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Microbiological Quality of Ready-to-eat Salads from Processing Plant to the Consumers

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Abstract This study aimed to assess the microbiological quality of ready-to-eat salads (Aerobic Colony Count, *E. coli*, yeasts and moulds, *S. aureus*, *Salmonella* spp., *L. monocytogenes*, *C. perfringens*) and the effect of temperature abuse on the microbial count. Ready-to-eat salads samples were produced and commercialized in Italy and sampled, from January 2017 to January 2018, both at different steps of the production process in an industry (n = 300) and in different supermarkets (n = 270). The pathogenic foodborne microorganisms *Salmonella* spp., *Listeria monocytogenes*, *S. aureus* and *Clostridium* spp. were not detected and only 2.98% of the 570 samples were contaminated by *E. coli*, a good hygiene indicator of fecal contamination. Ready-to-eat salads samples from the industry were less contaminated, both in percentage and concentration, than the supermarket ones, particularly due to high Aerobic Colony Count values: on the day of collection, 80% samples from the industry were satisfactory, opposed to 8.3% from the retailers; at the end of shelf life, 20% samples from the industry were unsatisfactory, opposed to 80% from the retailers. Although washing salads before consumption is not effective to eliminate pathogens internalized within the plant's tissues, our results showed that it was useful in reducing the microbiological load, especially *E. coli* count. This study revealed that high microbial content in retail ready-to-eat salads samples was principally due to microbial multiplication occurring during storage and transportation from industry to retailers and then at home. More frequent monitoring of storage and transport temperatures would be necessary to ensure the required hygienic quality, as well as it should be clear the writing on the packaging that "products must be kept at a maximum temperature of 8°C".

Keywords: *ready-to-eat salads, food safety, microbiological quality, temperature abuse, foodborne pathogens*

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1. Introduction

Over the last few years the per-capita consumption trend of ready-to-eat salads (RTES) has been characterized by an increase in Europe, particularly in Italy [1]. RTES offer many advantages because they satisfy the need to time saving and the importance to eat food with very good nutritional properties. They are trimmed, washed, dried and packed in bags or plastic containers (often in a modified atmosphere) [2] that must be stored at refrigeration temperatures lower than 8°C [3,4] and they are not exposed to further processing before consumption.

Plants, growing in the soil, are normally colonized on their phyllosphere by a variety of bacteria, largely belonging to *Enterobacteriaceae* and *Pseudomonadaceae* families [5,6]. Bacteria can infiltrate plants tissue via roots and stomata (natural apertures) or through wounds or cut surfaces [7,8,9,10], and in these ways circumvent the antimicrobial effect of surface treatments [11,12]. Recent outbreaks of food poisoning have been associated with consumption of salads contaminated by *Yersinia enterocolitica* [13], *Salmonella* [14], *Listeria monocyt*ogenes [15,16] and *E. coli* O157: H7 [17] and evidence suggests that such outbreaks are increasing [18]. Furthermore, RTES seem to be involved in the spread of bacteria carrying acquired antibiotic resistance genes [19].

The number and type of microorganisms contaminating salads are not predictable nor standardized: it depends on various physicochemical environmental factors as well as on the characteristics of the phyllosphere [10], meaning that different leaves of the same plant can differ considerably in terms of microbial content. Microorganisms, including yeasts and moulds, can contaminate vegetables both before harvesting, through various vehicles such as manure and irrigation water, and in each RTES production phase: harvest, transport, processing and distribution of the product [20].

Many reports have described the contamination of RTES by *Escherichia coli*, coliforms, total aerobic and spoilage bacteria (Aerobic Colony Count, ACC), yeasts and moulds [21-27], and the most reported microbial contamination ranged between 2 and 9 Log_{10} CFU g^{-1} for ACC, between 2 and 8.8 Log_{10} CFU g^{-1} for coliforms, between 2.9 and 6.5 Log_{10} CFU g^{-1} for yeasts and above

5.8 Log_{10} CFU g^{-1} for moulds. *E. coli* and other pathogens such as *Salmonella*, *Listeria monocytogenes* and *E. coli* O157: H7 have occasionally been detected, and when present they were in low concentration.

To determine if each production stage is being controlled, it is therefore very important to respect hygienic Good Manufacturing Practices (GMPs) and to perform microbiological analysis at each Critical Control Point (CCP) identified in the "Hazard Analysis and Critical Control Point" (HACCP) plan. HACCP is a systematic and preventive approach used for the identification, assessment and control of biological, chemical and physical hazards in the food processing chain, from the raw material sourcing to final consumption [28]. It provides an effective way to advance food quality and safety, focusing on preventing hazards and improving processes [29]. The effectiveness of HACCP depends on the correct application of its principles combined with other programs, including GMPs, that are the basic operational and environmental conditions required to produce safe foods. They ensure that ingredients, products and packaging materials are handled safely and that food products are processed in a suitable environment, necessary conditions for the prevention of potential contamination and cross-contamination of food [30].

To decrease the microbial content in salads, leaves must be washed during the production phases with chlorinated potable water conforming to the Italian Legislative Decree n. 31 [31] and to the European Directive 98/83 [32]. Furthermore, the machinery used must be sanitized daily, since the cutting operation is one of the Critical Points of the industrial process of RTES production. Cutting causes an increase of respiratory activity and metabolic reactions, furthermore the release of leaves cellular fluids from the damaged tissues provides a nutrient-rich medium and an ideal substrate for the growth of microorganisms. Bacteria can penetrate the tissues through the cut surfaces, that are hydrophobic, so it becomes difficult to reach the microorganism during the subsequent washing phases [12,23]. Microbial multiplication after the cutting operation depends mainly on the time between cutting and washing and on the temperature of processing: short time and low temperatures inhibit bacterial multiplication [33].

