



Seasonal Variation of the Microbial Quality of Local Vegetables in Giresun, the Northern Province of Turkey

Atnan Uğur^{1,a,*}, Hilal Yıldız^{2,b}, Olcay Kavgacı^{3,c}

¹Department of Horticulture, Faculty of Agriculture, Ordu University, 52200 Ordu, Turkey.

²Department of Food Engineering, Faculty of Engineering and Architecture, Nevşehir Hacı Bektaş University, 50300 Nevşehir, Turkey.

³Provincial Food Control Directorate, 28100 Giresun, Turkey.

*Corresponding author

ARTICLE INFO

ABSTRACT

Research Article

Received : 23/09/2021

Accepted : 05/11/2021

Keywords:

Consume
Contamination
Health
Bacillus cereus
Salmonella spp

Consumption of vegetables, having a significant place in nutrition of humankind, is increasing day by day as their health-improving effects have been better understood nowadays. The main threat to food safety is pathogenic bacteria that cause several foodborne outbreaks. The microbial quality of vegetables are affected by certain criteria such as organic production, season production, regional production. The objective of this study is to examine the presence of *Bacillus cereus* and *Salmonella* spp. in the vegetables sold in markets grown locally in different seasons. The 89 samples, including parsley, lettuce, spinach, turnip, carrot, chard, Brussels sprouts and radish produced in the province of Giresun were analyzed in the study. The presence of *Bacillus cereus* and *Salmonella* spp. was determined according to the International Organization for Standardization procedure. *Salmonella* spp. was not determined in any of the analysed samples. On the other hand, *B. cereus* was detected in 84 samples with microbial load ranging between 1.0×10^2 and 4.9×10^4 CFU/g. Among the samples, the highest *B. cereus* contamination was observed in lettuce with a 3.1×10^3 CFU/g. The results showed that *B. cereus* counts varied by seasons, and the highest count was obtained from the samples grown in spring seasons. Determination of the sources of contamination for the vegetables in pre-harvesting and post-harvesting periods, provides information about the condition of production. The results will help raise the awareness of the farmers about the contamination risks from production to marketing for the prevention of microbial contamination.

^a atnanugur@gmail.com
^c olcay-mavi@hotmail.com

^{ID} <https://orcid.org/0000-0001-6015-3146>
^{ID} <https://orcid.org/0000-0002-9954-220X>

^b h09yildiz@gmail.com

^{ID} <https://orcid.org/0000-0002-7966-455X>



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Introduction

Vegetables are plants of which different parts such as roots, stems, leaves, bears, flowers, seeds and shoots have been consumed since the early ages. In time, many of them were cultivated depending on needs and consumption. Though different factors affect vegetable consumption, vegetable production and consumption cultures have developed based on ecologies (Ugur et al., 2014). Nowadays, the main goal of vegetable consumption is to satisfy the need for carbohydrates, vitamins and minerals of humans. In addition to their essential nutrients, increasing and popularizing consumption of vegetables are considered to be a viable strategy due to their mineral and secondary metabolite content (Barminas et al., 1998; Sevindik et al., 2017; Pehlivan et al., 2021). Pulses, thanks to their high protein content, are included in diets due to the limited means of access to meat and meat products (Pekşen and Artık, 2005). Fresh vegetables are rich in both water-soluble vitamins and other nutrients required for

improving nutritional status and reducing the risk of cardiovascular diseases (Alissa and Ferns, 2017). Moreover, it is stated that fresh vegetables are effective for prevention of many chronic diseases and ailments thanks to their rich content of polyphenols, carotenoids, alkaloids, flavonoids, secondary metabolites and antioxidants (Steinmetz and Potter, 1996; Baslam et al., 2011; Mohammed et al., 2020). It is observed that many diseases and outbreaks occur in regions with limited vegetable consumption because of malnutrition and unhygienic living conditions (Ignarro et al., 2007; Ronto et al., 2018).

