



## 61st JECFA - Chemical and Technical Assessment (CTA), 2003

**QUILLAIA EXTRACTS****Type 1 and Type 2****Chemical and Technical Assessment (CTA)****First draft prepared by Silvia Resnik****© FAO 2004****1. Summary**

Quillaia extracts (synonyms: quillaja extracts, bois de Panama, Panama bark extracts, quillai extracts, Quillay bark extracts, soapbark extracts) are obtained by aqueous extraction of the milled inner bark or wood of pruned stems and branches 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. The word “quillay” is derived from the native Mapuche word “quillean” that means “to wash”.

Quillaia extract (Type 1) contain over 100 triterpenoid saponins, consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are also major components. Some simple sugars and calcium oxalate are also present. Quillaia extract (Type 1) is treated with “stabilizing agents” such as egg albumin and polyvinylpyrrolidone and then filtered through diatomaceous earth. The stabilizing agents remove substances that would probably precipitate during storage, such as protein–polyphenol complexes. After filtration, the liquid is concentrated, and the concentrate may be sold as such or be spray–dried and sold as a powder containing carriers such as lactose and maltodextrin.

Quillaia extract (Type 2) is produced by subjecting Quillaia extract (Type 1) to ultra-filtration or affinity chromatography to remove unwanted solids, such as polyphenols and has higher saponin concentrations and better emulsifying properties than Quillaia extract (Type 1).

Quillaia extract (Type 1) contain 20-26 % g of saponins, whereas Quillaia extract (Type 2) generally contain 75-90 % of saponins.

The Quillaia extracts (Type 1 and 2) are used in food applications, primarily for their foaming properties. Moreover, many products can be diluted with high amounts of carriers such as lactose, maltodextrin or maltitol, reducing significantly their saponin concentration. Depending on the manufacturing process, some extracts may contain preservatives.

**2. Description**

QE (synonyms: quillaja extracts; bois de Panama, Panama bark extracts, quillai extracts, Quillay bark extracts, soapbark extracts, C.A.S. N<sup>a</sup> 68990-67-0, INS N<sup>a</sup> 999.) are obtained by aqueous extraction of the milled inner bark or wood of pruned stems and branches 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, especially Bolivia, Chile and Peru. The word “quillay” is derived from the Mapuche word “quillean” that means “to wash”.

Quillaia saponins are structurally different from the saponins derived from other plant species. Two structural features that distinguish *Quillaja saponaria* saponins from those of other plant species are a fatty acid domain and a triterpene aldehyde at carbon 4 of the triterpene (Kensil et al., 1995). The chemical structures of the *Quillaia* saponins are highly complex with many opportunities for diversity. Reverse phase-high performance chromatography (RP–HPLC) has revealed up to 30 components in preparations of *Quillaia* saponins, such as Quil A (So et al., 1997). It is likely that the true number of variants would exceed 100 if all conformational isomers were considered (Barr et al., 1998). Higuchi et al. (1988) carried out the first complete structural analysis of a *Quillaia* saponin, which they designated QSIII (Fig. 1). QSIII was shown to be identical to QS-17 (Jacobsen et al., 1996) 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 1.

**Table 1. 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)

*Quillaja* saponins have a five-ringed quillaic acid backbone with small carbohydrate chains, consisting of two to five sugar units, attached at the 3' and 28' carbons of quillaic acid and are frequently branched (Bomford et al., 1992). Attached to the fucose first sugar unit at the 28' position of the carbohydrate chain is an 18 carbon acyl chain with a small carbohydrate chain at its terminal end, which consists of one or two sugar units (Figure 1, Table 2). The molar relation between monosaccharide and saponins in some *Quillaja* saponins is shown in Table 3.

