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Seasonal Variation in Microbial Contamination of Various Food Items in Karachi

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Abstract. The present study was carried out to investigate the effects of seasonal variation on the microbiological quality of various food items collected from Karachi, Pakistan. A total of two thousand food samples were collected during summer, winter, spring, and autumn season and analyzed for total bacterial count (TBC), total *Coliform* count (TCC), *Fecal coliform* count (FCC), mould and yeast count (MYC) and *Salmonella* spp. The highest percentage of unfit samples was recorded during summer *i.e*. 25.95%, while the lowest value 11.24% and samples were found unfit in terms of total bacterial count during winter. Furthermore, 24.25% of samples were unsatisfactory during the autumn season followed by spring *i.e*. 14.54%. Moreover, findings further demonstrated that MYC was observed higher in all seasons as compared to TBC. None of the samples was found positive for *Salmonella* spp.

Keywords: seasonal variation, microbial contamination, food commodities

Introduction

The basic right of every human being is healthy and nutritions food studied by (Ayala and Meier, 2017). Seasonal variations play a key role in the survival and multi-plication of microbes in food products (Nalepa *et al*., 2018; Suriyasathaporn and Nakprasert, 2012). Seasonal variability not only affects food quality but also the accessibility of food (Zimba *et al*., 2019). Consequently, there are major economic, public, social and environmental consequences. Several findings have shown that seasonal variation e.g. temperature and humidity affect food safety and also have negative effects impacts on local and international food trade (Ali *et al*., 2017).

In Pakistan seasonal pattern is characterised by four seasons, winter (December to February), spring (March to May), summer including rainy season or monsoon period (June to September) and autumn (October to November). The higher temperatures and humidity in summer support bacterial and fungal growth in food commodities (Koluman *et al*., 2017). In fact, for their growth and multiplication, different groups of micro-organism need different ranges of optimum temperature. It has been reported that bacteria, especially in food, prefer to grow in the temperature range between 32 to 43 °C (Mercier *et al*., 2017). In a recent study, it was found that the prevalence of some of the food diseases is higher in summer than in winter seasons (Gong *et al*., 2018; Burkart *et al*., 2011).

In fact, food losses caused negative impacts not only on food quality and safety but on economic development and the environment (Sheahan and Barrett, 2017). Unfortunately, in Pakistan, limited research has been done on food contamination and related topics. Therefore, it is an exigent need to identify those factors that can minimize food losses and have valid information on food quality and safety.

Materials and Methods

Two thousand food samples (raw, cooked, uncooked, and frozen) were collected from different industries of Karachi, Pakistan. They included herbs and spices (492), cereals and cereal products (463), Sugar and confectionaries (190), sauces, pulp and pastes (175), dry fruits (140), fruits and vegetables (fresh/frozen/processed) (119), ready to eat products (106), juices and syrups (68), fat, oil and dressings (52), meat and poultry (52), dry mixes (47), milk and dairy products (42), fish and

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other seafood (33), bakery products (13), egg and egg products (08) (Fig. 1). The samples were taken aseptically in labelled polyethylene bags and carried out for quantitative and qualitative microbiological examination to the laboratory. These samples were collected during a period of about 12 months (2015 to 2016). Frozen samples were stored at -20 °C for less than 24 h and perishable food samples were stored at 0 to 4 °C, while none perishable food samples were stored for less than 24 h at room temperature.

Sample preparation. About 50 g of each sample was placed in the blender jar and added 450 mL of sterile Butterfield's phosphate buffer (pH 7.0) and mixed well, for at least 2 min (this will be 1: 10 dilution). Powder samples were added in small portion, mixed thoroughly after each addition to homogenize the sample. Then serial dilutions were made by transferring of 10 mL of successive dilution to a universal bottle containing 90 mL of sterile Butterfield's phosphate buffer (pH 7.0). In according with specific standard methods described by BAM (Bacteriological Analytical Manual, online, 2006; USFDA, Chapter # 05) these suspensions were seeded in various culture media under incubation parameters. Standard methods were adapted for the detection of aerobic plate count (Andrews *et al*., 2006), mould and yeast count, *Coliform*, *Fecal coliform* (AFNOR/NF BIO 12/20-12/06) and *Salmonella* (ISO 6579:2002; AFNOR BIO 12/01-04/94 protocol).

