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Behavior of *Salmonella* during fermentation, drying and storage of cocoa beans

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ABSTRACT

Due to cocoa being considered a possible source of *Salmonella* contamination in chocolate, the behavior of *Salmonella* during some cocoa pre-processing stages (fermentation, drying and storage) was investigated. The fermentation process was carried out on a pilot scale (2 kg beans/box) for 7 days. Every day a fermentation box was inoculated with a *Salmonella* pool (ca. 4 log MPN/g). The results showed that *Salmonella* did not affect ($P > 0.05$) the growth of the main microorganism groups involved in cocoa fermentation. On the other hand, the pathogen was influenced ($P < 0.05$) by yeast, acetic acid bacteria and pH. In spite of *Salmonella* showing counts ≤1 log MPN/g in the first days, at the end of fermentation it grew in all samples, reaching counts as high as 7.49 log MPN/g. For drying and storage, cocoa beans were inoculated during the fermentation (experiment A) or during the drying (experiment B). In these stages the decline of the water activity affected the pathogen behavior. In experiment A during the drying, *Salmonella* count increased in most of the samples. In experiment B either a slight growth or no growth in the samples inoculated up to 48 h was observed, whereas the other samples showed reductions from the initial count. After 30 days of storage at room temperature, the water activity decreased to 0.68, and reductions of *Salmonella* ranged from 0.93 to 2.52 log MPN/g. Despite the reductions observed during the storage, the pathogen was detected even after 120 days. Therefore, the results showed that *Salmonella* growth or survival depends on when the contamination occurs.

1. Introduction

Salmonella is a common enteropathogen responsible for foodborne disease that results in hundreds of deaths annually worldwide (ICMSF, 2011; Li et al., 2013). Foodborne outbreaks due to consumption of *Salmonella* contaminated chocolate products have been reported since the 1970s (D'Aoust, 1977; Werber et al., 2005). Dried cocoa beans and chocolate have characteristics such as low water activity and high fat content that result in *Salmonella* viability for long periods of time (Tamminga et al., 1976; Komitopoulou and Peñaloza, 2009). In addition, the high fat content of chocolate contributes to the protection of *Salmonella* in the alimentary tract after ingestion (D'Aoust, 1977) and, therefore, the low infectious dose of *Salmonella* in chocolate products (less than 1 CFU/g) (Hockin et al., 1989; Werber et al., 2005; Scott et al., 2009).

Although cocoa products are not the only ingredients that may introduce *Salmonella* into chocolate, they have been implicated as the potential source of some outbreaks (Werber et al., 2005). Cordier (1994) points out cocoa beans as a major source of *Salmonella* contamination throughout the manufacturing of chocolate and in cocoa-based ingredients, such as liquor. Contamination with *Salmonella* during cocoa pre-processing (harvest, pod breaking, fermentation, drying and storage) is not unexpected, due to poor hygiene conditions (Cordier, 2000). After breaking of the pods, a wide variety of microorganisms are transferred to cocoa beans from an array of sources, such as soil, insects, laborers' hands, tools, and installations (Ostovar and Keeney, 1973; Schwan and Wheals, 2004). Part of this microbiota is responsible for the natural fermentation that lasts up to 7 days (Schwan et al., 1995; Nielsen et al., 2007; Thompson et al., 2013). However, pathogens can be also introduced into the material (Cordier, 2000).

Upon completion of fermentation, the drying process begins in order to reduce the moisture of the beans from 40–50% to 6–8% (ICMSF, 2011; Thompson et al., 2013). For sun drying, the beans are placed on wooden platforms, mats, polypropylene sheets or concrete floors (Beckett, 2009; Thompson et al., 2013). The drying time depends on the weather conditions; in rainy periods, it may be prolonged for several weeks. After drying, the beans are graded by hand, packed and stored for up to 12 months (Thompson et al., 2013). As previously mentioned, the sanitary conditions of these stages are not always the most appropriate, subjecting the beans to contamination by vectors, dust and worker

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manipulation (Nascimento et al., 2010). de Smedt et al. (1991) detected *Salmonella* in dust from the cocoa bean cleaning operation. In Brazil, *Salmonella* was isolated from cocoa beans stored on farm (Nascimento et al., 2010). Further, Komitopoulou and Peñaloza (2009) reported the ability of *Salmonella* to survive in inoculated cocoa beans at ambient conditions over 4 weeks.

