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Kinetics of Microorganisms in Ready-To-Eat Salads Stored at 4°C Sold in Supermarkets in the City of Abidjan (Côte d'Ivoire)

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A B S T R A C T

Consumption of ready-to-eat salads is increasingly observed in large African cities, in particular Abidjan (Côte d'Ivoire). Thus, monitoring the microbiological quality of these salads packaged in polyethylene terephthalate (PET) plastics is a necessity in order to guarantee their compliance and prevent occurrence of food poisoning. The objective of this work was to assess microbiological quality of fourth-range salads sold in supermarkets in the City of Abidjan during the storage period at 4°C after opening the packages. Our study focused on 4 types of salads, Spinach, Lamb's lettuce, Lamb's lettuce and Arugula and Young shoots (lettuce, arugula, spinach, red chard, bull's blood) bought in Abidjan's supermarkets. We proceeded to an enumeration of mesophilic and psychrophilic aerobic bacteria as well as yeasts and molds by an interval of 3 days. The results revealed the presence of these microorganisms as soon as the packages were opened. Most of ready-to-eat salads were of satisfactory quality from the opening of the packaging but not on the 2nd and 3rd day of analysis. However, composition of salads and shelf life favored the growth of microorganisms. Good practice guides for the purchase, storage and consumption of these foods must be put in place to prevent poisoning.

Keywords

Fourth range salads, Mesophilic and psychrophilic bacteria, Fungal flora

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Introduction

Fourth range of food products concerns products that are pre-cleaned, mainly packaged and ready to be eaten without preparation or prior cooking (Paudya *et al.*, 2017). Nowadays, the changing lifestyle of consumers and the great attention paid to healthy and nutritious foods have widened the demand for fourth range products (Cofelice *et al.*, 2019). Fourth range therefore plays an important role in the food industry because it meets the demand of consumers, who have little time to cook, but who want to follow a

healthy and balanced diet, without having to spend a lot of time in the kitchen. In addition, salads of fresh fruits and vegetables are the most preferred because the WHO, FAO and the World Cancer Research Fund recommend the consumption of 400 to 600g of fruits and vegetables per day (Pollard *et al.,* 2009; Adjrah *et al.,* 2011). However, the production and distribution of these products requires the use of a specific manufacturing process, all phases of which are governed by common health and safety rules (OESAAS, 2018). Despite the many advantages of fourth-range salads, several studies have shown their major involvement in food-borne epidemics (Zhang *et al.,* 2012; MacDonald *et al.,* 2016; Espenhain *et al.,* 2019). In Africa, the prevalence of foodborne pathogens in foods in some African countries is the high prevalence of major pathogens in ready-to-eat foods, almost as high as in raw foods (Paudyal *et al.,* 2017).

In Côte d'Ivoire, the trend to move towards a healthy lifestyle is pushing the population to take more and more interest in ready-to-eat salads (fourth range). We are therefore witnessing a flood of several types of salads of various origins in supermarkets in Abidjan. However, consumers sometimes lack knowledge about adequate refrigeration (Marklinder *et al.,* 2004). Anin *et al.,* (2016) assessed the microbiological contamination of certain artisanal fourth range products sold on local markets in Abidjan.

Also, Toe & Dadié (2018) determined the prevalence and potential virulence of *Escherichia coli* in ready-to-eat raw vegetable salads in collective catering in Abidjan. However, scientific data on kinetics of microflora in fourth range salads are not yet available. That's why we determine devolution of the microflora growth according to conditions of domestic storage over time after packaging opening.

Materials and Methods

This study took place over a three-month period from July to September 2020. Collection of salads packaged in polyethylene terephthalate (PET) plastics was purchased in supermarkets of Abidjan (Côte d'Ivoire). Indeed, the salads selected are of 4 types (2 from one type of salad, one with 2 types of salads and last one with 5 salads) and have foreign origins (Europe). After collection, samples were stored at -80 ° C. Microbial evolution was determined by time interval (Day 0; Day 3; Day 7).

