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Research article

Microbiological quality of raw berries and their products: A focus on foodborne pathogens

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ABSTRACT

Berry samples (n = 316; strawberries, raspberries, blackberries and blueberries) obtained from a fruit processing plant were examined regarding bacteriological quality and their potential public health risk. Three types of berry products were analysed including raw material, product from the mixing step and final product. *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Bacillus cereus*, sulphite-reducing clostridia spores and coagulase-positive staphylococci were the parameters investigated. *Salmonella enterica* serovar Braenderup and *L. monocytogenes* were isolated from one fruit sample of raw material each. Two samples harboured *E. coli* between 0.7 and 0.9 log cfu g^{-1}, not exceeding the hygienic criteria. Coagulase-positive staphylococci were not detected in the studied samples; however, coagulase-negative staphylococci (CNS) were isolated from a small proportion of samples mainly raspberries. Presumptive *B. cereus* were isolated from a relatively large proportion of the samples, raspberries and blackberries being the most contaminated fruits. The absence of pathogenic microorganisms in the final product as well as the low prevalence of presumptive *B. cereus* and CNS indicates proper implementation of good manufacturing and hygiene practices (GMPs/GHPs) by the food industry. Nevertheless, the results indicate that the raw material examined may contain pathogenic bacteria and thereby represent a risk to consumers regarding the manifestation of foodborne diseases.

1. Introduction

Fresh fruit and vegetables are essential components of a healthy and balanced diet; their consumption is encouraged by different organizations (e.g. WHO, FAO, USDA, EFSA) and nutrition experts to protect against a range of diseases (Regmi, 2001; World Health Organization (WHO), 2003; FSA (Food Standards Agency), 2006; FAO/WHO (Food and Agricultural Organization of the United Nations/World Health Organization), 2008; Berger et al., 2010). The nutritional value of berry fruits is widely recognized and is highly sought by consumers, especially because of their remarkably high levels of antioxidant phytonutrients, polyphenol content and for general health benefits (Milivojević et al., 2011). Strawberries, raspberries, blackberries and blueberries are the most commonly consumed in the EU. Moreover, berries are significantly consumed fruits worldwide and their increasing consumption has led to the need for improved food safety in the berry fruit industry (Sospedra et al., 2013; Kong et al., 2014).

Epidemiological surveys of fresh produce and the occasional outbreaks, demonstrate the potential for a wide range of these products to become contaminated with pathogenic microorganisms. Bacterial pathogens such as *Salmonella enterica* (Oliveira et al., 2010; Holvoet et al., 2014), *Escherichia coli* O157:H7 (Ethelberg et al., 2010), *Bacillus cereus* (Valero et al., 2002), *Listeria monocytogenes* (Jofré et al., 2016) and *Pseudomonas* spp. (Hamilton-Miller and Shah, 2001; Viswanathan and Kaur, 2001) are especially of major concern due to the environmental occurrence of these bacteria.

Fresh and frozen berries are being increasingly involved as a vehicle of foodborne diseases (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards) 2013, 2014). Although these outbreaks are commonly linked to viral (Norovirus and Hepatitis A) and parasitic (*Cyclospora cayetanensis*) pathogens, outbreaks of bacterial origin are also documented (Palumbo et al., 2013).

The last reported outbreak in 2011 which caused at least 15 cases of illness including one death, traced back to a farm source of contaminated strawberries in Oregon, United States, and implicated *E. coli* O157:H7

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(OHA (Oregon Health Authority), 2011). An outbreak of *E. coli* O26 associated with consumption of raw blueberries was also reported, where six people were sickened and one was hospitalized (Goetz, 2011). In 2003, an outbreak of salmonellosis, linked to contaminated strawberries, involved 13 illnesses and two hospitalizations in California (CDC (Centers for Disease Control and Prevention), 2011). In 2009 and 2010, blueberries contaminated with *Salmonella* Muenchen and Newport resulted in 14 and six illnesses, respectively (CDC(Centers for Disease Control and Prevention), 2011; Miller et al., 2013). Additionally, in 1984, an outbreak of listeriosis was associated with fresh blueberries in Connecticut (Ryser, 1999).