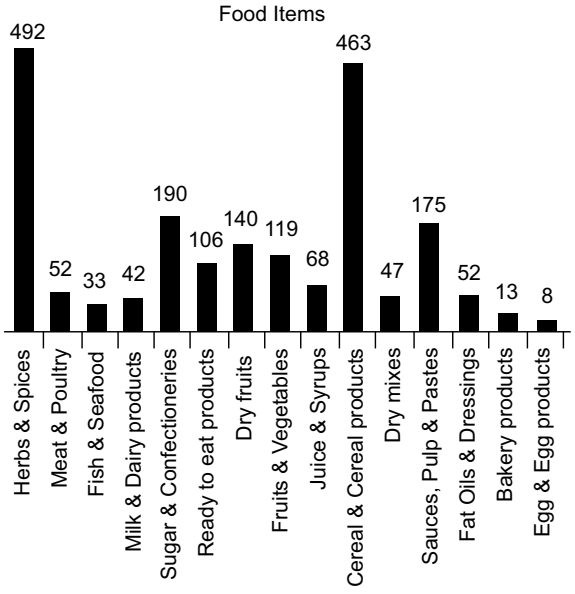

Fig. 1. Frequency of food products analyzed in the present study.

Total bacterial count (TBC). TBC was determined by pour plate method, briefly 1 mL portion from three dilutions *i.e*. 1:10, 1:100 and 1:1000 were transferred to the respective sterile petri plates. Plate Count Agar (Oxoid) was then poured in each petri dish and incubated at 35 °C for 48 h. After incubation colonies were counted in plates having colonies in the ranges of 25-250. The observed values in cfu/mL. The blank control was un-inoculatd plate count agar plate (Smith and Townsend, 1999).

Total *Coliform* count (TCC) and *Fecal coliform* count (FCC). MPN method was employed for the estimation of TCC and FCC. An aliquot of 1 mL of each dilution (1:10, 1:100 and 1:1000) was placed into three sets of 10 mL Lauryl Sulphate Broth (Merck) containing inverted Durham's tubes. These were incubated over 24 and 48 h at 35±0.5 °C. After incubation period tubes were observed for turbidity and gas production. For the confirmation of *Coliform*, tubes with gas and turbidity were sub-cultured into Brilliant Green Lactose bile broth (Merck) and incubated at 35±0.5 °C for gas production at 48±2 h. For the confirmation of *Fecal Coliform*, 10 mL of EC broth (Oxoid) with inverted Durham's tubes was inoculated by a loopful of each suspension. Inoculated tubes were incubated at 44.5±0.2 °C for 24±2 h and examined for gas output (Reddy *et al*., 2009). Total *Coliform* and *Fecal coliform* were calculated from MPN Tables. The blank control was Lauryl Tryptone broth (LTB) (Oxoid). The test tubes with *Escherichia* (E) *coli* were used as a positive control, while *Staphylococcus (S) aureus* were used as negative control.

Enumeration of mould and yeast. 1 mL from the dilutions *i.e*. 1:10, 1:100 and 1:1000 was placed in each petri dish followed by pouring and mixing Dichloran Rose Bengal Chloramphenicol Agar (Merck). After solidification, plates were incubated at 25 °C for 5-7 days. The *Candida* or *Aspergillus niger* were used as a positive control, while *Staphylococcus aureus* were used as negative control (Spangenberg and Ingham, 2000).

