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Foaming and Emulsification Properties of Surface Active Saponin Extracts from *Balanite aegyptiaca* Delile and *Securidaca longepedunculata* Fresen

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Abstract

The Foamability and emulsification capacity was analyzed for the Enriched surface active saponins of HBAR, HSLR, HSQER, HSQE25-30%, synthetic surfactant SDS, and Tween 80. The enriched saponin extracts showed positive results for enriched saponins presence. The results for foam height showed the enriched saponins HBAR with the highest foam height of 23.6 cm - 17.6 cm. HBAR, being the highest foamability saponin and SDS, 17.6 cm and T80 the least, 17.3 cm.HBAR and HSLR indicated stable R% values at R5% and R15% at 86.5% - 81.2% and SDS, and T80 at 56% and SDS at 55.5%. R15% revealed decreased values at 57.3% to 27.6% for the saponins, SDS and T80 34.6%-32.9%. The EI_0 and EI_{24} showed that saponins values ranged from HBAR 79% to HSQE 25-30% 30% and HSLR 32% to HSQE 25-30% 25%.The nanoemulsion polydispersity index, except for the saponins crude extract BAR 905.7 nm and SLR 629.5 nm, the hydroxylated saponins HBAR, HSLR, HSQER, HSQE 25-30% z-average were between 8.29 nm to 99.35 nm. The studied low-concentration saponin solution has shown viable and efficient foamability, foam stability, emulsification and alternative applicable surface properties.

Keywords: Foam, Foamability, Saponin extract, Foam stability, Surfactant

Introduction

Foam is a substance trapping air or gas bubbles inside a solid or liquid. Typically, the volume of gas is much larger than that of the liquid or solid, with thin films separating gas pockets. A foam system consists of spherical gaseous voids in a dense matrix (Lee *et al.*, 2007). Foaming takes place when free gas molecules are transformed into spherical bubbles. It typically occurs when the surrounding conditions change too drastically to permit a smooth response from the system (Petkova *et al.,* 2020). Saponins are trendy surface active glycosides which are present in a wide range of plant species (Oakenfull, 1991; Sparg *et al.,* 2004; Balakrishnan *et al.,* 2006; Vinken *et al.,* 2007; Guclu-Ustundag & Mazza 2007; Hostettmann & Marstom 1995; Muntaha *et al.*, 2015). A common structural feature of all saponins is a triterpene or steroid aglycon part, decorated with typically one or two oligosugar groups, linked to the aglycone via ether or ester bonds. The steroidal-aglycone saponins are typical for monocotyledon plants, while dicotyledons produce mainly triterpenoid saponins. The sugar groups typically comprise arabinose, glucose, rhamnose, xylose and galactose (Sparg *et al.,* 2004). With various chemical structures of both parts, many combinations of hydrophilic glycols and hydrophobic aglycons give rise to an amphiphilic character of the whole molecule, providing several distinct saponins with distinct surface activity (Sparg *et al.*, 2004). The surface activity of the saponins results from the presence of a hydrophobic scaffold which comprises a triterpenoid, steroid or steroid-alkaloid group and a hydrophilic part which consists of different saccharide residues which are linked to the hydrophobic scaffold via glycoside bonds. Various molecular structures are found within different plant species and often within the same plant species (Vinken *et al*. 2007). This wide range of molecular structures gives rise to various physicochemical properties, biological activity and function. The intrinsic surface activity of the saponins is the basis of their use as foam stabilizers in beverages such as beer and soft drinks (Oakenfull 1991; Guclu-Ustundag & Mazza 2007; Cheeke 1999), in emulsion stabilization (Oakenfull 1991; Cheeke 1999), and solubilization of additives in foods (Jenkin & Atwal 1994). Different aspects of their biological activity are the basis of their use as natural medicines (Liu & Henkel, 2002; Fukuda *et al.*, 2000). However, saponins exhibit anti-inflammatory, anti-fungal, anti-bacterial, anti-viral, and anti-cancer properties and cholesterol-lowering potential. These properties make them increasingly important in applications in cosmetics, shampoos and conditioners, and skin anti-ageing products (Brown 1998; Sirtori 2001). The surface activity and the wide range of complementary properties of saponins make them potentially crucial for an even more comprehensive range of applications (Guclu-Ustundag & Mazza, 2007). In light of these adsorption properties, saponins have been extensively studied (Van-Wazer, 1947; Stanimirova *et al.*, 2011; Golemanov *et al.*, 2012; Wojciechowski 2013; Golemanov *et al.*, 2013; Golemanov *et al.*, 2014; Wojciechowski 2014; Pagureva *et al.*, 2016; Bottcher & Drusch 2016; Tippel *et al.*, 2016, Tucker *et al.*, 2020); and in addition saponins self-assembly in solution were characterized (Mitra & Dungan 1997; 2000;2001; Tykaraska *et al.* 2012; Kitamoto *et al.*, 2009; Piexto *et al.*, 2011; Liu *et al*., 2013; Wojciechowski *et al.*, 2016; Matsuoka *et al.*, 2016). Furthermore, saponins mixing properties with various proteins have been studied (Matsuoka *et al.*, 2016; Wojciechowski *et al.*, 2011; Piotrowski *et al.*, 2012; Kerwon & Wojciechowski 2014; Wojciechowski *et al.*, 2014; Bottcher *et al.*, 2016). Foams are systematic hexagonal textures formed due to gas dispersion through a continuous surfactant solution. It is thermodynamically unstable, and surfactants stabilize them to prevent bubble coalescence. Foams are also described in terms of their characteristic foamability, defined as the capacity of the surfactants to form foam irrespective of the unique foam properties, and foam stability describes the variations of foam height or volume with time, immediately after foam generation (Malysa & Lunkenheimer, 2008). Foamability and foam stability are interrelated; the more stable the foam films, the greater the solution's foamability. This work aimed to evaluate efficiencies of foaming properties such as Foamability, foam stability and the resultant effect of saponins and synthetic surfactant concentration influencing the interfacial properties.

