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The physico-chemical and microbiological properties of wheat flour in Thrace

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Abstract: The physico-chemical and microbiological properties of wheat flour samples (type 650) obtained from 7 different locations in the Thrace region (Turkey) were assessed. Totally 142 wheat flour samples were analysed physico-chemically in terms of ash, moisture, fat acidity, and protein amounts, and microbiologically in terms of total mesophilic aerobic bacteria (TMAB), *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, moulds, rope-spore counts, and *Salmonella* spp. In the physico-chemical parameters investigated, only the ash levels of flour samples were higher than the limit laid down in the Turkish Food Codex and Codex Alimentarius. In some locations, the levels of TMAB, *E. coli*, and rope spores were found to be higher than the legal limits in Turkish legislation. On the other hand, the differences among investigated areas were significant physico-chemically in terms of ash, moisture, fat acidity, and protein parameters, and microbiologically in terms of TMAB, *E. coli*, *C. perfringens*, and moulds ($P < 0.05$). It is concluded that the physico-chemical and microbiological properties of flours sampled at various collection points were variable and in some locations their microbiological properties did not fulfil the legal requirements.

Key words: Physico-chemical quality, microbiological quality, wheat flour, Thrace

Trakya bölgesi buğday unlarının fiziko-kimyasal ve mikrobiyolojik özellikleri

Özet: Trakya (Türkiye) bölgesinin 7 farklı noktasından temin edilen Tip 650 un numunelerinin mikrobiyolojik ve kimyasal durumları araştırılmıştır. 142 un örneği kimyasal olarak kül, rutubet, yağ asitliği ile protein miktarları ve mikrobiyolojik olarak da Toplam Mezofil Aerob Bakteri (TMAB), *E. coli*, *B. cereus*, *C. perfringens*, küf, rope-spore sayısı ile *Salmonella* spp. analiz edilmiştir. Buna göre TMAB, *E. coli* ve rope-spor sayısı bazı bölgelerde Türk standartlarının legal limitlerinin üzerinde tespit edilmiştir. İncelenen kimyasal parametrelerden ise sadece kül düzeyleri Türk Gıda Kodeksi ve Codeks Alimentarius'a ait limit değerler üzerinde bulunmuştur. Diğer taraftan, incelenen lokasyonlar arasında TMAB, *E. coli*, *C. Perfringens* ve küf sayısı gibi bazı mikrobiyolojik ve kül, rutubet, yağ asitliği ve protein miktarı gibi bazı kimyasal parametreler açısından önemli farklılıklar bulunmuştur ($P < 0.05$). Sonuç olarak, buğday unu numunelerinin mikrobiyolojik ve kimyasal durumları temin edildikleri lokasyonlara göre değişkenlik göstermiş ve bazı noktalarda mikrobiyolojik sonuçlar legal standartların üzerinde bulunmuştur.

Anahtar sözcükler: Fiziko-kimyasal kalite, mikrobiyolojik kalite, buğday unu, Trakya

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Introduction

Cereals and cereal products constitute a significant food resource for the world's population. Various spoilage micro-organisms can proliferate on cereal grains and finished products held under improper storage properties (Deibel and Swanson 2001).

Flour is generally regarded as a microbiologically safe product as it is a low water activity commodity (ICMSF 1998). Although the growth of pathogenic bacteria may not be supported under such properties, pathogens that contaminate flour may survive for extended periods (Berghofer et al. 2003). There are reported cases of food poisoning resulting from contaminated flour. Australian, European, and US studies indicate that *Salmonella* spp., *Escherichia coli*, *Bacillus cereus*, and other spoilage micro-organisms are present in wheat and flour at low levels (Cicognani et al. 1975; Ottogalli and Galli 1979; Spicher 1986; Eyles et al. 1989; Richter et al. 1993). In addition, mould growth in flour is known to decrease its quality significantly. Mould contamination on cereals, which can exist at the farm or at the site of storage, affects the yield, quality, and nutritional value of the products (Aran and Eke 1987). When the water content exceeds the alarm level for wheat flour (13%-15%) moulds start growing (Jay 1996). This situation illustrates how some chemical parameters could affect microbiological parameters. Fungal contamination of flour has been the subject of various investigations (Aran and Eke 1987; Beuchat, 1992; Weidenbörner et al. 2000).

