

Quality control of commercial quillaja (*Quillaja saponaria* Molina) extracts by reverse phase HPLC

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Abstract: Saponin-rich extracts of the Chilean indigenous tree *Quillaja saponaria* Molina are widely used as natural foaming agents in foods and beverages, food emulsifiers, photographic emulsions, vaccine adjuvants, etc. However, with the exception of the vaccine industry, saponin concentration is not adequately quantified. Normally, the quality and price of the extracts are determined using simple foam tests. This may not be adequate, since similar foam levels can be obtained by blending quillaja extracts with other low-cost saponin sources (eg *Yucca schidigera* extracts). Also, many products are diluted with high amounts of carriers, reducing significantly their saponin concentration. To overcome these problems and standardise the quality of commercial extracts, the use of reverse phase HPLC techniques is explored. It is shown that RP-HPLC yields consistent and repetitive results and can be easily implemented to control the quality of quillaja extracts. Commercial non-refined extracts contain 190–200 g saponins kg⁻¹ solids, while semi-refined extracts contain 750–800 g saponins kg⁻¹ solids. Also, extracts derived from quillaja bark (traditional raw material) and whole quillaja wood (novel ecological production method) have similar saponin composition and concentration.

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Keywords: *Quillaja saponaria*; *Yucca schidigera*; saponin

INTRODUCTION

Saponin-rich extracts derived from the Chilean tree *Quillaja saponaria* Molina are extensively used as natural foaming agents in food and beverages, food emulsifiers, cosmetics, photographic applications, vaccine adjuvants, etc. Quillaja extracts (QE) are produced from the aqueous extraction of bark (traditional process) or whole wood obtained from pruning operations of existing forests (novel ecological process).¹

Aqueous QE contain saponins, polyphenols, proteins, sugars and salts. They are commercialised as liquid concentrates (550 g solids l⁻¹) or spray-dried powders with three different degrees of purification. (1) Non-refined quillaja extracts contain all water-soluble quillaja solids. These extracts are extensively used as foaming agents in beverages. For some important applications they are blended with extracts of the Mexican plant *Yucca schidigera* (YE) to attain synergistic effects (eg slush-type drinks);² typical blends contain 440 g l⁻¹ QE and 110 g l⁻¹ YE. (2) Semi-refined extracts contain a higher saponin content owing to partial removal of non-saponin compounds via ultrafiltration or affinity chromatography. (3) Highly purified extracts are used as vaccine adjuvants.

At present, the largest use of QE is as foaming agents

in the food and beverage industry. However, saponin concentration is rarely measured, and specifications for commercial products only include foam tests, colour of the preparation and ash concentration. Foam tests are inadequate, since the products can be blended with other low-cost sources of saponins (eg *Y schidigera*, *Saponaria officinalis*) to attain similar foaming properties. Also, QE are frequently diluted with carriers such as lactose, maltodextrin or maltitol (Japanese products), reducing their natural saponin concentration.

Fortunately, quillaja saponins have been thoroughly studied and identified owing to their importance as adjuvants in human vaccines.³ Saponin analysis using RP-HPLC is well established in the vaccine industry and yields repetitive results.⁴ In addition, the chromatographs can be used to identify individual saponins that are characteristic of quillaja extracts. These saponins have been properly identified using MS techniques, and their structures published.⁵

Based on the above, the aim of this work is to extend the use of RP-HPLC techniques to quantify quillaja saponins in commercial products used in foods and beverages, cosmetics, etc. A comparison of extracts derived from bark and biomass as well as saponin concentration in QE/YE blends is presented. Also, the

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Table 1. Commercial quillaja extracts analysed by RP-HPLC