Materials and methods

Extracts of enriched saponins were prepared from *Balanite aegyptiaca* and *Securidaca longepedunculata*. Commercial saponins extracts of Quillaja HSQE 25-30% and HSQER were obtained from Teledyne Inc. USA. The synthetic surfactants, Sodium dodecyl sulfate and Polysorbate monooleate (Tween 80), were obtained from Fischer Scientific, USA, and used without further purification. Milli-Q water was prepared from the laboratory using the Millipore Milli-Q Ultra-Filtration Plus water purification system.

Foaming Index

The saponins' foaming ability was determined by applying the method of Reza *et al.* (2016) with slight changes in concentration; 3% w/v of all experimental and reference samples were prepared in Milli-Q water. Then 3 ml of each surfactant solution was placed in a test tube and vortex for 5 seconds at 25 ^{0}C. The foaming height for each Surfactant was measured for the first 7 minutes and between 2 minutes intervals ranging from 0 and 15 minutes. The measurement obtained average values recorded from triplicate readings for each Surfactant.

Foam Stability

The foam stability was determined by adopting the method of Reza et al. (2016) with modifications for saponins, SDS and Tween 80 3 % w/v surfactant solution samples by generating foaming capacity through 5 seconds vigorous shake of 3 mL surfactant solution, and allowed to observe the stability countdown for the foam height collapsed as measurements are taken between 2 minutes intervals in 12 minutes.The R5% parameter was used to validate the surfactants stability Lunkenheimer & Malysa (2015).

$R5 = h_5/h_0 \text{ X } 100$……………………………..(1)

Emulsion Index (EI_{24})

The emulsifying capacity of saponins surfactant solutions was evaluated by an emulsification index (EI_{24}). As determined by adding 1.5 mL of crude oil and 1.5 mL of the surfactant solutions into a test tube, vortexed for 2 min spin at 300 rpm, and allowed to stand for 24h, 48h and 72h with repeated spinning to begin a cycle. The average EI_{24} was given as a percentage of the height of the emulsified layer formed divided by the total height of the liquid column (cm) of the entire solution. The emulsification index percentage is calculated using the following equation EI_{24} = height of emulsion formed x 100 / total solution height (Ebrahimi *et al.* 2012), as adopted by (Reza *et al.* 2016) with slight modifications.

$$EI_{24} = \frac{height\ of\ emulsion\ formed}{total\ height\ of\ surfactant\ solution} \text{ x } 100 \quad \ldots\ldots (2)$$

Results and Discussions

Foaming ability

Foaming ability is a fundamental property of saponins and surfaces active agents performing efficiently and effectively. Figure 1 displayed various saponin extracts of HBAR, HSLR, HSQE 25-30% and HSQER that showed appreciable foam heights and stability with maximum foam height between 23.6 cm of HBAR and 20 cm HSLR. This characteristic foaming capability of the enriched saponins extracts has demonstrated foaming ability, hence assumed to contain high saponins as shown by the results of the foaming stability. Foaming influence requires rapid adsorption, high surface elasticity and viscosity (Piispanen *et al.* 2004). Therefore the highly dense foam produced by hydroxylated saponins of HBAR 23.6 cm and HSLR 20 cm solutions may be due to a large amount of saponin present. The commercial saponins extract HSQE 25-30%, and HSQER indicated moderate foam heights of 18 cm and 19.6 cm, while the synthetic Tween 80 and SDS showed 17.6 cm and 17.3 cm. The saponins sample foamability performance showed HBAR>HSLR>HSQER>HSQE 25-30%>T80>SDS, respectively. A high foaming power requires rapid adsorption, high surface elasticity and viscosity (Piispanen *et al.* 2004). In addition, Birdi (2010) stated that saponins resulted in high dynamic surface tension reduction that helps generate large surface areas required for foaming. Pradhan and Bhattacharyya (2017) reported high foaming power of plant-based surfactant extracts of Henko between 2 cm – 46 cm and Ritha 6 cm – 33 cm, while that of Pyagi Phool was comparatively lower between 2 cm - 21 cm. Foaming increases with increasing concentration due to the availability of more surfactants in the films to stabilize the foam (Pradhan & Bhattacharyya, 2014).

