APPLICATION FOR THE AMENDMENT OF THE AUSTRALIA/NEW ZEALAND FOOD STANDARDS CODE 1.3.1 WITH QUILLAJA EXTRACT

22 May 2012
Prepared by National Starch Pty Ltd

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PART 1
GENERAL REQUIREMENTS

1.1 EXECUTIVE SUMMARY

Quillaja extract is cited in the Codex Standard 192-1995 revision 2010 as approved food additives (Foaming agent, Emulsifier) and it has been approved food additive in the European Union, United States, Canada, China, Japan, India, Singapore, Thailand, Taiwan and Vietnam.

The U.S. Food and Drug Administration (FDA) approved the use of *Quillaja* as a natural substance used in conjunction with flavors (21 CFR 172.510). Moreover, *Quillaja* extracts are considered Generally Recognized as Safe (GRAS) for use as a foaming agent in semi-frozen carbonated and non-carbonated beverages, based on information provided by the American Beverage Association (ABA; GRAS Notice No. GRN 000165).

In addition to the food additive approvals listed above, National Starch has completed a self affirmed GRAS determination for quillaja extract in the form of Q-Naturale as an emulsifier in beverage products and dietary supplement applications. Q-Naturale will be used for the delivery of fats, nutrients, vitamins, color and clouding agents in these applications subject to the use-level limitations.

National Starch Food Innovation (hereafter NSFI), through a business partnership with an industry leading supplier of quillaja, Desert King International, has obtained global exclusive rights to sell quillaja-based products, Q-Naturale® 100, Q-Naturale® 200 and Q-Naturale® 300, for food and beverage applications as a replacement for emulsifiers such as gum Arabic and modified food starch. In Australia, we intend to market only Q-Naturale® 200

In this document the application of Quillaja extract is described. The document has been written in the format of the Food Standards Australia New Zealand, Application Handbook, issued as at 1 August 2011. The application is related to the Standards for food production and is addressed to the information requirements of Section 3.1 (General requirements) and Sub-section 3.3.1 (Food additives).

The purpose of this application is to amend the Food Standard Code 1.3.1 Food Additives in order to obtain approval the use of new food additive (Quillaja extract) as emulsifier in beverages.

The amendment is supported in this document by:

- Part 1, containing general information
- Part 2, containing technical information
- Part 3, containing safety data on quillaja extract
- Part 4, containing dietary exposure to quillaja extract
- All appendixes, containing the technical comparison test on benefit of use, regulatory status in various countries and literatures.

1.2 APPLICANT DETAILS

(a) Applicant's name/s: [redacted]

(b) Company/organisation name: National Starch Pty Ltd

(c) Address (street and postal):
Unit 5A, 167 Prospect Highway,
Seven Hills
NSW 2147

[redacted]

[redacted]

(f) Nature of applicant's business:
Manufacturer/importer/distributor of native and modified food starches and other food additives.

(g) Details of other individuals, companies or organisations associated with the application.
Not applicable.

1.3 PURPOSE OF THE APPLICATION
The purpose of this application is to vary the Food Standard Code 1.3.1 Food Additives in order to obtain approval for the use of new food additive (Quillaja extract) as emulsifier in beverages.

1.4 JUSTIFICATION FOR THE APPLICATION
(a) the need for the proposed change;
The need for the proposed change is to provide beverage emulsion manufacturer a natural emulsifier alternative to high oil load emulsions and clear beverage emulsion. The ability of quillaja extract to emulsify at high oil load enables emulsion manufacturers to achieve cost savings and expand existing emulsion manufacturing capacity. Although food additives such as polysorbates can be used to prepare clear beverage emulsion, quillaja extract as a natural emulsifier, extracted by water purification, provides a cleaner method of preparing such emulsions

(b) the advantages of the proposed change over the status quo, taking into account any disadvantages.
The technological function proposed for Quillaja extract in this application is Emulsifier. In particular, quillaja extract provides a natural option to prepare high oil load emulsion and clear beverage emulsion.
See Appendix A: Technical Comparison Test on Benefit of Use

Application in other countries:

Country	Date applied	Status
1. Malaysia	Feb 2011	The MOH committee has agreed for addition quillaja extract. Waiting for official gazette.

A. Regulatory impact information

1. Costs and benefits

(a) the cost and benefits to the consumer e.g. health benefits;

Quillaja extract can be used to prepare high oil load emulsions. Currently, the typical oil load in emulsions is 12% and the emulsifier used can be either modified starches or gum Arabic. When quillaja extract is used as emulsifier, a maximum oil load of 50% can be achieved in the emulsion. This brings about several advantages for the emulsion and beverage manufacturers. They are cost savings in terms of reduced transportation costs and reduced inventory. These cost savings could eventually be passed to consumers as manufacturers have reduced manufacturing costs.

(b) the costs and benefits to industry and business in general, noting any specific effects on small businesses e.g. savings in production costs;

Quillaja extract can be used to prepare high oil load emulsions. Currently, the typical oil load in emulsions is 12% and the emulsifier used can be either modified starches or gum Arabic. When quillaja extract is used as emulsifier, a maximum oil load of 50% can be achieved in the emulsion. This brings about several advantages for the emulsion manufacturers. They are cost savings in terms of reduced transportation costs and reduced inventory costs and also enable the emulsion manufacturers to expand their existing emulsion manufacturing capacity without capital investment.

(c) the costs and benefits to government e.g. increased regulatory costs.

No cost involved to the government.

2. Impact on international trade

The potential impact on trade is estimated at US$ 2 million

1.5 INFORMATION TO SUPPORT THE APPLICATION

(a) any public health and safety issues related to the proposed change including details of target groups and population groups that may be adversely affected

Quillaja extract has been an approved food additive in other countries and is not known to pose any public health and/or safety issues when used below its proposed limit

(b) any consumer choice issues related to the proposed change

There has been growing consumer interest in clear beverages such as flavored waters. This emulsifier can be used to prepare clear beverages and hence enables manufacturer to produce flavored waters using natural emulsifier.

