ISTANBUL AYDIN UNIVERSITY
ENGINEERING FACULTY
INTERNATIONAL JOURNAL OF FOOD ENGINEERING RESEARCH
(IJFER)

Year **2** Number **2 - 2016**

International Journal of Food Engineering Research (IJFER) is peer-reviewed journal which provides a platform for publication of original scientific research and applied practice studies. Positioned as a vehicle for academics and practitioners to share field research, the journal aims to appeal to both researchers and academicians.

Language
English

Publication Period
Published twice a year
April and October

Academic Studies Coordination Office (ASCO)

Administrative Coordinator
Nazan ÖZGÜR

Technical Editor
Hakan TERZİ

Visual Design
Nabi SARIBAŞ

ISSN: 2149-5777
Year: **2015** Volume: **1** Number: **1**

Printed by
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Aims and Scope

International Journal of Food Engineering Research (IJFER) is an international , peer-reviewed journal devoted to the publication of high quality original studies and reviews concerning a broad and comprehensive view of fundamental and applied research in food science&technology and their related subjects as nutrition, agriculture, food safety, food based diseases and economic aspects.

IJFER is an international periodical published quadrate a year (April and October). The journal is published in both print and electronic format.

From The Editor

Istanbul Aydın University Faculty of Engineering has started to publish an international journal on Food Engineering denoted as "International Journal of Food Engineering Reseach (IJFER)". We have especially selected the scientific areas which will cover future prospective food engineering titles such as Food Processing, Food Safety.. etc. and their related subjects as nutrition, agriculture, food safety, food-based diseases and economic aspects.

We have selected only a few of the manuscripts to be published after a peer review process of many submitted studies. Editorial members aim to establish an international journal IJFER, which will be welcomed by Engineering Index (EI), Science Citation Index (SCI) in short period of time.

Prof. Dr. Osman N. UCAN
Editor in Chief

International Journal of Food Engineering Research (IJFER)

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EVALUATION OF TURKISH OLIVE OIL QUALITY: SOME QUALITY CHARACTERISTICS AND TURKISH FOOD CODEX

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Abstract

Olive oil is a significant food product that is directly consumed and highly recommended for health. Therefore it must be produced at right and hygienic conditions. This study aimed to evaluate some quality and purity criteria of olive oil samples from different regions and different olive types around Turkey according to regulations of Turkish Food Codex. Free acidity, peroxide number and oil acid compositions of samples were studied for evaluation of quality and purity. According to the results, although free acidity results of all Riviera olive oil samples were in conformity to those in standards; 46% of extra virgin olive oil samples were out of the acceptable limits. Peroxide values of both types (22% of virgin olive oil and 15% of Riviera oil samples) were above the limits given in standards. Fatty acid compositions of linolenic, heptadecanoic and heptadecenoic acid values were not within the legal limits. According to overall results; 46%, 20% and 9% of all samples were out of the standard limits, for free acidity, peroxide value and distribution of fatty acids, respectively. These data provide evidence of the variation in olive oil quality around Turkish olive oil samples.

Keyword: ?

Introduction

Olive oil is extracted from olive tree *Olea europaea L.* fruits and consumed directly. It is nutritionally beneficial for health, comprising nutritional compounds of oil acids, vitamins, sterols and phenolic compounds. Since it has some unique antioxidant compounds (phenolic compounds, tocopherol, other aromatic components), high amount of mono-unsaturated fatty acid (oleic acid), high oxidative stability and it is processed only physically (press, centrifugation, percolation), it is considered as a natural fruit oil or oily

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fruit juice and has numerous different properties in comparison to other edible vegetable oils [1,2]. It is one of the most significant crops in Mediteranean countries and a very important contributor of the Mediterranean diet [3].

Positive effects of extra virgin olive oil on health comprise cardiovascular health, and cancer [4]. Previous studies clearly revealed the antioxidant, inflammatory, and chemotherapic effects as well [5]. Epidemiological and nutritional studies mainly focused on PUFAs [6], phenolic compounds and tocopherols [6, 7]. Other clinical studies presented the positive effets of contiunuos olive oil consumption and its effects on coronary heart diseases, [8], oxidative damage on DNA and RNA [9] and risk of Alzheimer disease [10, 11].

Global olive production has been made mainly around Mediterranean region (90% of all global production). Annual olive production is around 17 million tons and countries of Spain, Italy, Greece, Tunisia, Portugal, and Turkey are among the olive produces. Annual amount of olive production in Turkey was around 1 676 000 tons for the season 2013-2014, with more than 167 millions of trees. Production of olives is quite common for manty different regions of Turkey such as; Aydın, İzmir, Muğla, Balıkesir, Bursa, Manisa, Çanakkale, Gaziantep and İçel; in the Aegean, the Marmara, the Mediterranean and the Southeast regions [12]. In Spain, world leader olive oil producer, total amount of oli oil produced was around 825.7 thousand ton in season 2014-2015, while in Turkey it was around 190 thousand ton. USA and European Unioun member countries are significant world olive oil traders [12].

Olive oil that is unable to be consumed directly is processed into as refined olive oil. Refining may comprise processes such as removal of aroma and resin, blanching, in addition to decreasing the free acidity level to below 0.3%. This type of olive oil is so called as refined olive oil. Although undesired properties are eliminated, the specific aroma and flavor of oil is also lost during refining process. Riviera type oil is a mixture of refined olive oil having changing ratios (5-20%) and virgin olive oil [13].

Legal status of olive oil in Turkey is determined according to the rules specified by the Turkish Food Codex standard [14]. High

quality olive oil is hard to produce and expensive and the high costs related to olive trees growing, olive harvest and oil extraction are among the main reasons behind this fact. Therefore adulteration of this valuable product in such approach as blending changing quality olive oil types and marketing them as extra virgin olive oil, quite commonly exists [14]. For prevention of adulteration, it is significant to determine the quality and purity properties of olive oil samples.

Olive oil quality is related to many different factors such as olive variety, geographic region, climate conditions, tree properties, olive fruit maturity and harvest time, way of olive collecting, storage conditions, oil processing methods, and mechanical properties of press machines [15]. This study aimed to investigate the quality and purity criteria of some common olive oil samples of extra virgin olive oil and Riviera type olive oil according to Turkish Food legistations.

Materials

50 different extra virgin olive oil and 20 different rafined type samples from producers at 4 different growing regions of Turkey comprising the Marmara region, the Aegean region, the Mediterranean region and the Southeast region, in two consecutive harvest years of seasons 2012-2013 and 2013-2014. Different regions and provinces were depicted in Table 1**.** The predominant olive varieties of Gemlik, Ayvalık and Memecik which are grown in a number of regions were chosen as monovarietal types whereas Karma (mixed) types were made by blanching different varieties. List of all olive oil samples used in the study was given in Table 2.

Table 1. Provinces at different growing regions

Growing Region	**Provinces**
The Marmara region	Çanakkale (Küçükkuyu), Balıkesir (Burhaniye, Edremit, Ayvalık) ve Bursa (Orhangazi, Mudanya, Gemlik)
The Aegean region	İzmir (Ödemiş, Bornova), Aydın (Nazilli, Karacasu, Köşk), Manisa (Akhisar)
The Mediterranean region	Muğla (Fethiye), Hatay (Samandağ), Kilis
The Southeast region	Gaziantep

Table 2. Olive oil samples used in the study

Olive Oil Sample	Provinces	Type	Variety	Season
N1	Kilis	Extra	*Mixed*	2012-2013
N2	Kilis	Extra	*Mixed*	2013-2014
N3	Samandağ/Hatay	Extra	*Gemlik*	2012-2013
N4	Samandağ/Hatay	Extra	*Gemlik*	2013-2014
N5	Samandağ/Hatay	Extra	*Mixed*	2012-2013
N6	Samandağ/Hatay	Extra	*Mixed*	2013-2014
N7	Küçükkuyu/Çanakkale	Extra	*Ayvalık*	2012-2013
N8	Küçükkuyu/Çanakkale	Extra	*Ayvalık*	2013-2014
N9	Altınoluk/Edremit/Balıkesir	Extra	*Ayvalık*	2012-2013
N10	Altınoluk/Edremit/Balıkesir	Extra	*Ayvalık*	2013-2014
N11	Nazilli/Aydın	Extra	*Memecik*	2012-2013
N12	Nazilli/Aydın	Extra	*Memecik*	2013-2014
N13	Nazilli/Aydın	Extra	*Mixed*	2012-2013
N14	Nazilli/Aydın	Extra	*Mixed*	2013-2014
N15	Burhaniye/Balıkesir	Extra	*Ayvalık*	2012-2013
N16	Burhaniye/Balıkesir	Extra	*Ayvalık*	2013-2014
N17	Tarsus/Mersin	Extra	*Mixed*	2012-2013
N18	Tarsus/Mersin	Extra	*Mixed*	2013-2014
N19	Ödemiş/İzmir	Extra	*Memecik*	2012-2013
N20	Ödemiş/İzmir	Extra	*Memecik*	2013-2014
N21	Köşk/Aydın	Extra	*Memecik*	2012-2013
N22	Köşk/Aydın	Extra	*Memecik*	2013-2014
N23	Aydın	Extra	*Memecik*	2012-2013
N24	Aydın	Extra	*Memecik*	2013-2014
N25	Aydın	Extra	*Memecik*	2012-2013
N26	Aydın	Extra	*Memecik*	2013-2014
N27	Gemlik/Bursa	Extra	*Gemlik*	2012-2013
N28	Gemlik/Bursa	Extra	*Gemlik*	2013-2014
N29	Bornova/İzmir	Extra	*Mixed*	2012-2013
N30	Bornova/İzmir	Extra	*Mixed*	2013-2014
N31	Ayvalık/Balıkesir	Extra	*Ayvalık*	2012-2013

N32	Ayvalık/Balıkesir	Extra Virgin	*Ayvalık*	2013-2014
N33	Mudanya/Bursa	Extra Virgin	*Gemlik*	2012-2013
N34	Mudanya/Bursa	Extra Virgin	*Gemlik*	2013-2014
N35	Küçükkuyu/Çanakkale	Extra Virgin	*Ayvalık*	2012-2013
N36	Küçükkuyu/Çanakkale	Extra Virgin	*Ayvalık*	2013-2014
N37	Ayvalık/Balıkesir	Extra Virgin	*Ayvalık*	2012-2013
N38	Ayvalık/Balıkesir	Extra Virgin	*Ayvalık*	2013-2014
N39	Ayvalık/Balıkesir	Extra Virgin	*Ayvalık*	2012-2013
N40	Ayvalık/Balıkesir	Extra Virgin	*Ayvalık*	2013-2014
N41	Aydın	Extra Virgin	*Memecik*	2012-2013
N42	Aydın	Extra Virgin	*Memecik*	2013-2014
N43	Fethiye/Muğla	Extra Virgin	*Memecik*	2012-2013
N44	Fethiye/Muğla	Extra Virgin	*Memecik*	2013-2014
N45	Karacasu/Aydın	Extra Virgin	*Memecik*	2012-2013
N46	Karacasu/Aydın	Extra Virgin	*Memecik*	2013-2014
N47	Küçükkuyu/Çanakkale	Extra Virgin	*Ayvalık*	2012-2013
N48	Küçükkuyu/Çanakkale	Extra Virgin	*Ayvalık*	2013-2014
N49	Orhangazi/Bursa	Extra Virgin	Gemlik	2012-2013
N50	Orhangazi/Bursa	Extra Virgin	Gemlik	2013-2014
R1	Bornova/İzmir	Riviera	*Mixed*	2012-2013
R2	Bornova/İzmir	Riviera	*Mixed*	2013-2014
R3	Gemlik/Bursa	Riviera	*Mixed*	2012-2013
R4	Gemlik/Bursa	Riviera	*Mixed*	2013-2014
R5	Akhisar/Manisa	Riviera	*Mixed*	2012-2013
R6	Akhisar/Manisa	Riviera	*Mixed*	2013-2014
R7	Akhisar/Manisa	Riviera	*Mixed*	2012-2013
R8	Akhisar/Manisa	Riviera	*Mixed*	2013-2014
R9	Gaziantep	Riviera	*Mixed*	2012-2013
R10	Gaziantep	Riviera	*Mixed*	2013-2014
R11	Nizip/Gaziantep	Riviera	*Mixed*	2012-2013
R12	Nizip/Gaziantep	Riviera	*Mixed*	2013-2014
R13	Bursa	Riviera	*Mixed*	2012-2013
R14	Bursa	Riviera	*Mixed*	2013-2014
R15	Bornova/İzmir	Riviera	*Mixed*	2012-2013
R16	Bornova/İzmir	Riviera	*Mixed*	2013-2014
R17	Bornova/İzmir	Riviera	*Mixed*	2012-2013

R18	Bornova/İzmir	Riviera	*Mixed*	2013-2014
R19	Ayvalık/Balıkesir	Riviera	*Mixed*	2012-2013
R20	Ayvalık/Balıkesir	Riviera	*Mixed*	2013-2014

Methods

3.2.1 Olive Oil Quality Tests

3.2.1.1 Free acidity (oleic acid, %)

Method of Turkish Standards Institute (Number: TS EN ISO 660) [16] was used to determine the free acidity of the olive oil samples. Free acidity is defined as percent free fatty acid (FFA) content and given as a percentage of oleic acid in Eq. 1.

$$w_{FFA} = V \times c \times \frac{M}{1000} \times \frac{100}{m} = \frac{V \times c \times M}{10 \times m} \quad (1)$$

Where;

V = Amount of potassium hydroxide with ethanol consumed (mL)

c = Normality of potassium hydroxide solution with ethanol (N),

M = Molecular weight of oleic acid (= 282g/mol);

m = Sample weight (g)

3.2.1.2 Peroxide value

Determination of Peroxide Number was made according to TS EN ISO 3960 [16] and result given as mili equivalent weight of active O_2/kg oil [16]. The equation used is given in Eq. 2.This value is accepted as a real indicator for oil oxidation [17]. Sample dissolved in acetic acid- isooctane solution is mixed with potassium iodine solution to form iodine which is titrated with sodium thiosulfate [16].

$$PV = \frac{1000 \times (V - V_0) \times c}{m} \quad (Eq.\ 2)$$

Where;

V = Sodium thiosulfate solution volume consumed, (mL)

V_0 = Sodium thiosulfate solution volume consumed for blank, (mL)

c = Exact molarity of sodium thiosulfate solution consumed;

m = Sample weight, (g)

3.2.2 Olive oil purity analyses

3.2.2.1 Analysis of fatty acids and isomers

Measurements were made using gas chromatography (GC) according to the conditions given standard [18]. GC details were depicted in Table 3. Results were given as methy ester %.