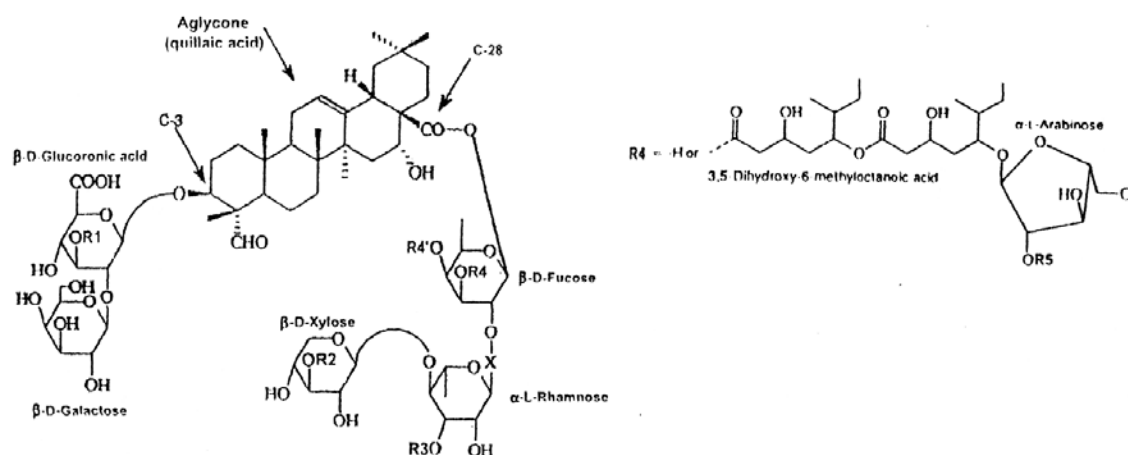


Figure 1: Molecular structures of saponins described on table 1

**Table 2: Molecular structures of some saponins from *Quillaja saponaria* Molina. (adapted from van Setten and van de Werken, 1996)**

Saponin	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>4</sub> '	R <sub>5</sub>	X
DS-1	$\beta$ -D-Xylp	$\beta$ -D-Apif	-H	-H	-H	absent	absent
DS-2	$\beta$ -D-Xylp	$\beta$ -D-Apif	$\beta$ -D-Glcp	-H	-H	absent	absent
QS-7	**	**	**	**	**	**	**
QS-17	$\beta$ -D-Xylp	$\beta$ -D-Apif	$\beta$ -D-Glcp	Figure 1	-H	$\alpha$ -L-Rhamp	absent
QS-18	$\beta$ -D-Xylp	$\beta$ -D-Apif	$\beta$ -D-Glcp	Figure 1	-H	-H	absent
QS-21	$\beta$ -D-Xylp	$\beta$ -D-Apif	-H	Figure 1 or -H	-H	-H	absent
QS-21 V1	$\beta$ -D-Xylp	$\beta$ -D-Apif	-H	Figure 1	-H	-H	absent
QS-21 V2	$\beta$ -D-Xylp	$\beta$ -D-Apif	-H	Figure 1	-H	-H	absent

\*\* linkage not found

**Table 3: molar relation between monosaccharide and saponins in major saponins from *Quillaja saponaria* Molina**

Monosaccharides	Saponin			
	QS-7	QS-17	QS-18	QS-21
Rhamnose	2.22	2.34	1.15	1.27
Fucose	0.90	0.96	0.88	0.91
Arabinose	Trace	0.98	0.74	0.77
Xylose	1.28	1.33	1.34	1.44
Galactose	1.00	1.00	1.00	1.00
Glucose	1.35	1.23	1.16	0.35
Glucuronic acid	0.65	0.64	0.72	0.74

For some QE, isolated fractions have been found to contain more than one saponin. In particular, Quil A could be divided in three fractions after purification by RP-HPLC. The first fraction eluting was designated QH-A while two of the more hydrophobic fractions eluted later and were designated as QH-B and QH-C (Rönnerberg et al., 1995).

The major points of chemical diversity are related to: a branched carbohydrate at the C 3 position; the carbohydrate chain at C 28; the nature of attachment of the acyl chain to fucose; the length of the acyl chain; the carbohydrate moieties on the acyl chain; and the active aldehyde at C 4 on the quillaic acid.

The branched carbohydrate at the C 3 position was thought to be a constant feature. However, Guo et al. (1998) identified two structures in which xylose is either absent or replaced with rhamnose. These were isolated from QH-A or from a mixture of QH-A and QH-C after strong alkaline hydrolysis. The three variants were present in approximately equal quantity. Although the disaccharide might possibly be a breakdown product, the replacement of a pentose with a deoxyhexose must occur during synthesis.

Considerable variation has been reported in the carbohydrate chain at C 28 of the quillaic acid. Kensil et al. (1993a, b) and Soltysik et al. (1993, 1995) identified QS-21-V1 and QS-21-V2 as two different compounds which were copurified by RP-HPLC (Kensil et al., 1988) but also could be separated by hydrophilic interaction chromatography. Cleland et al. (1996) found a tetra-saccharide chain terminating with either apiose in QS-21-V1 or xylose in QS-21-V2.