Commission Regulation No 2073/2005 on microbiological criteria for foodstuffs [34] and Regulation No 852/2004 [35] on the hygiene of foodstuffs have been issued in Europe in order to limit foodborne diseases. In particular, Regulation No 2073/2005 lays down food safety and process hygiene criteria for specific combinations of foodstuffs and microorganism, their toxins or metabolites, while Regulation No 852/2004 requires retailers to adopt hygiene measures and to put in place, implement and maintain a permanent procedure based on HACCP principles. The fourth principle of HACCP shows the importance of the identification and application of control measures and monitoring the CCP identified in each phase of food preparation procedures, with the aim to reduce or remove bio-hazards. One of the main CCPs, identified in almost all the industry flow charts, is the food storage at temperatures lower than 8°C or, otherwise, as low as possible compatibly with the necessary presence of operators during food handling, conservation in refrigerators and transportation phases. Cold-chain compliance is, in fact, fundamental to limit microbial multiplication in perishable foods [11,36] and temperature control has a key role in preventing the multiplication of mesophilic pathogens. Since vegetables are perishable foods and good substrates for the proliferation of microorganisms, especially after cutting, it is clear that the cold-chain must be maintained: the processing stage must be <14°C, transport and preservation temperature should not exceed 8°C [4]. Conservation post-sales is usually a condition underestimated [37] and it becomes a Critical Point that cannot be easily monitored since it depends on the consumers awareness of food conservation.

The objective of this manuscript was to evaluate the microbiological quality and the impact of temperature abuse to microbiological quality of ready-to-eat salads distributed in Central Italy, and to assess if the household washing before consumption can reduce the microbial content.

2. Materials and Methods

2.1. Sample Collection

The samples were collected from an industry and different supermarkets in Central Italy from January 2017 to January 2018. A total of 570 samples of RTE mixed salad were analyzed: 300 belonging to the examined industry [60 samples of raw material cleansed of non-edible parts, 60 of mixed salad leaves after the second washing phase and 60 after the fifth one, 60 samples of packaged ready-to-eat salads and 60 of packaged ready-to-eat salads at the end of the shelf life (ESL)] and 270 bagged RTES collected from different supermarkets [90 were analyzed as such, 90 were washed before the analysis and 90 were examined at the end of their shelf life].

The samples from the industry were collected at 15 different times, four samples for each of the five stages analysed (total of 20 samples per day of collection) with the aim of following the entire process flow.

The samples from the supermarkets were collected at 15 different times, for a total of 18 bags per day of collection all belonging to the same batch, and then splitted randomly into the three different groups of analysis (as such, washed and at the end of shelf life).

Two hundred grams of each sample were collected aseptically from the examined industry, put in sterile polyethylene carrier bags, transported to the laboratory in refrigerated bags (about 4°C) and analyzed on the day of collection. In Figure 1 the stages of ready-to-eat salads production of the industry involved in the study are presented.

Samples from retailers were brought to the laboratory in refrigerated bags (about 4°C) and preserved at room temperature for 30 minutes before the analysis (some were performed on the day of collection, others at the end of shelf life, i.e. 7 days after the sampling) to mimic the

temperature environmental abuse during transportation home of buyers.

2.2. Microbiological Analysis

Samples (25 g) were blended for 60 s in 225 mL of 0.1% (w/v) Buffered Peptone Water. Decimal dilutions were carried out using the same diluent and were used to inoculate agar media (all from Thermo Scientific - Oxoid Ltd., Hampshire, UK) in agreement with specific standard methods for ACC [38], *E. coli* [39], yeasts and moulds [40], *Pseudomonas* spp. [41]. *Staphylococcus aureus* count was obtained in conformity with UNI EN ISO 6888-1 [42], and the identification of suspected colonies was performed through Api Staph (bioMérieux Italia Spa, Florence, Italy). *Salmonella* detection was performed in conformity with ISO 6579-1 [43]. *Listeria* spp. strains were isolated in accordance to UNI EN ISO 11290-1 [44] and characterized through Gram stain, haemolysis test on Columbia blood agar, catalase production (Bactident Catalase Merk) and at last API Listeria kit (bioMérieux Italia Spa, Florence, Italy). *Clostridium perfringens* and other *Clostridium* Sulphite-Reducing bacteria were detected following ISO 15213 [45].

For the interpretation of results (Table 1), the microbiological limits mentioned in the Commission Regulation n. 1441 [46], amending Commission Regulation n. 2073 [34] in Europe on ready-to-eat vegetables within the period of maximum shelf life, and the reference standard values proposed in Guidelines of Health Protection Agency [47] and in the Italian Guidelines of Ce.I.R.S.A. [48] were used.

2.3. Temperature and Free Chlorine Measurement in the Industrial Production

Temperature of 6 CCPs was determined (HI 92810, Hanna Instruments) three time each: environmental temperature was measured at the centre of the room, while water temperature of the five washing tanks was measured at the centre of the tank, at the end of the washing process.

Water used in the five washing tanks (Figure 1) was treated with hypochlorite (8 ppm), final concentration near 1.5 mg L^{-1}, to reduce the microbial concentration of the salad. Free chlorine detection was determined (Chlorine pocket colorimeter, Hach) in 7 water CCPs: tap water at the entrance of the establishment, after chlorination and before each of the five washing tanks.

2.4. Statistical Analysis

Microbial counts were analyzed in log scale (Log_{10} CFU g^{-1}), attributing one to observations where no colonies were obtained at any dilution (limit of detection is 10 CFU g^{-1}). The effectiveness of the washing phases was calculated using the logarithmic reduction rate between the various steps of production. Differences were considered statistically significant when P-values were lower than 0.05. All statistical calculations were performed using Epi Info 3.5.1. 2008.

