International Proceedings of Chemical,
Biological & Environmental Engineering

Nutrition and Food Sciences

Volume 39

Edited by
Yang Dan

IACSIT PRESS
www.iacsitp.com

2012 International Conference on Nutrition and Food Sciences
IPCBEE vol. 39 (2012) © (2012) IACSIT Press, Singapore

Microbiological Quality of Mixed Fresh-Cut Vegetable Salads and Mixed Ready- to-Eat Fresh Herbs in Mashhad, Iran

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Abstract. This study aimed to determine the microbiological quality of mixed fresh-cut vegetable salads and mixed ready-to-eat fresh herbs produced in Mashhad, Iran. A total of 174 samples including 89 mixed fresh-cut vegetable salads and 85 mixed ready-to-eat fresh herbs were collected between July 2010 and March 2011. Samples were analyzed for aerobic plate counts, coliforms, enterobacteriaceae, Escherichia coli, Escherichia coli O157:H7, Salmonella spp., Staphylococcus aureus, and yeast and mold counts.
The incidence levels of aerobic plate count bacteria indicated that 50.6% of mixed ready-to-eat fresh herbs and 49.4% of mixed fresh-cut vegetable salads contained less than 107 cfu/g. Enterobacteriaceae and total coliform levels ranged from 3 log cfu/g to 8.3 log cfu/g. Lactic acid bacteria were present in mixed fresh-cut vegetable salads and mixed ready-to-eat fresh herbs at 5.9 log cfu/g and 4.88 log cfu/g respectively. Yeasts and molds were found in mixed fresh-cut vegetable salads and mixed ready-to-eat fresh herbs at 5.68 log cfu/g and 5.78 log cfu/g respectively. Yeasts and molds at $\leqslant$ 5 log cfu/g were recovered from 42.7% (38 of 89 samples) and 40% (34 of 85 samples) of mixed fresh-cut vegetable salads and mixed ready-to-eat fresh herbs respectively. While 19.1% of mixed fresh-cut vegetable salads and 27.8% mixed ready-to-eat fresh herbs contained E. coli, only 6.3% of all samples were contaminated with the microorganism at $\geqslant$2 log cfu/g. E. coli O157:H7 was detected in mixed fresh-cut vegetable salads and mixed ready-to-eat fresh herbs with an incidence of 6.5% and 11.4% respectively. Staphylococcus aureus was found in 94.9% of samples, whereas coagulase-positive staphylococci were detected in 23.6% of samples. Our results also exhibited that 9.4% of mixed ready-to-eat fresh herbs and 5.6% of mixed fresh-cut vegetable salads were contaminated with Salmonella spp.

Keywords: Mixed fresh-cut vegetable salads; Minimally processed vegetables; Microbiological quality; Incidence levels; Enteric pathogens.

1. Introduction

Demand for fresh-cut and mixed ready to eat vegetables has been increased in recent years, due to th nutritional value as well as health benefits. Different organizations (WHO, FAO, USDA, EFSA) recommer the increasing fruits and vegetables consumption to decrease the risk of some diseases such as cardiovascul and cancer (1). Fresh-cut vegetables are considered to be as a vehicle for the transmission of food-bor pathogens and a number of reports refer to raw vegetables harbouring potential food borne pathogens (2, 3 There have been a number of reports on microbiological quality of fresh-cut and mixed ready to e vegetables from different parts of the world (4, 5, and 6). To the best of authors' knowledge, there have be no previous studies examined the microbiological quality of mixed fresh-cut vegetables and mixed ready-t eat fresh herbs in Iran, despite the fact that the number of vegetable processing plants have rapidly bee increased. The aim of this study was to investigate the microbiological quality of mixed fresh-cut vegetab salads and mixed ready-to-eat fresh herbs in Mashhad, Iran.

2. Materials and Methods

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1. Sampling

Totally 174 samples of mixed fresh-cut vegetables and mixed ready-to-eat fresh herbs were analyzed ween July 2010 and March 2011. Samples were transported in an ice box to the laboratory where they re analyzed for spoilage and pathogenic microorganisms within 2 h of sample collection.

Twenty five grams of each sample was added to 225 ml of buffered peptone water (BPW) and blended gorously for 2 min at normal speed in a kitchen blender (Sanyo, Japan). Serial decimal dilutions of the spension were then prepared in BPW before performing the analysis.