Figure 1. Foaming of saponins extracts, SDS and Tween 80

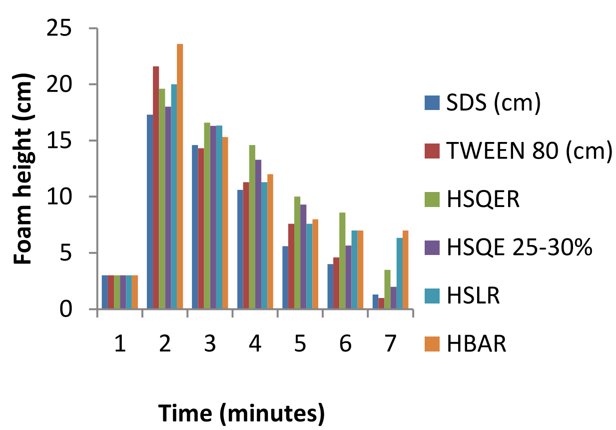

Figure 2. Foam height of HBAR, HSLR, HSQE 25-30%, HSQER, SDS and Tween 80.

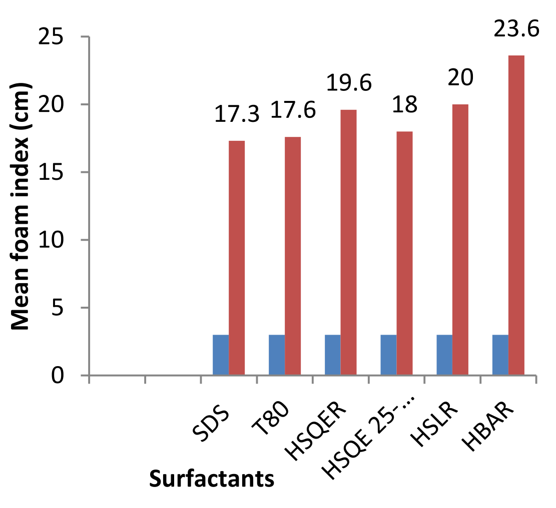

Figure 3. Maximum foam index height of saponins and synthetic surfactant solutions.

Foam Stability

Figure 4 – 9 showed foam stability for the saponin samples and synthetic Surfactant studied. The result showed foam stability in 15 min observation for HBAR 23.6 - 6 cm, HSLR 20 - 6 cm, HSQER 19.6 - 4.3 cm, Tween 80 17.6 - 3.3 cm, HSQE 25-30% 18 - 3 cm and SDS 17.3 - 2.3 cm. The R5 % stability value for the saponins showed HBAR 86.5%, HSLR 81.2%, HSQER 75.3 %, HSQE 25-30% 58.4%; and the synthetic surfactants indicated 56% for Tween 80 and 55.5% for SDS. The foam stability was analyzed using the parameter relating the ratio of initial foam height and that of 5 min and 15 mins observations as R5% and R15%. The saponins' stability showed a better R5 % efficiency in the order HBAR>HSLR>HSQER>HSQE 25-30%>Tween 80>SDS. Further observation for R15 % for 15 min stability, the stability order revealed decreased R15% values with HBAR 57.3%>HSLR 55.5%>HSQER 54%>SDS 34.6>Tween 8032.9%>HSQE 25-30% 27.6%. Therefore the parameter R5, defined as the ratio of the foam height at 5 min after formation to the initial height, is proposed to evaluate foam stability Lunkenheimer & Malysa, (2015). Lunkenheimer and Malysa also maintained that foams with R5 values higher than 50% are considered metastable, while lower R5 values indicate low stability.