(c) any evidence that the food industry generally or other specific companies have an interest in, or support, the proposed change to the Code (this item is mandatory for applications relating to food additives, processing aids, nutritive substances, novel foods, irradiated foods).

Health and wellbeing is a growing trend in Australia. There are also more people seeking natural label.

There are some enquiries and keen interests from food manufacturers in Australia on this natural emulsifier to meet this growing trend. Since quillaja extract is not an approved food additive in Australia, National Starch is proactive in promoting this product in the country. International flavor houses outside of Australia have worked with quillaja extract and found unique benefits for this product. They have further identified

Australia as one of the key markets for the flavor emulsion made with quillaja and hence we are filing this application.

1.6 ASSESSMENT PROCEDURE

This application is intended for adding a new food additive (quillaja extract) for listing in the Food Standard Code 1.3.1 Food Additives therefore the appropriate procedure to be adopted in assessing the application is General Procedure.

1.7 CONFIDENTIAL COMMERCIAL INFORMATION (CCI)

The manufacturing process of Q-Naturale® 200 is considered as CCI and it is separated into APPENDIX H.

1.8 EXCLUSIVE CAPTURABLE COMMERCIAL BENEFIT (ECCB)

This application is not expected to confer an Exclusive Capturable Commercial Benefit (ECCB) as Quillaja extract is not a novel food additive and the result in an amendment to the Code does not provide exclusive benefits to the applicant.

1.9 INTERNATIONAL AND OTHER NATIONAL STANDARDS

A. International Standards

The Codex Standard relevant to this application is listed below.

CODEX STAN 192-1995 REVISION 2010 (page 139)
CODEX GENERAL STANDARD FOR FOOD ADDITIVES (See Appendix B)

QUILLAIA EXTRACTS

INS	999(i)	Quillaia extract type I	Functional Class: Emulsifier, Foaming agent
INS	999(ii)	Quillaia extract type 2	Functional Class: Emulsifier, Foaming agent

FoodCatNo	FoodCategory	MaxLevel	Notes	Year Adopted
14.1.4	Water-based flavoured drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks	50 mg/kg	132 & 168	2007

Note 132 Except for use at 130 mg/kg (dried basis) in semi-frozen beverages.

Note 168 Quillaia extract type 1 (INS 999(i)) only. Acceptable maximum use level is expressed on saponin basis.

B. Other National Standards or Regulations

Other national standards or regulations are listed below (The details of regulations are in Appendix C).

European Union: Food additive: Quillaja extract; E999

- Permitted for use in water-based flavoured non-alcoholic drinks and cider (excluding cidre bouché) to a maximum level of 200 mg/l as an anhydrous extract

Technical use: emulsifier, stabiliser, foam stabiliser, encapsulent in water-based flavoured non-alcoholic drinks and cider only, provided the use level is not exceeded.

Label Declaration: Label with food additive category followed by its specific name i.e., Quillaja extract, or E-number

United States:

Technical use:

- Food additive status: 21 CFR §172.510 "Natural flavoring substances and natural substances used in conjunction with flavors", **and** 172.515 "Synthetic flavoring substances and adjuvants" [172.515 (b) references adjuvants regulated by an appropriate section (172.510) in this part]
  - Quillaja can be used as an adjuvant (emulsifier, stabilizer or foam stabilizer) in conjunction with natural **and** synthetic flavors.
  - Use at a minimum quantity to achieve the intended physical or technical effect and in accordance with GMP.

- GRAS approval: Subject of GRAS petition GRN 000165
  - No Objection by FDA to GRAS petition
  - Use as a foaming agent in semi-frozen carbonated and non-carbonated beverages at levels not to exceed 500 mg/kg (dry basis) in beverage concentrate prior to the incorporation of water and carbon dioxide or air in retail establishments.

- FEMA GRAS: FEMA GRAS number 2973 for quillaja extract
- Self Affirmed GRAS: In addition to the food additive approvals listed above, National Starch has completed a self affirmed GRAS determination for quillaja extract in the form of Q-Naturale for use as an emulsifier or encapsulation agent in beverage products and dietary supplement applications. Q-Naturale is permitted for the delivery of fats, nutrients, vitamins, colors and clouding agents in these applications subject to the use-level limitations listed below.

Food Category	Current Food-Uses	Quillaja Extract Use-Level (%)
Beverages and Beverage Bases	Semi-Frozen Carbonated and Non-Carbonated Beverages	0.05
	Brewed Sodas	0.01
Alcoholic Beverages	Flavored Alcoholic Beverages	0.0870
Beverages and Beverage Bases	Carbonated Beverages (RTD)	0.0167
	Energy, Sport, and Electrolyte Drinks (powder)	0.0167
	Energy, Sport, and Electrolyte Drinks (RTD)	0.0870
	Enhanced Water (RTD)	0.0870
Coffee and Tea	Iced and Instant Tea Products (powder)	0.0167
	Iced and Instant Tea Products (RTD)	0.0167
Processed Fruits and Fruit Juices	Fruit Drinks and Ades (powder)	0.0167
	Fruit Drinks and Ades (RTD)	0.0335
	Fruit Juices	0.0335
Processed Vegetables and Vegetable Juices	Vegetable Juices and Blends	0.0335

Labeling:

- Sale as an ingredient: Use the common and usual name – quillaja, quillaja extract

- As part of a flavor sold to a food manufacturer (not a consumer product): Several choices:
  - Declare each flavor ingredient (including quillaja) by its common or usual name in descending order of predominance, or
  - Use the following statement: All flavor ingredients contained in this product are approved for use in a regulation of the Food and Drug Administration". Any flavor ingredient or non-flavor ingredient not contained in one of these regulations shall be separately listed on the label.
  - In cases where the flavor contains a solely natural flavor(s), the flavor shall be labeled e.g., "strawberry flavor" or "natural strawberry flavor". If the flavor contains both a natural and artificial flavor, the flavor shall be labeled, e.g., "natural and artificial strawberry flavor".

- As part of a flavor in a consumer product: Quillaja would be exempt from ingredient declaration labeling provide it qualifies as an "incidental additive" that is present at insignificant levels whereby it does not have technical or functional effect in the finished food.