After pre-processing, the cocoa beans are subjected to roasting. This process normally reaches temperatures between 110 and 140 °C (Beckett, 2009), and is considered the main step responsible for the reduction of microbial contamination (ICMSF, 2005; Thompson et al., 2013). However, thermal resistance of *Salmonella* in low-moisture foods is well-known (D'Aoust, 1977; Podolak et al., 2010; Beuchat et al., 2013). In a previous study, we observed a high heat resistance of *Salmonella* during cocoa hot air roasting ($D_{110\ °C}$ of 4.8–8.9 min and $D_{140\ °C}$ of ca. 2.5 min). The results indicated that depending on the initial load and the process parameters cocoa roasting cannot ensure the complete elimination of *Salmonella* (Nascimento et al., 2012).

Due to all these facts, control of raw material is considered essential to prevent *Salmonella* in the cocoa and chocolate industries (Cordier, 1994; ICMSF, 2011). However, there is no data published on *Salmonella* behavior during cocoa fermentation or drying, and hardly any studies on bean storage with a exception being Komitopoulou and Peñaloza (2009). Hence, studies are needed to supply data for risk assessments and for the establishment of appropriate processing conditions to prevent or minimize *Salmonella* contamination throughout the cocoa supply chain. For this reason, the aim of this study was to evaluate *Salmonella* growth and survival during fermentation, drying and storage of cocoa beans.

2. Material and methods

2.1. Salmonella strains

A pool of five *Salmonella enterica* serotypes was used as an inoculum (*S.* Typhimurium ATCC 14028, *S.* Enteritidis ATCC 13076, *S.* Oranienburg IAL (Instituto Adolfo Lutz) 1203, *S.* Senftenberg IAL 1235 and *S.* Eastbourne IAL 1131). The serotypes were chosen according to the following criteria: *S.* Typhimurium and *S.* Senftenberg due to their high thermal resistance, *S.* Oranienburg and *S.* Eastbourne for being serotypes that have already been involved in outbreaks resulting from consumption of chocolate (although not the same strains), and *S.* Enteritidis for being frequently isolated from food. All strains were stored at −80 °C, and maintained on tryptic soy agar (TSA, Difco) slants at 4 °C.

2.2. Preparation of inocula

Each *Salmonella* strain was propagated twice in tryptic soy broth (TSB, Difco) at 37 °C for 18–24 h. Then, a pool of the 5 strains was prepared as follows: after centrifugation at 3000 ×*g* for 10 min at 20 °C (centrifuge PK 121R, ALC, Italy), the cells obtained were rinsed twice and resuspended in phosphate-buffered saline (PBS) plus 1.5% Tween 80 (Merck). The initial concentration of the inoculum in the cocoa samples was ca. 4 log of most probable number per gram (MPN/g).

2.3. Evaluation of Salmonella growth during cocoa pre-processing

2.3.1. Fermentation

Cocoa pods (*Theobroma cacao* L.), without injury and mold contamination from a Brazilian mixed hybrid plantation, were used in this study. The fermentation process was performed three times, according to Efraim et al. (2010). 2 kg portions of cocoa pulp–bean mass were placed in seven 3-l-capacity polystyrene boxes, which had holes drilled in the base to allow aeration and drainage of liquids. The boxes were covered with plantain leaves and left to ferment for 7 days in a temperature and humidity-controlled room (25–35 °C and 60–80%). The aim was to simulate the environmental conditions of the Brazilian production area. To increase aeration, 48 h after the beginning of fermentation the cocoa mass was transferred from each box onto a sterile stainless steel tray, mixed with a sterile spatula and then returned to the box. Thereafter, this same procedure was repeated once a day up to the end of the process.

