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ORIGINAL ARTICLE

Physicochemical properties of saponin containing *Acanthophyllum laxiusculum* extract: example application in foam stability and qualitative parameters for malt beverage industry

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Abstract Aqueous Extract of *Acanthophyllum laxiusculum* root (AE) exhibited remarkable foaming profile, emulsification properties and antifungal activity due to the presence of high concentration of total saponins. Total phenolic compounds, another main component, accounted for the AE antioxidant activity. Spray drying of AE, as a thermal process, did not affect the foaming indices of *A. laxiusculum* Spray-dried Extract (SE) and is a recommended alternate for convenient application in food industry. Addition of SE to the malt extract at accepted levels of total saponin daily intake (below 50 mg/kg) showed positive attribution of malt and carbonation on the foaming quality of SE. Meanwhile, antioxidant activity of commercial malt beverage was enhanced by addition of phenolic compounds containing SE. The 20–60% antifungal inhibitory ratio of the SE developed here is within the applied range of total saponin that supports its application to inhibit the growth of *Saccharomyces cerevisiae* after malt beverage production. The bitter taste of SE was not sensed in malt beverage at 30 mg/kg total saponin content and suggested *A. laxiusculum* root extract as a natural additive in malt industry.

Keywords *Acanthophyllum laxiusculum* · Saponin · Foam stability · Antioxidation · phenolic compounds

Abbreviations

AE	Aqueous extract of *A. laxiusculum* root
SE	Spray-dried extract of *A. laxiusculum*
CWSs	Carbonated water saponin solutions with SE of *A. laxiusculum* samples
CMSs	Carbonated malt saponin beverages with SE of *A. laxiusculum* samples
NWSs	Non-carbonated water saponin solutions with SE of *A. laxiusculum* samples
NMSs	Non-carbonated malt saponin beverages with SE of *A. laxiusculum* samples
Bx	Brix, total reduced soluble sugar
bw	Body weight

Introduction

Acanthophyllum is a genus of the *Caryophyllacea* family which are commonly recognized in Turkmenistan, Afghanistan and Iran (Karazhiyan 2019). To date, 61 plant species have been identified for this genus, from which 33 species are cultivable and 23 species are endemic in Iran (called "chubak") (Karazhiyan 2019; Soltaninejad et al. 2016). Noticeably, in some publications, the term "soapwort" has been considered equivalent to generally named chubak (*Acanthophyllum* genus), *Saponaria officinalis* (*Saponaria* genus) or *Gypsophila arrostii* (*Gypsophila* genus) (Ayres and Loike 2005; Çelik et al. 2007;

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Karazhiyan 2019) where scientifically this term should be specific for *Saponaria officinalis*.

Acanthophyllum genus generally contains triterpenoid saponins and exhibits properties related to the saponin application including emulsification, surface active agent, foam formation and antimicrobial activity (Ayres and Loike 2005; Reichert et al. 2018; Sezgin and Artik 2010).

Plant saponins are glycosides containing two groups of glycons and aglycons. Glycons are 1, 2 or 3 sugar chains linked to the aglycones (steroidal or triterpene structure) to produce mono, bi or tridesmoside glycosides. (Ayres and Loike 2005). The toxicity of saponins is an extremely important issue due to their widespread occurrence in foods. Although, this component has been used in some products such as "Halva Ardeh", reports regarding its toxicity effects have been neglected (Soltaninejad et al. 2016).

Hemolytic saponins interact with the cholesterols in the erythrocytes membrane, which lead to generation of pores on the membrane and release of hemoglobin from cytosol to the extracellular solution. Considering this hemolytic activity, Zheng et al (2019), created a software named "e-Hemolytic-Saponin" which evaluates and predicts the hemolytic activity of saponins according to its database. Generally, the oral toxicity of saponins is relatively low due to the weak absorption of saponins in the body and steroidal structure have more toxic effect relative to triterpenes (Ayres and Loike 2005). However, structural and active surface properties of saponins have led to their widespread applications in fields of medicine and packaged food.

Among the most important industrial applications of saponins, is their foaming attribute which has been applied in the production of malt beverages (Camacho and Lobo 2016).

The formation and quality of foam in malt beverages are influenced by malt's intrinsic constituents including amount and the type of polypeptides (especially proteins with MW > 5000 Da and high number of hydrophobic regions), high molecular weight non-starch oligosaccharides and extrinsic constituents such as polyphenols especially hop's iso-alpha-acids and their oxidized or reduced derivatives (Evans and Bamforth 2011). According to Camacho and Lobo (2016), application of *Quillaja* saponin within the range of 0–200 mg/kg in beer induced more stable foam formation for maximum of 274 s (Camacho and Lobo 2016).

Surface-active plant components, such as total saponins in *A. laxiusculum* root, with foaming properties and antifungal attributes are good candidates for application in related food formulation. The aim of this study is to investigate the physicochemical foaming properties, antimicrobial and antioxidant activities of *A. laxiusculum* root extract and its application in malt beverage industry. Nonetheless, by perceiving the characteristic of this plant extract, its further application in food industry is envisaged.

