

Impact of the HACCP System on a Catering Facility: Special Reference to Ready-to-Eat Salads

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Abstract

The consumption of ready-to-eat salads (RTE) in developing countries has increased considerably in a time where the food borne disease linked to vegetable salads poses serious safety threats. Most methods have been used to increase the microbiological safety of RTE salads. This study aims to evaluate the impact of the HACCP system on a catering facility for RTE salads such as nicoise salads (NS), Tunisian salads (TS) and green salads (GS). A total of 238 samples were tested to determine the microbiological quality and identify possible correlations between them. 118 samples were evaluated before the implementation of the HACCP system and 120 samples after the application of the HACCP approach. The microbiological evaluation was focused on the total mesophilic flora (TMF), Staphylococcus aureus (SA), Escherichia coli (EC) and Salmonella spp. The obtained results indicated that the implementation of the HACCP system had no effect on the general microbiological profile since the general unsatisfactory analysis rate was 85% before HACCP implementation system and remained unchangeable after the HACCP application. However, a significant difference between NS, TS and GS where distinguishable with NS presenting the best ranking (35%) of satisfactory analysis. For EC profile, a significant association between the EC deterioration state of RTES and the HACCP application was found. While, a significant association between TMF classifications and the three RTE categories was noted. However, no significant changes of SA profile between the three RTES categories were observed. Besides, no Salmonella spp was detected during the current analysis (n= 238). Thus, the binary logistic regression on microbial profiles revealed high correlation between the SA and EC classification outcomes. Hence, through this study a personalized and operational microbial model was presented which leads to better prediction of failures leading to inspire professionals to adopt the present statistical model for identifying the contamination and to explore appropriate solutions.

Keywords: RTE Salads; Microbiological Safety; HACCP; Total Mesophilic Flora; Staphylococcus Aureus; Escherichia Coli; Salmonella Spp

Introduction

Consumption of fresh vegetables is highly increasing [1] in a time where the number of reported foodborne illnesses linked to fresh vegetables has been amplified where etiological agents range from pathogenic bacteria [2].

The ready-to-eat vegetable salads (RTE) have shown the

presence of a wide range of microbial foodborne pathogens including *E. coli*, coliforms, Salmonella, L. monocytogenes, total aerobic and spoilage bacteria, yeasts and fungi responsible of serious threats inducing illnesses associated to their intakes contamination [3,4].

According to Mir, et al. [5] contamination of RTE vegetable salads may occur through various means from farm to fork. Therefore, maintaining the microbial safety of RTE salads requires a systematic approach that includes all aspects of their way to consumer dishes [5]. The HACCP method is a scientific approach to assess hazards associated with food production and establish control systems to ensure food safety [6].

Some studies noted the benefits of using the HACCP assessment systems in standardized food safety management, quality control and several food businesses [7,8]. Maldonado-siman, et al. [9] specified that the reduction of microbial counts is considered among the major benefit of the HACCP concept. In contrast, various studies have been carried out to exhibit the difficulties and the barriers of the implementation of the HACCP approach. For argument's sake, a study carried out by Murat, et al. [10] determined the barriers of implementing HACCP and concluded that the lack of preventive actions/universal procedures of re-requested program (PrP) is the main barrier representing around 92.5% while, other limits such as the lack of knowledge on HACCP represented 83.5%, the lack of time around 88.7% and the lack of employee motivation about 83.5%.

According to EU Commission [11] and Ricci, et al. [12]

the burdensome of the system is identified as barriers to the implementation of the HACCP concept. However Azanza & Myrna Benita [13] consider the non-awareness to HACCP guideline as a major barrier for implementing the HACCP system, besides, the lack of traceability noted among the deficiencies of the HACCP system [14,15]. However Eves A, et al. [16] & Panisello JP [17] reported the employees believe about the HACCP system which was reported to be quite complicated, especially because of its many documents. Furthermore, currently, the application of the HACCP system has been imposed because of international competition and stricter regulations [18].

Inappropriately, generally the Maghreb countries and in Tunisia particularly, industries are not all certified according to HACCP requirements, although, few studies have offered a comprehensive account of its impact on served meals. Therefore, this study aims to provide a structured model for the HACCP system evaluation in a large-scale catering unit with a full screening of the microbial profiles of RTE salads before and after implementation of the HACCP approach.

Materials and Methods

This study was carried in the region of the Sahel in Eastern center of Tunisia. A typical catering unit with a capacity of 3000 meals served per day was chosen. In brief, after an initial field evaluation of the facility, an establishment of PrP program which focused on the kitchen's agronomy and equipment, the reconsideration of the sanitation plan, and the increase of the personnel knowledge and hygiene awareness.

Salad type	Ingredients	Preparation of ingredients
Tunisian salad	Cucumber peeled, seeded and diced in small pieces Ripe tomatoes diced in small pieces corn kernels from 1 ear of corn Red or green pepper, minced and seeded for milder salad, Fresh lemon juice Extra virgin olive oil Coriander	Mix fresh decontaminated vegetables together, season with olive oil, pinch of salt, powdered black pepper, and lemon to taste.
Niçoise salad	Tomatoes sliced Small Shallot, very thinly sliced Fresh parsley leaves Celery stalks, very thinly sliced Radishes, very thinly sliced Fennel bulb Teaspoon drained capers	Slice each tomato crosswise into ¼ inch slices. Cut up shallot, parsley, celery radishes and fennel bulb. Sprinkle the mixture with oil, vinegars, salt and pepper. Marinate 10 minutes, turn over and marinate two minutes on the other side. Keep refrigerated until consumption.
Green salad	Lettuce, tomatoes, cucumbers, parsley,	Boil cubed potatoes and carrots and
Vegetables salad	green peppers, salt, lemon, and vegetable oil boiled peas,	mix with boiled peas and mayonnaise
(cold appetizer)	mayonnaise	Ingredients are mixed in a bowl.

 Table 1: Food description.