Various economic, sociological and ecological factors are influential on vegetable consumption (Ugur et al., 2014). Despite ecological factors' drawing interest as the most effectual ones, we find different forms of vegetable consumption in different societies due to cultural factors. On the other hand, socioeconomic factors affect the type and quantity of the vegetables consumes. As low-income

families generally consume vegetables for their needs of nutrition/energy, potato consumption is more common in such families. In high income families, vegetables are considered as means of diversifying the diet and supermarkets and greengrocers are preferred despite selling vegetables for higher prices (Uğur et al., 2014). As the level of awareness and income of societies has increased, vegetable consumption has been regarded as a way of healthy nutrition instead of an activity for putting on the feed bag and nutritional and bioactive agent contents of the vegetables have been started to be taken into consideration (Baslam et al., 2011; Yen and Tan, 2012). Furthermore, factors such as organic production, season production, being hormone-free, being locally-grown products and packaging/labelling have gained significance while assessing the production and marketing process of the vegetables (Mie et al., 2017). Locally-grown products have been preferred for reasons such as compatible with traditional appetite, freshness and non-use/limited use of synthetic inputs (Avgoustaki and Xydis, 2020). However, use of animal manure instead of chemical fertilizers in local vegetable growing and the possibility of use of pathogenic water mixed with sewage water may pose a risk in terms of safety of such products (Beuchat and Ryu, 1997). Pathogenic bacteria in both the natural flora of the soil and intestinal system of humans and animals such as *Clostridium botulinum*, *B. cereus*, *Listeria monocytogenes* and *Salmonella* spp., *Shigella* spp., *E. coli*, *Campylobacter* spp. as well as parasites may contaminate the raw vegetables (Bergwerff and Debast, 2021). In raw products, contamination with such pathogenic microorganisms may occur throughout the entire food transport and processing procedure from the farm up until the consumption (Zhao et al., 2009). Microbial contamination may also arise during the processing and packing procedures following the harvesting. Moreover, the raw vegetables may be exposed to pathogenic contamination when not cooked carefully and may be hazardous to human healthy especially when they are consumed raw (Balali et al., 2020). Therefore, food safety is sought for the products consumed and it is expected to minimize the possible dangers and risks associated with the raw vegetables from their harvesting till their consumption by the end user within the frame of the applicable legislations and standard measures determined by the authorities (Gil et al., 2015). It is aimed to bring the infections related with food to a level that will not harm the public health and/or totally eliminate such infections (Giray and Soysal, 2007). *Salmonella* spp. and *B. cereus* are significant bacteria that may be infected with food and endanger public health. *Salmonella* spp. are moving, asporous, unencapsulated gram negative bacteria in the form of basil. *Salmonella* spp. may colonize in gastrointestinal system of numerous animals including all mammals, birds and insects (D'aoust and Maurer, 2007). *Salmonella* spp. may reproduce greatly in 24 hours under a temperature of 37°C. They can live for a long time in humid environments out of sunlight, sewage water, well water and soil. Infection generally happens by consuming the water and food infected by these bacteria. *Salmonella* infections occur through oral rote with contaminated food and water (Kenney, 2019). Being protected from *Salmonella* requires caring for personal hygiene rules,

consuming clean water and food, eradicating the carriers and installing proper sewage system (Yücel, 2020).

B. cereus is a food-originated pathogen generally contaminating the vegetative food (Valero et al., 2003). *B. cereus* can be found in naturally-decomposing organic matters, fresh water and sea water, vegetables and intestinal flora of different animals. Due to viscid nature of endospores, *B. cereus* may hold on to food production environments frequently. It can infect different types of food such milk and dairy, vegetables, rice and rice food, meat and meat products, soups and spices (Kumari and Sarkar, 2016; Organji et al., 2015). Food poisoning associated with *B. cereus* may be observed throughout the whole globe in any season. Its prevalence is closely associated with preparing and storing food under improper conditions. Consumption of food containing 10^6 CFU *B. cereus* per gram is sufficient for development of clinical picture. *B. cereus* produces toxins in the body upon consumption of infected food and may lead to two gastrointestinal diseases namely emetic (vomiting) and diarrhea (Kim et al., 2010). When emetic toxin (cereulide) is produced in food, the infected person vomits after swallowing the contaminated food. Diarrhea syndrome arises when enterotoxins are produced in bowels following swallowing of food contaminated with *B. cereus* (Kumari and Sarkar, 2016). And this threatens the human life. The objective of this study is to identify the microbial contamination in the vegetables grown in the province of Giresun, the Northern Province of Turkey, and offered in local bazaars by the farmers.

