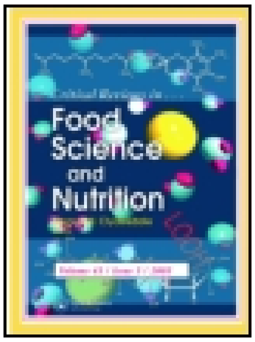

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Benzoic Acid and Its Derivatives as Naturally Occurring Compounds in Foods and as Additives: Uses, Exposure and Controversy

Ana del Olmo, Javier Calzada & Manuel Nuñez

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ANA DEL OLMO, JAVIER CALZADA, and MANUEL NUÑEZ*

Departamento de Tecnología de Alimentos, INIA, Carretera de La Coruña Km 7, Madrid, 28040 Spain

* Corresponding author: Manuel Nuñez E-mail: nunez@inia.es Phone: +34 913476799 Fax: + 34 913572293

ABSTRACT

Benzoic acid is an aromatic carboxylic acid naturally present in plant and animal tissues which can also be produced by microorganisms. Benzoic acid and a wide range of derivatives and related benzenic compounds, such as salts, alkyl esters, parabens, benzyl alcohol, benzaldehyde and benzoyl peroxide, are commonly used as antibacterial and antifungal preservatives and as flavouring agents in food, cosmetic, hygiene, and pharmaceutical products. As the result of their widespread occurrence, production and uses, these compounds are largely distributed in the environment being found in water, soil, and air. Consequently, human exposure to them can be high, common and lengthy. This review is mainly focused on the presence and use of benzoic acid in foods but it also covers the occurrence, uses, human exposure, metabolism, toxicology, analytical methods for detection and legal limits for benzoic acid and its derivatives. Their controversial effects and potential public health concerns are discussed.

Keywords

benzoic acid, benzoates, foods, health, parabens, toxicology

INTRODUCTION

Benzoic acid ($C_7H_6O_2$) is the simplest aromatic carboxylic acid, with a carboxylic group directly bonded to the benzene ring. It is naturally present in plant and animal tissues, and can also be generated in fermented products through microbial metabolism. It is industrially synthesized and included as preservative and/or flavouring agent in food, cosmetic, hygiene and pharmaceutical products. It is also used as additive, nucleating agent, intermediate, stabilizer and/or catalyst in coolant, solvent, photography, plastic, textiles, pesticide, paper, and dye industries. More than 90% of commercial benzoic acid goes to phenol production, by oxidative decarboxylation, and then to caprolactam and nylon production, by hydrogenation, oximation and Beckmann rearrangement. Derivatives of benzoic acid and related benzenic compounds, such as sodium, potassium and calcium benzoates, alkyl benzoate esters, hydroxybenzoate esters (parabens), benzyl alcohol, benzaldehyde and benzoyl peroxide, can be naturally found and/or chemically synthesized, and are widely used in different industrial sectors. However, adverse reactions, potential toxicological effects and public health concerns have been reported and discussed despite being considered as harmless substances when used under legal limits or Good Manufacturing Practices (GMP). Controversy remains nowadays as shown by the FAO/WHO withdrawal of the approval for propylparabens in foods, hygiene products and cosmetics in 2006, after more than 85 years of extended use of those products. This paper provides a multidisciplinary overview of benzoic acid and its derivatives, primarily focused on their use and presence as additives in foods. Natural generation, uses, occurrence, toxicological effects, human

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exposure and metabolism, analytical methods for detection, legal limits and potential public health risks are also discussed.

CHARACTERISTICS, PRODUCTION AND USES

Benzoic acid and its derivatives are widely used as antibacterial and antifungal preservatives (Table 1) in foods, cosmetics, hygiene products, and oral, parenteral and topic medicines (SCCNFP, 2011). Benzoic acid has even been described to exert activity against picornavirus like bovine enterovirus type 1 and used as veterinary biocidal hygiene product (EU Regulation 528/2012). Their minimum microbicidal concentrations range from 20 to 1200 mg/l at pH 6.0 for different bacterial or fungal species, and their minimum inhibitory concentrations range from 50 to 1000 mg/l (FAO/WHO, 2000). Benzoic acid and its derivatives are also used as flavouring agents (Table 2) in foods, cosmetics, and hygiene products (CIR, 2001).

Benzoic acid is also known as phenylformic acid, benzene carboxylic acid, benzene formic acid, carboxybenzene or phenylcarboxylic acid. Its name derives from "benzoin", a balsamic resin from *Styrax* trees used as flavouring agent, fixative and medicine, which contains up to 20% benzoic acid. It is also found in the castoreum, the exudate from castor sacs of beaver, used in perfumery, drugs and foods as natural flavouring. Its structure was determined by Justus Von Liebig and Friedrich Wehler in 1832 and its excretion in urine as hippurate was firstly described by Ure in 1841 (Badenhorst et al., 2014). It is a colourless aromatic compound, with a molecular weight of 122.13, a melting point of 122.4 ºC, a boiling point of 249.2 ºC, an acidic character due to its carboxylic group (pK = 4.19 at 25 ºC), and solubility in water and/or in

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organic solvents depending primarily on its degree of protonation. Overall, its solubility in water is low (0.29 g in 100 ml at 20 ºC), it is soluble in ethanol and slightly soluble in benzene and acetone. Benzoic acid was originally obtained by sublimation of benzoin resin, but currently it is industrially produced by partial oxidation of toluene with oxygen catalyzed by cobalt or manganese naphthenates.

The main benzoic acid salts, sodium, potassium and calcium benzoates, are white or colourless, hygroscopic, crystalline powder, odourless, with a sweetish astringent taste. They are industrially produced from benzoic acid by neutralization with their corresponding hydroxides or by heating with their corresponding concentrated carbonates. Sodium benzoate ($C_7H_5O_2Na$) has a molecular weight of 144.1, is freely soluble in water (66 g in 100 ml at 20 ºC) and sparingly soluble in ethanol. Potassium benzoate ($C_7H_5O_2K$) has a molecular weight of 160.2, is freely soluble in water (71 g in 100 ml at 20 ºC) and ethanol, slightly soluble in methanol and insoluble in ether. Calcium benzoate ($C_{14}H_{10}O_4Ca$) has a molecular weight of 282.3 and is sparingly soluble in water (2.7 g in 100 ml at 20 ºC). The melting point of benzoates is over 300 ºC and their boiling point is close to 465 ºC. Benzoic acid and its salts are widely used as plasticizer agents in the manufacturing of plastic packaging material including packaging for foods such as yogurt, butter and margarine. They are also used as corrosion inhibitors in coolants, active ingredients in pesticide products, biocidal products in veterinary hygiene, pediculicide agents, hydroxyl-radical scavengers with antioxidant activity, diuretics and therapeutic agents in the treatment of some urea cycle disorders and other genetic enzymopathies, facilitating an alternative pathway of nitrogen excretion in the body (FAO/WHO, 2000; SCCNFP, 2011). Benzoates have been also proposed as a novel therapeutic approach in schizophrenia disorders, at

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oral doses of 1000 mg/day, by decreasing the severity of clinical symptoms and improving cognitive functions, probably due to their activity as D-amino acid oxidase inhibitors (Lane et al., 2013).

Benzyl alcohol (C_7H_8O), also known as phenylmethyl alcohol, phenylmethanol, benzenemethanol, phenylcarbinol or α-hydroxytoluene, is a clear colourless liquid with a mild pleasant aromatic odour and sharp burning taste, a molecular weight of 108.1, a melting point of -15.2 ºC and a boiling point of 204.7 ºC. It is partially soluble in water (4.3 g in 100 ml at 25 ºC) and completely miscible in alcohols, ether, chloroform, benzene and acetone. Benzyl alcohol is industrially produced by the action of sodium or potassium carbonate on benzyl chloride. It is used as fragrance component in cosmetics, solvent and viscosity-decreasing agent in cosmetics and hygiene products, preservative and/or flavouring agent in foods, cosmetics and pharmaceutical products, local anaesthetic or external analgesic, topical antiseptic, pediculicide, skin protector, embedding material in microscopy and chemical agent in photographic developing systems (CIR, 2001; Sudareva and Chubarova, 2006; SCCNFP, 2011).

Benzaldehyde (C_7H_6O), also known as benzoic aldehyde, benzenecarbonal, benzenecarboxaldehyde, benzenemethylal, phenylformaldehyde, phenylmethanal, benzenecarbaldehyde or artificial almond oil, is a clear colourless to slightly yellowish oily liquid with an odour of bitter almonds and burning aromatic taste, with a molecular weight of 106.1, a melting point of -26 ºC and a boiling point of 179 ºC. It is scarcely soluble in water (0.3 g in 100 ml at 20 ºC) but completely miscible in oils, alcohols, ether and most organic solvents, and when exposed to air it readily oxidizes to benzoic acid. It is industrially produced by direct

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oxidation of toluene with a cobalt catalyst. It is used as flavouring agent in foods, cosmetics and pharmaceuticals, fragrance component in perfumery, denaturing agent and solvent.

Benzoyl peroxide ($C_{14}H_{10}O_4$), also known as benzoperoxide, benzoyl superoxide, benzoyl dioxide, benzoyl benzenecarboperoxoate or dibenzoyl peroxide, is a colourless crystalline solid with a faint benzaldehyde-like odour, a molecular weight of 242.2 and a melting point of 103 º C, which decomposes explosively at temperatures higher than 105 ºC. It is sparingly soluble in water (less than 1 g in 100 ml at 25 ºC), alcohols and oils, but soluble in benzene, chloroform and ether. It is industrially produced by interaction of benzoyl chloride, sodium hydroxide and hydrogen peroxide. It is used for hair and teeth bleaching, in the pulp and paper industry, as antiseptic mainly in acne treatment, as radical initiator for industrial polymerizations and synthesis of polyester and resins, as catalyst in the plastic industry, and as bleaching agent in foods such as flour, whey and some Italian and Swiss cheeses (Hamdu, 2014). It can react with oxidizable substances such as annato or carotenoid pigments yielding benzoic acid and peroxides, and increasing benzoic acid levels in food products (CAC, 2008; Listiyani et al., 2011).

Alkyl benzoate esters include compounds such as methyl benzoate, ethyl benzoate, propyl benzoate, butyl benzoate, amyl benzoate, lauryl/myristyl benzoate, C12-15 alkyl benzoate, C16-17 alkyl benzoate, stearyl benzoate, isostearyl benzoate, ethylhexyl benzoate, butyloctyl benzoate, hexyldecyl benzoate and octyldecyl benzoate. The shorter chain alkyl benzoates are colourless liquids, but viscosity, melting point and boiling point generally increase with chain length, and longer chain alkyl benzoates are poorly soluble or completely insoluble in water. Alkyl benzoates are industrially produced by esterification of benzoic acid and alkyl groups, generally via strong acid-catalysis with sulphuric or hydrochloric acid. They are used in foods, cosmetics and hygiene products as flavouring or fragrance ingredients, skin-conditioning agents, emollients, preservatives, solvents and plasticizers (Becker et al., 2012).