In the second stage, the HACCP team was assembled, protocols were described (Table 1) and flow charts were constructed. In the last stage, the 7 HACCP principles were fully implemented leading to the establishment of the HACCP control plan which gathered both an operational control program and Critical Control Points (CCPs) (Figure 1).

Food Analysis

Sampling Method: The experimental design was executed comparatively before and after the implementation of the HACCP system. Every week, 3 salads were pooled randomly in three different days of the experiment, corresponding respectively to 25 weeks before the HACCP implementation and to 29 weeks after the HACCP implementation. Each sample contained at least one salad category which may belongs to TS, GS or /and GS, according to the food available in the day of the sampling. In total, 238 samples were assessed, 118 samples before the HACCP implementation and 120 samples after the declaration of the HACCP conformity. All sampling processes were in complete compliance of the sampling standards [19].

Samples Preparation: Upon arriving at the Laboratory of Food Analysis, food samples were spread out on a stainless steel tray and 10 g of mixed portions were collected aseptically. The sample was placed in a sterile stomacher bag, in which we added 90 ml of buffered peptone water. The sample was milled using a stomacher for 2 to 3 minutes. The obtained homogenate was 10-1; then we conducted a series of decimal dilutions up to 10-7. To search for Salmonella spp., 25 g in each salad was sampled, to which we added 225 ml of buffered peptone water.

Microbiological analysis and classification: The enumeration of total mesophilic flora (TMF) was done according to the International Standard ISO 2293 [20] by counting the observed microorganisms at 30°C. The evaluation of Staphylococcus aureus (SA) was made according to ISO 6888-1 [21]. A special interest was given to the coagulase positive Staphylococcus by using a specific medium (Baird-Parker solid medium) at 37°C. Escherichia coli (EC) analysis was conducted following the ISO 16649-1 requirements. Whilst, the detection of Salmonella was performed according to ISO 6579 [22]. Incertitude errors were evaluated for each of the bacteria separately, except for Salmonella, where no reliable method of calculation is currently available. In fact, the error significance was estimated by computing the standard derivation of the reproducibility, according to the ISO Standard 19036 [23] as recommended by the General Directorate of Health and Consumer [11]. The error rates were estimated from 0.5 to 1 log10 reduction. The classification of the microbiological counts was based mainly on the recommendations of AFSSA [24] founded on the European food safety regulations (food law) and the recommendations of Balzaretti & Marzano [25].

Data Processing: As a primary treatment, a lognormal transformation was performed on the microbial counts to ameliorate their characteristics [26,27]. After that, a series of nonparametric tests were carried out to determine the HACCP application impact on the Microbial profiles of the studied RTES and to highlight the significant differences between them.

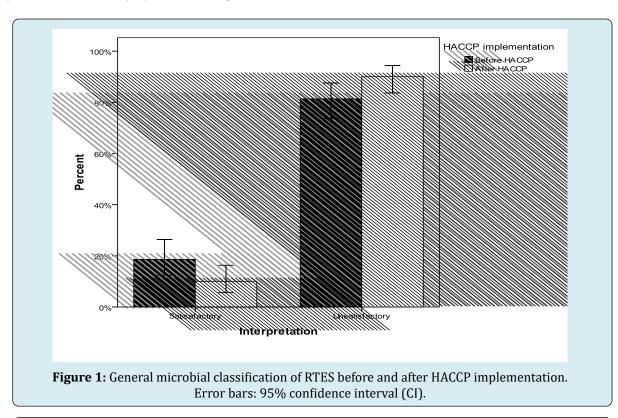
Results and Discussion

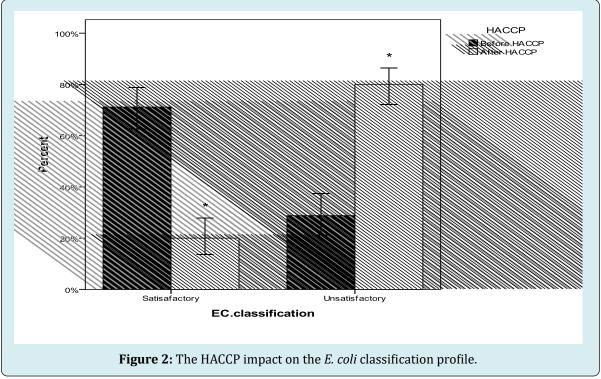
General Microbiological Profiles

The implementation of the HACCP system had no effect on the general microbiological classification because the general unsatisfactory analysis rate was 85% before implementation of HACCP system and remained unchangeable after the HACCP application, Chi square (1, N=238) =3.6, p=0.057 (Figure 1). The majority of RTE samples were not in conformity with the quality requirements. Similar observations were noted in the Italian Canteens, where high unsatisfactory rate of the salad samples (100%) seemed to be the usual contamination factor [28]. For the evaluation of HACCP system implementation on the quality of mixed fresh-cut salad prepared in a university canteen Osimani A, et al. [29] reported that a high number of samples exceeded the mandatory or suggested limits for food processing hygiene (mesophilic aerobes, coliforms, Staphylococcus aureus) and no E. coli and Salmonella spp strains were detected in the tested samples. On their side, Campos J, et al. [3] reported that 86% of salad samples analysed from Brazilian supermarkets where all major brands and considered unsatisfactory. Moreover, an unsatisfactory rate (76%) was reported by Marzano & Balzaretti [30] after analysing samples from mass catering establishments. Equally, 53 % of raw fruits and vegetables did not comply with the quality requirements in an Italian airport [29]. After using the HACCP system and appreciated the role of training in the success of the self-monitoring system, Cenci-Goga, et al. [31] succeeded in keeping the food spoilage within safe proportions and effectively improved the microbial quality and safety of the meal served. According to Kafetzopoulos, et al. [32] the simply implementing of the HACCP food safety system or conforming to its requirements does not ensure the highest level of product safety performance. To continue the current investigation, several comparisons of microbial profiles between salad categories was performed, Chi square (2, N=238) = 17.7, p=0.00. The comparison of the results of this classification allowed to distinguish a significant difference between the NS, TS and GS where NS had the best ranking with 35% of satisfactory analysis while the TS had 11%, Chi square (1, N = 200)=13.4 p=0.00, and GS had only 5%, Chi square (1, N=78)=10.5 p=0.00 (Figure

2). To further scrutinize the obtained data, the samples specific microbial profiles were analysed and the significant differences among the bacterial evaluations was determined on namely Escherichia coli (EC), Total mesophilic flora

(TMF), *Staphylococcus aureus* (SA) and Salmonella spp. In this assessment the microbial contamination concentration loads and the classification outcomes were investigated.



