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Research article

Microbiological quality in convenient ready-to-eat vegetables during shelf life

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Abstract: Minimally processed vegetables and fruits are the main ingredients of ready-to-eat salads, often sold in convenient packaging. The increased consumption of ready-to-eat products is a result of a fast lifestyle and awareness on their nutritional attributes. Additionally, the processing technology is well documented. This study aimed to determine the microbiological quality of shredded Iceberg lettuce (*Lactuca sativa var. capitata*), sliced tomato (*Solanum lycopersicum*) and diced mango (*Mangifera indica L.*). From each batch, five random units were selected, one of which was analyzed on the day of production (initial sample—IS) and the other four after their shelf life (final sample—FS). The samples were tested for aerobic colony count (ACC) 30 ℃, Enterobacteriaceae, *Enterococcus* spp., *Pseudomonas* spp., *Aeromonas hydrophila*, molds and yeasts. The results showed that ACC of diced mango and shredded lettuce had a similar median of 6.20 and 6.08 log g^{-1}, respectively, and sliced tomato had the lowest one (5.40 log g^{-1}). The median value found for Enterobacteriaceae was 3.47 log g^{-1}. The FS for *Enterococcus* of diced mango and shredded lettuce had a similar median (1.00 log g^{-1}) and sliced tomato had the highest (2.54 log g^{-1}). Both IS and FS had higher unsatisfactory rate for *Aeromonas hydrophila*. All samples revealed acceptable rate for molds and yeasts. This study found high microbial loads in ready to eat vegetables. Data showed that this type of product should be subject to a more stringent quality control, so that the consumers could be provided not only with easy to consume products but also with microbiological quality products during their shelf life.

Keywords: ready to eat vegetables; hygiene; microbiology quality; convenient packaging

Abbreviations: ACC: Aerobic Colony Count; CFC: Cetrimide, Fucidin, Cephaloridine; GMP: food Manufacturing Practices; FS: Final Sample; GV: Guidance Values; IS: Initial Sample; PCA: Plate Count Agar; REV: Ready to Eat Vegetables; TBX: Tryptone Bile X-Glucuronide; VRBG: Violet Red Bile Glucose

1. Introduction

Minimally processed vegetable products are all those that have undergone physical changes in their original form [1], such as being peeled, chopped, shredded, among other transformations [2]. Also known as ready to eat vegetables (REV), it preserves the freshness and quality of the original products [3]. Studies in this area are increasingly important and justified by a growing public interest in minimally processed products and the growing interest for food products that are easy and quick to consume (the "to go" concept), as well as those products presented together (the "all in one" concept).

REV represents a constantly increasing segment where the major objectives of producers are to generate convenient products with the same quality and nutritional value as fresh products [4]. The technological processes used aim to maintain a high sensorial and nutritional quality [2,5]. Therefore, in order to fulfil these objectives and combine them with convenience, an adequate package, such as hurdle technology is needed.

The convenience and health benefits are two of the main motivations leading consumers to purchase these products [6]. In addition, consumers value the sanitary and hygienic quality, low residues food additives, a decrease of the volume to be transported, as well as the waste volume [7].

REV have a shorter shelf life as their storage is limited to days, due to the technological processing, which accelerates their degradation rate [1]. The microbiota of these products is very variable and complex [8] once they suffer different types of contamination during the processing [9]. The microbial activity raises as a consequence of the increase of contact surface and by the increase of tissue exudates, which provides adequate conditions for microbial growth [9,10].

When cell integrity disappears, either due to aging of the tissues or due to mechanical damage caused by processing, the susceptibility of contamination by pathogens increases [2]. The type of vegetable product, cultivation practices and hygiene conditions during production and handling, storage temperature, among other factors, will influence the type of microorganisms and their level of contamination [10].

On one hand during storage, the presence of air, high humidity, and high temperature increases the rate of degradation of these type of products [11]. The degradation of these products is due to different types of molds such as those of the genera *Penicillium, Alternaria, Botrytis* and *Aspergillus*, and bacteria of the genera *Pseudomonas, Erwinia, Bacillus* and *Clostridium* [11].

On the other hand, vegetable products may be a vehicle for pathogens of human and animal origin, as a result of fecal contamination during farming and processing [11]. Therefore, vegetable products are also of great epidemiological importance and have been responsible in recent years for occurrences and food-borne outbreaks [12].