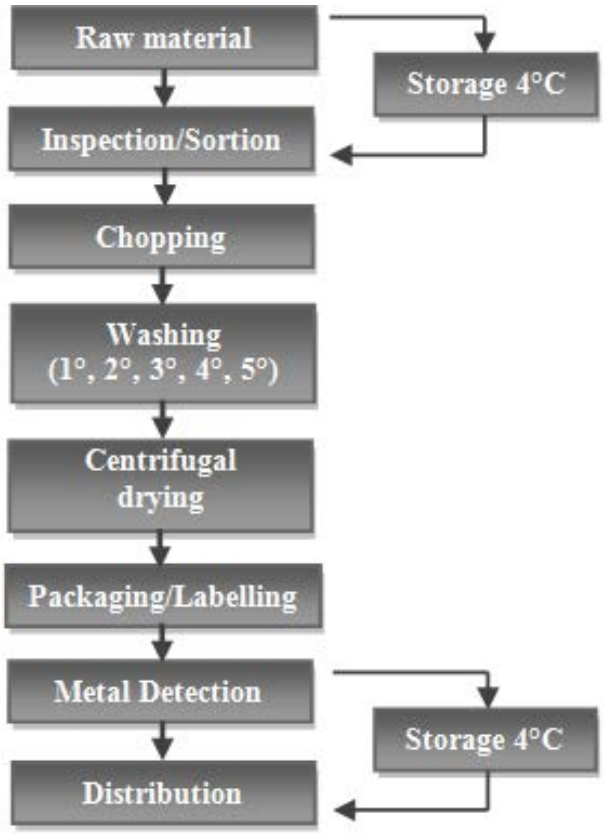

Figure 1. Process flow diagram for the production of ready-to-eat salads in the industry involved in the study

Table 1. Microbiological limits for certain pathogens and indicator microorganisms in ready-to-eat salads

Microorganism	Result (CFU/g)			Method	Normative References
	Satisfactory	Borderline	Unsatisfactory		
Aerobic Colony Count	$<5x10^5$	$5x10^5$ - $<5x10^7$	$\geq 5x10^7$	UNI EN ISO 4833-1, 2013	Ce.I.R.S.A. (2013)
E. coli	$\leq 10^2$	10^2 - $<10^3$	$\geq 10^3$	ISO 16649-2, 2001	E.C. n. 1441 (2007)
Yeast and Moulds	$<10^4$	10^4 - $\leq 10^6$	$>10^6$	ISO 21527-2, 2008	Ce.I.R.S.A. (2013)
S. aureus	<20	20 - $\leq 10^4$	$>10^4$	UNI EN ISO 6888-1, 2004	Health Prot. Ag. (2009)
Salmonella spp.	not detected in 25 g	-	detected in 25 g	ISO 6579, 2002	E.C. n. 1441 (2007)
L. monocytogenes	<10	10 - $\leq 10^2$	$>10^2$	UNI EN ISO 11290-1, 2005	Health Prot. Ag. (2009)
C. perfringens	<10	10 - $\leq 10^4$	$>10^4$	ISO 15213, 2003	Health Prot. Ag. (2009)

3. Results

3.1. Temperature and Free Chlorine Detection

Environmental temperatures measurements ranged from 11 to 12°C (mean 11.5±0.81°C). The water in the washing tanks had temperatures ranging from 9.3 to 12 °C. The mean temperature of the first and fifth tanks (9.4±0.91°C and 10.5±1.27 °C respectively) were slightly lower than the others, probably due to the presence of refrigerated bubbling air which had the purpose of moving the salad leaves and favor the detachment of bacteria.

The concentration of free chlorine detected in the five tanks gave quite similar results (mean=0.8 mg L^{-1}, SD=0.17 mg L^{-1}).

3.2. Microbiological analysis, samples collected from the industry

Figure 2 shows the means and standard deviations (SD) of the microbial counts for ACC, *Pseudomonadaceae*, and yeasts and moulds of the analyzed samples.

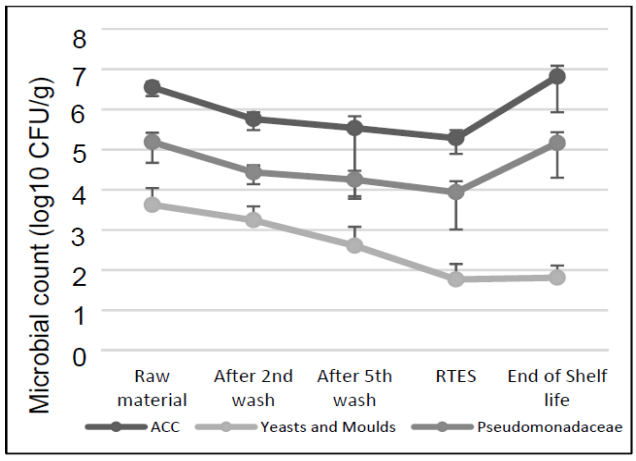

Figure 2. Microbial Counts of the ready-to-eat salads (RTES) at various steps of production. Data represents the mean of 60 replicates and their standard deviations. ACC, Aerobic Colony Count

In raw material the ACC ranged from 6.3 to 6.7 Log_{10} CFU g^{-1} with a mean of 6.5 Log_{10} CFU g^{-1}, yeasts and moulds ranged from 1.8 to 4.2 Log_{10} CFU g^{-1} with a mean of 3.6 Log_{10} CFU g^{-1}, and *Pseudomonas* spp. ranged from 4.4 to 5.4 Log_{10} CFU g^{-1} with a mean of 5.2 Log_{10} CFU g^{1}.

The five washing phases caused a gradual loss of microbial load, similar for ACC, yeasts and moulds and *Pseudomonas* spp. (Figure 2 and Table 2). Total logarithmic reduction of microbial count during the entire production process of RTES (RM-RTES in Table 2) was greater than 1 Log_{10} CFU g^{-1} for all the three groups considered. The pathogens *S. aureus*, *Salmonella* spp., *L. monocytogenes*, *Clostridium* spp. and *E. coli* were never detected.