Cereals have been grown in Anatolia (Turkey) for thousands of years and they constitute a part of life in rural areas. Wheat, barley, maize, oats, rye, rice, millet, spelt, canary grass, and mixed grain are the main types of cereals grown in Turkey. Wheat is an important crop that covers 9.5 million ha, making Turkey the eighth largest wheat producing area worldwide (FAO 2006). Likewise, wheat is a significant component of the Turkish diet, its annual consumption being approximately 250 kg per head (Akova 2006). The major wheat growing regions in Turkey are Central Anatolia, Thrace, and South-Eastern Anatolia. Thrace is a region with a tremendous agricultural potential due to its suitable climate and soil characteristics. The statistics show that, in 2005, 10%-15% of the wheat, 55% of the rice, and 60%-65% of the sunflower production in Turkey was from this region (Inciroglu 2006). Furthermore, the Thrace region has a geographical importance due to its location in the south-east of Europe and represents a transit corridor between Europe and Asia.

The aim of this study was to determine the physico-chemical and microbiological properties of wheat flours produced in Thrace, and to assess whether or not their quality complies with the both Turkish Food Codex and Codex Alimentarius Standards.

Materials and methods

Materials

A total of 142 wheat flour samples (Type 650) obtained from 7 locations (1. Tekirdağ, 2. Malkara, 3. Keşan, 4. İpsala, 5. Uzunköprü, 6. Edirne, 7. Lüleburgaz) in Thrace (Figure 1) were analysed in the summer (June-August) of 2006. The samples of were obtained from factories in the immediate vicinity of the harvesting area. A 1000 g portion of each sample was immediately transported to the laboratory in chilled containers at 4-6 °C and subsequently analysed in terms of physico-chemical and microbial parameters.

Physico-chemical analysis

Wheat flour samples were analysed for ash, moisture, and fat acidity according to ISO 2171 (1993), ISO 712 (1985), and ISO 7305 (1998), respectively. Nitrogen content of samples was determined by using a combustion method (AOAC 2000), and Leco FP-528 (USA) nitrogen analyser. In addition, crude protein was calculated by using a multiplication factor of N × 5.83 (Watt and Merril 1975).

Microbiological analysis

For microbiological analysis, a 10 g portion of wheat flour was placed in a sterile stomacher bag and homogenised for 2 min in 90 mL of sterile peptone saline solution [Oxoid CM 733 (Basingstoke, UK)] using a Stomacher 400 (Seward Medical Ltd, London, UK). Serial decimal dilutions were prepared with the same diluents. Agar plates were inoculated in duplicate (Thaddeus et al. 2001).