The objective of this study was to determine the microbiological quality of iceberg lettuce (*Lactuca sativa var. capitata*), sliced tomato (*Solanum lycopersicum*) and sliced mango (*Mangifera*

indica L.) sold in convenient packages and to understand its evolution during shelf life. The aim of the research was accomplished.

2. Materials and methods

2.1. Samples preparation and characterization

Products were selected from two ready-to-eat vegetables (REV) production companies: lettuce cut in 5 cm/5 cm shreds and packaged in polypropylene film (air permeability), with a pH ranged from 5.85 to 5.88 and aw from 0.858 to 0.899; tomato cut in 0.5 cm slices and packed in a polystyrene material tray wrapped with a plastic film (air condition), pH values ranging from 3.94 to 4.1 and aw from 0.880 to 0.917; and mango cut in 2 cm/2 cm dices and packed in a polyethylene terephthalate cup with a lid, providing a low oxygen transmission rate, pH ranged from 3.16 to 3.19 and aw from 0.863 to 0.898. These products represent different types of REV, with different processing, different final presentations and different physical-chemical properties - Iceberg lettuce for its resistance to refrigeration temperatures and to degradation processes; tomato for its high exudation rate and mango for having different characteristics from the previous vegetables.

From each batch, five random units were selected, one of which was analyzed on the day of production (initial sample—IS) and the other four after their shelf life (final sample—FS), after four to five days, meaning, after having cycled the commercial distribution, and therefore, subject to the real storage conditions.

A data logger was placed on the FS packing, in order to register the temperature of the products during storage, obtaining a total of 29,904 temperatures records.

The total number of collected samples was 40 distributed by: tomato—6 IS and 11 FS; mango—2 IS and 9 FS; lettuce—3 IS and 9 FS. For all IS, pH and aw values were measured.

2.2. Microbial analysis

Ten grams of each sample was weighed into sterile stomacher bags and homogenized with 90 mL of sterile peptone buffered water (BPW, Biokar, BK131HA) for 2 min at medium speed in a stomacher (Seward, 400 Circulator 88).

The aerobic colony counts (ACC) were performed using Plate Count Agar (PCA Biokar, BK144HA) according to ISO 4833:2003. Enterobacteriaceae were enumerated using Violet Red Bile Glucose (VRBG Biokar, BK011H) according to ISO 21528-2:2004. Escherichia coli were determined using the medium TBX (Biokar, BK146HM), with incubation at 44 ℃ for 18 to 24 hours, according to ISO 16649-2:2001. The isolation and enumeration of Enterococcus spp. were carried out in Kanamicina aesculina azida agar base (Oxoid, CM591) supplemented with Kanamycin Sulfate (Oxoid, SR0092), incubated at 37 ℃ for 18 to 24 hours. The enumeration of Pseudomonas spp. was carried out on Pseudomonas agar base (Oxoid, CM559), added with selective CFC supplement CFC (Oxoid, SR0103E), incubated at 25 ℃ for 24 to 48 hours. For the enumeration of Aeromonas hydrophyla the base medium of Aeromonas (Ryan) (Oxoid, CM0833) was used supplemented with Ampicillin (Oxoid, SR0136E) and incubated at 30 ℃ for 72 h. For molds (NF V 08-59:1995) and yeasts (NF V 08-59:2002), Rose Bengal Chloramphenicol agar (Biokar, BK151HA) was used in spread plate technique and incubated at 22 ℃ for 120 h long.

Guidance values (GV) (Table 1) for ready-to-eat food, from the Instituto Nacional de Saúde Doutor Ricardo Jorge, Portugal [13] were used to determine the sample evaluation (Satisfactory-S; Acceptable-A; Unsatisfactory-US), except for Pseudomonas, Aeromonas and Enterococcus. The guide values are not mandatory in Portugal.

To evaluate the results regarding Pseudomonas and Aeromonas a parallelism with the ACC was defined. For determination of Enterococcus, a two-class criterion based on the presence or absence (similar to what is used in water analysis) was used [14].

Table 1. Guide Values for ready-to-eat food Adapted from: Santos et al. [13].

