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Quillaja Saponin Characteristics and Functional Properties

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Quillaja saponins, interfacial behavior, colloidal properties, technofunctionality, complexation

Abstract

Consumer concerns about synthetically derived food additives have increased current research efforts to find naturally occurring alternatives. This review focuses on a group of natural surfactants, the *Quillaja* saponins, that can be extracted from the *Quillaja saponaria* Molina tree. *Quillaja* saponins are triterpenoid saponins comprising a hydrophobic quillaic acid backbone and hydrophilic sugar moieties. Commercially available *Quillaja* saponin products and their composition and properties are described, and the technofunctionality of *Quillaja* saponins in a variety of food, cosmetic, and pharmaceutical product applications is discussed. These applications make use of the biological and interfacial activities of *Quillaja* saponins and their ability to form and stabilize colloidal structures such as emulsions, foams, crystallized lipid particles, heteroaggregates, and micelles. Further emphasis is given to the complexation and functional properties of *Quillaja* saponins with other cosurfactants to create mixed surfactant systems, an approach that has the potential to facilitate new interfacial structures and novel functionalities.

SAPONINS

Saponins are secondary plant metabolites that are widely found in more than 500 plant species, with 0.1%–10% saponins present in the plant extracts (Güçlü-Üstündağ & Mazza 2007, Oakenfull 1981, Pagureva et al. 2016). Saponins can be derived from various parts of plant materials such as seed, root, leaf, fruit, stem, and bark (Cheok et al. 2014), and they can be extracted from soybean, chickpea, spinach, ginseng, sugar beet, sunflower, oats, and *Quillaja saponaria* Molina trees (Güçlü-Üstündağ & Mazza 2007, Oakenfull 1981). Further saponin sources have been identified and include, for example, marine animals such as starfish (Oakenfull 1981). The word saponin is derived from the Latin word sapo, which translates to soap, and it refers to the interfacial activity observed for this class of molecules (Hostettmann & Marston 1995). Saponins are also often sensory active, i.e., they often have a characteristic bitter taste, albeit some sweet and neutral-tasting saponins have also been reported (Güçlü-Üstündağ & Mazza 2007).

On the basis of their chemical structure, saponins can be classified by their hydrophobic backbone, which is either a steroid (furostan; **Figure 1*a***) or triterpene (β-amyrin type; **Figure 1*b***) sugar-free aglycone; also often referred to as sapogenin. One or more sugar moieties are typically attached to the sapogenin giving rise to a specific amphiphilic nature of the molecule (Hostettmann & Marston 1995). The varying structures of sapogenins and attached sugar moieties have been described in detail by Hostettmann & Marston (1995) and give rise to a sugar-based classification scheme. One sugar moiety, for example, attached at the C-3 position (**Figure 1**) denotes a monodesmosidic saponin, whereas bidesmosidic saponins contain two attached sugar chains and tridesmosidic saponins contain three attached sugar chains. For bidesmosidic saponins, the sugar moieties of steroid saponin structures are usually attached at the C-3 and C-26 positions (**Figure 1*a***), whereas in triterpenes, sugars are often attached at the C-3 and C-28 positions (**Figure 1*b***). The most commonly known saponins are of a bidesmosidic nature (Güçlü-Üstündağ & Mazza 2007). However, there are also a few saponins that have more sugar chains attached (Güçlü-Üstündağ & Mazza 2007), such as the recently identified tridesmosidic saponins from the tree *Koelreuteria paniculate* Laxm. (Mostafa et al. 2016). The sugar moieties are usually composed of two to five monosaccharides such as D-glucose, D-galactose, D-glucuronic acid, D-galacturonic acid, L-rhamnose, L-arabinose, D-xylose, and D-fucose (Hostettmann & Marston 1995).

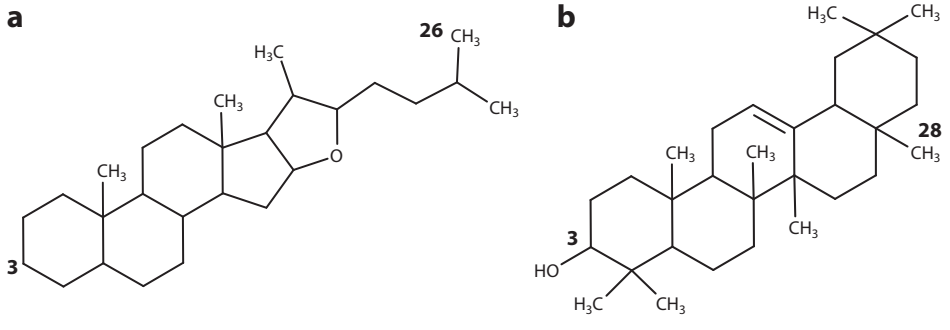

Figure 1

Basic chemical structure of the hydrophobic backbone of saponins that have either a (*a*) steroid (furostan) or (*b*) triterpene (β-amyrin type, oleananes) aglycone. Modified from Hostettmann & Marston (1995).

QUILLAJA SAPONINS

Currently, only a few commercially available saponin products are available, and these are almost exclusively derived from *Quillaja saponaria* Molina, a tree that is indigenous to Chile (Dalsgaard 1978). The growth of the evergreen *Quillaja saponaria* Molina trees is regionally limited. On the basis of specific climate requirements, the native *Quillaja saponaria* trees grow only between latitudes 30° S and 38° S (Donoso et al. 2011), as referenced by Schlotterbeck and coauthors (Schlotterbeck et al. 2015).

The name *Quillaja* comes from the Chilean word quillean, which means to wash, and it relates to the detergent activity of *Quillaja* saponins. The first enriched saponin extract from *Quillaja saponaria* Molina bark was obtained by Dalsgaard (1978), who subsequently named this extract Quil A. Since then, the demand for *Quillaja* saponins for industrial applications has greatly increased because of their wide applicability in the food, cosmetic, and pharmaceutical industries (see section titled Industrial Use of *Quillaja* Saponins). The estimated amount of biomass from *Quillaja* trees required for industrial applications by 2019–2020 ranges from 40,000 to 48,000 tons (Schlotterbeck et al. 2015). This high demand is currently fueling research to find alternative sources or develop formulations that require fewer *Quillaja* saponins as active ingredients.

Initially, only the bark of *Quillaja* trees was used as a saponin source, resulting in overexploitation of the *Quillaja* bark (Rundel & Weisser 1975). This led to a drastic reduction in the number of naturally growing *Quillaja* trees. In 1944, the Chilean National Forestry enacted a law to limit the exploitation of *Quillaja* trees (San Martín & Briones 1999). The limited availability of *Quillaja* bark saponins led to investigations of the use of other parts of the *Quillaja saponaria* Molina trees as saponin sources as well as research into improved extraction processes (Gaete-Garretón et al. 2011). The extracts of the wood as well as of the leaves from *Quillaja* trees were found to also contain high saponin concentrations (on average 2.58% saponins), and these saponins were found to have similar compositions and saponin patterns as extracts that had been obtained only from the bark of the *Quillaja saponaria* trees (San Martín & Briones 1999, 2000; Schlotterbeck et al. 2015). Today, along with the bark, the wood and leaves are also used as *Quillaja* saponin sources to reduce deforestation of *Quillaja* trees in Chile (San Martín & Briones 1999, Schlotterbeck et al. 2015) as well as to reduce costs (San Martín & Briones 2000).

Chemical Structure

To date, approximately 100 different *Quillaja* saponin structures have been identified (Nord et al. 2001) and in recent years many naturally occurring saponin structures (Guo & Kenne 2000), as well as new synthetically derived variants (Fernández-Tejada et al. 2015), have been characterized. The variety of *Quillaja* saponins consists of different triterpene structures (Guo & Kenne 2000) with varying molecular weights (~1,500–2,400 g/mol) and degrees of hydrophobicity (Tippel et al. 2016b). The structures of saponins in *Quillaja saponaria* bark extract were analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC). Results of these studies show that the extract contains a number of key saponin fractions, known as QS-7, QS-17, QS-18, and QS-21, that differ mainly in their monosaccharide composition (Kensil et al. 1991).

The basic chemical structure of *Quillaja* saponins comprises a hydrophobic triterpene quillaic acid and usually two attached sugar moieties (**Figure 2**) (Higuchi et al. 1986). In general, there is only one carboxylic acid group (β-D-glucuronic acid) attached at position R_1 (**Figure 2**) in the basic *Quillaja* saponin molecules, providing a single chargeable group. For instance, the *Quillaja* saponin fraction QS-21 has a pK_a value of 7.2 ± 0.1 (Pedebos et al. 2014), which is indicative of a weak or medium acid. Analyzing the saponin pattern of a *Quillaja saponaria* bark extract revealed a

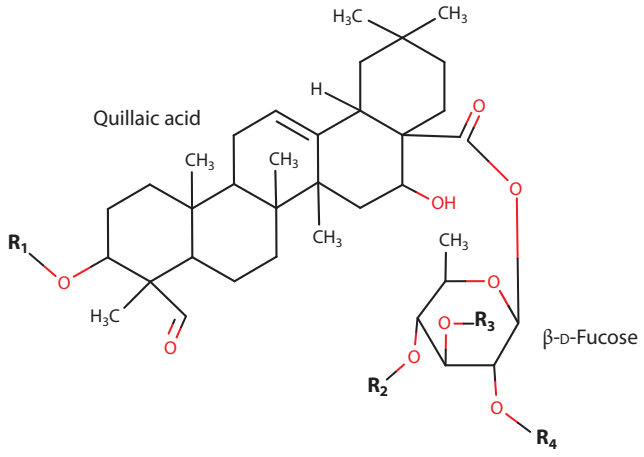

Figure 2

General chemical structure of *Quillaja* saponin with quillaic acid as aglycone. $R_{1–4}$ represent saccharide or acyl residues. Modified with permission from Nord et al. (2001).

similar peak pattern to the commercially available Quil A product, confirming that their extraction source stemmed from the *Quillaja saponaria* Molina bark (Kensil et al. 1991). Among the different saponin fractions, the saponin structure QS-18 predominates in *Quillaja saponaria* Molina extracts and can thus be used for authentication (Kensil et al. 1991, San Martín & Briones 2000). Another widely studied saponin fraction, QS-21 (see **Figure 3**), is composed of one branched trisaccharide and one unbranched tetrasaccharide residue (Sun et al. 2009). At residue R_3 of the basic *Quillaja* saponin structure (**Figure 2**), QS-21 has a fatty acid group attached to it (**Figure 3**) (Sun et al. 2009).

