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Microbiological Status and Quality Traits of Ready-to-Eat Minimally Processed Vegetables Sold in Córdoba, Argentina

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HIGHLIGHTS

- All 60 minimally processed vegetable samples were positive for total coliforms.
- Fecal coliforms counts ranged from 0.30 to 1.95 log Most Probable Number/g.
- Some minimally processed vegetable samples showed low microbiological quality and imperfect quality traits.

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Acronyms and abbreviations
CFU=Colony Forming Unit
FC=Fecal Coliforms
MPN=Most Probable Number
MPV=Minimally Processed Vegetable
TC=Total Coliforms
YM=Yeast and Molds

ABSTRACT

Background: The changes and the availability of processed foods have increased the demand for ready-to-eat foods, such as Minimally Processed Vegetables (MPVs). The purpose of this work was to evaluate the microbiological status and quality traits of the MPVs obtained from retail outlets of Córdoba, Argentina.

Methods: Totally, 60 MPVs of 12 brands (30 single-ingredient salads and 30 mixed salad trays) were randomly sampled from different retail outlets of Córdoba, Argentina. The samples were analyzed according to international standards for Total Coliforms (TC), Fecal Coliforms (FC), *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and Yeast and Molds (YM). The quality traits (respect to physical and sensory properties) of the MPVs were evaluated. Statistical analyses were performed with InfoStat.

Results: All 60 MPV samples were positive for TC, ranging from 1.32 to 3.38 log Most Probable Number (MPN)/g. FC counts ranged from 0.30 to 1.95 log MPN/g. Out of 60 samples, 15 (25%) were positive for *E. coli*. Three brands of mixed trays were positive for *S. aureus*. Regarding sensory characteristics, the parameters such as freshness, turgid, normal color, decay, and dehydration were compliance in 42 out of 60 (70%) samples.

Conclusion: Some MPVs sampled from Córdoba, Argentina showed low microbiological quality and imperfect quality traits.

Introduction

In recent years, demographic and lifestyle changes have led people to spend less time for preparing meals and consuming healthy foods such as vegetables (Schuh et al., 2020). The changes and the availability of processed

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foods have increased the demand for ready-to-eat foods, such as Minimally Processed Vegetables (MPVs; Moubarac et al., 2017).

The elaboration of MPVs includes different stages such as selection, prewashing, cutting, disinfection, rinsing, spinning, packaging, and refrigeration (Zambrano-Zaragoza et al., 2017). These minimal processing methods have the virtue of slightly modifying the original characteristics of the fresh product and give it a sufficient shelf life, which allows it to be transported from the processing plant to the consumer in good conditions. However, the production of this type of food may accelerate the rate of deterioration and favors the development of microorganisms (Siroli et al., 2015). With a high surface/weight ratio and a relatively high pH, salad could host a large microbial population, particularly bacteria, which may contribute to the natural decay of vegetative organs detached from the plant (Ragaert et al., 2007). Various food-borne pathogens like *Salmonella* and *Escherichia coli* O157:H7, *Staphylococcus aureus*, etc. may contaminate the product during plant cultivation and processing (Meireles et al., 2017).

The incidence of enteric pathogens in food is a serious problem that has been associated with organic waste and irrigation water contaminated with fecal matter, livestock, and wild animals; and inappropriate conditions during storage (Mir et al., 2018). Compliance with Good Manufacturing Practices (GMP) is essential throughout the food production, processing, and marketing chain to guarantee food safety and to protect the population's health.

In Argentina, there is no enough information on the frequency of pathogen bacteria in MPVs salads. Hence, the major purpose of this work was to evaluate the microbiological status and quality traits of the MPVs obtained from retail outlets of Córdoba, Argentina.

Materials and methods

Samples

During August 2018 to November 2019, a total of 60 samples of MPVs (100 g trays) were collected, including 30 single-ingredient salad samples (arugula) and 30 mixed trays (white and purple cabbage, carrots, lettuce, or chicory). The samples consisted of 12 brands (M1-M12) of MPVs sold in Córdoba City (Argentina) that were randomly purchased from different supermarkets in their original packages. All samples were purchased before their best-before date, transported to the laboratory, and refrigerated (4-5 °C) until the expiration date when they were analyzed.