Every day during fermentation one box was inoculated with *Salmonella* pool (0.2% v/w) i.e., box 0 at the beginning of fermentation (time zero), box 1 on the 1st day and so on. Daily, immediately after the *Salmonella* inoculation, samples (75 g) were taken randomly from three different points of each fermentation box for microbiological and pH analyses.

For pH analysis the surface washing technique was used. 10 g of cocoa (pulp + bean) was superficially washed with 100 ml of distilled water. Then, the pH of this solution was measured using a digital pH-meter UP-25 (Denver, CO, USA) (Zenebon and Pascuet, 2005). Temperature of the cocoa samples was measured once a day using a digital thermometer (Testo). Temperature and humidity of the fermentation room were also monitored throughout the process by a Testo 615 portable meter (Testo, Germany).

2.3.2. Drying

Two drying experiments were performed, A and B, with each experiment being replicated twice. In experiment A the samples were inoculated with the *Salmonella* pool during the fermentation in the same way as described in Section 2.3.1. In experiment B, every 24 h during the drying process, one blank fermented sample was inoculated with 0.5% (v/w) of the *Salmonella* pool. For each experiment, 1.5 kg of fermented cocoa beans was spread on sterile stainless steel trays. Then they were left to dry naturally in a temperature and humidity-controlled room (25–35 °C and 60–80%) for 6–7 days until the moisture content reached 7–8%. The cocoa beans were turned over every day with a sterile spatula to ensure homogenized drying. Temperature and humidity of the room were monitored by a Testo 615 portable meter (Testo).

Salmonella enumeration was performed at the beginning and end of drying in experiment A. In experiment B it was carried out immediately after the inoculation of each sample and at the end of the process. The water activity of samples was measured once a day using an AquaLab 3TE hygrometer (Decagon Devices, Pullman, WA). The moisture contents were determined in a 315 SE oven (Fanen, Brazil) at 105 °C (Horwitz, 2006).

2.3.3. Storage

At the end of drying, the samples were transferred to sterile bags and stored at room temperature (22–32 °C and 50–90%). *Salmonella* count was determined after 30 days for both experiments (A and B), and also after 120 days in experiment B.

2.4. Microbiological analysis

2.4.1. Detection and enumeration of Salmonella

Salmonella enumeration was performed by the MPN method adapted from ISO (2007). To prepare the first dilution, 10 g of each sample was mixed with 90 ml of 0.1% peptone water (Merck) in a Stomacher bag, which was massaged and shaken by hand for 2 min to give a uniform homogenate. Subsequent serial decimal dilutions were prepared in the same medium. Following this, three 1 ml portions from each dilution were transferred to tubes containing 10 ml of buffered peptone water (BPW, Difco) and incubated at 37 °C for 18–24 h. Afterwards, 0.1 ml from each BPW tube was added to 10 ml of Rappaport–Vassiliadis modified broth (Difco), with incubation at 41.5 °C for 24 h, and 1.0 ml to 10 ml tetrathionate broth (Difco), with incubation at 37 °C for 24 h. Thereafter, cultures were streaked on xylose lysine deoxycholate agar (XLD, Difco) and bismuth sulfite agar (BS, Difco) and the plates were incubated at 37 °C for 24–48 h. Presumptive *Salmonella* colonies were confirmed by biochemical tests (triple sugar iron, lysine decarboxylase,

Voges–Proskauer, urease and indole). Afterwards, the serotype of each confirmed colony was identified by serological tests using somatic and flagellar antiserum groups.

When the *Salmonella* count was below the detection limit (<0.48 log MPN/g), presence/absence in ca. 25 g was determined, according to ISO (2007).