		Microbiological quality (cfu g^{-1} when not indicated)			
Microorganism	Food Group	Satisfactory	Acceptable	Unsatisfactory	Unacceptable/potentially hazardous
Aerobic colony count 30 ℃	3	$\leq 10^4$	$>10^4 \leq 10^6$	$>10^6$	NA
Yeasts	3	$\leq 10^2$	$>10^2 \leq 10^5$	$>10^5$	NA
Molds	3	$\leq 10^2$	$>10^2 \leq 10^3$	$>10^3$	#
Total coliforms	3	$\leq 10^2$	$>10^2 \leq 10^4$	$>10^4$	NA
E. coli	3	≤ 10	$>10<10^2$	$\geq 10^2$	NA

*-Applicable to products kept in the refrigerator

#-evaluated on a case by case basis

NA-Not applicable

2.3. Statistical analyses

All data were subjected to analysis of descriptive statistics using Excel (Microsoft).

3. Results and discussion

The results were analyzed by microbiological parameter and by type of product. Microbiological quality of the samples and of the different products was also evaluated, according to the Portuguese GV. This evaluation corresponded to a comparison between IS and FS.

When analysing the overall microbiological results, regarding to ACC a 6.12 log g^{-1} median was registered, and the evolution from the IS to FS (Table 2) was observed. Among the products, tomato had the lowest mean and median for the IS, presenting a better initial quality (Table 3). However, there was no difference for the ACC in the FS between the different type of products, showing that during the storage the nature of the products has low or no influence on the microbial evolution.

Table 2. Statistical measures of the sample by microbiological parameter (log g^{-1}).

	Aerobic colony count 30 ℃			*Enterobacteriaceae*			*Enterococcus spp.*			*Pseudomonas spp.*			*Aeromonas hydrophila*			Molds			Yeasts		
	Initial	Final	Total	Initial	Final	Total	Initial	Final	Total	Initial	Final	Total	Initial	Final	Total	Initial	Final	Total	Initial	Final	Total
N	11	29	40	11	29	40	11	29	40	11	29	40	11	29	40	11	29	40	11	29	40
Mean	4.43	6.34	5.82	2.84	3.71	3.47	4.54	1.76	1.70	4.16	5.06	4.81	36.40	3.77	3.66	1.34	1.02	1.11	2.62	4.13	3.72
Median	4.08	6.38	6.12	2.46	3.79	3.47	1.00	1.00	1.00	4.54	5.04	5.04	3.93	4.26	3.95	1.00	1.00	1.00	2.38	4.04	3.83
SD	1.41	1.28	1.56	1.73	1.73	1.75	1.22	1.13	1.14	1.88	0.98	1.33	1.73	1.35	1.45	0.55	0.11	0.33	0.75	0.86	1.07
Minimum	2.34	3.45	2.34	1.00	1.00	1.00	1.00	1.00	1.00	1.00	3.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.70	2.48	1.70
Maximum	6.78	8.76	8.76	5.89	6.28	6.28	5.00	5.36	5.36	5.90	7.79	7.79	5.81	5.82	5.82	2.70	1.60	2.70	4.08	5.82	5.82

Table 3. Statistical measures of the sample by microbiological parameter and product.

