BENZOE TONKINENSIS

Chemical and Technical Assessment (CTA)

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1. Summary

This Chemical and Technical Assessment summarizes data and information on Benzoe tonkinensis submitted to JECFA by Agroforex Company[1] in a dossier dated September 2010[2] and an Activity Report of May, 2011[3]. The intended use of Benzoe Tonkinensis is as a food flavour.

Benzoe tonkinensis is a balsamic resin from *Styrax tonkinensis* (Pierre) Craib ex Hartwitch tree, which belongs to the Styracaceae family. It is collected directly from the tree, cleaned and sorted into four grades according to size. The production is entirely manual, from tapping to packaging. It is variously referred to as Siam benzoin gum, Siam benzoin and Benzoin Laos or in a generic way as "benzoin gum". Laos is considered as the major producer of Benzoe tonkinensis, if not the only one at present.
The product can be described as white-yellow to reddish splits of flattened almond-like grains with a strong vanilla smell. The resin is composed mainly by benzoic acid (15-45 %) and coniferyl benzoate (15-60%), with lesser amounts of vanillin (<5%), benzyl benzoate (<2%), 2-hydroxy-1-phenylethanone and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone. The chemical composition is similar to the one described by Fernandez et al. (2003) and Castel et al. (2006).

Besides Benzoe tonkinensis, another type of benzoin is produced, Benzoe sumatranus, obtained from two other *Styrax* species, *Styrax benzoin* Dryander and *Styrax paralleloneurum* Perkins. These two resins differ in their botanical source, geographical origin and chemical composition. The term "benzoin gum" can include one or the other of the two sources or their mixtures.

In Siam benzoin the main constituents are benzoic acid and its esters (such as coniferyl benzoate and benzyl benzoate), while in Sumatra benzoin the major constituents are cinnamic acid and its esters (such as coniferyl cinnamate and cinnamyl cinnamate). Vanillin is present in both types of benzoin resins and gives rise to its familiar vanilla odour (most readily detected in the Siam type) (Kashio and Johnson, 2001).

This chemical and technical assessment only applies to Benzoe tonkinensis.

2. Description

Benzoe tonkinensis is commercialized after it has been cleaned and sorted into four grades, according to its size: grade 1 (BLG[4]1) - almonds, large tears; grade 2 (BLG2) - medium tears; grade 3 (BLG3) - small tears and grade 5 (BLG5) - very small tears, almost powder-like.

The production is distributed in the following way: 10% Grade 1, 10% Grade 2, 60% Grade 3 and 20% Grade 5. The various grades do not present any significant variation of the chemical composition, but are the result of the cleaning process. They can have only an impact on the colour of the product during the storage at ambient temperature (from beige to brown colour).

The product presents a strong vanilla smell, is insoluble in water and soluble in ethanol.

[1] Agroforex Company, Pierre Morin Road, 43-45, P.O.Box 6682, Vientiane, Laos.
[2] Agroforex, Report, September 2010.
[3] Agroforex, Activity Report, May 2011.
[4] BLG: Benjoin Laos Grade.

Ready processed for export, Benzoe tonkinensis appears as pieces which are opaque, grainy, ovoid, flattened (almonds), from few to 30 mm, white-yellow.

After storage at ambient temperature, Benzoe tonkinensis can slightly melt and appears as agglomerated brown-red mass. Its colour turns reddish outside due to air exposure, but stays white-yellow inside.

3. Manufacturing process

Styrax tonkinensis (Pierre) essentially comes from northern regions of Laos, on mountainous massifs. It grows naturally on paddy fields in the slash and burn system, but the current exploited trees have been planted and their quality was improved. Each tree correctly tapped will product during two-thirds of its life, which lasts around twenty years. The production is estimated to be 160 to 240 kg/ha, assuming 400 trees/ha, which is about 0.4 to 0.6 kg per tree per harvest, at the rate of one harvest per year (FAO 2001).

The harvest unfolds in two distinct stages. The first one, from June to August, is called the tapping. A multi incision is made on the trunk of the tree, limited to the bark. The resin will accumulate near the scarification and it will progressively harden.

