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Original Article

ESTIMATED DAILY INTAKE AND EXPOSURE OF SODIUM BENZOATE AND POTASSIUM SORBATE THROUGH FOOD PRODUCTS IN SCHOOL CHILDREN OF TIRUPATI, INDIA

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ABSTRACT

Objective: According to Food Safety and Standards Authority of India (FSSAI) the preservatives, sodium benzoate and potassium sorbate belong to permitted class II preservatives. The aim of this study is to determine the concentration levels of these preservatives in food products that are consumed by school children and to assess the chronic dietary exposure by conducting the Total Diet Study (TDS).

Methods: The quantitative determination was carried out by UV spectrophotometer. The absorbance for sodium benzoate and potassium sorbate were measured at 228 and 250 nm respectively. The 24-hour diet recall method was used to estimate the amount of food ate in last 24 hours. For estimation of preservative exposure dietary modelling techniques were utilized which combine the amount of preservative concentration present in that food with the amount of food consumed. Then the dietary exposure was assessed by considering the Acceptable Daily Intake (ADI).

Results: The results include chemical concentration levels of the foods analyzed as well as estimated dietary exposures and contributions to the exposure from different foods. The obtained mean concentration of sodium benzoate was found to be425 ppm for sauces, 161 ppm for pickles and 80 ppm for soft drinks. Potassium sorbate was found to be 130 ppm for fruit juices,302 ppm for jellies and 380 ppm for jams. The highest mean dietary exposure for both the preservatives was observed in children of 2-7 years age group, the percentage exposure of sodium benzoate was 33% of the ADI and potassium sorbate was 17 % of the ADI.

Conclusion: This study can enlighten the public on the consumption of preservative containing food products within the limit and encourages to eating fresh preservative free foods.

Keywords: Sodium benzoate, Potassium sorbate, Food products, Spectrophotometer, School children, Dietary exposure, Acceptable daily intake, Total diet study.

INTRODUCTION

Present days, we are consuming the pretreated and processed foods which contain several food additives such as artificial sweeteners, colourants, stabilizers and preservatives. Among these additives preservatives play a major role. The process of preservation was in practice from ancient days where some of the older methods are drying, salting, sugaring, and heating. But the present generation depended on the processed foods. These foods contain chemical preservatives as they are more effective to inhibit the growth of mould, yeast and a wide variety of bacteria [1-4]. In this study sodium benzoate and potassium sorbate were selected. Their usage was not only restricted to food products, but also used in cosmetic herbal formulations [5]. These preservatives are present in major food products consumed by school children. These compounds are more effective in foods with low pH value and ineffective at neutral pH value [6]. The most common analytical methods for determination of sodium benzoate and potassium sorbate are Reverse-phase HPLC [7, 8], Spectrophotometry [9], Capillary Electrophoresis [10], Gas Chromatography-Mass Spectrometry [11] have been reported. Food and Agriculture Organization (FAO) and World Health Organization (WHO) combine to form a Joint FAO/WHO Expert Committee on Food Additives (JECFA), it evaluated and established the acceptable daily intake (ADI) expressed in milligrams per kilogram of body weight per day (mg/kg, body weight/day). Benzoic acid and its calcium, potassium and sodium salts ADI were 0-5 mg/kg body weight. Sorbates and its calcium, potassium and sodium salts ADI was 0-25 mg/kg body weight [12, 13].

Adverse effects of sodium benzoate are skin reactions such as rash, non-immunological contact urticaria, metabolic acidosis, hyperpnoea and asthma [14-17]. Effects of potassium sorbate are genotoxic and mutagenic to human blood cells and also found to be toxic to human DNA in peripheral blood lymphocytes [18-22]. Some of the beverage companies are selling soft drinks at the very low price at Rupees 5/-for 250 ml and most of the fast food conglomerates were using preservatives in milkshakes and ice cream toppings and they are opening their stores even in small towns. The children were usually unaware of preservatives; just they get attracted to modern foods by looking at their colourful images and consume high quantities by neglecting traditional foods. In this study around 960 school children were enquired about their daily dietary habit, age and gender. The aim of the study is to analyze the preservatives in the simple method and to create awareness in the public of its usage.