Materials and Methods

Research Area

The research area lies between 37° 50' - 39° 12' east longitudes and 40° 07' - 41° 08' north latitudes in the province of Giresun. The coastal region of the province is formed of high-slope valleys split by streams. The settlement is dense on the small planes at the mouths of the streams. Population density is lower in mountainous areas and the population mostly deals with plant growing and livestock. Hazelnut is the most significant agricultural product and vegetables are grown on small yards with traditional methods by using local seeds to contribute in household economy. Such vegetables grown by women are also offered by them in local bazaars. Use of animal manure is common for vegetable growing and open system irrigation (surface irrigation) is preferred. There is no sewage system in mountainous areas.

Material

Total 89 vegetable samples grown in the province of Giresun and offered in local bazaars [parsley (28), lettuce (38), spinach (11), turnip (4), carrot (2), chard (2) radish (2) and Brussels sprouts (2)] form the main material of the research. No sampling was performed for the vegetables. Samples were taken from the vegetables offered in local bazaars in every season in every fifteen days. The samples were kept at 0-5°C and analyzed in 24 hours. In the samples of parsley, lettuce and spinach, bacteria were detected in autumn, winter and spring but they were only detected in autumn for the samples of turnip, radish, carrot, Brussels sprouts and chard.

Methods

To determine the microbial load in vegetable samples, *Salmonella* spp. and *Bacillus cereus* bacteria were detected.

Detection of *Salmonella* spp. in Vegetables

25 g of vegetable samples were homogenized with Buffered peptone water (225 mL) under aseptic conditions and then incubated for 18±2 hours at 37±1°C. After incubation, 1 mL of pre-enriched samples was added into the tubes containing 10 ml Rapaport Vassiliadis Soy Medium (RVS) and incubated for 24±3 hours at 41.5±1°C. 10 mL of pre-enriched samples were added into the bottles containing 100 mL of Muller-Kauffmanbroth Novobiocin Broth (MKTTn) and the bottles were incubated for 24±3 hours at 37±1°C. and for 24±3 hours at 37±1°C. Following the incubation, a streaking loop was dipped into the mediums of RVS and MKTTn separately and used to streak onto Xylose-Lysine-Desoxycholate Agar (XLD agar) and Chromogenic *Salmonella* agar plates. The plates were incubated for 24±3 hours at 37±1°C. The typical colonies grown on XLD agar and Chromogenic *Salmonella* agar were picked up and inoculated on Blood agar and Nutrient agar. Typical colonies of Chromogenic *Salmonella* agar were purple – dark pink, 1mm and smooth and colonies of XLD agar had a black center and slightly transparent reddish zones due to colour change of the indicator. Then, petri dishes incubated for 24±3 hours at

37±1°C. Following the incubation, the colonies multiplied on Blood agar and XLD agar were verified by gram negative kits of Vitek 2 system (National Standards Authority of Turkey, 2020).

Detection of *Bacillus cereus* in Vegetables

10 g of samples were taken from the vegetables under aseptic environment and transferred to stomacher bags containing 90 ml Maximum Recovery Diluent (MRD) and homogenized. A serial ten-fold-dilution was performed by pipetting. 0.1 mL was taken from the appropriate dilutions and inoculated on chromogenic agar (Bacara) using the surface spreading method. The plates were incubated for 22±2 hours at 30±°C and the orange colonies and those with surrounding white zones were counted (National Standards Authority of Turkey, 2009).

Analysis of the Data

No advanced statistics were used in the study, only the arithmetic mean of the samples according to the harvest season of the vegetables was taken. The measurements were taken using the spectroradiometer.

Results

In the study, we examined presence of *Bacillus cereus* and *Salmonella* spp. in 89 vegetable samples grown by the female farmers in autumn, winter and spring.