3.3. Microbiological Analysis, Samples Collected from the Retailers

The results obtained from the analysis of the 270 samples collected in the supermarkets of three Italian Regions were not statistically different (P>0.05) and they were grouped.

The pathogens *S. aureus*, *Clostridium* spp., *Salmonella* spp. and *L. monocytogenes* were never isolated in all the samples collected from the retailers.

Figure 3 shows that the ACC and yeasts and moulds mean values of the 90 unwashed RTES samples were slightly higher (7,1 Log_{10} CFU g^{-1} and 5,6 Log_{10} CFU g^{-1} respectively) than the 90 washed samples (7 Log_{10} CFU g^{-1} and 4.65 Log_{10} CFU g^{-1} respectively). The 90 samples analyzed at the end of the shelf life had 7.3 Log_{10} CFU g^{-1} as ACC mean value and 5.6 Log_{10} CFU g^{-1} as yeasts and moulds mean value. These results are really similar to the ones found in the not washed RTES ones. Results for *Pseudomonas* spp. count revealed that there were no relevant differences (P>0.05) among RTES, RTES washed and RTES-ESL samples. Differences (P<0.05) were only observed for *E. coli*, which was present only in 17 (6.3%) samples of the 270 total RTES, and never in the washed samples.

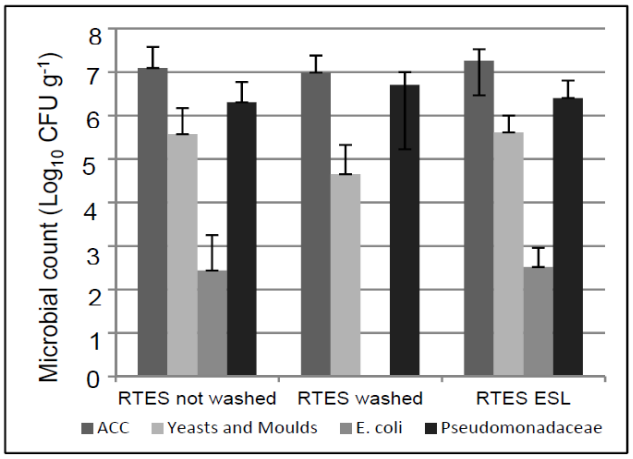

Figure 3. Microbiological quality of ready-to-eat salads (RTES) collected from the retailers. Data represents the mean of 90 replicates and their standard deviations. ACC, Aerobic Colony Count

3.4. Hygiene Indicators Results

The results obtained for ACC, yeasts and moulds and *E. coli* at the end of the production process (RTES) and at the end of the shelf life (RTES ESL) were used to evaluate the hygienic status of the production environment and processing conditions. Figure 4 shows that 80% and 20% of RTES belonging to the industry and analyzed on the day of collection were overall (TOTAL) satisfactory and acceptable, respectively. These percentages became 80% acceptable and 20% unsatisfactory in RTES ESL, prevalently due to ACC increment during conservation for 7 days at about 4°C.

Table 2. Microbial logarithmic reduction/increase during the production phases of ready-to-eat salads

	RM -2nd W	2nd W – 5th W	5th W - RTES	RM - RTES	RTES - ESL
Aerobic Colony Count	-0.79	-0.22	-0.25	-1.27	+1.53
Yeasts and Moulds	-0.38	-0.63	-0.84	-1.85	+0.04
Pseudomonas spp.	-0.75	-0.18	-0.31	-1.25	+1.22

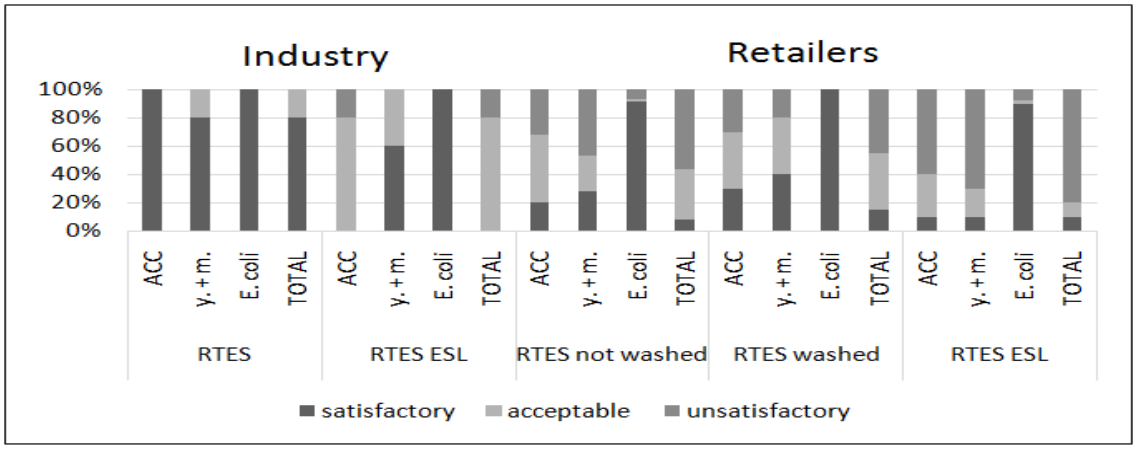

Figure 4. Results of hygiene indicator tests of the salads collected from the investigated industry and the retailers on the day of collection (RTES), and at the end of the shelf life (RTES ESL). Results obtained using microbiological limits of Table 1. ACC, Aerobic Colony Count; y. + m., yeasts and moulds

Low percentages of the samples collected from the retailers were overall judged as satisfactory (8.3% of RTES unwashed, 15% of RTES washed and 10% of RTES-ESL in TOTAL columns), the percentage of unsatisfactory samples at the end of shelf life was indeed very high (80%).

