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Short Communication

Food Quality Aspects of Fresh Green Salads in Selected Retail Stores in Los Baños, Laguna, The Philippines

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ABSTRACT

Introduction: Consumption of minimally processed vegetables has gained popularity due to consumer emphasis on convenience and healthy eating. However, much handling during processing poses health risks to the consumers. This study was aimed at determining the proximate composition and microbiological quality of minimally processed packed fresh green salads sold in seven different retail stores in Barangay Batong Malake, Los Banos, Laguna. **Methods:** Proximate composition, microbiological quality and presence of filth in the sampled salads were analysed using standard AOAC, BAM and floatation methods, respectively. **Results:** The proximate composition of the samples in percentage consisted of carbohydrates (3.07- 14.26), protein (0.95-11.79), fat (0.03-3.64), fibre(0.64-1.13) and moisture (73.27-92.77). Microbial analysis showed a mean total plate count of 2.4 x 107and a broad range of 2.7 x 104 – 6.6 x 107 CFU/g. Most probable numbers (MPN) of >11,000/g coliforms were found in four samples and *Escherichia coli* bacteria were detected in five samples but none of the *E.coli* count exceeded 9.2 MPN/g. Both insect fragments and textile fibre were detected in two samples. Based on the specifications by the Food and Drug Administrations of the Philippines, the levels of contamination found could lead to imminent spoilage andpose a potential health hazard. **Conclusion:** Although green salads contain fibre and low calories which are nutritionally important, the present findings in a Filipino location accentuates the need for more stringent enforcement of food safety measures to protect the consumers from possible occurrence of food poisoning.

Key words: Fresh green salads, microbiological load, proximate composition

INTRODUCTION

The popularity and demand for minimally processed fruits and vegetables have surged in recent years as a result of the rising health problems and time-crunched lifestyle of many people who make an effort to stay healthy while they are on the move. Because of such a need, different varieties of these products have mushroomed in the local markets.

By definition and product description, minimally processed fruits and vegetables are fresh produce processed to increase its functionality and provide convenience

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while maintaining its fresh like qualities (Siddiqui *et al.*, 2011). Fresh-cut products are fruits or vegetables that have been trimmed, peeled and/or cut into a fully usable product, which are subsequently packaged to offer consumers high nutrition, convenience and flavour while maintaining freshness (FAO, 2011). Although high in demand, a reduced shelf-life and rapid deterioration of products are the observed major drawbacks of minimal processing. The mere process of peeling, cutting and slicing intensifies its vulnerability to many forms of contamination and nutrient loss. In the market, a typical green salad package consists of lettuce, cabbage, spinach, and other leafy green vegetables.

Eating greens such as minimally processed and ready-to-eat green salads became a trend to address the health and wellness need of the population. Regular consumption of fruits and vegetables in general offers a lot of benefits such as a nice boost of fibre, vitamins and minerals. Moreover, they are low in fat and calories. Due to this enormous increase in demand, the development of new and improved methods for maintaining food quality are among the major concerns in the quality standards in food and microbial safety. The processing not only adds value to the product, but also makes the products more convenient to the consumers. On the other hand, much handling during processing intensifies the risk of contaminants, microorganisms in particular. *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Salmonella* and *Shigella* are just some of the microbial pathogens associated with minimally processed vegetables. Cutting and shredding vegetables for instance liberate some plant fluids that offer microbial contaminants a nutritious and moisture rich medium (Da Silva *et al.*, 2007, Oronusi *et al.*, 2013, Falomir, Gozalbo & Rico, 2010; Adjrah *et al.*, 2011). Moreover, the absence of heat treatment to eliminate the present microbes and a high possibility of temperature abuse between the time it is processed and consumed subjects consumers to food borne illnesses.

In the United States, reported multistate outbreaks of foodborne illness were associated with the increase in consumption of ready-to-eat bagged salads. Investigation on the quality of ready-to- eat green vegetable salads have been conducted in recent years in countries like the United States, United Kingdom, Spain and Brazil (Bon *et al.*, 2008) but in the Philippines, where there is increasing consumption and demand for ready to eat salads as it is conveniently marketed in various retail stores, there is hardly any recent information on the microbial quality and proximate composition of such food items. To contribute knowledge on its microbial and nutritional qualities in light of the commercial importance of these food items, the objective of this research then is to determine the microbiological and proximate analysis of minimally processed fresh green salad commonly sold locally in different retail stores.