Table 1. Existence of *Bacillus cereus* in parsley

Harvest season	Sample no	<i>Bacillus cereus</i> (CFU/g)
Autum	Sample 1	1.0×10 ²
	Autum mean	1.0×10 ²
Winter	Sample 1	<10
	Sample 2	1.0×10 ²
	Sample 3	1.0×10 ³
	Sample 4	3.0×10 ²
	Sample 5	1.0×10 ²
	Sample 6	1.3×10 ³
	Sample 7	5.0×10 ²
	Sample 8	1.0×10 ²
	Sample 9	4.0×10 ²
	Sample 10	<10
	Sample 11	4.0×10 ²
	Winter mean	3.5×10 ²
Spring	Sample 1	2.0×10 ²
	Sample 2	1.6×10 ³
	Sample 3	7.0×10 ²
	Sample 4	1.0×10 ²
	Sample 5	7.0×10 ²
	Sample 6	2.0×10 ²
	Sample 7	9.0×10 ²
	Sample 8	1.0×10 ²
	Sample 9	<10
	Sample 10	<10
	Sample 11	5.0×10 ²
	Sample 12	2.0×10 ²
	Sample 13	7.0×10 ¹
	Sample 14	6.0×10 ¹
	Sample 15	3.0×10 ³
	Sample 16	2.0×10 ²
	Spring mean	5.3×10 ²
General Mean		4.6×10 ²

Table 2. Existence of *Bacillus cereus* in lettuce

Harvest season	Sample no	<i>Bacillus cereus</i> (CFU/g)
Autum	Sample 1	8.0×10^2
	Sample 2	4.0×10^2
	Sample 3	7.0×10^2
	Sample 4	1.4×10^3
	Sample 5	1.4×10^3
	Sample 6	2.0×10^2
	Autum mean	8.2×10^2
Winter	Sample 1	6.0×10^2
	Sample 2	7.0×10^2
	Sample 3	4.0×10^3
	Sample 4	2.3×10^3
	Sample 5	9.0×10^2
	Sample 6	1.2×10^3
	Sample 7	7.0×10^2
	Sample 8	2.8×10^3
	Sample 9	4.0×10^2
	Sample 10	4.0×10^2
	Sample 11	4.8×10^3
	Sample 12	9.0×10^2
	Sample 13	1.5×10^3
	Sample 14	1.7×10^3
	Sample 15	6.0×10^2
	Sample 16	6.0×10^2
Winter mean	1.5×10^3	
Spring	Sample 1	1.9×10^3
	Sample 2	2.7×10^3
	Sample 3	4.7×10^3
	Sample 4	1.2×10^3
	Sample 5	9.0×10^2
	Sample 6	4.0×10^2
	Sample 7	<10
	Sample 8	1.2×10^3
	Sample 9	1.1×10^3
	Sample 10	4.0×10^2
	Sample 11	4.0×10^2
	Sample 12	1.2×10^3
	Sample 13	1.7×10^4
	Sample 14	1.9×10^3
	Sample 15	6.0×10^3
	Sample 16	4.9×10^4
Spring mean	5.6×10^3	
General mean		3.1×10^3

Table 3. Existence of *Bacillus cereus* in spinach

Harvest season	Sample no	<i>Bacillus cereus</i> (CFU/g)
Autum	Sample 1	9.0×10^2
	Sample 2	1.0×10^2
	Sample 3	1.0×10^2
	Autum mean	3.7×10^2
Winter	Sample 1	7.0×10^2
	Sample 2	2.0×10^2
	Sample 3	4.0×10^2
	Sample 4	3.4×10^2
	Sample 5	3.0×10^2
Winter mean	3.9×10^2	
Spring	Sample 1	3.0×10^2
	Sample 2	1.2×10^3
	Sample 3	4.3×10^2
	Spring mean	6.4×10^2
General mean		4.5×10^2

Table 4. *Bacillus cereus* numbers in parsley, lettuce and spinach by seasons

Harvest season	Minimum values (CFU/g)	Maximum values(CFU/g)	Mean values(CFU/g)
Autum	1.0×10^2	1.4×10^2	6.1×10^2
Winter	< 10	4.8×10^3	9.5×10^2
Spring	< 10	4.9×10^4	2.9×10^3

Table 5. Existence of *Bacillus cereus* in turnip, radish, carrot, brussels sprouts and chard