Production practices, growth conditions and the location of the berries on the growing plant (soil surface, aerial part) in combination with intrinsic and extrinsic factors as well as harvesting and processing, will affect the microbiological quality of berries at the time of consumption (Harris et al., 2003; Li and Wu, 2013). Since berries are a perishable fruit, they are generally consumed raw or minimally processed, as well as a frozen ingredient added to many foods. Berries are also consumed as highly processed products, such as components of jams, preserves, heat treated fruit juices or purées and dried fruits which can be shelf-stable, having undergone heating or drying (Milivojević et al., 2011; Hsu et al., 2014).

Berry fruits have high moisture content and a soft skin, making them vulnerable to physical damage and microbiological spoilage. However, due to the high acidity (pH 2.7 up to pH 4.5) of the internal tissues they are unlikely to support the survival or growth of foodborne pathogens over extended periods (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014). On the other hand, contamination and cross-contamination via equipment, water (i.e. if washing is applied) and particularly via food handlers may take place during minimal processing, which are considered risk factors for berries. They are not usually blanched or heat-treated unless they are used in processed products, and once contaminated with foodborne pathogens berries cannot be easily decontaminated for fresh consumption (Knudsen et al., 2001; Concha-Meyer et al., 2014).

To the best of our knowledge, little information exists about microbiological quality of berries during all stages of the fruit processing once it arrives at the processing plant. To this regard, the aim of this work was the microbial characterization of the raw materials and the company fruit products. This survey will provide more data about the microbiological quality of fruit products processed sequentially from raw fruit, intermediate fruit product (mixing step) and the final product to facilitate future risk assessments. In order to have a representative sampling, several batches from all company suppliers of the selected berries were analyzed. Additionally, the results obtained will allow the companies to set target microorganisms that must be controlled during processing.

2. Material and methods

2.1. Sampling

This study used frozen berry samples harvested and produced from three to four suppliers during one year, through winter and summer months in 2017. Strawberries, raspberries, blackberries and blueberries comprised the majority of the samples analysed (n = 316). Samples were collected from a fruit processing plant producing fruit flavourings for ice cream, yogurt, pastry, and other products. Three types of berry products were collected for analysis including raw material (RM), intermediate fruit product (IP) and final product (FP). The intermediate sugar-based product contains 30% of fruit, 10–30% of sugar, 2% of hydrocolloids and >1% of food colourants/aromas/citric acid, while the intermediate product with sugar substitute consists of 30% of fruit, 2% of hydrocolloids and >1% of food colourants/aromas/citric acid/sugar substitute. Both products were subjected to a preheating at 30 °C for about 1–2 h. The final product is the same as intermediate product after the pasteurization process at 85–97 °C for 5–10 min (pH < 4.5 and aw >0.95). The pasteurization process depends on several factors such as initial microbial load of the raw material, target microorganism, physic-chemical parameters and global and European guidelines.

Samples of strawberries (*n = 120; 41 RM, 39 IP and 40 FP*), blueberries (*n = 52; 17 RM, 18 IP and 17 FP*), raspberries (*n = 59; 19 RM, 21 IP and 19 FP*) and blackberries (*n = 85; 30 RM, 26 IP and 29 FP*) were included in this study. The samples were taken at the production plant level by the quality management team of the plant and sent frozen to the laboratory on the days following.

2.2. Sample preparation and microbiological analyses

25 g of each fruit sample were suspended in 225 mL of buffered peptone water (BPW, Biokar Diagnostics, Beauvais, France) and aseptically homogenized in a stomacher (BagMixer® 400 P, Interscience, France) for 2 min. Serial decimal dilutions were prepared from this initial dilution. Each of the different dilutions (1 mL or 0.1 mL) was transferred onto the surface of the plates containing appropriate culture media for each target microorganism. Another portion of 25 g of the sample was homogenized in 225 mL of Half-Fraser broth (BioMérieux, Marcy-l'Etoile, France) and incubated at 30°C during 24 h for the detection of *L. monocytogenes*.

