pre-processing of the cocoa pods, seeds and beans; (2) the primary cocoa processing plants or cocoa grinding facilities that transform cocoa beans into semi-finished products (cocoa liquor, cocoa butter, cocoa cake and cocoa powder and expeller cake or cocoa dust); (3) the *couverture* chocolate industry, which through appropriate production processes transforms semi-finished cocoa products into *couverture* chocolate; and (4) the chocolate industry, which uses *couverture* chocolate as raw material to manufacture finished chocolate products (chocolate candies, bars, tablets, figures, chocolate confectionery, etc.) (Garcia, 2003).

From a public health standpoint, the microorganisms most likely to adversely affect the quality of chocolate are toxigenic fungi and *Salmonella*. With regard to *Salmonella*, the European Community (EC, 2003) lists chocolate among the products associated with major salmonellosis outbreaks in humans that spread across several countries and affected large numbers of people. Though cocoa products are not the only ingredients that may introduce *Salmonella* into chocolate, they have been implicated as the most prominent potential source of some outbreaks (dried cocoa beans, cocoa powder).

The first step of cocoa pre-processing consists in opening the cocoa pods and fermenting the pulp and seeds. Before the ripe cocoa pods are opened, the pulp and seeds inside are virtually sterile. Contamination occurs when the fruits are cut open – typically with a machete – infecting the material with a wide variety of microorganisms. These microorganisms are transferred from an array of sources, including knives and machetes, the hands of workers, the baskets used to collect and transport the seeds, as well as dried mucilage left on the walls of boxes from previous fermentations. Part of this microbiota is responsible for the natural fermentation of the pulp, a process that usually lasts 6–7 days and follows a well-established microbial succession pattern. In the early stages of the fermenting process, yeasts produce ethanol and secrete enzymes that break down pectin. In the next phase, lactic bacteria proliferate with the production of lactic acid, followed by acetic bacteria that produce acetic acid and cause a rise in temperature. The next stage is typically characterized by the predominance of aerobic spore-forming bacteria that increase the pH, while in the final stages of the process filamentous fungi may appear at the surface. At the end of fermentation, the pH will be around 5.0 while during the process the temperature may go up as high as nearly 50 °C.

Upon completion of fermentation, the seeds are transferred to "drying barges" (broad, wooden-floored drying platforms with a retractable roof), where they remain until the sun-drying process is complete. The barges are covered with a roof to protect the beans from the rain and damp night air. The duration of this drying stage depends strongly on the weather conditions. In rainy periods, the drying barges are sheltered by the retractable roof structure and the drying time usually increases from 6 to 10 days. During this stage, the seeds are frequently moistened to help remove the remaining mucilage from their surface. This is done by workers

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who shuffle the cocoa beans about with their feet. Drying can also be done in driers, provided the temperature does not exceed 60 °C for a minimum drying time of at least 48 h – the minimum time required for excess acid to volatilize and a certain degree of oxidation to occur, both of which are desirable to produce a finished product with optimal quality characteristics (ICMSF, 2000).

After drying, the beans are selected by hand, packed in special protective bags and stored in sheds and barns before being shipped to the processing industries. The stored bags are placed directly onto the floor and the sanitary conditions of the storage area are not always the most appropriate, subjecting the beans to contamination by vectors such as insects, rodents and birds.

Control of raw material is considered essential to prevent *Salmonella* in chocolate (Cordier, 1994; D'Aoust, 1977; ICMSF, 1988). Nonetheless, there is still a huge gap in the scientific data and information available concerning contamination of primary ingredients. Even greater is the lack of information on the points of entrance for this pathogen to these ingredients. Contamination with *Salmonella* in the stage prior to fermentation is not unexpected, given the prevailing harvesting practices and the intensity with which the material is handled by hand. There are, however, hardly any studies available on contamination during this stage. For that reason, the objective of this study was to evaluate the presence of *Salmonella,* total coliforms and *Escherichia coli* throughout the different steps of coca pre-processing, from the moment the pods are cut open to when the dried fermented beans are ready to be shipped to chocolate manufacturers.

2. Material and methods

2.1. Sampling

Analyses were performed on 30 samples of coca seeds before fermentation, 30 samples of seeds during fermentation, 30 samples of beans during drying and 29 samples of stored dried fermented cocoa beans, totaling 119 samples.

The samples were collected from three different cocoa farms located in the southern part of the state of Bahia, Brazil. The samples were placed in sterile bags and subsequently stored in ice in expanded polystyrene boxes before being transported to the Food Technology Institute in Campinas/SP, Brazil, where they were analyzed.