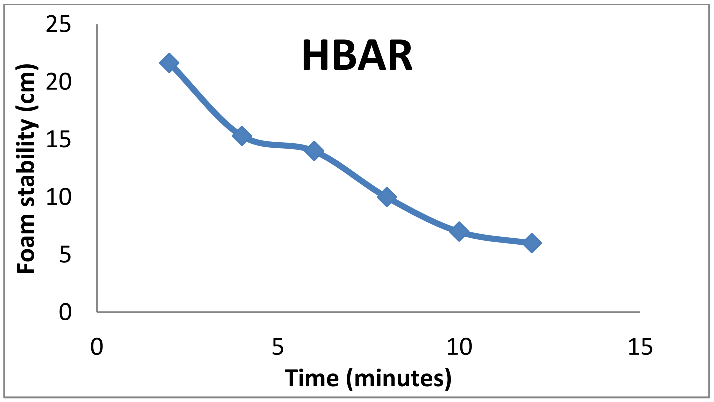

Figure 4. Foam stability of HBAR

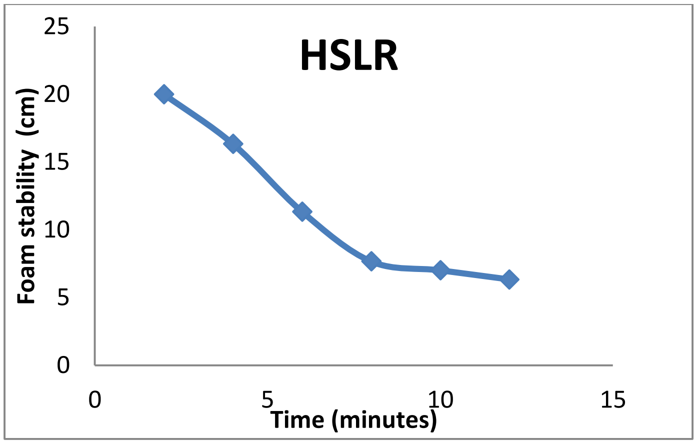

Figure 5. Foam stability of HSLR

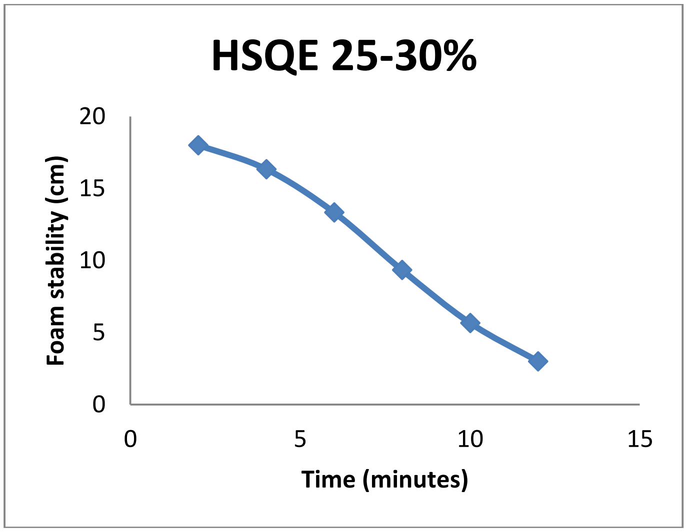

Figure 6. Foam stability of HSQE 25-30 %

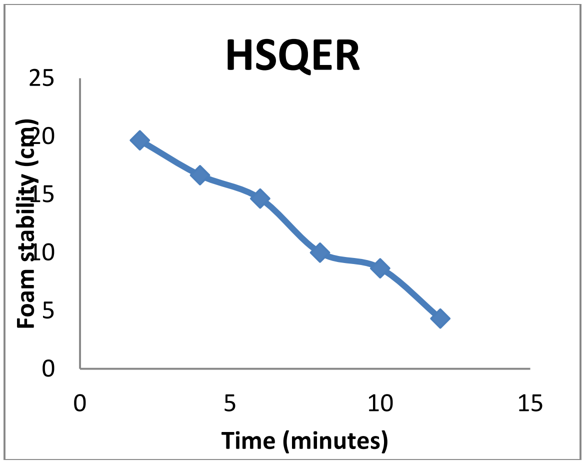

Figure 7. Foam stability of HSQER

