

MICROBIOLOGICAL QUALITY ASSESSMENT OF MIXED VEGETABLES SALAD COLLECTED FROM SELECTED AREAS OF PESHAWAR, KHYBER PAKHTUNKHWA-PAKISTAN

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Abstract

The current research was carried out to evaluate the microbiological quality of three (33) vegetable mixed salad procured from various vendors and areas in the Peshawar. The samples were assessed for mould count, coliform count and aerobic plate count (APC). The APC were range from 1×10^3 to 8×10^9 CFU/g, with 24 samples unacceptable, 4 samples marginally acceptable and 5 samples acceptable. The coliform bacteria were ranged from 1×10^2 to 6×10^8 CFU/g, with 6 samples marginally acceptable and 27 samples were unacceptable. The mould were counted and ranged from 800 to 3800 CFU/g, with 13 samples unacceptable and 20 samples were acceptable. Generally, the results exposed substantial microbial pollution in the examined samples, especially with respect to coliform count and APC. The findings specify insufficient sanitation practices and hygienic handling among some sellers and highpoint the essential for storage, proper handling, safe water and better hygiene of ready-to-eat vegetable salad in Peshawar.

Introduction

There are greater than two hundred ailments comprising from diarrhea to tumors/cancers are produced through dangerous food that is contaminated with synthetic compounds, parasites, viruses and microorganisms. It was counted that six hundred million in the globe drop infected afterwards consuming polluted diet and 420,000 people died annually, resultant in the harm of thirty-three million disability adjusted lifetime ages (Gizaw, 2019). Vegetables and fruits are the prime ingredients of a vigorous food. Many researches observe that the incidence of diet-borne disease linked to the feeding of vegetables and fruits have augmented (Ana *et al.*, 2009). In Okigbo's (1990) view, a vegetable is the succulent portion of the plant which is not sweet, and may be seasoned or flavoured with condiments prior to consumption. Plants or parts of plants can be consumed raw in salads or cooked in some dishes, such as rice. Vegetables have the attributes elements, oil, amino acids, Ca, Fe and vitamins. Yellow and green leaves vegetables have extremely palatable greens that are rich in vitamin A and iron. Vegetables have been found to be useful in neutralising the acids formed during ingestion of livestock products and thus used for example roughages to stop costiveness (Oyenuga and Fetuga, 1985). Salads are a food that is made with chopped vegetable and/or fruit and eaten raw. Mayonnaise and cheese (Hameed *et al.*, 2020) are the other additional ingredients in salads. Salad provides high level of protein, fat, carbohydrate, vitamins, minerals and antioxidants. Vegetable ingredients such as lettuce, cucumber, cabbage and carrot are used as components in vegetable salad (Hameed *et al.*, 2020). Cultural changes and improvements in processing, conservation, distribution and trading have resulted in the availability of almost any type of fresh fruit and vegetable, in good quality. Other technologies used, however, have added to health risks, primarily related to pathogenic micro-organisms. Gastroenteritis outbreaks has been attributed to eating of polluted vegetables (fresh), on several occasions. There have been reports of infection with toxin (inadequate hygienic handling of vegetables) (Silvia *et al.*, 2007). The vegetables salads become augmented source for bacteria, moulds and yeast leading to increase in the number of microorganisms that may spoil the quality of the salads (Hameed *et al.*, 2020). Salads are perceived as a means by which pathogens can be spread to humans. Most of the cases where microorganisms are

responsible for spoilage in salads, are not considered to be a food borne illness; therefore, people are eating the salad and causing food borne illnesses (Abaza, 2017). In light of this, microbiological quality evaluation of vegetable mixed salad samples was procured from various areas of Peshawar-Khyber Pakhtunkhwa-Pakistan was investigated.

Materials and Methods

Collection of Samples

The total 33 vegetables salad samples were procured randomly selected from various vendors of Peshawar in Khyber Pakhtunkhwa – Pakistan. The samples were transferred into sterile Schott Duran-Germany bottles (1 litre) in a cooled box with ice bags and were immediately stored in a cooled incubator in the laboratory for analysis on the same day.

Processing of Sample

Microbiological analysis was carried out by preparing stock suspensions of each sample by mixing 25g into 225mL of phosphate buffered saline (PBS) separately. Sterilized tissue homogenizer was used to homogenize it. The analysis was carried applying 10-fold consecutive dilution were produced in a test tubes through pipetting one milliliter from the uniform phase blend to the test tubes holding nine milliliter of autoclaved buffered saline (phosphate) and one milliliter of the blend was shifted to the subsequent test tubes holding nine milliliters of diluent. Using inoculating loop the culture was shifted for consecutive dilutions up to 10^{-12} (Hameed *et al.*, 2020).