2.2. Test methods

Salmonella analysis was performed according to the method of the Food and Drug Administration (Andrews & Hammack, 2005). Pre-enrichment was performed in buffered peptone water (BPW), taking great care not to break or fragment the seeds, since their inner part contains compounds that are toxic to *Salmonella*. Next, the sample materials were enriched in Rappaport–Vassiliadis and tetrathionate broth and subsequently plated onto Bismuth Sulphite Agar, Hecktoen enteric agar, and Xylose Lysine Deoxycholate agar. Suspect colonies were confirmed by biochemical and serological tests.

Total coliforms and *E. coli* counts were determined by the most probable number (MPN) method, described in the *Compendium of methods for microbiological examination of foods* (Downes & Ito, 2001). The presumptive test was performed using Lauryl Sulfate Tryptose broth. Confirmation of total coliforms was carried out in Brilliant Green Bile broth and that of *E. coli* in EC broth, followed by isolation on EMB agar and confirmation by biochemical tests (Indole, Voges–Proskauer, Methyl Red and citrate).

3. Results

3.1. Cocoa seeds before fermentation

The mean results of the analyses performed on cocoa seeds before fermentation are shown in Table 1 (average per producer and overall average).

Neither *Salmonella* (detection limit 1 CFU/25 g) nor *E. coli* (detection limit 3NMP/g) were detected in any of the samples examined. Total coliforms (detection limit 3NMP/g) were also not detected in any of the samples from farm 3, but, in contrast, 70% and 40% of the samples from farms 1 and 2, respectively, were

Table 1
Analyses results of *cocoa seeds before fermentation* (average per producer and overall average).

Producer	Contamination	Total coliforms (log MNP/g)	*E. coli* (log MNP/g)	*Salmonella* (in 25 g)
Producer 1	Positive samples (%)	07 (70%)	0	0
	Count of the positive samples (average)	0.6–1.6 (1.1)	–	–
Producer 2	Positive samples (%)	04 (40%)	0	0
	Count of the positive samples (average)	0.6–2.2 (1.6)	–	–
Producer 3	Positive samples (%)	0	0	0
	Count of the positive samples (average)	–	–	–
Average	Positive samples (%)	11 (37%)	0	0
	Count of the positive samples (average)	0.6–2.2 (1.2)	–	–

Table 2
Analyses results of *cocoa seeds during fermentation* (average per producer and overall average).

Producer	Contamination	Total coliforms (log MNP/g)	*E. coli* (log MNP/g)	*Salmonella* (in 25 g)
Producer 1	Positive samples (%)	06 (60%)	03 (30%)	0
	Count of the positive samples (average)	0.6 to >3.0 (>1.8)	1.4–1.5 (1.4)	–
Producer 2	Positive samples (%)	07 (78%)	02 (20%)	0
	Count of the positive samples (average)	0.6 to >3.0 (>1.7)	1.2–2.0 (1.6)	–
Producer 3	Positive samples (%)	02 (20%)	02 (20%)	0
	Count of the positive samples (average)	1.0–2.0 (1.5)	1.0–2.0 (1.5)	–
Average	Positive samples (%)	15 (50%)	07 (23%)	0
	Count of the positive samples (average)	0.6 to >3.0 (> 1.7)	1.0–2.0 (1.5)	–

found to be contaminated with counts varying between 0.6 and 2.2 log CFU/g and a mean value of 1.2 log CFU/g.

3.2. Cocoa seeds during fermentation

The mean results of the analyses performed on cocoa seeds during fermentation are depicted in Table 2 (average per producer and overall average).

Although *Salmonella* was not detected, seven (23%) of the 30 samples investigated showed *E. coli* contamination, with counts ranging from 1.0 to 2.0 log CFU/g and a mean value of 1.5 log CFU/g. On the other hand, total coliform counts varied between 0.6 and >3.0 log CFU/g, with a mean count of >1.7 log CFU/g.

3.3. Cocoa beans during drying

The mean results of the analyses performed on coca beans during drying are presented in Table 3 (average per producer and overall average).

The presence of *Salmonella* was not detected during drying, but the percentage of samples contaminated with total coliforms and *E. coli* had increased in comparison to the previous stage, from 50% and 23% positive samples to 93% and 47%, respectively.

All three cocoa farms had a greater percentage of samples testing positive for *E. coli* contamination during the drying stage.

- *Producer 1* – Increased from 30% during fermentation to 50% during drying. The average count changed from 1.4 to >2.4 log CFU/g.
- *Producer 2* – Increased from 20% during fermentation to 30% during drying. The average count of the samples was relatively little affected: 1.6 log CFU/g during fermentation and 1.1 log CFU/g during drying.
- *Producer 3* – Increased from 20% during fermentation to 60% in the drying stage. The average counts of the samples increased from 1.5 to >2.6 log CFU/g.