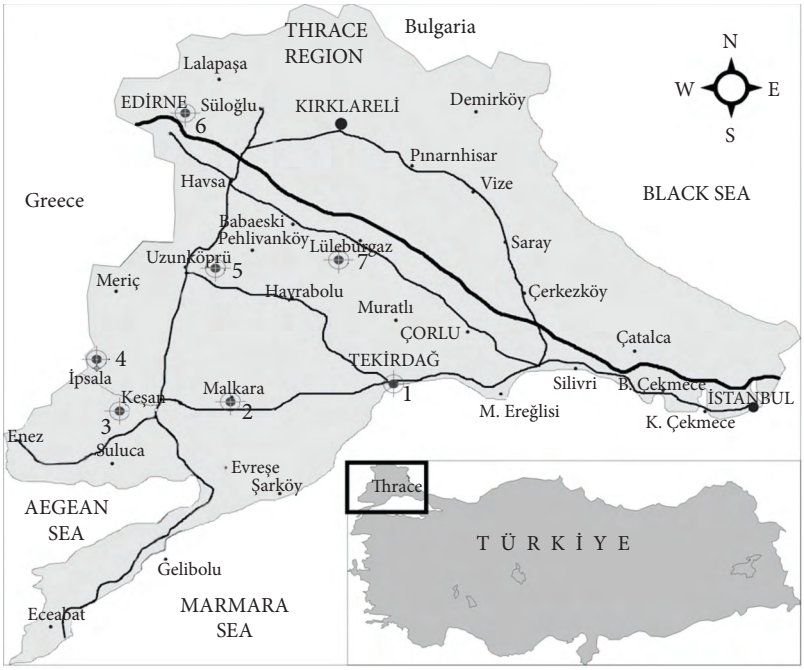

Figure 1. Location of study areas and wheat flour sampling points in Thrace, Turkey (1. Tekirdağ, 2. Malkara, 3. Keşan, 4. İpsala, 5. Uzunköprü, 6. Edirne, 7. Lüleburgaz)

For enumeration of TMAB, Plate Count agar (Oxoid CM 325) was inoculated by the pour plate method, and colonies were counted after inoculation for 48 h at 35 °C (Maturin and Peeler 2001).

To detect *E. coli*, the 3-tube MPN procedure was applied as specified by Feng et al. (2002). One millilitre aliquots of sample dilutions (1:10, 1:100, and 1:1000) were transferred to Lauryl tryptose broth (LST) (Oxoid CM 451) in glass tubes (3 each). Incubation was at 35 °C for 24 h. Tubes developing gas bubbles were considered presumptively positive. From those tubes, a loopful of the culture was transferred into a tube of *E. coli* (EC) broth (Merck 1.10765). The EC tubes were incubated for 24 h at 45.5 °C and examined for gas production. From EC tubes exhibiting gas production, a loopful was streaked out onto L-EMB agar (Oxoid CM 69), which was then incubated for 18-24 h at 35 °C. Finally, typical colonies on L-EMB agar plates were confirmed by biochemical tests (IMVIC tests and Gram straining).

To enumerate *B. cereus*, predried Mannitol Egg Yolk Polymyxin agar (Oxoid CM 929) plates containing egg yolk emulsion (Oxoid SR 047) were inoculated by evenly spreading 0.1 mL of the sample dilutions onto the surface. Incubation was for 24 h at 30 °C. Typical colonies were sub-cultured on Tryptone Soya Agar (Oxoid CM 131), and further confirmed by their morphological (gram straining) and biochemical properties (Voges Proskauer reaction, gelatine hydrolysis, nitrate reduction, tyrosine degradation, lysozyme test) as described by Rhodehamel and Harmon (2001a).

For detection of *C. perfringens*, 0.1 mL of the sample dilutions was spread on Tryptose-Sulfite-Cycloserine (TSC) agar (Oxoid CM 587) containing egg yolk emulsion (Oxoid SR 047). After the inocula had been absorbed (i.e. after about 5 min), the plates were overlaid with 10 mL of TSC agar without egg yolk emulsion. When the agar had solidified, the plates were placed in an upright position and incubated for 20-24 h at 35 °C under anaerobic conditions (Rhodehamel and Harmon 2001b).

Mould counts were determined on Dichloran Rose Bengal Chloramphenicol agar plates (Oxoid CM 727). Plates were inoculated by spreading 0.1 mL of sample

dilution on the surface of the agar and incubation was in the dark for 5-7 days at 25 °C (Beuchat 1992).

To determine the rope spores in wheat flour, 11 g of the sample was added to 99 mL of peptone saline solution, and homogenised for 1 min in a Stomacher 400. The suspensions were placed in a circulating water bath at 90-95 °C for 20 min, and then cooled in melting ice prior to preparing serial dilutions (1:100, 1:1000, and 1:10,000) in peptone saline solutions. Subsequently, a 1 mL aliquot was inoculated in 3 tubes containing Dextrose Tryptone Broth (Oxoid CM 73), which were incubated for 3 days at 32 °C (TS 1992).