2.4.2. Enumeration of the fermenting microorganism groups

For culture-based isolations, 10 g of cocoa beans was added to 90 ml of 0.1% peptone water (Merck) in Stomacher bags, which were massaged and shaken by hand for 2 min to give uniform homogenates. From this first dilution, serial decimal dilutions were prepared in the same medium. Subsequently, enumeration of specific microorganism groups was carried out as described below. For yeasts, potato dextrose agar (PDA, Merck) containing 10% tartaric acid (Merck) with 3–5 days of incubation at 25 °C (Mislivec et al., 1992) was used. Lactic acid bacteria (LAB) were enumerated on de Man–Rogosa–Sharpe (MRS, Merck) plus 400 mg/l cycloheximide (Sigma) (Camu et al., 2007) to inhibit yeasts, with anaerobic incubation for 3 days at 37 °C. Acetic acid bacteria (AAB) were counted on PDA containing 10% tartaric acid (Downes and Ito, 2001) and 400 mg/l cycloheximide (Camu et al., 2007) with 3–5 days of incubation at 30 °C. Morphologically different colonies from each sample and agar media were isolated and confirmed by Gram staining and catalase tests. Additionally, AAB were subjected to confirmation on ethanol agar (yeast extract 10 g/l (Merck), calcium carbonate 20 g/l (Merck), ethanol 20 ml/l (Merck), agar 20 g/l (Merck), pH 6.0) with oxidation of ethanol and calcium carbonate (Sievers and Swings, 2005). The results were reported as log CFU/g.

2.5. Statistical analysis

The analysis of variance (ANOVA) was performed using SAS software (version 9.1, SAS Institute, Cary, NC). The correlation analysis was carried out according to Pearson method (Steel and Torrie, 1980).

3. Results and discussion

3.1. Salmonella behavior in cocoa fermentation

The temperature of the cocoa mass increased from 23 °C at the beginning of fermentation to 30 °C after 24 h. The highest temperature recorded was 43 °C, on the 4th day. The increase in temperature is linked to the growth of AAB, which metabolizes ethanol produced by yeasts to acetic acid and water, releasing heat (Schwan et al., 1995). During this phase, the temperature of the cocoa mass can reach up to 50 °C (Ardhana and Fleet, 2003; Schwan and Wheals, 2004; Galvez et al., 2007). As the present study was carried out on a pilot scale (2 kg beans/box), the smaller amount of cocoa used may have influenced the aeration of the mass, the growth of microorganisms and, hence, the rates of the metabolic reactions, resulting in a smaller increase in the temperature. Camu et al. (2007) observed a similar maximum temperature (43.5 °C) when studying cocoa fermentation on a large scale in Ghana. After the 5th day, the temperature showed a declining tendency, recording 35 °C at the end of the process. This rapid decline is probably also related to the amount of fermenting mass.

In the first days of fermentation, a slight decline in the cocoa pulp pH from 3.7 to 3.2 was observed. It is probably due to the breaking down of glucose and ethanol into lactic acid and acetic acid, as reported by Schwan and Wheals (2004). An increase in pH was observed in most of the samples from the 4th day onwards, reaching values around 6.5 on the 7th day (Fig. 1). This final pH is consistent with that found by Rombouts (1952). However, Camu et al. (2007) observed a pH of 4.3 at the end of the fermentation. The factors that influence the evolution of pH throughout the fermentation are the variety and amount of the cocoa used, and the species of microorganisms present (Schwan and Wheals, 2004). Furthermore, the rise in the pH towards the end of fermentation is probably related to evaporation of volatile acids such as acetic acid (Nielsen et al., 2007), conversion of citric acid by LAB and yeasts into non-acid compounds (Carr, 1982), and growth of aerobic spore-forming bacteria (Ardhana and Fleet, 2003; Schwan and Wheals, 2004).