Microbiological analysis

The samples were processed as described by Abadias et al. (2008). At first, 25 g of each sample was diluted in 225 ml of peptone saline solution and homogenized for 5 min. Ten-fold serial dilutions of the suspension were made in peptone saline solution and analyzed for Total Coliforms (TC), Fecal Coliforms (FC), *E. coli*, and Yeast and Molds (YM). YM, TC, FC, and *E. coli* were quantified using classical methodologies, with the results being expressed as Most Probable Number (MPN)/g and YM was expressed as Colony Forming Unit (CFU)/g (De Oliveira et al., 2011; Mom et al., 2020). In addition, another 25 g were diluted in 225 ml of buffered peptone water (Merck, Germany) for detection of *Salmonella* (ISO 6579:2002). The samples were incubated at 37 °C for 24 h. After 24 h, an aliquot of each sample was taken and mixed with Rappaport Vassiliadis Soya broth (RVS; Oxoid, UK) and Müller-Kauffmann tetrathionate-novobiocin broth (MkTTn; Oxoid, UK). After the selective enrichment step, each sample was streaked on a differential medium (Xylose Lysine Desoxyscholate and Hektoen enteric agar). The isolate and determination of *S. aureus* was made according to Oh et al. (2007). To isolate *S. aureus*, 25 g of each sample was enriched with 225 ml of tryptic soy broth (TSB; Merck, Germany) with 10% NaCl at 37 °C for 24 h. Then, 0.1 ml of suspension was spread onto Baird-Parker (BP) agar (Britania, Argentina). Results were obtained as presence or absence of *Salmonella* spp. and *S. aureus* in 25 g of sample.

Quality traits

In all samples, the characteristic established by the regulation (AAC, 2008) were visually evaluated for quality traits (respect to physical and sensory properties). If the product did not comply with at least one of these characteristics (for example, it was not clean), it was categorized as 0=fail. If it complied with all the requirements, it was categorized as 1=complies.

Statistical analyses

The data obtained were statistically analyzed by (ANOVA) and the means were then compared by Fisher LSD test ($p \leq 0.05$), after testing the assumptions of normality and homoscedasticity. The numbers of YM, TC, and FC were log-transformed. Statistical analyses were performed using the InfoStat v.2018 program.

Results

All samples (n=60) were positive for TC. However, the TC counts showed considerable variation between the brands, ranging from 1.32 to 3.38 log MPN/g. FC were found in 11 out of 12 analyzed brands ranging from 0.30 to 1.95 log MPN/g. TC and FC counts were significantly ($p<0.05$) different among various 12 vegetable brands (Figure 1).

Out of 60 samples, 15 (25%) were positive for *E. coli*. YM counts varied greatly with range of 3.47-8.47 log10 CFU/g. Three brands of mixed trays (M2, M4, and M6) were positive for *S. aureus*. *Salmonella* spp. was not found in any of the analyzed samples.

Physical and sensory characteristics of samples are detailed in Figures 2 and 3. Regarding sensory characteristics, the parameters such as freshness, turgid, normal color, decay, and dehydration were compliance in 42 out of 60 (70%) samples. Refrigeration was the only parameter that was fulfilled in all the analyzed samples.

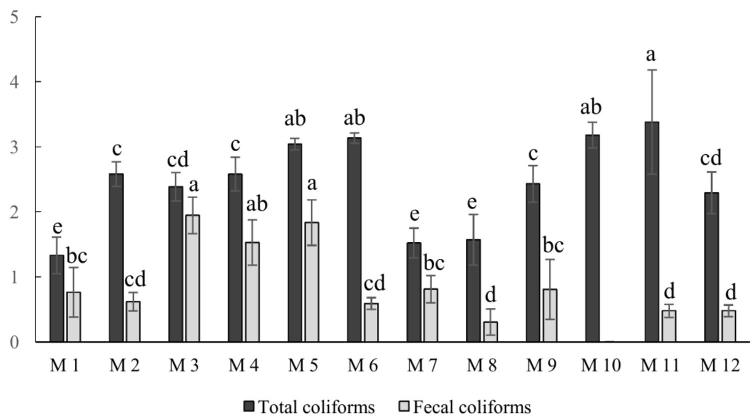

Figure 1: Abundance of total and fecal coliforms (log_{10} MPN/g) in arugula and mixed trays sampled during 2018-2019 obtained from different retail outlets of Córdoba, Argentina. M1, M3, M5, M7, M8, and M9: different brands of arugula trays. M2, M4, M6, M10, M11, and M12: brands of mixed trays. For each parameter, different letters indicate significant differences between brands (LSD; n=5; $p<0.05$)