Parabens are esters of *p*-hydroxybenzoic acid with methyl to butyl or benzyl groups and molecular weights comprised in the range of 152 to 228 or higher. Parabens are industrially produced by esterification of *p*-hydroxybenzoic acid with an appropriate alcohol in the presence of a catalyst like concentrated sulphuric acid or *p*-toluenesulfonic acid. They are used as preservatives in foods, cosmetics, pharmaceuticals, hygiene and personal care products, due to their broad spectrum of antimicrobial activity, solubility in water and high stability (Liao et al., 2013).

Leaking of benzoic acid and its derivatives into the environment happens as a consequence of their widespread production and utilization. Anthropogenic releases of benzoic acid are primarily emissions into water and soil from their uses as preservatives and from the pesticide, tincture, paper, bakelite and pharmaceutical industries. Concentrations of benzoic acid in ground water ranging from 0.01 to 27.5 mg/l have been reported (Razika et al., 2010) and it has even been detected in marine air (Lawler et al., 2014).

NATURAL OCCURRENCE IN FOODS

Benzoic acid and many of its derivatives occur as common metabolites in plants and animals, and they may also arise from microbial activity. Consequently, endogenous benzoic acid can be naturally found in a wide range of foodstuffs, as reported by different authors (Nagayama et al., 1983, 1986; Sieber et al., 1989, 1995; Heimhuber and Herrmann, 1990).

Plants, fruits, nuts, spices, vegetables and fungi contain benzoic acid. Its concentration is related to plant species, growing season, plant survival and reproduction, and plant-insect and plant-plant interactions (Long et al., 2009). Benzoic acid is formed in apples infected with *Nectria galligena* to inhibit fungal growth, reaching levels of up to 247.6 mg/kg (Brown and Swinburne, 1971, 1973) and in apples infected with *Pezicula malicorticis* (Noble and Drysdale, 1983). It can be biosynthesized in plants from L-phenylalanine by a β-oxidative pathway with CoA-esters as intermediates, by a non-oxidative pathway with benzaldehyde as intermediate, and by a combination of both routes. These processes take place at different subcellular compartments. The methylation of benzoic acid occurs in the cytoplasm of vegetal cells while its formation from benzaldehyde occurs in the mitochondria (Long et al., 2009). For its catabolism, benzoic acid is conjugated with low-molecular-weight peptides, accumulated in vacuoles, transformed via oxidative cleavage of the aromatic ring and the resulting aliphatic fragments are incorporated into the cell metabolism while the carbon dioxide is released to the external medium (Chrikishvili et al., 2006). Not only benzoic acid but also related compounds such as benzoates, benzyl esters, benzyl alcohol and benzaldehyde are natural flavour constituents in fruits and vegetables, involved in ripening and biological processes. Their biosynthetic pathways include the reduction of benzoic acid to benzaldehyde and then to benzyl alcohol, which is subsequently esterified to benzylbenzoate and other acylbenzoate esters (Croteau, 1977). Benzyl alcohol is naturally present in plants and fruits such as apricots, snap beans, cocoa, cranberries and mushrooms, in the essential oil of plants like jasmine, hyacinth and ylang-ylang, and in

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honey (CIR, 2001). Benzaldehyde is naturally present in plants and fruits, especially in those belonging to the *Rosaceae* family, like almonds, cherries, apricots and apples (CIR, 2006), and contributes to the fragrance of flowers like carnations.

The benzoic acid content of fruits and vegetables was determined by Heimhuber and Herrmann (1990). Apple, pear, melon, watermelon, pineapple, banana, quince, greengage, grape, common orange, lemon, grapefruit and kiwi contained less than 1 mg/kg, peach and navel orange 1-2 mg/kg, and strawberries up to 29 mg/kg. In vegetables, aubergine, cauliflower, cabbage, potato, cucumber, carrot, radish, lettuce, celery, onion and garlic contained less than 1 mg/kg, and pumpkin, spinach and soybean 1-2 mg/kg in. Cereals contained less than 0.5 mg/kg. In nuts, cashew, almond, hazelnut, pistachio and sesame had less than 1 mg/kg, and peanut and walnut 1-4 mg/kg. In spices, parsley had less than 1 mg/kg, paprika and white pepper 1-3 mg/kg, turmeric, coriander, laurel and black pepper 3-5 mg/kg, cayenne pepper and mustard seed 5-10 mg/kg, allspice 10-15 mg/kg, cloves, salvia, thyme, nutmeg 15-50 mg/kg, and cinnamon 336 mg/kg. In fungus, levels between 0.1 and 5.3 mg/kg were reported by Nagayama et al. (1986). Higher levels of benzoic acid have been found in certain fruits and plants, with up to 500 mg/kg in most berries, up to 1300 mg/kg in ripe fruits of *Vaccinium* species, and up to 200.000 mg/kg in resin from *Styrax* trees (FAO/WHO, 2000).

In animals, benzoic acid has been found in muscles, viscera, fluids, glands and secretions of omnivorous and herbivorous species, at levels usually lower than 1 mg/kg. Benzoic acid can be ingested with the diet, mainly fruits, vegetables and plants, and it may also come from the environment, drugs and feed additives (Chrikishvili et al., 2006). It can be present in animal tissues, including liver and kidney, as an intermediate of the metabolic and degradative pathways of biological substances and from aromatization reactions (Beer et al., 1951). In ruminants, it can arise from microbial degradation of tyrosine and other aromatic amino acids in the rumen (López et al., 1998). The presence of benzoic acid in most foodstuffs of animal origin is usually very limited, except if added as food preservative. However, milk and especially fermented dairy products may contain high levels. Natural concentration of benzoic acid in milk usually ranges between 2 and 5 mg/l (Obentraut, 1982), but up to 24.8 mg/l were reported in raw cow milk (López et al., 1998) and 28 mg/l in UHT cow milk (Javanmardi et al., 2015). Its natural presence in milk has not been related with mastitis or with the presence of pathogenic bacteria such as *Staphylococcus*, *Clostridium* and *Enterococcus* (López et al., 1998).

During milk fermentation, the concentration of some organic acids, such as benzoic, lactic, propionic, acetic, sorbic and nucleic acids increases while the concentration of others, like hippuric, orotic and citric acids, decreases (Urbienė and Leskauskaitė, 2006). In fermented milk, a sharp increase of benzoic acid concentration during fermentation and beginning of storage as well as a decrease at the end of the storage period has been reported. Concentrations of benzoic acid up to 18.8 mg/l (Garmiene et al., 2010) and 24 mg/l (Urbienė and Leskauskaitė, 2006) have been found in fermented cow milk, which were associated with the activity of lactic acid bacteria such as *Lactobacillus*, especially *Lactobacillus acidophilus*, and to a lesser extent with *Streptococcus*, *Lactococcus* and *Bifidobacterium*. Up to 25 and 36 mg/l have been found in goat or sheep fermented milk, respectively, with no differences in benzoic acid content attributable to starter cultures (Horníčková et al., 2014). Benzoic acid accumulation in cheese has also been reported (Sieber et al., 1995; Garmiene et al., 2011), with higher levels at the rind than in the centre (Sieber et al., 1995). Up to 12 mg/kg were reported for semi-hard cheese at 1 month

(Garmiene et al., 2011), 90 mg/kg for fresh farm Cottage cheese (Horníčková et al., 2014), 152 mg/kg for hard-cheese at 48 months (Garmiene et al., 2011), and 250 mg/kg for raw cow milk cheese at 6 months (Calzada et al., 2015). In a survey on the benzoic acid content of 100 different cheese samples, including hard, semi-hard, semi-soft and soft varieties made from raw and pasteurized cow, sheep and goat milk, levels ranged from 0 to 28.7 mg/kg with no differences ascribable to cheese typology, production technology, milk origin or production area (Iammarino et al., 2011). The natural presence and formation of benzoic acid in milk and dairy products interferes with the legal use of benzoic acid as food additive in these foodstuffs.

Production of benzoic acid in dairy products can occur by at least four routes (Kurisaki et al., 1973; Sajko et al., 1984; Sieber et al., 1995; Tavaria et al., 2002; Iammarino et al., 2011). The first one is the microbial enzymatic conversion of hippuric acid, naturally present in milk at values usually under 50 mg/l, which results into the formation of benzoic acid and glycine by lactic acid bacteria, *Escherichia coli* and *Pseudomonas*. The other three metabolic pathways were later proposed because the stoichiometric conversion of hippuric acid could not explain the high concentrations of benzoic acid found in some cheeses and other dairy products. One of these pathways is the enzymatic degradation and microbial breakdown of phenylalanine and phenylvaleric acid, yielding cinnamic, hydrocinnamic, β-phenyl-β-ketopropionic and β-phenyl-hydroxypropionic acids as intermediate products and benzoic acid, ammonia and acetophenone as final products. Benzoic acid can also derive from the auto-oxidation of benzaldehyde, which can be present at relatively high concentrations in cultured dairy products due to the activity of lactic acid bacteria aminotransferases on phenylalanine and/or to the phenylacetic acid oxidation by moulds like *Penicillium chrysogenum*. Finally, benzoic acid can result from the metabolic activity of bacterial contaminants such as *Pseudomonas* on phenol, produced through the degradation of tyrosine by lactic acid bacteria.

Organisms like the marine bacterium *Microbulbifer* and herbaceous plants like *Oxalix tuberosa* and *Andrographis paniculata* (Błędzka et al., 2014) have been found to produce parabens. These compounds have also been reported to naturally occur in serum and milk at concentrations of few micrograms per litre (Liao et al., 2013).

BENZOIC ACID AS FOOD AND FEED ADDITIVE

Benzoic acid, its salts and other derivatives are classified as food additives, mainly as preservatives and flavouring agents (Tables 1 and 2). The undissociated form of benzoic acid is more effective as antimicrobial agent, but its salts, especially sodium benzoate, are preferentially used because of their greater solubility, up to 200 times higher. Benzoic acid interacts with the cell membrane, altering its properties. It crosses inside the cell and subsequently dissociates at the higher intracellular pH, releasing charged anions and protons. Its antimicrobial activity has been reported to be exerted by membrane disruption, accumulation of toxic ions, alteration of intracellular pH homeostasis and suppression of metabolic routes by inhibition of some Krebs cycle enzymes such as oxoglutarate dehydrogenase and succinate dehydrogenase, glycolysis pathway enzymes such as phosphofructokinase, and enzymes involved in oxidative phosphorylation (Krebs et al., 1983; Brul and Coote, 1999; Iammarino et al., 2011). Benzoic acid and its salts exert their maximum antimicrobial activity at pH values between 2.5 and 4.5, and therefore they are preferentially used as preservative in acid foodstuffs. They can be directly added or used in edible coatings in a great diversity of foods, including fish, seafood, meat and egg products, sauces, soft drinks, beverages and juices, canned foods, chemical leavened baked goods, condiments, fruit and vegetable products. Mixtures of benzoic acid or benzoates together with other acids such as sorbic, citric, propionic, lactic, and ascorbic, or with nitrates or nitrites, are used in fermented vegetables (Casado et al., 2011).