Table 3. GC conditions

Instrument: Agilent 6890 GC
Detector: FID (Flame Ionization Dedector)
Coloumn: 100 m x 0.25 mm ID, 0.2 µm HP-88
Split Ratio: 1:50
Injector Temperature: 250 °C
Oven Temperature: 120 °C ⟶ 230 °C
Detector Tempearture: 280 °C
Detector Gases: Hydrogen (40 ml/min.), Air (450 ml/min.), Helium (30 ml/min.)

3.2.3 Statistical Analyses

The differences among extra virgn olive oil and among Riviera type samples were investigated by one-way analysis of variance (ANOVA) ($p<0.05$). Detailed examinations for significant differences were made using Duncan's New Multiple Range Test. SPSS 16.0 statistical software (SPSS, Inc., Chicago, IL, USA) was used in all statistical analyses.

4. RESULTS and DISCUSSION

4.1 Olive Oil Quality Tests

4.1.1 Free acidity and peroxide value

Overall results showed that 23 of all extra virgin olive oil samples were above the legal limit for free acidity given in Turkish Food Codex (0.8%, oleic acid). On the other hand, Riviera type olive oil samples were within the legal limits. Free acidity values of extra virgin olive oil and Riviera olive oil samples according to their growing regions were given in Tables 4 and 5, respectively. he free acidity values among extra virgin olive oil samples changed between 0.28 and 12.69 % oleic acid, while for Riviera type samples the results ranged between 0.11 and 0.96 %. Present findigns were slightly higher than the findings of a previous study [19], in which 10 samples of extra virgin olive oil and 8 samples of Riviera type olive oil had the free acidity levels of changing between 0.41 and 0.93%; and 0.14 and 0.69%, respectively.

Differences among the growing regions were only significant for extra virgin olive oil samples from The Mediterranean region ($p<0.05$). The Mediterranean region was previously determined as having the highest free acidity levels in a study comprising different growing regions of the North and

South Aegean, Mediterranean and Southeast Anatolia [20]. Early maturation and late harvesting of olives was proposed to be related with the higher acidity values [20, 21]. Higher acidity was also related with the increased enzyme activity as a result of higher than desired water content in olives, pathologic organisms and mechanical problems, and these factors were given as reasons for the hydrolysis of triglycerides, as well [22].

Table 4. Free Acidity (oleic acid, %) values of extra virgin olive oil samples according to their growing regions

Olive oil growing regions	n	Free Acidity (Oleic acid, %) X
The Mediterranean region	10	3.96[a]
The Aegean region	28	1.06[b]
The Marmara region	12	0.84[b]

*Different letters in the same coloumn (a,b) represent statistically significant differences in results ($p<0.05$)

Table 5. Free Acidity (oleic acid, %) values of Riviera olive oil samples according to their growing regions

Olive oil growing regions	n	Free Acidity (Oleic acid, %) X
The Aegean region	12	0.40
The Southeast region	4	0.46
The Marmara region	4	0.64

Changes in free acidity and peroxide number values of extra virgin and Riviera olive oil samples according to their harvest times were given in Table 6. Average free acidity values of 2012-2013 season extra virgin and Riviera type oil samples were found as 1.28 and 0.39, respectively. On the other hand, same measurements for seasons 2013-2014 were 1.89 and 0.50. Differences between different harvesting seasons were statistically insignificant ($p>0.05$).

Differences in free acidities of extra virgin and Riviera type olive oil samples due to packaging were statistically insignificant ($p>0.05$). However generally higher results

free acidity results in plastic bottle types were similar to previous literature findings [23, 24] Overall results for peroxide number values revealed that 11 of extra virgin olive oil samples were above the limit value (20 meq active O_2/kg) defined in Turkish Food Codex; whereas among the Riviera type olive oil samples; 3 samples were higher than the legal limit (15 meq active O_2/kg). The lowest peroxide values among extra virgin olive oil samples were in samples N8 of 2013-2014 and N15 of 2012-2013 seasons (7 meq active O_2/kg). The highest value was detected in sample N23 (51 meq active O_2/kg) of 2012-2013 season. Average peroxide value results were 19.32 ve 15.36 meq active O_2/kg for 2012-2013 and 2013-2014 seasons, respectively (Table 6).

Table 6: Average free acidity and peroxide number values according to seasons of harvest of extra virgin and Riviera olive oil samples

Type of Olive Oil	Seasons	n	Free Acidity (oleic acid, %) X	Peroxide Number (meq active O_2/kg oil) X
Extra virgin	2012-2013	25	1.28	19.32
	2013-2014	25	1.89	15.36
Riviera	2012-2013	10	0.39	11.50
	2013-2014	10	0.50	12.50

The lowest and highest values for Riviera type olive oil results were for sample R14 belonging to 2013-2014 season (5 meq active O_2/kg) and sample R2 (2013-2014 season), 26 meq active O_2/kg). On the other hand, the average peroxide values in Riviera type olive oil samples were 11.50 ve 12.50 meq active O_2/kg for 2012-2013 and 2013-2014 seasons, respectively. Differences in peroxide values among extra virgin olive oil samples and Riviera type olive oil samples were insignificant ($p<0.05$) when different growing regions were considered (Tables 7 and 8). Extra virgin olive oil samples from The Mediterrenean region and Riviera type olive oil samples from the Aegean regions had slightly higher average peroxide values than the other regions studied. Peroxide values determined in this current study, were generally higher than the results some

previous literature concerning the similar regions of growth and/or olive varieties [20, 25-27]; although some similar results were also found [28]. The main reason might be the enlonged storage periods between production and analyses in addition to negative storage conditions such as light [23, 29]. Average peroxide number values were higher in glass bottle types (19.38 meq active O_2/kg) in extra virgin olive oil samples, whereas plastic bottle types had higher average peroxide values (12.57 meq active O_2/kg) in Riviera type olive oil samples.

Table 7. Average peroxide number values of extra virgin olive oil samples according to their growing regions

Olive oil sample	n	Peroxide Number (meq active O_2/kg oil) X
The Mediterranean region	10	18.10
The Aegean region	28	17.32
The Marmara region	12	16.75

Table 8. Average peroxide number values of Riviera type olive oil samples according to their growing regions

Olive oil sample	n	Peroxide Number (meq active O_2/kg oil) X
The Aegean region	12	12.58
The Southeast region	4	10.50
The Marmara region	4	11.75

Olive Oil Purity Analyses

4.2.1. Fatty Acid Composition Results of Olive Oil Samples

The most common fatty acid found in olive oil is the oleic acid and according to the overall results, all samples provided the required legal minimum limits for oleic acid. Moreover, all samples are within the limits for linoleic, palmitic, stearic, arachidic and myristic acids. Average fatty acid % results according to different growing regions were given in Table 9. The highest average oleic acid was 71.34% in the Marmara region, while the lowest result was detected in the Mediterreanaen region

(65.34 %, $p<0.05$). This might be attributed to the argument that the cooler regions having higher amounts of oleic acid % [31]. Increase in unsaturated fatty acid content with the decrease in temperature was also reported by previous studies [32, 33].

Table 9. Average fatty acid % results of extra virgin olive oil samples according to different growing regions

Fatty Acid Composition, %	Growing region			
	The Mediterrenean region	The Aegean region	The Southeast region	The Marmara region
Oleic acid	65.34[b]	69.99[a]	69.59[a]	71.34[a]
Linoleic acid	9.95[a]	9.43[a]	9.47[a]	8.69[a]
Linolenic acid	0.65[a]	0.64[a]	0.59[a]	0.60[a]
Palmitic acid	15.88[a]	14.91[a]	15.68[a]	14.42[a]
Stearic acid	2.93[a]	2.51[b]	2.94[a]	2.85[a]
Heptadecanoic acid	0.11[a]	0.11[a]	0.11[a]	0.11[a]
Heptadecenoic acid	0.07[b]	0.16[ab]	0.06[b]	0.19[a]
Arachidic acid	0.11[a]	0.14[a]	0.24[a]	0.10[a]
Eicosenoic acid	0.29[a]	0.22[a]	0.06[a]	0.16[a]
Palmitoleic acid	1.16[a]	0.91[b]	0.99[ab]	0.90[b]
Myristic acid	0[b]	0[b]	0.01[a]	0[b]

*Different letters in the same line (a,b) represent statistically significant differences in results ($p<0.05$)

Results gathered around provinces in the growing regions, displayed that the lowest oleic acid content was in the extra virgin olive acid from Hatay-Samandağ (62.31%, product of season 2013-2014). The highest oleic acid content was also measured in the extra virgin olive oil belonging to 2013-2014 season, in Aydın-Nazilli (78. 02 %). Statistically significant and lower ($p<0.05$) steraic acid % was detected the samples from the Aegean region. Differences among different provinces and regions of growing were also common to previous findings [34].

Ten samples of extra virgin olive oil and 8 samples of Riviera type olive oil werestudied [19] and according to the results, oleic acid% of extra virgin olive oil samples changed between 71.09 and 74.82 %, while in Riviera type samples it was 70.72-73.23%. In the current study, except for the highest sample (78.02 %); oleic acid content in extra virgin olive oil samples anged between 62.31-74.94 % and for Riviera samples 67.44-72.41 %. Therefore the findings were close to the results of their study [19]. However, generally, the literature findings related to oleic acid were higher than the present findings; whereas the palmitic acid contents were generally lower [34, 35]. In samples with lower oleic acid content, the other fatty acids such as palmitic acid and linoleic acid were more dominant with higher values. Average fatty acid % results of extra virgin olive oil samples according to olive varieties in Table 10.

Table 10: Average fatty acid % results of extra virgin olive oil samples according to olive varieties

Fatty Acid Composition, %	Ayvalık	Gemlik	Memecik	Mixed
Oleic acid	68.87^{a}	71.08^{a}	69.72^{a}	69.56
Linoleic acid	9.76^{a}	7.23^{b}	10.03^{a}	9.30^{a}
Linolenic acid	0.57^{b}	0.58^{b}	0.72^{a}	0.63^{a}
Palmiticacid	15.63^{a}	14.72^{a}	14.27^{a}	15.10^{a}
Stearic acid	2.55^{bc}	3.03^{a}	2.46^{c}	2.75^{ab}
Heptadeccdanoic acid	0.15^{a}	0.11^{a}	0.06^{a}	0.12^{a}
Heptadecenoic acid	0.22^{a}	0.17^{ab}	0.11^{b}	0.13^{b}
Arachidic acid	0.12^{a}	0.07^{a}	0.15	0.15
Eicosenoic acid	0.38^{a}	0.12^{a}	0.22^{a}	0.14^{a}
Palmitoleic acid	0.88^{a}	1.09^{a}	0.94^{a}	0.96^{a}
Myristic acid	0	0	0	0

*Different letters in the same line (a,b) represent statistically significant differences in results ($p<0.05$)

Table 11: Average fatty acid % results according to the seasons of harvest

Fatty Acid Composition, %	Season of harvest	
	2012-2013	2013-2014
Oleic acid	69.56	69.67
Linoleic acid	8.95	9.73
Linolenic acid	0.63	0.62
Palmitic acid	15.12	14.85
Stearic acid	2.67	2.67
Heptadecanoic acid	0.12	0.11
Heptadecenoic acid	0.16	0.14
Arachidic acid	0.12	0.15
Eikosenoic acid	0.36	0.06
Palmitoleic acid	0.99	0.91
Myristic acid	0	0

As depicted in Table 11, seasons of harvest made only slight differences on the average fatty acid % results of the samples ($p>0.05$). The comparison of oleic acid % contents of two harvest seasons revealed that, Gemlik had the highest, while Ayvalık had the lowest content of oleic acid %, for both seasons. These findings were parallel to previous findings [36].

Generally type of packaging had almost no effect on the fatty acid compositions of olive oil samples ($p>0.05$) as given in Table 12.

Table 12: Average fatty acid % results according to the type of packaging

Fatty Acid Composition, %	Type of Packaging		
	Glass	Plastic	Can
Oleic acid	69.68	69.57	69.84
Linoleic acid	9.36	9.31	9.57
Linolenic acid	0.60	0.64	0.60
Palmitic acid	14.99	14.99	14.82
Stearic acid	2.68	2.64	3.02
Heptadecanoic acid	0.13	0.10	0.14
Heptadecenoic acid	0.16	0.14	0.21
Arachidic acid	0.16	0.12	0.21
Eicosenoic acid	0.39	0.13	0.22
Palmitoleic acid	0.91	0.97	0.84
Myristic acid	0	0	0

CONCLUSION

Results presented in this study, indicated that some of the olive oil samples were not within the required limits given in Turkish Food

Codex standard. Unsatisfactory results are thought to be related with the problems in storage conditions, olive quality, effect of geographical differences and process conditions. On the other hand, fatty acid compositions were within the limits of Turkish standards and these results were indicators of purity but not adulteration. This study was significant as giving idea about the quality problems present in the olive oil products found in the Turkish market and frequent occurance of unsuitabilities of these products in the legal limit values.

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DETERMINATION OF HYDROXYMETHYLFURFURAL CONTENTS OF SOME APPLE JUICES ON THE MARKET BY HPLC METHOD

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Abstract

Hydroxymethylfurfural (HMF) were formed when foods that contains sugar stored at improper temperatures or heat treatment applied to high temperature during production. In this research,the conformities of some of the apple juices sold in the Turkish market were estimated according to Food Regulations . For this purpos eight different companies essays in markets of Istanbul were coolected randomly in order to analysis of PH, hydroxylmethylfurfural (HMF) and water soluble dry matter of apple juices . HMF was analysed in the each sample was carried out by using High Performance Liquid Chromatography (HPLC). Result indicated that minimum and maximum values for HMF. PH, water soluble dry matter of apple juices were estimated as 1.77-7.73 mg/L, 2.88-3.83 and 11.25-11.90% respectively .