Producer	Commercial name	Raw material	Composition declared by producer
Natural Response, Chile	QL-1000	Quillaja biomass	Liquid, 550g solids l ⁻¹ , non-refined QE
Producer 1, USA	BF 3399	Quillaja bark	Liquid, 550g solids l ⁻¹ , non-refined QE
Natural Response, Chile	QP-1000	Quillaja biomass	Spray-dried powder, non-refined QE
Quest, Ireland	Saponin 5012	Quillaja bark	Spray-dried powder, non-refined QE, lactose
Natural Response, Chile	QL-Ultra	Quillaja biomass	Liquid, 200g l ⁻¹ partially purified QE
Maruzen Pharmaceuticals, Japan	Quillajinin C-100	Quillaja bark	Liquid, 250g l ⁻¹ partially purified QE, 100g l ⁻¹ maltitol
Natural Response, Chile	QP UF 300	Quillaja biomass	Spray-dried powder, 300gk ⁻¹ partially purified QE
Maruzen Pharmaceuticals, Japan	Quillajinin QP-20	Quillaja bark	Spray-dried powder, 50gk ⁻¹ partially purified QE, 950gk ⁻¹ maltodextrin
Schmittman, Germany	DAB 9	Quillaja bark	Spray-dried powder, purified QE
Desert King International, USA	QY-150	Quillaja biomass, yucca	Liquid, 440g l ⁻¹ non-refined QE, 110g l ⁻¹ non-refined YE
Producer 2, USA	BE 0799	Quillaja bark, yucca	Liquid, 440g l ⁻¹ non-refined QE, 110g l ⁻¹ non-refined YE

relation between saponin concentration and foam is discussed.

EXPERIMENTAL

Commercial products

Commercial quillaja extracts were obtained from the companies listed in Table 1. Products BF 3399 (US producer 1) and BE 0799 (US producer 2) have been coded, since the analysis showed possible adulteration. The list includes liquids and spray-dried powders of non-refined and semi-refined QE derived from biomass and bark. Commercial yucca/quillaja blends are represented by products BE 0799 (US producer 2) and QY-150 (Desert King International, USA). Also, to study saponin concentration and foam of yucca/quillaja blends, a quillaja liquid concentrate derived from biomass (QL-1000, Natural Response, Chile) and a yucca liquid concentrate (Foamation 50, Desert King International, USA) were mixed in different proportions on a weight-to-weight basis. Pure Foamation 50 was also analysed to detect chromatographic differences in relation to non-refined QE.

Foam

To study the relationship between saponin concentration determined by RP-HPLC and foam, a simple test was performed. Liquid samples were diluted 10 times in water (1g of sample was added to 9g of distilled water), while powders were diluted at 0.5g in 9.5g of distilled water. A 1ml aliquot of this mixture was added to 350ml of distilled water in a graduated cylinder (diameter 7.6 cm, height 25 cm). After covering with Parafilm, the cylinder was shaken vigorously 30 times and allowed to settle. The foam level in ml was recorded after 30 min.

RP-HPLC analysis

RP-HPLC was carried out based on previous reports.⁴ A Shimadzu model LC-6 chromatograph equipped

with a Shimadzu model SPD-6A UV detector and a C₄ column was used (Vydac, 4.6 cm id × 25 cm length, 5 μm, pore size 300 Å, cat #214TP54). The sample loop was 20 μl. Solvent A was ultrapure water with 1.5 g kg⁻¹ TFA, and solvent B was ultrapure acetonitrile with 1.5 g kg⁻¹ TFA. The gradient was started with 30% of B in A, increasing to 45% in 35 min (linear gradient), and maintained for 5 min at 45%. The flow rate was 1 ml min⁻¹ and detection was performed at 220 nm.

Highly purified QE used as adjuvants in animal vaccines can be used as standards. Based on previous reports,³ the saponin concentration of these preparations can be approximated as 900 g kg⁻¹. Two commercial products can be used for this purpose: Supersap (Natural Response, Chile) and Quil-A (Superfos, Denmark). In this work, Supersap was used at 15 g l⁻¹, ie 13.5 g saponins l⁻¹. Concentrated liquid quillaja samples such as QL-1000 were diluted 10 times with distilled water (1 ml of sample per 9 ml of distilled water) and filtered through 0.2 μm filters before injection. Dry weight of samples was determined in duplicate at 110 °C overnight.