The antimicrobial activity of benzoic acid and benzoates has been probed against a wide range of microorganisms involved in food poisoning and spoilage. They are effective against yeasts and moulds belonging to the genera *Aspergillus*, *Penicillium*, *Eurotium*, *Debaryomyces*, *Saccharomyces*, *Pichia*, *Candida*, *Kloeckera*, *Kluyveromyces* and *Zygosaccharomyces* (Sieber et al., 1995; Praphailong and Fleet, 1997; Palou et al., 2002; Guynot et al., 2005; Heydarynia et al., 2011), and against bacteria belonging to the species *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Leuconostoc dextranicum* and *Leuconostoc mesenteroides* (Rushing and Senn, 1963; Sieber et al., 1995). Tolerance mechanisms and resistant strains to benzoic acid and benzoates have been reported. In this context, *Gluconobacter oxydans*, an acetic acid bacterium responsible of spoilage in acid foods, fruit products and fermented beverages, has been described to rapidly develop adaptation and tolerance when exposed to benzoic acid, requiring concentrations of the antimicrobial much higher than those permitted in foods (Eyles and Warth, 1989). Among yeasts, resistance to benzoic acid and benzoates has been found for *Zygosaccharomyces bailii*, *Yarrowia lipolytica*, *Issatchenkia occidentalis*, *Saccharomyces cerevisiae*, *Cryptococcus curvatus*, *Pichia farinosa*, *Candida blankii*, *Debaryomyces hansenii* and *Trichosporon brassicae* (Mihyar et al., 1997; Praphailong and Fleet, 1997; Arroyo-López et al., 2006). There are microorganisms such as *Acinetobacter*, *Alcaligenes*, *Azotobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Rhodopseudomonas* and *Vibrio* capable of using benzoic acid as carbon source by reduction of the aromatic ring and/or oxidation to catechol by the β-ketoadipate pathway (Evans, 1947; Guyer and Hegeman, 1969; Reiner, 1971; Thayer and Wheelis, 1982; Razika et al., 2010).

Benzoic acid may influence taste perception when used as preservative and/or flavouring agent in foods. It is able to stimulate trigeminal innervation of taste cells, cause oral prickling, increase sweetness perception, weaken sourness and saltiness perception, and strongly reduce bitterness (Otero-Losada, 2003).

Another approved use for benzoic acid is as a feed additive, at levels between 0.5 and 1% in fattening diets (EFSA, 2007). It is mainly based on benzoic acid metabolic pattern and its antibacterial activity in the gut. Beneficial effects in piglets, such as enhancement of body weight and average daily gain together with reduction of *E. coli* counts and diarrhoea frequency, were reported for diets supplemented with 0.5% benzoic acid (Papatsiros et al., 2011), although in a previous study diet supplementation with 1% benzoic acid had shown no effects on gut microbiota, morphological and histological parameters, or health of piglets (Dierick et al., 2004). In broiler chickens fed diets supplemented with 0.25-0.75% benzoic acid, ileal coliform counts decreased but growth rate declined and feed conversion ratio increased (Józefiak et al., 2007). Benzoic acid added at 0.2% to the diet decreased body weight gain and impaired feed conversion ratio in broiler chickens, while added at 0.1% it increased body weight gain and improved feed conversion ratio, although the positive effects ceased after day 14 of the trial (Józefiak et al., 2010). Supplementation of broiler chicken diet with 0.2% benzoic acid decreased digesta pH in all sections of the gastrointestinal tract, resulted in longer and wider intestinal villi, and increased apparent metabolizable energy, but had no effect on growth performance (Olukosi and Dono, 2014). Differences in the mechanism of benzoic acid detoxification and excretion between animal species may influence its performance as growth promoter. Benzoic acid is excreted in mammals after conjugation with glycine in the liver and kidneys (Józefiak et al., 2007) while in the domestic fowl it is mostly excreted as the ornithine conjugate, with dietary arginine probably serving as the only substrate for ornithine synthesis (Nesheim and Garlich, 1963). According to these authors, 1.5% benzoic acid in the diet of chickens markedly depressed their growth rate, a detrimental effect which was mitigated by the addition of ornithine and arginine, but not of glycine, to the diet.

TOXICOLOGY AND ADVERSE EFFECTS

The toxicology and adverse effects of benzoic acid and its derivatives and their safety levels have always been controversial. Currently, benzoic acid and a great variety of related compounds are generally recognized as safe (GRAS) substances and their use as additives and/or flavouring agents in foods (Tables 1 and 2), cosmetics, pharmaceutical and hygiene products, is permitted by FAO/WHO and FDA/USDA. In this context of controversy, the European Food Safety Authority (EFSA) recommended in 2006 the withdrawal of approval for propylparaben (E216) and its sodium salt (E217) after more than 85 years of extended use, while for the rest of parabens the acceptable daily intake (ADI) of 10 mg/kg body weight/day remains and its use in cosmetics is permitted with a maximum concentration for each compound of 0.4% and a total

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maximum level of 0.8% (FAO/WHO, 2006). More recently, propylparabens have been shown to cause adverse effects such as endocrine disruption, estrogenic activity and reproductive disorders in rats at dietary doses of down to 10 mg/kg body weight/day and these compounds have been related to skin melanomas, testicular and breast cancer (Darbre and Harbey, 2008; Błędzka et al., 2014).

According to the European Commission (2005), benzoic acid and its salts are considered with benzyl alcohol, benzyl acetate and benzaldehyde as a single category group from the human health point of view by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), as they are rapidly metabolized and excreted in urine within 24 hours. These substances are absorbed from the gastrointestinal tract, reaching their maximum peak concentration in plasma within 1-2 hours, and they can be also partially absorbed (22-89%) by dermal route and inhalation (FAO/WHO, 2000). Benzyl alcohol is degraded by oxidation to benzaldehyde in liver and kidneys, mediated by alcohol dehydrogenases and cytochrome P450, and benzaldehyde is further oxidized to benzoic acid by aldehyde dehydrogenases (Sudareva and Chubarova, 2006). Alkyl benzoate esters are degraded to benzoic acid and the corresponding alcohols by enzymatic activity in different tissues and locations like the respiratory tract, skin, blood and gastrointestinal tract (Becker et al., 2012). Similarly, parabens are degraded by non-specific esterases widely distributed in the body, in skin, subcutaneous fat tissue, liver and gastrointestinal tract, yielding alcohols and *p*-hydroxybenzoic acid which is thereafter conjugated with glycine, glucuronide or sulphate and excreted in urine (Abbas et al., 2010; Błędzka et al., 2014). The fate of orally administered benzoic acid in humans and 20 other animal species which included mammals, birds and reptiles was investigated by Bridges et al. (1970). In mammals,

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benzoic acid is rapidly detoxified in liver and kidneys by conjugation with glycine (80%) in the mitochondrial matrix and to a lesser extent with glucuronic acid (20%) in the endoplasmic reticulum, and excreted in urine as hippuric acid and benzoyl glucuronide (SCCNFP, 2011; Badenhorst et al., 2014). Cats are defective in the glucuronide pathway and especially sensitive to benzoic acid, which can exert acute and cumulative effects at levels of 0.5 g/kg body weight and induce convulsions, aggression, hyperaesthesia and even death (Bedford and Clarke, 1972; Janz, 1989; FAO/WHO, 2000). In some birds and especially in reptiles, benzoic acid is mainly excreted as ornithuric acid (Bridges et al., 1970) and its detoxification is relatively slow, lasting more than 2-3 days. The metabolism of benzoic acid and its related compounds, which greatly varies depending on the intake, the animal species and even among individuals, has been widely reviewed by Tremblay and Qureshi (1993) and Badenhorst et al. (2014).

In spite of benzoic acid and its salts and derivatives being considered as non-harmful substances to humans, some toxic and adverse effects have been reported. Benzoic acid is slightly irritant to the skin and eyes, and sodium benzoate only to eyes (FAO/WHO, 2000). Topical application of benzoic acid can cause urticaria, erythema and stinging, probably by inducement of large quantities of prostaglandin D2 in the skin and cutaneous vasodilatation (Downard et al., 1995). Benzoic acid and its salts have also been related to exacerbation of orofacial granulomatosis and therefore benzoate-free diet is being recommended in clinical practice for patients affected by chronic inflammatory diseases (Campbell et al., 2011). Some cases of urticaria, asthma, rhinitis, hives and anaphylactic shock have been reported following oral, dermal or inhalation exposure to benzoic acid and its salts, even at doses lower than ADI (Michils et al., 1991; FAO/WHO, 2000), and sodium benzoate has been described to induce chronic generalized pruritus (Asero, 2006). Benzoic acid can also cause irritation of the digestive mucous membrane, and doses higher than ADI (i.e. 1000 mg/kg body weight/day for 5 consecutive days) can induce nausea, headache, oesophagus burning and reduction of the digestive usage coefficient by inhibition of enzymes such as pepsin, trypsin, polypeptidases and D-amino acid oxidases (FAO/WHO, 2000; Iammarino et al., 2011). Other adverse effects attributed to benzoic acid include diarrhoea, muscular weakness, metabolic acidosis, convulsions, tremors, hypoactivity and emaciation, which could be related to an excessive utilization of glycine for detoxification of benzoic acid, resulting in the reduction of glycine levels and interfering with the metabolic processes in which it is involved, leading to the reduction in creatinine, glutamine, urea and uric acid production (Tremblay and Qureshi, 1993; FAO/WHO, 2000). Benzoic acid and its salts have been also linked to childhood hyperactivity or hyperkinetic syndrome (Egger et al., 1985; Tuormaa, 1994; McCann et al., 2007).

Benzoic acid and benzoates are not only used as preservatives in foods and cosmetics but also in medicines and other pharmaceutical products, and in this context the main safety concern is their ability to displace bilirubin from albumin, which can lead to hyperbilirubinemia, encephalopathy and kernicterus (bilirubin induced brain dysfunction) especially in neonates (EMA, 2014). Besides this, sodium benzoate is used for treatment of urea cycle disorders, at doses of 180-650 mg/kg body weight/day over up to several years, which could induce adverse effects such as hyperactivity, impulsiveness and inattentive behaviour, especially in children. In this context, the intake of sodium benzoate at doses of 200 mg/kg body weight/day for 4 weeks in rats has been reported to induce anxiety, behavioural changes and motor impairment, probably by reduction of glycine levels in the body, depletion of glutamine, inhibition of the expression of

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inducible NO synthesis in astrocytes and microglia and reduction of Zn levels in the brain (Noorafshan *et al*., 2014).