3.4. Stored dried cocoa beans

The mean results of the analyses performed on the fermented and dried cocoa beans stored at the farms are depicted in Table 4 (average per producer and overall average).

Salmonella was detected in one (3%) of the 29 samples. The percentage of samples contaminated with *E. coli* remained constant in comparison to the previous stage. As for contamination with total coliforms, a reduction was observed in the percentage of positive samples from 93% to 72%. However, the mean counts were relatively little changed between the two stages (>2.9 and >2.4 log CFU/g).

The results show that, compared to the previous stage, the percentage of samples contaminated with *E. coli* increased on the first farm and decreased on the other two.

- *Producer 1* – Increased from 50% during drying to 89% during storage. The sample contaminated with *Salmonella* came from this farm.
- *Producer 2* – Decreased from 30% during drying to 10% during storage.
- *Producer 3* – Changed from 60% during drying to 50% during storage.

4. Discussion

According to Jay (2005), contamination with *Salmonella* species via the environment as well as workers' hands, equipment or utensil surfaces is possible, since, although their primary habitat is the intestinal tract of man, animals and insects, they can spread throughout the environment through vectors and feces-contaminated soil and ground water.

In this study, *Salmonella* was detected in only one of 119 samples analyzed. Due to this low incidence, it was not possible to use these data to assess the risk that each stage represents for the introduction of the pathogen into the process. For this reason,

Table 3
Analyses results of *cocoa beans during drying* (average per producer and overall average).

Producer	Contamination	Total coliforms (log MNP/g)	*E. coli* (log MNP/g)	*Salmonella* (in 25 g)
Producer 1	Positive samples (%)	10 (100%)	05 (50%)	0
	Count of the positive samples (average)	2.2 to >3.0 (>3.0)	1.4 to >3.0 (>2.4)	–
Producer 2	Positive samples (%)	09 (90%)	03 (30%)	0
	Count of the positive samples (average)	1.4 to >3.0 (>2.6)	0.9 to 1.2 (1.1)	–
Producer 3	Positive samples (%)	09 (90%)	06 (60%)	0
	Count of the positive samples (average)	>3.0 (3.0)	1.6 to >3.0 (>2.6)	–
Average	Positive samples (%)	28 (93%)	14 (47%)	0
	Count of the positive samples (average)	1.4 to >3.0 (>2.9)	0.9 to >3.0 (>2.2)	–

Table 4
Analyses results of *stored dried cocoa beans* (average per producer and overall average).

Producer	Contamination	Total coliforms (log MNP/g)	*E. coli* (log MNP/g)	*Salmonella* (in 25 g)
Producer 1 (09 sample)	Positive samples (%)	08 (89%)	08 (89%)	01 (10%)
	Count of the positive samples (average)	1.3 to >3.0 (>2.6)	0.9 to >3.0 (>2.2)	ND[a]
Producer 2	Positive samples (%)	06 (60%)	01 (10%)	0
	Count of the positive samples (average)	0.9 to >3.0 (>2.2)	>3.0	–
Producer 3	Positive samples (%)	07 (70%)	05 (50%)	0
	Count of the positive samples (average)	1.0 to >3.0 (>2.4)	0.9 to >3.0 (>1.6)	–
Average (29 samples)	Positive samples (%)	21 (72%)	14 (48%)	01 (3%)
	Count of the positive samples (average)	0.9 to >3.0 (>2.4)	0.9 to >3.0 (>2.0)	ND[a]

[a] Not determined.

the authors opted to evaluate the evolution of the contamination of samples with *E. coli* as indicative of fecal contamination.

The results show that the seeds that came in from the fields – and before fermentation – were not contaminated with *E. coli*. This seems to indicate that the raw material is a less likely source of enteropathogens, such as *Salmonella,* in the pre-processing of cocoa.

During fermentation, the hot and acid environment is not favorable to the growth and multiplication of *Salmonella,* which has a growing temperature range from 5 to 7 and 46 °C (optimum growth temperature: 35–43 °C) and a growing pH range between 3.8 and 9.5 (optimum pH range 7.0–7.5) (ICMSF, 1996). However, the possibility of *Salmonella* to survive and even multiply, under the harsh conditions of temperature and pH that prevail during the fermentation of cocoa, should not be discarded. ICMSF (2000) reports that, without a rigorous control during the final stage of fermentation, with the increase of the pH to values in the 6.0–7.0 range, bacterial species such as *Enterobacter* and *Escherichia* may come to predominate, with the concomitant development of unpleasant odors. This finding was confirmed in this study, since *Salmonella* was not detected in any of the samples analyzed during the fermentation process and 20–30% of the samples from the three investigated cocoa farms were found to be contaminated with *E. coli*. However, no correlation was observed between the presence or counts of *E. coli* and fermentation time. This fact, along with the restrictive pH of the samples, the low counts observed and the percentage of non-contaminated samples, do not appear to indicate multiplication of *E. coli*, but rather contamination of the seeds during fermentation.