Extraction and Fractionation

Quillaja saponin extracts can be classified according to their saponin content into nonrefined, semirefined, and highly refined *Quillaja* saponin products (San Martín & Briones 2000). To obtain the saponins from *Quillaja* biomass, numerous extraction techniques can be used depending on the intended purity. Simple, nonrefined *Quillaja* saponin products are produced by coarsely shredding the biomass and using water as an extraction solvent (Schlotterbeck et al. 2015). On the basis of composition outlined in the following section, semirefined *Quillaja* products are obtained from the nonrefined ones by removing low-molecular-weight compounds, sugars, and salts via ultrafiltration or affinity chromatography (San Martín & Briones 2000).

Further extraction techniques include maceration, reflux, and Soxhlet extraction, ultrasound- and microwave-assisted extractions, and pulse electric field, enzyme-assisted (Cheok et al. 2014, Schlotterbeck et al. 2015), and solid-phase extractions (Nord & Kenne 1999). *Quillaja saponaria* Molina bark or biomass are extracted using a variety of solvents such as water or acids (Gaete-Garretón et al. 2011, Maier et al. 2015b), methanol (pure or diluted) (Higuchi et al. 1986, Nord & Kenne 1999), or mixtures thereof (Guo et al. 1998, Schlotterbeck et al. 2015). Aqueous extraction of *Quillaja* biomass can be enhanced by using ultrasound-assisted processes or by using smaller biomass chips to reduce the required time and temperature to obtain *Quillaja* bioactives (Gaete-Garretón et al. 2011). The authors showed that the extraction of *Quillaja* chips using an ultrasound-assisted aqueous extraction process at 20°C for 20 min yielded similar amounts of

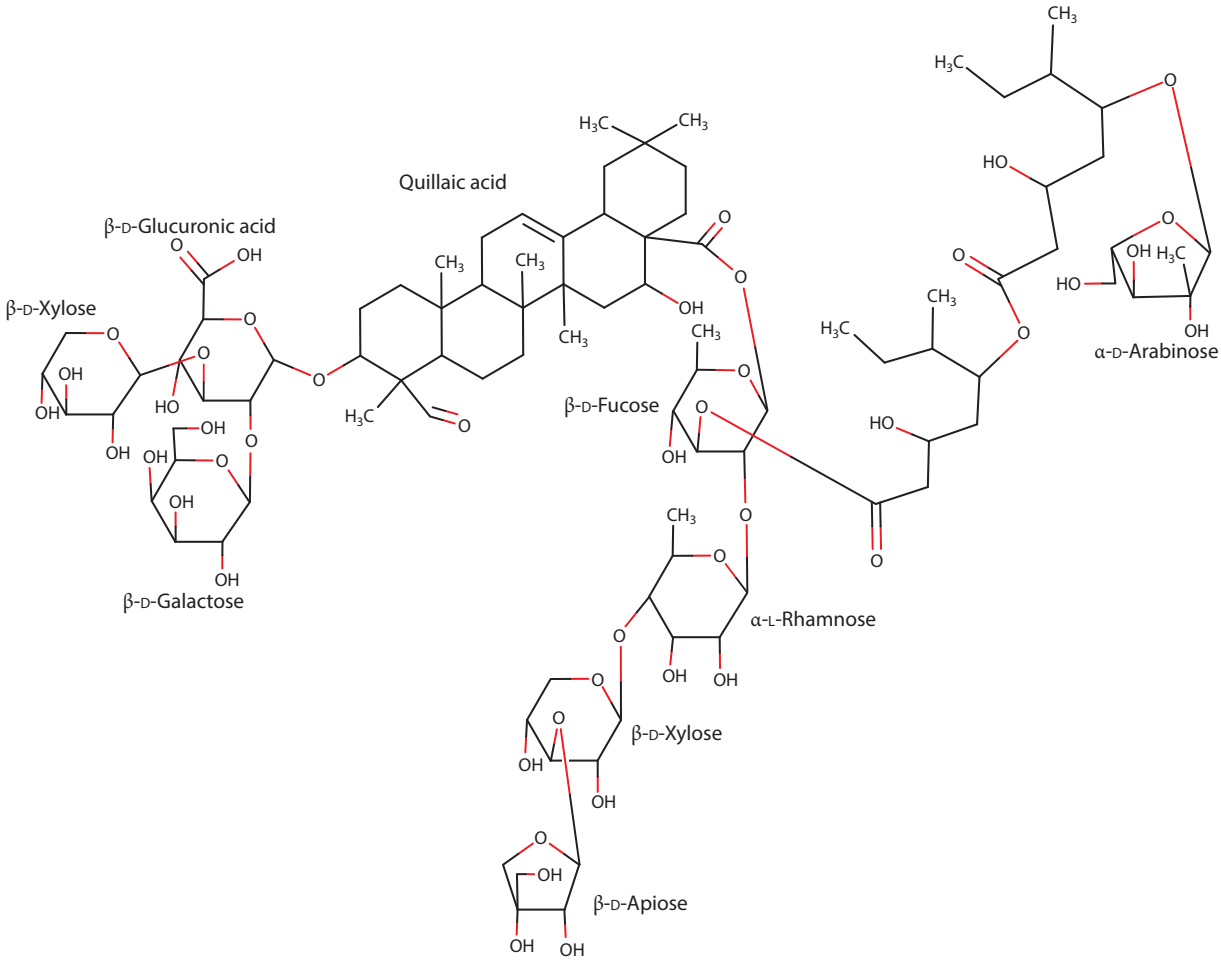

Figure 3

Chemical structure of QS-21, which is one of the main fractions of the commercial *Quillaja* saponin extract Quil A. Modified with permission from Sun et al. (2009).

bioactives from *Quillaja saponaria* Molina biomass to those yielded using the conventional method of using *Quillaja* chips extracted in water at 60°C for 3 h (Gaete-Garretón et al. 2011). The ultrasound effect was attributed to cavitation that led to bubble formation, facilitating transport of the hydrophobic saponins from cells into the solvent cells by increasing the permeability of the *Quillaja* plant tissue (Gaete-Garretón et al. 2011). An improvement in saponin refinery was also achieved by extracting the tree biomass in April because the trees naturally accumulate more saponins (Schlotterbeck et al. 2015).

Quillaja saponaria extracts can be further refined by fractionation; a useful tool to produce a range of *Quillaja* extracts that vary in composition and thus exhibit different technofunctionalities. Nord et al. (2001) studied the different *Quillaja* saponin structural variants, revealing theoretically 2,880 possible structures. Further separation of *Quillaja* extracts into fractions or individual saponins was done by column chromatography on silica gel (Guo et al. 1998, Higuchi & Komori 1987) and/or normal and reverse phase column chromatography (Guo et al. 1998,

Higuchi et al. 1986, Nord & Kenne 1999). The use of preparative HPLC as a common chromatographic method to separate individual fractions has been widely reported in the literature (Maier et al. 2015a,b; Nord et al. 2001; Nord & Kenne 1999). A new method to separate and identify *Quillaja* saponin structures was developed by Wang et al. (2008), who used a hydrophilic interaction chromatography (HILIC) system [HILIC × HILIC-Q-TOF-MS (quadrupole time-of-flight mass spectrometry)]. Surprisingly, highly purified *Quillaja* saponin products were found to often have reduced functional properties, including foam stabilization abilities, a fact that has been suggested to be due to the removal of other functional compounds during extraction and fractionation procedures that act synergistically, in particular polyphenols or proteins (San Martín & Briones 2000).

Composition

Quillaja saponin extracts have been made available as a number of different commercial products that vary in their purity, and thus, in their composition, color, and functionality (Copaja et al. 2003, Resnik et al. 2005, San Martín & Briones 2000, Wojciechowski 2013). The differences in *Quillaja* saponin products that are commercially available are summarized in **Table 1**. Previously, San Martín & Briones (2000) listed commercial *Quillaja* extracts of different producers; however, the products and producers have substantially changed since 2000. In previous years, the saponin content of plant extract solutions was approximated by the foamability of the respective extracts. More recently, spectrophotometric and chromatographic methods were established (Cheok et al. 2014, San Martín & Briones 2000). RP-HPLC (San Martín & Briones 2000) and ultrapressure liquid chromatography have been employed to provide a more accurate quantification of saponins (Cheok et al. 2014). In addition to saponins, tannins, sugars, calcium oxalates, phenolic compounds, salts, and other components are present in the extracts, depending on manufacturing procedures used (Nord et al. 2001, San Martín & Briones 2000). Product properties have been found to depend on the purity of the extract. For instance, a highly purified *Quillaja* saponin extract (e.g., SuperSap from Desert King, >90% saponins) has a white color, whereas a less refined *Quillaja* saponin product (e.g., *Quillaja* Dry 100 NP from Desert King, 26% saponins) has a reddish-brown color (Resnik et al. 2005, Stanimirova et al. 2011, Vinarova et al. 2015). The color is indicative of the presence of polyphenolic compounds (Maier et al. 2015a). Recently, the nature of these polyphenolic constituents in *Quillaja* saponin extract has been identified and the majority was found to be (+)-piscidic acid (75%–87% of total phenolics) (Maier et al. 2015a). Other minor phenolic compounds such as derivatives of *p*-coumaric acid accounted for approximately 8%–20% of the total phenolic profile in *Quillaja* saponin extract, whereas vanillic acid and glucosyringic acid derivatives constituted only up to 7% of the total phenolic composition (Maier et al. 2015a).

Furthermore, the degree of purification influences the ionic character of the *Quillaja* saponin products. A less refined *Quillaja* extract comprising 8%–25% sapogenins (Sigma 84510, Sigma-Aldrich) was found to behave much like an ionic surfactant, whereas a highly purified *Quillaja* saponin product (SuperSap, 91% saponin, Desert King) acted like a nonionic surfactant (Wojciechowski 2013). The authors attributed the difference in ionic character to the weak acidic behavior of the less refined *Quillaja* saponin extract Sigma, with half of the molecules having a proton-dissociable group. In comparison, the highly purified *Quillaja* saponin product SuperSap behaved like a nonionic surfactant in an acid–base titration test, indicating only a few proton-dissociable groups (Wojciechowski 2013).