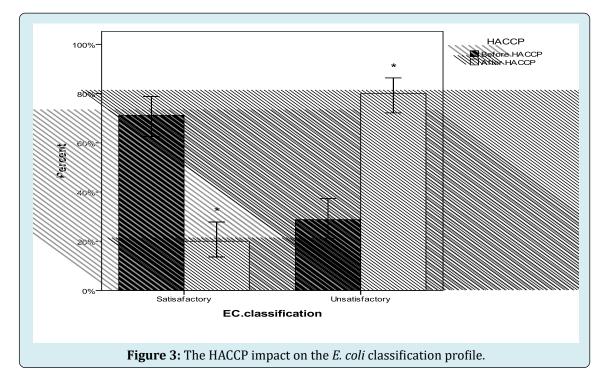


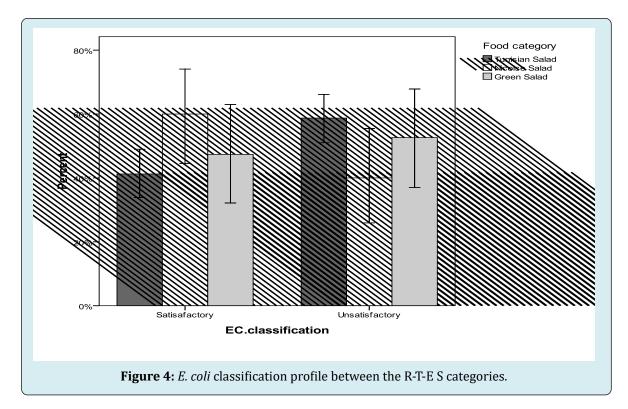
Escherichia Coli Profile

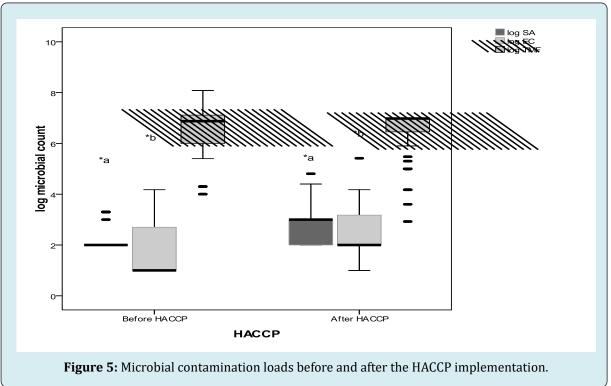
A significant association between the EC deterioration state of RTES and the HACCP application was observed. In fact, the EC contamination level increased from 28.8% to 80%, Chi square (1, N=237) = 62, 8, p = 0.00 at a significance level of 5% (Figure 3). Other studies have proved a high EC standard compliance of the RTES, where the abuse level was almost nullified in both Public and Private mass meal serving units [25,33]. Similarly, the abuse level barely exceeded 11% in other assessments carried out in Turkish and Brazilian Public restaurants [3,34]. Chellapandi, et al. [35] observed that 4.88 % (n=27) of fresh vegetables (tomato, potato and cabbage) contained E. coli. The study conducted by Shah, et al. [36] demonstrated that fresh RTE vegetable salads were contaminated with various types of E. coli pathotypes. Castro-Rosas, et al. [37] studied the microbial guality and prevalence of diarrheagenic E. coli in RTE salads of raw vegetables and among the salad's samples tested, 85 % (110/129) of harbored E. coli and 7 % (8/110) of diarrheagenic E. coli was noted. In a recent study [38], E. coli population was noticed in 16.70% of total samples analysed (n=480) and was present in 15% of tomato and 20% of carrot. More recently, EC was found in 11.57 from a total samples (n=229) of ready-to-eat salads and 2.62% of isolates were found to be able to produce extended spectrum β -lactamase (ESBL) [39]. According to the obtained data the quantitative assessment revealed that logEC counts were significantly affected by the HACCP application, Mann-Whitney U Test (MWUT) p = 0.00. The median passed from 1 log10 ufc g-1 in contrast before the HACCP application to 2 log10 ufc g-1 after the HACCP

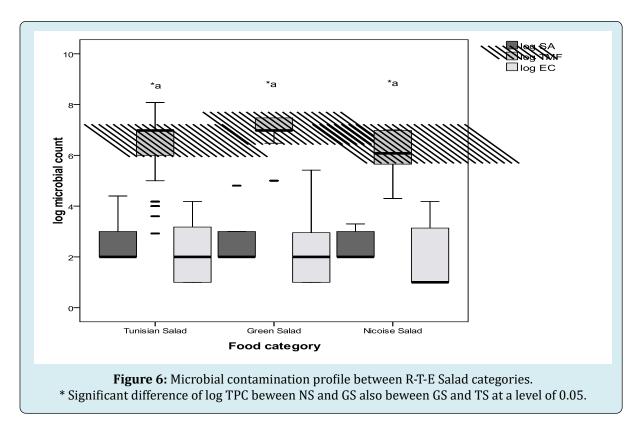
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implementation, median test Chi square 2 (1, N=237) = 8,45, p = 0.00 (Figure 4). However, no significant difference of EC state was noticed between the three sampled RTES. neither at the classification level, Chi square (2, N=237) =4.6, p=0.1 (Figure 5), nor at the contamination one Kruskal Wallis test (KWT), Chi square (2, N=237) = 1.13, p=0.5 (Figure 6), since EC contamination was highly and uniformly distributed within the salad categories. It is clear that EC is a faecal hygiene indicator normally absent in food stuffs and its presence indicates a manure contamination of vegetables [28] that could occur either at the pre-harvesting or at the post-harvesting stages. The joint Committee FAO/WHO [40] has assessed the risk of this particular contamination in vegetables and issued some critical recommendations such as the necessity to establish a water safety strategy for the horticultural irrigation. According to Pagadala S, et al. [41], Singh BR, et al. [42] the most possible causes of presence of E. coli are probably the contaminated soil with contaminated irrigation water. In this sense, Balkhair [43] proved that the subsurface drip irrigation system used was successful in reducing the contamination of all studied bacteria categories compared to surface drip system. On the other hand Yoon, et al. [44] have mentioned that EC is a frequent contamination of leafy vegetables and herbs. Nevertheless, the undamaged vegetables during the preparation may deteriorate their structure and increase the microbial infiltration [40,45], besides according to Beuchat [46] vegetables with damaged tissues should be removed at the reception in factories since they can provide nutrients for microbial proliferation and/or allow entry of pathogens to the interior of vegetable tissues.