Enumeration of microorganisms focused on mesophilic aerobic flora, psychrotrophic aerobic flora and fungal flora. Techniques used for enumeration refer to AFNOR standards and ISO standards. First, decimal dilutions were carried out in accordance with the AFNOR NF V 08 010 standard of March 1996. A quantity of 25 g of products was diluted in a volume of 225 mL of buffered peptone water, From the suspension obtained (stock suspension), a series of dilutions was carried out in the order of 10^{-1} to 10^{-6}. Enumeration of mesophilic aerobic flora was carried out on PCA Agar according to UNI EN ISO 4833-1: 2013 standard, incubated at 30 °C for 72 h. Psychrophilic aerobic flora was also counted on the PCA agar according to ISO 17410 (2001) incubated at 6.5 °C for 72 h. As for fungal flora, it was counted on Sabouraud-Chloramphenicol Agar according to ISO/IEC 17025 at 25 °C for 72.

Presumptive colonies are counted and then average concentration or bacterial load was reported according to ISO 7218 (2007). The data processing was carried out using two software programs, Excel 2016 and Statistica 7.1. Fisher's LSD Test was performed to distinguish differences between averages.

Results and Discussion

Microorganisms growth evolution during storage periods

Spinach

The average loads of microorganisms in spinach sample during storage period are listed in Table 1. On opening the packaging of these samples, the revivable microorganisms were counted. The results show that microbial loads varied between 6.80 10^2 CFU/g and 2.97 10^5 CFU/g. During storage period (4°C), mesophilic and psychrophilic aerobic flora developed slowly until the 3rd day (D3).However, after the 3rd day to the 7th day of storage, significant growth was observed in psychrophilic aerobicbacteria while mesophilic remained slow. This salad was also marked by a low load of yeast and mold when opening the package. But this flora underwent significant growth during storage at 4 °C.

Lamb's lettuce

Lamb's lettuce when opening the packaging had a very low average load of mesophilic aerobic flora (7.80 10^2 CFU/g) as did psychrophilic aerobic flora (5.90 10^2 CFU/g).

In this sample the number of mesophilic bacteria which was low when the packaging was opened reproduced slowly on the 3rd day and very rapidly on the 7th day of storage. Psychrophilic bacteria, also with a low initial load, experienced rapid growth from the 3rd day until the 7th day of storage. However, the count did not reveal the presence of mold and yeast in this type of salad (Table 2).

Lamb's lettuce and Arugula

Microbiological analyzes revealed low average microbial loads varying between 1.20 10^3 CFU/g and 1.8210^4 CFU/g. However, fungi flora was not detected. During storage at 4 °C, mesophilic aerobic flora reproduced significantly from day 3 to day 7.Psychrophilic flora also developed significantly throughout storage period (Table 3).

Mixture of young shoots

The enumeration of this salad gave very high bacterial loads, 1.73 10^8 CFU/g for the mesophilic flora and 2.81 10^7 CFU/g for the psychrophilic flora. Yeast and mold had a load of 4.20 10^2CFU/g.

During the storage period, psychrophilic bacteria developed significantly unlike the mesophilic flora and fungal flora which developed slowly (Table 4).

Comparison of the evolution of microbial growth in different salads

Mesophilic aerobic flora

With regard to the aerobic mesophilic flora, the salad consisting of lamb's lettuce and arugula showed a statistical difference in the average loads during storage time.

It follows that lamb's lettuce salad showed significant growth on the 7th day. Young shoots (although having high values) and spinach salads did not obtain significant growth over time (Figure 1).

Psychrophilic aerobic flora

Psychrophilic bacteria showed significant growth in all of our samples. The increase in average loads was a function of the number of components in the package. This is how the salad made from young people growing with 5 elements (lettuce, arugula, spinach, red chard, bull's blood) had the highest values (figure 2).

Table.1 Microbial evolution in spinach during storage time. MAF: Mesophilic Aerobic Flora, PAF: Psychrophilic Aerobic Flora, FF: Fungal Flora

Days Flora	D0	D3	D7
MAF (CFU/g)	$2.97\ 10^{5a}$	$3.2910^{6\ a}$	5.9310^{7a}
PAF (CFU/g)	$2.80\ 10^{5a}$	$3.3710^{6\ a}$	4.6410^{7b}
FF (CFU/g)	$6.80\ 10^{2a}$	1.5010^{3b}	2.7210^{3c}

Values followed by the same letter on the same lines are not significantly different at the 5% level according to Fisher's LSD test.