The second stage is the harvest, taking place from December to March, after the end of the monsoon, when the resin is dry. The resin has accumulated behind the bark where the incision was made 5 to 6 months before. The piece of bark containing dry Benzoe tonkinensis is cut and collected. This is the crude product, which undergoes further manual cleaning and sorting processes.

4. Chemical characterization

4.1 Composition

Benzoe tonkinensis is constituted of several compounds. The sponsor presented data using two different extraction solvents for the identification and quantification of the compounds in Benzoe tonkinensis: Method 1 and Method 2, whose procedures are summarized in Figure 1.

Using Method 1, compounds extracted in methanol:methylene chloride 50:50 v/v (denominated by the sponsor as “volatile fraction”) were identified by gas chromatography coupled to mass spectrometry (GC-MS) and quantified by gas chromatography with a flame ionization detector (GC-FID), using area normalization. The amount of the extract (ranging from 21-53%, n = 18 samples) was determined, using GC-FID by internal standardization with tridecane. The non-volatiles in the methanol:methylene chloride extract were not characterized.

With Method 1, data about the composition of volatile compounds were obtained in two studies carried out by the sponsor in 2000 (60 samples, 2 basins, 6 harvests: 1992/93, 1993/94, 1994/95, 1995/96, 1996/97, 1997/98) and 2007 (28 samples, 2 basins, 3 harvests: 2003/04, 2004/05, 2005/06).

The compounds present in the extract obtained with Method 1 of eighteen samples of grade 1, 3 and 5, harvested in 2003, 2004 and 2005 are presented in Table 1. The five major compounds identified were: benzoic acid, vanillin, benzyl benzoate, 2-hydroxy-1-phenylethanone and 1-(4-hydroxy-3 methoxyphenyl)-2 propanone. This chemical composition is similar to the one described by Fernandez et al. (2003) and Castel et al. (2006).

