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Microbiological Studies on Some Salad Vegetables in Local Markets

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Abstract. One hundred and forty-eight samples of five salad vegetables, namely: parsley, lettuce, green onion, carrots and cucumber, were collected from local markets and analyzed for total and faecal coliforms. In addition, three hundred and forty samples of the same kinds of vegetables were tested for prevalence of salmonellae and *Listeria monocytogenes*. Total coliform counts ranged from 1.4×10^4 CFU/g for cucumber to as high as 3.9×10^6 CFU/g for parsley. The mean faecal coliform counts ranged from 5.3×10^2 CFU/g to as high as 5.4×10^4 CFU/g for cucumber and parsley respectively. Salmonellae were detected in 6, 5, 4.8 and 4.5% of lettuce, cucumber, green onion and parsley samples, respectively. Presumptive *Listeria monocytogenes* was detected in parsley (44%), lettuce (12%) and green onion (8%). It was concluded that consuming these salad vegetables raw, and particularly the green leafy vegetables, without proper cleaning and sanitizing, presents a potential public health threat.

Introduction

Enteric pathogens and consequently their indicators the coliforms, in general have received considerable attention as contaminants of horticultural products [1–5]. Special attention has been paid to those vegetables that are usually eaten raw, since they can act as vehicles for transmitting pathogenic enteric diseases [6–8]. Different authors have proven that vegetables are contaminated with microorganisms when they are irrigated with sewage water and when the soil is fertilized with manure [9–11]. While salmonellae have been associated with animal products including poultry, meat, eggs and manure [12], their significance as vegetable borne bacteria is well documented [6; 13; 1]. *Listeria monocytogenes*, an important food born pathogen and widely distributed in nature, has been isolated from soil, water, sewage, silage and animals [14]. Thus it may contaminate fresh vegetables at their source or subsequently during handling and marketing [13; 15–17].

In Saudi Arabia, green salad is becoming one of the most popular food items. A recent nation-wide survey on the dietary habits of the Saudi population [18] revealed that eating green salad is a daily habit of about 50% of the individuals surveyed throughout the country. There is a growing concern among the public toward the safety of salad vegetables, due to the use of treated waste water for irrigation in some areas. Hence, the present study was undertaken to determine the bacteriological quality of 5 vegetables widely grown and commonly consumed raw as green salad components throughout the Kingdom.

Materials and Methods

Samples of five salad vegetables namely: carrots, cucumber, lettuce, green onion and parsley were colleted from local vegetable markets. Bulk samples were purchased in their original packages, put into plastic bags and transported to the laboratory. Samples were analyzed, as purchased at the same day or stored overnight at 4°C before testing. Representative samples were prepared by aseptically cutting and mixing 400 g of vegetables from each bulk sample. Duplicate subsamples were drawn and added to sterile peptone water or enrichment broth to make 10^{-1} dilution in a sterile 400 ml capacity stomacker bags. All samples were blended in a Stomacker 400 (Techmar Co., Cincinnati, OH) for 2 min. Further dilutions were prepared as needed.

Total coliforms (TC) were enumerated on violet red bile agar (VRBA; Oxoid) according to the procedure of APHA [19]. Faecal coliforms (FC) were directly surface plated onto eosin methylene blue (EMB; Oxoid), incubated at 44.5°C for 24 hr and differentially counted.

For salmonellae detection, they were pre-enriched into lactose broth (Oxoid), enriched in selenite cystine broth (Oxoid), isolated on bismuth sulfite agar (Oxoid) and confirmed according to AOAC methods [20].

For the detection of *Listeria monocytogenes,* samples were inoculated into enrichment broth (UVM formulation, Oxiod) and incubated for 2 days at 30°C [21;22]. A loopful of the enrichment broth was streaked onto *Listeria* selective medium agar (Oxoid formulation, Oxid) and incubated for 2 days at 30°C [23]. Suspect colonies were subjected to further tests for confirmation according to the scheme of Lovett [24].

Statistical Analysis

Data were subjected to statistical analysis for analysis of variance and Duncan's multiple range tests using SAS [25] general linear model procedure at KSU computer center.

