

Study of Biological Hazards Present on the Surfaces of Selected Fruits and Vegetables

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Introduction

Fruits and vegetables eaten raw are potential vehicles of pathogens due to the impossibility of applying thermal treatments, which would considerably reduce biological hazards that might be present (De Roever, 1998; Viswanathan and Kaur, 2001). Many of the contaminant microflora are part of the production environment or may also occur during harvesting, transportation, processing, storage, marketing or even in a consumer's home (Beuchat, 1998). Lettuce is one of the most consumed salad vegetables in Portugal and absorption of nutrients is favoured by the consumption of raw leaves, which remain uncovered from production to the distribution phase. Fruits have a physical barrier, the peel, which protects them from microbial contamination and proliferation. However, many fruits possess rough peels with very pronounced textures, like pineapples and melons, that can contribute to a significant increase in a microbial load carried, and can therefore result in contamination of the interior of the fruit during peeling (Altekruse *et al.*, 1997). These types of products are usually consumed raw, are grown in contact or near the soil, are exposed to intensive handling from harvesting until consumption and in the case of the fruits, they are not usually washed before peeling. Therefore, the aim of this study was to evaluate the microbial load on the surface of fruits with rough and very pronounced textured peels, namely pineapples and melons, as well as leaf lettuce, analyzing the varieties, countries of origin and distribution sites of these products.

Methods

Between June 2009 and June 2010, 120 samples (40 lettuces, 40 pineapples and 40 melons) were collected with different geographic origins and sold in several distribution sites in northern Portugal. Some lettuces were taken directly from the growing crop field. Two varieties of lettuce (*Lactuca sativa* L. var. *capitata* and *Lactuca sativa* L. var. *crispa*) and melons (*Cucumis melo* L. var. *cantalupensis* Naudin and *Cucumis melo* L. var. *reticulatus* Naudin) were studied (Fig. 1). Different varieties of pineapple were used, one of them from Azores (*Ananás dos Açores*) (Fig. 1). The fruits were peeled without prewash and the peels analyzed after storage at 4 °C overnight. Leaves of lettuces were analyzed directly without any treatment. Enumeration of aerobic plate count, *Enterobacteriaceae*, *Escherichia coli* and coagulase-positive staphylococci, as well as detection of *Listeria monocytogenes* and *Salmonella* spp., were performed for all samples. For characterization of coagulase-positive staphylococci isolates, a DNA extraction (Aires de Sousa *et al.*, 1996) and Multiplex PCR (Zhang *et al.*, 2004) were done to evaluate the presence of a set of *Staphylococcus* genus-specific primers and verify if *Staphylococcus aureus* were present in the samples. For characterization of *L. monocytogenes* isolates, a DNA extraction and a Multiplex PCR (Doumith *et al.*, 2004) were performed to identify the serovars present in the samples. The minimum inhibitory concentration (MIC) of several antibiotics was determined for coagulase-positive staphylococci and *L. monocytogenes* isolates by the agar dilution method (CLSI, 2007). An analysis of variance (one-way ANOVA) was performed to test significant differences in bacterial counts between varieties of the products, countries of origin and distribution sites.



Fig. 1. A. Lettuce *L. sativa* L. var. *capitata*. B. Lettuce *L. sativa* L. var. *crispa*. C. Melon *C. melo* L. var. *cantalupensis* Naudin. D. *C. melo* L. var. *reticulatus* Naudin. E. *Ananás dos Açores*. F. Pineapple from other varieties.

Results and Discussion

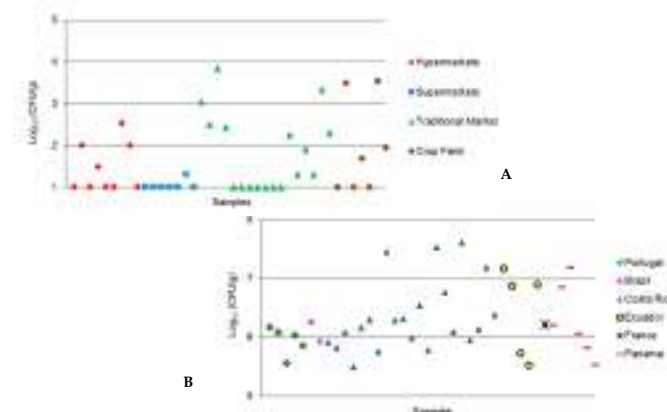


Fig. 2. A. *E. coli* counts in lettuce comparing distribution sites. B. Aerobic plate counts in pineapple comparing countries of origin.

There were no significant differences in the varieties of the products in this study ($p > 0.05$), but significant differences were observed in lettuce samples according to distribution sites ($p < 0.05$): samples purchased at supermarkets showed lower *E. coli* counts than those of other establishments (Fig. 2A). Faecal contamination (*E. coli*) was found in 47.5 % of lettuce samples, 15 % were contaminated with *L. monocytogenes* and 7.5 % with coagulase-positive staphylococci. Significant differences were observed between fruit peels according to country of origin and distribution sites ($p < 0.05$). Pineapples from Ecuador and melons from Morocco had higher aerobic plate counts (Fig. 2B) and *Enterobacteriaceae* counts (data not shown), respectively, than other countries. The fruits purchased in hypermarkets showed lower bacterial counts when compared with other establishments (data not shown). 5 % of pineapples presented faecal contamination, 2.5 % were contaminated with *L. monocytogenes* and 2.5 % with coagulase-positive staphylococci. 2.5 % of melon samples were contaminated with *L. monocytogenes* but neither *E. coli* nor coagulase-positive staphylococci were found in these samples. *L. monocytogenes* isolates belonged to serovars 2 (1/2c or 3c) or 4 (4b, 4d and 4e) and none were found to be resistant to antibiotics commonly used in therapy of listeriosis. None of the coagulase-positive staphylococci was *S. aureus* neither did they exhibit multidrug resistance. *Salmonella* spp. was not detected in fruits and vegetables analyzed in this study.

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Acknowledgements

This work was supported by National Funds from FCT – Fundação para a Ciência e a Tecnologia through project PEst-OE/EQB/LA0016/2011.

The accomplishment of this work was supported by QREN project - ADI 11493 "Probiofrut: Estudo de novas formulações e otimização tecnológica para o desenvolvimento e caracterização de novas matrizes de fruta com propriedade probióticas/simbióticas".