For the detection of *Salmonella* spp., 25 g of wheat flour samples were placed in sterile Stomacher bags, homogenized for 2 min in 225 mL of sterile Lactose broth (Oxoid CM 107), and pre-enriched for 24 h at 35-37 °C. Subsequently, 1 mL of the pre-enriched culture was transferred to Selenite Cystine Broth (SC) (Merck 1.07709) and incubated for 18-24 h at 35-37 °C. In parallel, 0.1 mL of the pre-enriched culture was transferred into 10 mL of Rappaport Vasiliadis Soy (RVS) Broth and incubated at 41 ± 1 °C for 18-24 h. After incubation, 1 mL of SC broth and 1 mL of RVS broth were added to separate tubes containing 10 mL of Mannose (M) Broth (Merck, 1.10658) and incubated for 16-20 h at 41 ± 1 °C. Both M-broths were combined, heated at 100 °C for 15 min, and subsequently cooled. From the heated broth mix, 0.5 mL was pipetted into a VIDAS Salmonella (SLM) (bioMerieux S.A., Marcy l'Etoile, France) reagent strip; detection of Salmonella antigens was performed in a mini Vidas analyser (bioMerieux). For samples with presumptive positive results, confirmatory testing was performed (AOAC 2004).

Statistical analysis

Physico-chemical and microbiological results were calculated based on the absolute values. Colony counts were converted to logarithmic values. One-way ANOVA and Duncan's multiple range tests were used to analyse microbial log counts. Statistical estimations were performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA, 1997).

Results

The results of physico-chemical analysis in wheat flour samples are shown in Table 1. The mean ash content, moisture and protein concentration in Turkish wheat flour samples (Type 650) varied with different sampling points i.e. from 0.54 ± 0.11% to

Table 1. Chemical quality parameters of wheat flour samples (n = 142) obtained from different locations[a] in Thrace, Turkey

Parameters and legal limits	Levels	Locations[a]						
		1	2	3	4	5	6	7
Total Ash (%) 0.65%[b]	**Mean**	0.54 ± 0.11[d*]	0.65 ± 0.07[b]	0.62 ± 0.06[c]	0.71 ± 0.08[a]	0.71 ± 0.05[a]	0.60 ± 0.08[c]	0.65 ± 0.09[b]
Moisture (%) Max. 14.5%[b] Max. 15.5%[c]	**Mean**	12.63 ± 0.76[c]	13.66 ± 0.61[b]	14.20 ± 0.65[a]	13.73 ± 0.33[b]	13.75 ± 0.70[b]	13.70 ± 0.63[b]	12.31 ± 0.92[d]
Fat Acidity (%) Max. 0.07%[b,c]	**Mean**	0.067 ± 0.001[a]	0.043 ± 0.001[e]	0.060 ± 0.001[b]	0.061 ± 0.001[b]	0.055 ± 0.001[c]	0.060 ± 0.001[b]	0.050 ± 0.001[d]
Protein[d] (%) Min. 7.0%[b,c]	**Mean**	11.30 ± 0.070[c]	12.00 ± 0.040[b]	11.50 ± 0.064[c]	12.56 ± 0.073[a]	12.76 ± 0.055[a]	11.45 ± 0.082[c]	11.54 ± 0.08[c]

[a]: Locations: 1. Tekirdağ, 2. Malkara, 3. Keşan, 4. İpsala, 5. Uzunköprü , 6. Edirne, 7. Lüleburgaz.
[b]: Maximum acceptable limits of wheat flour according to Turkish Food Codex (1999).
[c]: Maximum acceptable limits of wheat flour according to Codex Alimentarius Standard (1995).
[d]: Crude protein: Nx 5.83
[*]Means in a line with different letters are significantly (P < 0.001) different from one another.

0.71 ± 0.08%, 12.31 ± 0.92% to 14.20 ± 0.65%, and 11.30 ± 0.070% to 12.76 ± 0.055%, respectively. The lowest acidity level found was 0.043 ± 0.001% (location 2), while the highest acidity level was 0.067 ± 0.001% (location 1).

The results of the microbiological analysis of wheat flour samples are shown in Table 2. In this study, TMAB levels of 32 (22.5%) samples obtained from 4 locations in the Thrace region (i.e. areas 3, 4, 5, and 6; see Table 2) exceeded the Turkish legal limits (Turkish Food Codex 2001). The highest TMAB count in the samples was 1.6×10^7 cfu g^{-1} (location 3).