The acyl chain to fucose was initially identified (Higuchi et al., 1988) as a 3' attachment for QSIII and subsequently presumed to be the same for QS-18 and QS-21 (Kensil et al., 1992). More recently, two regioisomers, QS-21A and QS-21B with 4' and 3' attachment, respectively, have been described. These two isomers can be separated by RP-HPLC (Cleland et al., 1996; Jacobsen et al., 1996).

Higuchi et al. (1987) prepared a semi-purified *Quillaia* saponin mixture, which showed seven spots by HP-TLC, that then are subjected to mild alkaline hydrolysis (6% NH<sub>4</sub>HCO<sub>3</sub> in 50% methanol) to yield two products, DS1 and DS2. These fractions are identical to QS-21H, or Quadri-2A, and QS-18H, or Quadri-1A, respectively (Kensil et al., 1988; Dalsgaard et al., 1995). These structures result from ester hydrolysis and differ only by the presence, or absence, of glucose. DS-1 and DS-2 can also be identified in Quil A (Dalsgaard et al., 1995). The chemical characterization of QS-7 has not yet been described. However, its molecular weight of about 1870 (Kensil et al., 1993a), its substantially reduced hydrophobicity (Kensil et al., 1991) and lack of arabinose (Kensil et al., 1988) suggest it may be a partially deacylated QSIII or QS-18 with a hydrolysable ester function at position 3 (Fig. 1). Hydrolysis of this ester bond was described by Higuchi et al. (1987).

QS-17 has two carbohydrate molecules, QS-18 and QS-21 have one whilst QS-7 lacks any carbohydrate on the acyl chain.

Saponins are commercially obtained from four major sources: *Smilax ornata* (sarsaparilla), *Gypsophilla paniculata* (brides veil), *Saponaria officianalis* (soap root) and *Quillaia saponaria* Molina (soap bark). However, saponins obtained from *Quillaja saponaria* Molina alone or mixed with other low-cost saponin sources (eg *Yucca shidigera* extracts), are the most frequent compounds employed as food additives (San Martín, R. and Briones, R., 2000).

### 3. Manufacturing

For over 120 years QE have been produced from the aqueous extraction of the bark of *Quillaja saponaria* Molina tree belonging to the family *Rosaceae*. The wood is obtained from pruning operations (branches, limbs) that improve the quality of existing forests, without the need for deforestation.

### **Method of manufacture**

Historically, saponins are extracted mainly from the bark. Prior to debarking, the external part of the bark is removed with knives from the bark extract. The saponin content of bark is approximately 5 % w/w. By treating bark with hot water (70°-80°) 20 -25 % of extractives (w/w dry) can be obtained, with a saponin content in the extracted solids of about 20% (Kensil, 1996).

The ecological damage caused by the deforestation has stimulated the research on the use the whole quillaja 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 % (determined by RP-HPLC). The quality of the products derived from whole wood is as good as the commercial products derived from bark (San Martín et al, 1999).

QE are primarily commercialized with very little 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 also 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.

Hostettmann and Martson (1995) report that aqueous ethanol is used by Japanese companies to attain products of high purity. Wall and Rothman (1957) mentioned a process for extracting saponins by adding alcoholic solution as a solvent, then distilling off the solvent, acidifying and heating. In general, QE are produced with three different degrees of purification:

*Quillaia extract (Type1).* These products contain all water-soluble solids. The production process consists only 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, with preservatives as sodium benzoate (~0.5 g/l) or ethanol. The products have a typical red brownish color; however some extracts are bleached chemically to produce light color products. Quillaia extract (Type 1) contain 20-26 % saponins.

*Quillaia extract (Type 2).* These products are purified with ultrafiltration membranes or affinity chromatography to remove most non-saponin solids, such as calcium oxalate, sugars, tannins, polyphenols, etc. that may interfere in terms of color, chemical interactions, taste, and odor (Ogawa and Yokota 1985; Ogawa and Murakami 1987). They have a light color and are not bleached chemically. They have a higher saponin concentration than the quillaia extract (Type 1). Quillaia extract (Type 2) contain 75-90 % 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.