4. Discussion

Since fresh produce have been associated with 4,2% of total foodborne outbreaks in the European Union [18] and 14.8% of illness outbreaks that accounted for 22.8% of all foodborne illnesses in the US [33], this research was conducted with the objective of evaluating microbiological quality of RTES and of understanding the factors that can influence microbial quality of fresh produce.

As required by the EU "food safety criteria" at the market place [46], the pathogenic foodborne microorganisms *Salmonella* and *L. monocytogenes* were not detected in the analyzed samples, in accordance with the Brandao et al. study [3], as other bacteria such as *S. aureus* and *Clostridium* spp. These results were in contrast with other European studies where these bacteria were found, although at low levels [49,50,51]. Yeasts and moulds are widely distributed in the environment and can enter foods through inadequately sanitized equipment or as airborne contaminants. Due to their ability to produce toxic or allergenic substances, moulds are especially considered to be a health hazard for the consumers [26]. Therefore, they should be taken into account and added to the sampling plans of the general hygiene monitoring. During the production process yeasts and moulds count gradually decreased and it remained almost the same during refrigeration at about 4°C for 7 days, probably due to their reduced capacity to grow at low temperatures, differently from ACC and *Pseudomonas* spp. which, at the end of the shelf life, reached similar contamination levels of raw material. From results shown in Figure 2, it is possible to assume that main ACC count is due to *Pseudomonas* population. The progressive reduction of the microbial content observed during the processing phases was probably due to the mechanical action of the water flow, which removed the microorganisms. Moreover, the microbial reduction could be due to the low environmental and washing water temperatures, which did not permit multiplication, and to the five washing phases with chlorinated water, that inactivated the microorganisms. Nevertheless, residual chlorine concentrations were probably too low to obtain a great microbial decrease [52,53,54]: free chlorine detected values were similar to those found in potable water, being 0.5 mg L^{-1} [55], so it would be helpful the use of higher chlorine concentrations [56] or the introduction of different and efficacious water disinfection strategies [57]. RTES samples belonging to the industry were less contaminated both in percentage and in concentration than the supermarket ones, in which microbial multiplication was probably permitted during transportation and preservation [58,59]. During the entire production process of RTES (RM-RTES in Table 2), total logarithmic reduction of microbial count was greater than 1 Log_{10} CFU g^{-1} for all microorganisms considered. From these results, together with the absence of other bacteria such as *S. aureus* and *E. coli*, it is possible to hypothesize the correct application of Good Agricultural Practices (GAP) and the compliance of the personnel with the GMP during the different production phases of the industry involved in the study.

RTES collected from the retailers were analyzed after temperature abuse to mimic consumers behavior. Yeasts and moulds were found in concentrations higher than 2 Log_{10} CFU g^{-1}, as reported in other studies [26,49].

The pathogen *E. coli* is part of the *Enterobacteriaceae* family, it is a good hygiene indicator and his presence in foods can be indicative of fecal contamination, and so of the potential presence of enteric pathogens. Unsatisfactory results can indicate that the process should be revised because of a potential failure, such as cross-contamination, inadequate cleaning and sanitization, poor temperature and time control. Out of the 570 samples, only 17 (2.98%) were contaminated by this bacterium, demonstrating adequate hygienic practices [60] and adequate methods of cultivation and irrigation in field; as described previously, indeed, quality of irrigation water and type of irrigation system influence the microbial safety of fresh produce [61]. Thirteen of the 17 *E. coli* positive samples exceeded the maximum admitted of 10^2 CFU g^{-1} [46] for ready-to-eat vegetables. In other studies, the occurrence of *E. coli* was much higher, ranging from 26% to 32.9% [19,50,62].

The worse microbiological quality of RTES collected from the retailers was probably due to the temperature abuse occurring not only during their transportation home, but also from the production sites to wholesalers, and subsequently to retailers, as well as their exposure in refrigerated counters. These considerations could explain the different microbial load of ESL samples collected in the industry compared to the ones collected from the retailers.

This study shows that most RTES collected from the retailers near production date and analyzed on the day of collection presented overall high percentages of unsatisfactory (56.7%) or acceptable (35.0%) microbiological quality; these bad results in term of too high microbial contamination support the importance of temperature in influencing microbial growth [50].

During storage occurs a quick increment of the microbial load, so it is fundamental to obtain products at the end of the production chain with as low as possible microbial content. To achieve this result, it is also important to monitor the washing procedures, since different factors can affect the effectiveness of chlorine washing disinfection, such as the chlorine concentration, the pH, the organic material load, the temperature and the contact time.

Furthermore, to obtain RTES of good quality, it is very important that the microbial load in the raw material be low, even if it can be further lowered by industrial washing procedures. Therefore, the following GMPs and continuous control of CCPs in all production processes are of great importance.

Finally, although washing salads before consumption is not effective to eliminate pathogens internalized within the plant's tissues [11], bacteriological analysis showed that it was useful in reducing their microbiological load, especially *E. coli* count. Unlike the other microorganisms, *E. coli* concentration in the unwashed RTES was not very high, being 2,4 Log_{10} CFU g^{-1}, and its microbial decrease could probably due to the removal efficiency of free chlorine. Owoseni et al. [56] reported that a free chlorine concentration of 0.5 mg/L was able to reduce *E. coli* bacterial concentration within a range of 3.88-6.0 log, even if at higher doses a more marked reduction in the viability of *E. coli* isolates was achieved.