As of late, some commercial *Quillaja* saponin products are now being blended with cheaper and more readily available substances, for instance, a saponin extract from the Indian plant *Madhuca longifolia* L. In the respective study, the authors did not provide details about the product suppliers

Table 1 Composition of commercially available *Quillaja* saponin products

Producer	Commercial name	Raw material	Saponin content (%)	Composition	pH	Appearance	Reference
AppliChem GmbH	Saponin	*Quillaja* bark	10–14[a]	Not specified	4–7	Beige solid	**http://www.applichem.com**
Desert King	Andean QDP Ultra Organic	*Quillaja* wood	>60.0	0.01–0.5% fiber 0.05–0.5% fat 1.0–7.0% moisture <3.38% citric acid 3.5–7.0% protein 4.6% phenolic compounds 6.5–12.0% ash 73.0–87.9% carbohydrates	3.9–4.9[b]	Light amber	**http://desertking.com** Reichert et al. 2015 Maier et al. 2015a
Desert King	Vax Sap	*Quillaja* bark	>90	ND	5.5 ± 1.0[a]	White	**http://desertking.com**
Desert King	QL 1000	ND	9	ND	ND	Dark-brown liquid	Stanimirova et al. 2011
Desert King	QL Ultra	ND	13	ND	ND	Yellowish liquid	Stanimirova et al. 2011
Desert King	Quillaja Dry 100	ND	25.6	ND	ND	Brown solid	Golemanov et al. 2012
Desert King	SuperSap	ND	91	8% moisture, traces of electrolytes and other organic ingredients	ND	White solid	Stanimirova et al. 2011
Ingredion	Q-Naturale® 200V	Whole plant	13–16	78–80% moisture <5% ash on dry basis	3–4.2	Light amber liquid	**http://www.ingredion.us**
Sigma-Aldrich	S4521	*Quillaja* bark	20–35[c]	ND	ND	Solid	**http://www.sigmaaldrich.com**
Sigma-Aldrich	S7900	*Quillaja* bark	20–35[c]	ND	ND	Light yellow solid	**http://www.sigmaaldrich.com**

[a] 10% solution.
[b] 20% solution.
[c] Given as sapogenin content.
Abbreviation: ND, no data.

to avoid disclosure of confidential information (Thalhamer & Himmelsbach 2014). To identify blended *Quillaja* saponin products, a characteristic phenolic pattern was suggested as a control (Maier et al. 2015a,b). A unique phenolic composition and new components, namely Quillajasides A and B, were found to be specific for the *Quillaja saponaria* Molina source (Maier et al. 2015a,b). Further methods for authentication used the fatty acid domain (**Figure 3**) that had been reported to be present only in saponins from *Quillaja saponaria* Molina (Kite et al. 2004). In general, it should be noted that for scientific studies it is of substantial importance to know the purity and composition of commercial saponin products, especially when trying to assess technofunctional properties.

The processing and storage of the saponins also appear to have an influence on their composition because of potential degradation of extract compounds. For example, Gutbier et al. (1921) determined turbidity of a *Quillaja* solution during storage and reported increases over time. At the time, the authors did not provide any explanation for this phenomenon, nor did they record the time they stored the solution. Overall, there is little information available about the degradation or stability of saponins (Park et al. 2005). For instance, Park et al. (2005) opted to refer in their study to the datasheet of the saponin product supplier. The supplier Desert King International provides information about the heat stability conditions of a *Quillaja* saponin product (8 h at 100°C, 1–5 h at 120°C) and its pH stability (pH 2.5–8) (Park et al. 2005), but as of today there have not been any systematic investigations of potential polymerization or hydrolysis reactions that may occur. Clearly, there is a lack of systematic studies concerning stability and degradation of *Quillaja* extract ingredients, and this work needs to be carried out in the future to formulate and produce products in a consistent manner.

Biological Activity

The biological activity of *Quillaja* saponins was investigated as early as in 1976 by Ebbesen et al. (1976). The authors reported anticancer properties in mice. In the past few decades, several other biological activities of *Quillaja* saponins have been identified. For instance, *Quillaja* saponins exhibited a high hemolytic activity (Hassan et al. 2010) and required low minimum inhibitory concentrations (0.1 mg/mL) against *Staphylococcus aureus*, *Salmonella typhimurium*, and *Escherichia coli*. Notable differences in antibacterial activity among several saponin sources were observed, i.e., between saponins from guar, *Quillaja saponaria*, *Yucca schidigera*, and soybean (Hassan et al. 2010). In addition to their antimicrobial activity, *Quillaja* saponins can modify the surface of bacteria such as *Pseudomonas* sp. OS2, leading to enhanced bacterial metabolism of halogenated phenolic compounds such as 4-fluorophenol (Kaczorek et al. 2016). Further cell surface modifications were investigated with yeast cells. The authors reported that *Quillaja* saponins led to increased cell membrane permeability (Berlowska et al. 2015) that can be used to promote the release of amino acids (Berlowska et al. 2017).

Anti-inflammatory activities of *Quillaja* extracts (Sarkhel 2015) or individual components thereof such as quillaic acid were determined in in vivo studies with mice that had been previously inflamed by arachidonic acid or 12-*O*-tetradecanoylphorbol-13 acetate (Rodríguez-Díaz et al. 2011). However, aqueous *Quillaja* saponin extract fed to mice that had a carrageenan-induced paw edema showed an only slightly lower anti-inflammatory efficiency than the commonly given drug indomethacin (Sarkhel 2015).

The most well-established biological activities of *Quillaja* saponins are their adjuvant and immunostimulating properties (Güçlü-Üstündağ & Mazza 2007). Adjuvants are defined as substances that are added to vaccines "to enhance the immune response of an antigen" (Tharabenahalli-Nagaraju et al. 2014). Because of the adjuvant activity of *Quillaja* saponins, a large number of research studies have focused on this apparent immunomodulatory activity

(Dalsgaard 1978, Kersten & Crommelin 2003, Marciani 2015, Walkowicz et al. 2016). The adjuvant activity has been mainly attributed to the functional groups of *Quillaja* saponins. The central glycosidic linkage is a crucial factor, and any alteration of this group led to a reduction or complete elimination of all adjuvant activity. Changes in stereochemistry of saponin molecules resulted in efficient adjuvant activity but dose-limited toxicity. A changed conformational flexibility led to intermediate adjuvant efficiency as determined for modified QS-21 analogs in in vivo studies with mice (Walkowicz et al. 2016). The most promising form of *Quillaja* saponins as immunomodulators is a micellar assembly composed of Quil A, cholesterol, and phospholipids. These assemblies are also referred to as immunostimulating complexes (ISCOMs) (Morein et al. 1984). The amphiphilic structure of hydrophilic carbohydrates and hydrophobic quillaic acid was suggested to induce the formation of a nonbilayered structure characteristic of ISCOMs (Kersten & Crommelin 2003). In ISCOMs, immunostimulating components such as vaccines are incorporated into the hydrophobic core (Demana et al. 2004). These systems have been used for cancer immunotherapy since 2002 (Kenney et al. 2002).

For most applications, the concentration of *Quillaja* saponins administered is of importance because, at higher concentrations, *Quillaja* saponins may display toxic effects (Marciani 2015, Walkowicz et al. 2016). For example, the addition of 450 mg/kg *Quillaja* saponins to the dry diet of carp (~26 μg *Quillaja* saponin per carp) for four weeks did not have any toxic effect on the tested fishes (Serrano 2013). However, acute toxicity was determined against olive flounder in a dose-dependent manner with a mortality rate of 5% at low concentration (16 μg *Quillaja* saponin product/fish). The mortality rate climbed to 95% when injections of 500 μg *Quillaja* saponin extract were given to the fish (Tharabenahalli-Nagaraju et al. 2014). Analyzing the dead fish revealed damaged red blood cells and kidney tubule epithelium cells (Tharabenahalli-Nagaraju et al. 2014). Another instance of cell membrane damage in the midgut epithelium of aphids was attributed to the insecticidal activity of *Quillaja* extract (De Geyter et al. 2011). As for fish, a dose-dependent insecticidal activity was determined for *Quillaja* saponins with 46% and 100% mortality rate of aphids when 1 and 3 mg/mL of *Quillaja* saponins were added to their diet, respectively (De Geyter et al. 2011).

According to a meeting report by the World Health Organization (WHO), *Quillaja* saponins are promising substances for immunostimulation and adjuvant applications. Kenney et al. (2002) reported that human clinical testing was underway or to be conducted in the near future (Kenney et al. 2002). However, in our literature research, we did not find any in vivo human studies with *Quillaja* saponins yet. Nevertheless, there are a few studies investigating the biological activity of *Quillaja* saponins on human cells in in vitro studies (Roner et al. 2007) and in in vivo studies using animals (Tam & Roner 2011). Antiviral activity was reported at a concentration of 0.01 mg/mL *Quillaja* saponin extract that caused, for example, inactivation of 50% of vaccinia virus in human cells. A cell death rate of 50% was determined for human cells when 0.9 mg/mL of *Quillaja* saponin extract was added. The authors concluded that *Quillaja* saponin extract disrupted the viral envelope and the capsid proteins and thus provided antiviral activity at low concentrations below human cell toxicity levels (Roner et al. 2007). The inhibition of virus–host attachment by disrupting the cellular virus membrane by *Quillaja* saponin administration effectively reduced a rhesus rotavirus infection that would have induced diarrhea in mice and thus decreased the mortality of mice that were infected with the rhesus rotavirus (Tam & Roner 2011).

KEY INTERFACIAL PROPERTIES

The interfacially active saponins in *Quillaja* extracts are composed of two hydrophilic sugar chains and one hydrophobic aglycone, creating a three-block structure with distinct

hydrophilic–hydrophobic–hydrophilic properties (Kersten & Crommelin 2003). The block design provides the ability to self-assemble in the bulk phase and gives rise to other interfacial properties described in more detail below.

Interfacial Activity

The interfacial tension is indicative of a force that causes molecules that are at the interface to remain associated with the respective bulk phase. It is a direct result of the tendency to reduce the overall free energy of the system by keeping phase boundary areas at a minimum (Hiemenz & Rajagopalan 1997). The interfacial tension can be decreased by the presence of components that have a high affinity for interfaces, i.e., molecules that tend to adsorb to interfaces to optimize interactions of their hydrophilic blocks with hydrophilic phase molecules and their hydrophobic blocks with hydrophobic phase molecules (McClements 2005).

Quillaja saponins exhibit high interfacial activity and consequently decrease tensions efficiently at the air–water (Yang et al. 2013), tetradecane–water, and (olive) oil–water interfaces (Tippel et al. 2016b, Wojciechowski 2013). The rate of interfacial tension reduction increases the more polar the nonaqueous phase is (the polarity increases as follows: air < tetradecane < olive oil) (Wojciechowski 2013). The interfacial tension reduction of *Quillaja* saponins is summarized in **Table 2** for different *Quillaja* saponin products, the type of interface, concentration, and solution conditions such as pH, time, and temperature as well as the analytical method used.

The time needed for the interfacial tension to reach equilibrium values after an interfacially active compound has been added to the system is known as the adsorption kinetics, and thus represents an important parameter in interfacial film formation and surfactant performance (McClements 2005). The time needed for *Quillaja* saponin interfaces to reach an equilibrium interfacial tension has been reported to vary from a few minutes (determined via a Langmuir trough) to 45 min (determined via drop shape analysis) (Wojciechowski et al. 2014c). In comparison to proteins that typically require hours to achieve equilibrium adsorption conditions, saponins are therefore fast-adsorbing surfactants (**Figure 4*a***). The steady-state or equilibrium interfacial tension was found to vary between ~35.0 and 37.0 mN/m at the air–water surface (Wojciechowski et al. 2011) and was ~5.0 mN/m at a medium-chain triglyceride (MCT)–water interface (**Table 2**) (Yang et al. 2013). The values are substantially lower than those for proteins [e.g., 49.5 mN/m at the air–water interface (Bezelgues et al. 2008) and ~13.0 mN/m at the oil–water interface (Joshi et al. 2012) for whey protein isolate (WPI)] and are close to those of small molecule surfactants, e.g., polysorbates (Posocco et al. 2016).