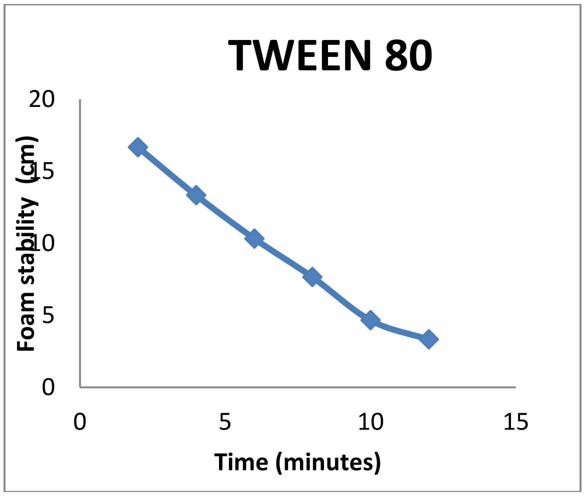

Figure 8. Foam stability of TWEEN 80

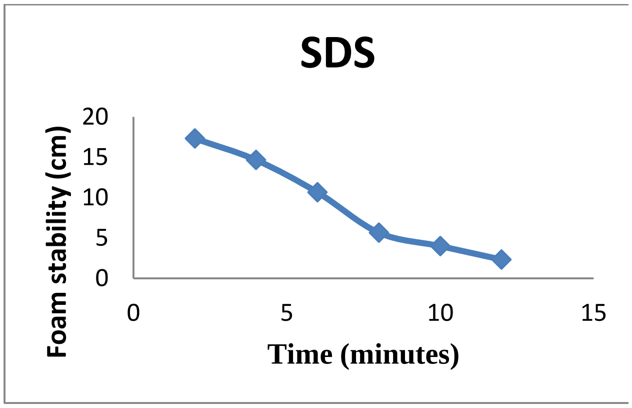

Figure 9. Foam stability of SDS

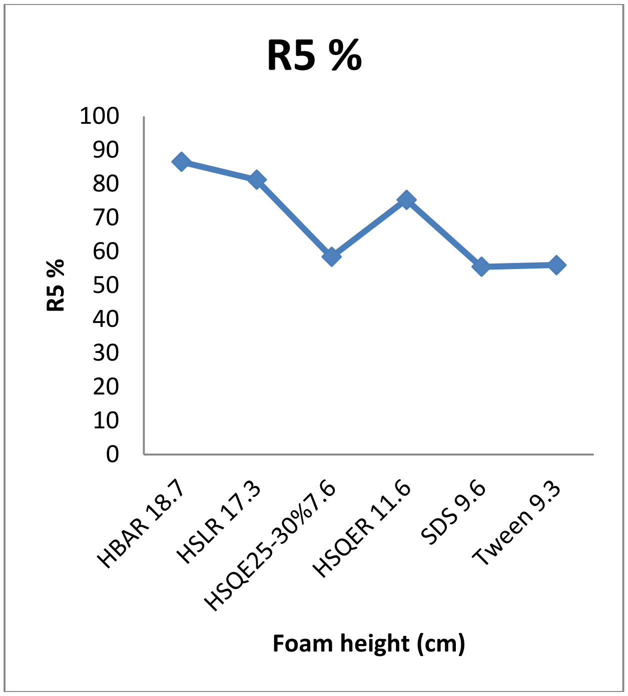

Figure 10. R5 values of surfactant stability

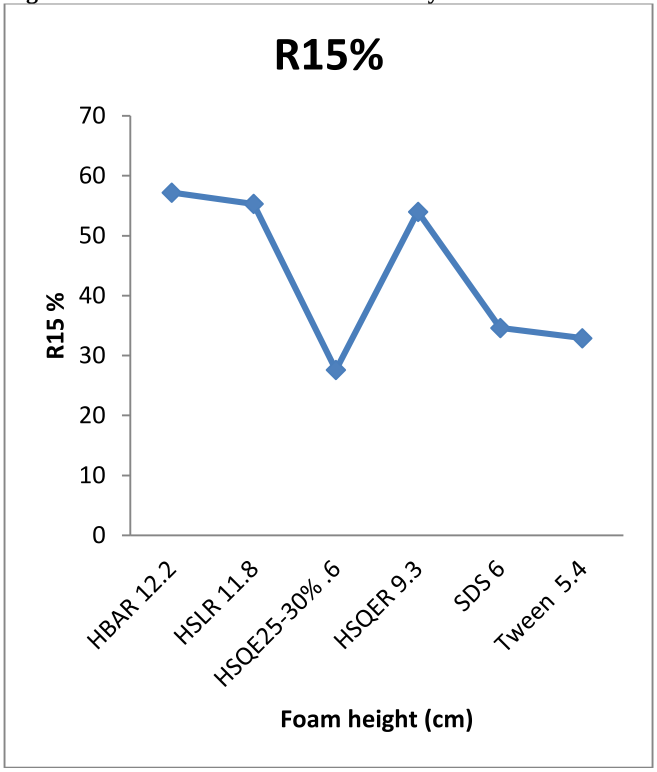

Figure 11. R15 % values of surfactant stability.