Results and Discussion

Table 1 shows the mean TC and FC counts in the five vegetables investigated. Table 2 shows the distribution of the samples among the different ranges of counts found. Parsley samples had the highest TC and FC counts ($P > 0.05$) reaching 3.9×10^6 and 5.7×10^4 colony forming units (CFU)/g, respectively. Out of thirty samples examined for TC, twenty-seven (90%) harbored more than 10^5 CFU/g, while FC count exceeded 10^4 CFU/g in 21 (70%) of the parsley samples. These relatively

Table 1. Mean TC and FC counts in the vegetable samples

Vegetable	No. of samples	Mean TC*	Mean FC*
Parsley	30	3.9×10^6 a	5.7×10^4 a
Lettuce	29	3.8×10^5 b	5.9×10^3 b
Green onion	22	2.7×10^5 b	1.1×10^4 b
Carrots	33	1.7×10^5 b	1.2×10^4 b
Cucumber	34	1.4×10^4 b	5.3×10^2 b

* Means within the same column with the same letters are not significantly different ($P \geq 0.05$).

Table 2. Distribution of total and fecal coliforms (TC&FC) counts in the vegetable samples

	No. of samples									
Coliforms	Carrots		Cucumber		Lettuce		Onion		Parsley	
CFU/g	TC	FC	TC	FC	TC	FC	TC	FC	TC	FC
10^2-10^3	1	3	7	29	0	14	0	8	0	0
10^3-10^4	11	25	11	5	1	12	1	6	1	9
10^4-10^5	20	0	16	0	7	3	9	8	2	17
10^5-10^6	1	5	0	0	19	0	11	0	6	4
$>10^6$	0	0	0	0	2	0	1	0	21	0
Total	33	33	34	34	29	29	22	22	30	30

high counts may be attributed to the large leaf surface area and the foliar folds which harbor microorganisms [11]. In addition, parsley and some other leafy vegetables are usually marketed in unhygienic way; i.e. they are wrapped in wet cloth, usually unclean, to preserve freshness. In a study conducted in Mexico by Rosas *et al.* (11) parsley was found to contain considerably lower TC and FC (3100 and 600 CFU/100g, respectively) even though waste water has been used for irrigation.

For lettuce, TC count averaged 3.8×10^5CFU/g. Out of twenty-nine lettuce samples tested, only one contained a TC count of $< 10^4$ CFU/g, while 21 (72%) samples harbored $> 10^5$ CFU/g. The FC counts of the lettuce samples averaged 5.9×10^3 CFU/g, and in three samples only, the count exceeded 10^4 CFU/g. Ercolani [1] reported average counts of TC and FC of 5.95×10^4 CFU/g and 6.13×10^3 CFU/g of lettuce, respectively with an overall average TC:FC ratio in the order of 10. Similarly Rosas *et al.* [11] reported TC and FC counts of 2.9×10^3 and 1.3×10^2 CFU/g of lettuce, respectively. Riser *et al.* [26] reported a TC count on a hydroponic grown lettuce of 1.7×10^6 CFU/g. These differences in microbial counts may be ascribed to the different cultivation practices including irrigation, fertilization and handling of the crops.

The TC count averaged 2.7×10^5 CFU/g in the green onion samples. In twenty-one samples (95%), the count exceeded 10^4 CFU/g. Furthermore, in twelve samples, the count exceeded 10^5 CFU/g. The FC count averaged 1.1×10^4 CFU/g.

The carrots gave mean TC and FC counts of 1.7×10^5 CFU/g and 1.2×10^4 CFU/g respectively. Out of the thirty-three carrot samples tested for TC, thirty-two (97%) harbored mean TC of more than 10^3 CFU/g. Twenty-five samples (78%) contained FC counts within a range of 10^3-10^4 CFU/g. Cucumber samples showed the lowest mean TC count (1.4×10^4 CFU/g) as well as FC count (5.3×10^2 CFU/g). All of the thirty-four samples tested for TC gave mean counts of less than 10^5 CFU/g. This could be attributed to the low surface to weight ratio [11] and to the better packaging and handling methods usually applied to cucumber and to the cultivation practices as well. Salmonellae (Table 3) were present in lettuce, cucumber, green onion and parsley at a rate of 6, 5, 4.8 and 4.5 percent of the samples tested, respectively. Irrigation water, fertilizer, handling and containers could have played an important role in contaminating the vegetables under study, with salmonellae [7, pp. 265-280]. Surprisingly, carrot was found to be free of salmonellae inspite of the fact that it comes in direct contact with the soil. In a study conducted in Egypt on salad vegetables by Saddik *et al.* [27] it was found that out of 36 samples analyzed, three were positive for salmonellae. A higher rate of prevalence was reported in Italy by Ercolani [1] who found that 68.3% of the lettuce samples contained salmonellae.

