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Full Length Research Paper

Bacteriological and fungal evaluation of some aromatic and taste giving herbs from Igdir region in Eastern Anatolia of Turkey

Zeynep Ulukanli[1]* and Ekber Karadag[2]

[1]Osmaniye Korkut Ata University, Department of Biology, Faculty of Science and Letters, Osmaniye, Turkey.
[2]Kafkas University, Department of Biology, Faculty of Science and Letters, 36100 Kars, Turkey.

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In this work, aromatic and taste giving herbs including thyme (n=23), basil (n=23) and mint (n=19) samples collected from retail outlets of Igdir were examined for the microbiological quality as well as the detection of some pathogenic bacteria. Total aerobic mesophilic counts varied from 10^2 to10^7 cfu/g in thyme, 10^3 to 10^7 cfu/g in basil and mint samples. Coliforms were present in 34.8% of thyme, 69.6% of basil and 36.4% of mint samples. *E. coli* was detected only in a mint sample. Detection rate for staphylococci and micrococci in thyme, basil and mint was 21.7, 69.4 and 79%, respectively. Enterococci were observed in 21.7, 26 and 26.3% of thyme, basil and mint samples, respectively. Samples revealed the absence of *Lactobacilli, S. aureus*. *E. coli* O157:H7 and *Salmonella* spp. Aerobic Spore Forming Bacteria (ASB) was present in 56.3% of thyme, 74% of basil and 94.8% of mint samples. Sulphide Reducing Clostridia (SRC) only occurred in 30.5% of thyme and 5.2% of mint samples included SRC. Total yeast and fungal counts indicated that 52% of thyme, 61% of basil, 58% of mint samples was > 10^4 cfu/g.

Key words: Thyme, basil, mint, herbs, microorganisms, Igdir.

INTRODUCTION

The addition of spices and/or herbs to various food preparations and products is fairly widespread in the world. Nowadays, the use of these natural materials supplies consumers with many health benefits. The modern food processor is also under increasing demands to produce natural, wholesome products in order to appease today's public health conscious and thus gain consumer acceptance [Moore, 2004; Nasar and Halkman, 2003].

Spices and herbs may be subjected to contamination and consequent colonization by microorganisms during the conventional process such as harvesting, washing, drying either as controlled dehydration or mostly by sun drying, packaging, storing and distribution [Kneifel et al., 2002] and may present a hazard to the consumers [Tekinsen and Ozdemir, 2006], and thus several hygiene parameters have to be considered in routine control [Kneifel et al., 2002]. Vast arrays of literatures in many countries as well as in Turkey have existed and mostly focused on the microbiological quality of some spices such as red pepper, black pepper, cumin, etc. due to the extensive use [Banarjee and Sarkar, 2003; Baxter and Holzapfel, 1982; Bhat et al., 1987; De Boer et al., 1983; Erol et al., 1999; Filiz, 2001; Garcia et al., 2001; Geeta and Kulkarni, 1987; Karapinar and Tuncel, 1986; Kneifel and Berger, 1994; Kivanc and Sert, 1989; Schwab et al., 1982; Tekinsen and Sarigol, 1982; Ulukanli et al., 2005; Uner and Ergun, 1999].

Many reports in countries like USA, South Africa, Netherlands, Australia and Austria have been presented on numbers and kinds of different microorganisms on some aromatic herbs used for culinary purposes [Baxter and Holzapfel, 1982; De Boer et al., 1983; Kneifel and Berger, 1994; Pafumi, 1986; Schwab et al., 1982],

*Corresponding author. E-mail: zeynepulukanli@osmaniye.edu.tr, zeynepulukanli@hotmail.com.

Table 1. The methods, media, incubation temperature and time used for the isolation and enumeration of microorganisms.

	Media used	Incubation temperature (ºC)	Incubation Time (h)
Total aerobic mesophiles	Plate count agar (Biolab PCA 20500)	30	72
(TAM) Coliforms	Violet red bile lactose agar (Merc 1.01406)	37	24
E. coli	Eosin methylene blue (EMB) agar (Merck 1.01347)	37	24-48
Enterococci spp.	Kanaymycin aesculine azide agar (Oxoid CM591)	37	24-48
Staph/Micrococci	Baird parker agar (Merck 1.05406)	35	24-48
Lactobacilli	MRS agar (Merck 1.10660)	37	24-48
Aerobic spore forming bacteria	Plate count agar (Biolab PCA 20500)	30	24-72
Sulphide reducing clostridia	Tryptose sulphite cycloserine agar (TSC) (Merck 1.11972)	30	24-72
Yeast/moulds	Acidified potato dextrose agar (Oxoid CM 139)	25	120
Salmonella spp.	Buffered Peptone Water (Merc 1.07228)	37	24
	Rappaport Vassiliadis Broth (Merck 1.07700)	42	24
	Salmonella-Shigella agar (Merck 1.07667)	37	24
E. coli H7:O157	Modified Novobiocin EC Broth (mEC+n, Merck 1.10765)	37	24
	CT-SMAC (Oxoid CM 813 with SR 172 E)	42	24-48