Microbiological analyses were carried out using the International Organization for Standardization (ISO) methodologies. The microbiological parameters of food safety and quality investigated were *E. coli* (according to ISO 16649–2:2001), *Salmonella* spp. (according to ISO 6579:2002), *L. monocytogenes* (according to ISO 11290–1:1996; ISO 11290–2:1998 and Amendment 1:2004), *B. cereus* (according to ISO 7932:2004), coagulase-positive staphylococci (according to ISO 6888–1:1999) and sulphite-reducing clostridia spores (according to IPQ, 1986, Portuguese Standard NP 2262:1986). Regarding sulphite-reducing clostridia spores detection, 10 mL of each sample in BPW solution were heat-shocked at 80–85 °C for 10 min before cultivation, so as to inactivate vegetative bacteria and enhance sporulation. Samples were then transferred to 10 mL of Differential Reinforced Clostridial Medium (DRCM) (peptone tryptic 10 g L^{-1}; meat extract 4 g L^{-1}; yeast extract 5 g L^{-1}; NaCl 5 g L^{-1}; starch 1 g L^{-1}; glucose 2 g L^{-1}; L-Cysteine hydrochloride 0.3 g L^{-1}; sodium sulphite 10 g L^{-1}; iron (III) citrate 0.5 g L^{-1}; agar 8 g L^{-1}) and incubated under anaerobic conditions at 37 °C for 5 days. This method relies on the reduction of ferric sulphite to iron sulphide and samples are considered positive when colour changes (i.e. presence of black spots).

Bacilli displaying typical growth for the *B. cereus* group, i.e. rough and dry colonies with violet pink background surrounding an egg yolk precipitation, were counted, isolated and chosen for haemolytic activity, tested on sheep-blood agar plates (BioMérieux) after a one day incubation at 30 °C. Black convex colonies showing lecithinase activity, with or without halo on Baird Parker medium (BPA, Biokar Diagnostics) were isolated and tested for coagulase, using rabbit plasma (Biokar Diagnostics). Colonies were also tested for catalase reaction. In the case of suspect colonies for *Salmonella*, biochemical confirmation was carried out with triple sugar iron agar, urea agar, and L-lysine decarboxylase medium. Confirmed isolates were serotyped at the Instituto Nacional de Saúde Doutor Ricardo Jorge (Portugal). Presumptive *L. monocytogenes* colonies on the chromogenic agar (ALOA, BioMérieux) were streaked onto sheep blood agar (BioMérieux) for appraisal of hemolysis, carbohydrate fermentation (i.e. rhamnose, xylose and mannitol) and Christie Atkins Munch-Petersen (CAMP) test according to the ISO 11290 standard, and they were further identified by multiplex PCR according to Doumith et al. (2004).

2.3. Genomic DNA extraction, PCR and sequencing

Colonies of staphylococci that appeared on the final product samples were isolated and identified by the sequence of the 16S rRNA. From cultures grown overnight in TSB (Tryptone Soy Broth, Biokar

Diagnostics) at 37 °C, DNA of each isolate was extracted using the GRS Genomic DNA Kit (Grisp, Porto, Portugal) according to the manufacturer's protocol for Gram-positive bacteria. PCR was performed in the final volume of 50 μL using: 5 μL of 10× Taq buffer + $(NH_4)2SO_4$, 5 μL of $MgCl_2$ (25 mM), 1 μL of dNTPs (10 mM), 0.5 μL of forward primer 27F 5′-AGAGTTTGATCCTGGCTCAG-3′ (100 μM), 0.5 μL of reverse primer 1492R 5′-GGTTACCTTGTTACGACTT-3′ (100 μM), 0.5 μL of Taq Polymerase (1U μL^{-1}), 2 μL of DNA and 35.5 μL of sterile ultrapure water. The amplification programme used in a thermocycler was initial denaturation at 95 °C for 5 min, followed by 30 cycles of as follows: denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1.5 min. This was followed by a final extension step at 72 °C for 10 min. Amplified products were run on 1% agarose gel at 80 V for 30 min. PCR products were purified, using the GRS PCR & Gel Band Purification Kit (Grisp), according to the supplier's instructions, and sent to Nzytech (Lisbon, Portugal) for sequencing. Sequences were identified in NCBI nucleotides databases using BLAST program.