Toxic levels for benzoic acid were established by the European Commission in 2005. Median lethal doses (DL_{50}) of 1700-2530 mg/kg, 5000-10000 mg/kg and higher than 0.026 mg/l/h have been reported for acute oral toxicity in rats, acute dermal toxicity in rabbits and acute inhalation toxicity in rats, respectively. In i*n vitro* studies on human erythrocytes, sodium benzoate decreased in a dose-dependent manner, from 1 g/l, the activity of enzymes like aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase (Monanu et al., 2005). In cultured human peripheral blood lymphocytes, benzoic acid and its potassium and sodium salts exerted genotoxic effects (clastogenic, mutagenic and cytotoxic) in a dose-dependent manner, from 100, 62.5 and 6.25 mg/l, respectively, with adverse effects including the increase of chromosomal aberrations, sister-chromatid exchange, micronucleus and DNA damages, and reduction of the mitotic index (Yilmaz et al., 2009; Zengin et al., 2011). In cultured peripheral blood mononuclear cells, sodium benzoate decreased in a dose-dependent manner, from 10 mM, the Th1-type response and γ-interferon activity, which could be related to immunosuppression, decreased efficacy to respond against pathogens and tumours, and development of allergic diseases (Maier et al., 2010). In *in vitro* cultures of human clonal epithelial cells (HeLa cells) and rat cortical neurons, sodium benzoate induced oxidative stress and cell death in a dose-dependent and cumulative manner, even at doses of 0.1% (Park et al., 2011). Sodium benzoate at levels higher than 1.5% decreased the release of leptin in *in vitro* cultures of murine adipocytes, which could be associated to a potential obesogenic effect (Ciardi et al., 2012). Rats fed for 35 days on a diet supplemented with 1.1% benzoic acid showed retarded growth and impairment of food utilization, and levels of 3% administered for only 5 days caused much more serious symptoms, including neurological disorders, ataxia and convulsions, which remained even 30 days after the treatment ceased (Kreis et al., 1967). Mice and rats fed for 10 days on a diet containing 2% sodium benzoate developed histological changes and increased liver weight, showed high blood levels of serum albumin, total proteins, cholesterol, triglycerides and cholinesterase activity, and some cases of hypersensitivity, convulsions and death also occurred (Fujitani, 1993). Rats fed for 7 weeks on a diet containing a mixture of permitted additives, including benzoic acid, sorbic acid, nitrites and glutamate, all of them at concentrations under the legal limits, showed increased diet intake but not body weight gain, and some haematological parameters, such as haemoglobin concentration, haematocrit value, erythrocyte count, serum iron concentration, iron binding capacity and transferrin saturation with iron, decreased significantly (Sadowska and Kuchlewska, 2011).

Besides these direct effects attributed to benzoic acid and related compounds present in foods, cosmetics or medicines, some indirect effects associated to their degradation and/or biochemical reactions and generation of new toxic compounds have been suggested. Benzyl alcohol, commonly used at levels of 3 to 5% as preservative in pharmaceutical and medicinal solutions, is known to oxidize slowly in the presence of air to benzaldehyde, which can be further oxidized to benzoic acid or react with the remaining benzyl alcohol moieties yielding benzaldehyde dibenzyl acetal, which can react with alcohols and form other type of acetals in the product (Abend et al., 2004; Sudareva and Chubarova, 2006). Benzoic acid and its salts might be decarboxylated in the presence of ascorbic acid in some drinks and products, yielding small amounts of benzene (Lachenmeier et al., 2008), which is classified into the group 1 of carcinogenic compounds to humans by the International Agency for Research on Cancer (IARC). Currently, there exists no standard for benzene in beverages other than bottled drinking water for which a maximum of 5 ppb was established by the FDA. However, in a survey on 86 non-alcoholic beverages performed by the FDA in 2007, benzene levels ranged from non-detectable to 88.9 ppb, with concentrations above the maximum level allowed in water for 8 of the beverages. The relation of benzene with the presence of benzoic and ascorbic acids and with factors that can influence its formation, such as Cu^{2+} and Fe^{3+} ions, pH, UV light and temperature, requires further research (FDA, 2013).

LEGAL LIMITS

Benzoic acid, its salts and parabens are included in the type 1 GRAS list of substances of FDA, which considered that "there is no evidence in the available information on substance that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or might reasonably be expected in the future, and they can be used as antimicrobials or preservative agents, flavouring agents and adjuvants with a maximum level of 0.1% in food, and their presence must be declared on the label" (CFR, 1999; U.S.FDA, 2015). In Europe, benzoic acid, its salts and many of its derivatives are considered as harmless compounds and used as preservatives and/or flavouring agents (Tables 1 and 2) in foods, cosmetics and medical preparations, but some regulations limiting their maximum levels have been established (European Commission, 2005). FAO/WHO (2014) establish different limits as food preservative or additive according to different foodstuffs, as shown in Table 3. Overall, benzoic acid and its salts are permitted in most foodstuffs with a maximum level of 1000 mg/kg. Higher limits have been established for certain foods, such as 6000 mg/kg for cooked shrimps, 5000 mg/kg for liquid-egg products, 3000 mg/kg for vegetable pulps, 2000 mg/kg for cooked seafood other than shrimps, semi-preserved fish and seafood products, brined vegetables, soy sauce, table-top sweeteners and food supplements, and 1500 mg/kg for salads, sandwich spreads and dietetic-medical foods. On the contrary, lower limits have been set for some products such as the 800 mg/kg for dried fruits, 600 mg/kg for vegetable-nectar concentrates, water-based and soft drinks, 500 mg/kg for soups and broths, and 200 mg/kg for smoked, dried, salted or fermented fish and seafood products. In dairy products, the addition of benzoic acid or benzoates is not allowed excepting for buttermilk and dairy fat spreads for which a limit of 1000 mg/kg is set, and for acidulate milk, acidified milk, yogurt and kefir for which a limit of 300 mg/kg is permitted. Regarding benzoyl peroxide, its use as bleaching agent is allowed at levels lower than 100 mg/kg in whey and whey products and lower than 75 mg/kg in flours, excluding cheeses and infant formulas where it is not permitted with the exception of some regional or typical uses, including the addition of benzoyl peroxide at levels of 20-40 mg/kg to bleach milk used for the production of certain Italian cheeses such as Asiago fresh, Asiago soft cheese, Asiago medium cheese, Asiago old cheese, Caciocavallo siciliano cheese, Gorgonzola cheese, Parmesan and Reggiano cheese, Provolone cheese and Romano cheese, and some Swiss cheeses like Emmental cheese (FAO/WHO, 2004). Contrarily, in USA this compound is admitted as bleaching agent in foods for which no limits exist further than good manufacturing practices.

Most food regulations in other countries are aligned with Codex Alimentarius Commission Guidelines (FAO/WHO, 2014). In Turkey, benzoic acid and its derivatives are

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accepted as food preservatives with a maximum overall level of 1000 mg/kg but they are not allowed in milk or dairy products (TFCR, 2011). In Australia, benzoic acid and its salts can be used as preservatives of rennet enzymes at maximum levels of 9000 mg/kg, and some more restrictive levels than Codex Alimentarius regulations can be found for solid supplementary sports food, alcoholic and non-alcoholic beverages (400 mg/kg), mushroom conserves (500 mg/kg), and dairy and fat based desserts and snacks (700 mg/kg), while benzoic acid, its salts and parabens can be used for tabletop sweeteners at levels considered in Good Manufacturing Practices (ANZ-FS, 2008). In Canada, benzoic acid, its salts and parabens are classified as permitted preservatives class 2, for which "particular authorised uses are specified in each case and includes preservatives to potentially control the growth of *Listeria monocytogenes* in RTE (ready-to-eat) foods sold in Canada", and a maximum overall level of 1000 mg/kg is considered (CFIA, 2014). In Taiwan and Hong Kong, benzoic acid, its salts and parabens are permitted preservatives in specified food items (DOH, 1993; CFS-HK, 2008), such as maximum levels of 2000 mg/kg in liquid rennet enzymes or 3000 mg/kg in another liquid enzyme preparations, with a wide range of limits in foods and drinks such as beer and malt beverages (70 mg/kg), vegetable juice or nectar and water based flavoured drinks (160 mg/kg), curry paste (350 mg/kg), soy sauces (550 mg/kg), cocoa mixes or syrups (700 mg/kg), fruit juice or nectar and canned or bottled fruit (800 mg/kg), instant noodles (1000 mg/kg), grape juice products (2000 mg/kg), horseradish pulp (3000 mg/kg) and liquid foam headings (10,000 mg/kg). In India, benzoyl peroxide can be used as preservative and flour treatment agent at levels lower than 40 mg/kg, and benzoic acid, its salts and parabens are permitted food additives but some more restrictive levels than Codex Alimentarius regulations can be found for certain products (FSSAI, 2010), like

Danish tinned caviar (50 mg/kg), alcoholic and non-alcoholic beverages (120 mg/kg), jams, jellies and marmalades (200 mg/kg), pickles (250 mg/kg), coffee (450 mg/kg), vegetable puree and flour for baked products (500 mg/kg), fruit products (600 mg/kg) and sauces (750 mg/kg). In Russia, benzoyl peroxide is accepted as preserving agent and flour processing aid at levels lower than 75 mg/kg, and benzoic acid, its salts and parabens are "food additives allowed for use in production of food products as preserving agents" (TRRKB-CU, 2012), but some differences regarding the Codex Alimentarius regulations can be found for products like flavoured non-alcoholic drinks (150 mg/kg), non-alcoholic beer and alcoholic beverages with alcohol content lower than 15% (200 mg/kg), jams, jellies, marmalades, sauces based on vegetable oil and black olives and products thereof (500 mg/kg), and boiled table beet (2000 mg/kg). In South Africa, benzoic acid, its salt and parabens are used as preservatives in certain products but there is no list of permitted additives, and additives regulations are categorized according to the type of foodstuffs (DHR-SA, 1977), with maximum allowable levels for fresh fish (100 mg/kg), jams and jellies (400 mg/kg), fish pastes (500 mg/kg), prepared fresh fruits or pulp, mayonnaises and dressings (600 mg/kg), fish sausages and manufactured fish products (700 mg/kg), manufactured meat products and fish roe (750 mg/kg), marinated fish or fish products (1000 mg/kg), sacramental wine prepared from unfermented grape juice (2750 mg/kg), processed blended cheese, cheese spreads, cheese preparations and soft cheese (600 mg/kg) and dietary supplements (levels as considered in Good Manufacturing Practices). On the other hand, a much more restrictive and specific legislation than Codex Alimentarius regulations is applied in Japan (JETRO, 2011). Alkyl esters of benzoic acid, benzaldehyde and benzyl alcohol are classified as flavouring agents in foods, benzoic and castoreum oil as natural flavouring agents, and benzoic acid, its salts, parabens and benzoyl peroxide as "designated food additives with standards of use and with conditions of use preventing importation from the European Union". Benzoyl peroxide is permitted as flour treatment agent at levels lower than 300 mg/kg, sodium benzoate as preservative in fruit products and juices at levels lower than 1000 mg/kg, benzoic acid and sodium benzoate as preservatives in non-alcoholic beverages (600 mg/kg), margarine (1000 mg/kg) and caviar (2500 mg/kg), and parabens as preservatives in fruit or vegetable products (12 mg/kg), vinegar, non-alcoholic beverages and syrups (100 mg/kg), fruit sauces (200 mg/kg) and soy sauces (250 mg(kg).