Detection of *Salmonella*. The 25 g of homogenized sample was transferred aseptically in a 225 mL of sterile Lactose broth (Merck) and incubated for 24±2 h at 35 °C. After incubation, 0.1 mL of the pre-enriched culture then transferred to 10 mL of Rappaport-Vassiliadis (Merck) medium and again incubated for 24±2 h at 42±0.2 °C. In analogous, 1 mL of pre-enriched culture was inoculated into 10 mL of Tetrathionate Broth (Merck) and incubated for 24±2 h at 43±0.2 °C. Further isolation was carried out on Bismuth Sulphite Agar

(Merck), Xylose Lysine Desoxycholate Agar (Merck) and Hektoen Enteric Agar (Merck). The plates were incubated at 35 °C for 24±2 h and were observed in normal *Salmonella* colonies. For identification, biochemical tests were also carried out. Mini Vidas analyser (bio Merieux) was used for the detection of *Salmonella* antigens. Confirmatory testing was performed by (Kebede *et al*., 2016). For *Salmonella* detection, *Salmonella typhi* were used as a positive control, while *Staphylococcus aureus* or *E. coli* were used as negative control.

Statistical analysis. Mean values were calculated from three replicates. The observed data subjected to analysis of variance and *t*-test by using software (IBM SPSS STATISTICS 20). Significance was considered at P = <0.05.

Results and Discussion

To determine microbial contamination a total of 2000 food samples were collected during different seasons from Karachi region, in summer season 678 samples collected and examined. 176 samples were found highly contaminated and 26 samples for TBC, 78 samples for TCC, 25 samples for FCC and 47 samples for MYC. In autumn season, the total 503 food samples were collected and analysed from which 122 samples were unfit for human consumption as per PS shown in (Table 1), the 16 for TBC, 57 for TCC, 16 for FCC and 33 for MYC. While *Salmonella* spp. were not detected in any sample. During spring season, 330 food samples were collected and analysed. Out of these, 48 samples were declared as unacceptable and 9 samples for TBC, 19 samples for TCC, 9 samples for FCC and 11 samples for MYC. *Salmonella* spp. were also found absent. During winter season 489 food samples were collected and examined, total 55 samples were recorded as unsatisfactory, while 9 for TBC, 20 for TCC, 8 for FCC and 18 for MYC, whereas none of the sample was found to carry *Salmonella* spp. shown in Table 2.

Data clearly showed that the summer season (25.95%), the highest percentage of unsatisfactory samples was found, where herbs and spices commodities were fall into highest percentage of contamination then dry mixes and cereal and cereal products, followed by autumn (24.25%), spring (14.54%) and winter (11.24%). The growth and survival of micro-organisms and temperature fluctuations have already been reported to be directly proportional to each other (Tshikantwa *et al*., 2018). Comparatively higher counts were observed in summer (25.95%) and autumn (24.25%), while lower counts were observed in spring *i.e*. 14.54% and in winter 11.24% (Fig. 2). Statistically, a significant difference (P<0.05) was observed between level of contamination in food samples collected during summer and winter seasons. Various researchers from other parts of the world have also claimed the effect of seasonal variation on the microbiological quality of food items (Nalepa *et al*., 2018; Denis *et al*., 2016; Akil *et al*., 2014; Eltigani *et al*., 2013; Suriyasathaporn and Nakprasert, 2012).

Previously reported studies (Denis *et al*., 2016; Hammond *et al*., 2015) found a higher incidence rate of microbial contamination in food products during the summer compared to the winter season. The same pattern has also been noticed in the present study which suggested that high microbial load in summer could may be due to availability of favourable environmental conditions such as temperature and humidity. In another study, it has been documented that higher ambient air temperature is directly associated with increased incidence of food spoilage (Dominianni *et al*., 2018; Moh *et al*., 2017). For instance, in Karachi summer season (June to September) has been characterized by temperature in between 30-35 °C along with heavy rainfall. Therefore, combination of warm weather and sufficient humidity support the growth of variety of microbes.

Findings of the current study revealed the lowest percentage of unsatisfactory food samples during winter season. Like other regions winter season has been characterized by low temperature, humidity and precipitation in Karachi. It has been reported that low temperatures during winter season is a limiting factor for variety of microbes (Kim and Ndegwa, 2018). The findings by (Duvenage and Korsten, 2016; Ashenafi, 2012) also justified that low temperature during winter season negatively affects microbial growth.