3. Results

The microbiological quality of berries (strawberries, raspberries, blackberries and blueberries) was determined by detection of *Salmonella* spp., *L. monocytogenes*, sulphite-reducing clostridia spores, and by enumeration of *E. coli*, presumptive *B. cereus* and coagulase-positive staphylococci.

The overall results indicated that the bacteriological quality of the berries sampled and tested during the three production steps (raw material, intermediate and final product) was satisfactory. The occurrence of *Salmonella* spp., *L. monocytogenes*, *E. coli* and sulphite-reducing clostridia spores are presented in Table 1.

No *Salmonella* spp. was detected in any of the 104 intermediate products analysed, as well as in the 105 final product samples. However, *Salmonella enterica* subsp. *enterica* serovar Braenderup was isolated in one out of 107 raw material samples. This pathogen was isolated from blackberries. Regarding the occurrence of *L. monocytogenes*, a strain serotype 1/2a was isolated after a two-step enrichment process in one out of 52 blueberry raw material samples. The contamination level was lower than 100 cfu g^{-1}; furthermore, this pathogen was not detected in any of the other fruit samples.

Generic *E. coli* was found in two (blackberries; raw material) out of 316 tested samples with low contamination level, between 0.7 and 0.9 log cfu g^{-1}.

Sulphite-reducing clostridia spores were found in berry fruits, blackberries being the fruit with the highest prevalence of these microorganisms with 35 positive samples in total. Only blackberries showed contamination of the final product.

Table 2 reports the results for the prevalence of presumptive *B. cereus* and coagulase-negative staphylococci (CNS) in berry fruit samples. Since the coagulase-positive staphylococci were negative in the analysed samples, the prevalence of CNS was reported. *Staphylococcus* spp. were recovered from 10.1, 11.5, 44.9 and 14.4% of strawberry, blueberry, raspberry and blackberry samples, respectively. The highest prevalence of CNS in the samples (7.5, 11.5, 39.6 and 11% from strawberry, blueberry, raspberry and blackberry samples, respectively) was found between 1.7 and 2.5 log cfu g^{-1}. Staphylococci populations >2.5 log cfu g^{-1} were only found in 2.6, 5.3 and 3.4% of strawberries, raspberries and blackberries samples, respectively. However, these counts were found in the final product of raspberries and blackberries. Staphylococci were also found in the final product of blueberries and raspberries (5.9 and 5.3%, respectively) in the range between 1.7 and 2.5 log cfu g^{-1}. All the staphylococci present in the final product of the berry samples were coagulase-negative, as mentioned above, and these isolates were identified as *S. epidermidis*, *S. warneri* and *S. lugdunensis*.

Samples yielding growth on *B. cereus* selective medium and hemolytic greenish colour with clearing zone on sheep-blood agar were taken as positive. Presumptive *B. cereus* were isolated from most of the fruit samples, with populations ranging from <0.7 to 2.0 log cfu g^{-1}, while apart from raspberries, very few samples presented counts >2.0 log cfu g^{-1}. On the other hand, raspberries presented the highest prevalence of bacilli in the samples (84.2 and 90.5% from raw material and intermediate product samples, respectively) with counts >0.7 log cfu g^{-1}. More specifically, bacilli populations >2.0 log cfu g^{-1} were found in 26.3 and 52.4% of raw material and intermediate product from raspberries samples, respectively. The prevalence of presumptive *B. cereus* in the final product was low and counts ranged from 0.7 to 2.0 log cfu g^{-1}, with percentage occurrence of 5.0 and 6.9% in strawberries and blackberries, respectively.

Table 1. Occurrence of *Salmonella* spp., *L. monocytogenes*, *E. coli* and sulphite-reducing clostridia spores detected in berry fruit samples (n = 316; strawberry, blueberry, raspberry, blackberry).