Aerobic Plate Count Determination

One milliliter of every dilution was appropriately blend with autoclaved melted Nutrient Agar (NA) and decanted into disinfected Petri plates at 45 °C. It was permitted to harden and these Petri plates were incubated for twenty-four at 37°C in an incubator (Mettler-Germany). Colony on the plate was counted with colony counter (Hameed *et al.*, 2020) and plates with the range of colonies that can be counted (30-300 colonies) were selected.

Coliform Bacteria Measurement

The MacConkey's culture media was produced as per instruction of the producer in a conical flask. Subsequent the autoclaving and dropping the media temperature to 45°C, one milliliter from individually dilution was blend and dispensed into decontaminated Petri plates. The Petri plates were solidified and kept for 24 hrs in incubator (Mettler- Germany) at 37°C. Plates with countable range were used for counting the colonies (Hameed *et al.*, 2020).

Mould Determination

To determine the mould count in the samples Sabouraud Dextrose agar (SDA) Petri plates were produced and appropriately categorized for particular 10-fold consultative dilution. Using the sterile glass spreader 1mL was taken from each dilution which was spread on the agar surface. Succeeding the storage incubation time 72 hours for twenty-five-degree centigrade mould colonies were calculated (Hameed et al., 2020).

Statistical Analysis

Table-1: Microbiological Analysis of mixed vegetable salads.

Areas	Vendors	APC (CFU/g)	Coliform (CFU/g)	Fungal (CFU/g)
Board Bazar	Vendor-1	8×10^{7d}	3×10^{5b}	300 ^a
	Vendor-2	6×10^{7d}	4×10^{6c}	200 ^a
	Vendor-3	4×10^{7c}	2×10^{4a}	800 ^b
Hayat Abad	Vendor-4	1×10^{6b}	1×10^{5b}	400 ^a
	Vendor-5	3×10^{7c}	4×10^{5b}	600 ^a
	Vendor-6	1×10^{3a}	180 ^a	80 ^a
Kohat Road	Vendor-7	5×10^{8e}	2×10^{7d}	3300 ^c
	Vendor-8	3×10^{6b}	6×10^{5b}	800 ^b
	Vendor-9	2×10^{4a}	7×10^{3a}	340 ^a
Chargano	Vendor-10	8×10^{9f}	4×10^{6c}	1800 ^b
Chowak	Vendor-11	6×10^{3a}	2×10^{2a}	760 ^b
	Vendor-12	7×10^{8e}	7×10^{7e}	3324 ^c
General Bus Stand	Vendor-13	4×10^{9f}	6×10^{8f}	3000 ^c
	Vendor-14	5×10^{4a}	1×10^{2a}	320 ^a
	Vendor-15	6×10^{8e}	2×10^{7d}	1900 ^b
Kharhano Market	Vendor-16	3×10^{8e}	5×10^{7e}	2300 ^b
	Vendor-17	4×10^{5a}	3×10^{2a}	630 ^a
	Vendor-18	7×10^{8e}	4×10^{7e}	2600 ^c
Warsak Road	Vendor-19	6×10^{7d}	2×10^{7d}	2800 ^c
	Vendor-20	8×10^{8e}	3×10^{8f}	3600 ^c
	Vendor-21	9×10^{7d}	9×10^{6c}	470 ^a
Khyber Bazar	Vendor-22	1×10^{5a}	8×10^{2a}	380 ^a
	Vendor-23	4×10^{8e}	2×10^{8f}	3800 ^c
	Vendor-24	8×10^{6b}	7×10^{6c}	570 ^a
University Road	Vendor-25	2×10^{6b}	3×10^{5b}	760 ^b
	Vendor-26	9×10^{5a}	8×10^{5b}	367 ^a
	Vendor-27	3×10^{6b}	2×10^{6c}	870 ^b
Nair Bagh Road	Vendor-28	8×10^{7d}	6×10^{7e}	2900 ^c
	Vendor-29	2×10^{4a}	3×10^{2a}	120 ^a
	Vendor-30	6×10^{6b}	2×10^{4a}	765 ^b
Ring Road	Vendor-31	2×10^{9f}	8×10^{7e}	3300 ^c
	Vendor-32	9×10^{8e}	2×10^{6c}	3500 ^c
	Vendor-33	7×10^{6b}	4×10^{5b}	823 ^b

Results nominated with identical superscripts are non-significantly different ($p \geq 0.05$). CFU= colony forming unit, APC= aerobic plate count.