Canada

Status: Approved as a miscellaneous food additive

- Table VIII lists permitted uses as: beverage bases, beverage mixes, soft drinks
- Permitted use: foaming agent
- Use level: GMP
- Broader food use for emulsification would require petitioning Health Canada for expanded food additive approval.

Labeling: quillaja extract

China: Food additive: Quillaja extract

- GB2760 Hygienic Standards for Use of Food Additives. Permitted as a natural substance used in conjunction with flavors without use limitation.

Technical use: Permitted as a natural substance used in conjunction with flavors.

Label Declaration: Final foods require label as "Flavor name". No need to declare quillaja extract in a final food ingredient labeling.

Japan: Food additive: Quillaja Extracts

- The Japanese Standards of Food Additives (JSFA) VIII Quillaja Extracts.

Technical use: Approved as a food additive without a use limitation in any kind of foods except alcoholic beverage.

Label Declaration: A label declaration as "Quillaja Extract or Quillaja Saponin or Emulsifier, only when using as emulsifier" in Japanese language in final food products.

India: Food additive: Quillaja extract; INS No.999

- Permitted in beverages without a use limitation.

Technical use: Approved as a food additive as a foaming agent.

Label Declaration: Label with food additive category followed by its specific name i.e., Quillaja extract, or INS number.

Singapore: Food additive: Quillaja extract; INS No.999

- Permitted in soft drinks to a maximum use level 200 mg/kg expressed as saponin basis in accordance with international practice.

Technical use: Approved as a food additive as an emulsifier or stabiliser.

Label Declaration: A label declaration as "emulsifier or stabiliser" can be used when quillaja is used as an emulsifier or stabiliser in final food products.

Thailand: Food additive: Quillaja extract; INS No. 999

- Permitted in water based, flavoured drinks, including sport, energy or electrolyte drinks and particulated drinks to a maximum use level 50 mg/kg based on saponin level and 130 mg/kg (dried basis) in semi-frozen beverages

Technical use: Approved as a food additive as foaming agent or emulsifier.

Label Declaration: A label declaration as "foaming agent" or "emulsifier" can be used when quillaja extract is used as a foaming agent or emulsifier in final food products.

Taiwan: Food additive: Quillaja extract

- Permitted in water based, flavoured drinks to a maximum use level 0.2 g/kg or 0.2 g/l.

Technical use: Approved as a food additive as an emulsifier.

Label Declaration: Label with food additive category followed by its specific name i.e., Quillaja extract, or INS number.

Vietnam: Food additive: Quillaja Extract; INS No.999

- Permitted in beverages to a maximum limit use level 1,500 mg/kg based on quillaja extract. ADI is 0-5 ppm.

Technical use: Approved as a food additive as a foaming agent.

Label Declaration: A label declaration as E999 when quillaja is used as a foaming agent in final food products.

1.10 STATUTORY DECLARATION

See Appendix F

PART 2
TECHNICAL INFORMATION

2.1 NATURE AND TECHNOLOGICAL FUNCTION

(a) each of the technological functions listed in Schedule 5 of Standard 1.3.1 – Food Additives that the additive fulfils;

Quillaja extract fall under the Schedule 5 functional class of emulsifier.

(b) the reason why the food additive is needed to fulfil these functions in each of the foods in which it is proposed to be used;

Quillaja extract is used to emulsify oil soluble ingredients such as flavours and natural colours such as beta carotene and turmeric. In the proposed food categories, the function of the quillaja extract is to emulsify and provide stability to flavour and colour components present in the food categories.

There are existing food additives that can emulsify flavour and natural colours in the proposed food additives. Unlike quillaja extract, these existing food additives, such as modified starch and gum Arabic, were not able to emulsify effectively at high oil loading in the emulsion. The advantages of high oil load emulsions are that the emulsion use level in end applications is low, emulsion manufacturers can achieve cost savings and expand existing emulsion manufacturing capacity without capital investment. Besides high oil load emulsion, Quillaja extract can be used to prepare emulsions for clear beverages. Although food additives such as polysorbates can be used to prepare clear beverage emulsion, quillaja extract as a natural emulsifier, extracted by water purification, provides a cleaner method of preparing such emulsions. Based on our best knowledge, quillaja extract is the only natural emulsifier for this application.

Therefore, the need for the proposed change is to provide beverage emulsion manufacturer a natural emulsifier alternative to high oil load emulsions and clear beverage emulsion.

(c) evidence that the amounts proposed to be added are consistent with achieving the technological function; and

The use levels of Quillaja extract depend on the flavour and colour use levels in end application. The evidence to show that the Quillaja extract use level is sufficient to achieve its technological function in high oil load and clear beverage emulsion was cited in Appendix A: Technical Comparison Test on Benefit of Use

There are two sets of experiments described in Appendix A, namely high orange oil load emulsion and emulsion for clear beverages.

In high orange oil load emulsion, the Quillaja extract use level was 10% for emulsion containing 50% orange oil and this use level was required to achieve stability in both emulsion and final beverages. Since the typical emulsion use level in beverage ranges from 0.02% to 0.15% for 50% oil load emulsion, the resultant quillaja extract use level ranges from 0.002% to 0.015%. Considering that the quillaja extract contains 13% saponins, the saponin content in beverage ranges from 0.0002% to 0.0019% and this falls within our proposed limit of 0.0050% or 50mg/kg.

In emulsion for clear beverages, the required Quillaja extract use level was 10% for emulsion containing 10% orange oil to achieve beverage clarity and stability. Typical emulsion use level in such beverages ranges from 0.05% to 0.3% and hence the quillaja

extract use level ranges from 0.005% to 0.03%. Considering that the quillaja extract contains 13% saponins, the saponin content in beverage ranges from 0.0006% to 0.0039% and this falls within our proposed limit of 0.0050% or 50mg/kg.