The presence of *Salmonella* was not detected during drying. Smedt et al. (1991) reported the occurrence of high numbers of *Salmonella* in samples of dried cocoa beans, with 258 (79%) contaminated samples among a total of 325 samples analyzed. On the other hand, the percentage of samples contaminated with *E. coli* increased on all three cocoa farms (30–60% of the samples were contaminated) in comparison to the previous stage. No correlation was observed between the presence or counts of *E. coli* and the drying time. This stage is, probably, the most critical for the introduction of *Salmonella.* The seeds are left exposed in the open for several days and hence may become contaminated by dust (unpaved areas adjacent to the drying platforms), animals (particularly domesticated and wild birds) and insects. Furthermore, the beans are trodden on and shuffled about by farm workers with their feet to remove the mucilage adhered to their surface thereby increasing the opportunities for contaminants to find their way onto or into the beans.

Once dried, the cocoa beans are stored in protective bags, which makes it more difficult for birds to get access to the stored material. However, these bags are placed directly onto the floor and are often not appropriately closed and/or sealed, allowing the entrance of vectors, or transmitting agents, such as insects and rodents. On farm 1, the percentage of samples contaminated with *E. coli* increased compared to the previous stage and one sample tested positive for the presence of *Salmonella*. The production of this property is smaller and, at the same time, the beans are kept in storage in the barns for longer periods of time compared to the other two farms investigated. Farm 2, which exhibited the lowest percentage of *E. coli* contaminated samples, has a large production, rapid turnover and shorter storage time.

For that reason, based on the results obtained and the production conditions observed on the cocoa farms, it is possible to conclude that birds (chickens, pigeons, sparrows and other birds) and insects frequently come into direct contact with cocoa seeds/beans in the process of drying in the open and are chronic vectors of fecal contamination of cocoa beans, introducing *E. coli* and, possibly, *Salmonella*. Other critical factors that may contribute to contamination of cocoa by these enteropathogens include: the common practice of treading and shuffling beans during the drying stage of cocoa pre-processing, the prolonged periods of time the product is kept in storage on the farms and environmental storage conditions. There are some actions that could be used to minimize this contamination, such as: the use of screens to protect the seeds during the drying process thus avoiding contact of birds and small mammals; prohibit farm workers from the practice of treading on the beans to remove mucilage; store the dried cocoa beans in bags appropriately sealed and on pallets; reduce the cocoa bean storage time on the farms; carry out pest control close to the installation used for cocoa pre-processing.

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References

Andrews, W. H., & Hammack, T. S. (2005). *Salmonella*. In *Bacteriological analytical manual online*. FDA (Food and Drug Administration). <http://www.cfsan.fda.gov/ebam/bam-5.html>.

Cordier, J. L. (1994). HACCP in the chocolate industry. *Food Control, 5*, 171–175.

D'Aoust, J. Y. (1977). *Salmonella* and the chocolate industry a review. *Journal of Food Protection, 40*(10), 718–727.

Downes, F. P., & Ito, K. (2001). *Compendium of methods for the microbiological examination of foods*. Washington: American Public Health Association (APHA).

EC (European Commission) (2003). *Salmonella* in foodstuffs: Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health. <http://ec.europa.eu/food/fs/sc/scv/out66_en.pdf>.

Garcia, A. E. B. (2003). O Brasil e as exportações mundiais de derivados de cacau. *Informações Econômicas, 33*(7), 47–61.

ICMSF (International Commission on Microbiological Specifications for Foods) (1988). *Microorganisms in foods 4: Application of the hazard analysis critical control points (haccp) system to ensure microbiological safety and quality*. London: Chapman and Hall.

ICMSF (International Commission on Microbiological Specifications for Foods) (1996). *Microorganisms in foods 5: Microbiological specifications of food pathogens*. London: Chapman and Hall.

ICMSF (International Commission on Microbiological Specifications for Foods) (2000). *Microrganisms in foods 6: Microbiological ecology of food commodities*. Gaithersburg: Chapman and Hall.

Jay, J. E. (2005). *Microbiologia de alimentos*. Porto Alegre: Artmed.

Smedt, J. M., Chartron, S., Cordier, J. L., Graff, E., Hoekstra, H., Lecoupeau, J. P., et al. (1991). Collaborative study of the international office of cocoa Chocolate and sugar confectionery on *Salmonella* detection from cocoa and chocolate processing environmental samples. *International Journal of Food Microbiology, 13*(4), 301–308.