however, literature surveys have revealed a limited information on the incidence and distribution of different microorganisms in aromatic culinary herbs in Turkey [Temelli and Anar, 2002]. The leafy part of plants, such as thyme, mint, oregano, belonging to the *Labiatae* family, is well known aromatic and medicinal herbs, which are popularly used in many countries. It has been added to meat, fish and variety of food product for years. In addition to improving the flavor, certain herbs and essential oils prolong the storage of life of foods by inhibitory activity towards the growth of microorganisms [Bagamboula et al., 2004; Sagdic, 2003]. Like the antimicrobial properties, herbs such as thymus, ferula, mint have been naturally used in the production of some traditional Turkish cheese-making without any addition of starter cultures [Tekinsen and Ozdemir, 2006].

In this work, we examined our herb samples from the region of Igdir. Igdir, is a province in Eastern Anatolia Region of Turkey, located along the border with Armenia, Azerbaijan (the area of Nakchivan), and Iran. Its adjacent provinces are Kars to the northwest and Agri to the west and south [Anonymous, 2010a]. The main economic activities of the Igdir region depends on agriculture and animal husbandry and mainly border trade with Azerbaijan (the area of Nakchivan) and Iran [Anonymous, 2010b]. The present paper reveals the results of a preliminary quantitative and qualitative study concerning the microbiological quality of herbs sold in the retail outlets of Igdir.

MATERIALS AND METHODS

Sixty five samples of herbs (thyme, basil and mint) were randomly collected from retail shops in the city of Igdir, Turkey. The samples were analyzed immediately after arrival at the laboratory.

A 10 g of each sample was aseptically weighted and homogenised in 90 ml of peptone solution (0.1%, w/v). Then, decimal dilutions were prepared using the same diluent. The following culture media and incubation conditions were used (Table 1).

RESULTS AND DISCUSSION

Microbiological analyses of 65 samples were shown in Table 2. Thyme, basil, mint samples in the retail outlets of Igdir were heavily contaminated with total aerobic mesophiles (TAM): Out of 23 thyme samples, counts in all samples varied from 10^2 to 10^7 cfu/g. All basil and mint samples were also contaminated at high range. Twenty-three basil and nineteen mint samples contained 10^3 to 10^7 microorganisms per gram. TAM counts were $>10^7$ cfu/g in 13% of the thyme, 30.4% of basil and 15.7% of mint samples. In spices, counts $>10^6$ cfu/g have not been at the acceptable level according to Turkish Food Codex (TFC) [Anonymous, 2000]. A study of Schwab et al. [1982] reported total aerobic counts of thymes as <100 - 6.3 x 10^7 cfu/g. High levels detected by Schwab et al. [1982] appears to be similar to our findings. In contrast, our results are dissimilar to those obtained in other studies, where low levels of 10^5 cfu/g [Baxter and Holzapfel, 1982] and 5.1 x 10^6 cfu/g [Kneifel and Berger, 1994] in thyme samples were reported. Levels as high as $>10^7$ cfu/g [De Boer et al., 1983] or 2.6 x 10^6 cfu/g [Kneifel and Berger, 1994] were detected in basil samples. Levels of 10^5 to 10^7 cfu/g [Pafumi, 1986] and 5.1 x 10^6 cfu/g [Kneifel and Berger, 1994] were also reported in mint samples. Our results for basil and mint samples seemed to be lower than the results of Kneifel

Table 2. Microbiological results of herbs (*cfu/g).