*Other commercial forms of QE:* Many products are diluted with high amounts of carriers such as lactose, maltodextrin or maltitol, reducing significantly their saponin concentration. The quality of the final extracts is evaluated in terms of their clarity and color in solution and saponins content, as well as for their foaming properties (San Martín et al, 1999).

Young plants, less than 15 years old, exhibit less heterogeneous saponins profiles than those obtained from mature extracts (Barr et al, 1998). A screening of the extracts obtained from thirty different natural, not cultivated, trees was performed by Kamstrup et al(2000) identified two separate profiles in this random group of isolates of which one (profile A), showing two predominant saponin peaks, appeared to be a subset of the other (profile B). Both profile A and profile B were observed each in 50% of the trees sampled. The comparison with a saponin profile of a commercial extract revealed that the latter displayed a mixture of both profiles. This mixed profile was attributed to the mixing of barks from both tree types during processing. The observed variation of the saponin profiles between trees was attributed by the authors to genetic factors, as neither soil, altitude, or age of trees or sampled tissues correlated to the composition of saponins; in addition they observed that trees from the same location could display different profiles. (Kamstrup et al., 2000) study on the variability of saponins in quillaia extracts. In search of plant varieties that exhibit a specific saponin spectrum. For the two major peaks present in profile A the authors showed that those were identical with the saponins QS-18 and QS-21 which had been described ten years earlier (Keslin et al, 1990). Long-term storage may also lead to oxidative or other changes in the product composition (Kamstrup et al, 2000)

*Other potential sources of Quillaia saponins :* Dalsgard and Henry (1998) proposed that the quillaia saponins can be produced by cells culture of several species of Quillaia such as Quillaja saponaria, Quillaja smegmadermos and Quillaja brasiliensis .

## **4. Characterization**

The existence of many different saponins, which vary in their chemical or biological activities, makes the characterization of QE difficult. The variable content of the individual saponins in QE also contributes to the difficulty of characterizing them. Fuller characterization requires identification of individual saponins to assess the quality, purity and toxicity of QE. 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 total saponins content.

The saponins content and identification of unpurified, semi-purified and highly purified QE can be performed by RP-HPLC. At least 22 peaks (denominated QS-1 to QS-22) are separable. The individual components were identified by retention time on a Vydac C<sub>4</sub> HPLC column (Kensil and Marciani, 1991; San Martín and Briones, 2000). The major saponins, purified by HPLC and low pressure silica chromatography, were found to be adjuvant active, although they differ in biological activities such as hemolysis and toxicity in mice. In particular, QS-21 and QS-7 were found to be less toxic in mice. More recently, QS-21 was further purified using hydrophilic interaction chromatography (HILIC) and was resolved into two peaks, QS-21-V1 and QS-21-V2, which have been shown to be different compounds (Kensil et al., 1991).

Carbohydrate content can be used to quantify the saponins in some instances. Scott and Melvin developed a method for determining the carbohydrate concentration of saponins (Kensil et al., 1991). The carbohydrate concentration can be estimated by use of an anthrone assay, where a ratio of extent of anthrone reaction is expressed in glucose equivalents per mg of purified saponin (dry weight). Differences in reactivity with anthrone for different saponins may result from differences in carbohydrate composition rather than from differences in the relative amounts of the individual monosaccharide. Therefore, this methodology is not accurate enough to quantify commercial products.

Most saponin adjuvants are known to have detergent properties, such as the ability to hemolyze red blood cells. So, the retention of hemolytic activity is a rough indication of the retention of adjuvant saponins.

Quillaia extracts (Type 1 and 2) have been reported to contain as minor components: water soluble polyphenols, tannins, sugars and others compounds. Some polyphenols are removed during the production process, but an important fraction remains and imparts the characteristic deep reddish-brown color of aqueous quillaia solutions. Tannins can be determined gravimetrically by adsorption with polyvinyl polypyrrolidone (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 do not exceed 32 % (on the dried basis determined by the cupric ion test) and for quillaia extracts type 2: 5% on the dried basis.

Other minor components can be determined by chemical analysis but Nitrogen content is about 1 % (on a dried basis), while fat content is about 5% (on a dried basis). The fat content might be due to the presence of wood resins, since the extraction is normally performed at 70-80 °. The literature also reports the presence of starch (Leung and Foster, 1996; Wichtl, 1994).