METHODS

Ready to eat fresh green salads were randomly purchased from seven selected retail stores in Barangay Batong Malake, Los Banos, Laguna. The samples were transported in cold pack containers to the laboratory within 30 minutes of collection. The proximate composition of the samples were determined according to the Association of Official Analytical Chemists (AOAC, 1990) procedures.

Microbiological analysis

Aerobic plate counts of the samples were determined in plate count agar following the standard pour plate method of the Merk Bacteriological Analytical Manual (BAM, 2001). The number of total coliform and *E. coli* were determined by using three tube most probable numbers (MPN) method. Dilutions were inoculated into lauryl sulfate tryptose broth with Durham tube and incubated at 370C for 24 to 48 h. One loop-full culture from the tubes with gas formation after the incubation period was transferred to brilliant green bile broth (BGB) and again incubated at 370C for 24 to 48 h. The turbid BGB tubes with gas formation were used for the enumeration of coliform using the MPN table. The incubated gas positive and turbid LST broth tubes were examined for blue fluorescence at wavelength 366 nm using a hand held UV lamp. Indole test using Kovac's reagent was performed for the confirmation of *E. coli*. Using the MPN table, the gas, fluorescence and indole positive tubes were used to determine number of *E. coli*.

Filth determination

Filth assessment was conducted according to the floatation method of AOAC (2000). Twenty five (25) grams of samples were weighed into a 400 ml beaker. The weighted sample was crushed into small pieces using a spatula. The crushed sample was covered with petroleum ether and was allowed to stand for 5 min. The petroleum ether was decanted from the sample through the use of a 9 cm diameter filter paper (Schleicher & Schull, Inc. # 8). After allowing the petroleum ether to evaporate from the sample, the sample was transferred to a 500 ml trap flask to which was added 125 ml of 60% ethanol and left to boil for 30 min. At the end of the boiling process, the ethanol lost by evaporation was replaced and the set-up was left to cool in an ice water bath. After cooling, 9 ml of heptane was mixed and left to stand for 5 min. The heptane layer formed on top was collected and filtered in a Buchner funnel. The filter paper was placed on a Petri dish and examined microscopically.

Statistical analysis

Data were subjected to Multivariate Analysis of Variance and Least Significant Difference to determine if significant ($P<0.05$) differences existed among the samples in terms of its proximate composition.

RESULTS

Significantly, large variations in proximate composition in general were noted among the seven seemingly identical samples (Table 1). Energy content of one hundred (100) grams of fresh green ready-to-eat salads ranged from 24 -107 calories. Samples 3, 5 and 7 were noted to have the highest amounts of moisture. The high moisture contents of samples 3 and 7 were not significantly different. Likewise, the high ash and crude fat contents garnered by samples 1 and 6 were not significantly different. Significantly high crude protein and crude fibre were noted in samples 4 and 6, respectively. Sample 1 had the highest significant amount of carbohydrates and energy.

The aerobic plate count provides an estimate of the total number of bacteria to which the substance has been exposed in an aerobic environment. Among the samples, the highest aerobic count was found in sample 6 (6.6 x 107 CFU/g) and the lowest count was found in sample 2 (2.7 x 104CFU/g). Most probable numbers (MPN) of >11,000/g coliforms were found in four out the seven samples. Presence of large quantities of coliform bacteria and *E.coli* indicates the probability of pathogens. *E. coli* was detected in all five samples but in no sample did *E.coli* count exceed 10MPN/g (Table 2). Filth which consisted of insect fragments and textile fibres was found in five samples but the count did not exceed 3 (Table 2). Previous studies on the microbiological quality of bagged spinach and lettuce and on the microbial population of leafy vegetables reported similar findings (Bon *et al.*, 2008, Temiz *et al.*, 2011, Da Silva *et al.*, 2007; Avazpour *et al.*, 2013)