Calculations

In all chromatograms, saponins were considered to elute after 6 min. This is based on the fact that hydrophilic tannins and polyphenolics elute in the early part of the organic solvent gradient.³ The area of saponins in the sample was compared to that of the standard, and the concentration of saponins in g l⁻¹ was estimated as

$$13.5 \times (A_{\text{sample}}/A_{\text{standard}})$$

STATISTICAL ANALYSIS

Data were statistically analysed by an analysis of mean values and statistical significance by Student's *t*-test. For each group, 10 samples were analysed (*n* = 10).

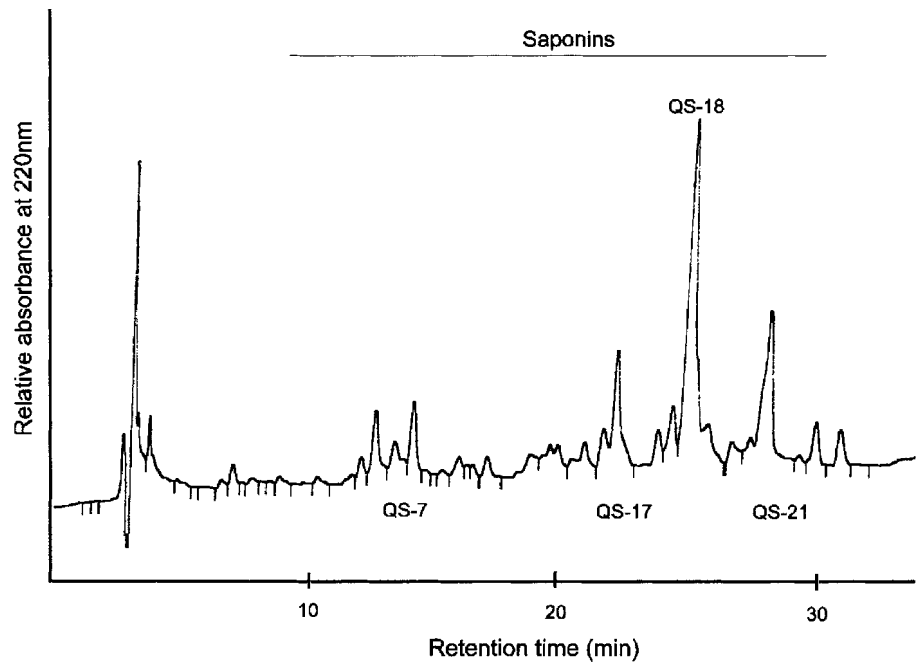


Figure 1. RP-HPLC profile of purified immunological saponins produced by Natural Response, Chile, Supersap 15 g l^{-1} . Characteristic quillaja saponins (QS-7, QS-17, QS-18 and QS-21) can be identified based on previous reports.⁴

RESULTS AND DISCUSSION

Saponin concentration of commercial OE

Fig 1 shows the RP-HPLC profile of Supersap at 15 g l^{-1} used as standard. Based on previous reports, it is possible to identify quillaja saponins QS-7, QS-17, QS-18 and QS-21.⁴ Also, in agreement with previous work, QS-18 is the dominant saponin in all samples analysed.⁴

In a first set of experiments, QE produced using whole biomass (novel ecological process) were compared to those derived from bark (traditional production process). Fig 2 shows a typical analysis for quillaja liquid concentrates derived from whole biomass and bark. The first large signal corresponds to polyphenols; it is followed by a sharp peak due to sodium benzoate used as a preservative. Although there is some