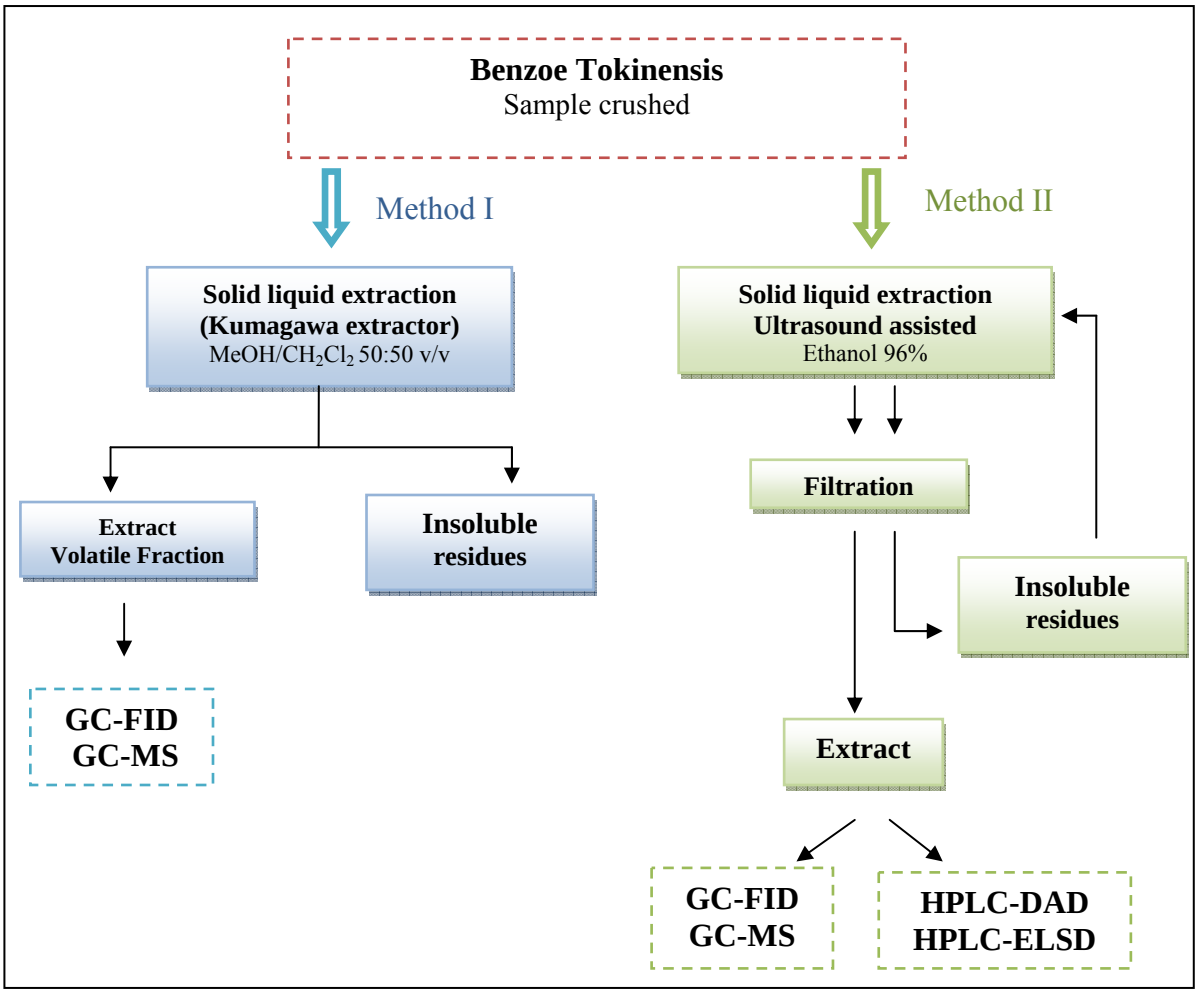

Figure 1. Scheme of Method 1 and Method 2 used for the identification and quantification of volatile and non-volatile compounds in Benzoe tonkinensis

Table 1. Main compounds identified in the methanol:methylene chloride extract of Benzoe tonkinensis (Method 1)

Compound	Mean concentration in the extract (%)	Minimum and maximum concentration in the extract (%)
Benzoic acid	80.34	68.60 - 87.90
Vanillin	5.15	2.05 – 13.96
Benzyl benzoate	4.18	2.84 – 6.82
2-Hydroxy-1-phenylethanone	2.15	1.09 – 6.72
1- (4-Hydroxy-3-methoxyphenyl)-2-propanone	1.48	0.48 – 3.84
Eugenol	0.9	
Isoeugenol	0.24	
Acetovanillone	0.17	
Benzylic alcohol	0.14	
Methyl benzoate	0.08	
Allyl benzoate	0.05	

The mean concentration and the range (minimum and maximum concentration) of benzoic acid, vanillin and benzyl benzoate in Benzoe tonkinensis (18 samples) are summarized in Table 2.

Table 2. Main volatile compounds determined in Benzoe tonkinensis

	Benzoe tonkinensis	
Compound	**Average[a] (%, w/w)**	**Range (%, w/w)**
Benzoic acid	27.3	17.5 - 46.3
Vanillin	1.8	0.7 - 3.5
Benzyl benzoate	1.5	0.8 - 2.4

a: average value of 18 samples analyzed in triplicate.

With Method 2, which comprises a simple ethanolic extraction assisted by ultrasound with further gas chromatographic analysis (GC-FID and GC-MS), it was also possible to quantify the three main volatile compounds in Benzoe tonkinensis: benzoic acid, vanillin and benzyl benzoate. The main non-volatile compound in the ethanolic extract was identified as coniferyl benzoate, which was quantified by high performance liquid chromatography coupled to a photodiode array detector or an evaporative light scattering detector. The extraction efficiency was higher than 98%.

