Microbiological and parasitological quality of different fresh-cut salads

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MICROBIOLOGICAL AND PARASITOLOGICAL QUALITY OF DIFFERENT FRESH-CUT SALADS

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ABSTRACT

Fresh-cut salads are "ready-to eat" food products intended for immediate consumption after minimal processing including washing, (cutting) and packaging. Although raw salads washing procedure reduces microbial load up to 90%, working surfaces contamination spreads through chopped salad while injured cell juices provide a favourable substrate for microbial growth. If present, higher loads or pathogenic microorganisms and parasites pose a serious health threat for consumers since these products are eaten fresh, without thermal treatment. The aim of this work was to examine the microbiological and parasitical quality of ready-to-eat salads from retail locations. In total 80 samples were collected from retail locations during February, May, June and July 2020. The presence of microorganisms in analysed samples was determined by standard microbiological methods while the presence of parasites was tested using sedimentation, differential staining and microscopy. Yeasts, moulds and *Enterobacteriaceae* as well as *Staphylococcus aureus* were detected in higher counts in expired salads, making them unsafe for consumption. *Salmonella*, coliforms, sulphite-reducing bacteria and parasites or their life forms (cysts/oocysts) were not detected. The presence of fungi *Alternaria* spp., possible mycotoxin producers, was higher in whole leaf salads, compared to chopped salads. Fresh-cut salads are perishable minimally processed products and it's microbiological and parasitical contamination should be closely monitored.

Keywords: fresh-cut salads, minimally processed, microorganisms, parasites, food safety

INTRODUCTION

Ready-to-eat salads are minimally processed products since their production includes mainly washing, cutting, peeling, etc. At the end of the production process they are packed in packaging that provides them longer storage time on the refrigerated shelves of shopping centres. Refrigeration ensures appropriate nutritional composition and the freshness of the product itself is maintained (Caradonna et al, 2017). Due quick and easy preparation and high nutritional value, demand, and production of ready-to-eat salads, has increased in recent years (Garreth, 2002).

Since these products are consumed raw, foodborne illnesses in consumers are more frequent. Salads can be contaminated by parasites and bacterial pathogens during primary production, processing, storage or transport. Additionally, pathogens can be found in irrigation water or manure as well as contamination occurs during transportation of raw materials or inappropriate manual harvesting (Garreth, 2002). The most common bacterial pathogens that can be found in ready-to-eat salads are enterobacteria (*Salmonella*), *Clostridia*, *Staphylococcus aureus*, and sometimes yeasts and moulds.

The aim of the experimental part of this paper was to determine the microbiological contamination and the parasite`s presence of in samples of ready-made salads ("fresh-cut" salads) from retail chains in the city of Osijek from February to July 2020. Furthermore, the diversity of the microbial population and the presence of pathogenic organisms was also determined in analysed samples.

MATERIALS AND METHODS

Random samples of ready-to-eat salads were collected from retail locations from February to July of 2020. A total of 80 samples were analysed. 60 g of the sample was washed in 300 mL of 0.85% NaCl solution and homogenized. Microbiological methods have been used to determine: bacteria of the *Salmonella* genus, *Staphylococcus aureus*, enterobacteria, sulphite-reducing clostridia, and yeasts and moulds. Microscopic examination of parasites was performed in the sediment by staining with 0.5% lugol solution (El Said Said, 2012) and the Kinyoun carbol fuchsin method. The presence of cysts or eggs was checked at magnifications of 200-10000×.

All samples were analysed for Salmonella spp., *Enterobacteriaceae*, Coagulase-positive staphylococci, sulphite-reducing *Clostridia*, yeasts and moulds, according to criteria from Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs and Croatian National Guidelines on microbiological criteria for foodstuffs.

Detection of Salmonella spp.

Detection and enumeration were performed according to the International Standard method ISO 6579 for detection of *Salmonella* spp. After homogenization in stomacher for 2 minutes, 25 g of sample was analyzed in: Buffered Peptone Water, Rappaport-Vassiliadis Soy (RVS) Broth medium, and XLD agar (Liofilchem, Italy).

Enumeration of Enterobacteriaceae, Coagulase-positive staphylococci, Sulphite-reducing Clostridia and Yeasts/Moulds

Tested microorganisms were analysed by the ISO 21528-2 for enumeration of Enterobacteriaceae, ISO 6888-1 for enumeration of Coagulase-positive staphylococci, ISO 15213 for enumeration of sulphite-reducing *Clostridia* and ISO 21527-1 for enumeration of yeasts and moulds. After homogenization in stomacher for 2 minutes, a 10 g of sample 10-fold serial dilutions were prepared and processed for enumeration of specified microorganisms using VRBG agar, Baird-Parker Agar, TSC agar and DRBC agar (all Liofilchem), respectively. Temperature and incubation period as well as identifications were performed according to specific ISO standard method.