It has been noticed that the number of samples contaminated with MYC was higher in all seasons as compared to the number of samples contaminated with bacteria. It is evident in Fig. 3 that in summer 6.93% samples were found positive for MYC, while 3.83% for bacteria. Likewise, 6.56% samples were found positive for MYC, whereas 3.18% for bacteria in autumn. In winter 3.68% and 1.84% samples were found positive for MYC and bacteria respectively and in spring moulds and yeasts and bacteria were detected in 3.33% and 2.72% samples respectively. Similar findings were also reported by Snyder and Worobo (2018), suggested that since fungal spores rapidly dispersed *via* air and water and survive over a wide range of environmental conditions. Moreover, some fungal species persist under

Table 1. Standard limits for food commodities as per Pakistan standards (PS)

Food items description	Pakistan standard limits (cfu/g or mL)									Pakistan standard Reference no.
	TBC			TCC			MYC			
	S	b	U	s	B	U	s	b	U	
Herbs and spices	10^4	$>10^4$ - $<10^6$	$>10^6$	10^2	$>10^2$ - $<10^3$	10^3	10^2	$>10^2$ - $<10^4$	10^4	3741-1996
Milk and dairy products	103	$>10^3$ - $<10^4$	$>10^4$	10^2	$>10^2$ - $<10^3$	10^3	10	>10 - $<10^2$	10^2	2835-1990, 363-1991, 2832-1-1990, 2027-1988
Chocolate confectionaries	10^3	$>10^3$ - $<10^6$	10^6	1.8	>1.8 - $<10^2$	10^2	10^2	$>10^2$ - $<10^3$	10^3	4715-2001, 4716-2001, 4557-2000/5243
Sugar confectionaries	10^4	$>10^4$ - $<10^6$	10^6	1.8	>1.8 - $<10^2$	10^2	10	>10 - $<10^2$	10^2	4717-2001
Egg & egg products	2.5×10^4	$>10^4$ - $<10^5$	10^5	10	>10 - $<10^3$	10^3	10	>10	-	FDA Circular 2013-010
Flour	10^2	$>10^2$ - $<10^5$	10^5	10	>10 - $<10^2$	10^2	10^2	$>10^2$ - $<10^4$	10^4	1931-2013
Baked goods	10^4	$>10^4$ - $<10^6$	10^6	50	>50 - $<10^3$	10^3	10^2	$>10^2$ - $<10^4$	10^4	761-2001, 382- 1964/ PS 4840
Chicken meat	5×10^3	$>10^3$ - $<10^7$	10^7	10	>10 - $<10^2$	10^2	---	---	---	4726 2001
Meat	10^4	$>10^4$ - $<10^5$	10^5	10	>10 - $<10^2$	10^2	---	---	---	2861-1990, 2826- 1990, 2827-1990, 2988 -1991,
Fats, oil and dressings	10	>10 - $<10^2$	10^2	---	---	---	10	>10 - $<10^2$	10^2	358-1997/2858,
Cereal and cereal grains	10^2	$>10^3$ - $<10^6$	10^6	10^2	$>10^2$ - $<10^4$	10^4	10^2	$>10^2$ - $<10^4$	10^4	1931-2013, 3342-1993, 154-1962
Dry mixes	10^4	$>10^4$ - $<10^6$	10^6	10	>10 - $<10^3$	10^3	10^2	$>10^2$ - $<10^4$	10^4	FDA Circular 2013-010
Dried fruits	$<10^5$	$>10^5$ - $<10^6$	10^6	3	>3 - <11	11	10^2	$>10^3$ - $<10^4$	10^4	1689-1985/2013
Cooked food/ready to eat	$<10^4$	$>10^4$ - $<10^5$	$>10^5$	10	>10 - $<10^2$	10^2	10	>10 - $<10^3$	10^3	FDA Circular 2013-010
Fruits and vegetables	---	---	---	3	>3	>10	10^2	$>10^2$ - $<10^4$	10^4	3945-1997, 510-1996, 520-1999, 4563-2000, 1923- 1987, 4569-2000
Juices	102	$>10^2$ - $<10^5$	105	01	>1	>10	10	>10 - <50	50	527-1992
Syrups	104	>104	5×104	10	>10	10^2	-	-	-	3114-1991
Sauces, pulp and pastes	10	>10 - $<10^2$	10^2	10	>10 - $<10^3$	10^3	10	>10 - $<10^2$	10^2	3947-1997, 512-1964
Fish and other seafood	5×10^5	$<10^5$ - $<10^7$	10^7	11	>11	500	---	---	---	2834

