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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). additives. Among antimicrobial substances that can be added to solid and liquid foods or to various beverages; are being used in order to efface ferment and every kind mold. 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 phydroxybenzoic Acid Esters. Liquid Chromatographic Determination in Foods' method was used (Anonymous, 1997).

As chromatographic condition; C-18 analytical column with the diameter of 5  $\mu$ m 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 bv 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	Rescript	Identified values	Its suitability
	Amount (n)	(limit)		
Chocolate cak	5	SA+BA=300	45,77-140,68	Suitable
		mg/kg	mg/kg	
Chocolate	5	SA+BA=300	0,00-456,08	1 unit sample
eclairs		mg/kg	mg/kg	is not suitable
Industrial	5	SA=1000	0,00-0,00 mg/kg	Suitable
Tomato Paste		mg/kg		
Traditional	5	SA=1000	508,91-1820,31	1 unit sample
Tomato Paste		mg/kg	mg/kg	is not suitable
Industrial	5	SA=1000	0,00-0,00 mg/kg	Suitable
Pepper Paste		mg/kg		
Traditional	5	SA=1000	113,92-1071,92	1 unit sample
Pepper Paste		mg/kg	mg/kg	is not suitable
Ketchup	5	SA+BA=2000	0,00-838,09	Suitable
		mg/kg	mg/kg	
Mayonnaise	5	SA+BA=1000	0,00-892,46	Suitable
		mg/kg	mg/kg	
Vegetable	5	SA=1000	399,76-1279,89	1 unit sample
Margarine		mg/kg	mg/kg	is not suitable
Carbonated	5	250SA+150BA	97,87SA+0,00BA	1 unit sample
Beverage		mg/kg	mg/kg -	is not suitable
			0,00SA+176,47	
			BA mg/kg	

## **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 0,00-100,21 mg/kg. 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).

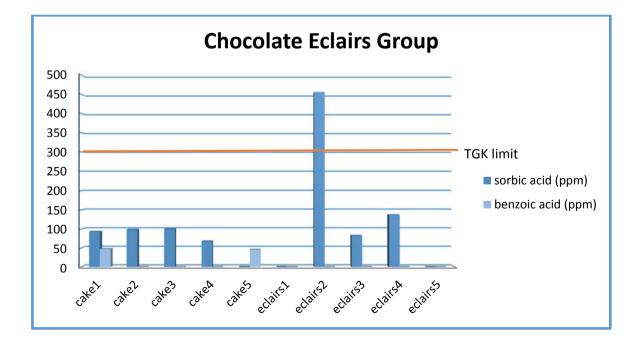


Figure 1: Sorbic acid and benzoic acid amount identified in chocolate cake samples

# 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).

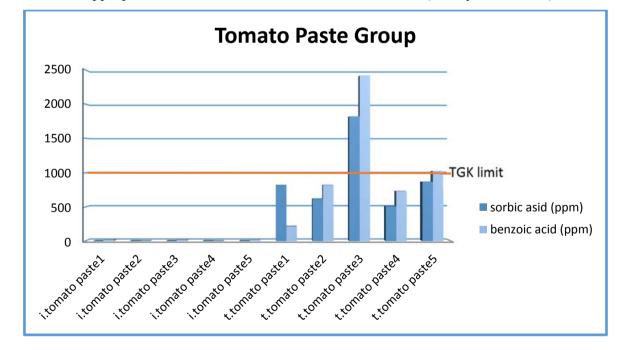


Figure 2: Sorbic acid and benzoic acid amount identified in tomato paste samples

## **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).

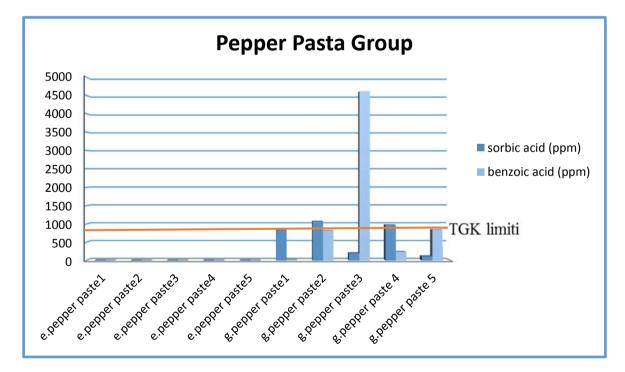


Figure 3: Sorbic acid and benzoic acid amount identified in pepper paste samples

# 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).

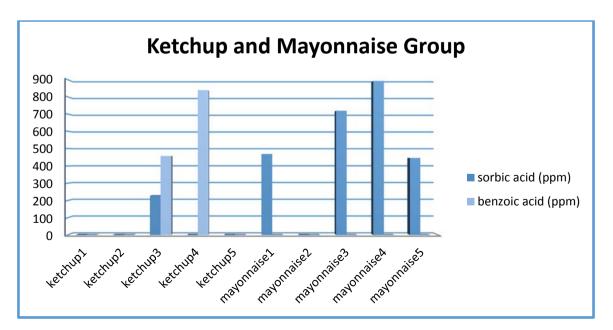


Figure 4: Sorbic acid and benzoic acid amount identified in ketchup and mayonnaise samples

## **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).

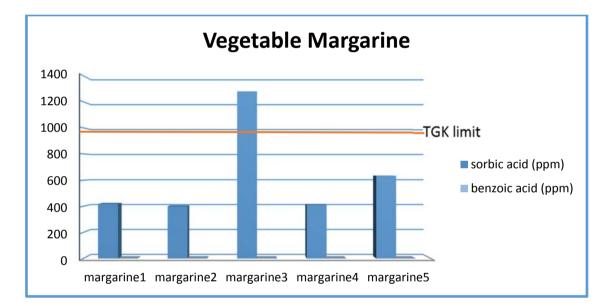


Figure 5: Sorbic acid and benzoic acid amount identified in vegetable margarine samples

## **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).

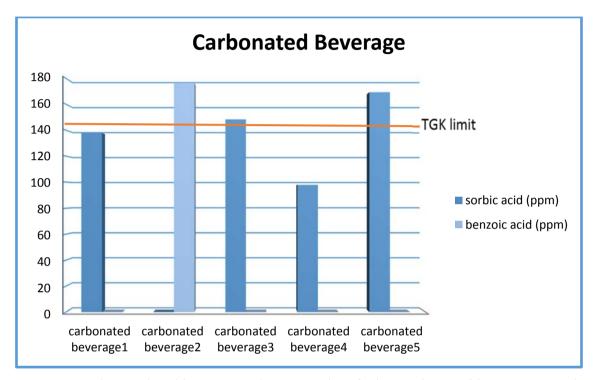


Figure 6: Sorbic acid and benzoic acid amount identified in carbonated beverage samples

# 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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