Keywords: *Apple juice, Hydroxymethylfurfural (HMF), HPLC,PH*

INTRODUCTION

There is an increased concern in the apple juice processing industry about quality maintenance and the avoidance of product adulteration . One of the major issues of adulteration is the use and substitution of fresh juice with apple juice concentrate.

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Since the production of concentrated apple juice involves heating, evaporation and storage, any changes in the compositional profile of phenolic com- pounds could be potentially used as a marker for monitoring any adulteration and, hence, to provide a reliable tool to distinguish between fresh and concentrated apple juices [1].

5-hydroxymethylfurfural (HMF) is important quality criteria in fruit juices.The presence of HMF is considered as an indication of quality deterioration.It is formed as a result of dehydration of ketopentoses, particularly in acidic or high-temperature environments [2].

HMF consist of an aromatic alcohol,an aromatic aldehyde and the furan ring. Molecular weight of HMF 126.11 g / mol, density 1.29 g / cm^3, in the form of the chemical formula C_6O_6 H_3. HMF is an intermediate product of the well-known Millard Reaction or is formed a result of dehydration of hexoses under acidic environments. HMF is used as an index the formation of juices, milk, honey, cereals, determination of storage time in many products such as jams, in order to understand whether or chemical appropriate heat treatment [3].

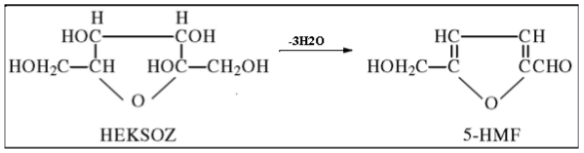

Fig. 1. Heating results in the acidic environment of 5-HMF formation of hexoses [3].

HMF is not present in fresh fruits,but it is naturally generated in sugar-containing food during heat-treatments such as drying or cooking. To date,the toxicological relevance of 5-HMF is not clearly documented. Nevertheless, 5-HMF in foodstuffs has attracted much interest because it exhibits mutagenic and DNA strand-breaking activity. Besides that, cytotoxic, nephrotoxic, carcinogenic, genotoxic are among other in vitro activities attributed to 5-HMF [4].

Maillard reaction (MR) may occur during food processing and/or storage, particularly at high temperatures, in carbohydrate containing and lysine-rich proteins foods. The reductor sugars and lysine are the main compounds involved in the initial states of the MR. In advanced states of MR,

undesirable compounds such as furfurals can be found [5].

If there is a level above a certain amount of HMF,the color is browning, significant deterioration in terms of taste and odor, lead to a reduction in nutrient levels of the product it is caused .For this reason, the possibility of marketing the product can eliminate partially or completely.Therefore, HMF in foods that are allowed are limited [6]. Apple juices are indicated that maximum limit of 10 mg /L [7].

HMF can be made of different analysis methods for quantitative analysis developed in the juice. These methods can be listed as spectrophotometric method, high pressure liquid chromatography (HPLC) method and the micellar capillary electro cromotografi (MECC) method. HPLC method is widely used in HMF analysis. The reason is that it is performed more quickly and easily than other methods. HPLC methods are routine and inexpensive way. It does not require any preparation except for sample dilution and filtration [8]. Purposes of this research were to investigate amount of HMF in apple juice and in Turkish markets in order to prevent maintenance and the avoidance of apple juice adulteration.

MATERIALS and METHODS

Apple juice samples were collected and selected radomly from the dominant firm productin Turkish market. The Company 1/1, 1/4 and 1/5 L-volume products were selected, each firm was represented one of the three different sample volumes.

Apple juice of different companies that make up packaging material research specimens of information is given in Table 1.

Product code	Production and Expiration date	Amount of product
A	17.09.14/17.09.15	200 ml
B	-/21.08.2016	1 L
C	23.12.14/23.12.15	200 ml
D	13.10.14/13.10.15	200 ml
E	20.06.15/20.06.16	200 ml
F	-/27.05.2017	250 ml (glass bottle)
G	22.08.15/22.02.17	1 L
H	-/12.06.15	1 L

Table 1. Packaging information of the examined apple juices.

From the information was given in the table in which shelf life of apple juices were written in the range of between 12 to 18 months. It was observed that on the number 3th and 8th samples expiried date and production date were not observed or unabled to readas shown in Table 1. Discussing the findings in each apple juice was considered separately and the evaluation was made according to the provisions of the applicable legislation or standards. Analysis were done two times for each product, the following analyzes were performed on the samples.

The pH value for the hydrogen ion concentration; PH meter (WTW pH İnolab 720 series) were determined.

Determination of soluble solid content; The ratio of water soluble dry matter of samples was determined after Refractometer (Leica Reichert Abbe Mark II) device wich wwas calibrated the sample with distilled water.

Hydroxymethyl furfural determination; HPLC method was performed by International Federation of Fruit Juice Producers 1996 [9]. Amount of HMF was determinated with the aid of reversed phase liquid chromatography on RP-18 column by using mobile phase water / methanol and was separated with the help of UV detector.

100 mg to 0.1 mg precision standard HMF were taken in to 100 ml flask and was completed in line with distilled water.For this investigation 10 mg / mL, 15 mg / L, 20 mg / L and 30 mg / L standard solutions for four different concentrations were estimated respectively. Solutions of different concentrations were injected seperately in doublicate by using of the 0.45 µm filter paper through via syringe into the device. Calibration curve were created according to area out put from the chromatogram which was obtained against each concentration. Apple juice sample was prepared from 25 ml each boxes and transferred into 50 ml volumetric flask then 1 ml Carez I, and then 1 ml Carez II solution were added into sample respectively [9]. The mixture were completed with distilled water up to 50 ml and filtered through coarse filter paper. 2 ml filtrate solution were diluted to 1:1 with water and then the diluted sample were filtered through 0.45 µm filter paper . 20 µm final filtrate solution were injected into the RP-18 column for HMF analysis.

RESULTS and DISCUSSION

PH value of apple juice samples analyzed were estimates between 2.88-3.83 the differences may be due to the cell sturcture of the apple varities. Water soluble dry matter (WSDM) were found between 11.25% and 11.90% which were specified with Food Regulations of Turkey as shown in table 2.

Sample	PH	WSDM(%)
A	3.40	11.70
B	3.72	11.45
C	3.33	11.25
D	3.36	11.60
E	3.50	11.75
F	3.58	11.90
G	2.88	11.40
H	3.83	11.55

Table 2. The pH and water soluble dry matter values of the apple juice samples.

HMF in apple juice sample that expressed to standart of HMF can be written;

$$HMF=(AS_A / AS_s) \times C_1 \times \text{Ratio of dilution}$$

Where AS_A is the peak area of HMF of the apple juice sample solution, AS_S is the peak area of the HMF standart solution and C_1 is concentration of HMF standart solution as shown in Fig.2.

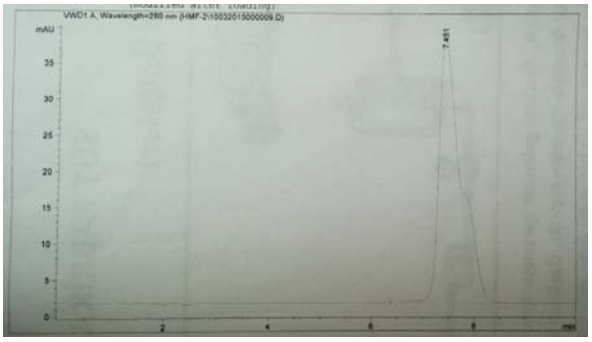

Fig. 2. HPLC chromatogram obtained 5-HMF standart (10 mg L^{-1}).

The HMF for all samples ranged from 1.77 to 7.73 (ppm) 10 mg L^{-1} depending on the type of companies product and varities of apples as shown in Table 3. These values are comparable to the TS 3633 which was given as up to 10 mg L^{-1} in which the amount of HMF present in apple juice samples were shown that there is no a significant different. This indicated that all samples were processed in good condition during concentration and filling .

Sample	The number of working examples	1	2	Average value	Standard deviation
A	2	1.49	2.06	1.77	0.16
B	2	5.69	5.48	5.58	0.02
C	2	5.37	4.27	4.82	0.60
D	2	2.20	2.30	2.25	0.01
E	2	3.20	3.62	3.41	0.08
F	2	8.53	6.94	7.73	1.26
G	2	5.74	6.56	6.15	0.34
H	2	2.42	2.13	2.27	0.04

Table 3. HMF amounts of apple juice samples.

These HMF are resonable agreement with the date presented by several other authers such as Telatar, during the processing of different varieties of apples and apple juice concentrate in work related to the formation of HMF, the HMF amount were found between 0-1.05 mg/L. The differences may be due to method and varities of applels investigation which were studied on the formation of HMF in the process of apple juice and during the process of evaporation of apple juice at three different temperature. Apple juices were extracted from Amasya, Golden Delicious and Hüryemez varieties which were largely used as raw material in the fruit juice industry. In his work, variety of apple and evaporation temperature were the efficient parameters on the formation of HMF. The HMF content of Hüryemez variety was found to be the most while Amasya variety was found to be the least. As a result, Amasya variety may be suggested as the most convenient one of fruit juice production [6].

Tüfekçi and Fenercioğlu, in their research, the conformities of some of the fruit juices (apple, pomegranate, orange and grape) sold in market to the Turkish Food Regulations were investigeted. The amount of HMF at work in apple juices were found between 1.62-7.49 mg/L. These HMF value are in agreement with our results while HMF of Pomegranate juice and grape juice samples

were above the limit of HMF value . This situation associated with was heat treated at a high temperature of this fruit juice concentrate or was stored in long been inappropriate temperature [10].

Elmastaş, in his study were determined amounts of HMF which forms during processing and storage of foods. The HMF amounts of apple juice were found between 1.01-2.70 mg/L. This result associated with a good production process and shorter duration of materials consumption of apple juices [11].

Lee et al., in their study, improved method for the simultaneous determination of patulin and 5-hydroxymethylfurfural (5-HMF) in sold fruit juices on the local market is described. The amount of HMF at work in apple juices were found between 0.08-14.5 mg / L. In this result, 5-HMF occurred in high incidence (100%) and 19% out of these samples exceeded the IFFJP (International Federation of Fruit Juice Producers) limits [4].

Results show that, the good precision and satisfactory recoveries with less generated makes this method useful for routine quality control of food products. This study is useful and provides the understanding of HMF levels in various apple juices.

CONCLUSION

In this study,samples of apple juice sold in the market was seen to vary the amount of HMF, PH and water soluble dry matter. Hydroxymethylfurfural (HMF) content of food is an important factor which should be taken into account in production. Simple, a precise and sensitive HPLC method which requires less chemicals and time for the determination of HMF. The method found useful for routine quality control of apple juice. Apple juices have emerged as a result of maintaining the proper storage temperature conditions as a result of the value of HMF content of less than maximum value of 10 mg/L. It is thought that the sensitivity achieved appears as an advantage particularly for the analysis of HMF in apple juices in which HMF concentration is relatively low. Also, well done production stage of the products and keep the short expiry date of the product consumption causes does not exceed the amount of HMF.

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EFFECT OF ENZYMES FOR INCREASING AMOUNT OF ANTHOCYANIN IN BLACK CARROT JUICE

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Abstract

In this study, the effect of two different enzyme, Natuzym DP+ (Weiss BioTech:10-A10166) and Rapidase Pac (DSM Pac: 414 294 401) on the black carrot juice were investigated for increasing amount of the anthocyanin content.

The black carrot juice were previcely heated at 85°C and cooled to 50°C than were added three different enzyme concentrations (25, 50, 75 µg/L) for one hour period at 50°C. At end of each experimental work the amount of anthocyanin content were determined by UV spectrophotometric method and compared with initial value.

The amount of anthocyanin content was increased from 831 mg/L up to 1337 mg/L and also It was found that the Natuzym DP+ was more effective than Rapidase Pac at 50°C for one hour period.

Keywords: *Black carrot juice, anthocyanin content, enzyme*

1. Introduction

Anthocyanins are members of the flavonoid group of phytochemicals, which is a well-known natural colorants and provide bright red colour in foods group such as teas, honey, wines, fruits, vegetables, nuts. (Kırca et al. 2007, Lila 2004). The sources include red cabbage, blueberries, cherries, raspberries, strawberries, black carrots, purple grapes and red wine (Mazza 2007). Because of their strong red to blue coloring,

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anthocyanins are the most recognized, visible members of the bioflavonoid phytochemicals (Ray et al. 2009).

Anthocyanins have been shown to be strong antioxidants and may be responsible for some biological activities including the prevention the risk of cardiovascular disease, diabetes, arthritis and cancer (Kahkonen 2003, Wang 1997). Anthocyanins have a good effects and interest to the artificial colorants The stability of anthocyanins is changed by different factors such as pH, temperature, light, pigment, metallic ions, the presenc of enzymes etc (Devi et al. 2012).

Black carrot is one of the most important root vegetable of *Apiaceae (Umbelliferae)* family (*Daucus carota* L.) originated from Central Asia, grown and consumed in Turkey, Afghanistan, Egypt, Pakistan and India ((Kaur et al. 2013, Elham et al. 2006). It is often used in juice, concentrate, shalgam i.e., food colorant in food sector (Türkyılmaz et al. 2012). It is among the fruits that contain high amounts of anthocyanins (Kırca et al. 2006) and other polyphenolics. Mazza and Miniati (1993) have reported a range of 1750 mg/100 g fresh weight and 45. 5 g/kg to 17.4 g/kg dry matter total anthocyanin amounts (Kammerer et al. 2004).

According to the investigations of scientists related to the black carrots, the results indicated that it contains high amounts of acylated anthocyanins such as sinapic, ferulic and p-cumaric acid (Dougall et al. 1998, Kırca et al. 2006). Because of the fact that related to the black carrots these starts to gain in the importance of further research activities of many scientists.

Black carrot is much more important in many cases due to its using mainly in food industries such as natural food coloring, drinks, beverage, confectionary, dairy, candies, yoghurt and although other like cosmetics, pharmaceutical starts to use the benefits of the black carrots.