Table 2. Data showing microbiological analysis of food samples with respect to seasonal variation

Seasons	n	Microbiological parameters									
		TBC		TCC		FCC		MYC		*Salmonella*	
		U	f	u	F	u	f	u	f	u	f
Summer, Jun.-Sep.	678	26	652	78	600	25	653	47	631	00	678
Autumn, Oct.-Nov.	503	16	487	57	446	16	487	33	470	00	503
Winter, Dec.-Feb.	489	09	480	20	469	08	481	18	471	00	489
Spring, Mar.-May	330	09	321	19	311	09	321	11	319	00	330

Key: n = total no. of samples analysed; u = unfit samples; f = fit samples; TBC = total bacterial count; TCC = total *Coliform* count; FCC = *Fecal coliform* count; MYC = mold and yeast count.

the most extreme physico-chemical processing employed in commercial food production. Anwer *et al*. (2017) also supported the significant role of fungi in the spoilage of various processed foods. It is probably due to the proliferation of fungi in foods containing limited moisture content.

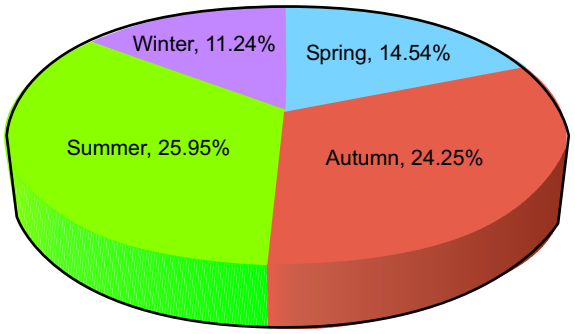

Fig. 2. Frequency of unsatisfactory samples in terms of seasonal variation.

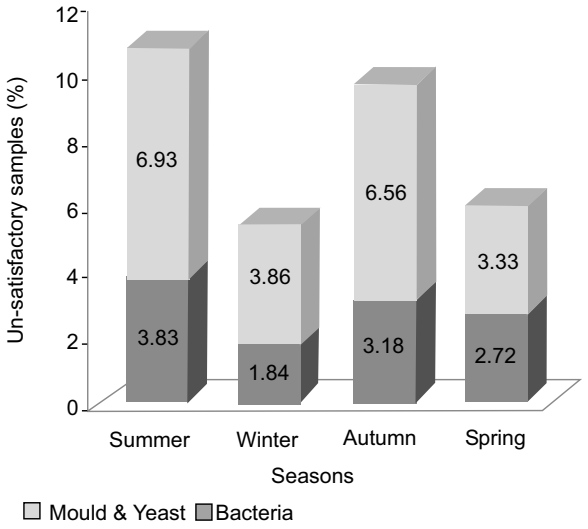

Fig. 3. Bars showing occurance of bacterial and fungal contamination in food samples during four seasons.

Keeping in view, the above findings it has been verified that the seasonal variation significantly affect microbial growth and multiplication. In odour to keep an eye on rising economical and health risks in terms of food preparation and consumption, this baseline study will provide sufficient data to step ahead the issues of food quality and safety.

Conflict of Interest. The authors declare no conflict of interest.

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