Fruit samples	N	*Listeria monocytogenes*	*Salmonella* spp.	*Escherichia coli*	Sulphite-reducing clostridia[d]
Strawberry	120	ND	ND	ND	4 (RM)
Blueberry	52	1[a]	ND	ND	10 (5/5; RM/IP)
Raspberry	59	ND	ND	ND	11 (7/4; RM/IP)
Blackberry	85	ND	1[b]	2[c]	35 (18/15/2; RM/IP/FP)[e]

N: number of samples analysed (*i.e.*, taken all samples together).
ND: not detected.
[a] serotype 1/2a; detected after a two-step enrichment process in the raw material.
[b] *Salmonella enterica* subsp. *enterica* serovar Braenderup detected in the raw material.
[c] counts between 0,7 – 0,9 log cfu g^{-1} in the raw material.
[d] positive samples in 1 g of product.
[e] RM: raw material; IP: intermediate product; FP: final product.

Table 2. Prevalence (%) of presumptive *Bacillus cereus* and coagulase-negative staphylococci in berry fruit samples in three production steps (RM: raw material; IP: intermediate product; FP: final product).

		Percentage of samples in the indicative interval					
		Presumptive *Bacillus cereus* (log cfu g^{-1})			coagulase-negative staphylococci (log cfu g^{-1})		
Fruit samples	N	<0.7[a]	0.7–2.0	>2.0	<1.7[a]	1.7–2.5	>2.5
Strawberry							
RM	41	65.9	34.1	0	97.6	2.4	0
IP	39	61.5	35.9	2.6	92.3	5.1	2.6
FP	40	95.0	5.0	0	100	0	0
Blueberry							
RM	17	5.9	94.1	0	100	0	0
IP	18	66.7	27.8	5.6	94.4	5.6	0
FP	17	100	0	0	94.1	5.9	0
Raspberry							
RM	19	15.8	57.9	26.3	89.5	10.5	0
IP	21	9.5	38.1	52.4	76.2	23.8	0
FP	19	100	0	0	89.5	5.3	5.3
Blackberry							
RM	30	23.3	73.3	3.3	96.7	3.3	0
IP	26	34.6	65.4	0	92.3	7.7	0
FP	29	93.1	6.9	0	96.6	0	3.4

N: number of samples analysed.
[a] Counts below limit of detection (i.e. 10 or 100 cfu g^{-1}) were reported as 5 and 50 cfu g^{-1}; correspondingly, a log cfu g^{-1} value of 0.7 and 1.7 represents a sample in which presumptive *B. cereus* and coagulase-negative staphylococci were not detected.

4. Discussion

The knowledge of the microbiological quality of the raw materials used in food industry is one of the most relevant tasks in the evaluation of the impact of all stages of the production process on the quality of finished product, and in particular in the definition of the time/temperature binomials of pasteurization processes. In this content, the aim of this work was the microbiological characterization of the raw materials and the company's fruit products. In order to have a representative sampling, several batches from all company suppliers of the selected berries were analysed.

Since there is no routine or regular monitoring of berries and the current European Union legal framework does not include microbiological criteria applicable for these fruits at the primary production stage, very limited information about the prevalence of foodborne pathogens is available. Additionally, most of the reported works refer to strawberries and decontamination strategies. Therefore, a complete comparison of the data presented herein is difficult, since there are no similar studies, but still comparisons can be made. Although outbreaks implicating berries and bacterial pathogens are not common, the potential for pathogenic contamination still exists due to berry agricultural practices and consumer preparation.

