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QUILLAIA EXTRACTS
Type 1 and Type 2

Chemical and Technical Assessment (CTA)

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1 Summary

Quillaia extracts (synonyms: quillaja extracts, bois de Panama, Panama bark extracts, quillai extracts, Quillay bark extracts, soapbark extracts; C.A.S. no. 68990-67-0, INS no. 999) are obtained by aqueous extraction of the milled inner bark or whole wood of *Quillaja saponaria* Molina (family *Rosaceae*), which is a large evergreen with shiny, leathery leaves and a thick bark, native to China and several South American countries, particularly Bolivia, Chile and Peru.

Quillaia extracts (Types 1 and 2) contain over 100 tri-terpenoid saponins, the glycosides of the aglycone quillaic acid. Other constituents are polyphenols, tannins, oxalate salts, simple sugars and trace amounts of fat and nitrogen compounds.

Quillaia extract Type 1 may be treated with stabilizing agents, such as egg albumin or polyvinylpyrrolidone, to remove substances that would precipitate during storage, such as complexes or polymers derived from tannins and polyphenols. The liquid is concentrated after filtration through diatomaceous earth. The concentrate may be sold in liquid form or as a powder after spray-drying. Carriers such as lactose and maltodextrin may be added to the powder forms. Type 1 extracts have been noted to contain 20 to 26% quillaia saponins on the dried basis, although new information indicates that the saponin content of Type 1 extracts varies more widely, between 10 and 30 %.

Quillaia extract Type 2 is derived from Type 1 extracts that are subjected to chromatography or ultra-filtration to reduce the amount of non-saponin soluble solids, such as polyphenols and tannins. Type 2 extracts may contain 65-90 % quillaia saponins on a dried basis.

Quillaia extracts (Type 1 and 2) are used in food applications, primarily for their foaming and emulsifying properties, which are attributed to their saponin content. Extracts may contain preservatives.

2 Description

Quillaia extracts (synonyms: quillaja extracts, bois de Panama, Panama bark extracts, quillai extracts, Quillay bark extracts, soapbark extracts; C.A.S. No. 68990-67-0, INS no. 999) are obtained by aqueous extraction of the milled inner bark or whole wood of *Quillaja saponaria* Molina (family *Rosaceae*), which is a large evergreen with shiny, leathery leaves and a thick bark, native to China and several South American countries, particularly Bolivia, Chile, and Peru.

Quillaia extracts (QE) are produced as reddish-brown powders or light reddish-brown liquids. QE are very soluble in water, and insoluble in ethanol, acetone, methanol, and butanol. In general, QE are produced with three different degrees of purification:

Quillaia extract (Type 1). The production process consists of treatment with stabilizing agents (e.g. egg albumin, PVP), followed by filtration with diatomaceous earth to remove compounds that tend to precipitate during storage (e.g. protein-polyphenol complexes). They are commercialized as concentrated liquids (normally 550 g/l solids) or spray-dried powders, which may contain preservatives, such as sodium benzoate (~0.5 g/l) or ethanol. The products have a typical red-brownish colour; however, some extracts are bleached chemically to produce light colour products. Quillaia extracts (Type 1) have been noted to contain 20 to 26 % saponins (FNP 52 Add 9). New information indicates that the saponin content of Type 1 extracts varies more widely, between 10 and 30%.

Quillaia extract (Type 2). These products are purified by chromatography or ultrafiltration to remove most non-saponin solids, such as calcium oxalate, sugars, tannins, and polyphenols, that may interfere in

terms of colour, chemical interactions, taste, and odour (Ogawa and Murakami, 1987; Ogawa and Yokota, 1985). They have a light colour and are not bleached chemically. They have a higher saponin concentration than quillaia extract (Type 1). Quillaia extract (Type 2) may contain 65-90 % saponins.

Highly purified extracts. These products are used as adjuvants in the production of animal vaccines and not as food additives. They are purified using ultrafiltration membranes, followed by column adsorption to remove polyphenols. The saponin identified as QS-21 is used in human vaccines for its lower toxicity and is isolated from the extracts by reverse phase HPLC (Kensil *et al*., 1991; Cleland *et al*., 1996).