QE foam abundantly when shaken in water. This property is critical for characterizing the product. However, it does not necessarily guarantee the purity of the product, since similar foam levels can be attained by mixing QE with other low cost saponin sources such as extracts of the Mexican plant *Yucca schidigera* (San Martín and Briones, 2000). Typical values have been reported for quillaia extracts type 1: 150ml and for type 2: 260 ml of foam

Current industrial practices require that QE do not exhibit precipitates or turbidity when diluted in water. Most manufacturing processes include a preliminary purification step to avoid the possibility of precipitation. Specifications for QE (FNP 52/add 9) have a limit for color (determined at 520 nm) to ensure that food colors are not affected. Normally, light colored QE are preferred, not more than 1.2 for quillaia extract type 1 and not more than 0.7 for quillaia extract type 2.

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 between 3.7-3.9 with phosphoric acid and to use sodium benzoate as a preservative, which exhibits optimum performance at a PH below 4.

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

Young plants, less than 15 years old, exhibit less heterogeneous saponins profiles than those obtained from mature extracts (Barr et al, 1998). A screening of the extracts obtained from thirty different natural, not cultivated, trees was performed by Kamstrup et al. (2000) identified two separate profiles in this random group of isolates of which one (profile A), showing two predominant saponin peaks, appeared to be a subset of the other (profile B). Both profile A and profile B were observed each in 50% of the trees sampled. The comparison with a saponin profile of a commercial extract revealed that the latter displayed a mixture of both profiles. This mixed profile was attributed to the mixing of barks from both tree types during processing. The observed variation of the saponin profiles between trees was attributed by the authors to genetic factors, as neither soil, altitude, or age of trees or sampled tissues correlated to the composition of saponins; in addition they observed that trees from the same location could display different profiles. (Kamstrup et al., 2000) study on the variability of saponins in quillaia extracts. In search of plant varieties that exhibit a specific saponin spectrum. For the two major peaks present in profile A the authors showed that those were identical with the saponins QS-18 and QS-21 which had been described ten years earlier (Keslin at al, 1990). Long-term storage may also lead to oxidative or other changes in the product composition (Kamstrup et al, 2000).

Information on QE for the 61<sup>st</sup> JECFA were submitted by manufacturers from different countries and cover more than 60% of the world production.

Table 4 summarizes information on various commercial preparations which compare the composition declared by the producer to 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 4. Commercial quillaia extracts analyzed by RP-HPLC**

Commercial name	Composition declared by producer	Saponin concentration determined by RP-HPLC
QL-1000	550g solids l <sup>-1</sup> , non-refined QE	106g l <sup>-1</sup>
BF 3399	550g solids l <sup>-1</sup> , non-refined QE	41.3g l <sup>-1</sup>
BE 0799	440g l <sup>-1</sup> non-refined QE, 110g l <sup>-1</sup> non-refined YE	71.5g l <sup>-1</sup>
QY-150	440g l <sup>-1</sup> non-refined QE, 110g l <sup>-1</sup> non-refined YE	82g l <sup>-1</sup>
QL-Ultra	200g l <sup>-1</sup> partially purified QE	160g l <sup>-1</sup>
Quillajinin C-100	250g l <sup>-1</sup> partially purified QE, 100g l <sup>-1</sup> maltitol	215g l <sup>-1</sup>
QP-1000	Non-refined QE	200g kg <sup>-1</sup>
QP UF 300	300g kg <sup>-1</sup> partially purified QE, 700g kg <sup>-1</sup> lactose	230g kg <sup>-1</sup>
Saponin 5012	Bleached non-refined QE and lactose	200g kg <sup>-1</sup>
Quillajinin QP-20	50g kg <sup>-1</sup> purified QE, 950g kg <sup>-1</sup> maltodextrin	50g kg <sup>-1</sup>
DAB 9	Purified QE	320g kg <sup>-1</sup>

YE: *Yucca* extract

Assays of QE are typically based on the sum of the contents of the four major saponins: QS-7, QS-17, QS-18 and QS-21. Reference values are expressed as follows: Saponin content of quillaia extract (Type 1) not less than 20 % and not more than 26 % on the dried basis, and semi-purified, quillaia extract (Type 2) not less than 75 % and not more than 90 % on the dried basis.