2. Microbiological Analysis

Aerobic mesophilic bacteria were counted by pour plating method on plate count agar and were cubated at 30° C for 48 h. Enterobacteriaceae and total coliforms were enumerated on violet red bile ucose agar (VRBG) and violet red bile lactose agar (VRBL), respectively, by pour plate method with an er layer, followed by incubation at 30° C for 24 h. The red colonies were counted.

Lactic acid bacteria count (LAB) was done by pour plating method with an over layer on MRS agar and ere incubated at 30° C for 48 h.

Yeasts and molds were counted by pour plating method on YGC agar, followed by incubation at 25° C r 3-5 days.

Staphylococcus aureus was isolated and enumerated by pour plating onto Baird-Parker agar containing gg yolk emulsion and incubated at 35° C for 24 h. Two or three of black colonies were selected and tested or catalase test, gram reaction and coagulase reaction.

E. coli was enumerated using choromagarECC (trypton bile X- glucouronide agar) (Choromagar Co., rance) by incubation at 30° C and 44°C for 24 h. The blue-green colonies were counted as E. coli. Isolated E. oli on choromagar ECC was sub-cultured onto Cefixime-Tellurite Sorbitol MacConkey agar (CT-SMAC) Merck Co., Germany) at 37°C for 24 h. Colorless colonies on the CT-SMAC agar were tested for E. coli)157:H7 by latex agglutination test.

To investigate of Salmonella spp., the blended samples in BPW were incubated at 37°C for 20-24 h. Salmonella spp. were then enrichment in Rappaport-Vassiliadis enrichment broth (RVS) and tetrathionat roth containing iodine solution and neobiocin (40 µg/ml) at 41.5°C for 24 h. After 18-24 h, samples were hen streaked on xylose lysine desoxycholate (XLD) (Merck co., Germany), Bismuth sulfite iron agar (BSI) Merck co., Germany) and brilliant green agar (BGA) and incubated at 37°C for 24-48 h. Two or more identical colonies were then transferred to lysine iron agar, triple sugar iron agar slants and urease broth. Each culture showing presumptive-positive results was maintained on Brain heart infusion agar. Cultures were further subjected to serological tests using polyvalent O and H antisera.

3. Results

Microbial quality and the distribution incidence levels of all analyzed samples are presented in Tables land 2. The aerobic plate count (APC) were ranged from 4.1 log cfu/g to 8.3 log cfu/g in mixed fresh-cut salads and from 4.3 log cfu/g to 8.3 log cfu/g in mixed green leaves vegetables. The microbial load of 44.9% of mixed fresh-cut salads and 43.5% of mixed green leaves vegetables was in the range of 7 log cfu/g to 8 log cfu/g.

Enterobacteriaceae counts were ranged between 3 log cfu/g to 8.3 log cfu/g for mixed fresh-cut salads and from 3.1 log cfu/g to 7 log cfu/g for mixed green leaves vegetables. The highest incidence levels on mixed fresh-cut salads and mixed green leaves vegetables were 42.7% and 32.9% respectively with the load between 5 log cfu/g to 6 log cfu/g.

Total coliforms were between 3.9 log cfu/g to 7.48 log cfu/g for mixed fresh-cut salads and 3.2 log cfu/g to7 log cfu/g for mixed green leaves vegetables. 50.6% and 49.4% of all mixed fresh-cut salads and mixed green leaves vegetables fell in a range between 5 log cfu/g to 6 log cfu/g.

Lactic acid bacteria (LAB) were ranged from 1 log cfu/g to 7.3 log cfu/g on mixed fresh-cut salads and from 1 log cfu/g to 6 log cfu/g on mixed green leaves vegetables. 87.8% of all mixed green leaves vegetables

were found to be in a range less than 5 log cfu/g, whereas only 44.4% of mixed fresh-cut salad samples have found in that range.

Yeasts and molds counts were between 3.85 log cfu/g to 6.7 log cfu/g for mixed fresh-cut salads and 2.04 log cfu/g to 6.6 log cfu/g for mixed green leaves vegetables. The highest overall incidence levels were ranged between 5 log cfu/g to 6 log cfu/g for mixed fresh-cut salads (43.8%) and mixed green leaves vegetables (36.5%).