E. coli was detected in all locations (50.7%). The highest *E. coli* counts > 1100 MPN g^{-1} were found in 14 samples (9.8%) originating from locations 3, 4, 5, and 6.

B. cereus counts were > 10^1 cfu g^{-1} in 4.2% of the samples; maximum counts, however, did not exceed 10^3 cfu g^{-1}.

For all samples, *C. perfringens* counts were below the maximum acceptable limit (1.0×10^4 cfu g^{-1}) specified in the Turkish Food Codex (2001).

The mean mould counts in all locations studied were 7.4×10^1 to 1.8×10^4 (location 3) cfu g^{-1}. All samples were below the maximum acceptable limits of the Turkish Food Codex (2009).

In 10 samples (7.0%), rope spore counts exceeded the maximum legal limit of 4500 MPN g^{-1} (locations 1, 3, 5, and 6).

Salmonella spp. were not detected in any of the wheat flour samples.

Discussion

Cereals or cereal products, in particular flour, constitute a large part of the daily Turkish diet, with ca. 250 kg per head annually (Akova 2006). Studies on the level of contamination and composition of the microflora of the Turkish flour are very valuable from the viewpoint of risk assessment.

The microflora of flour is composed of a variety of micro-organisms, including yeasts, moulds, psychotropic, thermophilic, and thermoduric bacteria, lactic acid bacteria, "rope bacteria", and pathogenic bacteria, more specifically *B. cereus*, *C. perfringens*, *C. botulinum*, and *Salmonella* spp. Although cereal grains and their milled products have rarely been implicated in foodborne disease (Deibel et al. 2001), it is the large quantity of flour annually consumed and the associated significant exposure to these micro-organisms that prompted the retrieval of data on the frequency of pathogenic bacteria and micro-organisms that would render the food unfit for the consumer.

Physico-chemical composition of wheat flour: Comparison of results from Turkey with other countries and compliance with legal standards

In this study for Thrace region wheat flour samples (Type 650), the mean ash values ranged from 0.54 ± 0.11% to 0.71 ± 0.08% (Table 1). This is in agreement with Ekinci and Unal (2002), who reported that Type 650 flour samples (n = 86) of the Marmara region (which includes Thrace) contained 0.57%-0.64% ash. In contrast, Alp et al. (2006) have reported the average ash level of Turkish wheat to be 1.36 ± 0.71%. Such high levels of ash are generally associated with increasing levels of bran in the wheat (Ekinci and Unal 2002). A regional effect cannot be excluded: only the flour samples obtained from locations 4 and 5 had higher ash levels than the maximum limit (0.65%) stated in the Turkish Food Codex (1999).

Moisture is an important parameter in flour that significantly affects shelf life and growth of microbial contaminants (ICMSF 1998). In the Turkish Food Codex (1999) as well as in the Codex Alimentarius Standard (1995), the maximum moisture in flour is defined as 14.5% and 15.5%, respectively. In our study the moisture in flour samples ranged from 12.31 ± 0.92% to 14.20 ± 0.65%, which for the Thrace region of Turkey approximates the range reported by Ekinci and Unal (2002) for Turkish wheat flour. These low mean moisture levels might be the reason for the low yeast counts in our samples. Although water activity of dry flour is too low to support growth or toxin production of contaminant moulds, changes in moisture contents of 1% or 2% have been reported to be sufficient for growth and toxin contamination (Eyles et al. 1989).

Fat acidity levels of the samples (sulphuric acid in dry matter) did not exceed the maximum legal limit of 0.07% (Codex Alimentarius Standard 1995; Turkish

Table 2. Microbial counts of wheat flour samples (n = 142) obtained from different locations in Thrace, Turkey