Data analysis

Parallels of microbial counts were analysed in log scale ($\log_{10}$ CFU g^{-1}), and Box plots were used for visualisation of results (MS Excel 2019, Microsoft Corporation, 2019).

RESULTS AND DISCUSSION

Fresh produce, like ready-to-eat salads have been associated with 4.2% of total foodborne outbreaks in the European Union (EFSA, 2017) and 14.8% of illness outbreaks that accounted for 22.8% of all foodborne illnesses in the US (Amin et al, 2012). This experimental work was conducted to evaluate microbiological quality and the presence of parasites in ready-to-eat salads obtained from retail centres in Osijek, Croatia. Microscopical examination of ready-to-use salads (Table 1 and 2) showed numerous artefacts (pollen, insect parts…) in soft leafed uncut salads (rocket, lamb`s lettuce and spinach) representing 96.52% of total samples analysed. Lamb`s lettuce was responsible detection of 94.12% fungal spores of *Alternaria* spp. Mentioned lettuce types have soft and fragile leaves so this could be the reason of increased *Alternaria* sp. growth. Every manipulation during processing causes the leaf tissue damage suggesting that washing procedures were not performed as they should. In cut salads (Table 2) although some artefacts and *Alternaria* spp. spores were detected, due to improved washing or the less fragile structure of plant parts (stronger leaves of cabbage or roots like carrot and beet) artefacts and spores were distributed somehow evenly. Still, inclusion of softer and more fragile lettuce in mixtures (rocket, spinach and lamb`s lettuce) increased artefact counts.

Table 1. Artefacts and spores of *Alternaria* sp. in uncut salads

Uncut salads			
Salad type	N	N (*Alternaria* sp.)	N artefacts
Rocket	15	1	24
Lamb's lettuce	14	16	48
Baby spinach	1	0	3
Spinach	2	0	39
Lamb's lettuce + radicchio	1	0	0
Misancija salad (spinach + radicchio)	1	0	1
Total	34	17	115
Artefacts (%)		29.57	
Alternaria sp. (%)		50	

Table 2. Artefacts and spores of *Alternaria* sp. in cut salads

Cut salads			
Salad type	N	N (*Alternaria* sp.)	N artefacts
Diet salad (carrot, red and white cabbage)	4	0	7
Red cabbage	4	0	12
Carrot and cabbage	1	0	2
Celery root	2	0	1
Carrot and apple	2	0	1
Iceberg, radicchio, carrot, spinach	2	0	0
Mix (endive, radicchio lamb`s lettuce, rocket spinach)	5	2	11
Romana (iceberg, endive, radicchio, carrot)	1	0	0
Rica salad (radicchio, lamb`s lettuce)	4	0	7
Carrot	1	0	3
Baby mix (spinach, swiss chard, kale)	1	0	7
Lamb`s lettuce, radish	1	0	2
Green and red lettuce	5	1	13
Cabbage (white)	2	0	8
Endive and radicchio	4	0	4
Iceberg mix (radicchio, carrot, endive, spinach)	3	0	12
Rocket, lamb`s lettuce, red and green lettuce, spinach, beet	4	0	13
Total	46	3	103
Artefacts (%)		44.66	
Alternaria sp. (%)		6.52	