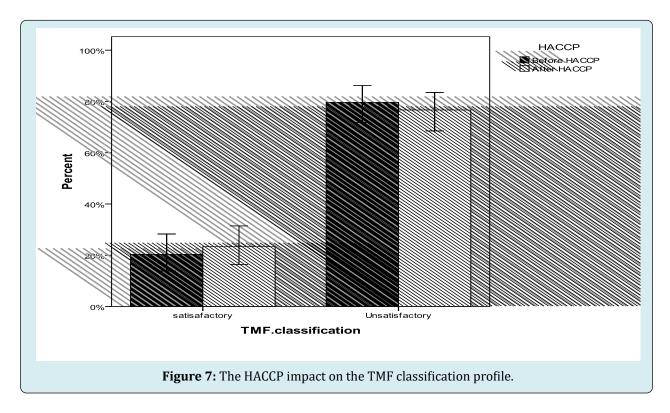




TMF Profile

The TMF classification did not change before or after the

HACCP implementation. The unsatisfactory rate of 79.50% remained relatively high in both cases. Chi square (1, N = 238) = 0.31, p=0.57 at a significant rate of 0.05 (Figure 7).



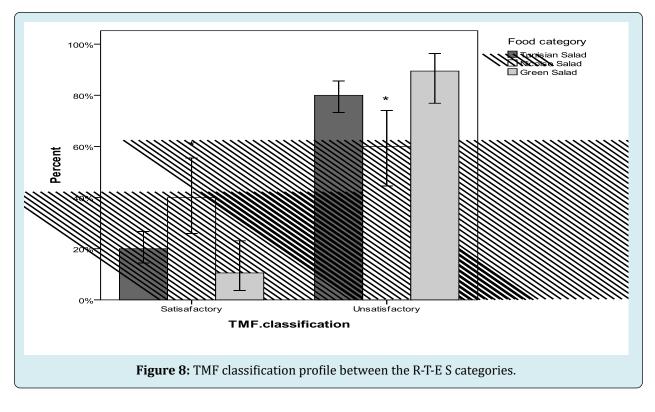
Salads. Food Sci & Nutri Tech 2021, 6(1): 000253.

This percentage was confirmed in other studies [28,3] and seems to be a usual baseline. A research estimated the contamination loads of different vegetables and indicated that 100 % of the bean sprouts and freshly cut salads were greater than 5 log10 cfu g⁻¹. Therefore, lettuces were classified 82 % unsatisfactorily, followed by carrots with 44% of unsatisfactory classification and the tomato was the least contaminated crop with 22 % [47]. Similarly study on prevalence of microbial contamination on the surface of raw salad vegetables conducted by Mritunjay & Kumar [38] revealed that 85.40 % of the samples (n = 480) exceeded the limits for aerobic bacterial count, indicating it's unacceptable for consumption. Therefore, unsatisfactory considerations are discussed since the aerobic bacterial count for food should not be higher than 5.0 log cfu g⁻¹ in India and several other countries [11,24]. For the Mritunjay & Kumar [38] study, the highest microbial counts were registered for spinach and cucumber, while cabbage had the lowest microbial counts. Likewise, after screening 154 vegetarian food stuffs, 30 % were unsatisfactorily classified in regard to TPC analysis. A recent study carried out by Zwe & Yuk [48] on characterizing the microbial quality of a total of 125 common fresh fruit and vegetable, showed that RTE salad have a mean aerobic mesophilic count of 6.5 log CFU/g. The current findings are in accordance with the results of Osimani A [29] who evoked the improvement of the TMF salad profile after the HACCP application in canteens where the frequency of the unsatisfactory analysis range from 25 % to 100 %. Moreover, in accordance with the present finding, other studies have not improved TMF's dissatisfaction rate through the application of the HACCP system [31]. In fact, according to the current outcomes, the HACCP system did not obviously reduce the TMF log after its application, MWUT p = 0.4, and the median remained identical to 6.9 log10 cfg g⁻¹, median of the chisquare test (1, N = 238) = 6.9, p = 0.7 (Figure 4).

