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Investigation of Microbial Quality of Some Leafy Green Vegetables

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Abstract: This study carried out to investigate the microbiological properties and probable public health risks in some leafy green vegetables sold in greengrocers and bazaars. Vegetables samples were collected and examined according to a standardized protocol for total aerobic mesophilic bacteria, coliforms, *Escherichia coli*, *Staphylococcus-micrococcus* sp., mold, yeast, *Clostridium perfringes* and *Bacillus cereus*. The average total aerobic mesophilic bacteria counts in parsley, lettuce, cress, watercress and dill samples were determined as 2.3×10^7, 4.7×10^6, 1.7×10^7, 1.1×10^7, 3.2×10^6 and 7.2×10^6 cfu g^{-1}, respectively. The high microbial contamination rates associated with these vegetable samples indicate that overall agricultural, hygiene, harvesting, production and sale practices are poor. The data presented here show that the analyzed samples had serious microbial load which could be hazardous for public health.

Key words: Vegetables, microbiological quality, mold, yeast, coliforms

INTRODUCTION

Food borne diseases originated from vegetables and fruits are being reported from many countries. Those viral, bacterial and parasitic diseases affect various numbers of people in population, either a few or thousands (De Rover, 1998; Anonymous, 2002).

The vegetables may be exposed to microbial contamination before, during or following harvest. The products may be contaminated by normal microflora microorganism which contains non-pathogen microorganism, besides pathogens of environmental, human or animal origin. Enteric pathogens can be found in soil, manure, urban wastes and in irrigation water, depending on relative moisture, microbial adhesion, rainfall and sunlight. The dominant microflora on vegetables consists of decomposed molds and yeasts; however, pathogens leading to bacterial, viral and parasitic human infections are also reported. Vegetables can be cross-contaminated following harvest by hand contacts, during storage, transport and cleaning, via other agricultural products or workers. Environmental conditions and transportation affect the hygienic quality of the products prior to processing or consumption. Breaking or surface corruption of the vegetables also prepare a suitable condition for microorganism contamination and proliferation (Beuchat, 1996, 1998; De Rover, 1998; Francis *et al*., 1999).

The aim of this study is to determine the microbiological quality of freshly consumed parsley, lettuce, cress, watercress, mint and dill samples and to demonstrate the potential risk for public health.

MATERIALS AND METHODS

Collection of the samples: A total of 106 samples of parsley (n:22), lettuce (n:20), cress (n:16), watercress (n:18), mint (n:16) and dill (n:14) were taken into sterile sample bags, each containing 250 g. of sample. The samples were collected from various greengrocers and bazaars in Diyarbakır, Turkey. They were maintained in cold conditions (4°C), delivered to the laboratory immediately and analyzed.

Microbiological analyses: Leafy green vegetable samples (10 g per) collected aseptically were prepared for microbiological analyses by homogenizing them in 90 mL peptone water (Merck, Darmstadt, Germany). The decimal dilutions prepared were cultured in appropriate medium by using standard analyses methods (Table 1) (Lancette and Harmon, 1980; AOAC, 1995; The Oxoid Manual, 1998; Downes and Ito, 2001; The Merck Microbiology Manual, 2002). Microscopy, gram staining, lesitinase production, acid production from mannitol, anaerobic utilization of glycosis, nitrate reduction test, Voges-Proskauer test, motility, endospore formation and hemolysis formation tests were used in the identification of *B. cereus* (Nout *et al*., 1998; Pirttijarvi *et al*., 1998).