## 5. Functional use

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

The term “food grade” saponin is widely used by manufacturers and it is defined as any grade or preparation of saponin which is approved for use in food and beverages under the United States Food and Drug Administration (FDA) regulation 21 CFR 172.510. QE are FEMA GRAS with FEMA number 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 emulsifier and foaming agent) and for use in cosmetics. The C.A.S. number is 68990-67-0

The General Standard on Food Additives (GSFA) of the Codex Alimentarius Commission 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 used levels of QE in syrups intended for dispensable frozen beverages (FCBs) or frozen lemonades is higher than in other beverages, therefore the maximum required level to achieve the technological effect in these products may be up to 500 mg/kg on dry solid basis (International Soft Drinks Council, personal communication, 2003).

Quillaia saponins 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 Jimenez-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 flavors for candies (Toya et al., 1994), dissolving of propolis (Kawai et al., 1994) and red coloring material (Oono and Higashimura, 1995), for soy sauce (Murakami and Watanabe, 1988a) and whipping cream (Murakami and Watanabe, 1988b). Other functional uses are mentioned in the References as antioxidants (Hisayuki and Takashi 1987; Kooryama and Chiba 1996) and leavening agents (Watanabe et al. 1989).

## 6. Reactions and fate in foods

Mitra and Dungan (1997, 2000) report some physicochemical properties of QE. The interaction of Quillaja saponins and cholesterol in foods result in the formation of monolayer and micelles, whose critical micelle concentration (CMC) depends on the salt concentration and temperature (Mitra and Dunga, 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 Jimenez-Flores 1991; Sundfeld et al, 1994).

## 7. References

- Barr, I.G., Sjolander, A. & Cox, J. C.** 1998. ISCOMs and other saponin based adjuvants. *Adv. Drug Deliver. Rev.*, 32: 247–271.
- Bomford, R., Stapleton, M., Winsor, S., Beesley, J.E., Jessup, E.A., Price, K.R. & Fenwick, G.R.** 1992. Adjuvanticity and iscom formation by structurally diverse saponins. *Vaccine*, 10: 572–577.
- Brennan, F. & Hamilton, W.** 2001. Food Grade Saponins as Oral Adjuvants. PCT International Application Number: PCT/GB00/03492. *International Publication number: WO 01/17555 A2*.
- Chino, Y., & Wako, M.** 1992. Manufacture of transparent emulsions for foods and beverages. *JP 04 51,853*.
- Cleland, J.L., Kensil, C.R., Lim, A., Jacobsen, N.E., Basa, L., Spellum, M. Wheeler, D.A., Wu, J.-Y. & Powell, M.F.** 1996. Isomerization and formulation stability of the vaccine adjuvant QS-21. *J. Pharm. Sci.*, 85: 22–28.
- Dalsgaard, K., Henry, M., San Martin Gamboa, R.M., Grande, H.J. & Kamstrup, S.** 1995. Compounds with adjuvant activity. *Patent application WO 95/09179*.
- Dalsgaard, K. & Henry, M.** 1998. Cultured Cells of *Quillaja sp.* U.S. *Patent 5,716,848*
- Guo, S., Kenne, L., Lundgren, L.N., Ronnberg, B. & Sundquist, B.** 1998. Triterpenoid saponins from *Quillaja saponaria*. *Phytochem.* 48:175-180.
- Higuchi, R., Tokimitsu, Y., Fujioka, T., Komori, T., Kawasaki, T. & Oakenful, D.G.** 1987. Structure of desacylsaponins obtained from the bark of *Quillaja saponaria*. *Phytochem.*, 26: 229–235.
- Higuchi, R., Tokimitsu, Y. & Komori T.** 1988. An acylated triterpenoid saponin from *Quillaja saponaria*. *Phytochem.*, 27: 1165–1168.
- Hisayuki, K., & Takashi, I.** 1987. Antioxidant. *JP 62243681 A*.
- Hostettmann, K. & Martson A.** 1995. *Saponins*, Cambridge University Press, UK.
- Jacobsen, N.E., Fairbrother, W.J., Kensil, C.R., Lim, A., Wheeler, D.A. & Powell, M.F.** 1996. Structure of the saponin adjuvant QS-21 and its base-catalysed isomerization product by <sup>1</sup>H and natural abundance <sup>13</sup>C NMR spectroscopy. *Carbohydr. Res.*, 280: 1–14.
- Kamstrup S., San Martin R., Doberti A., Grande H. & Dalsgaard K.** 2000. Preparation and characterisation of quillaja saponin with less heterogeneity than Quil-A. *Vaccine*, 18: 2244-2249.
- Kawai, M., Hirashita, N. & Kanae, J.** 1994. Manufacture of propolis compositions for health foods. *JP 94 197734*.
- Kensil, C. & Marciani, D.** 1991. Saponin adjuvant. *U.S. patent 5,057,540*.
- Kensil, C.A., Marciani, D.J., Beltz, G.A & Hung, C.H.** 1988. Saponin adjuvant. *Patent application WO 88/09336*.
- Kensil, C., Patel, U., Lennick, M. & Marciani, D.** 1991. Separation and characterization of saponins with adjuvant activity from *Quillaja saponaria* Molina cortex. *J. Immunol.* 146: 431–437.
- Kensil, C.A., Soltysik, S., Patel, U. & Marciani, D.J.** 1992. Structure / function relationship in adjuvants from *Quillaja saponaria* Molina. In F.Brown, R.M. Channock, H.S. Ginsberg & R.A. Lerner. eds. Cold Spring Harbor Press, pp. 35–40. Cold Spring Harbor, New York.
- Kensil, C., Soltysik, S. & Marciani, D.** 1993a. Enhancing drug delivery with modified saponins. *U.S. patent 5273965*.
- Kensil, C.A., Soltysik, S. & Marciani, D.J.** 1993b. Saponin-antigen conjugates and the use thereof. *Patent application. WO 93/05789*.
- Kensil, C., Soltysik, S. & Marciani, D.** 1996. Saponins as vaccine adjuvants. *Crit. Rev. Ther. Drug*, 13: 1-55.
- Kensil, C., Wu, J.-Y. & Soltysik, S.** 1995. Structural and immunological characterization of the vaccine adjuvant QS-21. In M. F. Powell & Newman M. J., eds., *Vaccine Design: the subunit adjuvant approach*. pp. 525–541. Plenum Press, New York.
- Kooryama, T., & Chiba, I.** 1996. Oxidation-stable emulsions of oil and fats with transparent appearance and food