The variance analysis (ANOVA) was carried out at significant degree (0.05) applying the Statistical Package for Social Sciences (SPSS) 15.0 (Inc., Chicago, Illinois, USA).

Results

Mixed vegetable salads were collected from 11 different locations of Peshawar and evaluated based on mould count, coliform bacterial count and aerobic plate count. There was a significant difference between the microbial counts for the samples collected from varying sources of vendors (Table 1).

The APC ranged from 1×10^3 to 8×10^9 CFU/g. The highest APC was noted from the specimen collected from Chargano Chowak of Vendor-10 with value 8×10^9 CFU/g and Vendor-13 at General Bus Stand

4×10^9 CFU/g. The Vendor-6 of Hayat Abad had the lowest APC value of 1×10^3 CFU/g. based on the quality criteria in Table 2, 5 samples came out permissible, 4 were marginally permissible and 24 were not permissible based on APC. The results revealed that the microbiological quality was poor as several samples in the Board Bazar, Kohat Road, Chargano Chowak, General Bus Stand, Kharhano Market, Warsak Road, Khyber Bazar and Nair Bagh Road and Ring Road had APC counts above 10^6 CFU/g.

There was a wide range of the coliform counts being from 1×10^2 to 6×10^8 CFU/g. The highest coliform count was recorded in Vendor-13 at General bus stand (6×10^8 cfu/g) while the lowest count was recorded in Vendor-14 at General bus stand (1×10^2 cfu/g). On the basis of the criteria given in Table 2, 27 samples were classified as unacceptable and 6 as marginally acceptable. All samples were in the acceptable range of $\leq 10^2$ CFU/g coliform count. Samples collected from General Bus Stand, Khyber Bazar, Kohat Road,

Kharhano Market, Warsak Road and Ring Road had coliform contamination which was particularly high. The level of yeast/mold counts varied from 80 to 3800 CFU/g. The highest fungal count was observed in Vendor-23, Khyber Bazar (3800 CFU/g), followed by Vendor-20, Warsak Road (3600 CFU/g), and Vendor-12, Chargano Chowak (3324 CFU/g). The lowest fungal count (80 CFU/g) was found in Vendor-6 at Hayat Abad. From the Table 2, it is observed that 20 samples were acceptable ($\leq 10^3$ CFU/g) while 13 samples were above the acceptable limit and were considered as unacceptable. The microbiological acceptability thresholds is shown in Fig.1

The overall results showed that large percentage of the mixed vegetable salad samples were contaminated with APC and coliform bacteria with comparatively lesser contamination with fungi. When considering all three microbiological parameters, a significant percentage of the salads tested were not considered to be of good quality as defined in Table 2.

Table-2: *Microbiological Quality Evaluation of salads.*

Parameters	CFU/g	Status
Yeast/mold count	$\leq 10^3$	Acceptable
	$> 10^3$	Unacceptable
Coliform count	$< 10^2$	Acceptable
	$\geq 10^2 - \leq 10^3$	Marginally acceptable
	$> 10^3$	Unacceptable
Aerobic plate count	$< 10^5$	Acceptable
	$\geq 10^5 - \leq 10^6$	Marginally acceptable
	$> 10^6$	Unacceptable

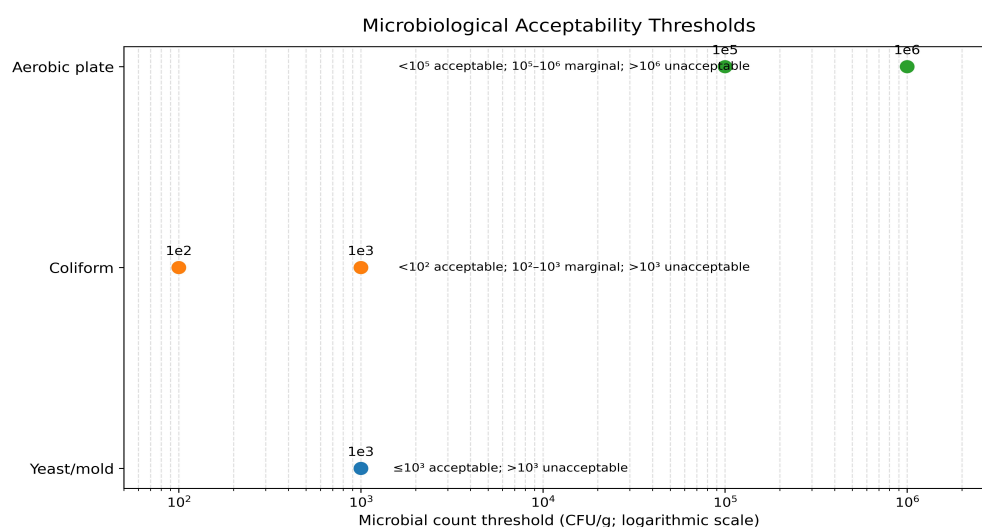


Fig.1 Microbial Acceptability Thresholds.