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Table 1: Mediums used in microbiological analyses and incubation conditions

Microorganism	Mediums	Incubation conditions: Temperature (°C)	Time (h)	Aerob/Anaerob
Aerobic mesophilic bacteria	Plate count agar (Oxoid CM 325)	35	48	Aerob
Coliforms	Violet red bile lactose agar (Oxoid CM 107)	35	24	Anaerob
Escherichia coli	TBX agar (Oxoid CM945)	30	4	Anaerob
		44	18	
Staphylococcus-micrococcus	Baird parker agar base + egg yolk tellurite emülsion (Oxoid CM 275 + SR54)	35	48	Aerob
Mold	Potato dextrose agar (Oxoid CM 139)	25	120	Aerob
Yeast	Potato dextrose agar (Oxoid CM 139)	25	120	Aerob
Clostridium perfringes	Perfringes selective agar base (OPSP) + supplements A ve B (Oxoid CM543 +SR76+SR77)	35	18-24	Anaerob
Bacillus cereus	*Bacillus cereus* Selective Agar Base + supplements (Oxoid CM617+ SR99+SR47)	30	18	Aerob
Salmonella sp.	Buffered peptone water (Merck 1.07228)	35	24	Aerob
	RVS broth (Merck 1.07700)	42	24	
	Selenite cystine broth (Merck 1.07709)	35	24	
	BPLS agar (Merck 1.10747)	35	24	
	Salmonella shigella agar (Merck 1.07667)	35	24	
	XLD agar (Merck 1.05287)	35	24	

Statistical analysis: SPSS software and variance analysis methods (ANOVA) were used in interpretation of analyses results. T-test was used in the evaluation of the significance of the difference between the groups. The significance between the values was evaluated at 95% confidence $p<0.05$ (SPSS, 1999).

RESULTS AND DISCUSSION

The numbers of the microorganisms determined on leafy green vegetables are shown in Table 2. *E. coli*, *C. perfringes* and *B. cereus* were identified in all of the samples analyzed (100%), while *Salmonella* sp. could not be isolated from any of them. The maximum and minimum values were found to be 2.2×10^6-4.3×10^7 cfu g^{-1} for TAMB, 1.6×10^3-8.2×10^5 cfu g^{-1} for coliforms, 4.0×10^2-1.9×10^5 cfu g^{-1} for *E. coli*, 1.7×10^3-2.7×10^5 cfu g^{-1} for *Staphylococcus-micrococcus* sp., 2.0×10^2-1.6×10^4 cfu g^{-1} for mold, 4.7×10^2-2.0×10^6 cfu g^{-1} for yeast, 1.0×10^1-1.8×10^2 cfu g^{-1} for *C. perfringens* and 3.3×10^2-2.7×10^4 cfu g^{-1} for *B. cereus* in the samples analyzed.

Green vegetables are included in daily menus either raw or cooked, alone or together with other foodstuffs. Particularly freshly consumed vegetables or those which are used in salad mixtures can be hazardous for health since they are not subjected to any thermal process. The microorganism numbers in vegetables can vary between 10^4-10^8 g^{-1}, depending on the season and the climate (Lund, 1992). The wide surface of vegetable leaves is suitable for water contact and microbial contamination (Anonymus, 2002). Molds are responsible for the ⅔ of the corruptions in vegetables (ICMSF, 1998).

Viswanathan and Kaur (2001) reported total aerobic microorganism number as 10^5-10^{10} cfu g^{-1} and coliform number as 10^6-10^9 cfu g^{-1} in raw vegetables used in salad mixture. Patterson and Woodburn (1980) determined the number of aerobic mesophyl and number of coliforms as 10^8 and 10^6 cfu g^{-1}, respectively. Saddik *et al.* (1985) reported the number of aerobic bacteria as 10^6 cfu g^{-1}. The numbers of TAMB (2.2×10^6-4.3×10^7 cfu g^{-1}) in this research are in accordance with those reported in other studies. Ayçiçek *et al.* (2006) reported the average TAMB and coliform bacteria count in head lettuce, lettuce, iceberg lettuce, parsley and dill samples as 4.8-5.5, 4.6-5.8, 3.9-6.0, 5.8, 5.7, 5.6, 4.3-5.3, 4.2-5.3, 3.4-5.8, 5.7, 5.6, 5.3 cfu g^{-1}, respectively while *E. coli* contamination rates were 10-13.3, 0-20, 3.3-10, 70 and 40% (29.4% av.), respectively. The number of coliforms was 1.6×10^3-8.2×10^5 cfu g^{-1} and the number of *E. coli* was 4.0×10^2-1.9×10^5 cfu g^{-1} in our research. The highest average number of coliforms and *E. coli* was determined in watercress and cress samples which were collected from bazaar, while the lowest was determined in the samples collected from greengrocers. Some *B. cereus*, which caused food poisoning in home-grown vegetables were also reported (Portnoy *et al.*, 1976). The number of *B. cereus* in this research was determined as 3.3×10^2-2.7×10^4 cfu g^{-1}.