MATERIALS AND METHODS

Sample collection

Around 65 samples of different companies in which the permitted class II preservatives such as sodium benzoate and potassium sorbate was used were selected and purchased from local market Tirupati, India. The samples include sauces, pickles, soft drinks, fruit juices, jellies, and jams. They were tested for two preservatives, sodium benzoate and potassium sorbate.

Chemicals and reagents

Sodium benzoate, potassium sorbate, hydrochloric acid and petroleum ether were purchased from Merck Ltd., (Mumbai).

Instrumentation

The analysis was carried out by UV-Spectrophotometer of Analytical Technologies Limited SPECTRO 2060 Plus. The sample data collection was optimized with UV detection at 227 nm for sodium benzoate and 250 nm for potassium sorbate. The UV spectrophotometric method was, according to the Association of Official Analytical Chemists (A. O. A. C*) Official Methods of Analysis* [23] and an International Organization for Standardization (ISO) guidelines [24].

Preparation of standard calibration curve

Standard stock solutions of sodium benzoate and potassium sorbate were prepared at 1000 mg/l with distilled water. In a series of six

100 ml volumetric flasks 10, 20, 30, 40 and 50 ml of diluted standard of sodium benzoate and potassium sorbate solutions were introduced and diluted up to the mark with distilled water.

Then 0.4 ml of hydrochloric acid (6M) was added to 5 ml of each standard solutions and the compound was extracted with 45 ml of petroleum ether. The absorbance of standard samples sodium benzoate and potassium sorbate were detected at 228 and 250 nm respectively. The absorbance was mentioned in [table 1, 2].

Table 1: Calibration data for Sodium benzoate at 228 nm

Concentration (mg/ml)	Absorbance [n] average	Standard deviation
0.1	1.22	0.33
0.2	1.89	0.75
0.3	2.36	0.92
0.4	2.91	1.26
0.5	3.58	1.57

n: absorbance is the average of three determinations

Table 2: Calibration data for Potassium sorbate at 250 nm

Concentration (mg/ml)	Absorbance [n] Average	Standard deviation
0.1	1.72	0.45
0.2	2.51	0.68
0.3	3.00	0.83
0.4	3.72	1.16
0.5	4.54	1.74

n: absorbance is the average of three determinations

Sample preparation

Thick and solid products

The samples like jams, sauces, pickles and jellies were homogenized by blending 10 g of the sample with 90 ml of distilled water for 2 min and immediately filtered through Whatman No: 3 paper. Then 0.4 ml of hydrochloric acid (6 M) was added to 5 ml of the filtrate and extracted with 45 ml of petroleum ether in a separating funnel.

Liquid samples

The samples like juices, pulpy fluid, syrups and soft drinks were thoroughly mixed and degassed for the removal of carbon dioxide. Then 0.4 ml of hydrochloric acid (6 M) was added to 5 ml of the filtrate and extracted with 45 ml petroleum ether in a separating funnel.

Total diet study (TDS)

For assessing the dietary exposure, TDS has conducted. It includes some of the parameters such as determining food preservative concentration data, mode of expressing and variability in preservative concentration data, classification of school children into different age groups and conducting 24-hour diet recall method. The 24-hour diet recall method involves step by step procedure starting from interviewing the children, collecting the amount of food intake in detail, collecting meal information such as name, time and location, reporting food additions, reviewing the collected information and ending the interview. Then, the individual consumption data were reported and estimated the mean daily intake of these preservatives. The chronic dietary exposure was calculated on per kilogram of body weight basis. Each individual's total dietary exposure to food chemical preservatives from all foods is divided by their own body weight. After estimation, these were compared to reference health standards to assess the potential risk to children by considering the acceptable daily intake (ADI) [25].

Equation for dietary exposure:

$$\text{Dietary exposure} = \frac{\Sigma(\text{concentration of preservative in food} \times \text{food consumption})}{\text{Body weight(kg)}}$$

RESULTS AND DISCUSSION

Determination of sample concentration by calibration curve method

The five-point calibration graph was drawn by plotting absorbance v/s concentration. The graph achieved was linear, it showed an acceptable correlation between absorbance and concentration with the correlation coefficient R^2=0.99 and the regression equation (y= 5.74x+0.644) for sodium benzoate and the correlation coefficient R^2=0.99 and the regression equation (y=6.85x+1.043) for potassium sorbate. It was shown in [fig. 1, 2]. The precision and accuracy of the method have been considered within the day assay and between days assay for both the preservatives. The reported standard deviation was less than 2 which indicates that the method was precise.