containing them. *JP* 9651928 A2.

**Kudo, S., & Nishi, E.** 1992. Surfactant and method for producing the same. *U.S. patent* 5,152,928.

**Leung, A. & Foster, S.** 1996. Encyclopedia of Common Natural Ingredients used in food, drugs and cosmetics. 2<sup>nd</sup> Edition. Jhon Wiley & Sons (USA), pp.432-433.

**Maeda, K., Tanada, T. & Akita, S.** 1989. Manufacture of improved mayonnaise. *JP* 01,108,956.

**Makkar, H.P.S., Blümmel, M., Borowy, N.K. & Becker, K.** 1992. Gravimetric Determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agric*, 61: 161-165.

**Mitra, S., & Dungan, S. R.** 1997. Micellar properties of quillaja saponin. 1. Effects of temperature, salt and pH on solution properties. *J. Agr. Food Chem.* 45: 1587-1595.

**Mitra, S., & Dungan, S. R.** 2000. Micellar properties of quillaja saponin. 2. Effect of solubilized cholesterol on solution properties. *Colloids and Surfaces B: Biointerfaces*, 17: 117-133.

**Mukai, S., Matusuda, T. & Kanazawa, Y.** 1993. Manufacture of foaming beverages with saponins, saccharides and carbon dioxide. *JP* 05 38,275.

**Murakami, F. & Watanabe, T.** 1988a. Production of emulsified fat and oil-containing soy sauce. *JP* 63 233761

**Murakami, F. & Watanabe, T.** 1988b. Manufacture of stabilized cream using quillaja saponin. *JP* 63,109,735.

**Murakami, F.** 1988. Emulsifying properties of quillaja saponins. *Gekkan Fudo Kemikaru* 4:47-53.

**Murakami, F.** 1996. Properties and applications of food emulsifiers. Saponins. *Bessatsu Fudo Kemikaru* 8: 52-56.

**Nayyar, D., Schulok, W., Saleeb, F. & Modesta, E.** 1998. Dry mix for producing a slush beverage. *U.S. patent* 5,853,785.

