Food Control 73 (2017) 775–783

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Microbiological quality and safety of minimally processed fruits in the marketplace of southern Portugal

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ARTICLE INFO

Article history:
Received 3 July 2016
Received in revised form
15 September 2016
Accepted 16 September 2016
Available online 13 October 2016

Keywords:
Fresh-cut fruit
Microbiological quality
Safety
Spoilage
Salmonella
Listeria monocytogenes

ABSTRACT

The availability of fresh-cut fruit (FCF) in the marketplace has been increasing in Portugal, although reports of its microbial quality are not known. Due to the growing concerns of these commodities over their microbial safety, the objectives of this work were to study the microbiological quality and prevalence of *Salmonella* and *Listeria monocytogenes* on fresh-cut fruits sold in southern Portugal. A study to examine the changes in pH and microbial counts, before and after the expiration dates, was also made. A total of 160 samples was purchased in the local grocery stores between September 2011 and August 2014, before their sell-by date. These samples were assayed for aerobic mesophilic (AM) and psychrotrophic (AP) microorganisms, yeasts and molds (YM), lactic-acid bacteria (LAB), coliforms (TC), *Escherichia coli* and coagulase positive staphylococci as well as *L. monocytogenes* and *Salmonella*. The microbiological counts ranged from 3.0-9.2 lg cfu/g (AM); 2.2–10.7 lg cfu/g (AP); 2.3–10.4 lg cfu/g (YM); 1.9–9.0 lg cfu/g (LAB) and less than 1–9.1 lg cfu/g (TC). The melons and watermelon presented the highest levels of the microbial quality parameters studied. However, no *E. coli*, staphylococci, *Salmonella and L. monocytogenes* were detected in any of the samples. After the sell-by date, an increase of the AM, AP, LAB and YM values was observed in all fruits. Conversely, the differences found in TC counts before and after the best-before date had no statistical significance. A decrease in pH was observed in all fruits except pineapple whose pH slightly increased after 14 days of storage. The results highlight the importance of preventing contamination and cross contamination, selecting adequate decontamination technologies and maintaining a strict temperature control during processing, distribution and selling of FCF.

1. Introduction

During the last few decades, fresh produce, including fruits, have emerged as a new vehicle for the transmission of food-borne diseases associated with etiological agents that in the past were ascribed to animal reservoirs. An increase in outbreaks has been reported all over the world (Denis, Zhang, Leroux, Trudel, & Bietlot, 2016; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). Some of the last outbreaks attributed to *Salmonella enterica* in fresh fruit include *Salmonella* Braenderup in mangoes (CDC, 2012a), *Salmonella* Typhimurium and *Salmonella* Newport in cantaloupe (CDC, 2012b), *Salmonella* Agona in papayas (CDC, 2011a) and *Salmonella* Newport in watermelon ready to eat (Byrne et al., 2014). Pathogenic *Escherichia coli* has also been found in outbreaks involving fresh produce through the consumption of raw sprouts (CDC, 2014), ready-to-eat salads (CDC, 2013), organic spinach (CDC, 2012c) and romaine lettuce (CDC, 2010; 2012d). Additionally, listeriosis is among the most important cases of outbreaks in the United States involving the consumption of cantaloupe (CDC, 2011b). The factors that may have contributed to the emergence of fresh produce as a cause of outbreaks are the natural evolution of microorganisms, the changes in the industry, in the population characteristics and in consumer behavior. On the other hand, the enhanced epidemiological surveillance and the application of new techniques to identify and track the pathogens have increased, thus resulting in a greater awareness of food borne infections worldwide (Beuchat, 2002; Brandl, 2006; Tauxe et al., 1997). The emergence of

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http://dx.doi.org/10.1016/j.foodcont.2016.09.046
0956-7135/

pathogens with low infection doses and the ability of microorganisms acquiring virulence or pathogenicity factors through natural evolution mechanisms are fundamental aspects in understanding the rise of fresh food products as responsible for diseases associated in the past to animals' reservoirs (Croxen et al., 2013; Melo, Andrew, & Faleiro, 2015; van Elsas, Semenov, Costa, & Trevors, 2011). Within the industry, mass production and centralization of the production/processing systems generates large distribution networks implying an increase in volumes of importation/exportation of fresh fruits and vegetables and the rising of the fresh-cut industry, which may contribute to these problems (Denis et al., 2016). The increase in the at-risk populations (elderly, immune-compromised), and the consumption of minimally processed fruits and vegetables may also have led to increased outbreaks (Beuchat, 2002; Brandl, 2006; Denis et al., 2016; Tauxe et al., 1997).