E. coli was detected in 19.1% of mixed fresh-cut salads and 27.8% of mixed green leaves vegetables. 6.3% of all mixed green leaves vegetables and mixed fresh-cut salads (five samples each), had E. coli counts $\geqslant$ 2 log cfu/g. However, mixed green leaves vegetables had higher incidence levels than the mixed fresh-cut salads.

E. coli O157:H7 was detected in 6.5% of mixed fresh-cut salads and 11.4% of mixed green leaves vegetables. S. aureus was found in 94.9% of all samples. 77.1% of the positive samples had less than 4 log cfu/g and 23.6% of samples were positive to coagulase test. Salmonella spp. was detected in 5.6% and 9.4% of mixed fresh-cut salads and mixed green leaves vegetables, respectively.

4. Discussion

There is insufficient information available on the incidence levels and microbial quality of fresh-cut vegetables worldwide rather than Iran. This article is the first report on the incidence levels and microbial quality of mixed fresh-cut salads and mixed green leaves vegetables of Mashhad, Iran.

All examined samples were contaminated with total aerobic mesophiles (APC). The results showed that APC were less than 107 cfu/g in 49.4% mixed fresh-cut salads and 50.6% of mixed green leaves vegetables. Abadias et al. reported the similar results (4). Vegetable samples examined by Nguz et al., Johnston et al. and Seo et al. (5, 6, and 7) reported the means of APC ranged from 3.0 to 7.8 log cfu/g on restaurant prepared lettuce, 4.5 to 6.2 log cfu/g on fresh produces and 5.4 log cfu/g to 8.9 log cfu/g in mixed salads. Our results were almost in agreement with other researchers. High aerobic mesophilic counts found in samples may reflect the fact that poor handling, inappropriate processing or a general lack of hygiene is the main cause.

Our results revealed that 100% of mixed fresh-cut salads and mixed green leaves vegetables were contaminated with Enterobacteriaceae and total coliforms, and the level of contamination varied from 3 log cfu/g to 8.3 log cfu/g for Enterobacteriaceae and 3.2 log cfu/g to 7.48 log cfu/g for total coliforms. Abadias et al. and Nguz et al. found that the incidence levels of Enterobacteriaceae in whole vegetables and fresh-cut vegetables were 78.6% and 73.3% respectively (4, 5). Johnston et al. showed that total coliforms ranged from 1 to 4.3 log cfu/g on green leaves and herbs (6). Little et al. reported levels of total coliforms on fresh lettuce up to 5 log cfu/g (8) and Seo et al. found the range of total coliforms in mixed salad vegetables was from 2.7 log cfu/g to 8.2 log cfu/g (7).

Our data indicated that 19.1% of mixed fresh-cut salads and 27.8% mixed green leaves vegetables contained E. coli. But only 6.3% of samples are at higher than 2 log cfu/g. Abadias et al. reported that in 7.1% whole vegetables, 11.4% fresh-cut vegetables and 40% sprouts E. coli was presented (4) and Soriano et al. showed that 25.7% of restaurant prepared lettuce contained E. coli (9). Nguz et al. and Johnston et al. reported 40% fresh cut vegetables and all of leafy green and herbs contaminated with E. coli (5,6).

Many authors have not reported the presence of E. coli O157:H7 on fresh-cut vegetables, whereas we were able to detect it on fresh-cut and green leaves vegetables. The incidence levels of E. coli O157:H7 on mixed fresh-cut salads and mixed green leaves vegetables were 6.5% and 11.4%, respectively.

S. aureus is a pathogen known to be carried by food handlers (10). The presence of S. aureus in mixed fresh-cut vegetables indicated the poor hygiene practices and levels higher than 4 log cfu/g are potentially hazardous. According to our data, S. aureus was detected in 94.9% of samples and the levels of S. aureus in 77.1% samples were less than 4 log and coagulase-positive staphylococci were detected in 17.1% of samples. A few studies have reported the isolation of S. aureus from fresh-cut vegetables. Nguz et al. showed the incidence levels of S. aureus were 83.9% of products analyzed (5). Soriano et al. reported that coagulase-positive staphylococci were detected in approximately 22.9% of the samples (10).

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