		Aerobic colony count 30 ℃			Enterobacteriaceae			Enterococcus spp.			Pseudomonas spp.			Aeromonas hydrophila			Molds			Yeasts		
		Initial	Final	Total	Initial	Final	Total	Initial	Final	Total	Initial	Final	Total	Initial	Final	Total	Initial	Final	Total	Initial	Final	Total
CUTTED MANGO	N	2	9	11	2	9	11	2	9	11	2	9	11	2	9	11	2	9	11	2	9	11
	Mean	5.12	6.31	6.09	3.45	3.49	3.49	1.30	1.00	1.05	4.54	5.35	5.20	5.04	4.61	4.69	1.20	1.00	1.04	2.44	4.23	3.91
	Median	5.12	6.32	6.20	3.45	3.41	3.41	1.30	1.00	1.00	4.54	5.51	5.51	5.04	5.00	5.00	1.20	1.00	1.00	2.44	4.43	3.17
	SD	1.46	0.80	0.98	3.46	1.59	1.79	0.42	0.00	0.18	1.90	0.63	0.89	0.06	0.83	0.76	0.28	0.00	0.12	1.04	1.08	1.26
	Minimum	4.08	5.20	4.08	1.00	1.00	1.00	1.00	1.00	1.00	3.20	4.34	3.20	5.00	3.45	3.45	1.00	1.00	1.00	1.70	3.17	1.70
	Maximum	6.15	7.86	7.86	5.89	6.00	6.00	1.60	1.00	1.60	5.88	6.46	6.46	5.08	5.82	5.82	1.40	1.00	1.40	3.17	5.82	5.82
ICEBERG LETTUCE	N	6	11	17	6	11	17	6	11	17	6	11	17	6	11	17	6	11	17	6	11	17
	Mean	5.00	6.42	5.92	3.41	4.97	4.42	1.67	2.05	1.91	4.84	5.47	5.25	4.05	3.86	3.93	1.10	1.05	1.07	2.80	4.06	3.61
	Median	1.17	6.18	6.08	3.01	5.11	4.30	1.00	1.00	1.00	5.13	5.36	5.36	3.95	4.26	3.96	1.00	1.00	1.00	2.56	4.04	3.91
	SD	3.62	1.03	1.26	1.24	1.03	1.32	1.63	1.43	1.47	1.16	0.89	1.00	0.96	1.08	1.01	0.15	0.18	0.17	0.83	0.80	1.00
	Minimum	3.85	4.90	3.62	2.38	3.36	2.38	1.00	1.00	1.00	3.30	4.57	3.30	3.08	1.00	1.00	1.00	1.00	1.00	2.00	2.48	2.00
	Maximum	6.78	7.96	7.96	5.49	6.28	6.28	5.00	5.36	5.36	5.90	7.79	7.80	5.81	5.11	5.81	1.30	1.60	1.60	4.08	5.57	5.57
SLICED TOMATO	N	3	9	12	3	9	12	3	9	12	3	9	12	3	9	12	3	9	12	3	9	12
	Mean	2.83	6.29	5.43	1.30	2.40	2.13	1.43	2.18	2.00	2.54	4.25	3.83	1.00	2.80	2.35	1.91	1.00	1.23	2.38	4.15	3.71
	Median	2.93	6.49	5.40	1.00	1.70	1.59	1.00	2.54	2.24	1.00	4.15	3.97	1.00	2.98	1.77	2.04	1.00	1.00	2.38	3.98	3.81
	SD	0.45	1.94	2.08	0.52	1.56	1.44	0.75	0.95	0.93	2.67	0.96	1.60	0.00	1.53	1.54	0.86	0.00	0.55	0.60	0.78	1.07
	Minimum	2.34	3.45	2.34	1.00	1.00	1.00	1.00	1.00	1.00	1.00	3.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.78	2.79	1.78
	Maximum	3.23	8.76	8.76	1.90	4.87	4.87	2.30	3.28	3.28	5.62	5.76	5.76	1.00	4.70	4.70	2.70	1.00	2.70	2.98	5.20	5.20

When analysing the results for conformity according to GV, the rate of US increased up to 68.97% (Figure 1).