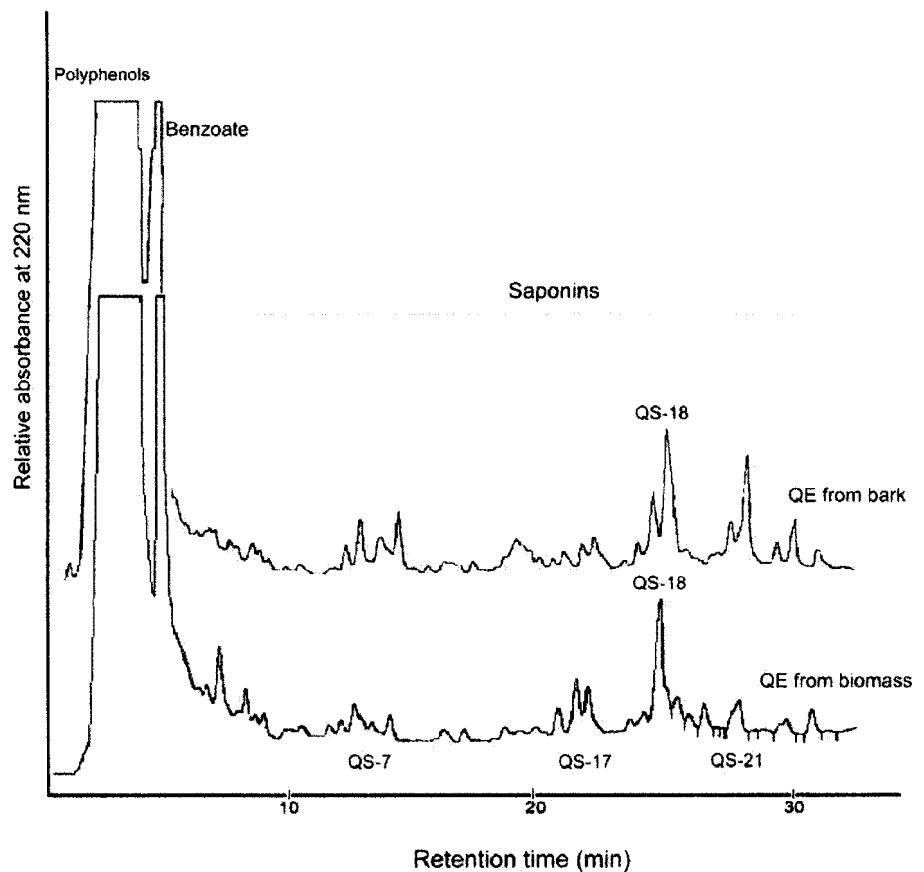


Figure 2. RP-HPLC profiles of quillaja liquid concentrates derived from biomass (QL-1000, Natural Response, Chile) and bark. Reported to contain 550 g l^{-1} non-refined QE. Injected at 55 g l^{-1} .

variability in saponin composition, the dominant saponin is always QS-18 for both biomass and bark extracts. However, bark extracts consistently contain more QS-21 and closely related saponins than biomass-derived products. This is of importance for immunological applications, since QS-21 is used as an adjuvant in human vaccines. Regarding saponin concentration, both biomass- and bark-derived products contain an average of 190 g saponins kg⁻¹ quillaja solids ($P = 0.95$). These results are in agreement with a concentration of 200 g saponins kg⁻¹ quillaja solids reported for non-refined commercial QE derived from bark.³ Thus QE derived from whole biomass and bark have a similar saponin concentration, and differences in saponin composition are only relevant to the production of vaccine adjuvants. This has important ecological considerations, since the use of quillaja biomass renders significantly higher amounts of saponins per tree than bark, ie an average tree yields 0.6 kg of saponins from bark versus 5.1 kg of saponins when all the biomass is used. This is based on yields of 16 kg of bark and 320 kg of biomass per tree and a concentration of soluble solids of 180 and 80 g kg⁻¹ for bark and biomass respectively.

In the case of semi-refined products, both biomass- (QL-Ultra) and bark-derived (Quillajanin C-100) products have a saponin concentration of 750–800 g kg⁻¹ refined solids ($P = 0.95$). This enhancement in saponin purity is due to the use of ultrafiltration membranes or affinity chromatography to remove low-molecular-weight compounds such as sugars and salts. Also, since the products have been treated with food-grade additives to partially remove the polyphenols, the chromatograph shows a smaller pre-saponin peak than that of non-refined QE (chromatograph not shown).