In another assessment, herein, it was found a significant association between TMF classifications and the three RTE categories, Chi square (2, N=237) = 10.8, p=0.04. A comparison was performed to point out the differences in the TMF classification profiles. The GS and the TS both have a similar unsatisfactory classification rate, 89.5% and 80%, correspondingly. However, the NS had the lowest unsatisfactory rate (60 %) and was significantly different from the two other salad categories (Figure 8). In fact, there was a significant difference between the TS and the NS, Chi square (1, N=200) = 7, p=0.008. Notably, the most important difference of TPC classification was attributed to the GS and the NS, Chi square (1, N=78) = 8.8, p=0.003, Cramer's V = 0.33, a medium effect size. At the quantitative assessment, there

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was a significant difference of the log TMF counts between the three RTES categories, KW Chi square (2, N=238) = 20.9, p=0.00, (Figure 6). The comparison demonstrated that NS contamination profile differs significantly from the GS, MWUT p=0.00, with lower contamination loads in the NS (median equal to 6 log10 ufc g-1) than in GS one (median equal to 7 log10 ufc g⁻¹), Chi square (1, N=78) =11.6, p=0.001. The log10TMF contamination count of the GS is significantly higher than the TS, (MWUT), p=0.000. Even if their medians are the same and equal to 7 log10 ufc g^{-1} , this could be explained by the opposite (skewness) of their corresponding distributions (Figure 6). However, no significant difference was found between the TS and the NS, which both had a similar TMF contamination load equal to 6,9 log10 ufc g^{-1} , p=0.028, with an adjusted significance level of 0,016, Median test Chi square (1, N=200) = 2.27, p=0.13. Briefly, the TMF contamination profile assessment confirmed the difference of the contamination distribution among the RTES categories and pointed out the higher contamination profile of the GS. The TS was followed by the NS, which had the best contamination profile. Similar contamination loads were reported by Christison, et al. [49] which evaluated the TMF count to 7 log10 ufc g⁻¹ in assorted salads. Except they did not specify the nature of the salad samples. The high contamination load of the current GS samples may be attributed to the presence of the carrots, lettuces, onions, and sprouts, which all carry high microbial counts. Many authors agreed with the initial high microbial contamination of those crops [3,49]. Scientific validations indicated that it's also important to mention the impact of irrigational water and soil to be highly contaminated with TMF and may constitute a microbial reservoir for the vegetables such as Salmonella and EC [44]. All points considered, those results are unacceptable as the unsatisfactory analysis was highly prevalent, the average of unsatisfactory TMF classifications reached 80%, and the HACCP system application had not corrected the situation. However, high TMF counts alone do not make foods unsafe, but they indicate poor handling, storage or inadequate general hygiene [50], which may explain the absence of food poisoning outbreaks notification in the current study. Evidently, it is necessary to go over the system and highlight the weakness, especially at the prerequisite program level where urgent control measurements were to be undertaken. Osimani, et al. [28] mentioned that the temperature control was one of the most critical preventive measurements to consider. Recent studies revealed that the food storage temperatures complied better with the legal requirements after the HACCP implementation and all food samples were satisfactorily classified [51,52].



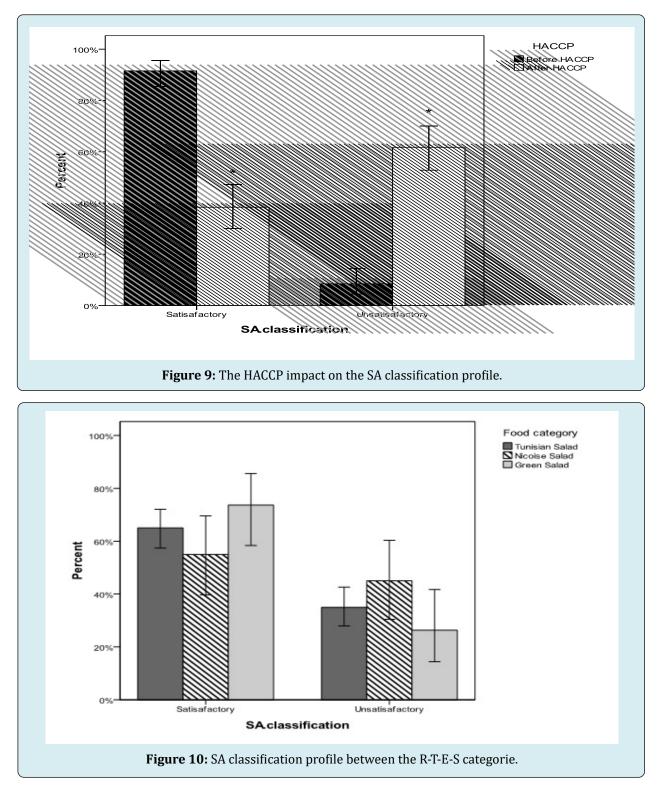
Staphylococcus Aureus Profile

The HACCP application has negatively impacted the SA profile with a significant manner. In fact, the satisfactory classified analysis has decreased from 91.5% to 38.3 % with a substantial increase of the unsatisfactory analysis from 8.47 % to 61.60 % (Figure 9). Furthermore Aycicek, et al. [34] found that salad meals were the most highly contaminated food in a military cafeteria with a prevalence of 37% mean while the contamination loads varied from 2.3 to 4.3 log10 ufc g⁻¹. The same authors also attributed the food spoilage rates to the same food matrixes that need a particular handling. In similar studies [32] a significant reduction of unsatisfactory analysis was recorded after the HACCP implementation, from 75 % to 0 %. Although the microbial interpretation was different than the already adopted in our study, the selfcontrol system with an adequate training program was able to warrant a high food safety standard. The authors pointed out the higher microbial count in vegetable-derived products. Similar observations were recorded in three Italian caterings where meals have been delivered according to "online serving" methods and the personnel and environmental hygiene were satisfactorily applied [30]. Besides, in hospital restaurants almost all samples complied with the safety requirements due to the strict hygienic food conditions in that kind of sensitive establishment [33,34]. The log SA has known a significant increase MW UT, p=0.000, and its median passed from 2 to 3 log10 ufc g⁻¹, median test Chi squared (1, N=238) = 73.70, p=0.00, (Figure 4). The degradation of the