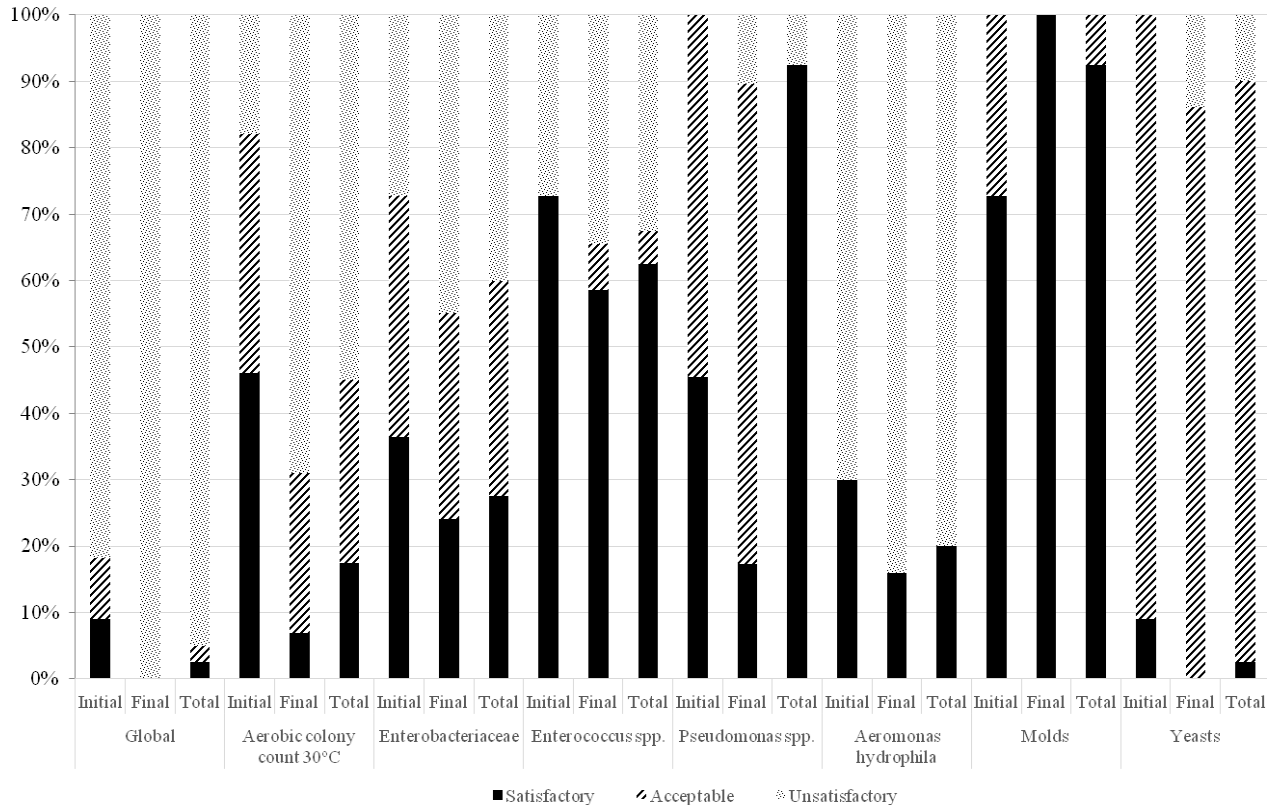

Figure 1. Global microbiological acceptance.

The results from iceberg lettuce and sliced tomato (Figures 3 and 4) indicate that their storage conditions were anomalous, since their FS had high rates of US, while not having any IS evaluated as US. The US rate for mango, reflects flaws and a weak application of the good manufacturing practices (GMP) (Figure 2). Samples with high initial counts are indicative of low quality products, which is reflected in a shorter shelf life, representing economic loss to stakeholders. In this way, the high rate of US in mango FS was expected due to microbiota progression during storage (Figure 2).

Regarding *Fungi*, on one hand, FS of molds counts were inferior to the IS, representing a 1.1 log g^{-1} decrease in the maximum values, but maintaining the same median. On the other hand, yeasts FS had more counts in comparison to IS, having increased 1.74 log g^{-1}; FS registered a similar maximum (Table 2).