Table 2 shows saponin concentration and foam of liquid and powder commercial samples. The expected saponin concentration for non-refined extracts based on the content of QE reported by the manufacturers and an average concentration of 190 g saponins kg⁻¹ solids is also included. It can be seen that for products

derived from quillaja biomass, as well as those of European and Japanese origin produced using bark, saponin concentration agrees well with the specifications of the producers. However, the RP-HPLC profile in Fig 3 of sample BF 3399 derived from bark (US producer 1) shows that it contains a much lower saponin concentration than a comparable commercial product derived from biomass such as QL-1000, suggesting adulteration with extraneous compounds. This is probably done to reduce costs and compete with extracts derived from quillaja biomass that are significantly cheaper. This is not the case for Japanese or European bark-derived products, which traditionally have been offered at higher prices for applications such as food emulsifiers or surface-active ingredients in photography.

Foam and saponin concentration

The relationship between foam and saponin concentration is shown in Fig 4. For non-refined QE, foam is directly proportional to the concentration of quillaja saponins. It is important to note that the foam test was designed to work at saponin concentrations of 25–30 mg l⁻¹ (concentration of saponins in the test cylinder), which is well below the critical micelle concentration (CMC) of quillaja saponins, estimated as 300–800 mg l⁻¹ depending on the purity of the preparation.⁶ Below the CMC, saponins exist as single molecules that tend to locate at the air–water interface. In this region the amount of foam produced, as well as the reduction of surface tension, is proportional to saponin concentration. Above the CMC, saponins aggregate in micelles of at least 50 molecules each, and the relationship between saponin concentration and foam is no longer linear.

In the case of purified quillaja products such as QL-Ultra or Quillajanin C-100 the foam produced is lower than that of non-refined extracts at the same saponin concentration. For example, QL-Ultra has a saponin concentration of 160 g l⁻¹ and is expected to foam about 240 ml, but it only foams 170 ml. Similarly, Quillajanin C-100 has a saponin concentration of 215 g l⁻¹ and is expected to foam 320 ml, but it

Table 2. Saponin content determined by RP-HPLC of commercial quillaja extracts. Foam values in ml are also included

Commercial name	Composition declared by producer	Saponin concentration determined by RP-HPLC	Expected saponin concentration ^a	Foam height (ml)
QL-1000	550 g solids l ⁻¹ , non-refined QE	106 g l ⁻¹	105 g l ⁻¹	160
BF 3399	550 g solids l ⁻¹ , non-refined QE	41.3 g l ⁻¹	105 g l ⁻¹	60
BE 0799	440 g l ⁻¹ non-refined QE, 110 g l ⁻¹ non-refined YE	71.5 g l ⁻¹	84 g l ⁻¹	100
QY-150	440 g l ⁻¹ non-refined QE, 110 g l ⁻¹ non-refined YE	82 g l ⁻¹	84 g l ⁻¹	130
QL-Ultra	200 g l ⁻¹ partially purified QE	160 g l ⁻¹	—	170
Quillajanin C-100	250 g l ⁻¹ partially purified QE, 100 g l ⁻¹ maltitol	215 g l ⁻¹	—	210
QP-1000	Non-refined QE	200 g kg ⁻¹	190 g kg ⁻¹	240
QP UF 300	300 g kg ⁻¹ partially purified QE, 700 g kg ⁻¹ lactose	230 g kg ⁻¹	—	230
Saponin 5012	Bleached non-refined QE and lactose	200 g kg ⁻¹	190 g kg ⁻¹	260
Quillajanin QP-20	50 g kg ⁻¹ purified QE, 950 g kg ⁻¹ maltodextrin	50 g kg ⁻¹	—	60
DAB 9	Purified QE	320 g kg ⁻¹	—	320

^a For non-refined QE the expected saponin concentration is based on an average content of 190 g saponins kg⁻¹ QE.