Environmental conditions have been reported to influence the adsorption kinetics of *Quillaja* saponins (**Table 2**). The use of either a phosphate buffer (pH 7.4, ionic strength: 0.1 M) or acidified water (pH 2.6) was found to increase the interfacial activity of *Quillaja* saponins, leading to a decrease in interfacial tension compared to ultrapure water (pH 6.2). The enhanced interfacial tension decrease was attributed to screened charges of the glucuronic acid moieties of *Quillaja* saponins, leading to a decreased electrostatic repulsion between the saponin molecules and thus enhancing their adsorption properties (Wojciechowski et al. 2011). Crude as well as highly purified *Quillaja* saponin products had a similar adsorption behavior indicative of the saponins being the dominating surface-active components in *Quillaja* extracts (Mitra & Dungan 1997, Pagureva et al. 2016, Yang et al. 2013). The low diffusion coefficient (D) of *Quillaja* saponins in short-term air–water surface adsorption studies (<30 s, $D = 1.8 \times 10^{-12}$ m²/s) led the authors to suggest that *Quillaja* saponin adsorption is both diffusion and barrier controlled; i.e., a mixed mass transport mechanism is responsible. The diffusion coefficient of *Quillaja* saponins increased by screening the electrostatic repulsion between the saponin molecules through increases in ionic strength, a

Table 2 Interfacial activity of commercially available *Quillaja* saponin products

Quillaja saponin product (producer)	Interface	γ^a (mN/m)	c^b (%)	Conditions			Method	Reference
				t (min)	pH	T (°C)		
Andean QDP Ultra Organic (Desert King)	Air–water	55 36	0.00003 5.5	5	7	25	Wilhelmy plate	Ralla et al. 2017b
	MCT–water	21 4	0.00003 5.5	5	7	25	Drop shape	
Andean QDP Ultra Organic (Desert King)	MCT–water	13 8	0.005 0.5	5	7	25	Drop shape	Reichert et al. 2018
Sigma 84510 (Sigma-Aldrich)	Air–water	69 36	0.000005 0.01	45	7	21	Drop shape	Piotrowski et al. 2012
Sigma 84510 (Sigma-Aldrich)	Air–water	72 37.5	0.0000825 0.165	45	7	21	Drop shape	Wojciechowski et al. 2014a
	Tetradecane–water	49 7	0.0000825 0.165					
	Olive oil–water	25 4	0.0000825 0.165					
Sigma 84510 (Sigma-Aldrich)	Air–water	70[c] 38	0.0000825 0.165	45	7	21	Drop profile	Wojciechowski 2013
SuperSap (Desert King)	Air–water	41	0.1[d]	10	5.5–6.5	25	Wilhelmy plate	Stanimirova et al. 2011
Q-Naturale® (Ingredion)	Corn oil–water	18 5	0.001 1.0	15	ND	ND	Drop shape	Bai et al. 2016
Q-Naturale® 200 (National Starch LLC)	MCT–water	25 5	0.0002 1.0	15	7	ND	Drop shape	Yang et al. 2013
Quillaja saponins (Sigma Chemicals, Acros Organic, Penco of Lyndhurst)	Air–water	50 36	0.004 0.05–1.2	ND	ND	25	Wilhelmy plate	Mitra & Dungan 1997
Quillaja bark saponin (Ingredion)	MCT–water	12.6	0.005	20	7	ND	Drop shape	Böttcher et al. 2017
Quillaja bark saponin (Ingredion)	Air–water	39	0.005	30	4.1	21.5	Drop profile tensiometer	Tippel et al. 2016a
Quillaja bark saponin (Ingredion)	Air–water	38.2	0.016	30	5	20	Wilhelmy plate	Böttcher & Drusch 2016

[a] γ denotes surface tension and interfacial tension at the air–water interface and oil–water interface, respectively.

[b] c denotes the concentration of the *Quillaja* saponin product in the water phase.

[c] For calculation, a γ of 72.75 mN/m (20°C) at the air–water interface was used (Vargaftik et al. 1983).

[d] Solution contained 10 mM NaCl.

Abbreviations: MCT, medium-chain triacylglyceride; ND, no data.

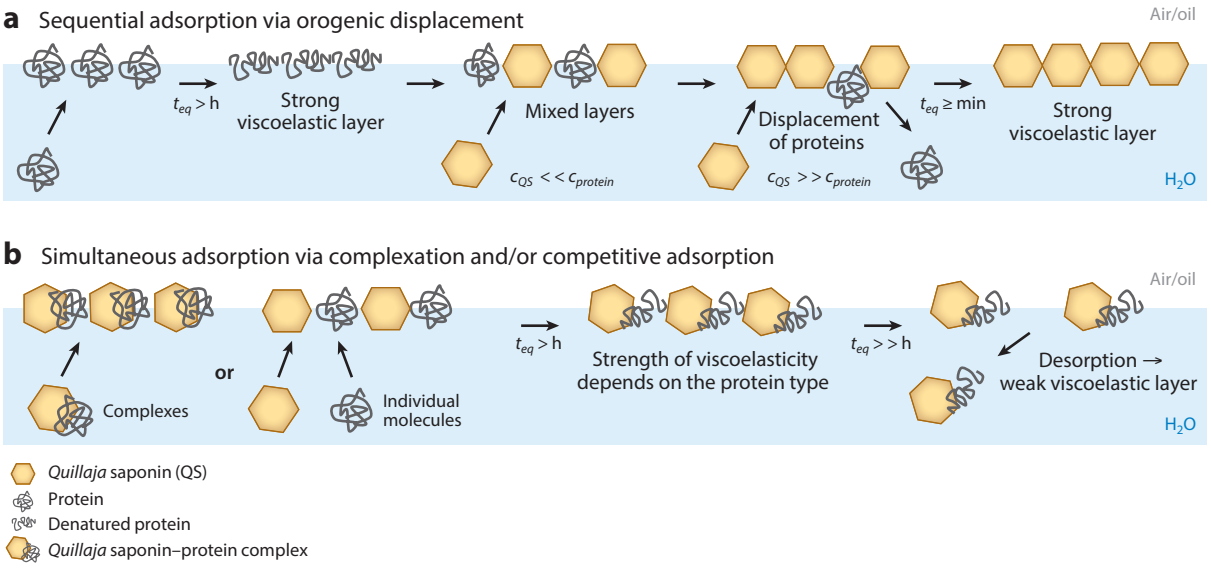

Figure 4

Schematic representation of adsorption mechanisms and adsorption layers for mixtures of *Quillaja* saponins and proteins. Abbreviations: t_{eq}, equilibration time; c, concentration.

lowered pH, or the addition of a protein such as lysozyme (Wojciechowski et al. 2011). The packing of saponin molecules depends on the orientation of saponin molecules at the interfacial layer. Possible orientations of saponin molecules have been schematically described as end-on, lay-on (Stanimirova et al. 2011), or side-on configurations (Böttcher & Drusch 2017). The interfacial area that saponins occupy depends on their aglycone and sugar moieties, with monodesmosidic saponins usually taking a smaller interfacial area than bidesmosidic saponins (Böttcher & Drusch 2017). In the literature, the interfacial area of bidesmosidic triterpenoid *Quillaja* saponins has been shown to vary from 0.30 nm² (Wojciechowski et al. 2011) and 0.43 nm² (Böttcher & Drusch 2016) to 0.92–1.14 nm² (Stanimirova et al. 2011) based on different interfacial tension isotherms and varying degrees of purification of *Quillaja* saponin products. So far, however, there is no clear rule as to how the aglycone or number of sugar moieties impacts the interfacial area upon adsorption. For example, the predominantly monodesmosidic triterpenoid saponins derived from horse chestnut (*Aesculus hippocastanum*) and tea oil (*Camelia oleifera* Abel) can occupy an interfacial area between 0.5 and 0.7 nm², whereas monodesmosidic steroidal saponins from *Tribulus terrestris* and fenugreek (*Trigonella foenum graecum*) can cover areas of 0.4–0.5 and 0.7–0.8 nm², respectively (Pagureva et al. 2016). Bidesmosidic triterpenoid ginsenosides from *Panax ginseng* were reported to occupy an interfacial area of 0.4–0.5 nm² (Pagureva et al. 2016).

The precise adsorption mechanisms of saponins in compositionally complex mixtures or in the presence of other surface-active components such as proteins or phospholipids are far from being fully clarified. Numerous studies have investigated the adsorption mechanisms of individual surfactants and proteins, and mixtures thereof, and these insights have also been applied to *Quillaja* saponins, as schematically illustrated in **Figure 4**. Comprehensive reviews on this topic as well as some current theoretical considerations can be found elsewhere (Fainerman et al. 2005, Kotsmar et al. 2009, Noskov & Krycki 2017, Pugnaloni et al. 2004). In short, the previous literature distinguishes two major mechanisms for mixed surfactant–protein systems: sequential and simultaneous adsorptions. In the sequential adsorption process (**Figure 4*a***), the interfacial layers

are initially covered with proteins, and the proteins are then displaced by either ionic or nonionic surfactant molecules via a mechanism known as orogenic displacement (Mackie et al. 1999, Mackie et al. 2000). There, the low-molecular-weight surfactants penetrate into openings in the protein mono- or multilayers, leading to an increase in surface pressure, further compressing the protein networks, which finally leads to a desorption of proteins and adsorption of the small molecule surfactants (Dan et al. 2012, 2013; Mackie et al. 1999, 2000). In the simultaneous adsorption process (**Figure 4*b***), the adsorption behavior is governed by the molecular interactions taking place between the molecules and the adsorption proceeds via complexation and/or competitive adsorption. In case there are no attractive forces between the molecules, the individual molecules are in competition to anchor themselves to the interfaces. If there are attractive interactions present, then surfactants and proteins may form electrostatic and/or hydrophobic complexes that may compete with any unbound surfactant (Fainerman et al. 2003; 2004a,b; 2005).