food quality to this proportion proportions would have put the consumer at risk of developing a food poisoning. Despite this microbial count rise after the HACCP application, there were no significant changes of SA profile between the three RTES categories, Chi square (2, N = 238) = 2.99, p = 0.22(Figure 10). All dishes had the same unsatisfactory percentage of 37%. Those findings were confirmed at the quantitative assessment level in which log SA mean ranks were the same for all dishes, KWT Chi square (2, 238) = 4.3, p=0.11, also the food samples had a near medians equal to 2 log10 ufc g⁻¹, median test Chi square (2, 238)=2.9 p=0.22 (Figure 6). This contamination level seems to be frequent in some vegetables. The strawberry contamination loads varied from 1.8 to 3 log10 ufc g⁻¹ with larger loads in leaf than in fruits [44]. Also, carrots and onions had high contamination prevalence, 73% and 62% respectively. Basically, SA contamination is linked to poor staff hygiene and poor food handling practices [44,50] . In this sense, researchers have claimed to fully comply with the legal requirements for SA in RTE because of the hygiene guidelines a good application [49]. Food handlers (FH) are one of the most important causes of food poisoning outbreaks either through the direct or the cross contamination of the food stuffs [53]. Around 94 % of food safety accidents were attributed to people mishandling food, and 10 % directly involved the hand-cleaning process [54]. In another study, 97 % of intoxication was directly related to FH. Many authors also pointed out the poor food-handling practices in Indian street vendors in our current study the microbial evaluations showed no salmonella contamination and all samples were

satisfactory classified. To further scrutinize our data, we have analyzed the specific microbial profiles of our samples and tried with a logistic regression model using as inputs

the different microbial profiles four bacterial evaluations, namely Escherichia coli (EC), total Mesophilic Flora (TMF), Staphylococcus aureus (SA) and Salmonella spp.



Food Safety Logistic Regression Model

Kapetanakou, et al. [55] have used mathematical modeling and quantitative microbial risk assessments to predict pathogen survival and evaluate the risk of contamination of ready to eat vegetable. Prediction model of growth of bacteria in leafy greens without temperature control was used by Mishra, et al. [56]. The Hurdle Approach-A Holistic Concept is adopted for controlling food safety risks of pathogenic bacterial contamination of leafy green vegetables [57] and for survey of foodborne Pathogens [58] as well as for validation of growth of Salmonella Enteritidis and background microorganisms in potato salad [59]. In the present work, a binary logistic regression on microbial profiles was performed in an attempt to explain and to predict the different microbial classification outcomes and the relationship that may connect them. The model was significantly accurate at a level of 78 %, Omnius tests of model coefficients p=0.00, test chi-square (4, N = 238) = 3.5, p = 0.58. The model calculated the odd ratio and we found that the SA abuse increases the probability of EC defaulting by 7, Wild statistic Chi square (1, N = 283) = 19.5, p = 0.00 and vice versa. As well, TMF abuse increases the probability of EC defaulting by 4, Wild statistic Chi square (1, N= 283) = 10.4, p = 0.00. However, no significant correlation was found between SA classification outcomes and TMF classification outcomes. Both variables are completely independent, Wild statistic Chi square (1, N = 283) = 0.01, p = 0.9. In accordance to the present results, the HACCP system in its current state negatively impacts the microbial classification of both EC and SA. Indeed, the probability of EC defaulting increases 4 times with the application of the HACCP, Wild statistic Chi square (1, N= 283) = 7.7, p=0.005. The negative impact of the HACCP system is more conspicuous in the case of SA classification, where the abuse frequency increases10 times over, Wild statistic Chi square (1, N= 238) = 30.7, p=0.00. It appears from the logistic regression model that SA and EC classification outcomes are the most highly correlated elements and after checking off the absence of suppressive effects. It is concluded that the unsatisfactory classification of SA increases the risk of having an unsatisfactory EC classification. In addition, all the microbial classifications are correlated in the same direction (odds ratio > 1), it is to determine that the presence of an unsatisfactory analysis in a given microbial analysis increases the risk of having at least 4 times the EC classification unsatisfactory. Herein, EC could be the most important and representative microbe in the present classification. It can predict the classification of TMF and SA. This conclusion must be taken with caution, because each process has its own microbial contamination profile and the microbial ecology is randomly distributed. Martínez-Tomé, et al. [60] did not isolate any sample of EC positive in its evaluation of food despite the fact that other microbial counts (MFC and Enterobacteriaceae) gave very

positive results. On the other hand Campos, et al. [3] found no correlation between different microbial assessments.

Conclusion

Overall, the HACCP system is a very complex system. However, without proper microbial checks, it could lose most of its effectiveness and objectivity. In the current study, food safety managers are provided with a scientific assessment tool for their self-monitoring system that could highlight weaknesses. Proposition for a customized and operational microbial assessment models are developed to leads to better failure prediction as well as a computer-assisted surveillance model. It is worthy to encourage professionals to adopt the current statistical model (logistic regression model) to identify proper contamination and undertake suitable conclusions. As well it could be very useful for government food safety agencies and certification bodies; whenever traceability and accurate quantitative microbial evaluation are required such was recommended [15].

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no competing interests.

References

- 1. FAO STAT (2015) Food and agriculture organization of the united nations, Statistics division.
- Callejo RM, Ubeda C, Rodríguez-Naranjo MI, Hornedo-Ortega R, Garcia-Parrilla MC, et al. (2015) Reported Foodborne Outbreaks Due to Fresh Produce in the United States and European Union : foodborne Pathog Dis 12: 32-38.
- Campos J, Mourão J, Pestana N, Peixe L, Novais C, et al. (2013) Microbiology Microbiological quality of readyto-eat salads : An underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. Int J Food Microbiol 166(3): 464-470.
- Pothakos V, Snauwaert C, De Vos P, Huys G, Devlieghere F (2014) Monitoring psychrotrophic lactic acid bacteria contamination in a ready-to-eat vegetable salad production environment. Int J Food Microbiol 185: 7-16.
- 5. Mir SA, Shah MA, Mir MM, Dar BN, Greiner R, Roohinejad S (2018) Microbiological contamination of ready-to-eat vegetable salads in developing countries and potential solutions in the supply chain to control microbial pathogens. Food Control 85: 235-244.