5. Conclusions

Ready-to-eat salads are convenience foods consumed by millions of people either at home or in schools and university canteens, hospitals and care homes for elderly. During their life, people can be susceptible of infections due to their immune system state, and they would expect to ingest a healthy kind of aliment with RTES. Current study revealed that high microbial content of RTES analyzed were mainly caused by microbial multiplication which occurred during storage and transport from the producer onwards, till home transport, and not by high microbial concentration in just-packed RTES. Hence, more frequent monitoring of storage and transport temperatures would be necessary to ensure the necessary hygienic quality of this kind of "convenience food".

To increase the awareness of the risk of microbial growth in consumers, it would also be helpful to write clearly on the packaging that the product should be kept at refrigeration temperatures lower than 8°C until use. Furthermore, in Italy on the packaging of ready salad it is written "already washed, ready for consumption" providing an indication of total safety of the product which does not always correspond to reality, as shown in this study. Keeping salads at low temperature, together with rinsing them before their consumption, will ensure most safety for consumers especially for people such as elderly, children and those with immune deficiencies.

Statement of Competing Interests

The authors have no competing interests.

References

[1] Pilone, V., Stasi, A. and Baselice, A., "Quality preferences and pricing of fresh-cut salads in Italy: new evidence from market data", *British Food Journal*. 2017, 119(7): 1473-1486.

[2] Pinela, J., Barreira, J.C.M., Barros, L., Antonio, A.L., Carvalho, A.M., Oliveira, M.B.P.P. and Ferreira, I.C.F.R., "Postharvest quality changes in fresh-cut watercress stored under conventional and inert gas-enriched modified atmosphere packaging", *Postharvest Biology and Technology*. 2016, 112*:* 55-63.

[3] Brandao, M.L.L., Almeida, D.O., Bispo, F.C.P., Bricio, S.M.L., Marin, V.A. and Miagostovich, M.P., "Assessment of Microbiological Contamination of Fresh, Minimally Processed, and Ready-to-Eat Lettuces (*Lactuca sativa*), Rio de Janeiro State, Brazil", *Journal of Food Science*. 2014, 79(5): M961-M966.

[4] Italian Ministerial Decree n. 3746, "Attuazione dell'art. 4 della legge 13 maggio 2011 n. 77, recante disposizioni concernenti la preparazione, il confezionamento e la distribuzione dei prodotti ortofrutticoli di quarta gamma", *Gazzetta Ufficiale* n. 186, 2014. [Online]. Available: https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/7763 [Accessed 08 April 2019].

[5] Hunter, P.J., Hand, P., Pink, D., Whipps, J. M. and Bending, G.D., "Both leaf properties and microbe-microbe interactions influence within species variation in bacterial population diversity and structure in the lettuce (Lactuca Species) phyllosphere", *Applied Environmental Microbiology*. 2010, 76: 8117-8125.

[6] Jay, J.M., Loessner, M.J. and Golden D.A., *Modern Food Microbiology*, 7th ed., Springer, 2009.

[7] Erickson, M.C., Webb, C.C., Diaz-Perez, J.C., Phatak, S.C., Silvoy, J.J., Davey, L., Payton, A.S., Liao, J., Ma, L. and Doyle, M.P., "Surface and internalized *Escherichia coli* O157: H7 on field-grown spinach and lettuce treated with spray-contaminated irrigation water", *Journal of Food Protection.* 2010, 73(6): 1023-1029.

[8] Klerks, M.M., Franz, E., Van Gent-Pelzer, M., Zijlstra, C. and van Bruggen, A.H.C., "Differential interaction of *Salmonella enteric* serovars with lettuce cultivars and plant-microbe factors influencing the colonization efficiency", *The ISME Journal*. 2007, 1(7): 620-631.

[9] Klerks, M.M., Van Gent-Pelzer, M., Franz, E., Zijlstra, C. and Van Bruggen, A.H.C., "Physiological and molecular responses of *Lactuca sativa* to colonization by *Salmonella enteric* serovar Dublin", *Applied and Environmental Microbiology*. 2007, 73(15): 4905-4914.

[10] Meyer, K. M. and Leveau, J.H.J., "Microbiology of the phyllosphere: a playground for testing ecological concepts", *Oecologia*. 2012, 168: 621-629.

[11] Erickson, M.C., "Internalization of Fresh Produce by Foodborne Pathogens", *Annual Review of Food Science and Technology*. 2012, 3: 283-310.

[12] Niemira, B.A., "Relative efficacy of sodium hypochlorite wash versus irradiation to inactivate Escherichia coli O15: H7

internalized in leaves of Romaine lettuce and baby spinach", *Journal of Food Protection*. 2007, 70(11): 2526-2532.

[13] MacDonald, E., Einöder-Moreno, M., Borgen, K., Thorstensen Brandal, L., Diab, L., Fossli, Ø., Guzman Herrador, B., Hassan, A.A., Johannessen, G.S., Johansen, E.J., Jørgensen Kimo, R., Lier, T., Paulsen, B.L., Popescu, R., Tokle Schytte, C., Sæbø Pettersen, K., Vold, L., Ørmen, Ø., Wester, A.L., Wiklund, M. and Nygård, K., "National outbreak of Yersinia enterocolitica infections in military and civilian populations associated with consumption of mixed salad, Norway, 2014", Euro Surveillance. 2016, 21: 34.

[14] Vestrheim, D.F., Lange, H., Nygård, K., Borgen, K., Wester, A.L., Kvarme, M.L. and Vold, L., "Are ready-to-eat salads ready to eat? An outbreak of Salmonella Coeln linked to imported, mixed, pre-washed and bagged salad, Norway, November 2013", *Epidemiology and Infection*. 2016, 144(8): 1756-60.