In previous studies, the interfacial activity of mixed surfactant systems comprising *Quillaja* saponins and a second surface-active agent (e.g., a protein or a phospholipid) has been analyzed. These studies revealed synergistic effects in lowering the interfacial tension at low saponin:protein ratios, whereas at intermediate ratios, the protein had a detrimental effect. At high saponin:protein ratios, the effect of the protein was negligible and the saponin governed the interfacial tension reduction (Kezwon & Wojciechowski 2014; Piotrowski et al. 2012; Reichert et al. 2018; Wojciechowski et al. 2011, 2014a). This was reported for *Quillaja* saponin–lysozyme (Kezwon & Wojciechowski 2014, Piotrowski et al. 2012, Wojciechowski et al. 2011), *Quillaja* saponin–β-lactoglobulin (Piotrowski et al. 2012), *Quillaja* saponin–β-casein (Kezwon & Wojciechowski 2014, Wojciechowski et al. 2014a), and *Quillaja* saponin–pea protein systems (Reichert et al. 2018). The synergistic effect at low saponin:protein ratios was explained by a reduced adsorption barrier of *Quillaja* saponins caused by the presence of or complexation with the proteins (Kezwon & Wojciechowski 2014, Piotrowski et al. 2012, Wojciechowski et al. 2011). The interactions between saponins and proteins were suggested to occur in the bulk phase (**Figure 4*b***) and not only at the interface (Böttcher et al. 2016, Kezwon & Wojciechowski 2014, Reichert et al. 2015, Wojciechowski et al. 2014a), and further changes may occur at the interface (Kezwon & Wojciechowski 2014; Reichert et al. 2017, 2018). Furthermore, *Quillaja* saponins were suggested to change the tertiary structure of β-lactoglobulin such that its rigid structure was lost and an unfolding occurred. In turn, there was an increase in the rate of interfacial tension decay (Piotrowski et al. 2012).

In contrast to proteins, combined systems of *Quillaja* saponins and phospholipids formed mixed adsorption layers (Luo et al. 2017, Reichert et al. 2018). The interactions between phospholipids and *Quillaja* saponins were extensively investigated in monolayers of the model phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (Wojciechowski et al. 2014b). The DPPC monolayers were compressed to various densities with a liquid-expanded (5 and 20 mN/m), liquid-condensed (32.5 mN/m), or solid (47 and 51 mN/m) interface. In all types of monolayers, the subphase exchange with *Quillaja* saponins led to an increase in dilatational viscoelastic properties indicative of incorporation of the saponins into the DPPC monolayer. At the highest surface pressure of DPPC monolayer tested, *Quillaja* saponin additions induced collapse of the monolayer (Wojciechowski et al. 2014b). However, subsequent increases in surface pressure suggested that a reconstruction of DPPC monolayers took place. On the basis of the rebuilding of DPPC monolayers after collapse by *Quillaja* saponins, Wojciechowski et al. (2014b) argued that strong interactions between the phospholipids and *Quillaja* saponins were responsible for not completely displacing DPPC. The results were found to be in contrast to common small molecule surfactants, e.g., sodium dodecyl sulfate, where collapse of DPPC layers was irreversible (Wojciechowski et al. 2014b). In addition, adding soy lecithin to *Quillaja* saponin further reduced the interfacial tension

compared to using only *Quillaja* saponin in oil-in-water emulsions. This effect was suggested to be related to a more efficient packing of mixed saponin–lecithin interfacial layers (Luo et al. 2017).

Interfacial Rheology

In interfacial rheology measurements, the mechanical properties of an interface are characterized by conducting shear or compression studies on a two-dimensional flat layer distinctly different from the three-dimensional bodies described by bulk phase rheology (Fuller 2003). In general, interfacial rheological behavior can be divided into two types, namely dilational and shear interfacial rheological behavior. In dilational interfacial rheology, changes in interfacial area are induced through compression or expansion of the interface. In shear interfacial rheology, the area is kept constant but the shape of the area is changed (Fuller 2003, Maldonado-Valderrama & Rodríguez Patino 2010). The main parameters governing the rheological behavior of interfacial layers are the composition and the structure of the adsorbed material (Maldonado-Valderrama & Rodríguez Patino 2010). In interfacial shear experiments, the lateral forces between the molecules are probed (Maldonado-Valderrama & Rodríguez Patino 2010), whereas in dilational interfacial rheology the migration and adsorption characteristics of the interface-forming components are investigated.

For *Quillaja* saponin interfaces, high dilational elasticities and low dilational viscosities were reported (Pagureva et al. 2016, Stanimirova et al. 2011, Wojciechowski et al. 2014c). A viscoelastic interface with high elasticity was also found in shear surface rheological experiments (Golemanov et al. 2012, Reichert et al. 2017). Thus, layers composed of *Quillaja* saponins can be described as purely elastic bodies at low deformation (<1%) (Golemanov et al. 2012, Pagureva et al. 2016, Wojciechowski et al. 2014c). Strong hydrogen bonding between the sugar moieties requires high energy for desorption of *Quillaja* saponins from the interface (Pagureva et al. 2016). The fast adsorption of *Quillaja* saponins to an interfacial layer was also investigated by interfacial shear measurements. A highly viscoelastic interfacial layer is formed by *Quillaja* saponins already after 5 min (**Figure 4*a***). The layer's viscoelasticity only slightly increased when the *Quillaja* saponin interface was aged for up to 12 h (Reichert et al. 2017). Furthermore, the surface load of *Quillaja* saponins at saturation (2.3 mg/m^2) (Bai et al. 2016) and the area per saponin molecule at the critical micellar concentration (CMC) (1.13 $\pm$ 0.04 nm^2) (Stanimirova et al. 2011) indicated that a formation of a thin interfacial layer took place. The orientation of *Quillaja* saponin molecules in the adsorption layer was reported to be in the lay-on instead of the end-on configuration, meaning that the hydrophobic triterpene rings were oriented toward the hydrophobic phase, whereas the hydrophilic sugar moieties interacted with the water phase (Stanimirova et al. 2011).

Combining *Quillaja* saponins with other surface-active agents led to a change in interfacial rheological behavior (Böttcher et al. 2016, Reichert et al. 2017). For instance, combining *Quillaja* saponin and β-lactoglobulin led to enhanced viscoelasticity of mixed interfaces as determined by interfacial shear rheology compared to β-lactoglobulin-only covered interfaces. This points to the formation of mixed saponin–protein interfaces with increased mechanical strength (**Figure 4*b***). However, the highest viscoelasticity was found for purely *Quillaja* saponin–covered interfaces (Böttcher et al. 2016) (**Figure 4*a***). In contrast, reduced viscoelasticity was reported for a mixed *Quillaja* saponin–pea protein interfacial layer compared to each of the individual components in interfacial shear rheological measurements (Reichert et al. 2017). This clearly suggests that the effects of protein–saponin combinations on interfacial rheology are highly specific to the compounds involved. In both studies, the mixed systems (i.e., *Quillaja* saponin–β-lactoglobulin and *Quillaja* saponin–pea protein) formed complexes that desorbed from the interface (**Figure 4*b***)

during storage (Böttcher et al. 2016, Reichert et al. 2017), indicating that a hydrophilization and a more particulate interfacial layer formation took place during aging for up to 12 h (Reichert et al. 2017). This was explained by the lower surface activity of saponin–protein complexes compared to the saponins (Böttcher et al. 2016) and increased stiffness of the saponin–protein interfacial layer (Böttcher et al. 2017).

Self-Assembled Structures

The amphiphilic nature of saponins is the basis for the formation of self-assembled structures in the bulk phase and the ability to act as surface-active agents at interfaces. Self-assembled structures are the consequence of the tendency of the system to further reduce the free energy and are primarily the result of enthalpy contributions derived from optimization of molecular interactions. Structures formed include micelles, liquid crystals, bilayers, vesicles, and microemulsions (Hiemenz & Rajagopalan 1997). For *Quillaja* saponins, most research articles about self-assembled structures focused on their ability to form micelles (Mitra & Dungan 1997, Ribeiro et al. 2013). The amphiphilic nature of *Quillaja* saponins was found to be suitable to generate micellar structures above a CMC of 0.14–0.77 g/L *Quillaja* saponins in aqueous solution at room temperature (Bai & McClements 2016, Luo et al. 2017, Mitra & Dungan 1997, Ribeiro et al. 2013, Wojciechowski et al. 2014c). The CMC varied depending on the composition of the *Quillaja* saponin product, the environmental conditions, and the method of analysis used.

Quillaja saponins were further studied for their complexation with proteins, an investigative line that began as early as 1993 with the study of Potter et al. (1993). There, the researchers reported an increase in molecular weight of *Quillaja* saponin–casein complexes in aqueous solution with increasing heating time (Potter et al. 1993). This corroborated with the formation of aggregated structures of mixed *Quillaja* saponin–Na-caseinate bulk systems at pH 7 at specific concentration ratios upon heating to 75°C (Reichert et al. 2016). The interactions and structure formation of *Quillaja* saponins and phospholipids (Demana et al. 2004) were found to be highly dependent on the nature of the sugar moieties of the saponins (Fukuda et al. 1985). *Quillaja* saponins were found to form varying colloidal structures with phospholipids whose behaviors were summarized in pseudoternary phase diagrams by Demana et al. (2004). With varying concentration ratios of Quil A, cholesterol, and phosphatidylcholine, the formation of worm-like micelles, lipidic particles, liposomes, cholesterol crystals, ring-like micelles, and ISCOM structures was reported (Demana et al. 2004).

APPLICATIONS IN DISPERSED SYSTEMS

The consumer demand to replace synthetically derived compounds with naturally occurring compounds to stabilize colloidal structures has greatly increased in recent years, and natural alternatives were extensively reviewed as recently as 2016 (McClements & Gumus 2016). Among the natural alternatives proposed, saponins from the *Quillaja saponaria* Molina tree were suggested to be highly promising because of their high surface activities, which may aid in the formation and stabilization of dispersed systems (McClements & Gumus 2016). Properties of interest include their emulsifying or foaming capabilities and the ability to form carrier systems such as lipid nanoparticles that could be used for encapsulation. Furthermore, some recent studies have investigated the use of *Quillaja* saponins in the formation of novel food structures, such as heteroaggregated emulsions. An overview of general and specific applications of *Quillaja* saponins that make use of their functional properties is shown in **Table 3**.