. FAO/WHO (1996) established an ADI of equal or less than 5 mg/kg body weight/day (expressed as benzoic acid equivalents) for benzoic acid and its salts, benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, that was latterly confirmed (FAO/WHO, 2001) and remains nowadays. For parabens, an ADI equal or less than 10 mg/kg body weight/day was established, excepting for the withdrawn propyl parabens (FAO/WHO, 2006).

DAILY EXPOSURE TO BENZOIC ACID IN FOODS

One of the major routes of exposition to benzoic acid is oral ingestion with foods as a naturally present compound and, more important, as an additive. As described, the FAO/WHO (2014) has established maximum legal limits for benzoic acid as additive in different foodstuffs and an ADI of 5 mg/kg body weight/day. However, benzoic acid can be present in a wide variety of commercial food products, in some cases at considerable levels (Tables 4 to 8). The intake of high amounts of benzoic acid over long periods could represent a threat to consumers, especially to the more vulnerable groups of population like children, patients and sensitive persons. Many studies about the estimated daily intake (EDI) in different groups of population and the main foodstuffs contributing to benzoic acid intake have been carried out, some of which are here described.

On the one hand, some studies have evaluated the presence of benzoic acid in different foodstuffs and found that its levels were under the legal limits and that EDI was much lower than ADI. In a survey on 61 samples of Chinese cured meat products from Taiwan markets, just 8% of the samples were found to contain detectable amounts of benzoic acid, under the Chinese legal limits of 1000 mg/kg (Lo et al., 2001). In 100 cheese samples from Italian markets and factories, benzoic acid was detected in 18% of samples, at levels ranging between 11.3 and 28.7 mg/kg (Iammarino et al., 2011). In a Serbian study on 748 samples of alcoholic and non-alcoholic beverages, ketchup and tomato products, pickled vegetables, olives, syrup and marmalade, detectable amounts of benzoic acid were found in 88.6% of samples but all of them were under the legal limits and an EDI of 0.32 mg/kg body weight/day, much lower than ADI, was calculated (Lazarević et al., 2011). In an study on 300 samples of alcoholic and non-alcoholic beverages, sweets, fruits, sausages and minced meat, noodles, pasta and cheeses from New Zealand markets, it was found that 98% of the samples contained benzoic acid levels under the regulatory limits and the unauthorised levels in the rest of the samples (cheese, apricots and fruit cakes) could be due to the natural occurrence of benzoic acid, with EDIs representing less than 18% of the ADI (Cressey and Jones, 2009). In a survey on 211 samples of non-alcoholic beverages from Hong Kong markets, including tea, coffee, juices, soy milk and soft drinks, just 17% of samples, mainly fruit juices and soft drinks, contained detectable amounts of benzoic acid and all of them were under the legal limits, with EDIs of 0.3 or 0.97 mg/kg body weight/day being calculated for children or high consumers respectively (CFS-HK, 2007). Other averaged EDIs for benzoic acid much lower than ADI such as 0.025, 0.31, 0.40, 0.50 and 0.96 mg/kg body weight/day have been reported in Korea, Hong Kong, France, UK and Australia, respectively (CFS-HK, 2007). In a Turkish study with 50 samples of different foodstuffs, including dairy products, beverages, sweets and pickles, the concentrations of benzoic acid and the EDIs were under the legal limits for all the products, with the only exception of yogurt rice dressing for which an EDI of 5.17 mg/kg body weight/day, slightly higher than ADI, was calculated (El-Ziney, 2009).

On the other hand, some studies have calculated EDIs lower than ADI and/or considered that benzoic acid in foods does not represent a public health risk, but detected some great amounts of benzoic acid and violation of maximum allowable limits or unauthorised uses. In a survey on 67 samples from Malaysian markets, including soft drinks, canned fruits and vegetables, jams, jellies and sauces, just 16.4% of samples contained detectable amounts of benzoic acid but all of them were over the Malaysian legal limits (Saad et al., 2005). In an Iranian study on 54 samples, including milk, ketchup, soft drinks and bread, 90.7% of samples were found to contain benzoic acid and more than half of the soft drinks presented concentrations higher than levels permitted by the European legislation and the Iranian Standards Organisation (Javanmardi et al., 2015). In an study on 11 quince jams from Portuguese markets, all of them contained detectable amounts of benzoic acid and 7 samples contained levels over the allowable legal limits (Ferreira et al., 2000), while in another Portuguese survey on 48 samples of beverages, including soft drinks and nectars, 50% of the soft drinks contained benzoic acid and 26% exceeded the maximum levels permitted by European and Portuguese legislations (Lino and Pena, 2010). In an Indian study with branded (118 samples) and non-branded (125 samples) tomato and chilli sauces, it was found that 100% of branded samples contained benzoic acid levels lower than legal limits while 59% of non-branded tomato sauces and 100% of non-branded chilli sauces had levels over the legal limits, although EDIs were much lower than ADI, with values equal or lower than 0.42 or 1.64 mg/kg body weight/day for branded or non-branded products, respectively (Dixit et al., 2008). Some Turkish surveys have also found benzoic acid levels in foods higher than the legal limits. Thus, in an study on 983 samples of fruit and vegetable products, sauces, cereals, bakery products, jellies, brines and food supplements, 2.3% of the samples, mainly sauces and fruit products, contained benzoic acid at levels higher than regulatory limits (Ulca et al., 2013), and the unauthorised use of benzoic acid was detected in 1.5% of the samples, mainly syrups and bakery products, while in another study on 109 samples, including sauces, yogurts, soups, juices, chips and chocolate, benzoic acid was found in 28.4% of the samples (Cakir and Cagri-Mehmetoglu, 2013) and 8.3% of the samples, mainly sauces, and to a lesser extent yogurts, juices and chocolate, contained levels higher than the legal limits. The Turkish Food Codex, in compliance with the European legislation, does not allow the addition of benzoic acid to dairy products, but in a study on 90 dairy products, 60% of the samples contained high amounts of benzoic acid, most of them at levels higher than 400 mg/kg (Koyuncu and Uylasser, 2009).

Research evaluating the presence of benzoic acid in different foodstuffs and taking into account the dietary habits, groups of population and more realistic or possible food intakes has suggested that benzoic acid could imply a risk to consumers and that a reduction of the maximum allowable limits should be considered. In a survey on 142 samples of milk and dairy products from Chinese markets, 77% of the samples contained detectable amounts of benzoic acid, with 86% of infant formula and 87% of milk powder products presenting high benzoic acid levels within the respective ranges 11-88 and 19-110 mg/kg, which might affect infants and sensitive individuals (Qi et al., 2009). In a Belgian survey on the consumption of 1245 food items, including wines, non-alcoholic beverages, juices, salads, jams, fish products, fermented vegetables, soups, sauces, dairy products and fruits, by an adult population group benzoic acid was found to be under the legal limits for all the samples and an EDI of 1.25-1.58 mg/kg body weight/day, much lower than ADI, was calculated (Vandevijvere et al., 2009). But these authors also found that the major contributor to benzoic acid intake were non-alcoholic flavoured drinks, which can be widely consumed, and suggested a possible risk to vulnerable groups of population. A Danish study performed on 1526 samples of foodstuffs, including dairy, fish, bakery, fruit, vegetable and meat products, jams, jellies, sauces and soft drinks, consumed by different groups of population showed that 31% of the samples contained detectable amounts of benzoic acid while transgressions of maximum limits, illegal uses or declaration faults were found in 3% of the samples (Leth et al., 2010). Two different EDIs were calculated in order to achieve more realistic food intake estimation. When assumed that population randomly consumed food products that do or do not contain benzoic acid (low intake calculation based on averaged content of benzoic acid in different food samples including those with no benzoic acid), it was found that more than 5% of population would have an intake higher than ADI for men up to the age group of 25-34 years and for women up to the age group of 19-24 years, and that the children could have an intake of more than three times their ADI. When assumed that population always consumed products containing benzoic acid (high intake calculation based on products that contain benzoic acid), 10% of both men and women would have a life-long intake of benzoic acid over the ADI. Similarly, in an Austrian survey on 2333 samples of foodstuffs, including alcoholic and non-alcoholic beverages, fish, fruit, vegetable, bakery and confectionery products, jams, jellies and sauces, and different age and sex groups of population, it was found that 14.3% of the samples contained benzoic acid (Mischek and Krapfenbauer-Cermak, 2012). Under the low intake calculation, all the population groups had an EDI below the ADI but under the high intake calculation the EDI exceeded the ADI in all the population groups, representing 135% of the ADI in children, 124% in females and 118% in males.

ANALYTICAL METHODS

Qualitative methods, just indicating the presence of benzoic acid, are the simplest techniques for benzoic acid determination. They include the ferric chloride test in solution, thin-layer chromatography (TLC) and the modified Mohler's test (FAO Manual, 1986; AOAC 2000, official method 910.02). The ferric chloride test involves the homogenization of the sample and acidification with HCl, extraction with ether, evaporation and dilution in water, and finally, addition of ferric chloride that gives a salmon-coloured precipitate of ferric benzoate. In the TLC technique, the sample is extracted by steam distillation and ether, evaporated and spotted on a silica gel plate, which is developed with ethanol:ammonia, air-dried and sprayed with peroxide ferric chloride, with benzoic acid appearing as mauve-coloured spot. In the modified Mohler's test, the aqueous solution resulting from the ferric chloride test is treated with NaOH, sulphuric acid and potassium nitrate, heated, boiled and then ammonium sulphide solution is added, with formation of a red-brown ring in the presence of benzoic acid.