Enzymes such as pectinase enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples and sapota. Pectinases have also been used in wine production (Anonim 2008). Enzymatic treatment alone or in combination with others, is one of the

potential pretreatment, which results in increased yield with better juice quality, colour and acceptability (Kaur and Sharma 2013). Khandare et al. (2011) indicated that enzyme-assisted black carrot juice processing significantly improved the antioxidant and total phenolics composition of black carrot juice. It was reported that overall increase of 33% in juice yield, 27% in total phenolics and 46% in total flavonoids. Khandare et al. (2011) reported that pre-press maceration treatment effected antioxidant composition of black carrot juice with different doses of cell wall degrading enzyme pectinase. Sharma et al. (2005) optimized the enzymatic process parameters for increased juice yield from carrot. In this study, it was reported that enzymatic treatment resulted in increase in juice yield by 13.95%. Kaur and Sharma (2013) reported that some factors affecting colour of carrot juice during storage are pH, acidity, processing temperature, duration and fruit cultivar.

Several researchers have recently reported that enzyme treatments significantly increase the juice yield, enhance the recovery of anthocyanins and phenolics and enhance the total anthocyanin content in black currant juice (Landbo and Meyer, 2004; Wang et al. 2009)

The aim of this research was to increase amount of pigments from black carrot juice by using a Natuzym DP+ and Rapidase enzymes at 50° C at an hour period time and also the effect of two different enzymes were investigated for increasing amount of the anthocyanin content

2. Materials and methods

2.1. Materials

Black carrots used in the study were obtained from the Büyükçekmece bazaar district of the city of Istanbul were processed immediately. A flow diagram of juice enzimatization and concentrate processing was shown in Fig. 1.

2.2. Juice Concentrate Processing

Carrots were washed and damaged parts were separated. Than grinding process of black carrot juice was made with juice extraction machine. Then, at 85 ºC for 10 minutes fruit juice is not boiled which is called blanching in order to inactivate enzymes. The purpose of this step was to provide the inactivation of enzyme naturally present in vegetables. If don't apply this

temperature enzymes can be effect on the depocosition of the phonelic compounds. The pure black carrot juice was processed by seperation of variable active substances. Black carrot juices was centrifuged at 40*100 rpm/rcf for 3 minutes. After centrifugation, carrot juice was hold 85-87 °C for 2-3 minutes in flash pasteurization, then cooling to 50-55 °C. Microorganisms in vegetable juice and other enzymes were inactivated and prevented quality loss in color and quality of mash.

The vegetable water that heated to 50 °C, enzyme was added to do experiment, the enzymatic fermentation was performed 60 minutes with Natuzym DP+ (Weiss BioTech:10-A10166) and Rapidase enzyme (DSM Pac:414294401) dosage. Thus, this process increased efficiency, vegetable juice color quality and color intensity. Undesirable particles in liquid was seperated with clarification. Coarse filtration with filter paper was preferred in carrot juices processing. They were analyzed for anthocyanins content

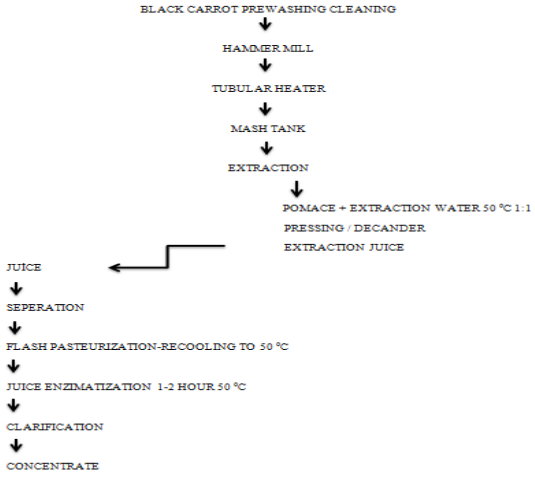

Fig. 1. Flow diagram for the processing of black carrot juice concentrate.

2.3. Methods

2.3.1. Other analyses

Brix was measured at 20°C using an Abbe's Refractometer and pH with pH meter (İnolab pH 720)

2.3.2. Enzymatic fermentation

In this research, Natuzym DP+ enzyme (25-50-75 µl enzyme solution/ L black carrot juice) and Rapidase Pac enzyme (25-50-75 µl enzyme solution/1 L black carrot juice) were added and kept at 50°C for 1 h which were filtrated and analyzed for total anthocyanins and other analyses. The effect of two different enzymes were investigated for increasing amount of the anthocyanin content.

2.3.2. Anthocyanin analysis

Total monomeric anthocyanins of samples were determined in duplicate using the pH-differential method as described by Giusti and Wrolstad (2001). For this reason, the aliquots of black carrot concentrate were first brought to pH 1.0 and 4.5 and were allowed to equilibrate 1 h at room temperature. The absorbance of equilibrated solutions was then measured at 560 (λ max) by using a UV-VIS spectrophotometer (JENWAY 6315). Pigment content was calculated based on cyanidin-3-glucoside (Alasalvar et al. 2005, Kirca 2007) with molecular weight of 449.2 and with extinction coefficient of 26,900 (Giusti and Wrolstad 2001). The difference in absorbance values at pH 1.0 and 4.5 was directly converted proportional to anthocyanin concentration. Quartz cuvettes of 1 cm pathlength were used and all measurements were carried out at room temperature. Absorbance readings were made against distilled water as a blank.

3. Results and discussion

While each application were processing, Brix and pH measurements were made. This measurements were made in raw material, after boiling, centrifuge, enzymation, pasteurization and after filtration. Table 1 and Table 2 shows that Brix and pH values of black carrots juices..

Respectively, stability of anthocyanins from black carrots was studied at various solid contents, °Brix and pH values in Natuzym DP+ and Rapidase Pac enzyme application as shown in Table 1 and Table 2.

Table 1. Brix and pH values of black carrots juices in Natuzym DP+ enzyme application.

Samples	Brix	pH
İn raw materials	10.9	6.15
After boiling	10.9	6.16
After centrifuge	9.5	6.22
After enzymation	9.5	6.08
After pasteurization	9.5	6.10
After filtration	9.5	6.11

Table 2. Brix and pH values of black carrots juices in Rapidase Pac enzyme application.

Samples	Brix	pH
İn raw materials	10.2	5.81
After boiling	10.2	5.86
After centrifuge	9.5	5.80
After enzymation	9.5	5.81
After pasteurization	9.5	5.80
After filtration	9.5	5.80

According to analysis; all the application and enzyme added processes didn't observed to exchange pH, but brix of the samples is changed by centrifugation.

It was observed, the implementation process did not changed the pH in carrot juice, but the centrifugation process was caused a decrease in the amount of dry matter. Enzyme application didn't change the pH at all. According to the readings, increasing concentrations of the Natuzym DP+ enzyme were found to be increased values of absorbance as shown in in Table 3.

Table 3. Total anthocyanins contents

Enzyme content	NATUZYM DP +	RAPIDASE PAC
Pre-enzyme treatment	831 mg/ L	1122 mg/ L
25 µl enzyme / 1 L black carrot juice	1177 mg/ L	1322 mg/ L
50 µl enzyme / 1 L black carrot juice	1235 mg/ L	1340 mg/ L
75 µl enzyme / 1 L black carrot juice	1337 mg/ L	1385 mg/ L

Initial anthocyanins contents were 831 mg/ L, 1122 mg/ L for Natuzym DP+ and Rapidase Pac respectively as shown in Table 3. These reference values were different each other due to several composition of carrot juices. The enzyme were used in this research was not affecton the pH of juice but the filtration and centrifugation were caused a reduction in degre of Brix content.

In this investigation was observed that the yield of anthocyanin depended on the additional amount of available enzyme as shown in Table 3. Effect of Natuzym DP+ enzyme related with concentration treatment for increasing anthocyanin concentration was shown in Fig 2 and also effect of Rapidase Pac enzyme treatment on increasing of anthocyanins concentration was shown in Fig 3.

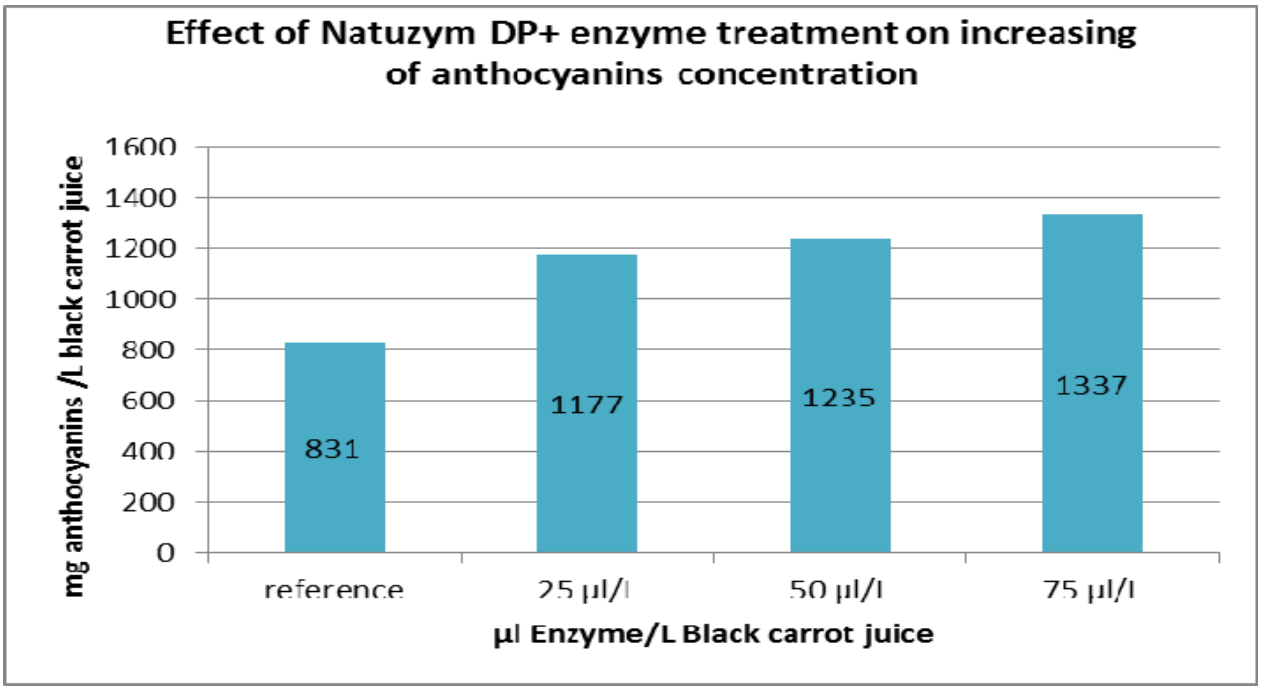

Figure 2. Effect of Natuzym DP+ enzyme treatment on the anthocyanins concentration

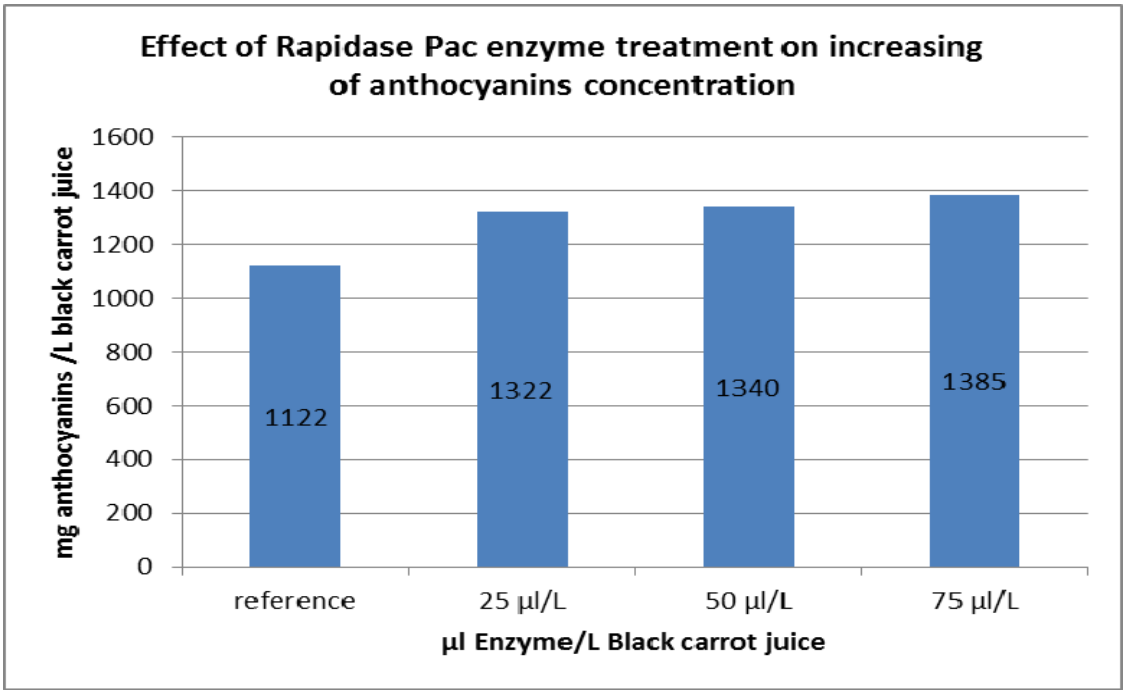

Figure 3. Effect of Rapidase Pac enzyme treatment on the anthocyanins concentration

Table 2. Enzyme yield depending on anthocyanins contents of black carrot juice

Enzyme used	Enzyme dosage (µl/l black carrot juice)	Yield of enzyme (%)
Natuzym DP+	25 µl	41.63
	50 µl	48.61
	75 µl	60.89
Rapidase Pac	25 µl	17.82
	50 µl	19.43
	75 µl	23.45

In Table 2 as can be seen that the yield of anthocyanins contents in black carrot juice depending on the enzyme concentration. According to Table 2, when enzyme dosage was increased, total amount of anthocyanins in black carrot juice were also increased. Thus, the result indicates that enzyme yield of Natuzym DP+ was more effective than Rapidase Pac enzyme were shown in Table 2. The highest enzyme yield of anthocyanin were obtained by additional of 75 µl Natuzym DP+ (60.89%), but the yield estimated with Rapidase Pac was 17.82%.