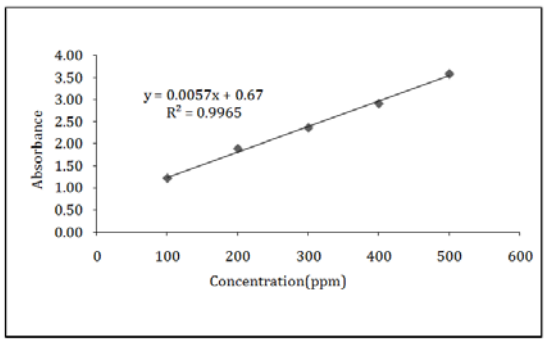

Fig. 1: Calibration curve for Sodium benzoate at 228 nm

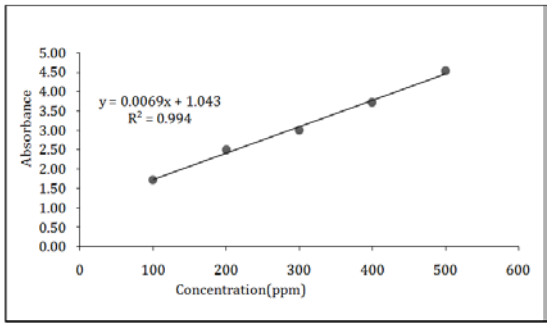

Fig. 2: Calibration curve for potassium sorbate at 250 nm

Preservative concentration data

The food regulatory body in India is Food Safety and Standards Authority of India (FSSAI) which established the Maximum Permitted Level [MPL expressed in ppm (mg/kg)] for preservatives added to food products [26]. The MPL for the sodium benzoate present in pickles was 250 ppm, for sauces 750 ppm and for soft drinks 120 ppm. The MPL for potassium sorbate in fruit juices was 200 ppm, for jams and jellies 500 ppm. In this study, ten products of different brands were selected for each kind of food sample that they declared the preservatives sodium benzoate or potassium sorbate on their labels. The concentration values of preservatives found in all analyzed food samples showed the difference in concentration levels between different brands and they did not exceed the permitted levels given in [table 3, 4]

Table 3: Concentration of sodium benzoate found in pickles, sauces and soft drinks

Food sample codes	Concentration(ppm)[a] mean±SD*		
	Pickles	Sauces	Soft drinks
X1	142±0.21	401±0.53	72±0.39
X2	135±0.47	456±0.48	63±0.26
X3	196±0.35	392±0.37	92±0.18
X4	168±0.42	493±0.59	87±0.24
X5	157±0.29	421±0.25	83±0.32
X6	138±0.31	355±0.33	57±0.27
X7	176±0.16	411±0.41	79±0.15
X8	184±0.22	373±0.39	96±0.23
X9	152±0.45	482±0.24	104±0.44
X10	163±0.14	476±0.46	66±0.36

a: average of five determinations, ppm: parts per million, SD: standard deviation

Table 4: Concentration of potassium sorbate found in fruit juices, jellies and jams

Food sample codes	Concentration (ppm)[a] mean±SD		
	Fruit juices	Jellies	Jams
X1	105±0.47	261±0.62	387±0.53
X2	93±0.35	293±0.58	418±0.86
X3	117±0.40	342±0.74	336±0.69
X4	132±0.29	314±0.46	379±0.47
X5	163±0.31	307±0.79	403±0.72
X6	144±0.52	280±0.41	364±0.51
X7	156±0.43	269±0.68	348±0.48
X8	129±0.22	356±0.54	357±0.45
X9	108±0.38	323±0.85	411±0.82
X10	149±0.26	278±0.44	395±0.77

a: average of five determinations, ppm: parts per million, SD: standard deviation

Sample weight assessment

In this study, the amount of marketed sample weights which were preferred by a majority of the population was considered. The preferred quantities of soft drinks are 750 ml to 1.5 liter, pickles 250 to 750 g, sauces 200 g to 1 kg, fruit juices 200 ml to 750 ml, jams 100 to 500 g, and jellies 50 to 200 g. Based on preferred quantities and one-time consumption amount the sample intake by 960 school children was estimated. It was given in [table 5].