Quantitative methods for benzoic acid in foodstuffs, developed or adapted in years 2001 to 2015, are shown in Tables 9 to 12. Most of these methods allow the simultaneous determination of other food additives, such as benzoates, parabens, sorbic acid, acetic acid, propionic acid, lactic acid, nitrites, nitrates and/or phosphates. A major problem for the determination of benzoic acid in foodstuffs is the presence of potentially interfering compounds due to the complexity of sample matrices. Extraction with solvents, distillation, clarification or solid-phase extraction techniques are usually required. Quantitative methods include a wide range of techniques, mainly spectrophotometry (Table 9), gas chromatography and flame ionization detection (GC-FID, Table 9), gas chromatography and mass spectrometry (GC-MS, Table 10), capillary electrophoresis and UV or amperometric detection (Table 10), proton nuclear magnetic resonance (Table 10), ion chromatography and conductivity detection (Table 11), and high performance liquid chromatography (HPLC), mainly reversed phase (RP) HPLC (Table 12). Currently, RP-HPLC with UV or diode array detection (AOAC 2000, official method 994.11; ISO 9231-2008) is the most widely used method for benzoic acid determination in foods such as milk, dairy products and juices in Europe and America, but also in other countries like China (GB21703-2010) and India (FSSAI, 2012). This method involves the dilution in water, sonication and filtration of the liquid sample (AOAC 2000, official method 994.11) or homogenization in water, basification with NaOH, sonication, heating, pH adjustment to 8 with sulphuric acid, clarification with Carrez reagents and methanol, and filtration (ISO 9231, 2008), and then RP-HPLC separation and UV detection at 227 or 230 nm. Other standardized techniques include the titrimetric method (AOAC 2000, official method 963.19) in which the sample is saturated with NaCl, acidified with HCl, extracted with chloroform, evaporated, dissolved in neutral alcohol and titrated against an standard alkali (0.05 N, NaOH), and the spectrophotometric method (AOAC 2000, official method 960.38) in which the sample is extracted in ether and the absorbance is measured at 272, 267.5 and 276.5 nm in the UV region. A GC-FID method after extraction with solvents and esterification was established by the Nordic Committee on Food Analysis (NCFA, 1984) for determination of benzoic and sorbic acids in different foodstuffs such as sauces, cheese and herring.

DISCUSSION

Benzoic acid and its derivatives are widely used as additives in the food and feed industries, as well in other sectors such as cosmetics, hygiene products and pharmaceutical industry. Although they are considered as harmless substances when used under legal limits, human exposure to them could be greater than expected due to their wide distribution in foodstuffs and environment, their high concentrations in some products and the various routes of absorption in the organism.

Non-alcoholic beverages, mainly fruit juices and carbonated soft drinks, are among the major contributors to benzoic acid intake, with differences depending on dietary habits and countries. In New Zealand, dietary benzoate exposure was reported to be mainly due to the consumption of carbonated non-alcoholic beverages (Cressey and Jones, 2009). In Austria, major contributors to benzoic acid dietary intake were reported to be fish and fish products (Mischek and Krapfenbauer-Cermak, 2012). In Denmark, the major contributor is the group of soft drinks and to a lesser extent the fat-based salads, sauces, dressings and pickled fish products (Leth et al., 2010). In Serbia, non-alcoholic beverages, ketchup and tomato products are the main contributors to benzoic acid intake and, to a lesser extent, pickled vegetables (Lazarević et al., 2011). In Romania major contributors are prepared salads, preserved vegetables, liquid egg products, cooked seafood and soft drinks (Violeta et al., 2007). Many reports have calculated benzoic acid EDIs for the whole population much lower than ADI and considered that benzoic acid does not represent a public health risk. However, some recent studies indicate that EDIs could be underestimated and that benzoic acid intake could represent a public health concern, being necessary to perform more precise intake estimations by using a wider range of population groups, more food items and more specific calculations based on the different dietary habits and preferences. Most EDI calculations are based on the averaged levels of benzoic acid within a range of foodstuffs including those that do not contain benzoic acid. But the real fact is that some people usually consume a higher proportion of certain types of food products and specific trademarks according to their preferences or possibilities, more than fixed proportions of different commercial items for the same product category. Exposure to benzoic acid would increase substantially if the selected commercial products contain high levels of benzoic acid. Thus, 200 g (or ml) of lemon juice (Table 4), surimi (Table 5), Arzúa-Ulloa cheese (Table 6), pickled vegetables (Table 7) or noodles rice spaghettini (Table 8), respectively containing 199.4, 178.0, 48.0, 151.0 or 921.4 mg of benzoic acid, would respectively represent 66.5, 59.3, 16.0, 50.3 or 307.1% of the ADI for a person of 60 kg. Since benzoic acid contents exceeded the European legal limits for some of these products, like lemon juice or noodles, calculations were redone excepting for cheese, for which addition is not allowed but there is no limit for naturally generated benzoic acid. On the basis of maximum legal concentrations (Table 3), ingestion of 200 g (or ml) of lemon juice, surimi, pickled vegetables or noodles rice spaghettini would respectively contain 120, 400, 400 or 200 mg of benzoic acid, which would respectively represent 40.0, 133.3, 133.3 or 66.7% of the ADI for a person of 60 kg. This suggests that the ingestion of just 200 g (or ml) of a single product could exceed the benzoic acid ADI in some cases.

Another food concern regarding benzoic acid and its derivatives (such as salts, parabens, alkyl esters and other related compounds) is the simultaneous presence and effects of other additives, including preservatives like sorbic acid (E200), sorbates (E201-E203), propionic acid (E280), propionates (E281-E283), dehydroacetic acid (E265), citric acid (E330), citrates (E331-E333), nitrates (E251, E252), nitrites (E249, E250), natamycin (E235), hexamethylenetetramine (E239) and lysozyme (E1105), antioxidants and acidity regulators like phosphates (E339-E343), artificial sweeteners like saccharin (E954), aspartame (E951) and acesulfame K (E950), and colorants like curcumine (E100), tartrazine (E102), quinoline yellow (E104), sunset yellow (E110), carmosine (E122), amaranth (E123), Ponceau 4R (E124), erythrosine (E127) and allura red (E129), which could also exert adverse effects or exacerbate among them (Parke and Lewis, 1992; Tuormaa, 1994; McCann et al., 2007; Carocho et al., 2014). Besides this, benzoic acid and some related compounds in foods and other products can react among them, resulting in the potential formation of compounds with new adverse effects. In this regard, benzoic acid and its salts can react, under certain conditions, with ascorbic acid and be decarboxylated to carcinogenic benzene, while benzyl alcohol can be oxidized to benzaldehyde, which can react

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with the remaining benzyl alcohol yielding benzaldehyde dibenzylacetal that can react with alcohols and form different acetals.

Oral intake of benzoic acid and its derivatives in foods is the major but not the only route of entrance into the organism, cutaneous and respiratory absorption being also possible. Benzoic acid and its derivatives are also widely used as flavouring agents and/or preservatives in cosmetics, oral and external hygiene products, and oral, topical and parenteral medicines and pharmaceutical products. High amounts of benzenic compounds are produced and used at the textile, plastic, paper, photographic, pesticides, tinctures and solvents industries. As a consequence of all these applications, benzoic acid and its related compounds are released into the environment and considered as common ubiquitous substances that can be found in air, water and soil.

The high occurrence and distribution of benzoic acid and related compounds in the environment, and their various routes of absorption, open the possibility that human exposure to these compounds could be considerably higher than the values estimated in most reports. Human exposure during long-life periods might represent a public health risk that should not be undervalued, especially for vulnerable groups of population such as children, sensitive or allergic individuals, and those suffering certain metabolic pathologies such as hepatopathies and defective conjugation processes.

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Table 1 Benzoic acid and some derivatives or related compounds approved as food additives by FAO/WHO (CAC/MISC 6-2013)

INS number	Compound
210	Benzoic acid
211	Sodium benzoate
212	Potassium benzoate
213	Calcium benzoate
214	Ethyl *p*-hydroxybenzoate
215	Sodium ethyl *p*-hydroxybenzoate
216*	Propyl *p*-hydroxybenzoate
217*	Sodium propyl *p*-hydroxybenzoate
218	Methyl *p*-hydroxybenzoate
219	Sodium methyl *p*-hydroxybenzoate
928	Benzoyl peroxide
1519	Benzyl alcohol

INS, International Numbering System defined by the Codex Alimentarius. *, currently withdrawn

Table 2 Benzoic acid and some derivatives or related compounds approved as flavouring agents by FAO/WHO (CAC/MISC 6-2013)

JECFA number	Compound	JECFA number	Compound
0022	Benzaldehyde	0860	Geranyl benzoate
0023	Benzyl acetate	0861	Glyceryl tribenzoate
0024	Benzyl benzoate	0862	Propylene glycol dibenzoate
0025	Benzyl alcohol	0863	2-methylbenzyl acetate
0460	Benzyl methyl sulphide	0864	*p*-isopropylbenzyl alcohol
0504	S-methyl benzothioate	0865	4-ethylbenzaldehyde
0525	Benzethiol	0869	2,4-dimethylbenzaldehyde
0526	Benzylmercaptan	0870	Butyl *p*-hydroxybenzoate
0579	Benzyl disulfide	0878	*p*-methoxybenzaldehyde
0670	Benzyl cinnamate	0879	*p*-ethoxybenzaldehyde
0751	2-benzofurancarboxaldehyde	0880	Methyl *o*-methoxybenzoate
0760	Cinnamyl benzoate	0881	2-methoxybenzoic acid
0799	α-methylbenzyl alcohol	0882	3-methoxybenzoic acid
0802	α-methylbenzyl propionate	0883	4-methoxybenzoic acid
0803	α-methylbenzyl butyrate	0898	2-hydroxy-4-methylbenzaldehyde
0804	α-methylbenzyl isobutyrate	0904	Benzyl salicylate
0805	*p*-α-dimethylbenzyl alcohol	0908	2,4-dihydroxybenzoic acid

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0823	α-ethylbenzyl butyrate
0830	3-benzyl-4-heptanone
0831	Benzophenone
0834	Ethyl benzoylacetate
0836	Benzoin
0837	Benzaldehyde dimethyl acetal
0838	Benzaldehyde glyceryl acetal
0839	Benzaldehyde propylene glycol acetal
0840	Benzyl 2-methoxiethyl acetal
0841	Benzyl formate
0842	Benzyl propionate
0843	Benzyl butyrate
0844	Benzyl isobutyrate
0845	Benzyl isovalerate
0846	Benzyl trans-2-methyl-2-butenoate
0847	Benzyl 2,3-dimethylcrotonate
0848	Benzyl acetoacetate
0849	Benzyl phenylacetate
0955	4-hydroxybenzyl alcohol
0956	4-hydroxybenzaldehyde
0957	4-hydroxybenzoic acid
0958	2-hydroxybenzoic acid
0959	4-hydroxy-3-methoxy benzoic acid
1040	Benzothiazole
1252	Benzyl ethyl ether
1253	Benzyl butyl ether
1256	Dibenzyl ether
1268	Isoeugenyl benzyl ether
1495	2,3-dimethylbenzofuran
1533	Eugenyl benzoate
1552	N-benzoylanthranilic acid
1557	2-methyl-4,5-benzo-oxazole
1562	Benzyl iosothiocyanate
1657	α,α-dimethylbenzyl isobutyrate
1878	4-hydroxy-3,5-dimethoxy benzaldehyde
1880	Sodium 4-methoxybenzoloxy