The significance of the average bacterial counts between the samples collected from greengrocers and bazaars was evaluated statistically. The difference between the numbers of *E.coli*, yeast and *C. perfringens* in watercress samples and the difference between the numbers of *Staphylococcus-micrococcus*, mold and *C. perfringens* in dill samples were statistically significant, while the differences between the other bacteria were not statistically significant. The differences between the numbers of *C. perfringens*, *E. coli* and mold in lettuce samples, *Staphylococcus-micrococcus,* mold and *B. cereus* in cress samples; *C. perfringens* in parsley

Table 2: The average microorganism counts in some green vegetable samples (cfu g^{-1})

	n	TAMB	Coliforms	*E. coli*	*Staphylococcus-Micrococcus* sp.	Mold	Yeast	*C. perfringes*	*B. cereus*
Parsley									
Greengrocers	8	3.7×10^{6a}	1.6×10^{3a}	1.6×10^{3a}	4.6×10^{4a}	1.4×10^{3a}	1.2×10^{4a}	2.5×10^{1a}	4.3×10^{2a}
Bazaars	14	4.3×10^{7b}	9.2×10^{4b}	1.5×10^{3a}	9.9×10^{4b}	1.3×10^{4b}	2.0×10^{6b}	4.0×10^{1a}	2.3×10^{3b}
Average	22	2.3×10^{7}	4.7×10^{4}	1.6×10^{3}	7.3×10^{4}	7.2×10^{3}	1.1×10^{6}	3.3×10^{1}	1.4×10^{3}
Lettuce									
Greengrocers	12	6.9×10^{6b}	1.0×10^{5b}	7.1×10^{2a}	1.1×10^{4b}	2.1×10^{2a}	5.4×10^{3b}	3.0×10^{1a}	8.0×10^{2a}
Bazaars	8	2.5×10^{6a}	9.7×10^{3a}	5.1×10^{2a}	1.7×10^{3a}	2.0×10^{2a}	6.5×10^{2a}	3.0×10^{1a}	4.6×10^{3b}
Average	20	4.7×10^{6}	5.5×10^{4}	6.1×10^{2}	6.4×10^{3}	2.1×10^{2}	3.0×10^{3}	3.0×10^{1}	2.7×10^{3}
Cress									
Greengrocers	6	3.1×10^{7b}	2.7×10^{4a}	3.9×10^{3a}	3.7×10^{4a}	1.6×10^{4a}	1.1×10^{6b}	1.0×10^{1a}	1.1×10^{3a}
Bazaars	10	3.8×10^{6a}	2.7×10^{5b}	1.9×10^{5b}	6.5×10^{4a}	8.7×10^{2a}	2.7×10^{4a}	4.5×10^{1b}	2.9×10^{3a}
Average	16	1.7×10^{7}	1.5×10^{5}	9.7×10^{4}	5.1×10^{4}	8.4×10^{3}	5.6×10^{5}	2.7×10^{1}	2.0×10^{3}
Watercress									
Greengrocers	8	1.2×10^{7a}	4.7×10^{5a}	1.0×10^{3a}	1.6×10^{5a}	2.2×10^{3a}	4.8×10^{4b}	1.0×10^{1a}	4.1×10^{3a}
Bazaars	10	1.0×10^{7a}	8.2×10^{5a}	1.0×10^{4b}	2.7×10^{5a}	6.9×10^{3a}	4.7×10^{2a}	4.0×10^{1b}	9.6×10^{3a}
Average	18	1.1×10^{7}	6.5×10^{5}	5.5×10^{3}	2.2×10^{5}	4.6×10^{3}	2.4×10^{4}	2.5×10^{1}	6.9×10^{3}
Mint									
Greengrocers	4	4.1×10^{6a}	6.0×10^{5b}	5.8×10^{3b}	2.9×10^{4b}	1.6×10^{3b}	1.4×10^{3a}	1.8×10^{2b}	2.7×10^{4b}
Bazaars	12	2.2×10^{6a}	1.8×10^{4a}	4.0×10^{2a}	3.4×10^{3a}	7.7×10^{2a}	8.3×10^{3b}	3.0×10^{1a}	3.3×10^{2a}
Average	16	3.2×10^{6}	3.1×10^{5}	3.1×10^{3}	1.6×10^{4}	1.2×10^{3}	4.9×10^{3}	1.1×10^{1}	1.4×10^{4}
Dill									
Greengrocers	8	7.9×10^{6a}	5.0×10^{5a}	2.4×10^{3a}	6.2×10^{4b}	9.0×10^{2a}	1.2×10^{3a}	3.6×10^{2b}	4.4×10^{3a}
Bazaars	6	6.4×10^{6a}	1.4×10^{5a}	2.1×10^{3a}	4.2×10^{4a}	4.6×10^{3b}	2.2×10^{3a}	5.0×10^{1a}	2.9×10^{3a}
Average	14	7.2×10^{6}	3.2×10^{5}	2.3×10^{3}	5.2×10^{4}	2.8×10^{3}	1.7×10^{3}	2.1×10^{2}	3.7×10^{3}