Micro-organisms and legal limits	Microbial Counts	locations [a] 1 (n: 22)	2 (n: 10)	3 (n: 30)	4 (n: 10)	5 (n: 36)	6 (n: 24)	7 (n: 10)
	10^1-10^2	2	-	-	-	2	-	-
	$>10^2$-10^3	8	-	-	-	8	-	4
TMAB[d]	$>10^3$-10^4	12	6	12	-	14	8	4
(cfu g^{-1})	$>10^4$-10^5	-	2	8	2	8	8	2
(1.0×10^5)[b]	**$>10^5$[c]-10^6**	-	2	4	4	2	-	-
	$>10^6$-10^7	-	-	4	2	2	8	-
	$>10^7$-10^8	-	-	2	2	-	-	-
log Mean (x ± Sx)		2.89 ± 0.12^{d}	4.22 ± 0.29^{bc}	4.71 ± 0.23^{b}	5.59 ± 0.34^{a}	3.66 ± 0.18^{cd}	4.69 ± 0.25^{b}	4.10 ± 0.11^{cd}
E. coli	<3	18	8	8	2	16	16	2
(MPN[e] g^{-1}) (<9)[b]	**>9[c]**	4	2	22	8	20	8	8
log Mean (x ± Sx)		0.33 ± 0.15^{b}	0.27 ± 0.18^{b}	1.37 ± 0.20^{a}	0.92 ± 0.41^{ab}	0.90 ± 0.16^{ab}	0.64 ± 0.21^{ab}	0.60 ± 0.78^{b}
B. cereus	$< 10^1$	22	10	28	10	34	22	10
(cfu g^{-1})								
(1.0×10^4)[b]	10^1-10^3	-	-	2	-	2	2	-
log Mean (x ± Sx)		0^{a}	0^{a}	0.20 ± 0.14^{a}	0^{a}	0.13 ± 0.90^{a}	0.17 ± 0.12^{a}	0^{a}
C. perfringens	$< 10^1$	22	10	28	10	30	22	6
(cfu g^{-1})								
(1.0×10^4)[b]	10^1-10^3	-	-	2	-	6	2	4
log Mean (x ± Sx)		0^{b}	0^{b}	0.13 ± 0.93^{b}	0^{b}	0.35 ± 0.13^{b}	0.25 ± 0.17^{b}	0.92 ± 0.38^{a}
	<10	4	6	2	-	4	-	-
	10^1-10^2	8	-	14	-	6	8	6
Mould	$>10^2$-10^3	10	2	6	4	12	10	4
(cfu g^{-1})	$>10^3$-10^4	-	2	6	2	14	6	-
(1.0×10^5)[f]	$>10^4$-10^5	-	-	2	2	-	-	-
log Mean (x ± Sx)		1.50 ± 0.17^{c}	1.96 ± 0.57^{bc}	2.14 ± 0.19^{bc}	3.21 ± 0.22^{a}	2.46 ± 0.18^{b}	2.43 ± 0.12^{b}	2.19 ± 0.15^{bc}
Rope spore	<30	-	2	26	8	12	16	6
(MPN g^{-1})	30-4500	18	8	2	2	22	6	4
(4500 MPN/g^{-1})[b]	**>4500[c]**	4	-	2	-	2	2	-
log Mean (x ± Sx)		2.36 ± 0.19^{a}	2.48 ± 0.91^{a}	0.38 ± 0.20^{a}	0.00 ± 0^{a}	1.43 ± 0.20^{a}	0.95 ± 0.27^{a}	0.28 ± 0.19^{a}
***Salmonella* spp.**	ND	ND	ND	ND	ND	ND	ND	ND
(Not detectable in 25 g)[b]								

[a]: 1. Tekirdağ, 2. Malkara, 3. Keşan, 4. İpsala, 5. Uzunköprü , 6. Edirne, 7. Lüleburgaz.

[b]: Maximum acceptable limits of wheat flour according to the Turkish Food Codex (2001).

[c]: Exceed Turkish Food Codex maximum acceptable limits.

ND: not detected

[d]: Total Mesophilic Aerobic Bacteria.

[e]: Most Probable Number.

[f]: Maximum acceptable limits of wheat flour according to the Turkish Food Codex (2009).

*Means in a column with different letters are significantly ($P < 0.001$) different from one another.

Food Codex 1999). Ekinci and Unal (2002) reported an average of 9.5% protein level of flour (Type 650) harvested from the Marmara region, with the highest concentrations found in Middle Black Sea and Aegean regions. In our study on the Thrace region, mean protein amounts were between 11.30 ± 0.070% and 12.76 ± 0.055%, which exceed the minimum legal limit of 10.5% stated in the Turkish Food Codex (1999). Reportedly, increments in ash and protein values are associated with yield increases as stated by Ekinci and Unal (2002) and Ercan (1986).