[15] Centers for Disease Control and Prevention (CDC), "Multistate Outbreak of Listeriosis Linked to Packaged Salads Produced at Springfield, Ohio Dole Processing Facility (Final Update)", 2016. [Online]. Available: https://www.cdc.gov/listeria/outbreaks/bagged-salads-01-16/index.html [Accessed 08 April 2019]

[16] Stephan, R., Althaus, D., Kiefer, S., Lehner, A., Hatz, C., Schmutz, C., Jost, M., Gerber, N., Baumgartner, A., Hachler, H. and Mausezahl-Feuz, M., "Foodborne transmission of *Listeria monocytogenes* via ready-to-eat salad: A nationwide outbreak in Switzerland, 2013-2014", *Food Control*. 2015, 57: 14-17.

[17] Centers for Disease Control and Prevention (CDC), "Multistate Outbreak of Shiga toxin-producing Escherichia coli O157: H7 Infections Linked to Ready-to-Eat Salads (Final Update)", 2013. [Online]. Available: http://www.cdc.gov/ecoli/2013/O157H7-11-13/index.html?s_cid=cs_002 [Accessed 08 April 2019].

[18] European Food Safety Authority and European Centre for Disease Prevention and Control, "The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016", *EFSA Journal*. 2017, 15(12): 5077.

[19] Campos, J., Mourão, J., Pestana, N., Peixe, L., Novais, C. and Antunes, P., "Microbiological quality of ready-to-eat salads: an underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes", *International Journal of Food Microbiology*. 2013, 166(3): 464-470.

[20] Mritunjay, S.K. and Kumar, V., "A study on prevalence of microbial contamination on the surface of raw salad vegetables", *3 Biotech*. 2017, 7(1): 13.

[21] Althaus, D., Hofer, E., Corti, S., Julmi, A. and Stephan R., "Bacteriological Survey of Ready-to-Eat Lettuce, Fresh-Cut Fruit, and Sprouts Collected from the Swiss Market", *Journal of Food Protection*. 2012, 75(7): 1338-1341.

[22] Becker, B., Stoll, D., Schulz, P., Kulling, S. and Huch, M., "Microbial Contamination of Organically and Conventionally Produced Fresh Vegetable Salads and Herbs from Retail Markets in Southwest Germany", *Foodborne Pathogens and Disease*. 2018.

[23] Bencardino, D., Vitali, L.A. and Petrelli, D., "Microbiological evaluation of ready-to-eat iceberg lettuce during shelf-life and effectiveness of household washing methods", *Italian Journal of Food Safety*. 2018, 7(1): 6913.

[24] Fiedler, G., Kabisch, J., Bohnlein, C., Huch, M., Becker, B., Cho, G.S. and Franz, C.M.A.P., "Presence of Human Pathogens in Produce from Retail Markets in Northern Germany", Foodborne Pathogens and Disease, 2017.

[25] Ilyas, S., Qamar, M.U., Rasool, M.H., Abdulhaq, N. and Nawaz, Z., "Multidrug-resistant pathogens isolated from ready-to-eat salads available at a local market in Pakistan", *British Food Journal*. 2016, 118(8): 2068-2075.

[26] Marinelli, L., Maggi, O., Aurigemma, C., Tufi, D. and De Giusti, M., "Fresh vegetables and ready-to eat salad: phenotypic characterization of moulds and molecular characterization of yeasts", *Annali di Igiene: medicina preventiva e di comunità*. 2012, 24(4): 301-309.

[27] Mogren, L., Windstam, S., Boqvist, S., Vågsholm, I., Söderqvist, K., Rosberg, A.K., Lindén, J., Mulaosmanovic, E., Karlsson, M., Uhlig, E., Håkansson, A. and Alsanius, B., "The Hurdle Approach–A Holistic Concept for Controlling Food Safety Risks Associated With Pathogenic Bacterial Contamination of Leafy Green Vegetables", *Frontiers in microbiology*. 2018, 9: 1965.

[28] Codex Alimentarius Commission, "Recommended International Code of Practice General Principles of Food Hygiene CAC/RCP 1-1969", Rev. 4-2003.

[29] Kafetzopoulos, D.P., Psomas, E.L. and Kafetzopoulos, P.D., "Measuring the effectiveness of the HACCP Food Safety Management System", *Food Control*. 2013, 33: 505-513.

[30] Raspor, P., "Total food chain safety: how good practices can contribute?", *Trends in Food Science & Technology*. 2008, 19: 405-412.

[31] Italian Legislative Decree n. 31, "Decreto Legislativo 2 febbraio 2001, n. 31. Attuazione della direttiva 98/83/CE relativa alla qualità delle acque destinate al consumo umano", Gazzetta Ufficiale 52, 41, 2001. [Online]. Available: http://www.camera.it/parlam/leggi/deleghe/01031dl.htm [Accessed 08 April 2019].

[32] European Commission, "Council Directive 98/83/EC, on the Quality of Water Intended for Human Consumption: calculation of derived activity concentrations", *Official Journal of the European Communities*. 1998, L330: 32-54.

[33] Amin, N., Olaimat, R. and Holley, A., "Factors influencing the microbial safety of fresh produce: A review", *Food Microbiology*. 2012, 32(1): 1-19.

[34] European Commission, "Commission Regulation No 2073 on microbiological criteria for foodstuffs", *Official Journal of the European Communities*. 2005, L338: 1-26.

[35] European Commission, "Commission Regulation No 852 on the hygiene of foodstuffs", *Official Journal of the European Communities*. 2004, L139: 1-54.

[36] Jol, S., Kassianenko, A., Wszol, K. and Oggel, J., "The Cold Chain, one link in Canada's food safety initiatives", *Food Control*. 2007, 18(6): 713-715.

[37] Nieri, D., Pesavento, G., Ducci, B., Calonico, C. and Lo Nostro, A., "Monitoring of the cold chain compliance in a meal-processing facility through the correlation study between the outdoor temperatures and coliforms counts on raw meat", *International Journal of Food Science and Technology*. 2014, 49: 928-935.