Vegetables	Sample no	<i>Bacillus cereus</i> (CFU/g)
Turnip	Sample 1	3.0×10^2
	Sample 2	5.0×10^2
	Sample 3	4.2×10^2
	Sample 4	7.0×10^2
	Mean	4.8×10^2
Radish	Sample 1	4.0×10^2
	Sample 2	4.0×10^2
	Mean	4.0×10^2
Carrot	Sample 1	5.2×10^2
	Sample 2	4.3×10^2
	Mean	4.8×10^2
Brussels sprout	Sample 1	4.0×10^2
	Sample 2	2.0×10^2
	Mean	3.0×10^2
Chard	Sample 1	2.0×10^2
	Sample 2	3.2×10^2
	Mean	2.6×10^2

Existence of *Bacillus cereus* in Vegetables

Existence of Bacillus cereus in Parsley

The data regarding detection of *Bacillus cereus* in parsley were given in the Table 1. In two parsley samples, *B. cereus* could not be detected in winter and spring. In autumn, 1.0×10^2 CFU/g bacteria were detected in only one sample. The highest bacteria level was measured to be 1.3×10^3 CFU/g and 3.0×10^3 CFU/g for the parsleys of winter and spring respectively. Considering existence of *Bacillus cereus* in parsley, the ranking was as following: autumn<winter<spring. *B. cereus* number detected in parsley is 4.4×10^2 CFU/g adjusting by average of all samples (Table 1).

Existence of Bacillus cereus in Lettuce

The data regarding detection of *B. cereus* in lettuce were given in the Table 2. *B. cereus* numbers varying in between 4.0×10^2 and 4.9×10^4 was detected in all lettuce samples except for the one taken in spring. *B. cereus* ranking by seasons was as following: autumn<winter<spring. The highest bacteria level was measured to be 5.6×10^3 CFU/g for the lettuce samples of spring. Average *B. cereus* number of lettuce samples were calculated to be 3.1×10^3 CFU/g (Table 2).

Existence of Bacillus cereus in Spinach

The data regarding detection of *B. cereus* in spinach were given in the Table 3. *B. cereus* numbers in spinach varied in between 1.0×10^2 and 1.2×10^3 CFU/g by seasons. The lowest *B. cereus* number of 3.7×10^2 CFU/g was detected in autumn and the highest level of 6.4×10^2 CFU/g was detected in spring according to average values. The highest value in the spinach samples in terms of *B. cereus* number was 1.2×10^3 and it was detected in spring (Table 3).

Existence of Bacillus cereus in Parsley, Lettuce and Spinach by Seasons

Minimum, maximum and average *B. cereus* numbers detected in the samples of parsley, lettuce and spinach by seasons were given in the Table 4. *B. cereus* numbers varied by seasons. While the highest bacteria number of 2.9×10^3 CFU/g was detected in spring, the lowest figure of 6.1×10^2 CFU/g was detected in autumn (Table 4).

Existence of Bacillus cereus in Turnip, Radish, Carrot, Brussels Sprouts and Chard

Sampling was only performed in autumn for Turnip, Radish, Carrot, Sprouts and Chard and the *B. cereus* numbers in these samples were given in the Table 5. It was identified that the *B. cereus* number in turnip, radish, carrot, brussels sprouts and chard was 2.0×10^2 - 7.0×10^2 CFU/g in autumn. Turnip and carrot yielded the similar values of 4.8×10^2 CFU/g in terms of average figures. Similarly, it was found out that the chard samples had the lowest *B. cereus* number of 2.6×10^2 CFU/g in this vegetable group (Table 5).

Discussion

Existence of *Salmonella* spp. and *B. cereus* were researched to determine the microbial quality of parsley, lettuce, spinach, turnip, carrot, chard, Brussels sprouts and radish offered in local bazaars in autumn, winter and spring. Although high level of bacteria detected in the vegetables does not imply existence of threat against human life, it can give us an idea about the sanitation conditions of the vegetables during the production and marketing process. *Salmonella* spp. infection is generally observed in areas which do not have a sewage system and poultry poop is mixed into the irrigation water. *Salmonella*