- 6. Tebbutt GM and Southwell M (1997) Compliance with recent food hygiene legislation and microbiological monitoring in cooked meat product plants. Int J Environ Health Res 7: 335-344.
- Dzwolak W (2019) Assessment of HACCP plans in standardized food safety management systems - The case of small-sized Polish food businesses. Food Control 106: 106716.
- Jan T, KC Y (2016) Study of HACCP Implementation in Milk Processing Plant at Khyber Agro Pvt. Ltd in Jammu & Kashmir. J Food Process Technol 7: 1000610.
- 9. Maldonado-siman E, Bai L, Ramírez-valverde R, Gong S, Lara RR (2014) Comparison of implementing HACCP systems of exporter Mexican and Chinese meat enterprises. Food Control 38: 109-115.
- 10. Murat Bao, Mariye Y, Tufba Ç (2007) Difficulties and barriers for the implementing of HACCP and food safety systems in food businesses in Turkey Food Control. Food Control 18(2): 124-130.
- 11. EU Commission (2016) Commission notice (2016/C 278/01) on the implementation of food safety management systems covering prerequisite programs (PRPs) and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food busi. Off J Eur Union C278: 1-32.
- 12. Ricci A, Chemaly M, Davies R, Fernández Escámez PS, Girones R, et al. (2017) Hazard analysis approaches for certain small retail establishments in view of the application of their food safety management systems. EFSA Journal 15(3): e04697.
- 13. Azanza MPV, Myrna Benita VZ (2005) Barriers of HACCP team members to guideline adherence. Food Control 16(1): 15-22.
- 14. Allata S, Valero A, Benhadja L (2017) Implementation of traceability and food safety systems (HACCP) under the ISO 22000 : 2005 standard in North Africa : The case study of an ice cream company in Algeria. Food Control 79: 239-253.
- 15. FAO/WHO (2004) Second FAO/WHO global forum of food safety regulators- Proceedings, Building effective food safety systems, Global Forum of Food Safety Regulators. FAO, Viale delle Terme di Caracalla, Rome.
- 16. Eves A, Dervisi P (2005) Experiences of the implementation and operation of hazard analysis critical control points in the food service sector. Int J Hosp Manag

24(1): 3-19.

- 17. Panisello JP, Quantick CP (2001) Technical barriers to Hazard Analysis Critical Control Point (HACCP). Food Control 12(3): 165-173.
- 18. Seydi M (2003) Problématique de la sécurité sanitaire des aliments dans les pays francophones au Sud du Sahara. Rev Africaine Santé Prod Anim 1: 86-94.
- 19. Vernozy-Rozand C, Servane R (2003) AFFSA, Bilan des conaissance relatives aux *E. coli* producteurs de shigatoxine (STEC).
- 20. ISO (1976) Meat and meat products-Aerobic count at 30 degrees, ISO 2293: International organisation for standardization. Geneva. Switzeland.
- 21. ISO (1999) Microbilogie-Directives générales pour le dénombrement de Staphylococcus aureus-Méthode de comptage des colonies, in: Iso 6888-1: International Organisation for Standardization. Geneva, Switzeland.
- ISO (2002) Microbiologie des aliments. Méthode horizontale pour la recherche des Salmonella spp, in: ISO 6579: International Organisation for Standardization. Geneva, Switzeland.
- ISO (2006) Microbilogie des denrees alimentaires et des aliments pour animaux- Guide pour l'estimation de l'incertitude des mesures pour les determinations quantitatives, in: ISO 19036 ISO/TC 34/SC 9: International Organisation for Standardization. Geneva, Switzeland.
- 24. AFSSA (2008) AVIS de l'Agence française de sécurité sanitaire des aliments relatif à la demande d'avis complémentaire concernant l'AVIS de l'Agence française de sécurité sanitaire des aliments concernant les références applicables aux denrées alimentaires en tant que c, Maisons-Alfort cedex.
- 25. Balzaretti CM, Marzano MA (2013) Prevention of travel-related foodborne diseases : Microbiological risk assessment of food handlers and ready-to-eat foods in northern Italy airport restaurants. Food Control 29(1): 202-207.
- 26. Martins RB, Hogg T, Otero JG (2012) Food handlers' knowledge on food hygiene: The case of a catering company in Portugal. Food Control 23(1): 184-190.
- 27. Santos-Fernández E, Govindaraju K, Jones G (2014) A new variables acceptance sampling plan for food safety. Food Control 44: 249-257.
- 28. Osimani A, Aquilanti L, Babini V, Tavoletti S, Clementi

F (2011) An eight-year report on the implementation of HACCP in a university canteen: Impact on the microbiological quality of meals. Int J Environ Health Res 21(2): 120-132.

- 29. Osimani A, Aquilanti L, Tavoletti S, Clementi F (2013) Evaluation of the HACCP system in a university canteen: Microbiological monitoring and internal auditing as verification tools. Int J Environ Res Public Health 10(4): 1572-1585.
- Marzano MA, Balzaretti CM (2011) Cook-serve method in mass catering establishments: Is it still appropriate to ensure a high level of microbiological quality and safety? Food Control 22: 1844-850.
- 31. Cenci-Goga BT, Ortenzi R, Bartocci E, Codega De Oliveira A, Clementi F, et al. (2005) Effect of the implementation of HACCP on the microbiological quality of meals at a università restaurant. Foodborne Pathogens and Disease 2(2): 138-145.
- Kafetzopoulos DP, Psomas EL, Kafetzopoulos PD (2013) Measuring the effectiveness of the HACCP Food Safety Management System. Food Control 33(2): 505-513.
- Rodriguez M, Valero A, Carrasco E, Pérez-Rodríguez F, Posada GD, et al. (2011) Hygienic conditions and microbiological status of chilled Ready-To-Eat products served in Southern Spanish hospitals. Food Control 22(6): 874-882.
- Aycicek H, Cakiroglu S, Stevenson TH (2005) Incidence of Staphylococcus aureus in ready-to-eat meals from military cafeterias in Ankara, Turkey. Food Control 16(6): 531-534.
- 35. Chellapandi K, Ralte L, Malsawmtluangi L, Masih LTKKS, Boro D (2015) Microbiology vegetables of Aizawl city. Malays J Microbiol 11: 40-46.
- 36. Shah MS, Eppinger M, Ahmed S, Shah AA, Hameed A, et al. (2015) Multidrug-resistant diarrheagenic *E. coli* pathotypes are associated with ready-to-eat salad and vegetables in Pakistan. J Korean Soc Appl Biol Chem 58: 267-273.
- 37. Castro-Rosas J, Cerna-Cortés JF, Méndez-Reyes E, Lopez-Hernandez D, Gómez-Aldapa CA, et al. (2012) Presence of faecal coliforms, Escherichia coli and diarrheagenic *E. coli* pathotypes in ready-to-eat salads, from an area where crops are irrigated with untreated sewage water. Int J Food Microbiol 156: 176-180.
- 38. Mritunjay SK, Kumar V (2017) A study on prevalence of microbial contamination on the surface of raw salad