3 Method of manufacture

Source material

For over 120 years QE have been produced from the aqueous extraction of the inner bark of the tree *Quillaja saponaria* Molina (fam. *Rosaceae*). More recently, the harvest of whole trees has been taking place and it is advocated as means of preserving the natural forests (Copaja *et al*., 2003; San Martin and Briones, 2000). The planting of trees in areas of lesser agricultural value is also advocated as the trees are tolerant to diverse climatic and altitude conditions. Indications of higher saponin production by trees grown under harsher conditions have been reported. The present world demand for Quillaia bark has been estimated to be 1,000 tons/year (Copaja *et al*., 2003).

Trees from the same location have been observed to display different saponin profiles. The variation in saponin profiles among trees has been attributed to genetic factors. However, the observed variability could not be correlated with soil type, altitude, age of trees, or sample tissues (Kamstrup *et al*., 2000). Young plants, less than 15 years old, exhibit less heterogeneous saponins profiles than those obtained from mature plants (Barr *et al*, 1998).

Manufacture

In the traditional method for obtaining QE, the external part of the tree bark is first removed with knives. The saponin content of the inner bark is approximately 5% w/w. By treating the inner bark with hot water (70-80^{o}), an extract with a saponin content in the extracted solids of about 20% can be obtained (Kensil *et al*, 1996).

The ecological damage caused by the deforestation has stimulated the research on the use of the whole quillaia wood (wood with bark, small branches), as a more stable supply of saponins. Whole wood contains about 8% water-soluble compounds, with saponin content in the solids of 20 %. The quality of the products derived from whole wood is as good as the commercial products derived from bark (San Martín and Briones, 1999).

QE are generally commercialized with little additional purification. Standard liquid products are prepared using water extraction after the raw material has been adequately milled. Following extraction, the liquid may be concentrated by evaporation to attain the desired concentration of solids. In some cases it is necessary to purify the extract (e.g. by treatment with activated charcoal, filtration) to remove compounds that tend to precipitate during storage. The final products contain saponins, protein, tannins, calcium oxalate and sugars.

QE intended for food applications are often diluted with carriers, such as lactose, maltodextrin, or maltitol, for purposes of standardization. The quality of the final extracts is evaluated in terms of their clarity and colour in solution and their saponins content, as well as for their foaming properties (San Martín and Briones, 1999). Long-term storage may lead to oxidative or other changes in the product composition (Kamstrup *et al*., 2000).

4 Characterization

Quillaia saponins are characterized by quillaic acid, a tri-terpene aglycone acting as the hydrophobic moiety, with several oligosaccharides attached. The sugar units are frequently branched (Bomford *et al*., 1992). A great number of structural variations are possible, and over 60 have been partly characterized. The common structure in most Quillaia saponins consists of the tri-terpene quillaic acid with a trisaccharide at the C-3 position and an oligosaccharide linked at C-28 through a fucose residue. The

oligosaccharide may be substituted with an O-acetyl group or with a dimeric C-9 acyl group. Some of the structures reported lack a substituent at C-28. Also some of the less abundant saponins have other aglycones or acyl groups (Nyberg *et al*., 2003; Nord *et al*., 2001; Nord and Kenne, 2000; van Setten *et al*., 1998). The molecular structures of some quillaia saponins are presented in Figure 1 and Table 1.