Table.2 Microbial evolution in Lamb's lettuce during storage time. MAF: Mesophilic Aerobic Flora, PAF: Psychrophilic Aerobic Flora, FF: Fungal Flora

Days Flora	D0	D3	D7
MAF (UFC/g)	$7.80\ 10^{2a}$	2.5510^{6a}	$3.16\times10^{7\ b}$
PAF (UFC/g)	$5.90\ 10^{2a}$	$3.04\times10^{6\ b}$	1.26×10^{7c}
FF (UFC/g)	< 1	< 1	< 1

Values followed by the same letter on the same lines are not significantly different at the 5% level according to Fisher's LSD test.

Table.3 Microbial evolution in spinach during storage time. MAF: Mesophilic Aerobic Flora, PAF: Psychrophilic Aerobic Flora, FF: Fungal Flora.

Days Flora	D0	D3	D7
MAF (UFC/g)	$1.20\ 10^{3\ a}$	$2.9510^{6\ b}$	1.6810^{7c}
PAF (UFC/g)	$1.8210^{4\ a}$	$3.2510^{7\ b}$	1.6010^{8c}
FF (UFC/g)	< 1	< 1	< 1

Values followed by the same letter on the same lines are not significantly different at the 5% level according to Fisher's LSD test

Table.4 Microbial evolution in young shoots during storage time. MAF: Mesophilic Aerobic Flora, PAF: Psychrophilic Aerobic Flora, FF: Fungal Flora

Days Flora	D0	D3	D7
MAF (UFC/g)	1.7310^{8a}	2.7710^{8a}	$2.9610^{8\ a}$
PAF (UFC/g)	$2.8110^{7\ a}$	1.2410^{8b}	2.6410^{8c}
FF (UFC/g)	$4.2010^{2\ a}$	$7.5010^{2\ a}$	1.02010^{3a}

Values followed by the same letter on the same lines are not significantly different at the 5% level according to Fisher's LSD test

Fig.1 Evolution of the mesophilic aerobic flora as a function of time in the different types of sample.* $p \leq 0.05$

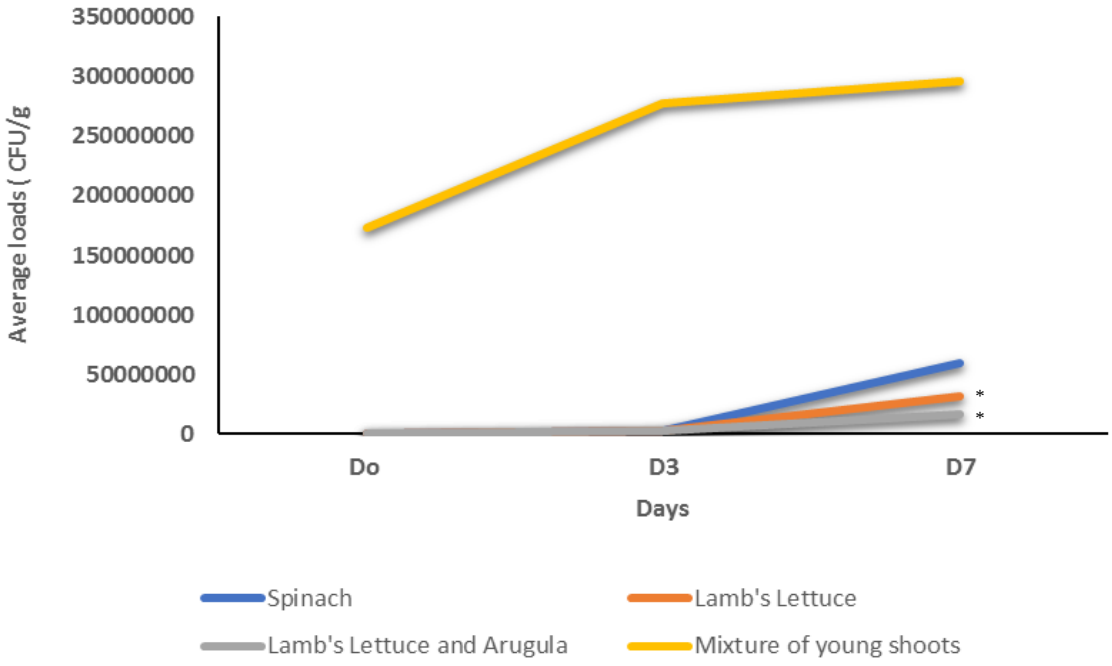


Fig.2 Evolution of the psychrophilic aerobic flora as a function of time of the samples from batch 1. * $p \leq 0.05$

