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BENZOE TONKINENSIS

Chemical and Technical Assessment (CTA)

Prepared by Susanne Rath, PhD, and reviewed by Harriet Wallin, MSc

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], an Activity Report of May, 2011[3], Complementary Data, December, 2012[4] and a Report of January 2014[5]. The intended use of Benzoe Tonkinensis is as a food flavouring agent.

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". Lao PDR 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.

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 from *Styrax tonkinensis* Pierre Craib ex Hartwitch tree.

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[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] Agroforex, Complementary Data, December 2012
[5] Agroforex, Report, January 2014.

2. Description

Benzoe tonkinensis is commercialized after it has been cleaned and sorted into four grades, according to its size: grade 1 (BLG[6]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.

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 Lao PDR, 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 sample preparation consisted of an ethanolic extraction of the compounds and identification and quantitation of the volatile compounds by gas chromatography coupled to a flame ionization detector and mass spectrometry (GC-FID-MS) and the non-volatile compounds by high performance liquid chromatography coupled to a diode array detector and an evaporative light scattering detector (HPLC-DAD-ELSD). Semi-preparative liquid chromatography was additionally employed for purification and characterization of some non-volatile compounds. The experimental procedure employed is summarized in Figure 1. For structure characterization mass spectrometry and nuclear magnetic ressonance were employed.

The resin is composed mainly by benzoic acid (15-45 %) and coniferyl benzoate (15-60%), with lesser amounts of vanillin (<5%) and benzyl benzoate (<2%). Other compounds namely p-coumaryl benzoate, siaresinolic acid, 3-oxo-siaresinolic acid, sumaresinolic acid, 3-oxo-sumaresinolic acid and morinol derivatives are also identified. This chemical composition is similar to the one described by Fernandez et al. (2003) and Castel et al. (2006).

[6] BLG: Benjoin Laos Grade.

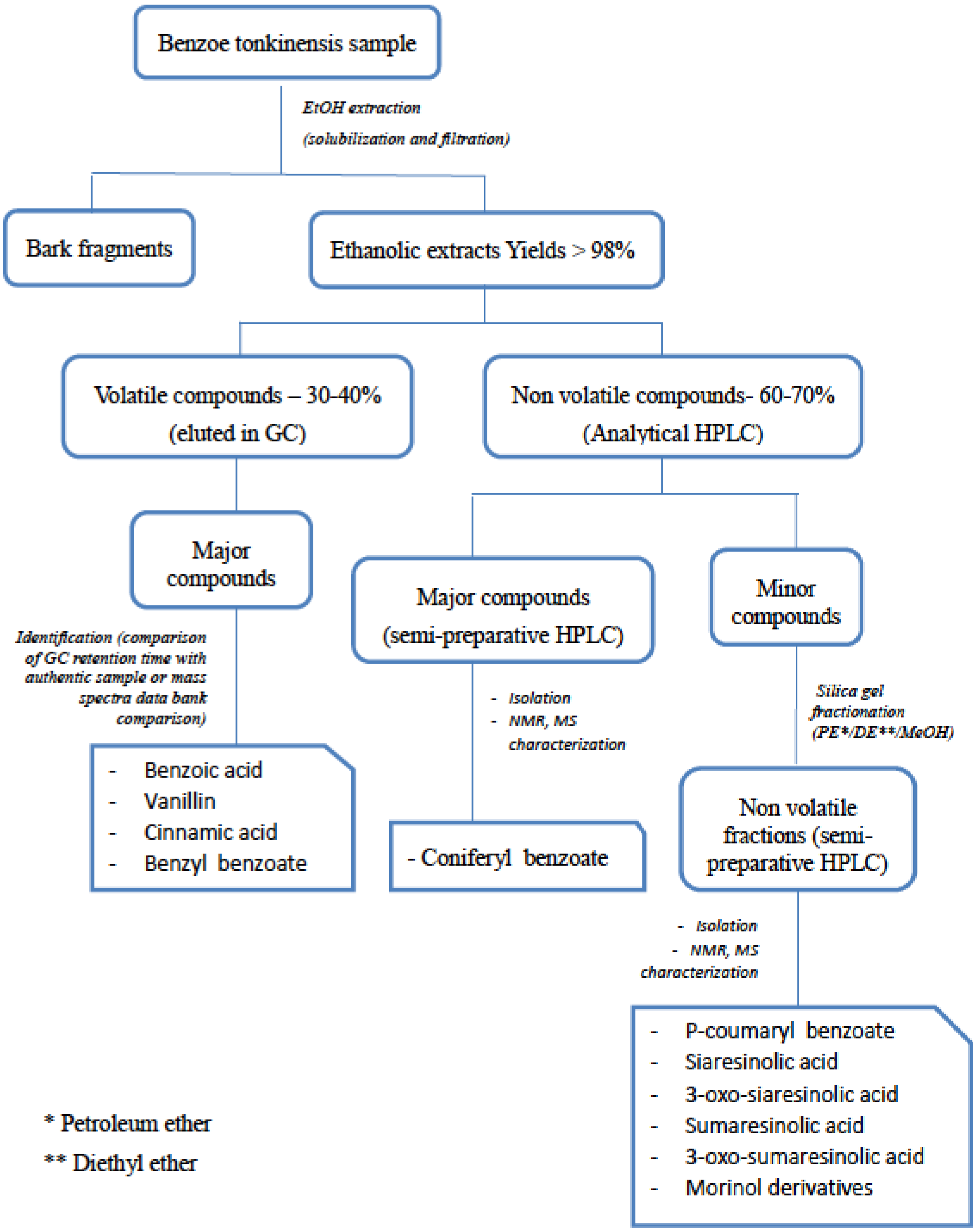
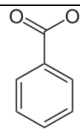
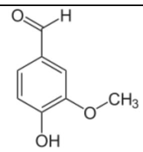
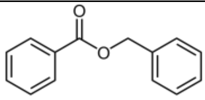
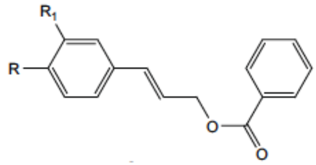