The *Salmonella* inoculation in the samples throughout the fermentation did not affect ($P > 0.05$) the population dynamic (yeast, LAB and AAB). Yeasts were the dominant group of microorganisms until the penultimate day. The population increased more than 5 log-units during the first 48 h, achieving an average count of 7.60 log CFU/g. Growth of the same magnitude was obtained by Ardhana and Fleet (2003) after 24–36 h. Yeast counts remained about 8 log CFU/g until the 7th day. According to Thompson et al. (2013) the yeast temporal distribution throughout the fermentation is related to ethanol concentration, pH and heat tolerance of the species present. LAB showed an initial growth rate slower than yeasts, with counts around 4 and 6 log CFU/g until the 4th day, achieving a maximum population of 8.5 log CFU/g at the end of fermentation. Camu et al. (2007) also observed high LAB counts on the last day of the process. In our study, AAB were detected from the 2nd day onwards. This group showed a similar evolution to LAB, and the highest count (7.7 log CFU/g) was obtained on the 6th day (Fig. 1).

Statistical analysis of the data showed that *Salmonella* behaved differently among the samples, being influenced ($P < 0.05$) by the fermentation stage in which it was inoculated. Yeasts and AAB had a negative correlation to 95% of significance on *Salmonella*. On the other hand, the pH value showed a positive correlation with the pathogen.

All the five *Salmonella* serotypes used in this study were recovered during the fermentation. In the sample inoculated at the beginning of the process (box 0), *Salmonella* count remained stable in the first 24 h. On the 4th day, a decrease to below the detection limit (<0.48 log MPN/g) was observed. Afterwards, on the last day of fermentation, there was a slight growth, with final population of 1.43 log MPN/g (Fig. 1A). According to Ardhana and Fleet (2003), Camu et al. (2007), and Galvez et al. (2007) an intense microbial activity with production of antimicrobial compounds (ethanol, lactic and acetic acid) takes place between 30 and 120 h of fermentation. This scenario characterizes an unfavorable environment for *Salmonella* growth, which could be seen in boxes 1, 2 and 3 where the initial count of *Salmonella* decreased to below the detection limit 24 or 48 h after the inoculation. The counts remained at this level until the 6th day. However, on the last day of fermentation, a growth tendency was observed (Figs. 1B, 1C and 1D). In boxes 4 and 5, although the *Salmonella* population declined 3.40 and 1.40 log MPN/g respectively, after 24 h of inoculation, the counts returned to the level of the initial inoculum at the end of the process (Figs. 1E and 1F). In box 6, *Salmonella* showed a significant growth within 24 h after inoculation, achieving a final count of 7.49 log MPN/g (Fig. 1G).

Salmonella growth observed in all samples during the last stage of fermentation coincided with the increase of pH to above 4.0. According to Li et al. (2013) *Salmonella* has the ability to proliferate at pH values ranging from 3.99 to 9.50. In addition, the pH increase probably had also influenced the dissociation of the organic acids, reducing their antimicrobial activity (Lund and Eklund, 2000), and consequently, resulting in *Salmonella* growth despite the high LAB and AAB counts. These data should be of particular concern to cocoa farms in some tropical areas, especially Ecuador, Venezuela and Guatemala, since cocoa beans usually show a high pH (5.5–5.8) at the end of fermentation (Beckett, 2009).

Therefore, *Salmonella* behavior in cocoa fermentation is closely related to the moment of contamination. Depending on the fermentation stage in which the contamination occurs, the *Salmonella* population may show a slight (1.40 log MPN/g) to a significant reduction (4.00 log MPN/g). However, at the end of the process, the pathogen is able to grow and achieve a level considered worrying from the viewpoint of public health, since such contamination may not be eliminated completely by cocoa roasting (Izurieta and Komitopoulou, 2012;