Table 3 Applications of *Quillaja* saponin products

Field	Application	Reference
Agriculture	Wastewater treatment	Kaczorek et al. 2016
	Soil regeneration	Pekdemir et al. 2005
Animal nutrition	Mortification of fish	Serrano 2013 Tharabenahalli-Nagaraju et al. 2014
	Enhanced nutrient utilization	Serrano 2013
	Insecticidal activity	De Geyter et al. 2011
Human nutrition	Aroma/amino acid release through lysis of yeast cells	Berlowska et al. 2015, 2017
	Beverages	Schultz & Monnier 2015 Tran & Li 2012
	Coffee creamer	Chung et al. 2017a,b,c
	Encapsulation/stabilization of bioactive compounds in emulsions or foams	Bouquerand et al. 2014 Chen et al. 2017 Schrader et al. 2011 Tippel et al. 2016b Weigel et al. 2018 Wiersma 1998 Yang & McClements 2013a
	Foam formation	Böttcher & Drusch 2016 Gillespie 2016 Park et al. 2005 Plahar et al. 2006
	Egg replacer	Gillespie 2016
	Aeration of food products	Park et al. 2005 Plahar et al. 2006
	Structure modulator emulsions	Maier et al. 2014, 2015c, 2016
	Improvement of bread dough fermentation and baking	Ogasawara et al. 2000
	Micellar formation	Tippel et al. 2016b
	Removal of turbidity-causing proteins	Alarcon Camacho & Sainz Lobo 2010
	Removal of cholesterol	Sundfeld et al. 1994
	Solubilizing agent	Riess et al. 2010
	Stabilization of crystallized lipid particles	Karthik et al. 2016 Salminen et al. 2014, 2016 Weiss et al. 2016
Cosmetics	Treatment of skin impurities	Neufang 2014
	Hair dye and hair coloring preparation	
Pharma	Adjuvant and immunostimulating activity	Dalsgaard 1978 Güçlü-Üstündağ & Mazza 2007 Kenney et al. 2002 Kensil & Marciani 1991 Kersten & Crommelin 2003 Marciani 2015 Morein et al. 1984 Morein & Loevgren 2012 Sun et al. 2009 Tharabenahalli-Nagaraju et al. 2014 Walkowicz et al. 2016

(*Continued*)

Table 3 (*Continued*)

Field	Application	Reference
	Anticancer activity	Ebbesen et al. 1976
	Hemolytic activity	De Geyter et al. 2011 Hassan et al. 2010 Tharabenahalli-Nagaraju et al. 2014
	Antimicrobial activity	Hassan et al. 2010
	Enhanced bacterial metabolism	Kaczorek et al. 2016
	Anti-inflammatory activity	Rodríguez-Díaz et al. 2011 Sarkhel 2015
	Improved bacterial growth	Patra et al. 2012
	Antiviral activity	Roner et al. 2007 Tam & Roner 2011

Emulsions

Emulsions are thermodynamically unstable dispersions composed of at least two immiscible liquid phases, typically oil and water (Dickinson 2009, McClements 2005). In an emulsion, one immiscible liquid (the dispersed phase) is dispersed in the other (continuous phase) in the form of droplets. In an oil-in-water emulsion, oil droplets are dispersed in an aqueous continuous phase (**Figure 5*a***), whereas in water-in-oil emulsions, water droplets are dispersed in an oil phase. The kinetic stability of emulsions can be improved by the use of emulsifiers adsorbing to the oil–water interface, thereby lowering the interfacial tension (see section titled Interfacial Activity) between the immiscible liquids, which reduces the thermodynamic driving force to minimize emulsion interfaces [$\Delta G = f(\gamma,\Delta A)$] (Dickinson 2009, McClements 2005). This also eases droplet breakup upon homogenization, resulting in the formation of small spherical droplets in the continuous phase (Walstra 1993). The properties of the emulsifier layer determine the emulsion's kinetic stability against gravitational separation, flocculation, aggregation, coalescence, and Ostwald ripening (Dickinson 2009, McClements 2005). Below, we focus on oil-in-water emulsions stabilized

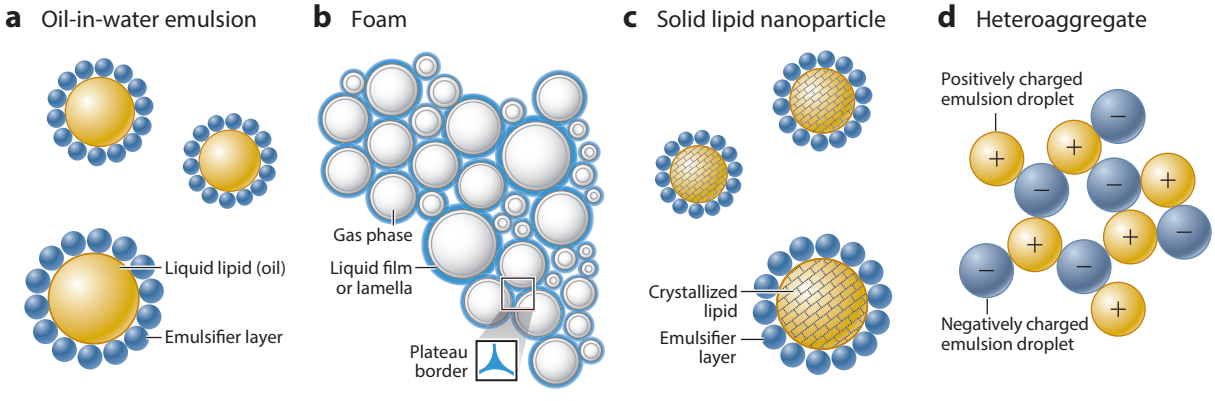

Figure 5

Schematic presentation of colloidal dispersions generated by applying *Quillaja* saponins.

by *Quillaja* saponins because to date—at least to the best of our knowledge—no data exist for water-in-oil emulsions stabilized by *Quillaja* saponins.

One of the first investigations of the emulsifying properties of *Quillaja* saponins was carried out by Pekdemir et al. (2005), who screened for a natural surfactant to be used for the emulsification of Ekofisk crude oil from the North Sea. The researchers found that *Quillaja* bark saponin was able to emulsify the crude oil even at low concentrations of 0.1% by shaking the mixture, albeit only to a limited oil:surfactant ratio (Pekdemir et al. 2005). In recent years though, many studies have been performed to elucidate the formation of emulsions stabilized by various *Quillaja* saponin products and manufactured with different homogenization techniques. For example, nanoemulsions (<200 nm) stabilized by *Quillaja* saponins can be prepared by dual-channel microfluidization (Bai et al. 2016), high-pressure homogenization (Chung et al. 2017a,c; Ozturk et al. 2015; Uluata et al. 2015; Zhang et al. 2015), and sonication (Chen et al. 2016, Piotrowski et al. 2012, Wojciechowski et al. 2014a) but not by phase inversion (Mayer et al. 2013). Mayer et al. (2013) attributed the poor efficiency of *Quillaja* saponins to manufacture nanoemulsions via the emulsion phase inversion method to its low oil solubility; a prerequisite for the spontaneous formation and stabilization of oil droplets. This may also be the reason why, to date, no study on water-in-oil emulsions is available, as oil solubility is often also a prerequisite there. Likely, saponins would need to be more hydrophobic (e.g., by having fewer sugar moieties attached to them) to function as water-in-oil emulsifiers.

Several studies have shown that *Quillaja* saponins have excellent emulsion-stabilizing properties (Bai & McClements 2016, Chen et al. 2016, Chung et al. 2017c, Uluata et al. 2015, Yang et al. 2013). For example, oil-in-water emulsions stabilized with *Quillaja* saponins showed high stability during heat treatment (30–90°C, 30 min) and storage (1 month at 5°C, 37°C, and 55°C), at varying pHs (pH 3–9) and high ionic strengths (up to 300 mM NaCl) (Bai & McClements 2016, Ralla et al. 2017a, Uluata et al. 2015, Yang et al. 2013), and when various oil types were used (long- and medium-chain triacylglycerides) (Yang & McClements 2013b). *Quillaja* saponin–stabilized emulsions were, however, not stable to the additions of calcium chloride (Ralla et al. 2017a). Long-term physical storage stability of fine-dispersed emulsions containing ω-3 long-chain polyunsaturated fatty acids was achieved for 60 days when stabilized by a commercial *Quillaja* saponin product, Q-Naturale® (Chen et al. 2016). Another *Quillaja* saponin product, Andean QDP Ultra Organic, was also reported to stabilize fine emulsions (160 nm) containing fish oil rich in long-chain ω-3 fatty acids such as eicosapentanoic and docosahexaenoic acids (Salminen et al. 2014). Physical stability toward heat and dilution was observed for a 10% oil-in-water emulsion stabilized by *Quillaja* saponins when added to a hot acid coffee solution (85°C) at sufficiently high *Quillaja* saponin concentrations (>0.5%) (Chung et al. 2017a,c). The observed emulsion-stabilizing properties were attributed to a strong electrostatic repulsion provided by *Quillaja* saponins because they have an unusually high negative ζ-potential of approximately −60 mV between pH 3 and 9 (Yang et al. 2013, Zhang et al. 2015). An additional contributor is their fast adsorption kinetics (Böttcher et al. 2017, Zhang et al. 2015).

Lower mean droplet sizes of emulsions stabilized with *Quillaja* saponins can be achieved at higher saponin concentrations (Reichert et al. 2018, Yang et al. 2013), at high homogenization pressures (Yang et al. 2013), and after several homogenization passes (de Faria et al. 2017, Yang et al. 2013, Zhang et al. 2015). In fine-dispersed flavor emulsions containing orange oil, the mean droplet size could be reduced by increasing the ratio of MCT in the MCT–orange oil dispersed phase (Zhang et al. 2015). The emulsifying properties of *Quillaja* saponins have also been of interest when designing encapsulated food ingredient systems. Several studies investigated the encapsulation efficiency of ingredients by *Quillaja* saponins stabilized in either micellar- (Tippel et al. 2016b) or emulsion-based systems (Chen et al. 2016, Tippel et al. 2016b, Uluata et al. 2015,

Weigel et al. 2018, Yang & McClements 2013a). For instance, *Quillaja* saponins can be used to efficiently encapsulate food colors such as lutein (Tippel et al. 2016b, Weigel et al. 2018), vitamins such as vitamin E (Yang & McClements 2013a), and ω-3 enriched oil (Chen et al. 2016, Salminen et al. 2014). Further examples of encapsulation systems of *Quillaja* saponins are described in sections Solid Lipid Nanoparticles and Nanostructured Lipid Carriers and Industrial Use of *Quillaja* Saponins.