vegetables. Biotech 7: 7-13.

- 39. Xylia P, Botsaris G, Chrysargyris A, Skandamis P, Tzortzakis N (2019) Variation of microbial load and biochemical activity of ready-to-eat salads in Cyprus as affected by vegetable type, season, and producer. Food Microbiol 83: 200-210.
- 40. FAO/WHO (2008) Microbiological hazards in fresh leafy vegetables and herbs. Microbiol. hazards fresh leafy Veg. herbs Meet. Report Microbiol Risk Assess Ser 14: 151.
- 41. Pagadala S, Marine SC, Micallef SA, Wang F, Pahl DM, et al. (2015) Assessment of region, farming system, irrigation source and sampling time as food safety risk factors for tomatoes. Int J Food Microbiol 196: 98-108.
- 42. Singh BR, Singh P, Verma A, Agrawal S, Babu N, et al. (2006) A study on prevalence of multi-drug-resistant (MDR) Salmonella in water sprinkled on fresh vegetables in Bareilly, Moradabad, and Kanpur (northern Indian cities). J Public Health (Bangkok) 14: 125-131.
- 43. Balkhair KS (2016) Microbial contamination of vegetable crop and soil profile in arid regions under controlled application of domestic wastewater. Saudi J Biol Sci 23(1): 83-92.
- 44. Yoon Y, Kim K, Nam M, Shim WB, Ryu JG, et al. (2010) Microbiological assessment in strawberry production and recommendations to establish a good agricultural practice system. Foodborne Pathog Dis 7: 1511-1519.
- 45. Hanning IB, Nutt JD, Ricke SC (2009) Salmonellosis Outbreaks in the United States Due to Fresh Produce : Sources and Potential Intervention Measures. Foodborne Pathog Dis 6(6): 635-648.
- 46. Beuchat LR (2006) Vectors and conditions for preharvest contamination of fruits and vegetables with pathogens capable of causing enteric diseases. Br Food J 108: 38-53.
- 47. Seow J, ágoston R, Phua L, Yuk HG (2012) Microbiological quality of fresh vegetables and fruits sold in Singapore. Food Control 25(1): 39-44.
- 48. Zwe YH, Yuk HG (2017) Food quality and safety in Singapore: microbiology aspects. Food Qual Saf 1: 101-105.
- 49. Christison CA, Lindsay D, Holy AV (2008) Microbiological survey of ready-to-eat foods and associated preparation surfaces in retail delicatessens, Johannesburg, South Africa. Food Control 19: 727-733.
- 50. Kotzekidou P (2013) Microbiological examination of ready-to-eat foods and ready-to-bake frozen pastries

from university canteens. Food Microbiol 34(2): 337-343.

- 51. Garayoa R, Díez-leturia M, Bes-rastrollo M, Garcíajalón I, Isabel A (2014) Catering services and HACCP : Temperature assessment and surface hygiene control before and after audits and a speci fi c training session. Food Control 43: 193-198.
- 52. Garayoa R, Isabel A, Díez-leturia M, García-jalón I (2012) Food safety and the contract catering companies : Food handlers , facilities and HACCP evaluation. Food Control 22(12): 2006-2012.
- 53. Sabbithi A, Naveen KR, Kashinath L, Bhaskar V, Sudershan RV (2014) Microbiological quality of salads served along with street foods of Hyderabad, India. Int J Microbiol 2014.
- 54. Todd ECD, Greig JD, Bartleson CA, Michaels BS (2007) Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 3. Factors contributing to outbreaks and description of outbreak categories. J Food Prot 70: 2199-2217.
- 55. Kapetanakou AE, Taoukis P, Skandamis PN (2019) LWT - Food Science and Technology Model development for microbial spoilage of packaged fresh-cut salad products using temperature and in-package CO 2 levels as predictor variables. LWT - Food Sci. Technol 113:

108285.

- 56. Mishra A, Guo M, Buchanan RL, Schaffner DW, Pradhan bani K (2017) Prediction of Escherichia coli 0157 : H7 , Salmonella , and Listeria monocytogenes Growth in Leafy Greens without Temperature Control J Food Prot 80(1): 68-73.
- 57. Mogren L, Windstam S, Boqvist S, Vågsholm I, Söderqvist K, et al. (2018) The hurdle approach-A holistic concept for controlling food safety risks associated with pathogenic bacterial contamination of leafy green vegetables. A review Front Microbiol 9: 1-20.
- 58. Zhang G, ChenY I, Hu L, Melka D, Wang HUA, et al. (2018) Survey of Foodborne Pathogens, Aerobic Plate Counts, Total Coliform Counts, and Escherichia coli Counts in Leafy Greens, Sprouts, and Melons Marketed in the United States. J Food Prot 81(3): 400-411.
- 59. Huang L (2016) Mathematical modeling and validation of growth of Salmonella Enteritidis and background microorganisms in potato salad-One-step kinetic analysis and model development. Food Control 68: 69-76.
- 60. Martínez-Tomé M, Vera AM, Murcia MA (2000) Improving the control of food production in catering establishments with particular reference to the safety of salads. Food Control 11(6): 437-445.