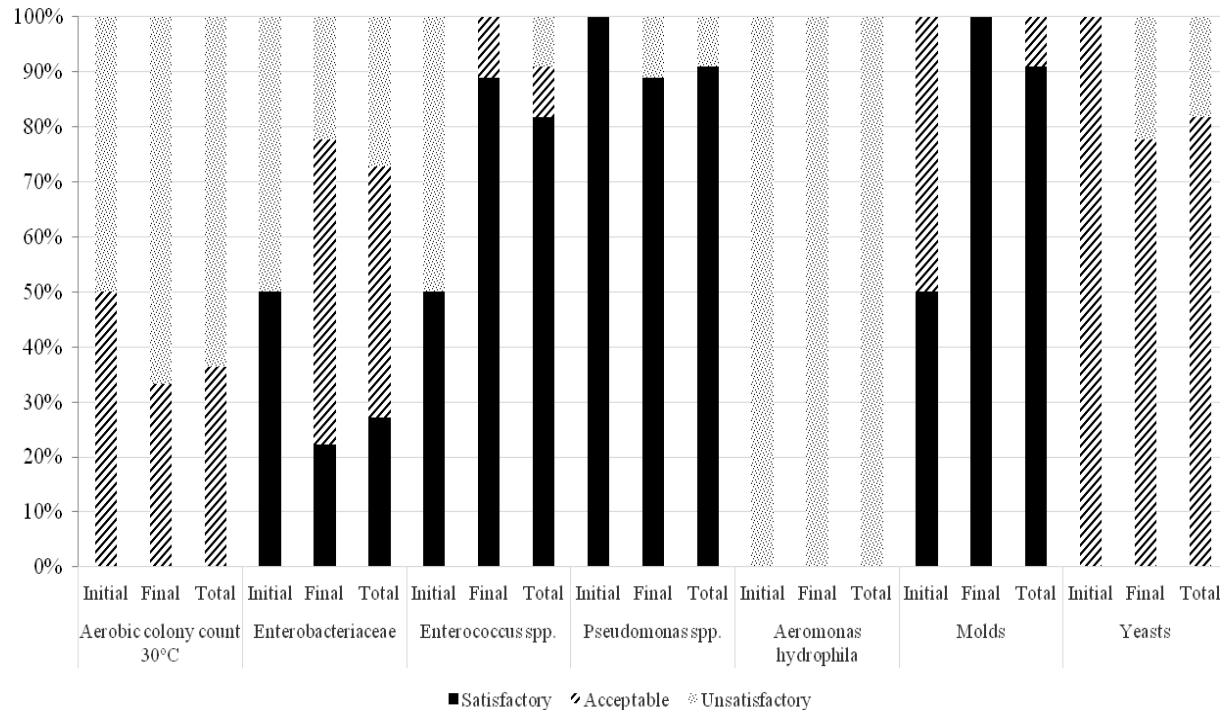

Figure 2. Microbiological acceptance for mango.

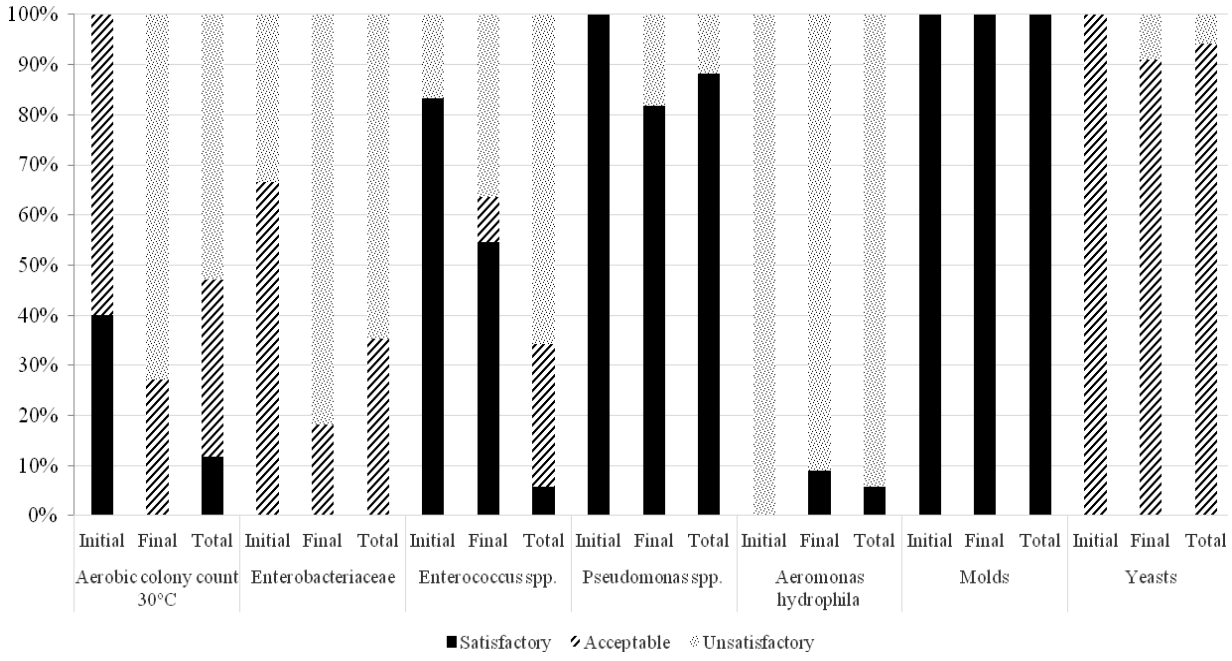

Figure 3. Microbiological acceptance for lettuce.