Table 3. Prevalence of salmonellae and *Listeria monocytogenes* (LM) in the vegetable samples

Vegetable	No. of samples	No. of positive samples (%) for	
		Salmonellae	LM
Parsley	66	3 (4.5)	29 (44)
Lettuce	68	4 (6.0)	8 (12)
Green onion	63	3 (4.8)	5 (8)
Carrots	63	0 (0.0)	0 (0)
Cucumber	80	4 (5.0)	0 (0)

Presumptive *Listeria monocytogenes* was found in parsley, lettuce and green onion at relativel high rates; 44%, 12% and 8% of the samples tested, respectively (Table 3). It was not detected in carrots or cucumber samples. Tiwari and Aldenrath [28] suggested a possible inhibitory effect of carrots on the growth of *Listeria monocytogenes*. In Spain [29], *Listeria monocytogenes* was detected in 7.8% of 103 vegetable samples, while in Japan [30], it was not detected in any of the 21 vegetable samples tested.

The relatively high TC and FC counts of the five vegetables studied, particularly the leafy vegetables, and the high association of *Listeria monocytogenes* with these salad vegetables coupled with its long term survival on vegetables [31] creates a potential public health concern. This concern is highlightened by the fact that these vegetables are used in combination to make green salads which are consumed raw. Therefore, in order to minimize the risk of infectious disease, it is advisable to follow better hygienic practices in irrigation, fertilization, harvesting, and handling of these crops [7, pp 265-284]. Trimming, peeling, rinsing with potable running tap water [11] and dipping in a sanitizer can all contribute to the reduction of pathogens on these vegetables.

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دراسات ميكروبيولوجية على بعض خضار السلطة في الأسواق المحلية

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(قُدم للنشر في ٢٣/١٠/١٤١٤هـ؛ وقبل للنشر في ٦/٨/١٤١٥هـ)

ملخص البحث. تم تجميع مائة وأربعين عينة من خضروات السلطة شاملة البقدونس والخس والبصل الأخضر والجزر والخيار من الأسواق المحلية. تم إخضاع هذه العينات لبعض التحاليل الميكروبيولوجية والتي شملت عدَّ بكتريا القولون الكلية والبرازية. وبالإضافة إلى ذلك تم إخضاع ثلاث مائة وأربعين عينة من الخضروات نفسها لتحليل السالمونيلا وليستريا مونوسيتوجينس. تراوحت أعداد بكتريا القولون الكلية من ١,٤×١٠^{٤} وحدة مكونة للمستعمرة (و م م) لكل جرام بالنسبة للخيار و٣,٩×١٠^{٦} و م م/جم في البقدونس. في حين تراوحت أعداد بكتيريا القولون البرازية من ٥,٣×١٠^{٢} و م م/جم للخيار و٥,٧×١٠^{٤} و م م/جم للبقدونس. وجدت السالمونيلا في كل من الخس والخيار والبصل الأخضر والبقدونس بنسب ٥,٦ و٤,٨ و٤,٥٪ على التوالي. في حين وجدت الليستريا في كل من البقدونس والخس والبصل الأخضر بنسب ٤٤ و١٢ و٨٪ على التوالي. ومن هنا جاء الاستنتاج على أن استهلاك هذه الخضروات التي تستعمل في السلطة الخضراء التي تؤكل نيئة. وبالذات الخضروات الورقية دونما تنظيف أو تطهير قد يشكل خطرًا بالغًا على الصحة العامة.