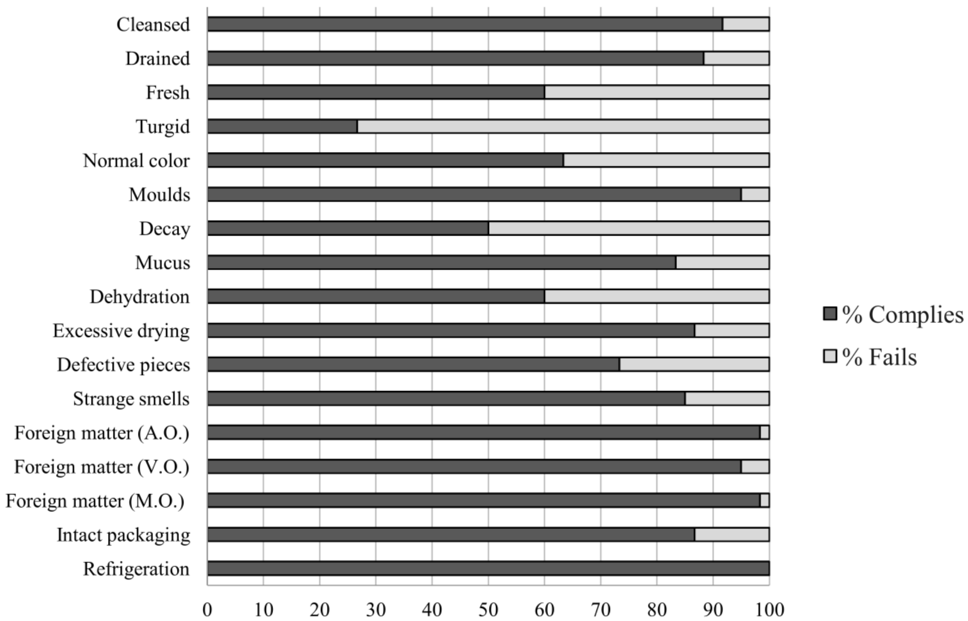

Figure 2: Compliance of quality traits of minimally vegetable processed samples according to regulations of the Argentine Alimentary Code (AAC). A.O.: Animal Origin; V.O.: Vegetable Origin; M.O.: Mineral Origin

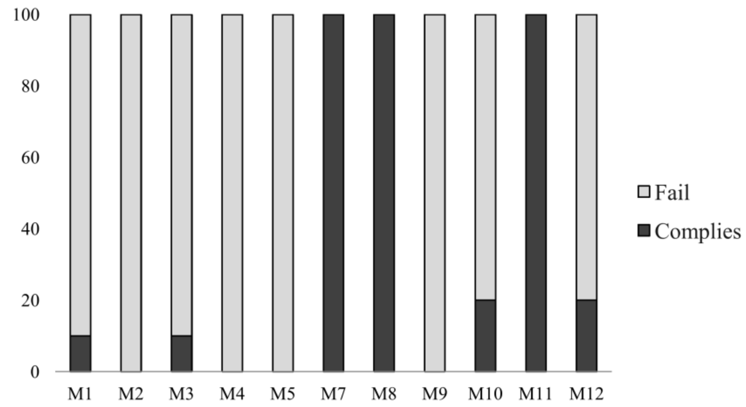

Figure 3: Compliance of quality traits of minimally vegetable processed according to regulations of the Argentine Alimentary Code (AAC) in vegetable brands. M1, M3, M5, M7, M8, and M9: brands of arugula trays. M2, M4, M6, M10, M11, and M12: brands of mixed trays.

Discussion

The results of the present work revealed that all samples of MPVs showed TC, which may also contribute to decreasing the shelf life of these products (Santos et al., 2020). The values found are like those mentioned by other authors (De Oliveira et al., 2011; Santos et al., 2020; Seo et al., 2010). The determination of FC is a useful microbiological parameter to evaluate the efficiency of the process and guarantee the safety of processed vegetables. In the current study, the presence of these microorganisms was identified in 80% of the samples, with populations higher than 2 log MPN/g for 75% of them. These data are in accordance with other authors. However, Santos et al. (2020) detected that only 20% of the analyzed samples were positive for FC in Brazil.

It should be indicated that the Argentine Alimentary Code like some international regulations does not determine criteria for TC and FC. However, the values obtained for FC are higher than the maximum value allowed by the Brazilian legislation for MVPs, which is 10^2 MPN/g for indicative samples (De Oliveira et al., 2011). Due to the vegetables "in natura" have high levels of microorganisms, the contamination of the raw material for MPVs is high (Santos et al., 2012). These results confirm the possible inefficient washing of these vegetables before they are packaged and marketed, which does not assure complete removal of the microorganisms (Zare Jeddi et al., 2014).