Table 5: Mean concentration and daily intake of food samples quantity in which sodium benzoate and potassium sorbate were present

Sample	Concentration[a] (ppm)	Intake (g or ml/day)	
		Mean	Standard deviation
Pickles	161.1±19.22	15	4
Sauces	426±45.72	30	9
Soft drinks	79.9±14.53	400(ml)	18
Fruit juices	129.6±22.29	300(ml)	15
Jellies	302.3±30.06	25	7
Jams	379.8±26.36	30	11

a: mean±SD, average of ten different brands of food sample

Age group assessment

As a part of the study, a survey was conducted during the period of November 2014 to February 2015 in around 12 different schools in Tirupati. About 960 students of different age groups were selected and their daily dietary information, age and their body weight were collected. Then the children were divided according to their age and categorized into three groups. They are Group-I consists of 2-7 years young boys and girls of the primary school with the mean body weight20 kg. Group-II consists of 8-14 years teenage boys and girls with the mean body weight of 35 kg. Group-III consists of 15-19 years adult boys and girls with the mean body weight of 50 kg. Diets for each individual in the representative age groups were derived for exposure estimations based on 24-hour diet recall food consumption data.

Food consumption data

This provides the best way to estimate actual consumption of food and estimated exposure to preservatives. However, there are some limitations related to the age group and changes in eating patterns. Generally, Indian traditional foods are preservative free but unlikely it has changed to modern processed foods which contain high quantities of chemical preservatives. Foods included in the study are those formed a major part of the diet, highly consuming and the foods which contain high concentration of chemicals although consumption of food may be low (Examples: Fruit Juices, Jams, Jellies, Soft drinks).

The most commonly eaten foods not included in the survey are fresh fruits, vegetables, milk, fresh meat, cereals and pulses. The sample intake necessary for estimating consumption was derived from [Table 5]. The mean daily intake was estimated by 24-hour diet recall method and dietary modelling techniques, that combine food consumption data with the preservative concentration present in that food. The estimated mean intake of sodium benzoate by Group-I was 30 mg, Group-II was 42 mg and Group-III was 53 mg. The mean intake of potassium sorbate by Group-I was 40 mg, Group-II was 60 mg and Group-III was 73 mg. The food that was the major contributor to dietary exposure to sodium benzoate and potassium sorbate for all groups of school children was soft drinks and fruit juices given in [table 6].

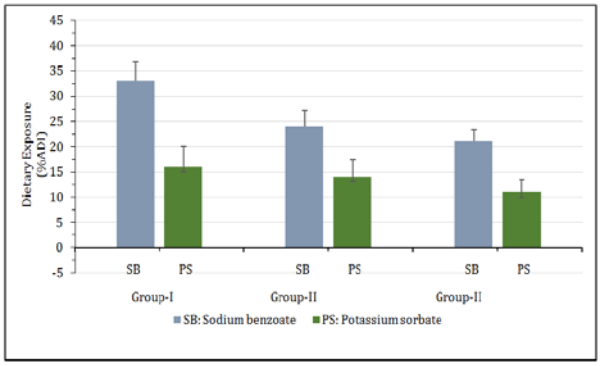

Fig. 3: Mean estimated dietary exposure of school children as a percentage of the ADI. For sodium benzoate ADI is (0-5 mg/kg body weight), for potassium sorbate ADI is (0-12.5 unconditional, 12.5-25 conditional). Error bars are the representation of standard deviation. Group I, II, III indicate the different ages of school children

Estimated percentage exposure

The mean percentage exposure to sodium benzoate by Group-I children was 33% of the ADI, Group-II children were 24% of the ADI and Group-III children were 21% of the ADI. The percentage exposure to potassium sorbate by Group-I children was 16% of the ADI, Group-II children were 14% of the ADI and Group-III children were 11% of the ADI.

The differences in exposure between population groups were due to different food consumption patterns. The percentage of estimated mean dietary exposure to foods containing sodium benzoate and potassium sorbate was found to be below the ADI for all assessed age groups as shown in [fig. 3].