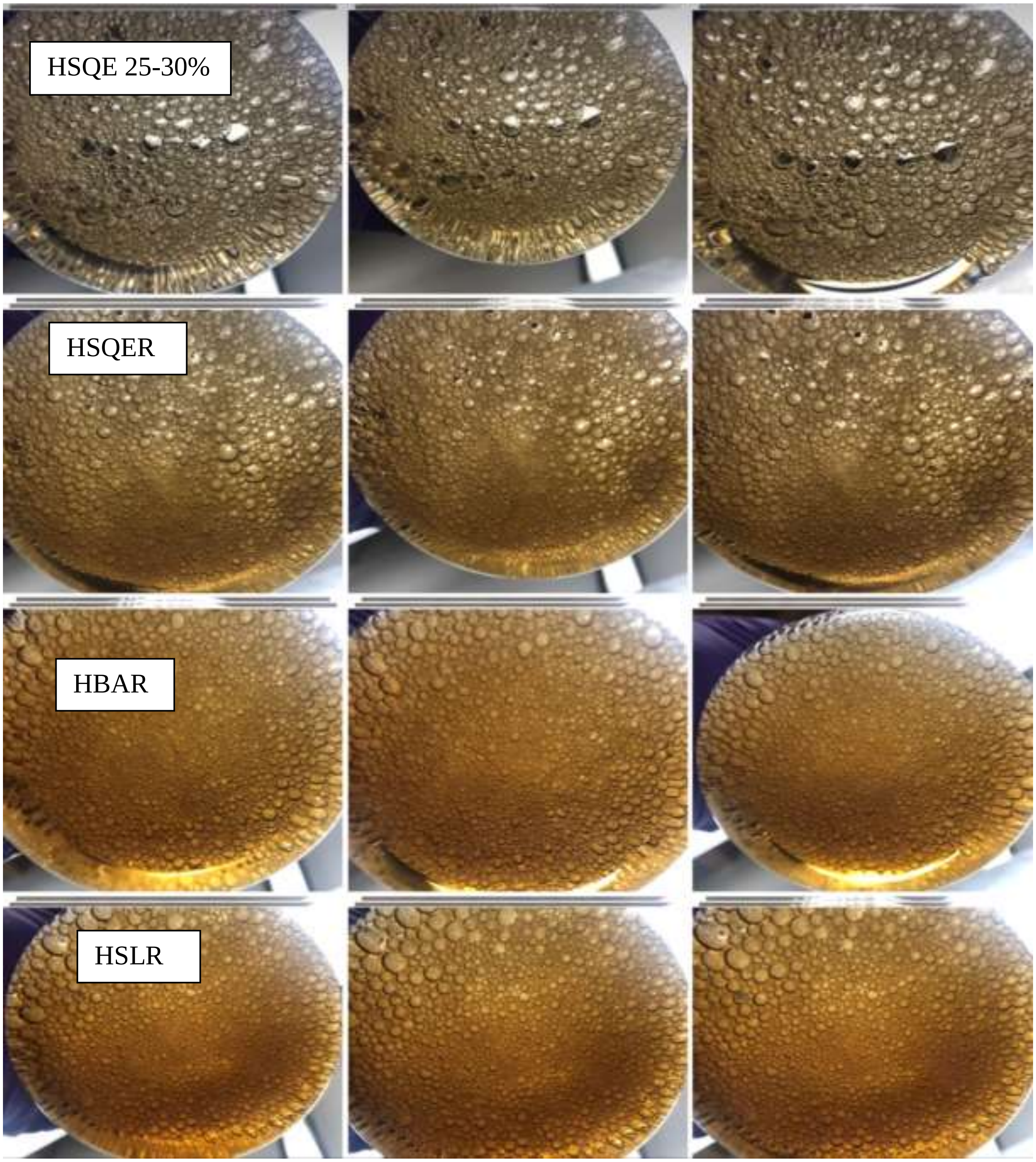

Figure 12. Photograph of saponins foam coalescence of HBAR, HSLR, HSQER 25-30%, HSQER, SDS and Tween 80.