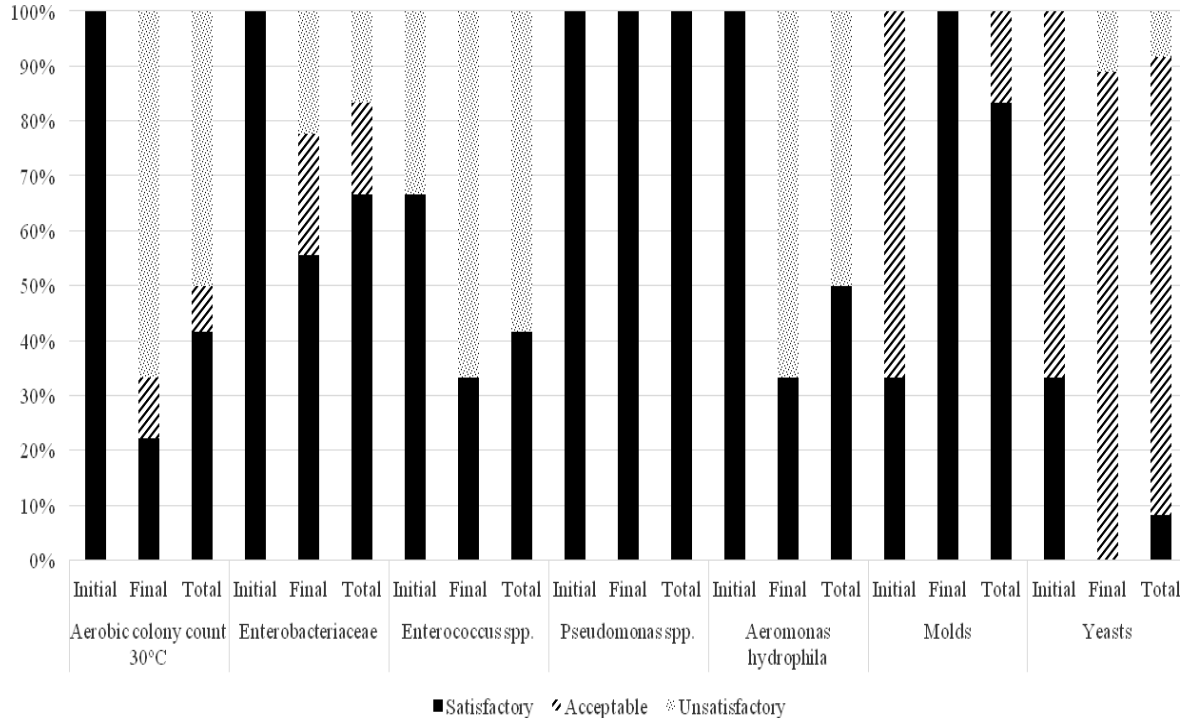

Figure 4. Microbiological acceptance for tomato.

This microbiological value (in this parameter) reflects more favourable acceptability rates since none of the products had US results. Therefore, this parameter does not seem useful for the quality evaluation and its evolution during storage (Figures 1–4).

Concerning yeasts an accentuated evolution of the median values, from IS to FS (Table 2) was observed. However, IS had no US, and in FS this value was relatively low (13.79%) (Figure 1). Addressing the type of product, tomato was the only one with S IS (Figures 2 and 4). The results ranged between 1.7 log g^{-1} and 5.82 log g^{-1} (Table 2), similar to those obtained by other authors who studied ready-to-eat fruits (among others) with results ranging from 1.0 log g^{-1} and 6.26 log g^{-1} [15].

Regarding *Pseudomonas* spp., this microorganism had similar results to ACC; FS counts were superior to IS (Table 2). Among the studied products, lettuce had the worst final quality, with a maximum value of 7.79 log g^{-1}. IS were all S, whilst FS had a 10.34% rate of US results (Table 2 and Figure 1). All tomato samples were S, both in IS and FS (Figure 4). Once again, tomato had the highest S rates, both in IS and FS. In the study described by Oliveira et al. [3] the mean for *Pseudomonas* spp. was 7.10 log g^{-1}, highly superior when compared to this study. A Brazilian study on leafy vegetables [16] showed results with a mean ranging between 6.90 log g^{-1} and 8.43 log g^{-1}. Also, the high levels of *Pseudomonas* spp., predominant alteration microbiota of products stored in the cold, indicates poor control of disinfection as well as a possible excess of moisture inside the packages. On the other hand, the cutting of the product, during its processing, favors the microbial growth since it allows the release of exudates [17].

Aeromonas hydrophila were responsible for higher US results, both in IS and FS (Figure 1), showing that the usage of this parameter as quality indicator should be better equated. Regarding the type of product, once again tomato had the lower microbiological results (Table 2). Nevertheless,

tomato had a wide maximum value progression from 1 log g^{-1} in IS to 4.7 log g^{-1} in FS (Table 3). All mango and lettuce IS were evaluated as US, just like mango FS. In lettuce this rate decreased to 90.91% in FS (Figures 2 and 3). Products had counts similar to Santos's et al. [18] study which results ranged between 3.15 log g^{-1} and 5.18 log g^{-1}.