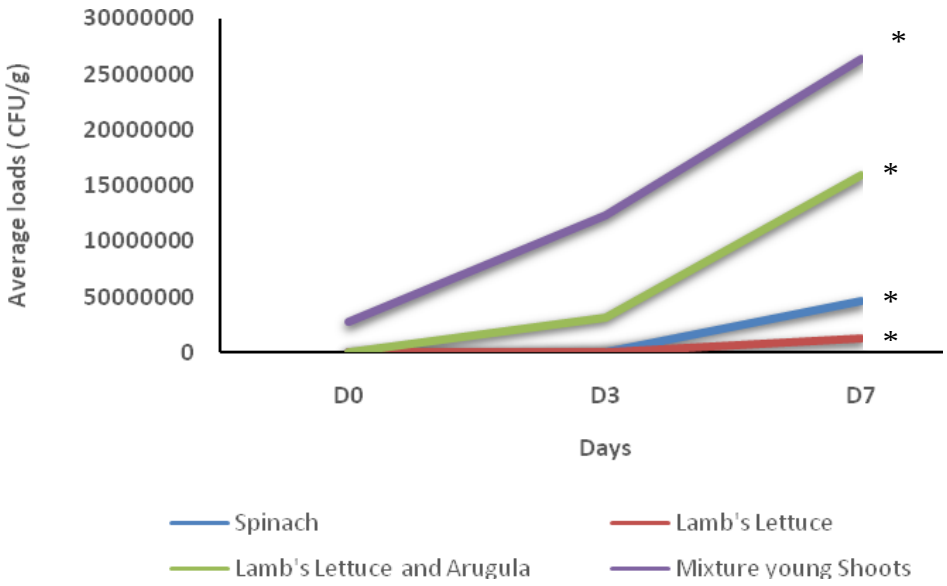
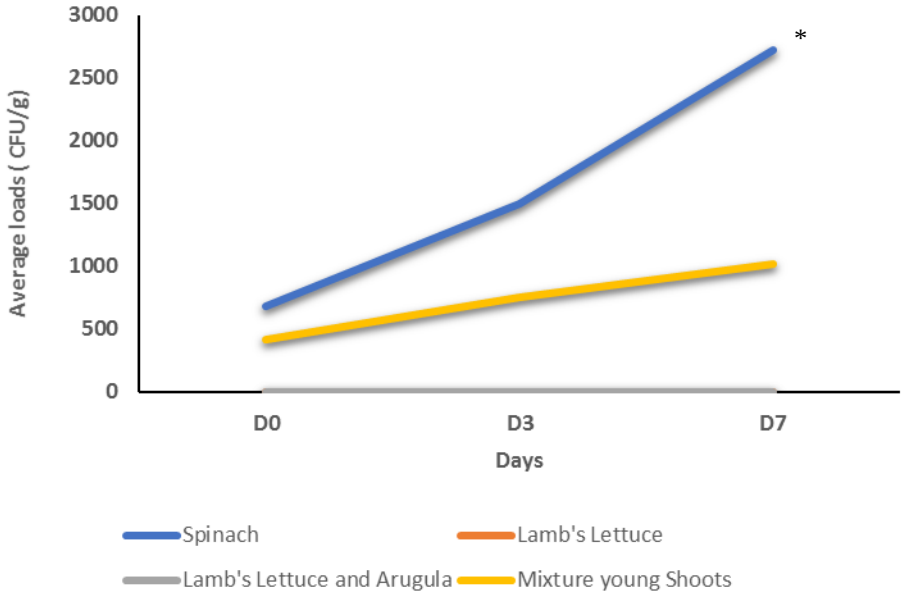


Fig.3 Evolution of the fungal flora as a function of the time of the samples. * $p \leq 0.05$



Fungal Flora (FF)

Only spinach had significant growth with yeasts and molds (figure 3). In general, the values with yeasts and molds were of the same order. Lamb's lettuce and Arugula had low values.