Materials and methods

Chemicals and materials

The 1,1-diphenyl-2-picryhydrazyl radical (DPPH), Folin-Ciocalteu reagent, sodium carbonate, gallic acid, isooctane, hydrochloric acid (HCl), sulfuric acid, ethanol, liquid paraffin and malt extract agar culture were obtained from Merck (Darmstadt, Germany). Glycyrrhizic acid ammonium salt as saponin standard was purchase from Sigma-Aldrich Chemical Co. (St. Louis, MO). *Saccharomyces cerevisiae* (PTCC-Code 5080) was provided by the Persian Type Culture Collection (Tehran, Iran).

Acanthophyllum laxiusculum roots were collected from rangelands in Yazd province (Iran). Malt beverages (carbonated and non-carbonated) were produced at Behnoush Company (Tehran-Iran).

Sample preparation

Aqueous Extract of *A. laxiusculum* (AE) was obtained by disintegration of the root and cutting it in approximately 10 cm pieces, followed by rinsing in cold water to remove any soil residue. Approximately 50 kg of the root was boiled in 150 kg of water for 5 h to reach 15°Bx (Brix, total reduced soluble sugar). The AE was sieved and filtered to separate the fine plant particles and create a clear solution for spray drying.

Approximately 20 L of AE was dried using a spray drier (Buchi mini spray dryer b290, Switzerland) to obtain the Spray-dried Extract (SE) of *A. laxiusculum*. The settings were adjusted as inlet: 140 °C, outlet: 80 °C at a feed rate of 5 mL/min. These samples (AE and SE) were chemically and physically analyzed and compared to investigate the effect of spray drying on *A. laxiusculum*.

Sets of Non-carbonated Water Saponin solutions (NWSs) and Non- carbonated Malt Saponin beverages (NMSs) were prepared by addition of SE at desired saponin concentrations to water and malt extract, respectively. According to JECFA, the saponin ADI is 0–1 mg/kg body weight (bw) and consumption up to 40 mg/kg is considered safe. Therefore, addition of SE to reach total saponin levels of 0 to 50 mg/kg at 10 mg/kg intervals was practiced in this study. These samples were prepared to investigate the physicochemical attributes of total saponin containing extract individually, and in the presence of malt.

Carbonated Water Saponin solutions (CWSs) and Carbonated Malt Saponin beverages (CMSs) were prepared by addition of 4.5 g dry ice to 1 L of NWS and NMS.

Proximate analysis of *A. laxiusculum*

Ash, fat, protein and reducing sugar content of *A. laxiusculum* root, AE and SE were measured in triplicates according to AACC methods 08–01.01, 30–20.01, 46–10.01 and 80–60.01, respectively (AACC 2000). A factor of 4.43 was considered to convert nitrogen determined by Kjeldahl to protein for plants product (Yeoh and Wee 1994).

Evaluation of total saponin content

Total saponin content of the *A. laxiusculum* root, AE (15° Brix) and SE (adjusted to 15° Brix) was measured by the vanillin-sulphuric acid assay (Tan et al. 2014) with modifications. A quantity of 0.25 mL sample was mixed with 0.25 mL of vanillin reagent (8% w/v in ethanol) and 2.5 mL sulphuric acid (72% v/v in water). Solutions were incubated at 60 °C for 15 min in a water bath, then cooled in cold water for 5 min. Absorbance at 560 nm was recorded using a spectrophotometer (Perkin Elmer lambda 2, USA). Glycyrrhizic acid ammonium salt was used as saponin standard to obtain a linear calibration curve according to Sezgin and Artik (2010). Total saponin was expressed as mg/g dry matter.

Determination of emulsification index

According to Soltaninejad et al (2016), AE and/or SE (adjusted to similar total reduced soluble sugar of AE) and liquid paraffin at a 1:1 ratio were added in a test tube and emulsified using a vortex for 2 min. The test tubes were stored at 25 °C for 24 h and emulsion layer thickness between the paraffin layer and sample solution was measured. The analysis was replicated six times and measurements recorded according to the following equation:

$$\text{Emulsification index} = \frac{\text{Emulsion layer height}}{\text{Total height}} \times 100 \quad (1)$$

Determination of antifungal activity

Antifungal activity of SE in specified quantity of 20, 30, 40 and 50 mg/kg total saponin content was evaluated based on colony formation assay (Lijima et al. 1995). *Saccharomyces cerevisiae* suspension was prepared based on 0.5 McFarland standard solution and serial dilutions technique. A 100 µL yeast cell suspension from the last dilution was spread over the surface of malt extract agar medium and incubated at 26 °C for 48 h. Colonies were counted in duplicate and inhibition ratio was calculated according to below formula:

$$\text{Inhibition ratio}\% = \frac{C - S}{C} \times 100 \quad (2)$$

where C is average number of colonies grown in the absence of SE and S is average of colonies grown at different total saponin levels.