In our work, *E. coli* was positive for 25% of the samples with populations and the contaminated vegetables were the arugula trays. These results agree with Zare Jeddi et al. (2014) who reported *E. coli* in 30% of the ready-to-eat salad samples in Iran. Abadias et al. (2008) found *E. coli* in 7.1% and 11.4% of whole vegetable and fresh-cut vegetable samples, respectively. Besides, higher incidence (40%) was reported in sprouts from main brands of MPVs in Lleida (Spain). In addition, Seo et al. (2010) found a similar pattern in MPVs purchased from a local supermarket in Seoul (Korea).

Zhang et al. (2020) reported that the presence of FC and *E. coli* indicates possible contamination by other microorganisms, such as enteric pathogens; and this is a hygienic criterion that should be applied during the manufacturing process. Faour-Klingbeil et al. (2016) detected *E. coli* in irrigated water from wells and samples of washing water from vegetables and corroborated that contamination persists in supermarkets, indicating possible sources of fecal contamination throughout the food chain. The results of the previous investigations about TC and enteric bacteria confirm the trend found in our study and indicate the need for constant monitoring of MPVs. TC bacteria are ubiquitous in soil and water but a high concentration of them can be considered as a potential risk factor. Their presence in MPVs could be related to an inefficient process of washing or washing with unsafe water.

In our work, only 23% of the total samples tested were positive for *S. aureus* so that the contamination rate was lower than that in study by Jung et al. (2005), who reported a high occurrence (37%) of *S. aureus* in lettuce salads from Korea. However, the values found were higher than other studies (Seo et al., 2010; Wu et al., 2018) than showed fewer levels of *S. aureus* in mixed trays. The arugula trays were free of *S. aureus*. The contamination with *S. aureus* can occur from contact with the nostrils of infected food handlers or workers during transportation or at the time of processing for sale (Sabbithi et al., 2014).

Salmonella is an important pathogen involved in large food-borne outbreaks worldwide. De Oliveira et al. (2011) found that 2 samples of wild chicories out of the 162 MPVs samples were positive for *Salmonella* in Spain. Sant'Ana et al. (2011) only detected *Salmonella* Typhimurium and *Salmonella enterica* subsp. Enterica in 4 out of 512 packages of MPV of lettuce as well as arugula. Contrary to our findings, Quiróz-Santiago et al. (2009) reported *Salmonella* spp. in 5.7% of MPVs from Mexico.

Schuh et al. (2020) found that YM counts were around 10^3 CFU/g in 21 out of 24 MPVs collected in Brazil. In addition, in this study, 50% of the total samples presented high numbers of fungi in ready-to-eat salads (in a range of 4.8×10^5 to 8.6×10^6 CFU/g). High contamination in MPVs was also reported by other researchers (De Oliveira et al. 2011; Tournas, 2005) who obtained similar results for samples of fresh and MPVs, and sprouts in Washington (USA) and Spain, respectively. In contrast, Kuan et al. (2017) reported values of YM ranged from 1 to >6 $\log_{10}$ CFU/g in organic and conventional produce vegetables at the retail in Malaysia.

Compared with molds counts, in our study, yeasts were the most prevalent microorganisms in the samples studied, which is consistent with the samples analyzed by Tournas (2005). These microbial groups are commonly used as standards for microbial quality estimation of MPVs (Szczech et al., 2018). The presence of YM in food can indicate poor hygienic handling and it is associated with food spoilage. Some of these organisms are potentially toxigenic and could produce mycotoxins which constitutes a danger to the health of the consumer (Maffei et al., 2013; Tournas, 2005).

Argentinean Alimentary Code also establishes criteria to assess the quality traits of MPVs. In our investigation, it was observed that only 2 (M7 and M8) of the 12 brands studied met all these criteria. Although quality attributes are scarce in MPVs, there is a greater emphasis on visual characteristics in the former. MPVs must have a consistent and fresh appearance, be of acceptable color, and be reasonably free of defects. Visual assessment by buyers and consumers is an important factor in the purchase decision.

Conclusion

Some MPVs sampled from Córdoba, Argentina showed low microbiological quality and imperfect quality traits. The results obtained in this study indicate the need for more exhaustive controls of the establishments where MPVs are produced. Also, the adoption of hygienic practices by food processors and consumers is necessary to minimize the risks of transmission of microbial pathogens.

Author contributions

O.F.C. and M.L.B. conducted the experimental work, analyzed the data, and wrote the manuscript; C.I.M. and L.E.R. conducted the experimental work and analyzed the data; C.V. and M.G.O. designed the study and wrote the manuscript. O.F.C. and M.L.B. contributed equally to this study. All the authors read and approved the final manuscript.

Conflicts of interest

The authors declared no conflicts of interests.

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