In this work, we analyzed the microbiological quality of ready-to-eat salads sold in supermarkets in Abidjan and the evolution of microbial growth monitored after opening the packages. Thus, the count of spinach salad showed that this salad was of satisfactory microbiological quality when packaging was opened due to the microbial load which varied between 6.80 10^2 CFU/g and 2.97 10^5 CFU/g. Indeed, according to microbiological criteria in accordance with regulation 2073/2005/EC, average acceptable loads in total aerobic flora and fungal flora for fresh little processed foods do not exceed 10^7 CFU/g and 10^4 CFU/g respectively (CMADA, 2018). Our results are different to Mritunjay & Kumar(2017) who showed the highest microbial counts in samples of spinach. According to them, this may be linked to poor handling practice during storage and at the point of selling. Valentin-Bon *et al.*, (2008) also demonstrated a strong microbial contamination of this food. The difference observed when opening the packaging of our spinach sample would probably be due to a more controlled situation in the production and distribution chain such as type of washing recommended, stability of the storage temperature, good packaging and shelf life of the product. Indeed, the commercial atmosphere modified by 0.25-3% O_2 and 3-12% CO_2 with a balance in N_2 (O'Beirne *et al.*, 2015), storage at a maximum temperature of 4 ° C and a shelf life between 7 to 10 days (De Giusti *et al.*, 2010) would be necessary to control spoilage of minimally processed foods. Soendjojo (2012), for its part, showed a low average load of yeast and mold in spinach from grocery stores, which confirms the low average loads of fungal flora obtained in our sample. During the storage period at 4 °C, the growth of the mesophilic aerobicflora was slow while this of aerobic psychrophilic flora was very significant. Garg *et al.*, (1990)

showed in their work that the storage of spinach salads at refrigeration temperature promotes the growth of psychrophilic microorganisms. However, mesophilic bacteria grew slowly at a low temperature which is in line with our results.

For lamb's lettuce, microbiological analyzes showed that it was also of satisfactory quality because of the absence of fungal flora and its very low average load in mesophilic aerobic flora (7.80 10^2 CFU/g) and aerobic psychrophilic flora (5.90 10^2 CFU/g) which comply with microbiological criteria. However, from the 3rd day of storage, an exponential growth was noticed in psychrophilic aerobic flora. Mesophilic aerobic flora multiplied slowly. This result is consistent with the work of Garg *et al.,* (1990).

Regarding the salad composed of lamb's lettuce and arugula, it was also of satisfactory quality when opening the packaging but from the 3rd day of storage it proved to be unsatisfactory due to a significant multiplication of the psychrophilic flora. This result is similar to those of Schuh *et al.,* (2020) on the evaluation of microbiological quality of minimally processed vegetables. The strong growth of the different bacterial flora conferred an unsatisfactory quality on these two salad samples from the 3rd day of storage. Our results are substantially similar to those of Arienzo *et al.,* (2020), who showed that after 2 days of storage at 4 °C, these same types of products analyzed presented an unsatisfactory microbiological quality.

Foryoung shoots, studies have shown high concentrations of mesophilic aerobic flora (1.7310^8 CFU/g) and psychrophilic aerobic flora (2.8110^7 CFU/g) when opening the packaging. Abadias *et al.,* (2008) produced similar results in salads from young shoots during their work on the microbiological quality of fresh and minimally processed fruits and vegetables, and sprouts from retail establishments.

This strong contamination could be linked to growing conditions and also to processing operations, such as delays in post-harvest refrigeration or processing after receipt of vegetables in the factory (Caponigro *et al.,* 2010). Which is in agreement with the work of Akoachere *et al.,* (2018) who showed that hygiene and poor storage practices for vegetables can aggravate bacterial contamination. Caleb *et al.,* (2012) also showed that the packaging of fresh, minimally processed products (4th range) must have a specific temperature. However, excessive storage temperature would lead to bacterial growth. Delibato *et al.,* (2018) also showed in their study on the persistence of microbial contamination of products that complete decontamination was not achieved in ready-to-eat fresh plant products despite the recommended types of washing. Low temperature storage during our analyzes could also inhibit the growth of the mesophilic aerobicflora as described by Garg *et al.,* (1990). In general, psychrophilic aerobic flora developed more rapidly in the mixed salad composed of young shoots than in the other samples. This result would probably be due to the composition of the salad which could act on the pH. Indeed, an acidic food promotes a significant growth of psychrophilic lactic acid bacteria. Garcia-Gimeno & Zurera-Cosano (1997), showed that the spoilage of mixed salads stored at a temperature of 4 °C was linked to lactic acid bacteria.