Compared with literature, Pectinex Ultra SP-L, Pectinex Smash, Pectinex BE 3-L and Biopectinase CCM were increased the total content of anthocyanins between 13–41% in the bilberry juices and 18–29% in the blackcurrant juices according to Buchert et al. 2005. In this study, It was reported that the most efficient enzyme preparation to increase the anthocyanin extraction was Pectinex BE-3L, increasing the yield by 41%. This result is in agreement with the results of our study.

The yield of enzyme found in black carrot juice in our study was higher than the values reported by author, such as Karadeniz and Ekşi (1999), who reported the effect of mash enzymation (Pectinex BE 3-L, Novo Nordisk Ferment) on juice yield and chemical composition of sourcherry juice, the increase in juice yield due to mash enzymation was found values of 6.25-9.0%. The contents of anthocyanins in the sourcherry juices ranged from 94 to 140 mg/l. It was caused differences so the structure of the fruit and enzymes used in this study than in black carrot juice. In our study, the total anthocyanins contents of black carrot juice were determined values of 831 -1337 mg/l. in Natuzym DP+ enzyme treatment, while the contents of total anthocyanins was 1122-1385 mg/l in Rapidase Pac enzyme treatment. Contents of total anthocyanins in juices of bilberry ranged from 1823 to 20174 mg/kg reported by Buchert et al. 2005. The differences may be due to the structure of juices.

Türkyılmaz et al. (2012) were found that depectinisation and bentonite treatments resulted in 7% and 20% were increased in monomeric anthocyanins content of black carrot juice respectively, but gelatine-kieselsol treatment and pasteurisation were caused to 10% and 3-16% reduction. Contents of total anthocyanins of two different the black carrot extracts value of 93.8 and 126.4 mg/ 100 g fw, were reported by Algarra et al. 2014. As comparing with our enzyme treatments were increased anthocyanin content than other researchers.

The authors Junker (1987) and Yücel (1993) were found that in apple juices by increased additional mash enzymes which were increased yield of anthocyanin between 5 to 30% . It also lower than our results. It was reported that a process for the increased juice extraction form carrot, enzyme

concentration changed from 50-650 mg/kg of grated carrot (Sowbhagya 2010).

Landbo and Meyer (2004) reported that juice yields ranged from 66.4% to 78.9% by wet weight of black currant mash in pre-press maceration treatments with 10 different pectinolytic enzyme preparations in experimental black currant juice production. The yields of anthocyanins in the juices were increased from 900 to 2200 mg/kg wet weight black currant mash. Similar results were also reported in black carrot juice. The differences may be due to cell structure of black carrot and black currant

Mieszcczakowska (2012) investigated that impact of enzyme on quality of balackcurrant and juices. Results were reported that the best pressing-yield for blackcurrant was achieved with polygalacturonase and pectin lyase, 65 g/100 g after 1 h and 74 g/100 g after 4 h of pectinolysis. The macerating mixture gave about 58-59 g/ 100 g yield, pressing-yield of plum juices was in the range of 94-97 g/ 100 g . This results associated and comparable with our results.

Tochi et al. (2009) reported that two commercial enzyme preparations, a pectinase and a liquid pectinase/hemicellulases were used singly or in combination at a rate 0.03% (w/w) in a two step extraction of pineapple juice. The concentrated juices were extracted twice treated with (Fluka) Rapidase enzymes. According the results of sensory evaluation, juice extracted using Rapidase scored better than that extracted with either Fluka. These observations were in agreement with our results.

4. Conclusions

The most efficient enzyme preparation to increase the anthocyanin extraction was 75 µl Natuzym DP+ enzyme dosage in black carrot juice, was increased the yield up to 60.89%. The enzyme treatments had quite significant effects on the total anthocyanins content in black carrot juice. This study indicates that the enzyme treatment were used in this processing strongly releated additional enzyme concentration, time and temperature on the anthocyanin extraction. It can be conculed that black carrot is a rich source of anthocyanins and an important colorant foods such as beverages, fruit juice

processing, confectionary, dairy products with good functional and nutritional value.

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INVESTIGATION OF SORBIC ACID AND BENZOIC ACID AMOUNT OF SOME FOOD EXPOSED FOR SALE IN ISTANBUL

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Abstract

Recently, interest in studies have aimed at preservation of foods, ensuring their safety and extending their shelf life span, which has lately intensified. Sorbic acid and benzoic acid which are also two of chemical preservatives, extend the shelf life span of food by preventing microbial deterioration. However, health problems such as urticarial reactions, asthma, hyperactivity in children and deterioration of hormone balance can occur by accumulation of these preservatives in the body.

In this study; the amounts of sorbic acid and benzoic acid were identified by using High Performance Liquid Chromatography (HPLC) in cake, industrial and traditional tomato paste, industrial and traditional pepper paste, ketchup, mayonnaise, vegetable margarine and carbonated beverage that is put on sale in Istanbul market, belonging to different companies. The obtained results were compared with maximum values that have been specified in Turkish Food Codex "Regulations of Food Additives except Colorants and Sweetening''. This in addition to the evaluation of the identified amounts of sorbic acid and benzoic acid according to food safety and public health.

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While sorbic acid amount was found between; 0.00-456,08 mg/kg in cake; 0.00-1820,31 mg/kg in traditional tomato paste; 0.00-1071,92 mg/kg in traditional pepper paste; 0.00-227,27 mg/kg in ketchup; 0.00-892,46 mg/kg in mayonnaise; 0.00-1279,89 mg/kg in vegetable margarine; 0.00-169,36 mg/kg in carbonated beverage, no sorbic acid was encountered in industrial tomato paste and industrial pepper paste.

At the time benzoic acid amount was recorded between; 0.00-47,42 mg/kg in cake; 0.00-2417,65 mg/kg in traditional tomato paste; 0.00-22,28 /kg in industrial tomato paste; 0.00-4613,84 mg/kg in traditional pepper paste; 0.00-838,09 mg/kg in ketchup; 0.00-176,47 mg/kg in carbonated beverage, no benzoic acid was encountered in industrial pepper paste, mayonnaise and vegetable margarine. In this study; sorbic acid and benzoic acid content in some of foods that people are consuming daily exceeded the limits that codex had specified.

Keywords: *Sorbic acid, benzoic acid, HPLC, food additive, food safety*

Introduction and Purpose

Currently food consumption rate proportionally increases in parallel with the growing population. Demand for fast food has increased more than past. With the effect of life conditions of humans and in this case additives are being added to foods in order to extend shelf life of food it even increased further

Food additives are substances that can be added to foods at any stage of production, processing, packaging, distribution or stocking. It is evident that the market of additives that are worldwide uses as either artificial or natural, had reached up to 10 billion dollars in 1900's, has a lot more increased nowadays (Randhawa and Bahna, 2009).

The reason of using additive in food is that it is a compulsory food preservation method. Food additives that are being used not only extend shelf life span of food, but also quality durability. The most important usage condition of these additives; is to keep health problems that may occur from its

consumption at minimum level (Sarıkaya and Solak, 2003). Among additives, antimicrobial substances that can be added to solid and liquid foods or to various beverages; are being used in order to efface mold, ferment and every kind of microorganism that is not desired in food however there is a probability of being existed, from environment or prevent their reproduction. Being able to perform their tasks that these materials, have depends on composition, amount of the material used and pH (Altuğ, 2001). It was observed that it has manifested toxic effect when used at higher amount than specified dosages in food. Place for acute, genetic and pharmacokinetic studies is being given according to toxicological evaluations of additives; studies are being conducted on sub-chronic experiments regarding their teratogenic effects, chronic researches regarding their carcinogenic effects (Çakır, 2010).

There are findings regarding the increase of consumption of food additives, which are allowed to be used, and they show toxic effect and caused worries. The uttermost seen have been eczema, asthma, headache, allergic itches, gastric discomforts, diarrhea (especially in children), hyperactivity and hypersensitivity etc. Even if dosages that will not harm health are utilized, subjects such as they might reach to amounts that can cause a problems in public health by gathering, these materials, in the body after a while, they might create damage to tissues. Briefly they might be mutagenic and carcinogenic for humans, must not be neglected (Brigs 1997, Koyuncu 2006).

In case of the additives added at the production stage of some food in İstanbul market and generally consumed by people (sorbic acid and benzoic acid) are used consciously or unconsciously, it has been aimed to detect whether they are appropriate for the limits indicated by Turkish Food Codex or not, and to evaluate the detected sorbic acid and benzoic acid amounts in terms of food safety and public health with this study.

Material and Method

1. Material

Some food products (cake, industrial and traditional tomato paste, industrial and traditional pepper paste, ketchup, mayonnaise, vegetable margarine, carbonated beverage) that are being sold in market of Istanbul, formed the research

material of this study. A total of 50 products were analyzed in order to identify the existence of sorbic acid and benzoic acid. Cakes such as chocolate cakes and chocolate eclairs belonging to different companies were chosen. Among pastes those that are produced industrially belongs to different brands. As for pastes produced traditionally, their treatment method is drying by sun. Ketchup, mayonnaise and vegetable margarine samples were also studied from different brands. Yet again carbonated beverages belonging to different brands were chosen and it was paid attention to be its variety be different.

2. Method

In this study HPLC device, Perkin Elmar Series 2000 brands were used in order identify the existence of sorbic acid and benzoic acid. As for sample preparation; 'Nordic Committee on Food Analysis, Benzoic acid, sorbic acid and p-hydroxybenzoic Acid Esters, Liquid Chromatographic Determination in Foods' method was used (Anonymous, 1997).

As chromatographic condition; C-18 analytical column with the diameter of 5 µm was used for chromatographic separation of sorbic acid and benzoic acid. As for mobile phase; acetate tampon and methanol mixture was used. Column conditioning process was done by passing mobile phase from the column before analysis.

2.1.Extraction Process

For extraction of sample, vegetable oil that is produced by thoroughly making homogeneous in homogenizer, and other products that are also produced by being mixed homogeneously, were prepared for subsequent process. 5 gr from solid samples and 5 mL from liquid samples were taken into 100 mL volumetric flask. The sample was made to mix with water by severe rinsing after adding 30 mL deionized water. 60 mL methanol was added into water-sample mixture and was once more rinsed severely. Meanwhile heated sample was fulfilled with methanol to 100 mL volume by cooling. About 20 – 30 mL of the 100 mL mixture was passed through filter paper. The obtained mixture was passed through a syringe-end filter that is 0,45 µm and has 13 mm diameter. During these processes sample was diluted 20 times and the result that was obtained during reading process of the sample in HPLC device, was set to multiplicate with 20. Final solution was placed in vial in order to

be measured in HPLC device that has UV detector, auto-sampler and pump.

2.2.HPLC Process

Chromatographic conditions for HPLC analysis; its flow rate is 1.2 mL/min, injection volume is 20 µL, determination wavelength is 238 nm and at room temperature. First of all calibration curve was drawn after preparing sorbic acid and benzoic acid standards with concentrations of 0,5-2,5-5,0-10,25,50,100. A standard sample was identified based on this curve and was being informed about accuracy of calibration, arrival time and fields of peaks. Exit time of standards from column were identified as for sorbic acid 5.14 min, benzoic acid 3.92 min. Extraction of samples was transferred into vial units just to make measurement, and then was placed in the auto-sampler part of HPLC device. Arrival times and spectrums of components were compared with standard. Peak purities checked existing spectrums. Thus, it was confirmed with identification, spectrums and arrival times of sorbic acid and benzoic acid, either exist or do not exist in the sample. Calibration curves were drawn with standard calibration solutions. According to calibration curve; results of the samples were identified from the device. Results; by multiplying with 20, basing on concentrations that come equivalent to fields of peaks that is read opposite to calibration graphic, value within the sample will found. Real value is obtained by multiplying the found results with recycle rate.

FINDINGS

From places where there are bakery, market, charcuterie etc. from various parts of Istanbul, examined samples; among foods produced as industrially wrapped or produced as traditionally, with the condition of being 10 gr from each, 50 units of samples were collected and examined in order to recognize the existence of sorbic acid and benzoic acid.

1. Sorbic Acid Findings

After making extraction process to the samples, reading process was done in HPLC device by transferring them into vials. After reading process in HPLC device; in 19 units out of 50 unit samples no sorbic acid was encountered. And in 31 unit samples sorbic acid was identified. Sorbic acid amount in 5 unit samples is not appropriate for the limits that Turkish Food Codex specified.

2. Benzoic Acid Findings

Following extraction process, samples were transferred into vials and reading process was conducted in HPLC device. No benzoic acid was encountered in 32 units out of 50 unit samples. And in 18 samples benzoic acid was identified. Benzoic acid amount in 2 unit samples is not appropriate for the limits that Turkish Food Codex specified.

Sample Type	Sample Amount (n)	Rescript (limit)	Identified values	Its suitability
Chocolate cak	5	SA+BA=300 mg/kg	45,77-140,68 mg/kg	Suitable
Chocolate eclairs	5	SA+BA=300 mg/kg	0,00-456,08 mg/kg	1 unit sample is not suitable
Industrial Tomato Paste	5	SA=1000 mg/kg	0,00-0,00 mg/kg	Suitable
Traditional Tomato Paste	5	SA=1000 mg/kg	508,91-1820,31 mg/kg	1 unit sample is not suitable
Industrial Pepper Paste	5	SA=1000 mg/kg	0,00-0,00 mg/kg	Suitable
Traditional Pepper Paste	5	SA=1000 mg/kg	113,92-1071,92 mg/kg	1 unit sample is not suitable
Ketchup	5	SA+BA=2000 mg/kg	0,00-838,09 mg/kg	Suitable
Mayonnaise	5	SA+BA=1000 mg/kg	0,00-892,46 mg/kg	Suitable
Vegetable Margarine	5	SA=1000 mg/kg	399,76-1279,89 mg/kg	1 unit sample is not suitable
Carbonated Beverage	5	250SA+150BA mg/kg	97,87SA+0,00BA mg/kg - 0,00SA+176,47 BA mg/kg	1 unit sample is not suitable

CONCLUSION AND DISCUSSION

1. CONCLUSION

1.1 Chocolate Paste Group

5 units of chocolate cake and 5 units of chocolate samples were analyzed. None of the samples that were not appropriate for Turkish Food Codex was identified in chocolate cake samples. While no sorbic acid was encountered in 1 sample of chocolate cakes, sorbic acid amounts of the remaining samples varies between 0,00-100,21 mg/kg. Yet again no benzoic acid was encountered in 3 samples, benzoic acid amounts of the remaining samples varies between 0,00-47,42 mg/kg.