TAM	$\angle 10^{2*}$	10^{2}-$\angle 10^{3*}$	10^{3}-$\angle 10^{4*}$	10^{4}-$\angle 10^{5*}$	10^{5}-$\angle 10^{6*}$	10^{6}-$\angle 10^{7*}$	10^{7*}
Thyme (n=23)	-	1 (4.3)	7 (30.4)	8 (34.7)	4 (17.3)	-	3 (13)
Basil (n=23)	-	-	2 (8.6)	7 (30.4)	7 (30.4)	-	7 (30.4)
Mint (n=19)	-	-	3 (15.7)	5 (26.3)	8 (42.1)	-	3 (15.7)
Coliforms							
Thyme (n=23)	15(65.2)	5 (21.7)	2 (8.6)	1 (4.3)	-	-	-
Basil (n=23)	7 (30.4)	6 (26)	5 (21.7)	4 (17.3)	1 (4.3)	-	-
Mint (n=19)	12(63.1)	1 (5.2)	3 (15.78)	2 (10.5)	1 (5.3)	-	-
Enterococci							
Thyme (n=23)	18(78.2)	5 (21.7)	-	-	-	-	-
Basil (n=23)	17 (74)	6 (26)	-	-	-	-	-
Mint (n=19)	14(73.7)	5 (26.3)	-	-	-	-	-
Stap/Microc.							
Thyme (n=23)	18(78.2)	5 (21.8)	-	-	-	-	-
Basil (n=23)	7 (30.4)	12 (52.1)	4 (17.3)	-	-	-	-
Mint (n=19)	4 (21)	9 (47.3)	3 (15.7)	1 (5.2)	-	1 (5.2)	1 (5.2)
ASB							
Thyme (n=23)	9 (39.1)	8 (34.7)	4 (17.3)	2 (8.6)	-	-	-
Basil (n=23)	6 (26)	7 (30.4)	7 (30.4)	2 (8.6)	-	-	1 (4.3)
Mint (n=19)	1(5.2)	9 (47.3)	5 (26.3)	4 (21)	-	-	-
SRC							
Thyme (n=23)	10(43.4)	1 (4.3)	2 (8.6)	3 (13)	1 (4.3)	-	-
Basil (n=23)	23 (100)	-	-	-	-	-	-
Mint (n=19)	18(94.8)	-	-	-	1 (5.2)	-	-
Yeast/mould							
Thyme (n=23)	-	-	4 (17.3)	7(30.4)	3 (13)	3 (13)	6 (26)
Basil (n=23)	-	2 (8.6)	3 (13)	4 (17.3)	10 (43.4)	4 (17.3)	-
Mint (n=19)	-	-	4 (21)	4 (21)	8 (42.1)	1 (5.2)	2 (10.5)

Abbreviations: TAM (Total Aerobic Mesophiles), ASB (Aerobic Spore forming Bacteria), SRC (Sulphide Reducing Clostridia).

and Berger [Kneifel and Berger, 1994], but were similar to those obtained by De Boer et al. [1983] and Pafumi [1986]. Plate count of aerobic mesophiles found in herbs is regarded as the indicator of general hygiene and quality parameter [Kneifel et al., 2002]. High aerobic mesophilic counts found in our samples may reflect that samples exposed to poor handling, inappropriate storage or a general lack of hygiene [Gillespie et al., 2000; Richardson and Stevens, 2003].

Coliforms were detected in 36.7% of mint, 69.6% of basil and 34.8% of thyme samples. Counts ranged from 10^{2} to 10^{4} cfu/g in thyme, 10^{2} to 10^{5} cfu/g in basil and 10^{2} to 10^{5} cfu/g in mint samples. Coliform counts in mint samples are similar to those reported by Pafumi [1986], whereas our result is not in agreement with the findings of Schawb et al. [1982] who reported coliform counts of thymes as <3-1.1 x 10^{6} cfu/g. Although coliforms were found at high levels in basil and thyme samples, *E. coli* was found in only one mint sample, whereas other samples examined gave no indication of this bacterium. The incidence of *E. coli* in mint samples is not similar to those reported by Pafumi [1986], who found that three mint samples were positive for *E. coli*. The detection of *E. coli* O157:H7 in herbs has received less attention. This may be related to the low occurrence of *E. coli* in herbs. Of the samples examined in this work, none revealed the presence of *E. coli* O157:H7. Similarly, *Salmonella* spp. was not isolated in any of the herbs analyzed. The

presence of *Salmonella* spp. in the similar types of herbs has not also been detected in other studies [Baxter and Holzapfel, 1982; De Boer et al., 1983; Kneifel and Berger, 1994; Pafumi, 1986]. In herbs, coliforms are an indicator of faecal contamination, but it is also ubiquitously present in herbs [Kneifel et al., 2002]. Although considerable level of coliforms in our samples, absence of *E. coli* (except for one mint sample), *E. coli* O157:H7 and *Salmonella* spp. may indicate good hygienic quality of the samples. The absence of these bacteria in the herbs examined may be due to the chemical composition of these herbs.