Table 1. Proximate composition (%) and energy content (kcal) of fresh green ready- to- eat salads

Composition	*Sample*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Moisture	73.27d	82.92c	87.83b	74.26d	92.77a	85.29cb	88.71b
Ash	3.11a	1.72dc	2.33bc	1.5d	0.46e	3.52ba	0.59e
Crude fat	3.20ba	1.98c	2.50b	2.97ba	0.03d	3.64a	1.85c
Crude protein	5.29b	2.71d	3.19c	11.79a	0.95f	1.49e	5.06b
Crude fibre	0.87dc	0.78de	1.06ba	0.64e	0.75de	1.13a	0.95bc
Carbohydrate	14.26a	9.89b	3.09d	8.84b	5.04dc	5.93c	3.38d
Energy	107.00a	68.22b	47.62c	102.25a	24.23d	62.44b	50.41c

Means with the same letter within a row are not significantly different

Table 2. Aerobic colony counts, coliform, E. coli and filth contents of seven (7) fresh green ready-to-eat salads

Composition	*Sample*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Aerobic Plate Count (CFU/g)	3.4 x 106	2.7 x 104	5.1 x 106	3.8 x 107	5.6 x 107	6.6 x 107	4.0 x 104
Coliform (MPN/g)	> 1100	1100	1100	>1100	>1100	>1100	1100
E.coli (MPN/g)	6.1	0	3	7.2	7.2	3	0
Filth	0	0	5	4	4	1	2

DISCUSSION

The sampled salads consisted mostly of lettuce, carrots, cucumber, tomatoes and onions.

The other salad samples had a mixture of parsley, radish, bell pepper, wedges of apples and peaches. Fruits and vegetables are low calorie food ingredients because these do not comprise fat, protein and carbohydrates which generally account for energy count. Regular consumption of green salads can help in maintaining weight and lessen the risks of chronic degenerative diseases. The significantly different protein content of salad samples may be attributed to the different amounts of protein ingredients like small chunks of chicken meat, slices of eggs and cubed cheese mixed in the salad to offer contrast in texture and flavour. Addition of bits of bread cubes called croutons may have contributed to the carbohydrate content of the salads. The highly variable chemical composition of the sample salads may be attributed to the different ingredients mixed in salads that were collected for this study. Likewise, the handling of these different ingredients contributed, to a large extent, to its microbial load.

Based on the Philippine Food and Drug Administration and ICMSF (1996), the microbiological recommendation for ready-to-eat fruits and vegetables is less than 104 and 107CFU/g, respectively. Microbial count exceeding the recommendation indicates a potential health hazard or imminent spoilage. Results higher than 1,000 MPN/g for total coliforms and greater than 3 MPN/g are considered as unsatisfactory and indicative of unhygienic practices (FDA, 2013; ICMSF, 1996). Taking follow up samples to verify the levels may be required to ascertain the causes and identify measures to improve its handling practices.

Raw vegetables carry high loads of microorganisms of diverse species. The high moisture content favours microbial growth. Green salads are consumed raw and thus no heat treatment is applied to reduce microbial load (Bon *et al.*, 2008, Temiz *et al.*, 2011; Adjrah *et al.*, 2011). Much handling in the preparation, packing, storage and assembly for service could have also contributed to a high level of contamination. Specifically, the unhygienic practices of the food handlers, water used for processing and the processing environment may have caused the unsatisfactory microbial load of the sample salads. Moreover, the holding temperature of salad during sale and service and the observed condensation moisture in samples that came in sealed plastic tray packs may also have promoted the proliferation of microorganisms. These findings are preliminary and additional studies are needed to fully establish the effects of handling practices, processes and the varied environmental conditions on the microbial counts.

CONCLUSION

The results obtained in this study showed high variability in the proximate composition of the seemingly identical salad samples conveniently sold in various retail stores. The microbial loads were of unsatisfactory quality based on local and international standards. It is suggested that further studies be undertaken to verify the sources and extent of contamination, and to identify measures to improve handling practices of fresh leafy vegetables.

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