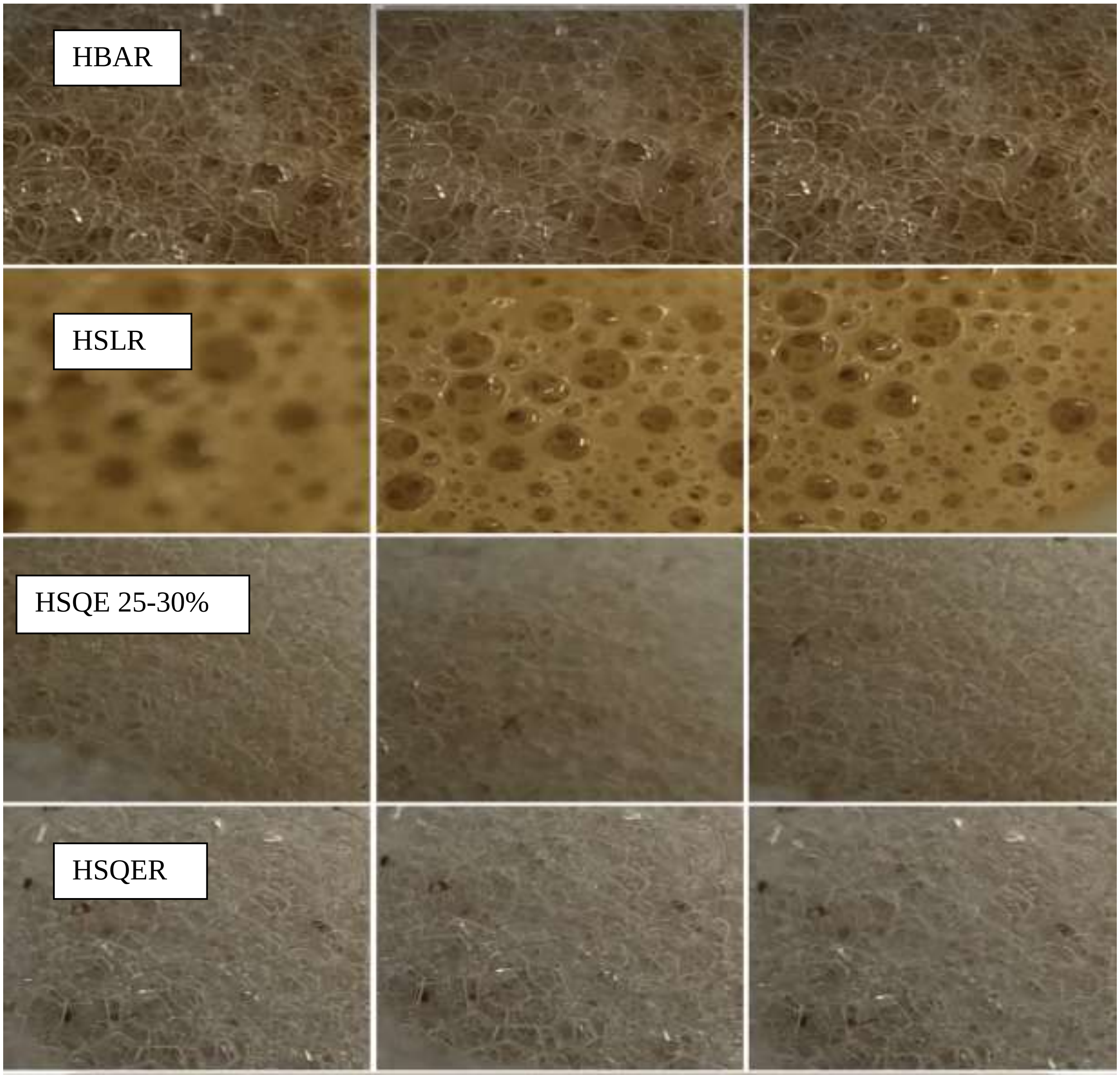

Figure 13. Photograph of Emulsions foam of HBAR, HSLR, HSQE 25-30% and HSQER

Emulsification Index EI$_{24}$

The emulsion index analysis performed for the modified surfactants showed the results in Figure 5. The hydroxylated enriched saponins of HBAR indicated viable emulsifying tendencies at 79% ±0.04 - 38% ±0.01 and HSLR 74 %±0.41 - 39 %±0.19, while the commercially obtained saponins of HSQE showed 30% ±0.12 - 25% ±0.31 and HSQER 59 % ±0.20 - 32% ±0.12 of 2 mg/mL emulsion formation of saponins in water solutions. The synthetic anionic and nonionic surfactants SDS and Tween 80 indicated a value slightly lower than the extracted saponins of plant 26% ±0.12 - 40 %±0.06 for SDS and 19% ±0.12 - 44% ±0.06 for Tween 80 in this work. Saponins extract have shown moderate emulsifying power from several established works of literature. Previous studies reported on surface active agents from plants showed excellent results. Jarzebski et al. (2019. 2020) reported addition of *Saponaria officinalis* and *Quillaja saponaria* saponins to extract that caused the survival of the higher emulsion layer, reaching up to 11% at an extract concentration of 2 g/L; and using the optimum conditions for preparing Hemp Seed Oil emulsions resulted in 98.63% ± 1.95 and 92.72% ± 2.21 entrapment efficiency based on the linoleic acid released in emulsion medium. Further coherent emulsification index of plant-based natural saponins was reviewed by Dammak et al. (2020). This study showed similar results to Schreiner et al. (2021) reported on the emulsion capacity of rich natural saponins of *Tribulus terrestris (TT)* 45-57%, *Trigonella foenum-graecum (FG)* 38-42%, *Ruscus aculeatus (RA)* 27-43% and a commercial pure saponin (PS) 49-74%, with the performance level of PS>TT>FG>RA.