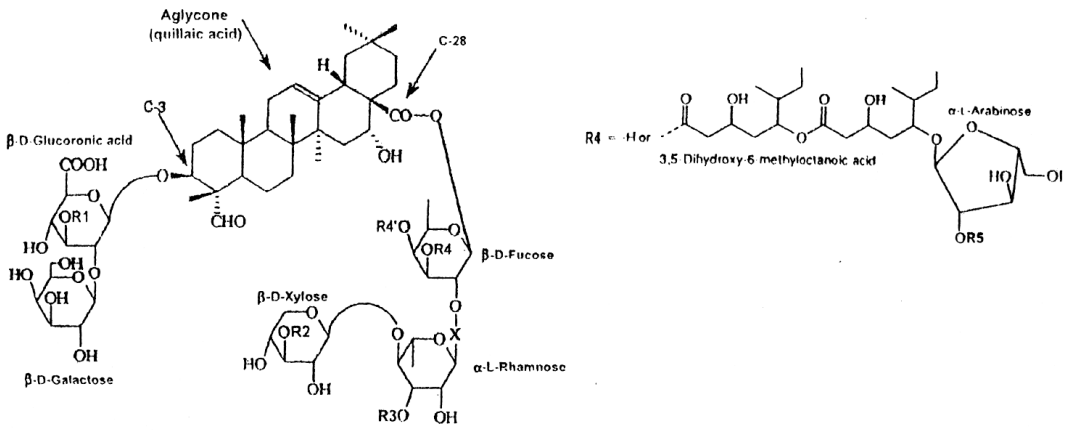

Figure 1: Molecular structure of quillaia saponins

Table 1: Molecular structures of some saponins from *Quillaja saponaria* Molina. (adapted from van Setten and van de Werken, 1996)

Saponin	R1	R2	R3	R4	R4´	R5	X
DS-1	β-D-Xyl*p*	β-D-Api*f*	-H	-H	-H	absent	absent
DS-2	β-D-Xyl*p*	β-D-Api*f*	β-D-Glc*p*	-H	-H	absent	absent
QS-7	**	**	**	**	**	**	**
QS-17	β-D-Xyl*p*	β-D-Api*f*	β-D-Glc*p*	Figure 1	-H	α-L-Ram*p*	absent
QS-18	β-D-Xyl*p*	β-D-Api*f*	β-D-Glc*p*	Figure 1	-H	-H	absent
QS-21	β-D-Xyl*p*	β-D-Api*f*	-H	Figure 1 or –H	-H	-H	absent
QS-21-V1	β-D-Xyl*p*	β-D-Api*f*	-H	Figure 1	-H	-H	absent
QS-21-V2	β-D-Xyl*p*	β-D-Api*f*	-H	Figure 1	-H	-H	absent

** linkage not found

It is likely that the true number of saponin variants would exceed 100 if all conformational isomers were considered (Barr *et al*., 1998). The reason for so many possible structures lies in the discovery of four different triterpenes as aglycones, three different di- or trisaccharides in the 3- position of the aglycone, eight different oligosaccharides in the 2-position of the fucose residue, six different substituents in the 3-position of the fucose residue, and five different substituents in the 4-position of the fucose residue. Taking into account all variations, 2880 possible structures for the quillaia saponins can be calculated, although many of them would be considered unlikely to occur (Nord *et al*., 2001).

Higuchi *et al*. (1988) carried out the first complete structural analysis of a quillaia saponin, which they designated QSIII. QSIII was shown (Jacobsen *et al*., 1996) to be identical to QS-17 (Table 1), as described by Kensil *et al*. (1988), based on chromatographic and carbohydrate analyses. A list of the most studied purified saponins, their synonyms, and some of their features are summarized in Table 2.

Table 2. The most studied saponins from *Quillaja saponaria* Molina.

Name	Synonym	Molecular weight
QSIII	QS-17, QA-17	2296
QS-7	B4B, QA-7	1862
QS-18	Quadri 1, B3, QA-18	2150
QS-21	Quadri 2, B2, QA-21	1988
DS-1 (Obtained by mild alkaline hydrolysis)	QS21H, Quadri2A	1590
DS-2 (Obtained by mild alkaline hydrolysis)	QS18H, Quadri 1A	1752
QS-957 (Obtained by strong alkaline hydrolysis)	Quadri 1B or 2BQS-L1	957

(Adapted from Higuchi *et al*., 1987 and 1988; Kensil *et al*, 1988; 1991 and 1992; Dalsgaard *et al*., 1995; Cleland *et al*., 1996; So *et al*., 1997)

The wide variety of possible molecular structures for quillaia saponins, together with variations in the saponin profiles of harvested trees, makes characterization of QE a complex task. However, in practice, the identification and quantification of the major saponins, QS-7, QS-17, QS-18 and QS-21, are adequate to express the saponins content of QE, because they represent up to 90 % of the total saponins present.