The findings showed that there were high variations in microbiological quality of the salads that were sold by

the vendors and those sampled from different areas with some salads being highly contaminated with

bacteria and fungi by the vendors and others being free of bacteria and fungi.

Discussion

It was noted that there were differences in the significance of differences between the sample collected from the greengrocer's market and the bazaars depending on the vegetable and the 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.) The potential for human disease from pathogens of edible plants is a serious problem. High levels of enteric pathogens can survive and grow on the surface. These dangerous microbes can cause fresh or lightly processed vegetables to cause food poisoning. Fresh vegetables and fruits can be contaminated at the production stage, during harvest, at the packing and distribution stages (Bartz, Wei, 2003). It was fact that vegetables and fruits are excellent foundations of functional foods may become polluted at any stage in the course of its final preparation, trade sale, supply, dispensation, harvesting and growth (USFDA, 2001). Generally, vegetables are cultivated in earth, manure and irrigated with bore and dam water. All of these are vital to the production of horticulture and can be sites of toxic microbes such as Salmonella and Escherichia coli (Wang *et al.*, 1996; Zhuang *et al.*, 1995). Trees (e.g., peaches, plums) can be a source of faecal microbes when harvesters and farmers in the cultivation fields come in contact with the fruit in the fields or harvesting and packaging equipment. The maximum index of coliform bacteria was reported in chilies, spinach, amaranthus and lucerene. While the fecal coliform bacteria were reported in maximum in gourd, maize and amaranthus. The highest count of streptococci was found in okra, maize and spinach. Minimum index of microbes was reported in gourd, luffa and bitter gourd (Alamgir *et al.*, 2016). The total aerobic plate amount CFU/g in uncooked/raw tomato, coriander, cucumber, carrot, cabbage, radish and blend vegetables samples were measured as 4.0×10^7 , 6.9×10^8 , 4.0×10^4 , 4.0×10^4 , 3.0×10^7 , 2.0×10^7 and 2.1×10^9 and the amount of coliform bacterial count were 3.0×10^8 , 3.5×10^5 , 5.0×10^3 , 3×10^4 , 9×10^2 , 5.2×10^3 and 3.2×10^7 respectively procured samples the city of Multan-Pakistan (Rabia *et al.*, 2014). Salads from road side vendors had the highest mean counts of bacterial (9.64), coliform bacterial amount (9.57) and mould amounts (2.51) (Hameed *et al.*, 2020). 315 street-

vended food samples were collected and 255 (81%) of the samples were contaminated with bacterial pathogens. Coliform bacteria were the most common contaminants found in 226 samples (88.6%), with Staphylococcus spp. and Salmonella/Shigella found in 20 samples each (7.9%). Asma *et al.* (2020) found that 100% of the rice, mixed vegetable salad and chicken soup were contaminated, while 86% and 80% of white kidney beans and chickpeas were contaminated, respectively. The foundations of coliform pollution could be H₂O utilized for rinsing instruments and vegetables. Coliform will definitely be passed on to human with uncooked food if the water is polluted with sewage water. The symptoms of diarrhea, abdominal pain, abdominal cramps, and nausea may be caused by presence of higher no. of coliform (Hameed *et al.*, 2020). The presence of faecal coliforms indicated that either there was Escherichia coli or faces pollution. Probable foundations of faces pollution may be practice of contaminated irrigation H₂O and compost putted to the field.

Nonetheless additional basis of contamination might be the treatment in the markets, specifically in the absence of good hygienic practices comprising pure H₂O for refreshment and vegetables and fruits rinsing (Amoah *et al.*, 2006). The other alarm area is the sanitary conditions of the fruits and vegetables sellers. Retailers are there from the beginning of the daytime till the end and can touch fruits and vegetables afterwards going to the washrooms without rinsing and cleaning their fingers which increase the hazard of contact with faeces. The peril may be created during merchandising accredited to the microbes spreading all through at the day time. Moreover, the vegetables and fruits (comprising extended food storage) globalization have led to a potent maximum menace of disease eruptions of fresh produce (Sivapalasingam *et al* 2004). The fruits and vegetables spread completed a higher area could lead to higher exposure to humans to a widespread range of food borne pathogens (USFDA 2001). When coliforms are present in high numbers, it is a sign of poor food handling or processing and is likely related to transmission of enteric pathogens (Hoonmo *et al.*, 2008).