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			acetate
0850	Benzoic acid	1883	4-methoxybenzoyloxyacetic acid
0851	Methyl benzoate	2061	Benzyl hexanoate
0852	Ethyl benzoate	2063	Prenyl benzoate
0853	Propyl benzoate	2064	Benzyl levulinate
0854	Hexyl benzoate	2065	4-methylbenzyl alcohol
0855	Isopropyl benzoate	2066	Benzyl nonanoate
0856	Isobutyl benzoate	2067	4-methylbenzaldehyde propylenglycol acetal
0857	Isoamyl benzoate	2068	2-ethylhexyl benzoate
0858	Cis-3-hexenyl benzoate	2105	2-methylbenzofuran
0859	Linalyl benzoate		

JECFA, Joint FAO/WHO Expert Committee of Food Additives

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Table 3 Maximum allowed limits (mg/kg) of benzoic acid and its salts (Na, K and Ca) as permitted food additives, according to Codex Alimentarius (FAO/WHO, 2014)

Product	Maximum level
*Beverages *[1]*	
Concentrates for fruit juice or nectar, fruit juice, fruit nectar, coffee or substitutes, tea, infusions, and cereal or grain beverages	1000
Concentrates for vegetable nectar, and water-based flavored drinks including “sport”, “energy” or “electrolyte”, particulated drinks and soft drinks with pH lower than 3.5	600
Water-based flavored drinks including “sport”, “energy” or “electrolyte”, particulated drinks and soft drinks with pH higher than 3.5	1000
Beer, wines, spirituous, refreshers, cider, perry and mead	1000
Bakery and sweets	
Bakery wares, cereal and starch-based desserts, fat-based desserts, fruit filling for pastries and fat emulsions	1000
Chewing gum, cocoa-based spreads and fillings, confectionery including candy and nougats, decorations, topping and sweet sauces, imitation chocolate and chocolate substitutes	1500
Table-top sweeteners	2000
Fruits and vegetables[2] and derivatives*	
Fruit: candied, cooked, fermented or in vinegar, oil or brine	1000

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Vegetables: cooked, fried, dried, fermented, purees, spreads	
Fruit pulp, fruit purees and fruit preparations	
Nut and seeds, coconut milk, seasoning and condiments	
Jam, jellies, marmalades, sauces, syrups and vinegars	
Dried fruit	800
Salads and sandwich spreads	1500
Vegetables in vinegar, oil or brine, and soybean sauce	2000
Vegetables pulps or preparations	3000
Eggs and derivatives	
Egg-based desserts	1000
Liquid-egg products	5000
Meat and derivatives	
Cured or salted and dried non-heat treated processed meat, poultry and game products in whole pieces or cuts (added on surface)	1000
Fish, seafood and derivatives	
Fish, mollusks, crustacean and echinoderms: smoked, dried, fermented or salted	200
Fish products: smoked, dried or salted	
Fermented fish products	1000
Fish, fish products, mollusks, crustacean and echinoderms: semi-preserved Mollusks, crustacean and echinoderms: cooked	2000
Caviar	2500

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Cooked shrimps	6000
Dairy products	
Dairy-based desserts	300
Dairy-fat spreads	1000
Other products	
Soups and broths	500
Pre-cooked pastas and noodles and like products	1000
Dietetic foods for medical purposes and dietetic formulas for slimming purposes	1500
Food supplements and dietetic foods	2000

*[1], subject to national legislation of the importing country. *[2], vegetables, including fungi, roots, pulses, legumes, aloe vera and seaweeds.

Table 4 Maximum levels (mg/kg or l) reported for benzoic acid in commercial beverages

Product	Maximum level	Reference
Apple juice	335	Lozano et al., 2007
Apricot juice	45	Ene and Diacu, 2009
Grape juice	971	Lozano et al., 2007
Lemon juice	997	Lozano et al., 2007
Orange juice	902	Lozano et al., 2007
Passion fruit juice	804	Tfouni and Toledo, 2002
Peach juice	63	Ene and Diacu, 2009
Ice tea	105	Lazarević et al., 2011
Fruit drink	170	Pant and Trenerry, 1995
Orange soft drink	338	Tfouni and Toledo, 2002
Cola drink	170	Morales et al., 2002
Cola light drink	160	El-Ziney, 2009
Energy drink	129	El-Ziney, 2009
Low alcohol wine	280	Pant and Trenerry, 1995
Cordial	440	Pant and Trenerry, 1995
Diet cordial	450	Pant and Trenerry, 1995
Thai rice wine	25	Techakriengkrai and Surakarnkul, 2007
Rice distillates	7	Techakriengkrai and Surakarnkul, 2007

Table 5 Maximum levels (mg/kg or l) reported for benzoic acid in commercial fish, seafood and meat products

Product	Maximum level	Reference
Canned fish	60	de Luca et al., 1995
Dried fish	2	Nagayama et al., 1986
Pickled herring	987	Leth et al., 2010
Danish caviar	1032	Leth et al., 2010
Prepared fish products	720	Mischek and Krapfenbauer-Cermak, 2012
Fish sauce	266	Lin and Choong, 1999
Tunny sauce	17	de Luca et al., 1995
Fish pâté	26	de Luca et al., 1995
Seafood salad	3	de Luca et al., 1995
Surimi	890	Leth et al., 2010
Shrimps, crustacean and mollusks	1040	Mischek and Krapfenbauer-Cermak, 2012
Boiled shrimps in brine	932	Leth et al., 2010
Cured duck meat	345	Lo et al., 2001
Cured pork meat	52	Lo et al., 2001
Cured pork liver	933	Lo et al., 2001
Paté	260	Leth et al., 2010

Table 6 Maximum levels (mg/kg or l) reported for benzoic acid in commercial dairy products

Product	Maximum level	Reference
Cow milk UHT	28	Javanmardi et al., 2015
Milk powder	110	Qi et al., 2009
Infant formula	88	Qi et al., 2009
Sour milk	23	Sieber et al., 1995
Sour cream	18	Sieber et al., 1995
Kefir	23	Sieber et al., 1995
Labneh	365	Tawalbeh et al., 2014
White yogurt	56	Sieber et al., 1995
Flavored yogurt	22	Horák et al., 1996
Fruit yogurt	72	de Luca et al., 1995
Buttermilk	19	Sieber et al., 1995
Whey	13	Sieber et al., 1995
Arzúa-Ulloa cheese	240	Calzada et al., 2015
Blue cheese	5	Kurisaki et al., 1973
Brie cheese	2	Kurisaki et al., 1973
Camembert cheese	4	Kurisaki et al., 1973
Cheddar cheese	20	Kurisaki et al., 1973
Cottage cheese	18	Sieber et al., 1995
Cream cheese	12	Kurisaki et al., 1973

Edam cheese	10	Kurisaki et al., 1973
Emmenthal cheese	3	Kurisaki et al., 1973
Esrom cheese	41	Kurisaki et al., 1973
Gouda cheese	15	Kurisaki et al., 1973
Gruyere cheese	18	Kurisaki et al., 1973
Havarti cheese	6	Kurisaki et al., 1973
Mozzarella cheese	28	Listiyani et al., 2011
Parmesan cheese	22	Kurisaki et al., 1973
Provolone cheese	41	Sieber et al., 1995
Quark cheese	48	Sieber et al., 1995
Samsoe cheese	4	Kurisaki et al., 1973
Whey cheese	219	Sieber et al., 1989

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Table 7 Maximum levels (mg/kg or l) reported for benzoic acid in commercial fruit and vegetable products

Product	Maximum level	Reference
Strawberry	29	Heimhuber and Herrmann, 1990
Dried apricot	30	Nagayama et al., 1983
Dried plum	13	Nagayama et al., 1983
Cranberry jam	181	Nagayama et al., 1983
Quince jam	1501	Ferreira et al., 2000
Light fruit jam	400	Pant and Trenery, 1995; Lazarević et al., 2011
Stewed fruit	377	Leth et al., 2010
Table olives	500	Lazarević et al., 2011
Packaged fermented olives	516	Casado et al., 2011
Packaged fermented cucumbers	1844	Casado et al., 2011
Packaged fermented caperberries	1763	Casado et al., 2011
Enokitake mushroom	5	Nagayama et al., 1986
Pickled vegetables	755	El-Ziney, 2009
Fermented soybean	18	Lee et al., 2013
Soy extract	18	Nagayama et al., 1986
Soybean sauce	450	Ohtsuki et al., 2012
Ketchup	1009	Dzięcioł et al., 2010
Mustard	196	Mischek and Krapfenbauer-Cermak, 2012

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Cacao nut	5	Nagayama et al., 1986
Peanut	4	Heimhuber and Herrmann, 1990
Nutmeg	217	Heimhuber and Herrmann, 1990
Cinnamon	461	Heimhuber and Herrmann, 1990
Red hot pepper traditional products	1716	Kongo et al., 2013

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Table 8 Maximum levels (mg/kg or l) reported for benzoic acid in other commercial food products

Product	Maximum level	Reference
Bread	229	Javanmardi et al., 2015
Cake caramel	9	El-Ziney, 2009
Cream caramel	2	El-Ziney, 2009
Honey	100	FAO/WHO, 2000
Syrup	87	Lazarević et al., 2011
Mayonnaise	586	Mischek and Krapfenbauer-Cermak, 2012
Margarine	721	Tfouni and Toledo, 2002
Vinegar	519	Lin and Choong, 1999
Filled pasta	47	de Luca et al., 1995
Noodles rice spaghettini	4607	Burana-osot et al., 2014
Noodles rice fettuccine	10499	Burana-osot et al., 2014
Potato “gnocchi”	26	de Luca et al., 1995

Table 9 Spectrophotometric and gas-chromatographic methods used for quantification of benzoic acid and related compounds or additives in foods, published in years 2001-2015

Compound	Food	Pre-treatment	Detection	Reference
Spectrophotometry				
Benzoic acid (Sorbic acid)	Fermented milk	Steam distillation	295 nm (258 nm)	Urbienė and Leskauskaitė, 2006
Benzoic acid and sorbic acid	Juices	Dilution in HCl	210-300 nm and PLS	Lozano et al., 2007
Benzoic acid	Beverages, jam, ketchup, pickled vegetables	NaCl saturation, acidification and ether extraction	267.5, 272, 276.5 nm	Lazarević et al., 2011
Gas- chromatography (GC)				
Benzoic, carboxylic and free fatty acids	Dairy products	Homogenization with solvents and SPE (de Jong and Badings, 1990)	FID	Huerta-Gonzalez and Wilbey, 2001
Benzoic, sorbic and dehydroacetic acids, and	Meat products	Steam distillation	FID	Lo et al., 2001

parabens				
Benzoic acid and sorbic acid	Food dressing and sauces	Headspace-SPME	FID	Dong et al., 2006
Benzoic acid and sorbic acid	Milk products	DLLME in acetone or octanal	FID	Abedi et al., 2014
Benzoic, carboxylic and free fatty acids	Raw cow milk cheese	Homogenization and SPE (de Jong and Badings, 1990)	FID	Calzada et al., 2015

DLLME, dispersive liquid-liquid microextraction; FID, flame ionization detector; PLS, partial least square regression; SPE, solid-phase extraction; SPME, solid-phase microextraction.