The saponins content and identification of QE can be determined by RP-HPLC. At least 22 peaks (called QS-1 to QS-22) can be distinguished. The individual components were identified by retention time on a Vydac C_4 HPLC column (Kensil and Marciani, 1991; San Martín and Briones, 2000). Saponin QS-21 can be further purified using hydrophilic interaction chromatography (HILIC) (Kensil *et al*., 1991). QS-21 can be resolved into two peaks, QS-21-V1 and QS-21-V2, which have been shown to be structural isomers due to intramolecular trans-esterification of the fatty acid moiety between the 3- and 4-hydroxyl groups of the fucose ring (Jacobsen *et al*., 1996). Storage of QS-21 in aqueous solution resulted in the interconversion of the isomers and the slow formation of degradation products due to ester hydrolysis (Cleland *et al*., 1996). Isomerization by trans-esterification has been observed in 14 other saponins all sharing the migration of an O-acyl group between two adjacent positions on the fucose residue (Nyberg *et al*., 2003).

Quillaia extracts (Type 1 and 2) have been reported to contain many minor components, among which are water-soluble polyphenols, tannins, and sugars. Some polyphenols are removed during the production process, but an important fraction remains and imparts the characteristic deep reddish-brown colour of aqueous quillaia solutions. Tannins can be determined gravimetrically by adsorption with polyvinylpolypyrrolidone (PVPP). The method is based on weighing the extract before and after treatment with PVPP and removal of the PVPP by centrifugation (Makkar *et al*., 1992). Typical values are not more than 8 % tannins on a dried basis. For quillaia extracts (Type 1), total sugars, determined by the cupric ion test, do not exceed 32 % on the dried basis, and for quillaia extracts (Type 2), 5% on the dried basis.

The nitrogen content of QE is about 1 %, while the fat content is about 5% both measured on the dried basis). The fat content might be due to the presence of wood resins, as the extraction is normally performed at 70-80°. The presence of starch is also reported in the literature (Leung and Foster, 1996; Wichtl, 1994).

Specifications for QE (FNP 52 Add 9) include limits for colour absorbance in order to ensure that food colours are not unduly affected by the presence of QE. The pH of fresh aqueous QE is between 5 and 5.5. However, for concentrated liquid extracts, a common industrial practice is to adjust the pH to between 3.7-3.9 with phosphoric acid, so that sodium benzoate, which exhibits optimum performance at a pH below 4, may be used as a preservative.

Specifications for quillaia extract (Type 1) (FNP 52/add 9) set a limit of no greater than 14 % for ash on the dried basis. This limit reflects the typically high levels of calcium oxalate in the extract (about 11% by weight, Lueng and Foster, 1996). Quillaia extract (Type 2), a more purified extract, has a specification limit for ash of not more than 5% on the dried basis.

Table 3 summarizes information on various commercial preparations that compares the composition declared by the producer for the sum of the four principal saponins (QS-7, QS-17, QS-18 and QS-21) determined by HPLC (San Martin and Briones, 2000).

Table 3. Commercial quillaia extracts analyzed by RP-HPLC

Commercial name	Composition declared by producer	Saponin concentration*
QL-1000	550 g solids l^{-1}, non-refined QE	106 gl^{-1}
BF 3399	550 g solids l^{-1}, non-refined QE	106 gl^{-1}
BE 0799	440 gl^{-1}, non-refined QE, 110 gl^{-1} non-refined YE	41.3 gl^{-1}
QY-150	440 gl^{-1}, non-refined QE, 110 gl^{-1} non-refined YE	71.5 gl^{-1}
QL-Ultra	200 gl^{-1} partially purified QE	160 gl^{-1}
Quillajanin C-100	200 gl^{-1} partially purified QE, 100 gl^{-1} maltitol	215 gl^{-1}
QP-1000	Non-refined QE	200 g kg^{-1}
QP UF 300	300 g kg^{-1} partially purified QE, 700 g kg^{-1} lactose	230 g kg^{-1}
Saponin 5012	Bleached non-refined QE and lactose	200 g kg^{-1}
Quillajanin QP-20	50 g kgl^{-1} purified QE, 950 g kg^{-1} maltodextrin	50 g kg^{-1}
DAB 9	Purified QE	320 g kg^{-1}