Microbial contamination of wheat flour: Comparison of results from Turkey with those from other countries and compliance with legal standards

TMAB is widely used to gain a general opinion about the hygienic quality and microbiological load of foodstuffs (Morton 2001). A number of studies on wheat flour report mean total aerobic counts in the order of 10^4 cfu g^{-1} or below (France: Potus and Suchet 1989; Germany: Spicher 1986; Italy: Cicognani et al. 1975, and Ottogalli and Galli 1979; USA: Richter et al. 1993; Australia: Berghofer et al. 2003). Although the number of samples exceeding total aerobic counts of 10^4 cfu g^{-1} in wheat flour can be very low (e.g. 6 out of 650 samples in Australia, Berghofer et al. 2003), it was found to be quite high in our study for the Thrace region (62 of 142 samples, or 43.6%). Nonetheless, only 22.6% of the samples exceeded the Turkish legal limits (1.0×10^5 cfu g^{-1}) (Turkish Food Codex 2001). On the other hand, regarding TMAB, the differences between areas were statistically significant ($P < 0.001$).

Coliform bacteria and E. coli counts are important as these are indicative of the general hygienic properties of foodstuffs. Moreover, the presence of *E. coli* in a finished, ready-to-eat product can be a public health concern, as this finding may indicate deficiencies in process control (inadequate processing or post process recontamination; Mossel et al. 1996; Deibel et al. 2001). For raw products, which are processed before consumption, the significance of *E. coli* counts in flour is largely determined by the intended use. Spicher (1986) found mean coliform counts of 10^2 cfu g^{-1} in German flour. Similar results were reported on Italian flour (Cicognani et al. 1975; Ottogalli and Galli 1979). Berghofer et al. (2003) detected only one flour sample containing *E. coli*, at the level of 9 MPN g^{-1}. In a US study, *E. coli* was found in 12.8% of flour and 22.0% of durum wheat flour (Richter et al. 1993). In our study, the *E. coli* count in 72 (50.7 %) of 142 wheat flour samples from the Thrace region exceeded the legal limits of the Turkish Food Codex (2001). Differences between locations 1, 2, 3, and 7 were significant in terms of *E. coli* counts ($P < 0.01$).

B. cereus can be isolated from many kind of foodstuffs, in particular from products rich in carbohydrates (boiled and fried rice, cooked pasta), puddings, soups, cooked meats, salads, and vegetable sprouts (Bennet and Belay 2001). Few data exist on the incidence of *B. cereus* in flour. Berghofer et al. (2003) reported low levels (0.3 MPN g^{-1}) of *B. cereus* in Australian flour. Similarly, Eyles et al. (1989) presented lower levels of *B. cereus* count in Australian flour. In our study, only 6 (4.2%) samples of a total of 142 wheat flour samples from the Thrace region contained *B. cereus* at the level of $>10^2$ cfu g^{-1}, whilst maximum counts were below the acceptable limit (1.0×10^4 cfu g^{-1}) of the Turkish Food Codex (2001).

C. perfringens (strains producing Type A toxin) at a level of 10^3 to 10^4 cfu g^{-1} can be found in soil. These are easily isolated from raw food samples (Ugur et al. 2001). In our study in the Thrace region, 9.8% (14 of 142) of the samples contained *C. perfringens* at levels above the detection limit of 10^2 cfu g^{-1}. On the other hand, *C. perfringens* counts were below the maximum acceptable limits (1.0×10^4 cfu g^{-1}) in the Turkish Food Codex (2001) in all wheat flour samples. In another Turkish study, Alp et al. (2006) did not find *C. perfringens* in a total of 27 wheat samples in Turkey. The mean values of *C. perfringens* at location 7 were the highest and significantly different from those at the other locations ($P < 0.01$).