The highest levels of total coliforms were found in parsley (3.9×10^6 CFU/g) and the lowest levels in cucumber (1.4×10^4 FU/g). The mean of the coliforms in the faeces was 5.3×10^2 CFU/g to 5.4×10^4 CFU/g (cucumber and parsley respectively). Of the samples of lettuce, cucumber, green onion and parsley, 6%, 5%,

4.8% and 4.5% contained *Salmonellae*, respectively. The presence of presumptive *Listeria monocytogenes* in parsley (44%), lettuce (12%) and green onion (8%) was observed (Ibrahim, 1996). Mesophilic and psychrotrophic microorganism counts of a entire of fifty six samples of vegetables (ready to consume) were screened. The microbial quality evaluation were carried out scheduled (monthly). The mesophilic counts (CFU/g) ranged from 4.7×10^5 in July to 1.6×10^8 in March. The counts of psychrotrophic microorganisms were from 7.9×10^6 to 2.7×10^8 CFU/g in the sample collected in July and January, respectively. Silvia *et al.*, 2007 have reported that higher temperature counts occurred after months of higher temperatures while lower temperatures led to lower counts for mesophilic and psychrotrophic microorganisms. The vegetables had high population of fecal coliform bacteria from geometric mean of 4.0×10^3 to 9.3×10^8 per gram wet weight of vegetables which are higher than the recommended level of 10^3 g^{-1} fresh weigh of vegetables (Amoah 2006). 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 should be made aware that fresh vegetables that are eaten may serve as a vector for contamination of the pathogens. The young and those with immune deficiency are known to be more susceptible to infection with bacteria, viruses and parasites than the others, and also pregnant women. Sanitation, stowage, washing, transport, finger interaction, hygienic production (cleanliness of labors, application at home, and biological control, farming chemicals, dung, compost, soil and H_2O) and environmental hygiene are vital to ensure food protection. After harvest washing of the vegetables not only removes the dust and dirt from the produce but also can be a cause of microbial contamination of the vegetables (Mehmet and Aydin 2008). It should be good enough in quality to make sure that it is not microbial contaminated for use in production, irrigation and washing.

It is important that good agricultural practices (GAP), good hygiene practices (GHP) and hazard analysis critical control points (HACCP) need to be carried out throughout the irrigation water in vegetable fields, from irrigation to harvesting, transportation, storage, sales and processing, as per (Blumenthal *et al.*, 2000; Anonymous, 2002). If it is possible to keep green vegetables in a cool place and in separate portions from other vegetables in the show, in addition to washing

them with clean water, it would be a good idea to reduce the bacterial content. To be able to be sold in a cold condition, vegetables need to be washed, cleaned and disinfected and then vacuum packed under modified atmospheric conditions, which extends the shelf life of vegetables and inhibits the growth of pathogens (Jacxsens *et al.*, 1999). The high levels of microorganisms may be due to a variety of factors including the use of impure water in the process of dilution (Lateef *et al.*, 2006). Microbial count is caused by the improper washing of the utensils, poor maintenance of the premises or domestic hygiene, the vegetables are peeled in advance, dust particles in the air and lack of good personal hygienic practices (Asma *et al.*, 2020). Food vendors tend to have more customers at the side of the busy road with a high traffic volume where the waste disposal system and the crowd of people also contribute to the contamination (Titarmare *et al.*, 2009). The presence of coliform in v ended foods could be a result of sewage water mixing with drinking water during food preparation, washing vegetables and food utensils (Asma *et al.*, 2020).

Conclusion

The microbiological quality of mixed vegetable salads from different vendors and sampling points was determined and it was seen that the quality is widely different from each other. Some samples, many samples, and a few samples met the criteria of being unacceptable, acceptable and marginally acceptable, respectively, in terms of counts of APC, Coliform and Fungi. Poor hygienic handling, poor sanitary condition and poor hygienic storage conditions can cause high microbial count. In general, the ready-to-eat salads could be a source of food safety concerns and the vendors need to practice good hygiene, use safe water, have proper handling, and storage procedures.

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