2.2 IDENTIFICATION OF QUILLAJA EXTRACT

Quillaja extract	Type I	Type II
Definition	Quillaja extract (Type 1) is obtained by aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family *Rosaceae*). It contains triterpenoid saponins (Quillaja saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are major components and some sugars and calcium oxalate will be present. Quillaja extract (Type 1) is available commercially as liquid product or as spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol.	Quillaja extract (Type 2) is obtained either by chromatographic separation or **ultrafiltration** of the aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family *Rosaceae*). It contains triterpenoid saponins (Quillaja saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are minor components. Some sugars and calcium oxalate will also be present. Quillaja extract (Type 2) is available commercially as a liquid product or as a spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol.
CAS Number	68990-67-0	68990-67-0
Formula weight	Monomeric saponins range from ca. 1800 to ca. 2300, consistent with a triterpene with 8-10 monosaccharide residues	Monomeric saponins range from ca. 1800 to ca. 2300, consistent with a triterpene with 8-10 monosaccharide residues
Description	Red-brownish liquid or light brown powder with a pink tinge	Light red-brownish liquid or powder
Solubility	Very soluble in water, insoluble in ethanol, acetone, methanol and butanol	Very soluble in water, insoluble in ethanol, acetone, methanol, and butanol
Foam	Dissolve 0.5 g of powder extract in 9.5 g of water or 1 ml of liquid extract in 9 ml of water. Add 1 ml of this mixture to 350 ml of water in a 1000-ml graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam level (ml) after 30 min. Typical values are 150 ml of foam	Dissolve 0.5 g of the powder form in 9.5 ml of water or 1 ml of the liquid form in 9 ml of water. Add 1 ml of this solution to 350 ml of water in a 1000-ml graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam volume (ml) after 30 min. Typical volumes are about 260 ml.
Color and turbidity	Powder form only: Dissolve 0.5 g in 9.5 g of water. The solution is not turbid. Determine the absorbance of the solution against water at 520 nm. The absorbance is less than 1.2.	Powder form only: Dissolve 0.5 g in 9.5 ml of water. The solution shall not turbid Determine the absorbance of the solution against water at 520 nm. The absorbance shall be less than 0.7.

Please note that Quillaja extract Type I is unpurified while Quillaja extract Type II is semi-purified.

Quillaja extract Type I and Type II meet the JECFA specifications for Quillaja extract Type I and Type II respectively (See Appendix D).

Our commercial product (Q- Naturale® 200) meets the JECFA specifications for Quillaja extract Type II.

2.3 CHEMICAL AND PHYSICAL PROPERTIES

Quillaja extract	Type I	Type II
Water	Powder form: not more than 6% (Karl Fischer Method)	Powder form: not more than 6% (Karl Fischer Method)
Loss on drying	Liquid form: 50 to 80% (2 g, 105°, 5 h)	Liquid form: 50 to 80% (2 g, 105°, 5 h)
pH	3.7-5.5 (4 % solution)	3.7-5.5 (4 % solution)
Ash	Not more than 14% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from loss on drying)	Not more than 5% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from Loss on drying)
Tannins	Not more than 8% on a dried basis	Not more than 8% on a dried basis
Saponin content:	not less than 20 % and not more than 26 % on the dried basis	not less than 65 % and not more than 90 % on the dried basis

Please note that Quillaja extract Type I is unpurified while Quillaja extract Type II is semi-purified.

Quillaja extract Type I and Type II meet the JECFA specifications for Quillaja extract Type I and Type II respectively (See Appendix D).

Our commercial product (Q- Naturale® 200) meets the JECFA specifications for Quillaja extract Type II.

2.4 IMPURITY PROFILE

Quillaja extract	Type I	Type II
Lead	Not more 2 mg/kg.	Not more 2 mg/kg.

Please note that Quillaja extract Type I is unpurified while Quillaja extract Type II is semi-purified.

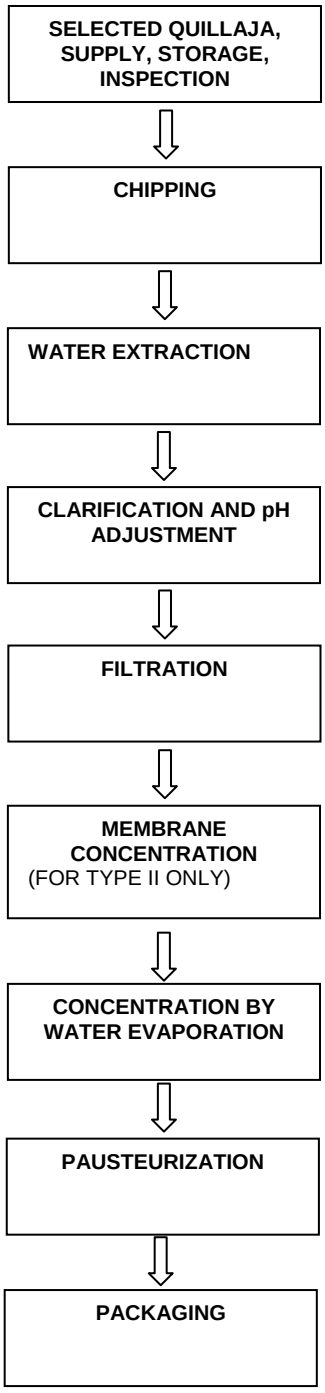
Quillaja extract Type I and Type II meet the JECFA specifications for Quillaja extract Type I and Type II respectively (See Appendix D).

Our commercial product (Q- Naturale® 200) meets the JECFA specifications for Quillaja extract Type II.

2.5 MANUFACTURING PROCESS OF QUILLAJA EXTRACT TYPE I AND TYPE II

The general manufacturing process is provided as below flow chart. The major difference between type I and type II is a membrane concentration process step. Type I has no membrane concentration process step while type II has to go through the membrane concentration process step.

The manufacturing process including processing aid for our commercial product (Q-Naturale® 200) is provided in the Appendix H.To the best of our knowledge and based on the processing aid that is used in the manufacturing process of our commercial product (Q- Naturale® 200), it should not give rise to any food safety issues.