The results of the microbiological study showed the presence of mesophilic and psychrophilic aerobic bacteria as well as fungal flora in the salads. When packaging was opened the greatest loads of Mesophilic (1.73 10^8 CFU/g) and psychrophilic germs (2.81 10^7 CFU/g) were found in young shoots

and fungal flora (6.80 10^2 CFU/g) in spinach leaves. Despite the adequate conservation conditions recommended after opening the packaging, the counts of microorganisms also showed that after opening the packaging, the imported 4th range salads, sold in supermarkets in Abidjan, are of poor microbiological qualities from the 3rd day of storage period. Thus, strict hygiene and safety measures must be taken in the production chain, in packaging and transport in order to considerably reduce the contamination of 4th range products which are not only an important source of nutrients but also contribute to the disease reduction.

References

Abadias, M., J. Usall, M. Oliveira, I. Alegre and Viñas, I. 2008. Efficacy of Neutral Electrolyzed Water (NEW) for Reducing Microbial Contamination on Minimally-Processed Vegetables. International Journal of Food Microbiology, 123 (1‑2): 151‑58.

Adjrah Y., D.S. Karou, B. Djéri, K Anani, Soncy, Y. Ameyapoh, C. de Souza and Gheassor M. 2011. Hygienicquality of commonly consumed vegetables, and perception about disinfecting agents in Lomé. International Food Research Journal, 18(4): 1499-1503.

Akoachere, JF.T.K., B. F Tatsinkou and Nkengfack, J. M. 2018. Bacterial and Parasitic Contaminants of Salad Vegetables Sold in Markets in Fako Division, Cameroon and Evaluation of Hygiene and Handling Practices of Vendors. BMC Research, 11(1): 100.

Anin L. A., 2016. Evaluation Microbiologique Et Origines De La Contamination Des Produits De 4ème Gamme Vendus Sur Les Marchés D'abidjan, Cote D'Ivoire. European Scientific Journal, 12(36): 1857 – 7881.

Arienzo, A., L. Murgia, I. Fraudentali, V. Gallo, R.Angelini andAntonini.G. 2020. Microbiological Quality of Ready-to-Eat Leafy Green Salads during Shelf-Life and Home-Refrigeration. Foods 9(10): 1421.

Caponigro, V., M. Ventura, I.Chiancone, L. Amato, E. Parente and Piro, F. 2010.Variation of Microbial Load and Visual Quality of Ready-to-Eat Salads by Vegetable Type, Season, Processor and Retailer. Food Microbiology 27 (8): 1071-77.

Caleb, O J., U. L. Opara and Witthuhn,C. R. 2012. Modified Atmosphere Packaging of Pomegranate Fruit and Arils: A Review. Food and Bioprocess Technology 5 (1): 15‑30.

Cofelice, M., L. Francesco and CuomoF. 2019. Quality Control of Fresh-Cut Apples after Coating Application. Foods8 (6).

Critères Microbiologiques Applicables aux Denrées Alimentaires (CMADA). Lignes directrices pour l'interprétation. Edition Août 2018

De Giusti M., F.Y. Castro, L. Marinelli, D. Tufi, D. De Medici, S. Di Pasquate, C. De Vito and Boccia A. 2010. The evaluation of the microbial safety of fresh ready-to-eat vegetables produced by different technologies in Italy. Journal of applied microbiology 109(2): 996-1006

Delibato, E., I. Luzzi, E. Pucci, Y. T. R. Proroga, F.Capuano and De Medici. D. 2018. Fresh Produce and Microbial Contamination: Persistence during the Shelf Life and Efficacy of Domestic Washing Methods. AnnaliDell'Istituto Superiore Di Sanita 54(4): 358‑63.