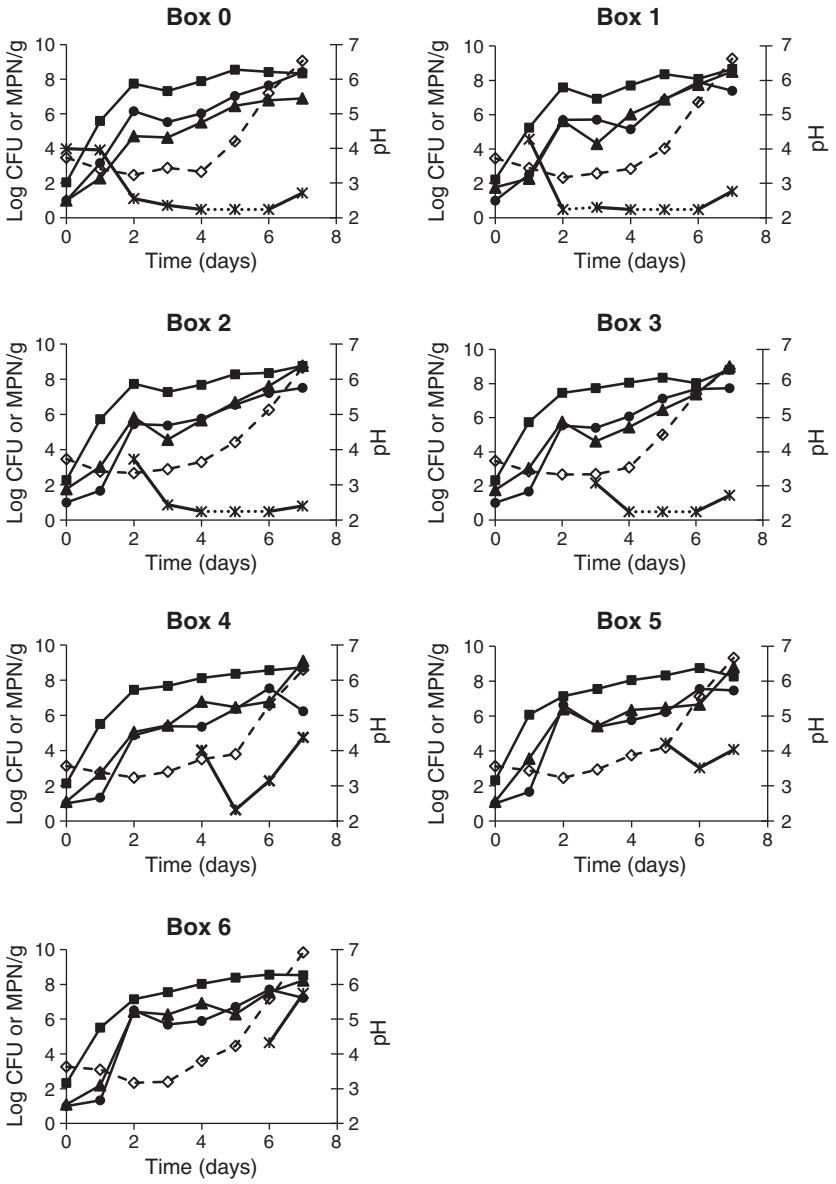

Fig. 1. Microbial count and pH evolution during cocoa fermentation. Yeast (■), lactic acid bacteria (▲), acetic acid bacteria (●), *Salmonella* (∗) and pH (-◇-). *Salmonella* count below the detection limit (···). Box 0 — *Salmonella* inoculated at the beginning of fermentation; Box 1 — Salmonella inoculated on the 1st day; Box 2 — *Salmonella* inoculated on the 2nd day; Box 3 — *Salmonella* inoculated on the 3rd day; Box 4 — *Salmonella* inoculated on the 4th day; Box 5 — *Salmonella* inoculated on the 5th day; Box 6 — *Salmonella* inoculated on the 6th day.

Nascimento et al., 2012) or chocolate conching (Goepfert and Biggie, 1968; Barrile and Cone, 1970; Krapf and Gantenbein-Demaechi, 2010; Nascimento et al., 2012).

3.2. Salmonella survival in cocoa drying and storage

Fig. 2 shows the reduction of water activity (aw) throughout the natural drying of cocoa beans inoculated with *Salmonella* during the stages of fermentation (experiment A) and drying (experiment B). After 6 days, the aw decreased from 0.98–0.99 to 0.72–0.73 and the moisture dropped from 40 to 7.5%.