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Table 10 Mass spectrometry, electrophoretic, enzymatic biosensors and magnetic resonance methods used for quantification of benzoic acid and related compounds or additives in foods, published in years 2001-2015

Compound	Food	Pre-treatment	Detection	Reference
Gas chromatography coupled to mass spectrometry (GC-MS)				
Benzoic acid and sorbic acid	Dairy and bakery products, sweets and beverages	Extraction (acid, ether, NaOH, NaCl and dichloro-metane), evaporation and derivatization	EIM	El-Ziney, 2009
Benzoic acid and sorbic acid	Drinks and water	Sonication and HS-LPME in toluene	EIM/SIM	Farahani et al., 2009
Benzoic acid	Fruit juices	Chloroform extraction and evaporation	EIM/SIM	Sen et al., 2011
Capillary electrophoresis (CE)				
Benzoic, sorbic and dehydroacetic acids, and parabens	Drinks, sauces and wine	Dilution and solid-phase extraction with ethanol	(MEEKC) UV/DAD at 200 nm	Huang et al., 2005
Benzoic acid and sorbic acid	Beverages, jams and soy sauce	Homogenization in sodium tetraborate and ethanol, filtration and EF-SPE	(CZE) UV at 214 nm	Han et al., 2008

Benzoic acid and benzoates (Sorbic and sorbates)	Beverages	Dilution in water	UV at 200 nm (254 nm)	Costa et al., 2008
Parabens, sorbates and lactates	Drinks and sauces	Solid-phase extraction	Amperometry	Wang *et* al., 2010
Benzoic acid and sorbic acid	Juices, drinks, wine and sauces	Dilution in phosphate and dynamic pH junction-sweeping	UV at 230 nm	Hsu et al., 2014
Biosensors based on tyrosinase inhibition				
Benzoic acid	Mayonnaise and beverages	Extraction in ethylacetate or filtration and sonication	Amperometric detector	Morales et al., 2002
Benzoic acid	Non-alcoholic beverages of guaraná	Homogenization with phosphate and L-tyrosine	Oxygen (consumption) electrode	dos Santos et al., 2013
Quantitative proton nuclear magnetic resonance (qHNMR)				
Benzoic acid	Sauces, margarine, caviar and beverages	Homogenization in NaCl, sulfuric acid and ether, centrifugation, extraction in ether and evaporation	Proton signals detector	Ohtsuki et al., 2012

CZE, capillary zone electrophoresis; DAD, diode array detector; EF-SPE, electrokinetic flow-ion pair extraction; EIM, electron impact ionization mode; HS-LPME, headspace liquid-phase microextraction; MEEKC, microemulsion electrokinetic capillary chromatography; SIM, selective ion monitoring; UV, ultraviolet detection.

Table 11 Ionic chromatographic methods used for quantification of benzoic acid and related compounds or additives in foods, published in years 2001-2015

Compound	Food	Pre-treatment	Detection	Reference
Ion exchange chromatography (IEC)				
IEC isocratic elution				
Benzoic acid	Cheeses	Homogenization in water, centrifugation and filtration	Conductivity	Iammarino et al., 2011
IEC gradient and isocratic elution				
Benzoic acid	Cheeses	Homogenization in water, centrifugation and filtration	Conductivity	Iammarino et al., 2011
IEC gradient-isocratic-gradient elution				
Benzoic, lactic, acetic and sorbic acids, nitrate and phosphate	Cheeses	Homogenization in NaOH, sonication, heating, centrifugation and filtration	Conductivity	Iammarino and Di Taranto, 2013
Reverse phase-liquid chromatography-tandem mass spectrometry and electrospray ionization (RP-LC/ESI-MS/MS)				
Benzoic, citric and sorbic acids, natamycin,	Cheeses	Sonication in NaCl, acetate and methanol,	PDA 200-400 nm and	Fuselli et al., 2012

lysozyme and hexamethylenetetramine	centrifugation and filtration	SRM

PDA, photodiode array; SRM, single reaction monitoring.

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Table 12 Reversed phase high performance liquid chromatographic (RP-HPLC) methods used for quantification of benzoic acid and related compounds or additives in foods, published in years 2001-2015

Compound	Food	Pre-treatment	Detection	Reference
Benzoic acid (Sorbic acid)	Drinks, juices, margarine, yogurt and cheeses	Homogenization in water or extraction with solvents (NaOH, ether or acetonitrile) and filtration	UV-DAD at 228 nm (260 nm)	Tfouni and Toledo, 2002
Benzoic acid and sorbic acid	Olives, jams, jellies, sauces, juices and wine	Extraction with methanol, centrifugation and filtration	UV at 235 nm	Mota et al., 2003
Benzoic and sorbic acids, and parabens	Drinks, fruits, jams, vegetables, jellies and	Extraction with methanol, sonication and filtration	UV at 254 nm	Saad et al., 2005

	sauces			
Benzoic acid	Drinks, juices, soy milk, tea and coffee	Acidification and extraction with alcohol	UV at 235 nm	CFS-HK, 2007
Benzoic acid and sorbic acid	Juices	Filtration and dilution in acetonitrile and acetate	UV at 234 nm	Lozano et al, 2007
Benzoic acid and sorbic acid	Rice wine and distillates	SPE in methanol	UV at 235 nm	Techakriengkrai and Surakarnkul, 2007
Benzoic acid	Tomato sauces and ketchup	Clarification with Carrez reagents and filtration	UV-DAD at 217 nm	Violeta et al., 2007
Benzoic acid	Tomato and chilli sauces	Extraction with methanol and centrifugation	UV-DAD at 230 nm	Dixit et al., 2008
Benzoic acid (Sorbic acid)	Beverages, cheese,	Dilution, acidification and	UV-DAD at 230 nm (264 nm)	Cressey and Jones, 2009

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	meat products, fruits and pasta	steam distillation		
Benzoic acid	Juices	Filtration, sonication and direct injection	UV-DAD at 228 nm	Ene and Diacu, 2009
Benzoic acid (Sorbic acid)	Dairy products	Homogenization in water, extraction with methanol and filtration	UV-DAD at 235 nm (254 nm)	Koyuncu and Uylasser, 2009
Benzoic acid	Milk and infant formula	Homogenization in water, clarification with Carrez reagents and filtration	UV-DAD at 230 nm	Qi et al., 2009
Benzoic, caffeine and aspartame (acesulfame-	Cola soft drinks	Degasification, filtration and dilution in water	UV-DAD at 217 nm (227 and 265 nm)	Trandafir et al., 2009

K and saccharin)				
Benzoic acid	Dairy and fish products, wine, drinks, juices, fruits, soups, vegetables, sauces and jams	Sonication in methanol and water, and SPE	UV-DAD at 235 and 255 nm	Vandevijvere et al., 2009
Benzoic acid and sorbic acid	Ketchup and beverages	Homogenization with NaCl, chloroform and methanol, and centrifugation	UV at 254 nm	Dzięcioł et al., 2010
Benzoic acid and sorbic acid	Dairy, bakery, meat, seafood	Extraction with oxalic acid, ethanol, propanol and	UV at 240 nm	Leth et al., 2010

	and fish products, sauces, jellies, sweets, salads and drinks	acetonitrile, and centrifugation		
Benzoic acid	Fermented milk	Sonication with NaOH, heating, clarification with Carrez reagents, dilution in methanol and filtration	UV at 227 nm	Garmiene et al., 2010
Benzoic and sorbic acids, saccharin and caffeine	Soft drinks and nectars	Centrifugation, filtration and sonication	UV at 220 nm	Lino and Pena, 2010
Benzoic acid	Ketchup, pickles, jams, chillies, ginger	Homogenization in MilliQ water, heating, centrifugation, filtration and sonication	UV at 230 nm	Rai et al., 2010
Benzoic acid	Cheeses	Sonication with NaOH, heating, clarification with Carrez reagents, dilution in	UV at 227 nm	Garmiene et al., 2011

		methanol and filtration		
Benzoic and sorbic acids, natamycin and lysozyme	Cheeses	Sonication in NaCl, acetate and methanol, centrifugation and filtration	UV-DAD at 227, 280 and 303 nm	Guarino et al., 2011
Benzoic acid	Cheeses	Homogenization in water, centrifugation and filtration	UV at 275 nm	Iammarino et al., 2011
Benzoic acid	Cheeses	Homogenization in water, clarification with Carrez reagents and filtration	UV-DAD at 230 nm	Listiyani et al., 2011
Benzoic acid (Sorbic acid)	Beverages, sauces, jams, jellies, bakery, fish, fruits, vegetables and food supplements	Sonication in sodium acetate, methanol and water, and clarification with Carrez reagents	UV-DAD at 230 nm (254 nm)	Mischek and Krapfenbauer-Cermak, 2012
Benzoic acid	Jams and sauces	Extraction with methanol	UV at 235 nm	Ohtsuki et al., 2012
Benzoic acid and sorbic acid	Sauces, margarine,	Steam distillation and filtration	UV-DAD at 230 nm	Kathriarahchi et al., 2012

	caviar and drinks			
Benzoic acid and sorbic acid	Sauces, juices, yogurt, soups, chips and chocolate	Extraction with methanol and filtration	UV-DAD at 200-400 nm	Cakir and Cagri-Mehmetoglu, 2013
Benzoic acid	Drinks and nectars	Centrifugation, filtration and sonication	UV at 235 nm	Diogo et al., 2013
Sodium benzoate (potassium sorbate)	Kashar cheese	Homogenization in acetate:acetonitrile and filtration	UV-DAD at 225 nm (255 nm)	Gul and Dervisoglu, 2013
Benzoic acid and sodium benzoate	Red-hot-pepper traditional products	Clarification with NaCl, Carrez reagents and methanol, and filtration	UV-DAD at 270 nm	Kongo et al., 2013
Benzoic acid and sorbic acid	Sauces, jams, jellies, beverages, cereals and bakery products	Extraction in methanol and filtration	UV at 235 nm	Ulca et al., 2013

Benzoic acid and hippuric acid	Milk, fermented milk, cheese and yogurts	Clarification with Carrez reagents and methanol, and filtration	UV-DAD at 280 nm	Horníčková et al., 2014
Benzoic acid and sorbic acid	Noodles	Pulverization, extraction with methanol :water and filtration	UV-DAD at 234 nm	Burana-osot et al., 2014
Benzoic acid and sorbic acid	Milk, soft drinks, sauces and bread	Dispersive liquid-liquid microextraction (DLLME)	UV at 254 nm	Javanmardi et al., 2015
Benzoic acid and sorbic acid	Dairy products	Extraction with water and methanol, and filtration	UV-DAD at 230 nm	Tawalbeh et al., 2014

DAD, diode array detector; SPE, solid-phase extraction; UV, ultraviolet detection.

DAD, diode array detector; UV, ultraviolet detection.

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