Enterobacteriaceae are important in terms of the fulfilment of the good manufacturing practices (GMP), as an indicator of the technological process efficiency, namely in washing/disinfection of raw material. In this way, the number of US IS (27.28%) (Figure 1) reflects GMP failures. Apparently, the products where it was more difficult to achieve S levels were mango and lettuce (Figures 2 and 3). This was already expected in lettuce, but not in mango, so it was probably due to cross contamination during processing. Once again, tomato showed progression in counts, from the IS to the FS (Figure 4). However, these results do not seem to be related to fecal contamination, once no *Escherichia coli* was found during this study, reinforcing the idea that the fecal connotation to Enterobacteriaceae should be relativized.

For *Enterococcus* a wide dispersion of the FS results was observed, when comparing to IS (Table 2). Regarding the products, lettuce had higher results, nevertheless, *Enterococcus* was present in variable levels in all products IS. Once more, this seems to reflect deficient washing/disinfection procedures. It is noteworthy that none of the mango FS had US results, indicating the microorganism mortality. This may be explained by the development of another microorganism, such as those belonging to genera Enterobacteriaceae,that were present in mango in large number. However, there are studies describing this fruit as having antimicrobial properties, that effectively decreases the number of *Enterococcus* in biofilm [19]. The study made by *Consumer Reports Magazine* [20] regarding to packaged salad reached a 23% US results. The presence of *Enterococcus* indicates the possibility of fecal contamination of the products (and also as a potential pathogen) as a result of using biological fertilizer or by contamination of the irrigation water, and which were not subsequently destroyed by the disinfection process at the processing level.

Based on the overall microbiological results, and assuming that one or more than one US parameter are sufficient to consider the sample US, 95% of the samples were US. However the percentage of US in IS was 81-82%, while in FS was 100% (Figure 1). One can consider that this aspect shows, for the diverse microorganisms, different behaviors, and different resistance and adaptation levels to the different food matrixes. Since these products are available for sale, and within their shelf life, the hygienic quality evaluation methodology should be reconsidered.

But, if not considering genera *Pseudomonas* e *Aeromonas* results, once there are no guide values to them, the final evaluation of the samples would be 45.45% US results in IS, and 86.21% US results in FS. This data shows clearly the importance of such parameters that are not usually included in guide values and microbiological criteria and which provides a good information about the spoilage suffered during cold storage of REV. Also, the results show that the microbiological quality had suffered a decrease during the storage ending in a total US evaluation. This can be explained by the registered storage temperatures (n = 29904), where 34.58% were above 5 ℃, of which 17.51% were ≥10 ℃.

The results were similar to those found in others studies made in Portugal [21], which although they also did not detect pathogens, exhibited higher values for ACC and Fungi, showing a low microbiological quality for these products, evidencing their ability to become transmission vehicles of pathogenic agents, representing a potential risk to consumers. The high values that were obtained were certainly due to a set of factors that worked cumulatively, with particular emphasis on the

washing and disinfecting processes of the different products, materials and equipment, evidenced by the presence of Enterobacteriaceae in the products [22].

Even though sliced tomato had the best quality results, this product, in comparison to the other products, had a wider microbiological progression from IS to FS, eventually due to cutting.

4. Conclusions

In terms of the microbiological quality evolution, all the products evolved negatively, with US results at the end of its shelf life. These results showed problems associated with production, distribution and storage circuit, specifically temperature control. For this reason, attention should be made to the monitoring of temperatures or to the reduction of the shelf life of these products.

The high levels of *Aeromonas hydrophila*, Enterobacteriaceae, *Enterococcus* and ACC, denounce flaws in the processes of washing and disinfection. The respective procedures should be reviewed and tested for the disinfectant to be used, the adequate concentration disinfectant and the contact time.

Data show that these type of products should be subject to more stringent quality control, in order for consumers to have convenient ready to eat fruits and vegetables with microbiological values within the acceptability range, defined for these products, during the established shelf life.

The results pointed out to a microbiological quality lower than expected. It is of the interest of all stakeholders (producers, distributers and consumers) that more work of this kind could be developed in order to assess the repeatability of the tendencies signalized in the present study.

In short, the majority of the analyzed products denounced a poor microbiological quality. This is indicative of the need to adopt more stringent hygiene practices, both by producers and consumers, minimizing the potential risk of transmission of pathogens via these products, such as a tight control of the cold chain to which they are exposed to.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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