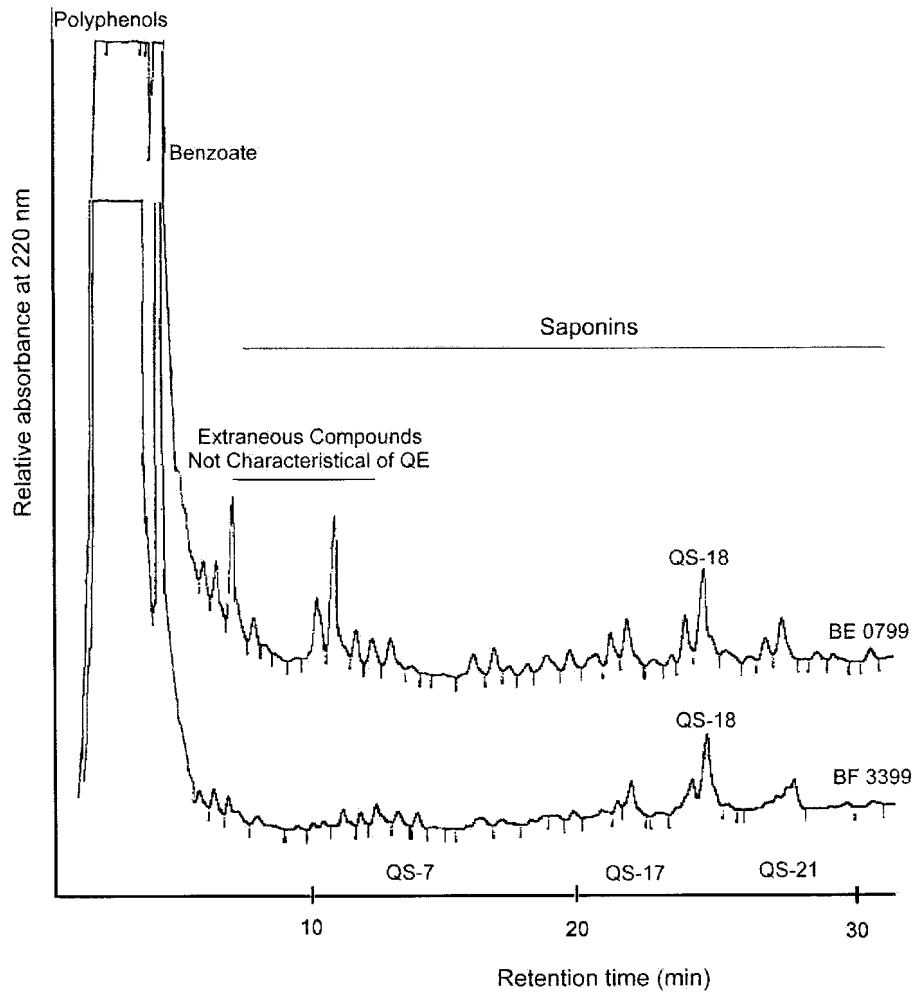


Figure 3. RP-HPLC profiles of sample BF 3399 (US producer 1), reported to contain 550 g l⁻¹ non-refined QE, and sample BE 0799 (US producer 2), reported to contain 440 g l⁻¹ QE and 110 g l⁻¹ YE. Both samples injected at 55 g l⁻¹.

only foams 210 ml. This suggests that some compounds that are removed in the purification steps also contribute to foam formation and stability (eg polyphenols, proteins).

Yucca/quillaja blends

For some important applications, QE and YE are

used together to attain synergistic effects. Since YE are cheaper than QE, it is very important to determine the exact concentration of quillaja saponins in the blends. This is not possible based on foam levels, since the foam produced by different yucca/quillaja blends remains relatively constant even at high levels of YE. For example, Table 3 shows that a