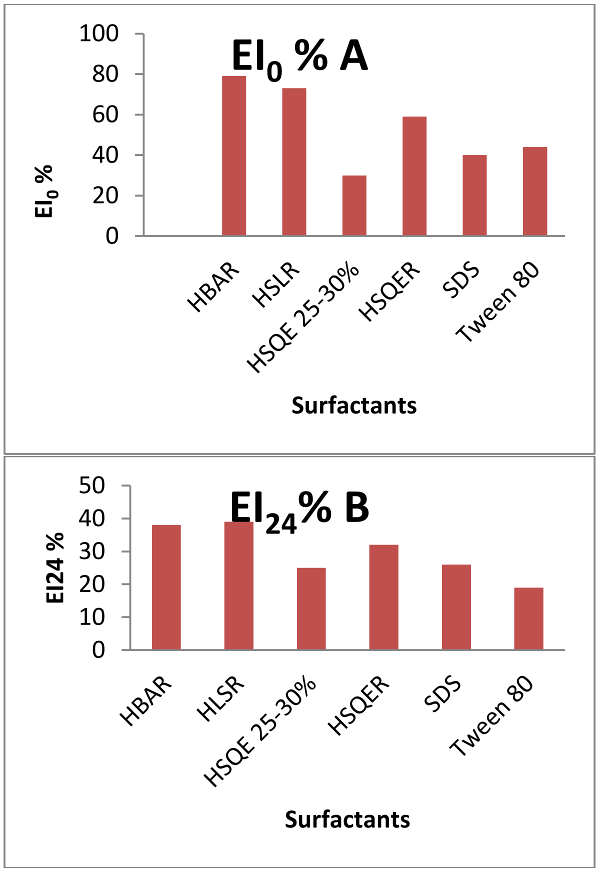

Figure 14. Emulsification index at initial (A) EI_0 and 24 hours (B) EI_{24}.

Polydispersity Index (PDI)

The emulsion values for the saponins and surfactants found in Table 12 showed the improved dispersant properties of the emulsion on the hydrodynamic (z-average) and polydispersity index recorded between the hydroxylated and un-hydroxylated saponins. The BAR, SLR, SQE25-30% values and SQER indicated 905.7 nm and 0.686, 629.5 nm and 0.791, 27.41 nm and 0.895 and 8.509 nm 0.704 were the z-average and PDI for the un-hydroxylated saponins respectively. The highest value was recorded for BAR z-average 905.7, while the SQE 25-30% has the highest PDI value for the un-hydroxylated saponins. The z-average and PDI values for Hydroxylated saponins HBAR, HSLR, HSQE 25-30%, and HSQER were 43.44 nm and 0.525, 53.19 nm and 0.379, 51.45 nm and 0.470, 99.35 nm and 0.431 respectively. The hydroxylated saponins z-average values for HBAR and HSLR showed a significant decrease from those for saponin standards (HSQE 25-30, HSQER) at 51.45 and 99.35 nm; alternatively, the PDI values for hydroxylated saponins showed decreased values across. The synthetic Surfactant recorded 4.294 nm and 86.32 nm; and 0.042 and 0.385 for Tween 80 and SDS z-average and PDI accordingly. However, the result of this study is similar to the reported values by Jarbesski et al. (2019) for lecithin and pea protein co-surfactant for Hempseed oil nanoemulsion at concentrations between 0.0% to 1.4% indicated z-average and PDI values range of 207 nm, 209 nm, 309 nm, 803 nm, and 0.233 to 0.787 accordingly. Interestingly, the work values reported have shown improved quality and properties of emulsion for efficient and optimal oil dispersion, as recorded. Thus, the Pdi influence smaller droplet formation and increases the stability. The interfacial tension between the droplet must be decreased (Jarzebski et al. 2019). A study on *V nigrum* as a natural emulsifier revealed Pdi of 133 nm and 92 nm (Jarzebski *et al*. 2018).

Table 1. Polydispersity Index

Dispersant solution	Intercept	Z- average (r.nm)	PDI
BAR	0.984	905.7	0.686
SLR	0.984	629.5	0.791
SQE 25-30%	0.732	27.41	0.895
SQER	0.848	8.509	0.704
HBAR	0.895	43.44	0.525
HSLR	0.972	53.19	0.379
HSQE 25-30%	0.936	51.45	0.470
HSQER	0.969	99.35	0.431
TWEEN 80	0.938	4.294	0.042
SDS	0.950	86.32	0.335

Conclusion

In conclusion, the foaming surface properties of the enriched saponin solutions of HBAR, HSLR, HSQE 25-30 % and HSQER showed effective foamability than the synthetic nonionic and ionic surfactants Tween 80 and SDS. In addition, HBAR and HSLR saponin solutions indicated the highest foam height and foaming stability. The results indicated that plant-based saponin of HBAR and HSLR have demonstrated significant amphiphilic properties relevant to its utilization as a foam stabilizer, emulsifier and dispersant.

Conflict of Interest

No author declares a conflict of interest.

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