Sorbic acid amount of 1 unit of chocolate eclairs samples was identified not to be appropriate for Turkish Food Codex. Total value of sorbic acid and benzoic acid of the sample that is not appropriate, is 456,08 mg/kg. No sorbic acid was encountered in 2 unit samples. Sorbic acid amounts of the remaining samples varies between 0,00-456,08 mg/kg. No benzoic acid could be identified in chocolate eclairs samples.

According to Turkish Food Codex "Regulations of Food Additives Except Colorants and Sweetening'', Sa + Ba value for milk based desserts that is not heat treated is 300 mg/kg (Anonymous, 2013).

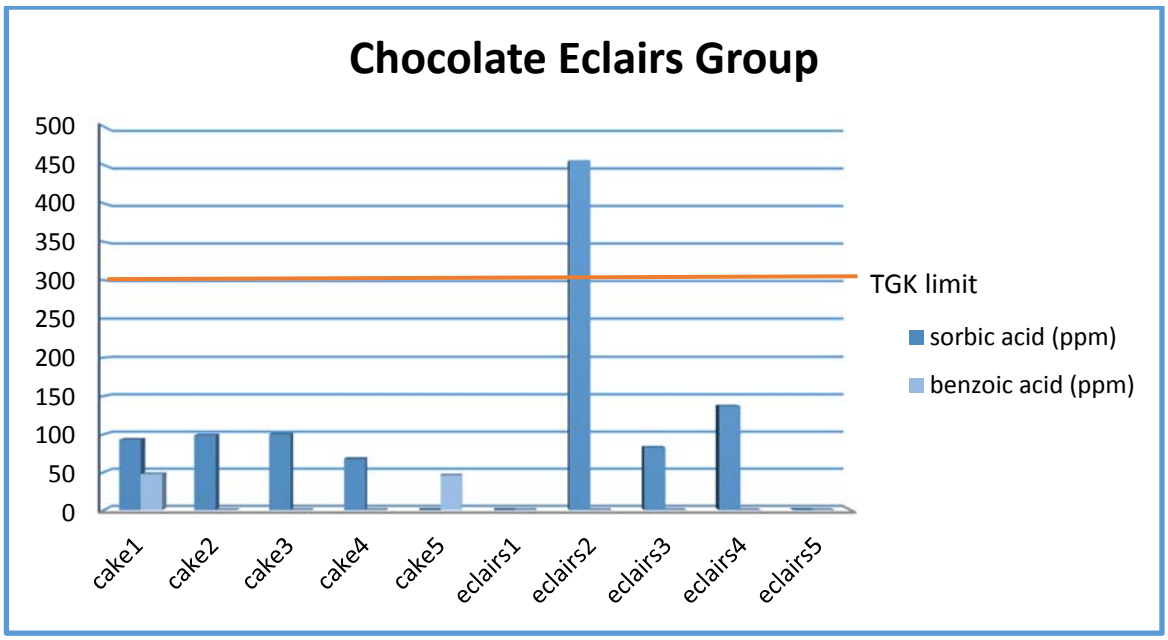

Figure 1: *Sorbic acid and benzoic acid amount identified in chocolate cake samples*

1.2. Tomato Paste Group

All the samples were appropriate to Turkish Food Codex and could be identified by analyzing 5 units of industrial tomato paste samples. No sorbic acid was encountered in any of the samples. Nonetheless, while no benzoic acid was encountered in 2 of the samples, benzoic acid amounts of the remaining 3 samples varies between 19,51-22,28 mg/kg.

Sorbic acid amount of 1 unit out of 5 traditional pepper paste units, was identified not to be appropriate for Turkish Food Codex. Sorbic acid amount of the sample that is not appropriate, is 1820,31 mg/kg. Sorbic acid amounts of the remaining samples varies between 508,91-867,51 mg/kg. Their benzoic acid amounts varied between 213,82-2417,65 mg/kg.

According to Turkish Food Codex ''Regulations of Food Additives except Colorants and Sweetening'', SA value for fruit and vegetable preparation (only paste, tomato mash and pepper mash) is 1000 mg/kg. No limit was specified regarding benzoic acid (Anonymous, 2013).

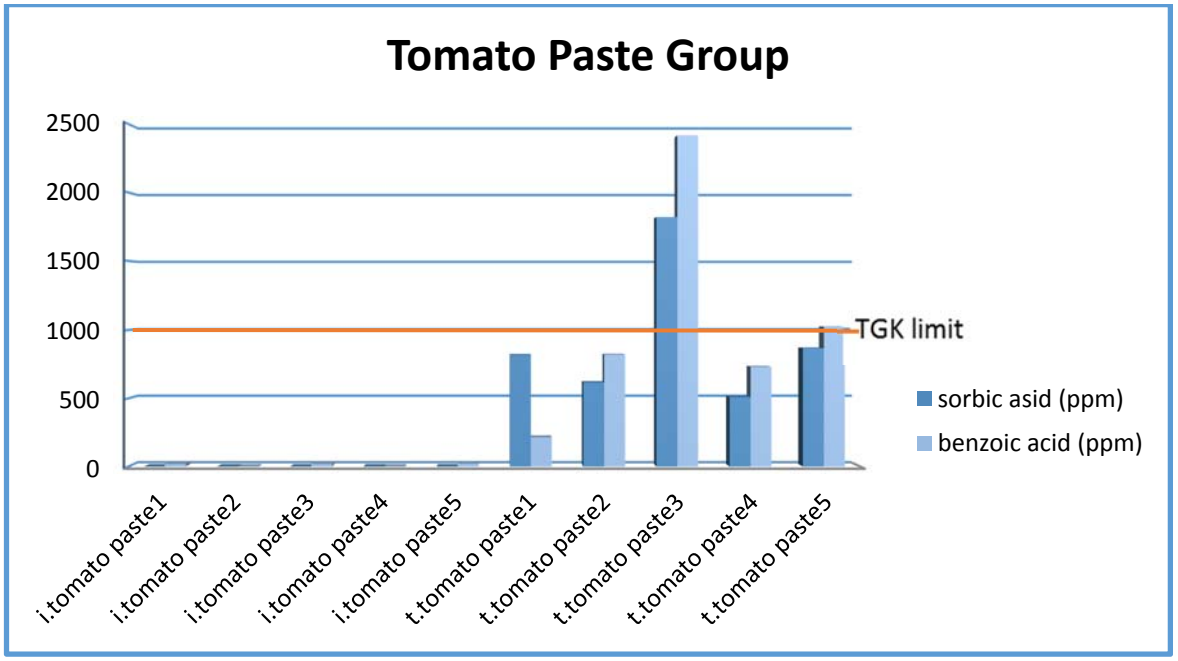

Figure 2: *Sorbic acid and benzoic acid amount identified in tomato paste samples*

1.3. Pepper Paste Group

All the samples were appropriate to the Turkish Food Codex and could be identified by analyzing 5 units of industrial pepper paste samples. No sorbic acid was encountered in any of the samples. Sorbic acid amount of 1 unit out of 5 traditional tomato paste units, was identified not to be appropriate for Turkish Food Codex. Sorbic acid amount of the sample that is not appropriate, is 1070,92 mg/kg. Sorbic acid amounts of the remaining samples varies between 113,92-971,71 mg/kg. Their sorbic acid amounts varied between 29,02-4613,84 mg/kg.

According to Turkish Food Codex "Regulations of Food Additives except Colorants and Sweetening'', SA value for fruit and vegetable preparation (only paste, tomato mash and pepper mash) is 1000 mg/kg. No limit was specified regarding benzoic acid (Anonymous, 2013).

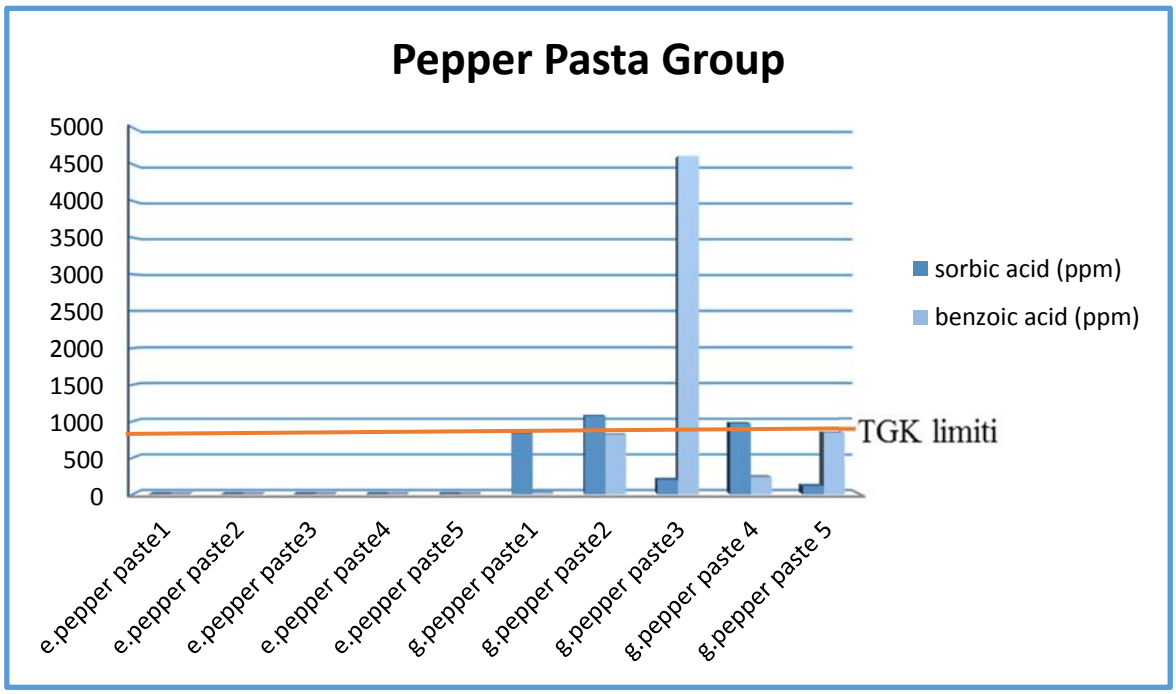

Figure 3: *Sorbic acid and benzoic acid amount identified in pepper paste samples*

1.4. Ketchup and Mayonnaise Group

In 5 analyzed Ketchup samples, all results match the standards fo Turkish Food Codex. No sorbic acid was encountered in 4 of the samples. Sorbic acid amount in 1 unit was identified as 227,27 mg/kg. However, while no benzoic acid was encountered in 3 of the samples, benzoic acid amounts of 2 samples varies between 0,00-838,09 mg/kg.

According to Turkish Food Codex "Regulations of Food Additives Except Colorants and Sweetening'', while sorbic acid amount for ketchup which is within emulsified sauces group that contains less than 60% fat, is 2000 mg/kg, benzoic acid amount is 1000 mg/kg. And its SA + Ba value is 2000 mg/kg (Anonymous, 2013).

Almost all the samples match the Turkish Food Codex and could be identified in 5 units of mayonnaise samples. No benzoic acid could be encountered in any of the samples. Yet again while sorbic acid could not be identified in 1 of them, sorbic acid amount in 4 samples varies between 0,00-892,46 mg/kg.

According to Turkish Food Codex "Regulations of Food Additives Except Colorants and Sweetening'', while sorbic acid amount for mayonnaise which is within emulsified sauces group that contains less than 60% fat, is 1000 mg/kg, benzoic acid amount is 500 mg/kg. And its SA + Ba value is 2000 mg/kg (Anonymous, 2013).

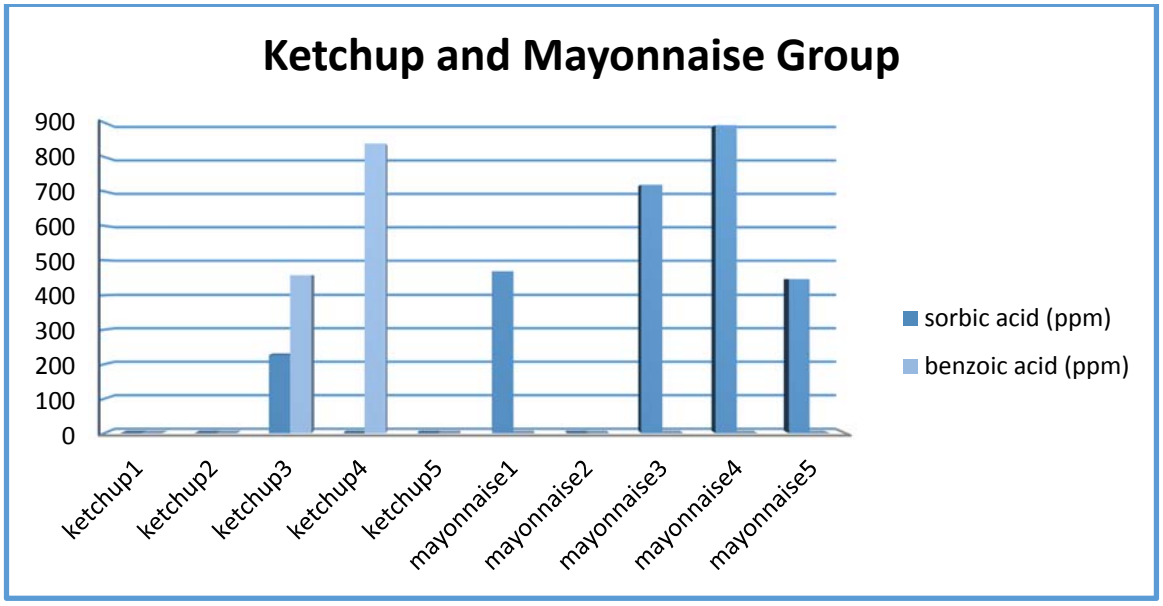

Figure 4: *Sorbic acid and benzoic acid amount identified in ketchup and mayonnaise samples*

1.5. Vegetable Margarine

1 out of 5 unit analyzed vegetable margarine samples is not appropriate for Turkish Food Codex. No benzoic acid could be encountered in any of the samples. And their sorbic acid amounts varied between 399,76-1279,89 mg/kg.