2.6 SPECIFICATION FOR IDENTITY AND PURITY

Quillaja extract	Type I	Type II
Identification:Chromatography	Determine as in METHOD OF ASSAY. The retention time of major peak of the sample corresponds to the major saponin peak (QS-18) of the standard.	Determine as in METHOD OF ASSAY. The retention time of major sample peak corresponds to the major saponin peak (QS-18) of the standard.
Saponin content:	not less than 20 % and not more than 26 % on the dried basis	not less than 65 % and not more than 90 % on the dried basis

Please note that Quillaja extract Type I is unpurified while Quillaja extract Type II is semi-purified.

Quillaja extract Type I and Type II meet the JECFA specifications for Quillaja extract Type I and Type II respectively (See Appendix D).

Our commercial product (Q- Naturale® 200) meets the JECFA specifications for Quillaja extract Type II.

2.7 FOOD LABELLING

Recommended food labeling is Emulsifier (999).

2.8 ANALYTICAL METHOD FOR DETECTION

HPLC method is used to determine the saponin content. The method is in Appendix D – JECFA specifications for Quillaja extract (Type I and II): Method of Assay.

2.9 POTENTIAL ADDITIONAL FUNCTIONS WHEN ADDED TO FOOD

Not Applicable.

PART 3
SAFTETY DATA ON QUILLAJA EXTRACT

3.1 TOXICOKINETICS AND METABOLISM STUDY

Not applicable because no absorption of saponins through the intestine[1].

There is limited information specifically on Quillaja saponins, but here are some published scientific literature addressing absorption and toxicity. According to the attached paper on soya saponins, there is no absorption of saponins through the intestine evidenced by the lack of presence of saponins or saponin-aglicones in the urine post ingestion. Moreover, the saponins are hydrolyzed up to aglicones since there is no presence of saponins in the feces either[2].

Please note that Quillaja saponins are larger than soy saponins and therefore even less likely to be absorbed by the intestine.

The four most abundant saponins in Quillaja extracts (QS-7, QS-17 (also known as QS III), QS-18 and QS 21) have molecular weights ranging between 1862-2296 g/mol (Resnick, 2004). Soy saponins have molecular weights ranging between 767-1437 g/mol (http://sites.google.com/site/masonaco/Home/applications/mass-spectrometry-lifescience/soy-saponines)

3.2 TOXICITY STUDY

Among the data supporting the safety of quillaja extract for human consumption is GRAS Notice No. GRN 000165, which showed no unresolved safety issues and generated no questions from the U.S. FDA. The GRAS Notice based on scientific principles, attached to the application as Appendix C-2, noted that the safety of Quillaja has been evaluated by the Joint Expert Committee on Food Additives (JECFA) and provided a copy of JECFA's safety evaluation. JECFA evaluated the publicly available scientific literature and determined an acceptable daily intake of 0 to 5 mg/kg bw/day. In addition, to its GRAS status, FDA has approved Quillaja extract as a food additive for use as a natural flavoring substance and natural substance used in conjunction with flavors in multiple food categories (21 CFR 172.510).

3.2.1 Nonclinical Safety

3.2.1.1 Subchronic Toxicity

Gaunt *et al*. (1974)[3] examined the short-term toxicity of Quillaja extract in weanling CFE strain rats. Groups of 15 male rats (130 – 175 g body weight) and 15 females (105 – 135 g body weight) were housed, five in a cage, and fed diets containing 0 (control), 0.6, 2.0, or 4.0% Quillaja extract for 13 weeks. Groups of five male and five female rats from the same batch were fed diets containing 0 (control), 2.0 or 4.0% Quillaja extract for 2 or 6 weeks. Animals were weighed and food intake was measured before the experimental diets were fed, and then weekly throughout the study. At the end of the appropriate feeding period, rats were killed by exsanguination under barbiturate anesthesia and an autopsy was conducted, during which brain, pituitary, thyroid, heart, liver, spleen, stomach, small intestine, caecum, kidneys, adrenals, and gonads were weighed. Blood collected at autopsy was examined for hemoglobin content, packed cell volume and counts of erythrocytes, reticulocytes, and leucocytes. Serum collected at autopsy was examined for contents of urea, glucose, total protein, and albumin, and for the activities of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and lactic

[1] Peter R. Cheeke. Dietary saponins are not absorbed.

[2] Oakenfull, D., and G.S. Sidhu. 1989. Saponins. Pg. 97-141 in: Cheeke, P.R. (Ed.), Toxicants of Plant Origin. Vol. II. Glycosides. CRC Press.

[3] Gaunt IF, Grasso P, Gangolli SD (1974). Short-term toxicity of Quillaja extract in rats. Food Cosmet Toxicol 12(5&6):641-650.

dehydrogenase. During the last week of the feeding period, urine was collected from all rats and examined for microscopic constituents and content of blood, bile, and ketones.
Results showed that there was a transitory reduction in the rate of bodyweight gain, associated with a reduced intake of food and water, but by the end of the study the weights of the treated rats did not differ significantly from those of the controls. The feeding of Quillaja saponin did not affect the results of hematological examinations (including erythrocyte fragility tests in hypotonic saline), serum and urine analyses, renal concentrating ability or the urinary cell excretion. The relative liver weight was reduced in males given 2.0 or 4.0% Quillaja and the relative stomach weight was increased in both sexes at the same levels. No histopathological effects attributable to treatment were found. The no-observed-adverse-effect level (NOAEL) in this study was 0.6% of the diet, equivalent to an intake of approximately 400 mg/kg/day.