**Ogawa, S., & Murakami, F.** 1987. Saponin purification. *JP* 62, 10,628.

**Ogawa, S., & Yokota, T.** 1985. Surfactants. *JP* 60,190,224.

**Oono, T., & Higashimura, Y.** 1995. Method for manufacturing of red coloring material. *JP* 9590188 A2.

**Pillion, D.J., Amsden, J.A., Kensil, C.R. & Recchia, J.** 1996. Structure- function relationship among *Quillaja* saponins serving as excipients for nasal and ocular delivery of insulin, *J. Pharm.Sci.*, 85: 518-524.

**Richardson, T., & Jimenez-Flores, R.** 1991. Saponin extraction of cholesterol from diary products. *PCT International Application* WO 91 05,836.

**Rönnerberg, B., Fekadu & Morein, M. B.** 1995. Adjuvant activity of non-toxic *Quillaja saponaria* Molina components for use in ISCOM-Matrix. *Vaccine*, 13: 1375-1382.

**San Martín, R. & Briones, R.** 1999. Industrial Uses and Sustainable Supply *Quillaja saponaria* (Rosaceae) saponins. *Econ. Bot.*, 53:302-311

**San Martín, R. & Briones, R.** 2000. Quality control of commercial quillaja (*Quillaja saponaria* Molina) extracts by reverse phase HPLC. *J. Sci. Food Agric.*, 80: 2063-2068.

**So, H.S., Yoon, H.S., Choi, D.Y. Kwon, Y.S., Sung, J.H., Lee, T.G., Park, E.S. Cho, H.S, Lee, B.M., Cho, J.M. & Ryu W.S.** 1997. Effect of a novel saponin adjuvant derived from *Quillaja saponaria* on the immune response to recombinant Hepatitis B surface antigen. *Mol. Cells*, 7: 178-186.

**Soltysik, S., Bedore, D.A. & Kensil, C.R.** 1993. Adjuvant activity of QS-21 isomers. *Ann. New York Acad. Sci.* 690: 392-395.

**Soltysik, S., Wu, J.Y., Recchia, J., Wheeler, D.A., Newman, M.J., Coughlin, R.T. & Kensil, C.R.** 1995. Structure / function studies of QS-21 adjuvant: assessment of triterpene aldehyde and glucuronic acid roles in adjuvant function. *Vaccine*, 13: 1403-1410.

**Sundfeld, E., Krochta, J. & Richardson, T.** 1994. Aqueous process to remove cholesterol from food products. *PCT International Application* WO 94 12,044.

**Toya, C., M. Fujita, & A. Toshio.** 1994. Enhancement of oil-soluble flavors in candies. *JP* 06 22,697.

**van Setten, D. C., & van der Werken, G..** 1996. Molecular structures of saponins of *Quillaja saponaria* Molina. In Waller, G. & Yamasaki, K. eds. Saponins used in traditional and modern medicine. Advances in experimental biology. Vol. 404. *Proceedings of the American Chemical Symposium on Saponins Chemistry and Biological Activity*, August 22-25, Chicago, IL. Plenum Press, New York.

**Wall, M.E & Rothman, E.S.** 1957. Process for extracting saponins from plant tissue. *U.S. patent* 2,791,581



**Waller, G. R., & Yamasaki, K.** 1996a. Saponins used in traditional and modern medicine. *In* Advances in experimental biology. Vol. 404. *Proceedings of the American Chemical Symposium on Saponins Chemistry and Biological Activity*, August 22-25, Chicago, IL. Plenum Press, New York.

**Waller, G. R., & Yamasaki, K.** 1996b. Saponins used in food and agriculture. *In* Advances in experimental biology. Vol. 405. *Proceedings of the American Chemical Symposium on saponins Chemistry and Biological Activity*, August 22-25, Chicago, IL. Plenum Press, New York.

**Watanabe, T., Murakami, F., Harada, S. & Watanabe, Y.** 1989. Leavening agents contain quillaja saponin and carrageenan for cake manufacture. *JP* 01 168,227.

**WHO. 1982.** Evaluation of certain food additives and contaminants (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683.

**WHO. 1986.** Evaluation of certain food additives and contaminants (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733 and corrigendum.

**Wichtl, M. 1994.** *Herbal Drugs and Phytopharmaceuticals*. pp 404-406. CRC Press, Stuttgart.