Table 6: Estimated daily intake of sodium benzoate and potassium sorbate through different food products by three age groups

Sample	Consumption data (mg/day)				
	Group-I	Group-II	Group-III	Mean	Standard deviation
Pickles	2	3	5	3.3	1.5
Sauces	8	14	19	13.6	5.5
Soft drinks	20	25	30	25	5
Fruit juices	22	28	33	27.6	5.5
Jellies	6	13	18	12.3	6
Jams	10	17	20	15.6	5.1

DISCUSSION

In this study, the preservative concentration found in all the analyzed food products was within the Indian official limits. Similar studies had conducted in other countries where benzoic acid was extensively used in their dietary food products. Some of them are, Belgium [27], Iran [28], Australia and New Zealand [29], Serbia [30], Korea [31], Denmark [32] and Japan [33]. The results from those countries showed great differences due to variability in dietary products from country to country. The toddlers are mostly affected in all the exposure studies. Hence, this study recommends using pasteurization, bio preservation and high-pressure food preservation by avoiding the chemical preservatives or else there is a chance of high deposition of preservatives in the human body.

CONCLUSION

India has the largest child population in the world, having 440 million children who are under 18years of age. Hence, this study focused on harmful chemical preservatives present in food products consumed by children. Based on obtained analytical results, the maximum permitted levels and individual consumption data, the exposure assessment for sodium benzoate and potassium sorbate showed the mean total child population exposure was below the ADI for all age groups.

However, some children of 2-7 years age group exceeded the ADI due to their high consumption relative to lower body weights. There will be no health-safety risk if we maintain the balanced preservative free diet. Thus, we hope that the health authorities of India should conduct the proper inspection of food industries, local markets and check the products whether they are following FSSAI regulations or not.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Saranraj P, Geetha M. Microbial spoilage of bakery products and its control by preservatives. Int J Pharm Biol Arch 2012;3:38-48.
2. Erich L, Martin J, Nico Raczek. Sorbic acid in Ullmann's Encyclopedia of Industrial Chemistry. Weinheim, Germany: Wiley-VCH Publishers; 2000.
3. Heydaryinia A, Veissi M, Sadadi A. A Comparative study of the effects of the two preservatives, sodium benzoate and potassium sorbate on *Aspergillus niger* and *Pencillium notatum*. Jundishapur J Microbiol 2011;4:301-7.
4. Sofos JN, Busta F. Antimicrobial activity of sorbate. J Food Prot 1991;44:614-21.
5. Yogesh Pounikar, Pushpendra Jain, Navneet Khurana, L Omray, S Patil, Asmita Gajbhiye. Formulation and characterization of Aloevera cosmetic herbal hydrogel. Int J Pharm Pharm Sci 2012;4:85-6.
6. Guynot ME, Ramos AJ, Sanchis V, Marin S. Study of benzoate, propionate, and sorbate salts as mould spoilage inhibitors on intermediate moisture bakery products of low pH (4.5-5.5). Int J Food Microbiol 2005;101:161-8.
7. Saad B, Bari MF, Saleh MI, Ahmad K, Talib MK. Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in food stuffs using high-performance liquid chromatography. J Chromatogr A 2005;1073:393-7.
8. Isabel MF, Eual IM, Paula B, Margarida AF. Simultaneous determination of benzoic and sorbic acids in quince jam by HPLC. Food Res Int 2001;33:113-7.
9. T founi SAV, Toledo MCF. Determination of benzoic acid and sorbic acids in Brazilian food. Food Control 2002;13:125-9.
10. Kuo KL, Hsieh YZ. Determination of preservatives in food products by cyclodextrin-modified capillary electrophoresis with multi wavelength detection. J Chromatogr A 1997;768:334-41.
11. Hsui Jung, Youk Meng Choong. A simple method for the simultaneous determination of various preservatives in liquid foods. J Food Drug Anal 1999;7:291-304.
12. World Health Organization. Evaluation of certain food additives forty sixth report of the joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series Geneva: WHO Press; 1997. p. 868.
13. World Health Organization. Evaluation of certain food additives seventeenth report of the joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series Geneva: WHO Press; 1974. p. 539.
14. Bronaugh RL, Stewart RF, Congdon ER. Methods for *in vitro* percutaneous absorption studies II Animal methods for human skin. Toxicol Appl Pharm 1982;62:481-8.
15. Coverly J, Peters L, Whittle E, Basketter DA. Susceptibility to skin stinging, non-immunologic contact urticarial and acute skin irritation. Contact Dermatitis 1998;38:90-5.
16. Lahti A, Maibach HI. An animal model for non-immunologic contact uriticaria. Toxicol Appl Pharmacol 1984;76:219-24.
17. Freedman BJ. Asthma induced by Sulphur dioxide, benzoate and tartrazine contained in orange drinks. Clin Allergy 1977;7:407-15.
18. Tulamait A, Laghi F, Mikrut K, Carey RB. Potassium sorbate reduces gastric colonization in patients receiving mechanical ventilization. J Crit Care 2005;20:281-7.
19. Mamur S, Yuzbasioglu D, unal F, Yilmaz S. Does potassium sorbate induce genotoxic or mutagenic effects in lymphocytes? Toxicol *In Vitro* 2010;24:790-4.
20. Kitano K, Fukukawa T, Ohtsuji Y, Masuda T, Yamaguchi H. Mutagenicity and DNA–damaging activity caused by decomposed products of potassium sorbate reacting with ascorbic acid in the presence of Fe salt. Food Chem Toxicol 2002;40:1589-94.
21. Hendy RJ, Hardy J, Gaunt IF, Kiss IS, Butterworth KR. Long term toxicity studies of sorbic acid in mice. Food Cosmet Toxicol 1976;14:381-6.
22. Gaunt IF, Butterworth KR, Hardy J, Gangolli SD. Long term toxicity of sorbic acid in rat. Food Cosmet Toxicol 1975;13:31-45.
23. Horwitz W. AOAC Official method 960.38 benzoic acid in non-solid food and beverages. 17thed. Gaithersburg, Md: AOAC Int; 2000.
24. International Organization for Standardization. Fruits, vegetables and derived products--Determination of sorbic acid content ISO 5519:2008. Geneva, Switzerland: ISO; 2008.
25. Food Standards Australia and New Zealand (FSANZ). Principles and practices of dietary exposure assessment for food regulatory purposes. Canberra: FSANZ. Available from: http://www.foodstandards.gov.au/science/exposure/documents/Principles%20_%20practices%20exposure%20assessment%202009. pdf. [Cited 2009 Aug 15].
26. Food Safety and Standards Authority of India (FSSAI). Food safety and standards (food products standards and food additives) regulations. New Delhi: Int Law Book Company; 2011. p. 449-97.
27. Vandevijvere S, Andjelkovic M, De Wil M, Vinkx C, Huybrechts I, Van Loco J, *et al.* Estimate of intake of benzoic acid in the