Quillaja saponins have also been investigated for their emulsifying properties in mixed *Quillaja* saponin–protein systems. The use of combinations was suggested to enhance their emulsification ability because of potential synergistic interfacial activities (see section titled Interfacial Activity). The effect of accelerated adsorption kinetics of *Quillaja* saponins after addition of even small amounts of β-lactoglobulin was, however, not observed in tetradecane-in-water emulsions (Piotrowski et al. 2012). The emulsifying properties of *Quillaja* saponins are known to be good (Yang et al. 2013), but in combination with macromolecules such as proteins they were sometimes found to be unfavorably altered (Wojciechowski et al. 2014a). On the one hand, the droplet size of 10% oil-in-water emulsions stabilized by mixed *Quillaja* saponin–β-lactoglobulin can be reduced with increasing homogenization passes (de Faria et al. 2017). On the other hand, mixed *Quillaja* saponin–β-casein stabilized emulsions were more prone to droplet growth than were *Quillaja* saponin– or β-casein-stabilized emulsions (Wojciechowski et al. 2014a). It is possible that complexes allowed for openings at interfaces that facilitated droplet coalescence or Ostwald ripening. Another detrimental effect of mixed *Quillaja* saponin–protein combinations was observed at high concentrations of β-lactoglobulin in MCT–water emulsions. There, emulsions aggregated after as little as one week of storage. In contrast, individual saponin- or protein-stabilized emulsions were stable for more than two weeks. This effect was suggested to be due to a displacement of β-lactoglobulin from the interface or structural changes of β-lactoglobulin induced by *Quillaja* saponins (Böttcher et al. 2017). Regardless of these somewhat mixed results, the use of *Quillaja* saponin–protein mixtures has been reported to improve bread dough fermentation and baking, leading to a patent (Ogasawara et al. 2000). The respective invention describes a new food emulsifier system composed of saponin–protein complexes with excellent emulsion-stabilizing properties and improved protein implementation in food products (Ogasawara et al. 2000).

Foams

A foam consists of a gas dispersed in a liquid, solid, or gelled matrix in the form of bubbles (Drenckhan & Saint-Jalmes 2015, Hill & Eastoe 2017). Basically, the gas bubbles (often air) are surrounded by a continuous thin liquid film, the so-called lamella, and thin film intersections (Plateau borders) (**Figure 5*b***), forming interstitial spaces between the bubbles and thus creating a three-dimensional network (Drenckhan & Saint-Jalmes 2015, Hill & Eastoe 2017, Rio et al. 2014). The formation of foams requires energy, and methods to manufacture foams include, e.g., whipping, shaking, or sparging of a solution (Drenckhan & Saint-Jalmes 2015). Depending on the chosen application, the continuous liquid phase can additionally be gelled or solidified after the foam has been generated (Rio et al. 2014). Foams are thermodynamically unstable and as such they are prone to different instability mechanisms induced by gravitational and van der Waals forces, namely drainage, coalescence, and coarsening (Hill & Eastoe 2017, Rio et al. 2014). Therefore, their stabilization requires surface-active compounds such as surfactants and (bio)polymers and particles such as silica and polystyrene latex particles (Binks 2002, Hill & Eastoe 2017, Wilde et al. 2004). These surface-active molecules adsorb to the gas–liquid interface and reduce the interfacial tension (see section titled Interfacial Activity), thus enabling the formation and stabilization

of foams (Hill & Eastoe 2017). The stabilization mechanisms and kinetic stability of the foams depend on the characteristics of the surface-active compounds used (Hill & Eastoe 2017, Wilde et al. 2004).

Quillaja saponins are well known for their foaming ability. In fact, indigenous people of Chile used the aqueous solution of *Quillaja saponaria* Molina bark to wash their hair and clothes precisely because they were known to foam (Güçlü-Üstündağ & Mazza 2007, Gutbier et al. 1921, San Martín & Briones 1999). The foaming properties can also be used to extract saponins or quantify them in extracts (San Martín & Briones 2000). Among several saponin types from different sources, *Quillaja* saponins provide one of the best foam-stabilizing properties, with 85% of the foam still intact after 1 h of storage (Böttcher & Drusch 2016). *Quillaja* saponin–stabilized foams were even found to be more stable at lower pH (pH 3) and higher ionic strength (500 mM NaCl). The authors suggest that the screening of charges at low pH or high ionic strength of bidesmosidic *Quillaja* saponins allowed for better packing at the surface, thus preventing destabilization of the membrane in foams (Böttcher & Drusch 2016).

Pickering-type foam stabilization was investigated for *Quillaja* saponin–stabilized fine-dispersed oil droplets that built networked lamellae between the bubbles, leading to improved foam stability compared to individually *Quillaja* saponin–stabilized foams (Chen et al. 2017). Alternatively, mixed *Quillaja* saponin–protein systems were studied for foam formation. At low and intermediate *Quillaja* saponin:lysozyme ratios (1:10, 1:5, and 1:1), foam height increased when compared to *Quillaja* saponin–stabilized foams, indicating synergistic saponin–protein foaming properties. However, at high *Quillaja* saponin:lysozyme ratios (>1:1), the foaming height was barely affected by the presence of lysozyme, and the saponin dominated the foam formation (Wojciechowski et al. 2011), which is in line with the interfacial activity discussed above (section titled Interfacial Activity).

Solid Lipid Nanoparticles and Nanostructured Lipid Carriers

Solid lipid nanoparticles (SLNs) are particles of solidified lipids that can be used as colloidal carrier systems for various functional ingredients such as pharmaceuticals or nutraceuticals (Müller et al. 2000). This has more recently led to them being investigated for their potential use in food applications (Salminen et al. 2014, 2016). Typical lipids used in generating SLNs include those that are solid at room temperature such as saturated tri-, di-, or monoacylglycerides, fatty acids, waxes, and mixtures thereof (Mehnert & Mäder 2012). SLNs can be prepared by hot high-pressure homogenization with (Karthik et al. 2016) or without subsequent ultrasonication (Salminen et al. 2014, 2016) or by cold homogenization (Van De Ven et al. 2009). In hot high-pressure homogenization, the only difference in emulsion formation is that the temperature is set above the melting temperature of the lipid(s) (Mehnert & Mäder 2012). The generated hot oil-in-water emulsion is then cooled down to solidify the lipid matrix, thereby creating the characteristic structure of SLNs (**Figure 5*c***).

Degradation of substances in SLNs was reported to depend on the emulsifier composition. For example, *Quillaja* saponins were able to prevent polymorphic transitions of less ordered crystallized tristearin matrices, thus providing physical and chemical protection of encapsulated ingredients (Salminen et al. 2016). Approximately 80% of β-carotene was preserved for 51 days when encapsulated in SLNs using *Quillaja* saponins. Using *Quillaja* saponins alone or in combination with high-melting-point lecithin yielded a rigid interface that could retain the less ordered α-subcell crystals and thus prevent polymorphic transition and provide protection of β-carotene against oxidation. In comparison, *Quillaja* saponins in combination with low-melting-point lecithin resulted in a more fluid interface that allowed crystals to polymorph into β-crystals with platelet-like shapes

and expelled the bioactive substances in the process. These systems were less capable of protecting β-carotene against oxidation (Salminen et al. 2016).

Besides the protection of sensitive ingredients, SLNs can also be used to enhance the biological activity of incorporated functional components. SLNs composed of stearic acid as the lipid core and *Quillaja* saponins with an incorporated anticancer drug (imatinib mesylate) were more effective in inactivating human breast cancer cells compared to an anticancer drug that had been directly added to the cell culture. The enhanced cytotoxicity of SLNs with the encapsulated anticancer drug was related to improved cellular uptake of SLNs and/or synergistic effects of *Quillaja* saponins with the anticancer drug (Karthik et al. 2016). An altogether different use of SLNs involves the encapsulation of saponins into nanoparticles. The incorporation of *Quillaja* saponins into SLNs was found to reduce the in vitro cytotoxicity of saponins compared to free saponins (Van De Ven et al. 2009).

In contrast to SLNs, nanostructured lipid carriers (NLCs) are composed of mixed solid and liquid lipids to generate an amorphous lipid matrix (Müller et al. 2002). NLCs can be used to encapsulate bioactive components to enhance their dispersibility in water as well as protect bioactive substances from environmental influences. Improved oxidative stability of ω-3 fish oil in NLCs composed of *Quillaja* saponins and high-melting-point lecithin as the emulsifier system was reported compared to fish-oil-in-water emulsions (Salminen et al. 2014). *Quillaja* saponins play a crucial role in the prevention of lipid oxidation in NLCs because they limit the polymorphic transition of the ω-3 fish oil–loaded tristearin carrier. A tristearin shell was formed through heterogeneous crystallization around the ω-3 fish oil, providing a physical barrier to oxygen and pro-oxidants. This physical tristearin barrier was not present in simple ω-3 fish-oil-in-water emulsions, and, consequently, lipid oxidation proceeded in these simple emulsions to a higher extent than in NLCs (Salminen et al. 2014).

Heteroaggregates

Heteroaggregation describes the behavior of binary dispersion of colloidal particles or droplets with different sizes, compositions, and charges to form aggregated structures with altered functional properties, e.g., increased viscosity or even solid-like behavior at low oil droplet concentrations (Islam et al. 1995, López-López et al. 2009). In the section below, the discussion is restricted to the electrostatic heteroaggregation of binary oil-in-water emulsions, in which one of the emulsions is stabilized by *Quillaja* saponins. Heteroaggregates or heteroaggregated emulsions are generally composed of two differently charged emulsion systems (**Figure 5*d***) that can be used to encapsulate two different lipophilic compounds (McClements 2014) or to form a network to increase the microstructure to enhance the viscosity (Maier et al. 2014).

A screening study to select appropriate emulsifiers for the formation of heteroaggregates revealed that of the many systems screened, a combination of positively charged Na-lauroyl-L-arginine ethyl ester (LAE) and negatively charged *Quillaja* saponin–stabilized emulsions was particularly able to form macroscopic aggregates without destabilization of the individual emulsion droplets (Maier et al. 2014). *Quillaja* saponins were reported to be suitable emulsifiers for the formation of heteroaggregates because of their steric droplet stabilization ability to maintain the droplet shape when combined with an LAE-stabilized emulsion (Maier et al. 2014). The heteroaggregated network could be further stabilized by cross-linking the electrostatically aggregated emulsion droplets. Cross-linking of the heteroaggregates composed of *Quillaja* saponin– and WPI-stabilized emulsions was achieved by enzymatic treatment with laccase or by chemical treatment with glutaraldehyde (Maier et al. 2015c) but not with the food-grade cross-linker genipin (Maier et al. 2016). The authors mentioned that the inability to efficiently cross-link *Quillaja*-based

heteroaggregates with genipin may have been due to the pH being too low for genipins' reactivity. Other modifications, such as using large (17–24 μm) instead of small (0.6–1.1 μm) emulsion droplets and thermal treatment of emulsions to increase hydrophobic interactions, did little to affect the gel strength of *Quillaja* saponin–WPI heteroaggregated emulsions (Maier et al. 2016).