The results from this study indicate that occurrence of the pathogens *L. monocytogenes* and *Salmonella*, as well as the hygiene indicator *E. coli* levels in the berries sampled are low. The few available studies and data suggest a low prevalence of these pathogens on berries in accordance with our study. In support of this view, several studies have reported minimal prevalence of *Salmonella* spp. and *E. coli* O157 on strawberries (0/173; Johannessen et al., 2002, 0/11; Mukherjee et al., 2004, 0/194; Mukherjee et al., 2006, 0/31; Bohaychuk et al., 2009, and 0/36; Yoon et al., 2010). In addition, a survey conducted by Delbeke et al. (2015) in Belgium reported that no *Salmonella* spp. or Shiga Toxin-producing *E. coli* (STEC) were isolated from strawberry samples (i.e. 0 out of 72). In the same report, generic *E. coli* was present in only two of 72 strawberry samples at levels of 1.0 and 3.0 log cfu g^{-1} (Delbeke et al., 2015). In another study, *E. coli*, *Campylobacter*, *Salmonella* and STEC on the strawberries sampled and tested were absent or at very low concentration; no pathogens were detected and only one out of 80 samples harboured *E. coli* with counts of 1.0 log cfu g^{-1} (Johannessen et al., 2015). Similarly, Macori et al. (2018) evaluated freshly harvested berries (raspberries, blueberries, blackberries and redcurrant) and found no *Salmonella* spp. and STEC in the tested samples. Blackberry was the only fruit found positive for *E. coli* which was detected in just two out of 75 berry samples. However, in another study a high proportion of positive samples was observed for *E. coli* both in field and purchased strawberries (up to 48.6%) but with counts less than 100 cfu g^{-1} (Dziedzinska et al., 2018). Levels of fecal organisms, such as *E. coli* are a better indicator of contamination (Nguyen-The and Carlin, 1994) and this could explain why this organism has been included as a hygienic criterion in the EU regulation (No 2073/2005) (Anonymous, 2005). In our study, none of the samples exceeded the hygienic criteria established by the Commission Regulation (EC) No 2073/2005 guidelines for vegetables and fruits (i.e. $>$ 100 cfu g^{-1}).

As far as *L. monocytogenes* is concerned, Johannessen et al. (2002) isolated this pathogen in one sample of domestically grown strawberries and Graça et al. (2017) reported no *L. monocytogenes* in the fresh-cut strawberries studied. Similar results were found by Dziedzinska et al. (2018), where *L. monocytogenes* detection was reported as sporadic with only one positive sample from all field strawberries (0.6%) and one positive sample of purchased strawberries (1.4%). The contamination level in both cases (our study and the referenced studies) was lower than 100 cfu g^{-1}, which rests within the food safety criteria for this pathogen defined by the Commission Regulation (EC) No 2073/2005 for ready-to-eat foods unable to support the growth of this pathogen (Anonymous, 2005). *Listeria* spp. are ubiquitous bacteria widely distributed in the environment and can be found in soil and water. Therefore, fruits and vegetables may easily become contaminated with this bacterium.

Sulphite-reducing clostridia species are ubiquitously distributed in the environment and can be readily isolated from decaying vegetation, marine sediments, soil and the intestinal tract of humans and other vertebrates (Palese et al., 2009). This can explain the presence of these microorganisms in the fruits analyzed as they traditionally grow in soil or in hydroponic cultivations and also the type of irrigation (i.e., lowered well-water or flooded surface channel water) can influence the transfer of contamination at pre-harvest level. The spores of this organism are more heat-resistant than most foodborne pathogens and can survive thermal processing conditions during manufacturing process of products. Hence, the presence of sulphite-reducing clostridia spores in the blackberry final product can be explained by the perceived heat-resistance of the spores of these microorganisms. *Clostridium* sp. includes pathogenic bacteria, such as *C. perfringens* and *C. botulinum*, constituting a serious public health problem since its spores are resistant to heat and persist in the environment.

Presumptive *B. cereus* was present in the samples analysed and ranged between 0.7 to 2.0 log cfu g^{-1} and some of the bacilli isolated even exceeded 2.0 log cfu g^{-1}. However, these microorganisms were only detected in the final product of strawberry and blackberry samples (5.0 and 6.9%, respectively) at the level of $<$2.0 log cfu g^{-1}. Similarly, low levels of *B. cereus* were detected in strawberries (1.1 log cfu g^{-1}) by Yoon et al. (2010). *B. cereus* is ubiquitous and can be found in a wide range of foodstuffs, soil, raw materials, raw fruits and vegetables, raw herbs, dry foods and processed foods (Park et al., 2018; Tango et al., 2018). *B. cereus* is a spore-forming bacteria and some strains are psychrotrophic and highly resistant to different environmental conditions (humidity, pH, temperature, etc.) (Kotiranta et al., 2000; Faille et al., 2002). The presence of presumptive *B. cereus* in the final product can be explained by the ability of spores of *Bacillus* spp. to survive pasteurization and may then be able to germinate and multiply (depending on pH, a_w, etc) at low temperatures due to their psychrotrophic nature (Christiansson et al., 1999). These characteristics make *B. cereus* an important bacterium in the heat-treated fruit based-products industry as high numbers of these bacteria and their toxins in foods pose a potential risk in terms of health and also food spoilage. It is generally believed that any food exceeding 10^4 to 10^5 cells or spores per gram may not be safe for consumption (Granum and Lund, 1997; Beattie and Williams, 2000). In our study, just four samples of raspberry showed presumptive *B. cereus* levels that were slightly higher than 3.0 log cfu g^{-1}. These samples were from raw material and intermediate product. The infective dose may vary from 10^5 - 10^8 viable cells or spores in part because of the large differences in the amount of enterotoxin produced by different strains. Hence food containing $> 10^4$ cells/spores per g may sometimes pose a risk (Granum and Lund, 1997). Therefore, the levels of presumptive *B. cereus* recorded from the samples in this study are not a cause for major concern.