According to Turkish Food Codex "Regulations of Food Additives Except Colorants and Sweetening'', while sorbic acid amount for vegetable margarine which is within fat emulsions (except butter) group that contains more than 60% fat, is 1000 mg/kg (Anonymous, 2013).

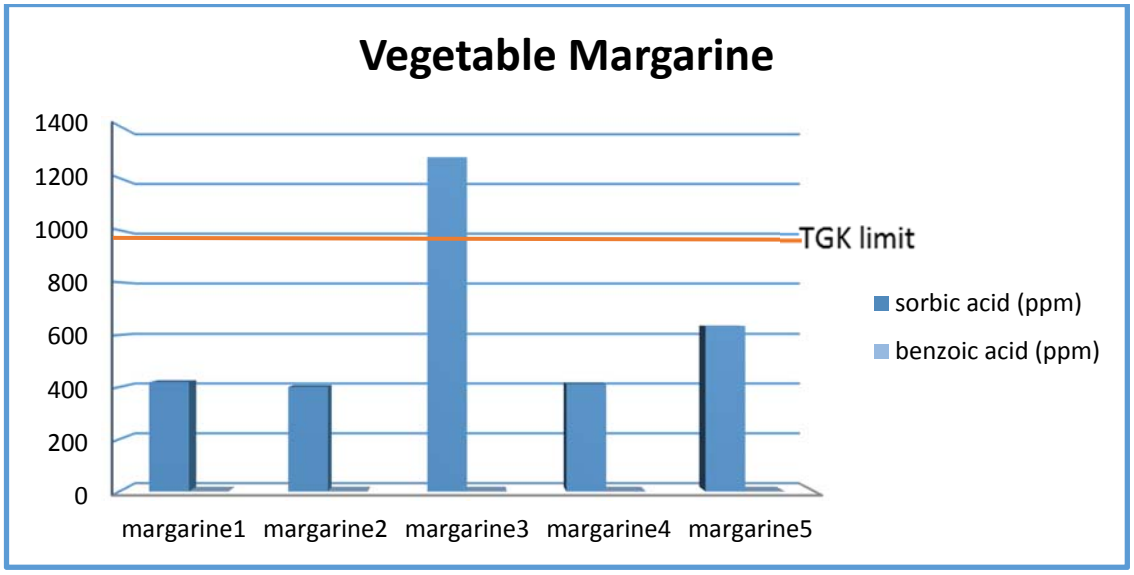

Figure 5: *Sorbic acid and benzoic acid amount identified in vegetable margarine samples*

1.6. Carbonated Beverage

1 out of the 5 tested units, carbonated beverage samples is not appropriate for Turkish Food Codex. While no sorbic acid was encountered in 1 of the samples, Sorbic acid amount of the remaining 4 samples varies between 97,87-169,36 mg/kg. Yet again while no benzoic acid was encountered in 4 of the samples, benzoic acid amounts of 2 samples were identified as 176,47 mg/kg.

According to Turkish Food Codex "Regulations of Food Additives Except Colorants and Sweetening'', sorbic acid amount for carbonated beverages which is

within aromatized beverages that does not contain alcohol group, is 300 mg/kg, benzoic acid amount is 150 mg/kg (Anonymous, 2013).

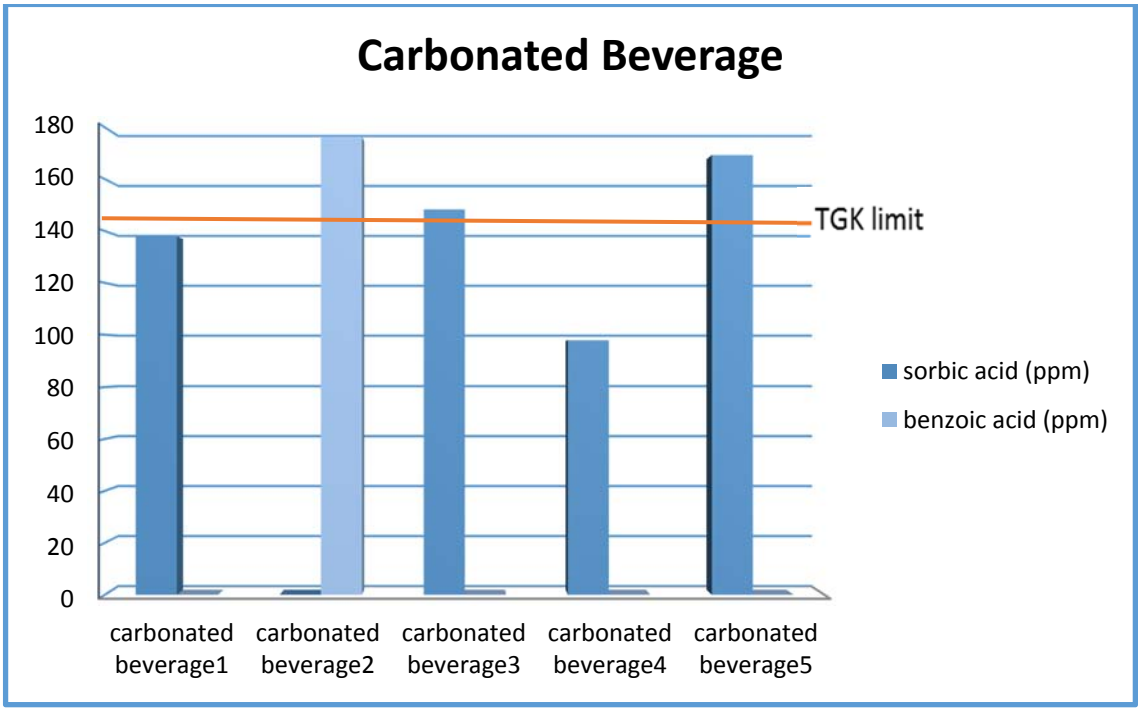

Figure 6: Sorbic acid and benzoic acid amount identified in carbonated beverage samples

2. DISCUSSION

Antimicrobial substances that can be added to food, are being used in order to efface mold, ferment and pathogen or non-pathogen microorganisms, that is not desired in food however there is a probability of being existed, from environment or prevent their reproduction. However pH and compound of the environment is an important factor in order these matters to perform their duties.

And primary compounds that are used for this purpose are materials such as sorbic acid, benzoic acid, nitrate, nitrite, salicylic acid (Yentür et al., 1995).

Sorbic acid and benzoic acid are kind of additivies that are used as antimicrobial preservative in food sector. If over taken into the body it may cause sicknesses such as urticarial reactions, asthma, hyperactivity in

children, hormone balance disorder (Erkmen, 2010). Especially benzoic acid; is being stated by literatures that it causes health problems such as trigger of neurotic disorder, weight loss, brain damage, rubescence and thickening in the skin, occurance of tumors (Wibbertman et al. 2000, Omaye 2004, Çakır 2011). In such case it forms great importance in regard of public health.

As Arda and Özşahin stated in their study, some chemicals when makes contact with the skin, may cause localized erythema and dropsy in the contact area within 15-60 minutes. This situation occurs depending on variances such as structure, concentration of chemical and exposed skin area. Urticarial reactions stay as localized and generally systemic symptoms do not occur. And these kind of reaction are; show up with benzoic acid, sorbic acid, alcohol, balsam of peru, metil salicylates (Arda and Özşahin, 2005).
Daily acceptable intake amount that JECFA had designated is 0-25 mg/kg for sorbic acid and its salts, and 0-5 mg/kg body weight for benzoic acid and its salts (Anonymous, 1996).

Sorbic acid and benzoic acid lately are widely used by producers as preservative food materials in the foods. Benzoic acid's specifications such as being low cost, being colorless and showing less toxic effect, has made it to take its place among uttermost used preservatives (Aktan et al., 1999). However it is being emphasized that it must be used at low level and with sorbates because of it being efficient at a narrow pH interval and forming undesired taste especially in fruit juices. And sorbic acid and its salts have a neutral taste and its difference from benzoates is that it improves by modifying the taste in fruits juices and some beverages (Altuğ, 2009).

Antimicrobial preservative additives that take place in Regulations, have been specified for some foods. But it is insufficient because this material threatens public health by indiscriminate usage by the producers, for the foods that their usage limits has not been specified (Koyuncu, 2006).

In this study; in the case of usage of, either conscious or unconscious, additives (sorbic acid and benzoic acid) added to some foods, that exist in market of Istanbul and people are generally consuming, during production stage; determination of them either being appropriate for the limits that Turkish Food

Codex had specified; had been identified using HPLC device and evaluation of identified sorbic acid and benzoic acid amounts in regard of food safety and public health was aimed.

Except for some paste and chocolate eclairs samples, in almost all product groups sorbic acid and/or benzoic acid was encountered; in the result of this study that contains different product groups, that was conducted in a total of 50 analyses belonging to different companies. Amounts of additives that was identified in some of these samples are at the level that Turkish Food Codex had specified and had been allowed. There is not a problem in such situation. However preservative amounts above the limits that regulations had specified, have been identified in 10% of total sample amount. And this situation especially forms threat in the regard of public health.

In a study conducted by Lino and Pina in 2010 in Portuguese, non-alcoholic beverages and some nectars were inspected. They had identified benzoic acid with the concentration of 158 mg/L and 148 mg/L, respectively, in the samples inspected as 11 traditional non-alcoholic beverage and 8 of them as non-alcoholic mineral beverage. Neveretheless, they had identified sorbic acid with the concentration of 172 mg/L and 188 mg/L, respectively, in the samples inspected as 11 traditional and 8 of them with mineral. Usage of sorbic acid and benzoic acid is allowed at specific limits in non-alcoholic beverages. However as it can be seen, the values they had obtained are above the specified limits (Lino and Pena, 2010). When this study conducted compared with other studies; one of carbonated beverage samples, in our study as well, contains disallowed amount (176,47 mg/L) of benzoic acid. Which means this study conducted is parallel with the study they had conducted. In such case supervision of food needs to increase.

Paste analysis had been done in study conducted by Çakır in the year 2011. A total of 23 paste samples were analyzed. He identified sorbic acid in 3 of them and benzoic acid in 6 of them (Çakır, 2011). In this studya total of 20 pieces paste samples correspondingly; sorbic acid were identified in 10 of the samples, benzoic acid in 13 of them. While there is a limit for sorbic acid amount in paste according to Turkish Food Codex, no limit regarding benzoic acid have been specified. However, in this study

benzoic acid at high rates have been recognized in paste and benzoic acid pose a problem over public health. Producers too, throw public health into risk by using benzoic acid at random rates. According to this supervision of food must be increased and the rescript needs to be recomposed.

Eventually, while food additives contains positive effects for the producers, it may cause a problem for the consumers especially when there is a carelessness at its usage. It is possible to reduce its harms to minimum level by paying attention to levels that regulations specifies, at its usage, in order to not cause a threat for public health.

And the most important factor that consumer needs to pay attention is; try to front to natural foods as much as possible, reduce fast food consumption to minimum and if it is being consumed, foods that contain no food additives as specified on its label or the foods that contains least should be preferred, by paying attention to additives in the food.

Producers must be warned about usage of food additives by raising awareness on consumers. Likewise government too, must arrange control mechanisms and laboratories that make production of food additives, appropriate for the standards. In addition, regulations relevant to the subject must be rearranged. As for its reason; some food materials that does not take place in the regulations, are being used unconsciously and in a wrong way by the producers (Yıldız, 2010).

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PHENOTYPIC DETERMINATION OF ESBL- and AmpC-PRODUCING ENTEROBACTERIACEAE IN CHEESE SAMPLES

Aylin ÖZADAM[1], Haydar ÖZPINAR[2*]

Abstract

The off-label over use of antibiotics results in development of antibiotic resistance in the bacteria. Beta-lactamase producing Enterobacteriaceae adversely affects the human health by leading to therapeutic failures against infections. Although microbiological criteria have been considered appropriate to the Food Codex, an inspection for antibiotic-resistant enterobacteria has not come into force yet. Therefore, the detection of foodborne beta-lactamases has gained significant importance for the human health. The objective of this study was to determine ESBL- and AmpC- producing *Enterobacteriaceae* in cheese phenotypically. In this study, a total of 83 cheese samples was examined by performing pre-enrichment, enrichment on selective media, and oxidase test according to the Criteria by ISO/DIS21528-2 microbiologically. Based on the microbiological results, a total of 18 isolates, including *Klebsiella pneumoniae* (27.8%), *Hafnia alvei* (27.8%), *Escherichia coli* (22.2%), *Klebsiella oxytoca* (11.2%), *Enterobacter cloacae* (5.5%), and *Citrobacter* spp. (5.5%) was identified by mass spectrometer. The phenotypic characterization of beta-lactamase type was conducted by disc diffusion, disc diffusion confirmation, and MIC determination according to the Guidelines of Clinical and Laboratory Standards Institute. The phenotypic results revealed that the most common beta-lactamase type was determined as ESBL in 9 isolates, followed by ESBL & AmpC in 4 isolates, and AmpC in 5 isolates, respectively. In conclusion, our study showed that ESBL- and Amp- type beta-lactamases were the most common phenotypes in Enterobacteriaceae from cheese. The cheese

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samples containing ESBL- and Amp- positive bacteria significantly presented a health risk for the consumers.

Keywords: *Antibiotic resistance, beta-lactamase, cheese, Enterobacteriaceae, food safety, public health.*

1. Introduction

The Global consumption of antibiotics in food-animals for growth promotion and disease prevention is twice that of humans [1]. The use of antibiotics cannot be controlled effectively due to economic concerns of the animal farming sector largely ignoring risks associated with human and animal health. Therefore, foods of animal origin are under suspicion for being transmission vectors for colonization and infection of the humans with antibiotic resistant bacteria [2].

Beta-lactamases are the most prevalent mechanism of antibiotic resistance that inactivate beta-lactam antibiotics, including penicillins, cephalosporins, and monobactams [3,4]. These enzymes are encoded by an extrachromosomal DNA fragment called plasmid. A plasmid can genetically be transferred between the same and/or different bacteria [5]. The beta-lactamases currently receiving the most attention are documented as extended spectrum beta-lactamases (ESBL) and aminopenicillin-deactivating cephalosporinase (AmpC), respectively [6].