Figure 1. Scheme of method used for the identification and quantification of volatile and non-volatile compounds in Benzoe tonkinensis.

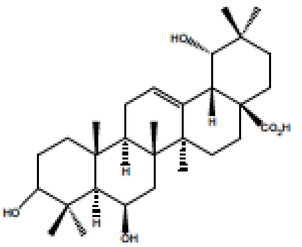
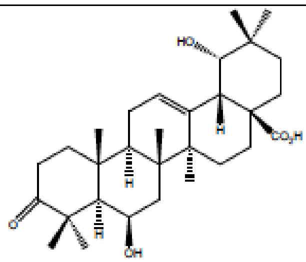
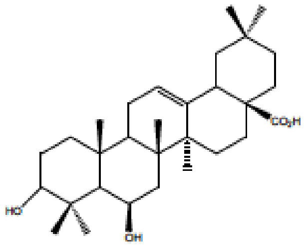
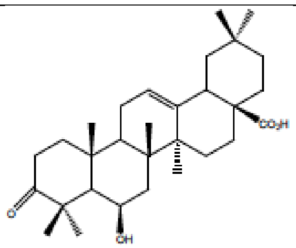
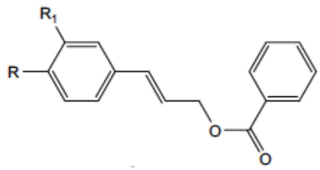
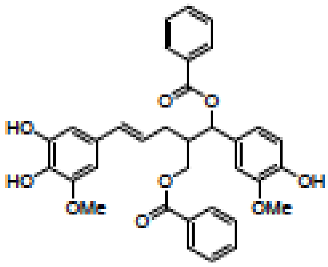
The average content of the main compounds identified and quantified in the ethanolic extract of samples of Benzoe tonkinensis are presented in Table 1. The chemical structures of other compounds identified, but not quantified, were: coumaryl benzoate, siaresinolic acid, 3-oxo-siaresinolic acid, sumaresinolic acid, 3-oxo-sumaresinolic acid and morinol derivatives (Table 2).

Table 1. Compounds identified and quantified[5] in the ethanolic extract of Benzoe tonkinensis.

Compound	Chemical formula	Average content, w/w (%)	Minimum and maximum concentration w/w (%)
Benzoic acid		28.3	22 - 36
Vanillin		1.8	<1 - 3
Benzyl benzoate		1.2	≈1
Coniferyl benzoate	 R=OH $R_1=OCH_3$	36.4	17 - 57

The main compounds identified and quantified in samples of different batches (Table 1) are benzoic acid (volatile compound) and coniferyl benzoate (non-volatile compound), whose concentrations varied between 22 to 36% (w/w) and 17 to 57% (w/w)[5].

Table 2. Compounds identied[5] in the ethanolic extract of Benzoe tonkinensis.

		
Siaresinolic acid	3-Oxo-siaresinolic acid	Sumaresinolic acid
	 R=OH R_1= H	
3-Oxo-sumaresinolic acid	p-Coumaryl benzoate	Morinol derivative

Castel et al. (2006) identified 42 volatile compounds in a sample of Siam benzoin gum harvested from *Styrax tonkinensis* trees in northern Lao PDR 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 (lead, cadmium, mercury, arsenic, chromium and antimony)[5] on five different batches of commercially available products. The analyses were carried out using acid digestion (nitric acid 70%) followed by quantitation using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). Results of the analysis are presented in Table 3.

Table 3. Inorganic contaminants determined in five samples of Benzoe tonkinensis[5]

Sample / Element	Concentration (mg kg^{-1})				
	BTL1	**BTL2**	**BTL3**	**BTL4**	**BTL5**
Lead	0.081	0.015	< 0.01	< 0.01	0.02
Cadmium	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Mercury	< 0.97	< 0.97	< 0.97	< 0.97	< 0.97
Arsenic	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Chromium	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04
Antimony	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002

Based on this information, the Committee established the specification limits for lead.

In addition, microbiological analysis were made and based on this information, the Committee set limits for *Salmonella* spp., Escherichia coli and yeast/moulds.

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%), loss-on-drying (not more than 5.0%) and microbiological criteria for *Salmonella* species, *Escherichia coli* and yeast and mouldes.

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. Due to its film forming and sequestrant capacities, Benzoe tonkinensis used as a flavour additive can be considered as: 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 proposed use and use levels for Benzoe tonkinensis, provided by the sponsor are listed in Table 4.

Table 4. 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. 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 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. Benzoe tonkinensis was not included in the Codex General Standard for Food Additives because JECFA could not complete the safety evaluation and establish full specifications.

In the United States of America, benzoin gum was notified for Generally Recognized as Safe (GRAS) status by the Flavour 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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