3.2.1.2 Chronic Toxicity

Phillips *et al*. (1979)[4] examined the long-term toxicity of Quillaja extract in mice. Mice of the TO strain obtained from a specified-pathogen-free breeding colony were used. Groups of 48 male and 48 female mice were fed diets containing 0, 0.1, 0.5, or 1.5% Quillaja extract for 84 weeks. The males were caged singly and the females in groups of four. The condition and behavior of the animals were observed frequently. At the end of the study the surviving animals were killed by exsanguination from the aorta under barbiturate anesthesia. At autopsy, all macroscopic abnormalities were noted and the brain, heart, liver, kidneys, spleen, stomach, small intestine, caecum, and testes were weighed. Blood samples were collected from a caudal vein of each of ten male and ten female mice from the control group and those on the two higher dietary levels at week 26 and 54, and from all surviving mice at week 84. The blood samples were examined for hemoglobin concentration and packed cell volume, and counts were made of reticulocytes and total erythrocytes and leucocytes.
Results showed that Quillaja extract had no adverse effect on the death rate or the incidence of histopathological findings, including tumors. However, there was a lower rate of body weight gain at the 1.5% dietary level, and there were isolated statistically significant differences between the treated and control animals, mainly at the 1.5% dietary level, in the hematological examinations and in some absolute and relative organ weights of both sexes. The authors concluded that, in mice, Quillaja extract fed at levels up to 1.5% in the diet (approximately 2.2 g/kg/day) did not exert a carcinogenic effect. The no-observed-adverse-effect level (NOAEL) from this study is considered to be 0.5% in the diet, giving an intake of approximately 0.7 g Quillaja extract/kg/day.

Drake *et al.* (1982)[5] assessed the long-term toxicity of the *Quillaja saponaria* extract in Wistar rats. Two short diet-acceptability studies were conducted in male rats. In the first study, animals had access to control and experimental diets, which contained 0.3, 1.0, or 3.0% Quillaja extract. The amount of each diet consumed was recorded for 21 days. In the second trial, animals were fed on either a control or experimental diet with 0.3, 1.0, or 3.0% Quillaja extract for 7 days, and daily food intakes were measured. In the long-term feeding study, groups of 48 male and female rats received diets containing 0, 0.3, 1.0, 3.0% Quillaja extract for 2 years. Rats were weighed at approximately 2-month intervals and food and water consumption were measured for the 24-hr period prior to body weight determinations. At week 15, 25, and 52 blood was collected from the tail

[4] Phillips JC, Butterworth KR, Gaunt IF, Evans JG, Grasso P (1979). Long-term toxicity study of Quillaja extract in mice. Food Cosmet Toxicol 17(1):23-27.
[5] Drake JJ-P, Butterworth KR, Gaunt IF, Hooson J, Evans JG, Gangolli SD (1982). Long-term toxicity study of Quillaja extract in rats. Food Cosmet Toxicol 20(1):15-23.

veins of 10 animals/sex/group. After 108 weeks, blood samples were taken from the aorta of all remaining animals during post-mortem examinations. Urine was collected and analyzed from 10 animals from the control and highest dose group on weeks 13, 24, and 78. Animals that survived during the study were autopsied unless prohibited by advanced autolysis or cannibalism. Surviving animals were sacrificed by exsanguination from the aorta under barbiturate anaesthesia. Results from the acceptability tests showed animals consumed an average of 23, 23.6, 27.4 g of the control diet with corresponding values of 1.3, 2.0, and 0.8 g of the test diet daily for 0.3, 1.0, and 3.0%, respectively, when offered a choice. When there was no choice of diet, the average intakes over 7 days were 25, 29, 26, and 21 g/day for rats given diets containing 0, 0.3, 1.0, and 3.0% Quillaja, respectively.
According to the authors, data from the long-term feeding study demonstrated that male rats fed the highest dietary level had significantly lower body weights compared to controls from months 10 to 22. Females on the lowest dietary level had significantly higher body weights during the first 6 months. The results of the renal concentrations and dilution tests at week 13 and 24 showed no statistically significant differences between control rats and those given 3% Quillaja extract. There were no significant differences between treated and control animals in serum analyses. The weights of the heart, kidneys, and thyroid were lower than the control values in male rats fed 3%, and thyroid weights were also lower among males fed 1% Quillaja extract. These differences

were statistically significant, but were not observed in females. Other significant changes included higher liver weights in females given the 3% level and lower liver weight in males given 1%. Histological findings were similar in treated and control animals. A small number of tumors were found in the treated groups and no relation to dosing was observed. Rats fed up to 3% of Quillaja extract in their diet appeared normal and did not exhibit any carcinogenic effect. The no-observed-adverse-effect level (NOAEL) in this current study was determined to be 3% in the diet, approximately equivalent to an intake of 1.5 g/kg/day.

3.3 SAFETY ASSESSMENT REPORT

See Appendix E –JECFA Evaluations, WHO Technical Report Series 934 and WHO Food Additives Series 48 and Series 17.

PART 4
DIETARY EXPOSURE TO QUILLAJA EXTRACT

4.1 A LIST OF FOOD GROUPS TO CONTAIN QUILLAJA EXTRACT

Proposed food groups:

14.1.1.2	Carbonated, mineralised and soda water
14.1.2.2	Fruit and vegetable juice products
14.1.3	Water based flavoured drinks
14.1.4	Formulated beverages
14.1.5	Coffee, coffee substitutes, tea, herbal infusions and similar products
14.2	Alcoholic beverages, including alcohol free and low alcohol counterparts

4.2 MAXIMUM PROPOSED LEVEL

Maximum proposed level of quillaja saponins (dry basis) in all proposed food groups in beverages = 50mg/kg

4.3 INFORMATION ON THE LIKELY LEVEL OF CONSUMPTION

Not Applicable.