INDUSTRIAL USE OF *QUILLAJA* SAPONINS

Quillaja saponins are increasingly used in a wide variety of industrial fields, including human and animal nutrition, pharmaceuticals, personal care products, and agriculture (**Table 3**). For food applications, *Quillaja* saponins were reported to efficiently increase the foaming capacity of aerated foods such as cowpea paste used for a traditional African dish called Akara (Park et al. 2005, Plahar et al. 2006). The addition of *Quillaja* saponins was used to enhance flavor. This was found to be due to their lysing effect of yeast cells, leading to a larger amount of amino acids released (Berlowska et al. 2015, 2017). There are several studies describing the efficient encapsulation of vitamins, colors, or other lipophilic substances in SLNs (Salminen et al. 2016), liposomes, or fine-dispersed emulsions prepared with *Quillaja* saponins (Tippel et al. 2016b, Yang & McClements 2013a). Other studies suggested that *Quillaja* saponin–stabilized emulsions could be used as liquid coffee creamer to replace Na-caseinate–stabilized coffee creamers to manufacture a vegan coffee whitener. However, the researchers did not compare the sensory properties of the saponin-based products to those of casein-stabilized liquid coffee creamers (Chung et al. 2017a,b,c). Other applications of *Quillaja* saponins in food products are based on their ability to form heteroaggregates, providing increased viscosity that might be useful in altering mouthfeel and organoleptic properties as well as reducing the fat content of food products (Maier et al. 2014, 2015c, 2016).

In addition to the *Quillaja* saponin use in food, a number of studies have sought to investigate their use as animal feed additives. *Quillaja* saponin added to animal diets resulted in enhanced nutrient utilization, e.g., in carp (Serrano 2013), and improved the growth of some rumen bacteria (determined in an in vitro study) (Patra et al. 2012). Another application that sought to utilize the effects of *Quillaja* saponin extract on bacteria was a wastewater treatment (San Martín & Briones 1999). In wastewater treatment experiments, an improved effect on the biodegradation of halogenated phenolic compounds (4-fluorophenol) by *Pseudomonas* sp. OS2 was achieved by supplementation with *Quillaja* saponins (Kaczorek et al. 2016). However, for other bacteria, such as *Raoultella planticola*, no effect of *Quillaja* saponin addition was found (Kaczorek et al. 2016). Furthermore, *Quillaja* bark saponin extracts were found to be useful for soil regeneration of Ekofisk crude oil–contaminated soil based on their emulsifying properties (Pekdemir et al. 2005). Finally, there are some useful biological activities of *Quillaja* saponins, such as their antiviral activity in mice (Tam & Roner 2011) and their insecticidal activity against aphids, that could give rise to the emergence of new pharmaceutical applications (De Geyter et al. 2011).

Future uses of *Quillaja* saponins may be in the cosmetic and personal care industry, e.g., as surfactants for shampoos and creams (San Martín & Briones 1999). To the best of our knowledge, there are no research articles available about cosmetic products containing *Quillaja* saponins; however, several studies suggest that they might be of use in cosmetic products (Bai & McClements 2016, Neufang 2014).

REGULATORY ASPECTS

Quillaja extract has been approved by the European Union as a food additive (emulsifier, stabilizer, foam stabilizer, and encapsulant) for water-based nonalcoholic drinks and ciders (excluding cider bouché) and perries to a maximum level of 200 mg/kg calculated as anhydrous extract (E 999)

under European Commission regulation number 1129/2011/EC. Its use is also allowed in cosmetic products as stated in European Commission regulation number 2006/257/EC.

In the United States, the US Food and Drug Administration (FDA) has permitted *Quillaja* extract as a food additive (21 CFR 172.510) for use as a natural flavoring substance and a natural substance used in conjunction with flavors in various food categories. The American Beverage Association filed an application in 2005 for GRAS (generally recognized as safe) status (GRAS Notice No. 165, FDA, 2005) for *Quillaja* extract, but the FDA has yet to grant it.

Quillaja extract is considered GRAS by the Flavor and Extract Manufacturers' Association (FEMA) when used as a flavoring ingredient in multiple food categories under FEMA number 2973 (Cohen et al. 2015, Hall & Oser 1965). The acceptable daily intake of *Quillaja* saponins has been set to 0–1 mg/kg body weight based on the exposure to *Quillaja* extract in semifrozen carbonated and noncarbonated beverages at a maximum level of 500 mg/kg on a dry basis in beverage concentrates (FAO-WHO 2005). The Codex Alimentarius states in its General Standard for Food Additives that *Quillaja* extracts can be applied as an emulsifier and foaming agent up to a maximum level of 50 mg/kg (on the saponin basis) in water-based flavored drinks. This includes sport, energy, and electrolyte drinks as well as particulated drinks, whereas the limit for use in semifrozen beverages is set to 130 mg/kg (on a dry basis) (FAO-WHO 2005).

In Canada, the Food and Drug Regulations state that *Quillaja* extract can be used as an emulsifier in various food formulations, with concentrations ranging from 25 to 130 mg/kg (calculated as saponin) (Can. Food Insp. Agency 2017). These food formulations include dry sauce mixes, dry soup mixes, marinades for meat and poultry, ready-to-drink tea, savory snack foods, vegetable juices (*Quillaja* saponin concentration ≤25 mg/kg), noncarbonated water-based flavored and sweetened beverages, flavored alcoholic beverages, chewing gums (≤50 mg/kg), and caffeinated energy drinks, sports drinks, and some beverages with added vitamins and minerals (≤130 mg/kg).

The use of *Quillaja* extracts is also allowed in China (Minist. Health People's Repub. China 2011), Japan (Jpn. Food Chem. Res. Found. 2018), Singapore [in alcoholic beverages (≤40 mg/kg) and soft drinks (≤50 mg/kg)] [Sale of Food Act, Chapter 283, Section 56(1)], and Vietnam (Vietnam Food Adm. 2012). It should be noted that this list is not exhaustive, and other countries may also have an existing regulatory framework that allows the use of *Quillaja* extracts.

REVIEW OF PATENTS

Numerous patents have been published that involve the use of *Quillaja* saponins, with a focus on their immunostimulating potential (e.g., Kensil & Marciani 1991, Morein & Loevgren 2012). Because the subject of the literature research was a review of the technofunctional properties of *Quillaja* saponins, only patents dealing with the use of saponins as a technical additive were reviewed, and they have been summarized in **Table 3**.

One patent described the efficient replacement of egg white in alcoholic and nonalcoholic beverages by a compound mixture containing saponins as foaming agents. This compound mixture was able to replace egg white in the original recipe (Gillespie 2016). Another patent protects the use of *Quillaja* extract in combination with oligosaccharides and/or polyols as additives in beer for foam stabilization. This invention includes a procedure of enzymatic treatment and filtration of the *Quillaja* extract to prevent precipitation in alcoholic beverages. By using this treated *Quillaja* extract, the turbidity-causing proteins in beer could be removed without adversely affecting beer foam quality and stability (Alarcon Camacho & Sainz Lobo 2010).

Many nonalcoholic beverages are composed of an emulsion that contains substances having a low water solubility. Often, such beverage emulsions are stabilized by weighing agents. As an

alternative, *Quillaja* extract alone or in combination with polysorbate was shown to efficiently stabilize beverages to maintain small droplet sizes and a clear appearance (Tran & Li 2012). Clear appearance of beverages was also the reason for a patented solubilization agent based on saponins. This solubilizing agent is composed of one or more emulsifying polymer(s) and saponin(s), e.g., *Quillaja* saponins to solubilize polyphenols or flavonoids and diterpenoid glucosides to disperse them homogeneously in a food or cosmetic product (Riess et al. 2010). Furthermore, the preparation of unpurified flavor oil nanoemulsions was achieved by using *Quillaja* saponins. This formulation was patented for the preparation of a flavored clear beverage concentrate (Schultz & Monnier 2015). The combination of saponin–protein (Ogasawara et al. 2000) and saponin–lecithin (Schrader et al. 2011) mixtures as emulsifier systems was also patented. The latter can be used to incorporate functional ingredients such as flavors, vitamins, food colors, or polyunsaturated fatty acids in concentrate or to dilute beverage emulsions (Schrader et al. 2011).

Another approach to implement and protect active ingredients was described by Weiss et al. (2016). The invention includes a composition and procedure for SLNs to encapsulate active ingredients for prolonged shelf-life by keeping the less ordered and more stable α-subcell crystal structure of the solid lipids for protection. For this, an emulsifier system was prepared comprising at least one saponin (e.g., *Quillaja* saponin) that crystallized prior core crystallization and thus provided physical and chemical stability of active ingredients (see section titled Solid Lipid Nanoparticles and Nanostructured Lipid Carriers) (Weiss et al. 2016). Another invention covers the preparation of encapsulated flavor or fragrance for powdered food products. By using a plant extract that contained saponins (e.g., *Quillaja* extract) and a biopolymer ($M_w < 100$ kDa), flavor emulsions with reduced viscosity could be prepared. This simplified the spray-drying process carried out afterward to obtain stable powders (Bouquerand et al. 2014). The encapsulation of capsicum oleoresin (e.g., pepper, red pepper, chili pepper, chili powder) in (*Quillaja*) saponin-stabilized emulsions was patented for snack foods to enhance the adhesion of flavors to food surfaces. That way, flavors remained on the food rather than adhering to the hands and fingers of consumers who ate the snacks (Wiersma 1998).

Finally, several applications of *Quillaja* saponins refer to their useful interactions with cholesterol. The complexation of *Quillaja* saponins and cholesterol was patented to remove cholesterol from food products, such as, e.g., dairy products (Sundfeld et al. 1994). The interactions between *Quillaja* saponins and cholesterol were suggested to be due to a binding of fatty acid chains of *Quillaja* saponins (as shown in **Figure 3** for QS-21) to cholesterol (Sun et al. 2009).

CONCLUSIONS AND PERSPECTIVES

This article reviewed the physical, chemical, and biological as well as technofunctional properties of *Quillaja* saponins. The literature compilation indicates that the purity and composition of *Quillaja* saponins are crucial for their interfacial activity and physicochemical characteristics and their subsequent ability to form and stabilize numerous colloidal structures in food, feed, cosmetic, and pharmaceutical sectors. *Quillaja* saponins are highly promising natural surfactants. The specific interactions between *Quillaja* saponins and proteins are extremely interesting and present novel opportunities to structure protein molecules. Combining *Quillaja* saponins with other cosurfactants also offers the chance to reduce the amount of *Quillaja* saponins needed and thus retain the ecological sustainability of the *Quillaja saponaria* Molina trees. Although *Quillaja* saponins have been extensively studied in the past years, as illustrated by this review, more studies focusing on molecular interactions with cosurfactants and their sensory impact, especially their bitterness, are still needed. An approach that has yet to be investigated and could be quite promising is the alteration of the chemical structure of *Quillaja* saponins, e.g., by cleaving one or more of the sugar

moieties and thereby changing the preference for particular interfaces such as water-in-oil interfaces. Likely, there exist suitable enzymes in nature, as the function of saponins in plants is to transport sugars from the root to the leaf system. The sugars are then cleaved off, which often renders the saponin bioactive, making it an effective defense agent for the plant. Taken together, *Quillaja* saponins are proving to be very valuable and highly functional naturally occurring compounds whose full potential is still to be explored.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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