The concentration of the main compounds identified and quantified in the ethanolic extract (Method 2) of five samples of Benzoe tonkinensis are presented in Table 3. The main compounds identified and quantified are benzoic acid and coniferyl benzoate, whose concentrations varied between 22 to 33% and 15 to 57% w/w, respectively. Considering the total amount of the four main compounds determined in the five samples analyzed by the sponsor (Table 3), it is worth noting that a significant quantity of unknown substances is present in the ethanolic extract. Based on this information, the JECFA requested additional information about the unknown substances present in the ethanolic extract of Benzoe tonkinensis.

Table 3. Main compounds identified and quantified in Benzoe tonkinensis (Method 2)

Sample identification	Benzoic acid (%)	Vanillin (%)	Benzyl Benzoate (%)	Coniferyl Benzoate (%)	Total (%)
B2	22 ± 3	3.0 ± 0.4	1.00 ± 0.08	15.0 ± 0.5	41 ± 5
B3	32 ± 1	<1	1.00 ± 0.02	35 ± 1	68 ± 2
B4	31 ± 2	<1	1.00 ± 0.07	46 ± 7	78 ± 10
B5	36 ± 1	<1	<1	57 ± 4	93 ± 5
B6	31 ± 1	<1	<1	27 ± 2	58 ± 3

Castel et al. (2006) identified 42 volatile compounds in a sample of Siam benzoin gum harvested from *Styrax tonkinensis* trees in northern Laos at the end of 2002. The determination was carried out on samples of grade 3 and 5 using three different headspace extraction techniques: static headspace, headspace-solid phase microextraction and headspace sorptive extraction. The quantitation was performed by gas chromatography with mass spectrometric detection. The main compounds identified were: benzaldehyde, methyl benzoate, benzoic acid, vanillin, benzyl benzoate, ethyl benzoate, isobutyl benzoate, methyl benzoate, benzyl formate, limonene, p-cymene, benzyl acohol, β-pinene, toluene, acetic acid, formic acid, hexanal, benzyl formate, propyl benzoate, eugenol, guaiacol and toluene.

4.2 Impurities

According to the sponsor, non removable residues, constituted of woody fragments and minerals have been quantified regarding the protocol of the European Pharmacopoeia (Eur.Ph.).

Benzoe tonkinensis grade 1 does present very few vegetal fragments or mineral and impurities in grade 3 does not exceed 2.5%. For both grades the rate is thus lower than 5%, the limit value defined by the European Pharmacopoeia.

The sponsor also provided information on other potential contaminants including heavy metals (cadmium, chromium, mercury and lead), barium, selenium, arsenic and antimony. The analyses were carried out using microwave assisted acid digestion (Ph.Eur. 2.4.27) followed by quantitation using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). Results of the analysis of two samples carried out in 2011 are presented in Table 4.

Table 4. Inorganic contaminants determined in two samples of Benzoe tonkinensis

Element	Average Concentration (mg kg^{-1})	
	Sample HP16/PS12	Sample HP18/PS14
Cadmium	<0.01	<0.01
Chromium	0.17	0.41
Mercury	<0.01	<0.01
Lead	0.05	0.03
Barium	4.75	10.39
Selenium	<0.03	<0.03
Antimony	<0.01	0.01
Arsenic	<0.03	<0.03

Based on this information, the Committee established the specification limits for lead and required data about the presence of lead, arsenic, antimony, chromium, mercury and cadmium on at least five different batches of commercially available products.

JECFA also requested information on microbiological contaminants.

4.3 Analytical methods

Analytical methods used to support the specifications for Benzoe tonkinensis are based on general tests in the *FAO Combined Compendium of Specifications* (FAO JECFA Monographs 1, vol. 4, 2006) and the European Pharmacopoeia for identity and purity. The specifications monograph cites specific tests for limits on lead (not more than 2 mg/kg), total ash (not more than 2.0%) and loss-on-drying (not more than 5.0%).

Benzoic acid is determined by gas chromatography using a flame ionization detector. The quantitation is performed after its extraction from Benzoe tonkinensis with boiling ethanol and through external standard calibration. Coniferyl benzoate is also determined in the ethanolic extract, through high performance liquid chromatography coupled to a photodiode array detector.