As previously observed in the fermentation, *Salmonella* behavior during the drying varied depending on the day/stage of contamination, being affected mainly by the aw. Indeed, in this study the drying was carried out in a temperature and humidity-controlled room (25–35 °C and 60–80%). In natural sun drying, other variables, such as UV rays and locally high temperatures, may also have an effect on *Salmonella* survival.

At the end of the drying, all samples from experiment A exhibited an increase in the *Salmonella* population between 0.49 and 1.28 log MPN/g, except for the sample from box 3, which remained stable (Table 1). In experiment B, growth was observed in the samples inoculated at the beginning of the process and after 24 h. During this initial phase, *Salmonella* growth was probably supported by the surrounding environment conditions, i.e., presence of residual mucilage, pH around 6.0 and especially aw ≥ 0.94. However, reductions in the initial count were observed when the inoculation was carried out in samples with aw ≤ 0.92 (Table 2). This result is in agreement with the literature that reports that aw threshold for *Salmonella* growth is 0.93–0.94 (Bell and Kyriakides, 2009; Li et al., 2013). In addition, in samples 3 and 4 from experiment B a significant decline in the initial count was observed immediately after the inoculation (1.5–2.0 log MPN/g). Other studies have also noted an immediate decrease in *Salmonella* population when hydrated cells are added to low-aw matrices (Keller et al., 2012; Kimber et al., 2012; Komitopoulou and Peñaloza, 2009), which may be attributed to an osmotic shock effect. Besides, when the drying process takes place quickly there is insufficient time to allow synthesis

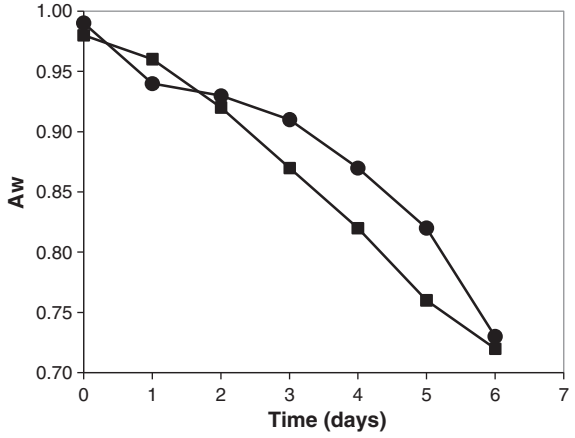

Fig. 2. Evolution of water activity during the drying of cocoa beans inoculated with *Salmonella* during fermentation (●) or drying step (■).

of protective osmolytes, resulting in cell death (Record et al., 1998). Compared to our study, Keller et al. (2013) reported greater sensibility of *Salmonella* to the decrease of aw in black pepper; the bacteria did not grow with aw less than 0.98 and was not detected below 0.96 after 5 days at 35 °C.

To evaluate the survivability of *Salmonella* in dried beans, the samples were analyzed after 30 days of storage at ambient temperature. At the end of this storage period, the aw was 0.68 and the *Salmonella* reductions ranged between 0.93 and 1.86 log MNP/g for experiment A and from 0.58 to 2.97 log MNP/g for experiment B. The pathogen was recovered in all samples that showed a count below the detection limit (<0.48 log MPN/g). In a previous study, *Salmonella* Enteritidis PT30 and Oranienburg showed reductions ranging from 1.50 to approximately 5 log CFU/g after storage of cocoa beans at 21 °C for 28 days (Komitopoulou and Peñaloza, 2009). Although the influence of temperature and humidity was not evaluated in our study, other researchers noted that they also affected *Salmonella* survival during the storage (Hiramatsu et al., 2005; Uesugi et al., 2006; Komitopoulou and Peñaloza, 2009; Keller et al., 2013).