4.4 THE PERCENTAGE OF THE MARKET LIKELY TO USE QUILLAJA EXTRACT

The proposed food group to incorporate quillaja extract is beverage segment. The percentage of the proposed food group of total beverage market is estimated to be about 40% in 2010 (data from www.euromonitor.com)

Statistic from subscribed market database (www.euromonitor.com)

Passport

Market Sizes | Historic/Forecast | Total Volume | mn litres

Geographies	Categories	2009	2010	2011	2012	2013
Australia	Alcoholic Drinks	2,732.9	2,826.1	2,911.9	3,005.1	3,102.9
Australia	Soft Drinks	3,693.6	3,750.6	3,799.8	3,841.6	3,873.5
Australia	Flavoured Bottled Water	85.0	86.8	88.9	91.4	94.0
Australia	Functional Bottled Water	32.7	38.4	42.7	46.6	50.1
Australia	Carbonates	1,926.4	1,923.2	1,911.7	1,895.1	1,874.3
Australia	Liquid Concentrates	133.9	132.6	130.6	128.0	125.0
Australia	Juice Drinks (up to 24% Juice)	1.4	1.4	1.4	1.4	1.4
Australia	Fruit-Flavoured Drinks (No Juice Content)	-	-	-	-	-
Australia	Nectars (25-99% Juice)	195.1	199.9	204.5	208.6	211.7
Australia	RTD Coffee	137.7	145.9	154.0	161.7	168.9
Australia	RTD Tea	38.7	44.5	48.8	53.1	57.2
Australia	Sports and Energy Drinks	168.2	186.5	206.3	224.9	240.5
New Zealand	Alcoholic Drinks	475.4	469.9	466.5	464.5	463.7
New Zealand	Soft Drinks	625.5	628.6	632.2	636.1	639.7
New Zealand	Flavoured Bottled Water	2.4	2.5	2.6	2.7	2.8
New Zealand	Functional Bottled Water	5.4	6.0	6.6	7.2	7.8
New Zealand	Carbonates	401.8	397.0	392.8	388.8	384.6
New Zealand	Liquid Concentrates	4.2	4.3	4.4	4.5	4.6
New Zealand	Juice Drinks (up to 24% Juice)	19.4	20.1	20.8	21.5	22.1
New Zealand	Fruit-Flavoured Drinks (No Juice Content)	-	-	-	-	-
New Zealand	Nectars (25-99% Juice)	2.4	2.4	2.5	2.6	2.7
New Zealand	RTD Coffee	0.9	0.9	0.9	0.9	0.9
New Zealand	RTD Tea	0.4	0.8	1.7	3.0	4.8
New Zealand	Sports and Energy Drinks	26.2	28.4	30.6	33.0	35.4

Research Sources:

Alcoholic Drinks: Euromonitor from trade sources/national statistics

Soft Drinks: Euromonitor from trade sources/national statistics

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Percentage of proposed food groups* to contain quillaja extract in ANZ

* Based on Australia Food Categories

Market Sizes | Historic/Forecast | Total Volume | mn litres											
Geographies	Categories	2010	Emulsion use level in beverage (%)	Emulsion market size (mn ltr)	Q-Naturale use level in emulsion (%)	Q-Naturale market size (mn ltr)	Remarks			
Australia	Alcoholic Drinks	2,826.1	0.064	1.81	3	0.05				
Australia	Soft Drinks	3,750.6	0	0.00	7	0.00	High oil load emulsion			
Australia	Flavoured Bottled Water	86.6	0.05	0.04	2.5	0.00	Clear beverage emulsion			
Australia	Functional Bottled Water	38.4	0.05	0.02	2.5	0.00	Clear beverage emulsion			
Australia	Carbonates	1,923.2	0.04	0.77	7	0.05	High oil load emulsion			
Australia	Liquid Concentrates	132.6	0.04	0.05	7	0.00	High oil load emulsion			
Australia	Juice Drinks (up to 24% Juice)	1.4	0.04	0.00	7	0.00	High oil load emulsion			
Australia	Fruit-Flavoured Drinks (No Juice Content)	0	0.04	0.00	7	0.00	High oil load emulsion			
Australia	Nectars (25-99% Juice)	199.9	0.04	0.08	7	0.01	High oil load emulsion			
Australia	Sports and Energy Drinks	186.5	0.05	0.09	2.5	0.00	Clear beverage emulsion			
Australia	RTD Coffee	145.9	0	0.00	3	0.00				
Australia	RTD Tea	44.5	0	0.00	3	0.00				
Australia	**TOTAL drinks able to use quillaja extracts**	2,759.2		**2.87**		**0.12**	121,356	litres QN	42%	=percent of proposed food groups to contain quillaja extract in Australia
New Zealand	Alcoholic Drinks	469.9	0.064	0.30	3	0.01				
New Zealand	Soft Drinks	628.6	0	0.00	7	0.00	High oil load emulsion			
New Zealand	Flavoured Bottled Water	2.5	0.05	0.00	2.5	0.00	Clear beverage emulsion			
New Zealand	Functional Bottled Water	6.0	0.05	0.00	2.5	0.00	Clear beverage emulsion			
New Zealand	Carbonates	397.0	0.04	0.16	7	0.01	High oil load emulsion			
New Zealand	Liquid Concentrates	4.3	0.04	0.00	7	0.00	High oil load emulsion			
New Zealand	Juice Drinks (up to 24% Juice)	20.1	0.04	0.01	7	0.00	High oil load emulsion			
New Zealand	Fruit-Flavoured Drinks (No Juice Content)	0	0.04	0.00	7	0.00	High oil load emulsion			
New Zealand	Nectars (25-99% Juice)	2.4	0.04	0.00	7	0.00	High oil load emulsion			
New Zealand	Sports and Energy Drinks	28.4	0.05	0.01	2.5	0.00	Clear beverage emulsion			
New Zealand	RTD Coffee	0.9	0	0.00	3	0.00				
New Zealand	RTD Tea	0.8	0	0.00	3	0.00				
New Zealand	**TOTAL drinks able to use quillaja extracts**	462.4		**0.49**		**0.02**	21,350	litres QN	42%	=percent of proposed food groups to contain quillaja extract in nz
ANZ Region	**TOTAL drinks able to use quillaja extracts**	**3,221.6**		**3.36**		**0.14**	142,706	litres QN	42%	=percent of proposed food groups to contain quillaja extract in ANZ

Total drinks market

Categories for quillaja extract to be incorporated

4.5 APPROVED USE LEVEL IN OTHER COUNTRIES

European Union: Food additive: Quillaja extract; E999

- Permitted for use in water-based flavoured non-alcoholic drinks and cider (excluding cidre bouché) to a maximum level of 200 mg/l as an anhydrous extract

Technical use: emulsifier, stabiliser, foam stabiliser, encapsulent in water-based flavoured non-alcoholic drinks and cider only, provided the use level is not exceeded.