Benzoe tonkinensis in food can be determined indirectly by its three main compounds: benzoic acid, vanillin and benzyl benzoate. Analytical methods for the determination of these compounds in food and beverages are well established and comprise mainly high performance liquid chromatography and capillary electrophoresis (Mota *et al*. 2003; Ying *et al*. 2007; Saad *et al*. 2005; Berzas *et al.* 2009; Jagerdeo *et al*. 2000 and Ali *et al.* 2008).

5. Functional uses

5.1 Technological function

Benzoe tonkinensis is intended for use as a food flavour. According to the Codex General Standard for Food Additives (GSFA) norm, dated 1989 and updated in 2009 during the 32nd session of the Codex Alimentarius, and due to its film forming and sequestrant capacities, Benzoe tonkinensis used as a flavour additive can be considered as: flavour enhancer, flavour stabiliser, flavour support and flavour fixer. Those functionalities are likely to improve the flavour stability and thus the organoleptic properties of the preparation.

5.2 Food categories and use levels

According to the literature (Burdock, 1995, 1997; Leung and Foster,1996 and Niederauer , 1994) benzoin gums have been incorporated in various food preparations, including baked goods, frozen dairy products, soft candies, gelatines, puddings, non-alcoholic beverages, alcoholic beverages, chewing gums, confections and frostings.

The food applications for which Benzoe tonkinensis is intended to be used by the sponsor, with their respective claimed doses, are listed in Table 5.

Table 5. Proposed uses and use levels of Benzoe tonkinensis in food preparations and beverages

Food preparation	Claimed doses ($mg\ kg^{-1}$ or $mg\ L^{-1}$)
Baked goods	10
Exotic meals	10
Oriental products	10
Oriental preparations	10
Chewing gums	10
Chocolate	10
Soft candies	6
Dairy products flavored jellified or not	6
Puddings	5
Sweets	4
Deserts	4
Viennese pastries	4
Frozen dairy products	3
Still flavour drinks	5
Non-alcoholic beverages	5
Alcoholic beverages	5

6. Regulatory status

Before the entering into force of the European Parliament and Council Directive 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners, benzoin gum (without any origin specification) was authorized in Europe referenced under EC number 906 for its coating and glazing functions, also presenting flavour support and flavour enhancer properties. It used to be an ingredient of soft candies, ice creams, deserts, pastries, base-gum for chewing-gums production, syrup basis, etc. During the preparation work of this directive, in 1990, the Scientific Committee on Food (SCF, 1990) did not evaluate this substance in absence of necessary information about its safety. Despite very old uses of Benzoe tonkinensis from Laos (whose brand name was Benzoin Siam), prior use was not considered sufficient justification. After the directive entered into force in 1997, a use of "Benzoin" (without specification) as flavouring was still possible in Europe in the framework of the "Blue book" (Flavouring substances and natural sources of flavourings, Council of Europe,1992, Volume 1, p.175).

JECFA, at its twenty-first meeting, prepared a tentative specifications covering the two forms of benzoin gum. However, in 1996, the Committee at its fifty-fifth meeting decided to withdraw the tentative specifications for benzoin gum as a food additive, due to the fact that the relevant toxicological information requested had not been provided. Thus Benzoe tonkinensis, despite a historical use in food for many years, could not enter in the step process for inclusion in the Codex General Standard for Food Additives, due to a lack of scientific data on its composition and toxicity to allow a full safety assessment.

In the United States of America, benzoin gum was notified for Generally Recognized as Safe (GRAS) status by the Flavor and Extract Manufacturers Association (FEMA) and was considered as a natural product. It is still used in many food categories: pastry (glazing agent), sweets

(flavour), exotic meals (flavour and savour, texture), drinks with or without alcohol (flavour, flavour support), dairy products jellified or not. The incorporation rate can vary and ranges, according to technological demands, between 0.015% (delicacies) and 4% for chocolate bars.

Canada has authorized the use of benzoin gum as glazing and polishing agent. In Japan, where its use is authorized in food, benzoin gum is used a chewing gum base. In France, benzoin gum was previously used in chewing-gum until 1991 as flavouring agent, at *quantum satis* dose.

7. References

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