After 120 days of storage, a qualitative analysis was performed in 25 g samples from experiment B to detect *Salmonella*. The pathogen was recovered in 100% of the samples inoculated on days 0, 1 and 2, and in 50% of the samples inoculated on days 3 and 5. However, it was not detected in the 4th day sample. This survival period was greater than that obtained by Juven et al. (1984) in cocoa powder. According to Janning et al. (1994) cells that survive the initial osmotic shock phase remain stable for a very long time. This phenomenon was also noted in other studies. After a rapid decrease observed in the initial count, Uesugi et al. (2006) recovered *Salmonella* in almonds after 175 days. In chocolate, Tamminga et al. (1976) detected *Salmonella*

Table 1
Salmonella behavior during drying and storage of cocoa beans inoculated during fermentation step.

Inoculation day	*Salmonella* (log MPN/g)[a]		
	At the beginning of drying[b]	At the end of drying	After 30 days of storage
0	3.32 ± 0.00	3.85 ± 0.27	2.92 ± 1.05
1	0.97 ± 0.00	1.46 ± 0.70	<0.48 ± 0.00
2	0.96 ± 0.00	1.50 ± 0.66	<0.48 ± 0.00
3	3.38 ± 0.00	3.37 ± 0.41	1.99 ± 0.26
4	6.04 ± 0.00	7.04 ± 0.00	5.18 ± 0.00
5	4.18 ± 0.00	5.04 ± 0.00	3.50 ± 0.19
6	6.38 ± 0.00	7.66 ± 0.00	6.42 ± 0.06

[a] Logarithm of most probable number per gram.
[b] Mean ± standard deviation.

Table 2
Salmonella behavior during drying and storage of cocoa beans inoculated during drying step.

Inoculation day	*Salmonella* (log MPN/g)[a]		
	Immediately after the inoculation[b]	At the end of drying	After 30 days of storage
0	4.85 ± 0.27	5.35 ± 0.97	2.38 ± 0.00
1	4.04 ± 0.00	4.52 ± 0.20	2.00 ± 0.14
2	4.02 ± 0.51	3.88 ± 0.71	1.98 ± 0.96
3	3.23 ± 1.20	1.06 ± 0.82	<0.48 ± 0.00
4	2.18 ± 0.28	1.47 ± 0.13	<0.48 ± 0.00
5	4.66 ± 0.00	4.01 ± 0.53	1.94 ± 0.54

[a] Logarithm of most probable number per gram.
[b] Mean ± standard deviation.

after 9 months of storage. Keller et al. (2013) recovered *Salmonella* in black pepper after 12 months.

Komitopoulou and Peñaloza (2009) reported that the ability of *Salmonella* to survive in dry conditions is strain dependent. In the present study, among the five *Salmonella* serotypes inoculated, only Senftenberg and Typhimurium were recovered at the end of the drying, whereas after 30 days of storage only Typhimurium was detected.

It is well-known that dried cells are more resistant to most stressors including dry heat, UV irradiation and sanitizers (Hiramatsu et al, 2005; Gruzdev et al, 2011). This information along with the data obtained in our study should be of particular concern to the cocoa and chocolate manufacturing industries, since cross-contamination (Cordier, 1994; Werber et al., 2005) or survival during the roasting process may occur (Izurieta and Komitopoulou, 2012; Nascimento et al., 2012). Furthermore, it is relevant to note that the possibility of *Salmonella* surviving during and after the drying process warrants caution in the handling of cocoa beans.

4. Conclusion

To the best of the authors' knowledge, this is the first study on the effects of cocoa pre-processing stages on *Salmonella*. This study sheds light on the role of primary processing steps on the fate of *Salmonella* in cocoa beans and is of foremost importance for risk assessment and to ensure production of safe chocolate. For the inoculum level and the experimental conditions evaluated in this study, the *Salmonella* behavior was dependent on the time that contamination occurred. It was affected mainly by pH during the fermentation and by aw during the drying and storage steps. The most critical period for *Salmonella* growth was between the end of the fermentation and the beginning of the drying. Thus, in order to minimize the risk of *Salmonella* contamination in the final product, a good agricultural practice program should be implemented on cocoa farms. This includes good hygiene and working practices, such as avoiding direct contact with rodents, birds and other vectors, using clean tools, and abolishing trampling of the beans (common practice in some production areas).

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