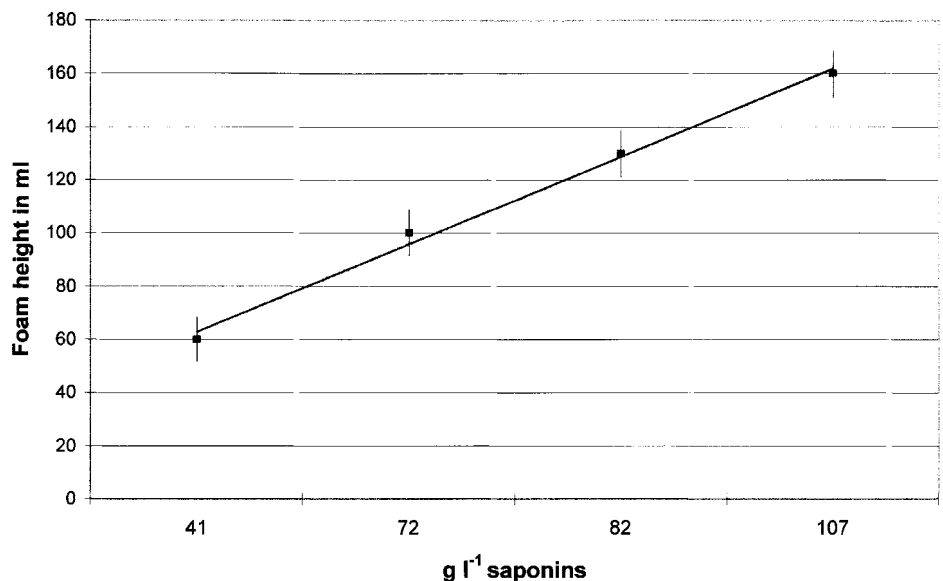


Figure 4. Foam produced by samples of non-refined QE with different contents of quillaja saponins.

Table 3. Foam produced by different quillaja/yucca blends: quillaja extract, QL-1000 (Natural Response, Chile); yucca extract, Foamation 50 (Desert King International, USA)

Product	Foam (ml)
<i>Pure products</i>	
Foamation 50, 550 g l ⁻¹ non-refined YE	120
QL 1000, 550 g l ⁻¹ non-refined QE	160
<i>Blends</i>	
440 g l ⁻¹ non-refined QE/110 g l ⁻¹ non-refined YE	150
330 g l ⁻¹ non-refined QE/220 g l ⁻¹ non-refined YE	140
90 g l ⁻¹ non-refined QE/460 g l ⁻¹ non-refined YE	120

product containing 440 g l⁻¹ QE and 110 g l⁻¹ YE has a foam level similar to that of a product containing 550 g l⁻¹ QE.

Conveniently, under the RP-HPLC conditions tested, yucca saponins are not detected, probably because they are steroidal and need different conditions to be adequately separated. Thus the chromatographs can be used to quantify exactly the concentration of quillaja saponins present in the products. For example, sample BE 0799 (US producer 2), reported to contain 440 g l⁻¹ of non-refined QE and 110 g l⁻¹ of non-refined YE, should contain 84 g l⁻¹ of saponins. However, the RP-HPLC profile shown in Fig 3 yields a concentration of only 71.5 g l⁻¹ of saponins, which corresponds to 376 g l⁻¹ of non-refined QE and 174 g l⁻¹ of non-refined YE. Again this is the case of a traditional US producer that uses bark as raw material. Another feature of the chromatograph of this sample is that the dominant saponins appear at lower retention times (eg 8 min) and not in the QS-18 region as expected. Based on previous reports, it seems that the sample may have been treated with a reducing agent or subjected to strong alkaline conditions.⁷ Under these conditions the sugar chains of the more hydrophobic saponins are hydrolysed and the saponin composition shifts to more hydrophilic saponins (lower retention times).

CONCLUSIONS

RP-HPLC can be used to characterise commercial QE based on their saponin concentration. Extracts derived from whole quillaja biomass have a similar saponin concentration and composition as those derived exclusively from bark. This is of ecological importance, since exploitation of whole quillaja biomass is a sustainable method of production of quillaja extracts.

The analysis of commercial samples shows that non-refined QE contain 190–200 g saponins kg⁻¹ solids, while semi-refined QE contain 750–800 g saponins kg⁻¹ solids. The analysis also shows that some US producers that use bark as their source of raw material adulterate their products with extraneous materials and/or low-cost saponin extracts.

Foam can be correlated with saponin concentration only for non-refined QE. However, if the products are blended with lower-cost saponins, foam remains relatively constant and is not a good indicator of quillaja saponin concentration. Also, if the products are purified to remove non-saponin compounds, part of the foaming capacity is lost. In both cases, only RP-HPLC can detect the exact amount of quillaja saponins.

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