S. aureus is one of the major causes of food poisoning. Intoxication is caused by the ingestion of enterotoxins within foods. Additionally, human skin and nasal membranes are the main reservoirs of these bacteria (Jablonski and Bohach, 1997), which may contribute to contamination of the products during handling and distribution. Since coagulase-positive staphylococci were not detected in the samples studied, it can be considered that our tested fruits would not lead to a health risk regarding the presence of these microorganisms. However, the CNS were recovered from 10.1, 11.5, 44.9 and 14.4% of strawberry, blueberry, raspberry and blackberry samples, respectively. Similarly, coagulase-positive staphylococci were also not detected in fresh-cut strawberries (Graça et al., 2017). In contrast, Johannessen et al. (2002) isolated *Staphylococcus* spp. from 15% of strawberry samples. Most of the staphylococci were coagulase-negative except for two out of 26 isolates that were *S. aureus* (non-toxinogenic). The CNS isolates were identified as *S. epidermidis*, *S. hominis*, *S. xylosus* and *S. capitis*. In another study, *S. aureus* was detected in strawberries at a level $<$2.0 log cfu g^{-1} (Yoon et al., 2010). In our study, the CNS isolates were identified as

S. epidermidis, *S. warneri* and *S. lugdunensis*. These microorganisms are reported as common commensal organisms found as part of the skin microbiota on humans. Hence their presence in the final product can be explained by the transfer of these bacteria from workers' hands during produce handling. Food handlers are well recognized as potential vectors for transferring microorganisms to food products; and thus, it's advisable to operate a periodic training program to assure maximum operational efficiency in food industry including hygienic practices.

In general, among all the berries analysed, raspberries and blackberries showed the highest presumptive *B. cereus* and coagulase-negative staphylococci counts; this could be explained by the different surface structure observed, which may affect the attachment of the bacterial cells.

Although spoilage microbiota predominates within raw material, the potential presence of pathogenic bacteria has been documented even though at low numbers. Therefore, to improve safety and extend product shelf-life, the fruit industry applies different thermal processes to kill pathogens and reduce spoilage microorganisms (Petruzzi et al., 2017).

The microbiological quality of raw material, the hygiene of the food contact surfaces, the manufacturing environment, the hygiene of food handlers and the efficacy of the product process, are major factors determining the microbiological quality and safety of the final product (Abadias et al., 2008; Lehto et al., 2011). The absence of pathogenic microorganisms in the final product of this study indicates that overall hygiene and processing practices carried out by the industry were good.

Although the numbers of bacterial outbreaks associated with berries are relatively low, the mentioned outbreaks involving strawberries and blueberries, indicates a gap in the production system. Considering the limited amount of post-harvest intervention strategies to kill pathogens on berries, along with a scarcity of published reports on prevalence of foodborne pathogens on these fruits, an understanding of the microbiological quality during pre- and post-harvest is crucial for developing strategies to prevent future foodborne outbreaks. Results from this study, therefore, may provide the fruit industries with important information for making recommendations on good agricultural and processing practices.

Declarations

Author contribution statement

Márcia Oliveira, Paula Teixeira: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Cristina Rodrigues: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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