[38] UNI EN ISO 4833-1, "Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Colony count at 30°C by the pour plate technique", International Organization for Standardization, Geneve, Switzerland, 2013.

[39] ISO 16649-2, "Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide", International Organization for Standardization, Geneva, Switzerland, 2001.

[40] ISO 21527-2, "Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds Colony count technique in products with water activity less than or equal to 0,95", International Organization for Standardization, Geneva, Switzerland, 2008.

[41] ISO 13720, "Meat and meat products – Enumeration of presumptive *Pseudomonas* spp.", International Organization for Standardization, Geneva, Switzerland, 2010.

[42] UNI EN ISO 6888-1, "Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium", International Organization for Standardization, Geneve, Switzerland, 2004.

[43] ISO 6579-1, "Microbiology of the food chain -Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Part 1: Detection of *Salmonella* spp.", International Organization for Standardization, Geneva, Switzerland, 2017.

[44] UNI EN ISO 11290-1, "Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1: Detection method", International Organization for Standardization, Geneve, Switzerland, 2005.

[45] ISO 15213, "Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions", International Organization for Standardization, Geneva, Switzerland, 2003.

[46] European Commission, "Commission Regulation No 1441 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs", *Official Journal of the European Communities*. 2007, L322: 12-29.

[47] Health Protection Agency, "Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods", 2009. [Online]. Available: http://webarchive.nationalarchives.gov.uk/20140714111812/http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1259151921557 [Accessed 08 April 2019].

[48] Ce. I. R. S. A., Centro Interdipartimentale di Ricerca e documentazione sulla Sicurezza Alimentare, "Linee Guida per l'analisi del rischio nel campo della microbiologia degli alimenti", 2013. [Online]. Available: http://www.ceirsa.org/docum/allegato_punto4.pdf [Accessed 08 April 2019]

[49] Abadias, M., Usall, J., Anguera, M., Solsona, C. and Viñas, I., "Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments", *International Journal of Food Microbiology*. 2008, 123: 121-129.

[50] Caponigro, V., Ventura, M., Chiancone, I., Amato, L., Parente, E. and Piro, F., "Variation of microbial load and visual quality of ready-to-eat salads by vegetable type, season, processor and retailer", *Food Microbiology*. 2010, 27(8): 1071-1077.

[51] De Giusti, M., Aurigemma, C., Marinelli, L., Tufi, D., De Medici, D., Di Pasquale, S., De Vito, C. and Boccia, A., "The evaluation of the microbial safety of fresh ready-to-eat vegetables produced by different technologies in Italy", *Journal of Applied Microbiology*. 2010, 109(3): 996-1006.

[52] Gómez-López, V.M., Marín, A., Medina-Martínez, M.S., Gil, M.I. and Allende, A., "Generation of trihalomethanes with chlorine-based sanitizers and impact on microbial, nutritional and sensory quality of baby spinach", *Postharvest Biology and Technology*. 2013, 85: 210-217.

[53] Losio, M.N., Pavoni, E., Bilei, S., Bertasi, B., Bovec, D., Capuano, F., Farneti, S., Blasi, G., Comin, D., Cardamone, C., Decastelli, L., Delibato, E., De Santis, P., Di Pasquale, S., Gattuso, A., Goffredo, E., Fadda, A., Pisanu, M. and De Medici, D., "Microbiological survey of raw and ready-to-eat leafy green vegetables marketed in Italy", *International Journal of Food Microbiology*. 2015, 210: 88-91.

[54] Weng, S.C., Luo, Y., Lib, J., Zhou, B., Jacangelo, J.G. and Schwab, K.J., "Assessment and speciation of chlorine demand in fresh-cut produce wash water", *Food Control*. 2016, 60: 543-551.

[55] Reynolds, K.A., Mena, K.D. and Gerba, C.P., "Risk of waterborne illness via drinking water in the United States", *Reviews of Environmental Contamination and Toxicology*. 2008, 192: 117-158.

[56] Owoseni, M.C., Olaniran, A.O. and Okoh, A.I., "Chlorine Tolerance and Inactivation of Escherichia coli recovered from Wastewater Treatment Plants in the Eastern Cape, South Africa", *Applied Sciences*. 2017, 7(8): 810.

[57] Ignat, A., Manzocco, L., Maifreni, M. and Nicoli, M.C., "Decontamination efficacy of neutral and acidic electrolyzed water in fresh-cut salad washing journal of food processing and preservation", *Journal of Food Processing and Preservation*. 2016, 40(5): 874-881

[58] Jevsnik, M., Hlebec, V. and Raspor, P., "Consumers' awareness of food safety from shopping to eating", *Food Control*. 2008, 19(8): 737-745.

[59] Pérez-Rodríguez, F., Castro, R., Posada-Izquierdo, G.D., Valero, A., Carrasco, E., García-Gimeno, R.M. and Zurera, G., "Evaluation of hygiene practices and microbiological quality of cooked meat products during slicing and handling at retail", *Meat Science*. 2010, 86(2): 479-485.

[60] Oliveira, M., Usall, J., Viñas, I., Anguera, M., Gatius, F. and Abadias, M., "Microbiological quality of fresh lettuce from organic and conventional production", *Food Microbiology*. 2010, 27(5): 679-684.

[61] Warriner, K., Huber, A., Namvar, A., Fan, W. and Dunfield, K., "Recent advances in the microbial safety of fresh fruits and vegetables", *Advances in Food and Nutrition Research*. 2009, 57: 155-208.

[62] Silva, S.R.P., Verdin, S.E.F., Pereira, D.C., Schatkoski, A.M., Rott, M.B. and Corcao, G., "Microbiological quality of minimally processed vegetables sold in Porto Alegre, Brazil", *Brazilian Journal of Microbiology*. 2007, 38(4): 594-598.