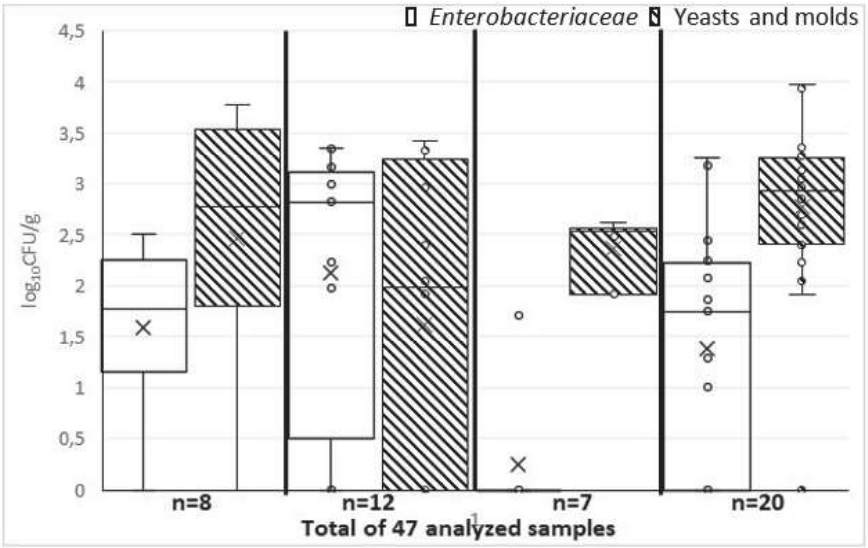

Figure 1. Contamination of ready-to-eat salads before expiration date

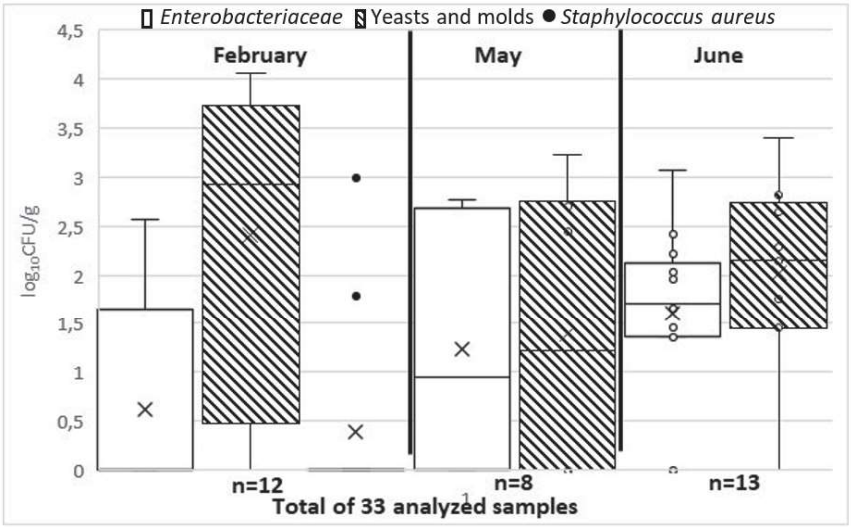

Figure 2. Contamination of ready-to-eat salads after expiration date

In total of 80 analysed samples, 43 was obtained from retail centres before expiration date (Figure 1), while 33 samples were on shelves of the same centres after expiration date (except in July). Conversely to expectation, counts of most tested microbes were lower in samples after expiration date. Ready-to-eat salads, specially cut, have very large surface area and probably samples with expired usage date were washed more thoroughly reducing thus the level of contamination. Additionally, washing water should be closely supervised for effective chlorine

concentration since it is unstable and reduces it`s microbicidal activity in the presence of organic matter. In February the number of yeasts and moulds was higher which could be connected with their ability to grow at lower temperature. On the other hand, warmer months had beneficial effect on *Enterobacteriaceae* multiplication. *Staphylococcus aureus* was detected only in one ready-to-eat salad from February, which passed expiration date. In total, 28 of 80 (35%) of analysed ready-to-eat salads did not meet the microbiological criteria (labelled as unsatisfactory samples), due to high levels of yeasts/moulds and *Enterobacteriaceae*.

Similar results were reported in several papers regarding microbiological quality of ready-to-eat salads (Calonico et al, 2019; Koushki et al, 2021; Ljevaković-Musladin et al, 2019) where *Salmonella* or *Listeria monocytogenes* were rarely detected although higher than legislation of coliforms, yeasts and moulds was the main reason of unsatisfactory microbiological quality of tested samples.

Salmonella and sulphite-reducing *Clostridia* or parasites or parasitic elements (oocysts) were not detected in any of analysed samples.

Ready-to-eat salads are raw and minimally processed products and washing is crucial step in production of safe products. Nevertheless, every washing procedure affects the quality of the vegetables, making them susceptible to contamination. Although chlorine products are usually used for washing, active chlorine is unstable and can be ineffective while residual of washing water offers more opportunity for microbial growth (Gill et all, 2009).

CONCLUSION

Our study showed that ready-to-eat salads can harbour spoilage microorganisms as well as unwanted and potentially hazardous ones. Pathogens like *Salmonella* spp. and *Staphylococcus aureus* as well as sulphite-reducing *Clostridia* were not isolated nor were parasite elements found, but there is high contamination of spoilage indicators, especially in cut salads and salads passed expiration date. Higher counts of spoilage indicators (yeasts and moulds) as well as *Enterobacteriaceae* suggests improper washing procedure and storage. Specially in ready-to-eat food products washing and hygiene are crucial in providing safe product for consumers. Additional research is needed as well as control since expired ready-to-eat products pose considerable threat for consumer`s health.

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