The resistance to beta-lactams has been identified in the family of *Enterobacteriaceae*, including *Klebsiella* spp., *Escherichia* (*E.*) *coli*, *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., and *Salmonella* spp. [7,8]. But, the patterns of resistance vary among the species [9]. The recent studies have indicated that E. coli has gained increasingly beta-lactam resistance, and frequently observed in foods of animal origin [10]. However, their impact on the human health still remains incomplete across the World, including Turkey [11,12]. In this study, we determined ESBL- and AmpC-producing *Enterobacteriaceae* in cheese samples phenotypically.

2. Material and Methods

Reference cultures

An ESBL positive strain *K. pneumoniae* ATCC 700603 and an ESBL negative strain *E. coli* ATCC 25922 were used for control testing, respectively.

Food samples

During the year 2014, a total of 83 cheese samples was randomly collected from public bazaars and food chain markets located in İstanbul. All samples were put into sterile sampling bags, and taken to the laboratory in a sample carry case (JPB, UK) at 4ºC. The microbiological evaluation was started in the same day.

Microbiological evaluation

25 g of cheese in 225 mL of *Enterobacteriaceae* Enrichment Broth (LABM, UK) was homogenized in a sterile bag (Interscience, France) for 2 min by a stomacher (EasyMix, France). The suspension was then incubated at 37°C for 18-24 h under aerobic condition. After that, 10 µL of the suspension was directly streaked onto an ESBL selective media (Liofilchem, Turkey) by a sterile loop. The plate was again incubated at 37°C for 18-24 h under aerobic condition. The colonies were then sub-cultured onto Tryptic Soy Agar (Merck, Turkey), and allowed for incubation at 37ºC for 18-48 h. The green-colored colonies indicated ESBL-positive *K. pneumoniae*, whereas pink-violet-colored colonies indicated ESBL-positive *E. coli* according to the manufacturer's instructions. The suspected isolates were tested for oxidase activity by Bactident Oxidase Testing Kit (Merck, Turkey). Finally, oxidase negative colonies were identified by a mass spectrometer (Vitek® MS bioMérieux, France).

Disc screening and confirmation of ESBL suspicious isolates

After identification, the isolates were suspended in a sterile salt solution (0.85% NaCl) to 0.5 McFarland-standardized density by a densitometer (bioMérieux, France). After that, they were transferred onto Mueller–Hinton agar (Liofilchem, Turkey) using sterile swabs. Cefpodoxime (CPD; 10 µg), cefotaxime (CTX; 30 µg), and ceftazidime (CAZ; 30 µg) containing antibiotic discs (CPD10 Mast Group, UK) were placed on the plate. Disc diffusion confirmation test was performed by a

combination of CPD, CTX, and CAZ±Clavulanate (CLA, 10 µg) (D67C MAST Group). The disc inserted plates were then incubated at 37°C for 18-24 h. The breakpoints with zone diameters and zones of inhibition were evaluated according to the criteria described by the Guidelines of CLSI (2013) [13].

Antimicrobial susceptibility based on minimal inhibitory concentration (MIC)

MIC determination was performed for ESBL- and AmpC-type beta-lactamases according to the manufacturer's instructions on Micronaut-S Beta-Lactamase VII plate (Merlin Diagnostika, Germany). A 50 µL aliquot of 0.5 McFarland-standardized suspension of the isolate was vortexed in 10 mL of Mueller Hinton Broth (Merck, Germany).

After that, 100 µL of this suspension was pipetted into each well of the 96-well plate, followed by an incubation at 37ºC overnight. The plates were then measured by ThermoScientific™ Multiskan FC spectrometer. The readings were automatically analyzed by the MCN6 Software (Sifin, Germany).

3. Results

Microbiological results

A total of 83 cheese was microbiologically examined according to the Criteria by ISO/DIS21528-2. A total of 18 isolates, including *Klebsiella pneumoniae* (27.8%), *Hafnia alvei* (27.8%), *Escherichia coli* (22.2%), *Klebsiella oxytoca* (11.2%), *Enterobacter cloacae* (5.5%), and *Citrobacter* spp. (5.5%) was identified by mass spectrometer (Figure 1).

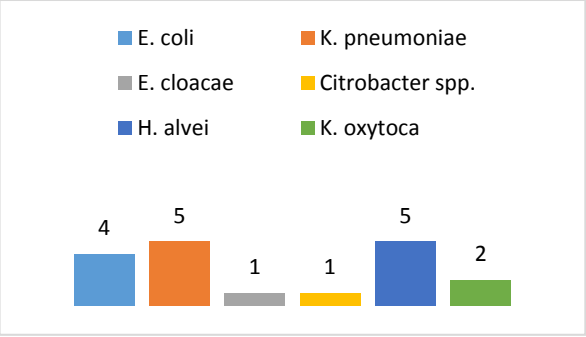

Figure 1. Types of ESBL- and AmpC-positive isolates based on cheese samples

4. *Phenotypic results*

The phenotypic characterization of ESBL- and AmpC- type beta-lactamases was conducted by disc diffusion, disc diffusion confirmation, and MIC determination, respectively according to [13]. The phenotypic results revealed that the most common beta-lactamase type was determined as ESBL in 9 isolates (5 *K. pneumoniae*, 2 *E. coli*, and 2 *K. oxytoca*), followed by ESBL &

AmpC in 4 isolates (2 *E. coli*, 1 *H. alvei*, and 1 *Citrobacter* spp.), and AmpC in 5 isolates (4 *H. alvei* and 1 *E. cloacae*), respectively. All the phenotypic results were presented in Table 1, Figure 1, Figure 2 and Figure 3.

Table 1. Species-based distribution of ESBL- and AmpC- positive isolates

Type	ESBL	ESBL and AmpC	AmpC	Total
K. pneumoniae	5	-	-	**5 (27.8 %)**
H. alvei	-	1	4	**5 (27.8 %)**
E. coli	2	2	-	**4 (22.2 %)**
K. oxytoca	2	-	-	**2 (11.2 %)**
E. cloacae	-	-	1	**1 (5.5%)**
Citrobacter spp.	-	1	-	**1 (5.5%**
				)
Total	**9 (50.0 %)**	**4 (22.2 %)**	**5 (27.8 %)**	**18 (100 %)**

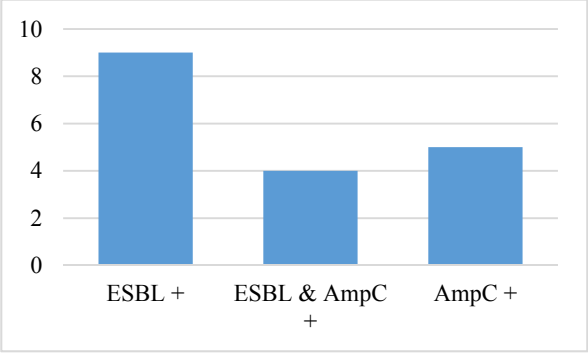

Figure 2. Distribution of ESBL- and AmpC type beta-lactamases in cheese samples

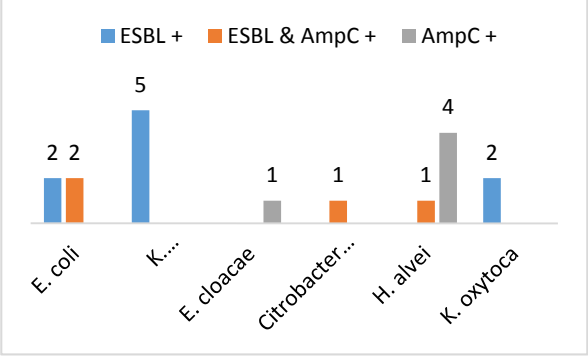

Figure 3. Dstributionj of beta-lactamases based on the type of Enterobacteriaceae

The average zone differences of CAZ±CVA, CTX±CVA and CPD±CVA were 26.3±3.2 mm, 28.6±7.4 mm and 24.4±11.7 mm in ESBL+ isolates, respectively, and 22.0±10.0 mm, 23.0±12.0 mm and 19.0±13.0 mm in

ESBL&AmpC+ isolates, respectively, while 15.5±1.9 mm for CPD±CVA in AmpC+ isolates.

Of 9 ESBL+ isolates, 2 were resistant to CTX (≥128 µg/mL), 2 to CAZ (MIC=16 µg/mL), 1 to COX (>32 µg/mL), 2 to CEP (=64 µg/mL), 1 to MER (MIC=8 µg/mL), and 1 to CMC (≤0.25/4 µg/mL).

Among ESBL & AmpC positive isolates, 4 were resistant to CTX (≥128 µg/mL), 4 to CAZ (MIC=16 µg/mL), 1 to MER (=64 µg/mL), 2 to COX (>32 µg/mL), 1 to ERT (>1 µg/mL), 3 to CEP (>128 µg/mL), and 2 to CMC (≤0.25/4 µg/mL), respectively.

The AmpC positive isolates showed resistance to CTX (=8 µg/mL) in 3, to CAZ (≥16 µg/mL) in 1, COX (≥32 µg/mL) in 4, and ERT (>1 µg/mL) in 1, respectively.

4. DISCUSSION

In this study, ESBL- and AmpC- type beta-lactamases were characterized in a total of 18 isolates phenotypically, including *K. pneumoniae* (27.8%), *H. alvei* (27.8%), *E. coli* (22.2%), *K. oxytoca* (11.2%), *E. cloacae* (5.5%), and *Citrobacter* spp. (5.5%) was microbiologically detected. The phenotypic results revealed that the most common beta-lactamase type was determined as ESBL in 9 isolates (5 *K. pneumoniae*, 2 *E. coli*, and 2 *K. oxytoca*), followed by ESBL & AmpC in 4 isolates (2 *E. coli*, 1 *H. alvei*, and 1 *Citrobacter* spp.), and AmpC in 5 isolates (4 *H. alvei* and 1 *E. cloacae*), respectively.

Many antibiotics that were formerly effective against bacterial infections are no longer effective because of resistant strains [14]. Off-label over use of antibiotics has fueled exchange of resistance-coding genetic elements making a bacteria resistant to antibiotics [15,16]. This situation contributes to circulation of antibiotic-resistant strains and resistance-coding genes among humans, animals, food, water and the environment [17]. The average consumption rate of antibiotics per kilogram for food animal produced annually will globally increase nearly double by 2030 [1,18]. By 2050, the infections associated with antibiotic resistant bacteria could kill 10 million people a year all over the World with a burden of $100 trillion: more than the size of the current World economy [19]. Despite of these facts, there is not actual data about the use of antibiotics in food animals in Turkey [17]. The related studies from Turkey in this area

are quite limited [18,20,21]. Our study, therefore, contributed to an underestimation of the antibiotic resistance patterns in foodborne Enterobacteriaceae.

The foods of animal origin easily gets contaminated by enterobacteria [22]. Their unhygienic consumption could be an important health issue in terms of food safety and antibiotic resistance. But, it should be essentially free from Enterobacteriaceae, including the resistant ones [23,24]. The beta-lactamase producing Enterobacteriaceae are considered as major agents of many foodborne infections, and confer to penicillins, 1st, 2nd and 3rd-generation cephalosporins, and aztreonam [25,26]. These strains may contaminate foods, and so colonize in the intestinal tract, or exchange their resistance-coding genes with commensal bacteria of the humans [27]. The recent studies indicated that beta-lactamases observed in human and foods of animal origin were the same to each other [28].

In this study, we phenotypically detected ESBL- and/or AmpC-type beta-lactamases in *K. pneumoniae, H. alvei*, *E. coli*, *K. oxytoca*, *E. cloacae*, and *Citrobacter* spp. The frequency rates of the beta-lactamase positive phenotypes in cheese were similar to Belgium [29], Germany [30], China [31], Holland [23], Poland [32], and Denmark [33], respectively. According to the Ministry of Health in Turkey (www.uhes.saglik.gov.tr), the antibiotic resistance patterns from clinical isolates have spread particularly in *E. coli* (33.2% in 2008 and 48.83% in 2013) and *K. pneumoniae* (40% in 2008 and 49.69% in 2013). But, the rapidly increasing frequency rate of beta-lactamase positive enterobacteria could be a result of the foods of animal origin, and this suspected risk factor has not been seriously addressed so far in Turkey [34]. Therefore, our study is extremely important for the purpose of detecting the presence of resistant bacteria in cheese.

AmpC-type beta-lactamase is associated with multiple antibiotic resistances, leaving limited therapeutic options [35]. In our study, we detected AmpC- production in *E. cloacae* and *H. alvei*. If raw milk flavor is required, the best culture to add is H. alvei. However, we determined that a flavoring food culture could even be resistant to anbiotics. Because co-existence of ESBL- and AmpC- is a growing concern all over the world [15]. Failure to detect these multi-resistance

pattern has contributed to their uncontrolled spread [2].

The MIC results revealed that ESBL positive isolates were resistant to CTX, CAZ, COX, CEP and CMC while ESBL & AmpC positive ones were resistant to CTX, CAZ, MER, COX, ERT, CEP and CMC. As seen the documented antibiotic agents, a co-existing pattern of ESBL with AmpC suggested two different agents, including MER and ERT. For alone AmpC producers, the antibiotic agents were CTX, CAZ, COX and ERT. All these beta-lactam agents are of importance in veterinary medicine [36]. Our results showed that the resistance patterns of these isolates were remarkable [29].

The detection of ESBL co-presence with AmpC in an isolate with ERT susceptibility could be considered as one of the indicators of KPC activity. But, we could not detect it by MIC determination. This means that carbapenem non-susceptible ESBL isolate is a potent problem in the future if it is not precisely detected.

5. CONCLUSIONS

Even though important food safety-indicator microorganisms are routinely checked by legal authorities based on directives, the inspection for antibiotic-resistant enterobacteria has not come into force yet [37,38]. There have been multiple studies reporting the spread of resistant bacteria from animals to humans through food [39]. Each transmission may not cause an illness, but it is still extremely important in mediating the spread of resistance-coding genes to humans [21].

In conclusion, our study revealed that ESBL- and Amp- were the most common phenotypes in Enterobacteriaceae from cheese samples, presenting a foodborne health risk for the consumers. Accordingly, excessive and/or unconscious use of antibiotics in farming animals should be considered, but there also is a need for advanced molecular studies to understand whether resistant bacteria are transferred from animals to humans or the other way around.

CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest.

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