Belgian adult population. Food Addit Contam Part A 2009;26:958-68.

28. Mostafa Delavar, Rita Ahmadi Araghi, Amir Mohammad Kazemifar, Mahdi Abdollahi, Behnoush Ansari. Determination of benzoate level in canned pickles and pickled cucumbers in food producing factories in markazi province and those that their products were sold in Arak City, Iran. Iranian J Toxicol 2012;6:686-90.
29. Food Standards Australia and New Zealand (FSANZ). Benzoates, sulphites and sorbates inthe food supply. Canberra: FSANZ. Available from: http://www.foodstandards.gov.au/scienceandeducation/factsheets/factsheets2005/benzoatessulphitesan2965. cfm. [Cited 2010 Dec 5].
30. Konstansa Lazarević, Dušica Stojanović, Nataša Rančić. Estimated daily intake of benzoic acid through food additives in adult population of south east Serbia. Cent Eur J Public Health 2011;19:228–31.
31. Yoon HJ, Cho YH, Park J, Lee CH, Park SK, Cho YJ, *et al.* Assessment of estimated daily intakes of benzoates for average and high consumers in Korea. Food Addit Contam 2003;20:127-35.
32. Leth T, Christensen T, Larsen IK. Estimated intake of benzoic and sorbic acids in Denmark. Food Addit Contam Part A 2010;27:783-92.
33. Ishiwata H, Yamada T, Yoshiike N, Nishijima M, Kawamoto A, Uyama Y. Daily intake of food additives in Japan in five age groups estimated by the market basket method. Eur Food Res Technol 2002;215:367–74.