spp. could not be detected in 89 vegetable samples analysed in our study. When the microbial load of 162 vegetable samples served raw in Brazil was analysed, *Salmonella* spp. was detected only in two wild chicory samples (1.2%) (De Oliveira et al., 2011). *Salmonella* spp. was detected in 7.5% of 345 leaf vegetables put in sale in Spain (Ruiz et al., 1987). Considering the vegetables in terms of existence of *B. cereus*, the number of this bacterium was counted to be <10 in five samples analysed, and the *B. cereus* numbers varied in between 1.0×10^2 and 4.9×10^4 CFU/g in remaining 84 samples. Considering the existence of *B. cereus* in terms of the sampling seasons, spring yielded the highest figures in terms of both average and maximum values. Spring had the highest *B. cereus* number of 2.9×10^3 CFU/g in terms of average values. Enan et al. (2012), in their study examining the existence of *B. cereus* by seasons in meat, corn snacks and cheese stated that the highest and lowest values were obtained in summer and spring and autumn respectively. An increase in the *B. cereus* values of the products was observed under higher temperatures. Sampling was not carried out in summer in our study but spring was identified to yield higher values compared to autumn. This might have been caused by the fact that our autumn samples were collected in November when it was generally colder. Bartoszewicz et al. (2008) state that *B. cereus* existence was more frequent in fresh and heat-treated milk in spring and summer when it is hotter than autumn and winter and this supports our suggestion. Autumn is the season when all vegetable samples can be analysed. It was found out by examining the *B. cereus* numbers of vegetable samples of eight vegetable types that lettuce yielded the highest value of 8.2×10^2 CFU/g and it was followed by carrot and turnip with an average number of 4.8×10^2 CFU/g. It was identified that parsley had the lowest value of 1.0×10^3 CFU/g. We believe that extreme values observed for lettuce and parsley are related to leaf structure and leaf layout of the vegetables. Hussain et al. (2019) mentioned that both substrate and cell surface composition might affect the bonding behavior. Kumar and Anand (1998) stated that cell surface properties such as adhesion proteins, capsules, surface load, flagella and pilus were important factors for active bonding of bacteria cells to substrate. In this vein, Elhariry (2011) set forth that lettuce and cabbage differed with regards to adherence surface and surface of lettuce supported adherence of spore and vegetative cells compared to cabbage. As a matter of course, hydrophobic *B. cereus* spores adhere and produce spores on hydrophobic structures (Kwon et al., 2017). In a study conducted by Rosenquist et al. (2005) in Denmark, 48,901 convenience food products including vegetables were analyzed in terms of existence of *B. cereus* and *B. cereus* numbers under 10^3 CFU/g, in 10^3 - 10^4 CFU/g range and over 10^4 CFU/g were detected in 98.7%, 0.7% and 0.5% of the samples respectively. Higher values were generally obtained in products containing fresh cucumber and tomato (2.6%), heat-treated rice (1.3%), cakes (1.2%), and puddings containing milk and rice (3.1%). In the same study, *B. cereus* number was measured to be under 10^3 CFU/g in 97.2% of vegetable and fruit products.

Conclusion

We find certain results interesting in this study in which the change of microbial quality in certain vegetables grown and marketed by local farmers by seasons. All vegetables were identified to be free of *Salmonella* spp. Detection of *B. cereus* in all but five samples indicate infection regarding this bacterium. Especially the *B. cereus* numbers of 10^3 - 10^4 CFU/g and over 10^4 CFU/g detected in 18 and 2 lettuce samples respectively indicate that it is the vegetable with the highest infection rate. On the other hand, parsley and spinach (two samples and one sample respectively) are other vegetables with high infection rates due to their *B. cereus* values of 10^3 - 10^4 CFU/g. The stages of soil, harvesting, shipping and marketing should be analyzed in detail for the harvested products. Utmost importance should be attached to sanitation rules during both cultivation stage and harvesting and post-harvesting process of the products to prevent microbial infection. To reduce microbial infection rates, the containers used in the post-harvesting period as well as stands and shelves on which vegetables are put should be kept clean and made of material preventing adherence of bacteria. Clean water should be used for washing vegetables and humidifying them on the bench. It is recommended training farmers on sanitation and raising their awareness in order to ensure microbial quality during all processes from cultivation to the market.

Declaration of Conflict of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Authors' Contributions

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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