Mean mould counts are usually around 10^3 cfu g^{-1} (France: Potus and Suchet 1989; Germany: Spicher 1986; Italy: Cicognani et al. 1975; Ottogalli and Galli 1979). Our results for the Thrace region are in accordance with these findings. Considerably higher levels of mould loads in cereal samples in Turkey, however, i.e. in the order of 10^5-10^6 cfu g^{-1}, have been reported by Aran and Eke (1987). There are different sources for moulds present in flour, for example fungi prevailing in the grain, the mill machinery itself, and/or a lower quality of sanitary control

(Christensen and Cohen 1950; Eyles et al. 1989; Berghofer et al. 2003). In addition, most of the moulds in the seed, present as mycelium in the outer layers of the pericarp and conidia, accumulate on the grain surface. Therefore, a higher contamination of whole wheat flour can be expected in milling procedures (Christensen and Cohen 1950; Christensen 1951). These differences in microbial load of whole wheat flour and wheat white flour are detectable, but can be quite low, as reported by Weidenbörner et al. (2000), with average total fungal counts of the whole wheat flour and the white wheat flour of 865 and 916 cfu g^{-1}, respectively. Regarding mould numbers, the differences between areas were significant ($P < 0.001$). The climate at location 4 was suitable for the occurrence of moulds due the heat and humidity, while mean values were lower in location 1, which has a similar climate, due to more suitable harvesting, drying, milling, and storage practices applied by more aware farmers and agriculturists.

Rope bacteria in food are generally generated by the spores of heat resistant *Bacillus subtilis* (Adams and Moss 1995). *Bacillus subtilis* is a soil-borne bacterium and its spores are frequently isolated from wheat flour but rather rarely from bakers' yeast. Beside its rope production, *Bacillus subtilis* ingested at high levels (i.e. corresponding to 10^8 cfu g^{-1} food) causes food poisoning symptoms including vomiting, nausea, diarrhea, and headache (Adams and Moss 1995). In our study in the Thrace region, rope spore levels in 70 wheat flour samples were below the minimum detection limit (30 MPN g^{-1}) and in 62 samples between the minimum detection limit and the maximum acceptable limit (4500 MPN g^{-1}), whilst 10 samples (7.4%) had, according to the Turkish Food Codex (2001), contamination levels higher than the maximum tolerable limit. To prevent the rope problem, the level of heat resistant rope spores must be reduced in wheat used as a material for commodities such as bread or other cereal products (ICMSF 1998).

Salmonella spp. cause conditions such as typhoid, paratyphoid, and food poisoning. Generally, *Salmonella* spp. should not be detectable in 25 g of foods (Mossel et al. 1996). In our study, *Salmonella* spp. were not detected in any of the wheat flour samples. Similarly, in other studies *Salmonella* spp. were either not detectable [in Australian (n = 650) and Turkish flour (n = 27) (Berghofer et al. 2003; Alp et al. 2006)] or reported to be present in low numbers (i.e. 1.32% of 3040 samples made from various type of US wheat (Richter et al. 1993). The wheat flour samples investigated in our study in the Thrace region were considered not to be hazardous in terms of *Salmonella* spp. contamination.

Factors associated with the extent of microbial contamination

From Table 2, it can be concluded that samples from areas 3, 4, 5, and 6 tend to have higher mean bacterial counts than those from other regions. Considering the results of the physico-chemical examination, higher average counts are more likely associated with higher average moisture (Table 1) rather than resulting from some spatial effect. Conversely, it is conceivable that process factors, such as grain type and processing technology, are the result of a spatial effect. Further analyses including more data on the raw materials used and the technology in the milling process are necessary to clarify this.

Compliance of Turkish wheat flour with legal standards

At some sampling points, TMAB, *E. coli*, and rope spore levels of flour produced in the Thrace region of Turkey were found to be higher than Turkish and International standards. As for the physico-chemical parameters, only ash level exceeds legal maximum standards. The relatively high air temperature in the region is probably the major reason for the low moisture level, which affects the growth of bacteria and more particularly moulds and yeasts on flour. Although our results do not suggest that flour from the Thrace region would represent clear and present microbiological health hazards, they do indicate that there is ample room for improvement in process hygiene in some areas.

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