Espenhain, L., M. Riess, L. Müller, S. Colombe, S. Ethelberg, E. Litrup, C. Jernberg, E. Litrup, C. Jernberg, S. Kühlmann-Berenzon, M. Lindblad, N. K. Hove, M. Torpdahl and Mörk. M. J. 2019. Cross-border outbreak of Yersinia enterocolitica O3 associated with imported fresh spinach, Sweden and Denmark, March 2019. Eurosurveillance, 24 (24): 1900368

Garcia-Gimeno, R. M., and Zurera-Cosano, G. 1997. Determination of ready-to-eat vegetable salad shelf-life. International Journal of Food Microbiology 36 (1997) 31-38

Garg, N., J. J. Churey and Splittstoesser, D. F. 1990. Effect of Processing Conditions on the Microflora of Fresh-Cut Vegetables. Journal of Food Protection 53 (8): 701‑3.

MacDonald, E., M. Einöder-Moreno, K. Borgen, L. Thorstensen Brandal, L. Diab, Ø. Fossli, B Guzman Herrador, A. A. Hassan, G. S. Johannessen, E. J. Johansen, R. JørgensenKimo, T. Lier, B. L. Paulsen, R. Popescu, C. TokleSchytte, K. SæbøPettersen, L. Vold, Ø. Ørmen, A. L. Wester, M. Wiklund and Nygård, K. 2016. National Out break of Yersinia Enterocolitica Infections in Military and Civilian Populations Associated with Consumption of Mixed Salad, Norway, 2014. Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin, 21 (34):30321.

Marklinder I. M., M.Lindblad, L. M.Eriksson,A. M. Finnson And Lindqvist, R. 2004. Home storage temperatures and consumer handling of refrigerated foods in sweden. Journal of Food Protection, 67(11): 2570–2577

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

O'Beirne, D., V. Gomez-Lopez, A. Juan, Tudela, A. Allende and Gil, M. I. 2015. Effects of Oxygen-Depleted Atmospheres on Survival and Growth of Listeria Monocytogenes on Fresh-Cut Iceberg Lettuce Stored at Mild Abuse Commercial Temperatures. Food Microbiology, 48 (June): 17‑21.

Paudyal N., V. Anihouvi, J. Hounhouigan, M. I.Matsheka, B. Sekwati-Monang, W. Amoa-Awua, A. Atter, N. B.Ackah, S. Mbugua, A. Asagbra, W.Abdelgadir, J. Nakavuma, M.Jakobsen and Fang, W.2017. Prevalence of Foodborne Pathogens in Food from Selected African Countries - A Meta-Analysis. International Journal of Food Microbiology, 249 (mai): 35‑43.

PollardC., M. Miller, R. J. Woodman, R. Meng and Binns, C. 2009. Changes in knowledge, beliefs, and behaviors related to fruit and vegetable consumption among western Australian adults from 1995 to 2004. American Journals of public health, 99(2): 355-361.

Schuh, V., J. Schuh, N. Fronza, F. B. Foralosso, S. Verruck, A. Vargas Junior and Silveira S. M.D. 2020. Evaluation of the Microbiological Quality of Minimally Processed Vegetables. Food Science and Technology, 40 (2): 290‑95.

Soendjojo, E., 2012. Is Local Produce Safer? Microbiological Quality of FreshLettuce and Spinach from Grocery Stores and Farmers' Markets. The Journal of Purdue Undergraduate Research, 2 (1): 54–63

Toe E., A. Dadié, E. Dako, G. Loukou, M. D. Koffi and Blé, Y.C. (2018). Prevalence and potential virulence of Escherichia coli in ready-to-eat raw mixed vegetable salads in collective catering in Abidjan, Côte d'Ivoire. British Food Journal, 20(1).

Valentin-Bon,I., A. Jacobson, S. R. Monday and Feng,P. C. H. 2008.Microbiological Quality of Bagged Cut Spinach and Lettuce Mixes. Applied and Environmental Microbiology, 74 (4): 1240‑42.

Zhang, Q., G. Q. Shi, G. P. Tang, Z. T. Zou, G. H. Yao and Zeng, G. 2012. A foodborne outbreak of *Aeromonas hydrophila* in a college, Xingyi City, Guizhou, China, 2012. Western Pacific surveillance and response journal, 3 (October): 39‑43.

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