Enterococci were found in 21.7% of thyme, 26% of basil, and 26.3% of mint samples, respectively. Counts of enterococci remained at lower levels of $>10^2$ cfu/g in all the samples examined. In contrast, Kneifel and Berger [1994] reported values lower than $<10^1$ cfu/g in thyme, basil and mint samples. The presence of enterococci in herbs may indicate either faecal contamination or is ubiquitously present in herbs [Kneifel et al., 2002]. Considering the rare occurrence of faecal indicator organism in our samples (except for one mint sample), enterococci in the samples examined may be contaminated via the air, water, soil, vegetation.

The distribution of staphylococci and micrococcus was found in the range of 10^2 to 10^3 cfu/g in 69.4% of basil samples, 10^2 to $>10^7$ cfu/g in 79% of mint samples and 10^2-10^3 cfu/g in 21.8% of thyme samples. *S. aureus* was not present in any of the samples. *S. aureus* has been reported in thyme, basil and mint samples in a previous study of Kneifel and Berger [1994]. Our findings are dissimilar to the findings of Kneifel and Berger [1994], but are similar to the data obtained by De Boer et al. [1983]. Staphylococci are commonly found on the skin of a wide variety of mammals and birds as well as on environmental surfaces [Aycicek et al., 2005]. The presence of coagulase-positive staphylococci in herbs is an indicator of human origin [Kneifel et al., 2002]. From our data, it can be said that high incidence and levels of staphylococci and micrococcus in thyme, basil and mint samples be considered as being part of its normal flora or via the environmental sources. Absence of *S. aureus* in herbs may indicate that herbs may appear not to be significant carrier of this pathogen due to the chemical composition of the herbs.

Lactobacilli may be an indicator of spoiled plant material [Kneifel et al., 2002]. In our study, lactobacilli were not detected in any of the samples examined. Results were not shown in Table. This finding is consistent with that of Kneifel and Berger [14] who reported similar results in retail thyme, basil and mint samples.

ASB (aerobic spore forming bacteria) were detected mostly in 94.8% of mint samples, but the presence in thyme and basil varied from 56.3 to 74% respectively. The counts for ASB in the samples of thyme and mint ranged from 10^2 to 10^4 cfu/g, whereas, an increase up to the level of 10^7 cfu/g was observed in 4.3% of basil samples. Our results could be comparable with the findings of Baxter and Holzapfel [1982] and Kneifel and Berger [1994]. Baxter and Holzapfel [1982] found 10^5 cfu/g in thyme samples. However, Kneifel and Berger [1994] reported counts of 4.3×10^4, 1.4×10^3 and 6.6×10^4 cfu/g in thyme, mint and basil samples, respectively.

As in Table 1, it seems that these aromatic culinary herbs analyzed in retail markets are exposed to low level of clostridial contamination than those obtained by aerobic spore forming bacterial contamination. SRC were not detected in the samples of basil. However, 5.2% of mint and 30.5% of thyme samples contained SRC. Counts of SRC exceeded the acceptable limits of TFC: $>10^4$ cfu/g in 4% of thyme and in 5% of mint. In a previous study, lower clostridial counts than those of our study were obtained for mint samples in the range of $<10^2$ to 10^3 cfu/g [19]. In other studies, clostridia were not detected in any thyme, basil and mint samples examined [Baxter and Holzapfel, 1982; De Boer et al., 1983; Kneifel and Berger, 1994].

Contamination with yeast and mould is common in herbs of all types. These are generally considered to be spoilage organisms of herbs. Yeast and mould counts ranged from 10^3 to 10^7 cfu/g in thyme, 10^2 to 10^6 cfu/g in basil and 10^3 to 10^7 cfu/g in mint samples, respectively. Yeast and mould counts above $>10^4$ cfu/g for spices were regarded as unacceptable according to the TFC [28]. Of the samples, 52% of thyme, 60.7% of basil, and 57.8% of mint samples had higher counts than the criteria set by TFC. In the present study, high levels of yeast and mould counts found in thyme, basil and mint samples are not similar to those found in samples analyzed by other workers samples [Baxter and Holzapfel, 1982; De Boer et al., 1983; Pafumi, 1986]. This may be due to the differences in cultivation, hygiene and other conditions like storage. The presence of aerobic sporeformers and moulds is important since their survival or the presence of their toxins after cooking process may result in food poisoning or deterioration of the product in which spices have been used. This is of vital importance for the microbiological quality and safety of the experimental shelf stable products [Baxter and Holzapfel, 1982]. It has been known that, antimicrobial substances have existed in herbs; however, it still may pose a health risk for consumers. We can conclude from this study that conventional means of buying herbs from retail outlets are specifically not healthy for the presence of spore forming microorganisms. Therefore, we emphasize that herbs before selling need to be produced under hygienic status as well as to preserve under proper conditions for possible detrimental health risks for consumers.

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