The minimally processed fruits are characterized by having non-sterile cut surfaces which are physiologically active and rich in nutrients and water. These foods are raw, ready-to-eat and not subjected to any thermal or chemical process of preservation (Berger et al., 2010; Nguyen-the & Carlin, 1994; Olaimat & Holley, 2012). For this reason, fresh-cut fruits are susceptible to microbiological contamination in the various stages, from food processing to distribution and commercialization (Barth, Hankinson, Zhuang, & Breidt, 2009; Francis et al., 2012). If the processing operations are improper and storage and distribution occur in inadequate conditions (temperature), some microorganisms from the initial population or resulting from cross contamination, can survive and multiply, accelerating the degradation processes (in the case of spoilage microorganisms) and/or increasing the risk of food becoming a danger to public health (in the case of pathogenic microorganisms). Additionally, the trend to increase the shelf-life of refrigerated foods can allow the growth of psychrotrophic pathogens such as *Listeria monocytogenes* (Melo et al., 2015). This trend and the use of packaging techniques, such as modified atmosphere, contributes to inhibit the growth of aerobic microbiota but does not inhibit facultative anaerobic and anaerobic pathogenic microorganisms that may have the ability to survive and multiply under these conditions. The microbial growth on food products during the chain of production and commercialization are believed to be one of the main causes of the majority of outbreaks (Codex Alimentarius Commission, 1999) and spoilage. Furthermore, the ability of microbiota to subsist and/or grow at different temperatures in fresh-cut fruits have been described by several researchers (Abadias, Usall, Anguera, Solsona, & Viñas, 2012; Alegre, Abadias, Anguera, Oliveira, & Viñas, 2010; Alegre, Abadias, Anguera, Usall, & Viñas, 2010; Dingman, 2000; Salazar, Lourenço, Graça, Quintas, & Nunes, 2015; Santo, Graça, Nunes, & Quintas, 2016). The microbial quality of fresh-cut fruit is of concern, not only from the food safety point of view, but also due to the spoilage involved in the reduction of shelf-life which results in huge economic losses (Johnston et al., 2006). The presence of spoilage microorganisms, mainly yeasts, lactic-acid bacteria and pectinolytic pseudomonas, may explain the off-flavor formation, slimy surface, wetness, soft rot, changing color and visual microbial growth/colonies (Nguyen-the & Carlin, 1994). Those spoilage microorganisms may have a mesophilic or a psychrophilic behavior such as lactic-acid bacteria (Pothakos et al., 2014).

The main objective of the present work was to study the microbiological quality of fresh-cut fruits in the marketplace in southern Portugal (Algarve), through the enumeration of aerobic mesophilic and psychrotrophic microbiota, total coliforms and *E. coli*, lactic-acid bacteria, coagulase-positive staphylococci and fungi. The detection of the food safety parameters *Salmonella* spp. and *L. monocytogenes* was also an objective of this work. In addition, a characterization of the microbiological parameters (aerobic mesophilic and psychrotrophic microbiota, total coliforms, lactic-acid bacteria and fungi) of the fresh-cut fruits before and after their sell-by date was performed.

2. Material and methods

A total of 160 packed samples of fresh-cut fruits were bought in retail markets in Algarve (Portugal) before their sell-by date during the period of 2011 and 2014. Samples of individual packs (70 g - 100 g) of fruit pieces were purchased throughout the year and transported to the laboratory in refrigeration conditions. For each sample, date, place of manufacture and purchase, lot number and best-before date were registered. One hundred and five fresh-cut fruit samples were examined on the day of buying before their best-before date (pineapple: 29; mango: 9; papaya: 10; green melon: 19; cantaloupe melon: 7; Galia melon: 12; watermelon: 8; strawberries: 4; fruit salads: 7) for mesophilic (AM) and psychrotrophic (AP) aerobic counts, yeasts and molds (YM), lactic-acid bacteria (LAB), total coliforms (TC), *E. coli*, coagulase positive staphylococci (STAPH) and for the safety parameters *Listeria monocytogenes* and *Salmonella* sp.. Fifty-five fresh-cut samples (pineapple: 10; mango: 9; papaya: 6; green melon: 9; cantaloupe melon: 7; Galia melon: 9; watermelon: 5) were analyzed before (5–6 days) and after their best-before date (when visible spoilage was detected). The microbiological parameters studied were AM and AP, TC, LAB and YM. The pH values of the fruits were also studied in the fresh-cut fruit before and after the expiration date. The set of 55 packed samples of fresh-cut fruit studied after their expiration date were stored, unopened, at 4 °C for 10–14 days when observable spoilage was noticed. Then the packages of these samples were opened and analyzed for the microbial parameters. The pH values measurements were performed, before and after the expiration dates, using a digital Crison instrument, GLP 21 pH meter, equipped with a penetration electrode, at 21 °C. For each sample, the pH was measured in 2 pieces of fruit in triplicate.

2.1. Microbiological analysis

The microbiological quality of fresh-cut fruit samples was studied according to standard methodologies (ISO) summarized in Supplemental Table S1 and using Chromocult Agar (Merck, Darmstadt, Germany) for coliform and *Escherichia coli* (González, Tamagnini, Olmos, & Sousa, 2003).

Subsamples of twenty-five grams of each fruit package were diluted in 225 mL of buffered peptone water (BPW, Oxoid) and homogenized in a stomacher (Model 400 Circulator, Seward, Norfolk, England) for 2 min, at regular speed. The enumeration of AM, AP, LAB, TC and *E. coli*, YM and STAPH was performed from this suspension. The remaining suspension was incubated at 37 °C for 18 h for the detection of *Salmonella* sp.. The accounting of total coliform and *E. coli* was performed by pouring plating aliquots of the serial dilutions in Chromocult agar. Another portion of 25 g of the sample was homogenized in 225 mL Half-Fraser broth (Oxoid, Basingstoke, England) and incubated at 30 °C during 24 h for the detection of *L. monocytogenes*.

2.2. Pathogen confirmation

Presumptive isolates of *Salmonella* spp. and *L. monocytogenes* were collected from 5 to 2 samples of fresh-cut fruits, respectively. Colonies were streaked onto TSA (Tryptic Soy agar, Scharlau, Barcelona, Spain) until their purification and were then tested for Gram stain, cytochrome C oxidase and catalase activities. Presumptive colonies of *Salmonella* spp. were cultivated in Brain Heart