[a, b, c] Means within the same column with the different superscript letter are significantly different ($p<0.05$)

samples and TAMB in mint samples were not statistically significant, whereas the numbers of the other bacteria were statistically significant. The difference of TAMB counts between parsley, lettuce and cress samples were significant, but the difference was not significant in the other samples.

It was observed that the significance of the difference of bacterial counts between the samples collected from greengrocers and bazaars depended on the vegetable analyzed and bacteria species investigated. These differences were probably due to the cultivation areas of the vegetables, transportation conditions, nonstandard storage and sales conditions as well as personal applications (such as washing vegetables, hand contacts etc.).

In raw vegetables ready-to-use, the number of coliforms must not exceed 210 MPN g^{-1}, while *E. coli* 95 MPN g^{-1} and *Salmonella* sp. should not be existent in 25 g. According to Microbiological Criteria Regulation of Turkish Food Codex, *Salmonella* sp. could not be isolated from any of the samples involved in this research although the numbers of coliforms and *E. coli* were above the legal limits (Anonymous, 2001).

It is concluded that the microbial load in parsley, lettuce, cress, watercress, mint and dill samples are extremely high and hence it poses an important public health risk. The community needs to be informed that freshly consumed vegetables can be a mediator for contamination of the pathogens. It is known that young and old people, pregnant women and people with immune-deficiency are more sensitive to bacterial, viral and parasitic infections than the other groups. Environmental hygiene, hygienic production (water, soil, manure, agricultural chemicals, biological control, applications at home, hygiene of workers), hand contact, transportation, cleaning, storage and sanitation are important in ensuring food safety. Washing the vegetables after harvest removes both the dust and dirt on them while can be a source of microbial contamination itself (NACMCF, 1999; CDC, 1999; Anonymous, 2002). The water used for production, irrigation and washing must be of enough quality to prevent microbial contamination. Good Agricultural Practices (GAP), Good Hygienic Practices (GHP) and Hazard Analyses Critical Control Points (HACCP) must be applied in all the stages from irrigation water in vegetable fields to harvesting, transportation, storage, sales and processing (Blumenthal *et al*., 2000; Anonymous, 2002). Keeping green vegetables in cool conditions and in separate sections from each other during sales and washing them by clean water can be considered to prevent the increase of bacterial load. It is necessary to offer the vegetables for sale in cold storage conditions after they are washed, cleaned, disinfected and vacuum packaged under modified atmosphere in order to avoid possible public health risk. Packaging under modified atmospheric conditions prolongs the shelf life of fresh fruits and vegetables and besides inhibits the proliferation of pathogens (Jacxsens *et al*., 1999).

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