* Determined by HPLC
YE – *Yucca* extract

5 Functional uses

Quillaia saponins have a wide range of industrial applications. The interest in these compounds has increased significantly in recent years due to their properties as foaming agents in beverages and emulsifiers in foods, as well as their applications in cholesterol-reduction and flavour enhancement (Murakami, 1988, 1996; Chino and Wako, 1992; Waller and Yamasaki, 1996 a, b; San Martin and Briones, 1999).

QE is recognized as a natural flavouring substance for use in food and beverages by the United States Food and Drug Administration (FDA), according to Title 21, section 172.510 of the US Code of Federal Regulations (21 CFR 172.510). QE are generally recognized as safe (GRAS) by FEMA (Flavour and Extract Manufacturers' Association); the FEMA number for QE is 2973. In the European Union, QE are approved for addition to water-based non-alcoholic drinks, cider, excluding "cidre bouché", and may be labeled as E999. In Japan, QE are allowed for human consumption (as emulsifiers and foaming agents) and for use in cosmetics.

The General Standard for Food Additives (GSFA) of the Codex Alimentarius Commission currently lists QE as suitable for use as a foaming agent in 'Water-based flavoured drinks', including 'sport' or 'electrolyte' drinks and particulated drinks (GSFA category 14.1.4; 500 mg/kg maximum use level) at Step 6 in the Codex process.

In soft drinks, unpurified QE are commonly used at concentrations up to 200 mg/kg (Mukai *et al*, 1993, Nayyar *et al*., 1998). In addition to minor uses in some soft drinks where slight foaming is desirable, e.g. root beers, QE are most commonly used in making dispensable frozen carbonated beverages (FCBs) or uncarbonated juice products (e.g. frozen lemonades). The use levels of QE in syrups intended for dispensable FCBs or frozen lemonades are higher than in other beverages. Therefore, the maximum required level to achieve the technological effect in these products may be as high as 500 mg/kg on dry solid basis (International Soft Drinks Council, personal communication, 2003).

QE can be used in cider, cream soda, cocktail mixes, baked goods, candies, frozen dairy products, gelatine and puddings. They can also be used for the production of low-cholesterol dairy food products (Richardson and Jiménez-Flores 1991; Sundfeld *et al*., 1994) and microemulsions (Kudo and Nishi, 1992). Some industrial applications include the production of mayonnaise (Maeda *et al*. 1989), enhancement of oil-soluble flavours for candies (Toya *et al*., 1994), solubilizing propolis (Kawai *et al*., 1994) and red colouring material (Oono and Higashimura, 1995), for soy sauce (Murakami and Watanabe, 1988a) and for whipping cream (Murakami and Watanabe, 1988b). Other functional uses mentioned in the References are antioxidants (Hisayuki and Takashi, 1987; Kooryama and Chiba, 1996) and leavening agents (Watanabe *et al*., 1989).

Current industrial practices require that QE do not form precipitates or exhibit turbidity when diluted in water. Mixtures of QE with saponins from the Mexican plant *Yucca shidigera* are also commercialized and used for specific applications such as slush-type drinks (San Martin and Briones, 2000).

6 Reactions and fate in foods

Mitra and Dungan (1997, 2000) report some physicochemical properties of QE. The interaction of Quillaia saponins and cholesterol in foods results in the formation of monolayers and micelles; the critical micelle concentration (CMC) depends on the salt concentration and temperature (Mitra and Dungan, 2000). Based on the ability of saponin micelles to form insoluble aggregates with cholesterol, Quillaja saponins can be used for the production of low-cholesterol dairy food products (Richardson and Jiménez-Flores 1991; Sundfeld *et al*., 1994).

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