United States:

Technical use:

- Food additive status: 21 CFR §172.510 "Natural flavoring substances and natural substances used in conjunction with flavors", **and** 172.515 "Synthetic flavoring substances and adjuvants" [172.515 (b) references adjuvants regulated by an appropriate section (172.510) in this part]
  - Quillaja can be used as an adjuvant (emulsifier, stabilizer or foam stabilizer) in conjunction with natural **and** synthetic flavors.
  - Use at a minimum quantity to achieve the intended physical or technical effect and in accordance with GMP.

- GRAS approval: Subject of GRAS petition GRN 000165
  - No Objection by FDA to GRAS petition
  - Use as a foaming agent in semi-frozen carbonated and non-carbonated beverages at levels not to exceed 500 mg/kg (dry basis) in beverage concentrate prior to the incorporation of water and carbon dioxide or air in retail establishments.

- FEMA GRAS: FEMA GRAS number 2973 for quillaja extract
- Self Affirmed GRAS: In addition to the food additive approvals listed above, National Starch has completed a self affirmed GRAS[7] determination for quillaja extract in the form of Q-Naturale for use as
- an emulsifier or encapsulation agent in beverage products and dietary supplement applications. Q-Naturale is permitted for the delivery of fats, nutrients, vitamins, colors and clouding agents in these applications subject to the use-level limitations listed below.

Food Category	Current Food-Uses	Quillaja Extract Use-Level (%)
Beverages and Beverage Bases	Semi-Frozen Carbonated and Non-Carbonated Beverages	0.05
	Brewed Sodas	0.01
Alcoholic Beverages	Flavored Alcoholic Beverages	0.0870
Beverages and Beverage Bases	Carbonated Beverages (RTD)	0.0167
	Energy, Sport, and Electrolyte Drinks (powder)	0.0167
	Energy, Sport, and Electrolyte Drinks (RTD)	0.0870
	Enhanced Water (RTD)	0.0870
Coffee and Tea	Iced and Instant Tea Products (powder)	0.0167
	Iced and Instant Tea Products (RTD)	0.0167
Processed Fruits and Fruit Juices	Fruit Drinks and Ades (powder)	0.0167
	Fruit Drinks and Ades (RTD)	0.0335
	Fruit Juices	0.0335
Processed Vegetables and Vegetable Juices	Vegetable Juices and Blends	0.0335

Canada:

Status: Approved as a miscellaneous food additive

- Table VIII lists permitted uses as: beverage bases, beverage mixes, soft drinks
- Permitted use: foaming agent
- Use level: GMP
- Broader food use for emulsification would require petitioning Health Canada for expanded food additive approval.

China: Food additive: Quillaja extract

- GB2760 Hygienic Standards for Use of Food Additives. Permitted as a natural substance used in conjunction with flavors without use limitation.

Technical use: Permitted as a natural substance used in conjunction with flavors.

Japan: Food additive: Quillaja Extracts

- The Japanese Standards of Food Additives (JSFA) VIII Quillaja Extracts.

Technical use: Approved as a food additive without a use limitation in any kind of foods except alcoholic beverage.

India: Food additive: Quillaja extract; INS No.999

- Permitted in beverages without a use limitation.

Technical use: Approved as a food additive as a foaming agent.

Singapore: Food additive: Quillaja extract; INS No.999

- Permitted in soft drinks to a maximum use level 200 mg/kg expressed as saponin basis in accordance with international practice.

Technical use: Approved as a food additive as an emulsifier or stabiliser.

Thailand: Food additive: Quillaja extract; INS No. 999

- Permitted in water based, flavoured drinks, including sport, energy or electrolyte drinks and particulated drinks to a maximum use level 50 mg/kg based on saponin level and 130 mg/kg (dried basis) in semi-frozen beverages

Technical use: Approved as a food additive as foaming agent or emulsifier.

Taiwan: Food additive: Quillaja extract

- Permitted in water based, flavoured drinks to a maximum use level 0.2 g/kg or 0.2 g/l.

Technical use: Approved as a food additive as an emulsifier.

Vietnam: Food additive: Quillaja Extract; INS No.999

- Permitted in beverages to a maximum limit use level 1,500 mg/kg based on quillaja extract. ADI is 0-5 ppm.

Technical use: Approved as a food additive as a foaming agent.

4.6 WHEN CONSUMPTION HAS CHANGED, INFORMATION ON LIKELY CONSUMPTION

Not Applicable.

PART 5
APPLICATION CHECKLIST

APPLICATION CHECKLIST

General Requirements (3.1)	
☑ Form of application ☑ *Executive Summary* ☑ *Relevant sections of Part 3 identified* ☑ *Pages sequentially numbered* ☑ *Electronic + 2 hard copies* ☑ *Electronic and hard copies identical* ☑ *Hard copies capable of being laid flat* ☑ *All references provided*	☑ Confidential Commercial Information ☑ *Confidential material separated in both electronic and hard copy* ☑ *Justification provided*
☑ Applicant details	☑ Exclusive Capturable Commercial Benefit
☑ Purpose of the application	☑ International and Other National standards
☑ Justification for the application	☑ Statutory Declaration
☑ Information to support the application	☑ Checklist/s provided with Application ☑ *Checklist* ☑ *Any other relevant checklists for Sections 3.3.1*
Assessment procedure ☑ *General*	

Food Additives (3.3.1)			
☑	Nature and technological function information	☑	Toxicokinetics and metabolism information (Not Applicable)
☑	Identification information	☑	Toxicity information
☑	Chemical and physical properties	☑	Safety assessments from international agencies
☑	Impurity profile	☑	List of foods likely to contain the food additive
☑	Manufacturing process	☑	Proposed levels in foods
☑	Specifications	☑	Likely level of consumption (Not Applicable)
☑	Food labelling	☑	Percentage of food group to contain the food additive
☑	Analytical detection method	☑	Use in other countries (if applicable)
☑	Additional functions (Not Applicable)	☑	Where consumption has changed, information on likely consumption (Not Applicable)