Measurement of total phenolic content

Total phenolic compounds in root, AE, SE (rehydrated to 15° Brix) and CMSs were measured by the Folin-Ciocalteu method (Tan et al. 2014) with modifications. For this purpose, 0.5 mL of sample was mixed with 0.5 mL of Folin-Ciocalteu (0.2 N) and 5 mL distilled water. After 8 min, 1.5 mL of sodium carbonate solution (2% w/v) and 2.5 mL distilled water were added and the mixture was stored at 25 °C for 2 h. The absorbance of the solution at 765 nm was recorded using a spectrophotometer (Perkin Elmer lambda 2, USA). Total phenolic content was expressed as mg/g dry matter using line equation, based on gallic acid standard serial dilution within the range of 10 to 500 mg/kg.

Determination of antioxidant activity

The DPPH method was employed to evaluate antioxidant activity of AE, SE and CMSs (Tan et al. 2014), with modifications. A quantity of 0.5 mL samples with 2.5 mL of DPPH (100 µM) solution was mixed and incubated for 20 min at 25 °C, followed by reading the absorbance at 515 nm in the dark using a spectrophotometer (Perkin Elmer lambda 2, USA).. Ethanol was used as blank and results were recorded according to the following equation:

$$\text{Antioxidant activity } (\%) = \frac{\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{DPPH}}} \times 100 \quad (3)$$

where Abs_{DPPH} is absorbance of ethanolic solution of DPPH at 515 nm and $\text{Abs}_{\text{sample}}$ is absorbance of samples at 515 nm.

Determination of surface tension and optical images of foam bubbles

Surface tension was determined according to Khosharay et al (2018). Experiments were carried out at a controlled temperature of 25 °C by circulating fluid. A glass capillary tube with inner and outer diameters of 1.2 mm and 1.587 mm, respectively, was used to suspend a pendant droplet. This apparatus was equipped by a digital camera

(Olympus, model C-2000Z, Germany) which was connected to a computer and a light source to obtain droplet images. Following equation was applied to calculate the surface tension:

$$\gamma = \frac{(\Delta\rho \times g \times R_0)}{\beta} \quad (4)$$

where γ is surface tension, $\Delta\rho$ indicates density difference between air and drop material, and g is the gravity acceleration. β and R_0 values are calculated using the equations below:

$$\beta = 0.12836 - \left(0.7577 \times \frac{D_s}{D_e}\right) + \left(1.7713 \times \frac{D_s}{D_e}\right)^2 - \left(0.5426 \times \frac{D_s}{D_e}\right)^3 \quad (5)$$

$$R_0 = \frac{D_e}{2 \times (0.9987 - (0.1971 \times \beta) - (0.0734 \times \beta^2) + (0.34798 \times \beta^3)} \quad (6)$$

where D_e and D_s are the highest diameter and the horizontal size of droplet, respectively.

Foam bubbles of CWSs and CMSs were transferred into petri dishes to capture optical images using an optical microscope (Ceti Magnum-T/Trinocular Microscope, UK) with magnification of 40× and a digital camera (Sony Cyber-Shot DSC-H50, Japan).

Measurement of foam formation

Foam formation ability was evaluated according to the procedure recommended by Dagan and Balaban (2006) with modifications. A quantity of 5 mL sample was transferred to a test tube and vortexed for 5 s, followed by resting at 25 °C for 1 min. Foam formation is reported as the ratio of formed foam volume to the volume of initial liquid sample.

Foam stability assay

Foam stability of AE and SE was measured at different time intervals of 1, 5 and 10 h according to an assay described by Dagan and Balaban (2006). The measurement was performed manually due to the failure of an instrument.

Foam stability of carbonated, non- carbonated water and malt solution samples were determined by NIBEM-T meter (Kunimune and Shellhammer 2008). Briefly, foam was formed by injecting carbon dioxide and its stability was measured by an apparatus equipped with standard glass. The time required by probe to travel 30-mm distance in foam was reported as Nibem-30.

Measurement of apparent viscosity

Apparent viscosity of AE, SE (adjusted to similar 15° Brix), CMSs and CWSs was determined by a Physica MCR 301 Rheometer (Anton-Paar, GmbH, Graz, Austria) with cone and cup system (probe CC27). A shear rate range of 120–1000 s^{-1} was employed at 25 °C for the measurement.

Color evaluation

The colorimetric properties of L*, a*, b* and total color changes (ΔE*) were recorded using Hunter Color lab (Color Flex EZ, USA) after standardization by white and black surfaces.

Analytical determination of bitterness

This method mainly measures iso-alpha-acids, which are identified as the main components responsible for the bitter taste of malt beverages. A quantity of 10 mL sample (AE, SE, CWS, CMS and/or commercial samples) was mixed with 0.5 mL of HCl (6 N) and 20 mL of isooctane at 20°C. Flask containing sample was placed on a stirrer at 20°C for 15 min. The upper phase was subjected to centrifuge (Hettich, Germany) for 3 min at 996×g (2982 rpm). A mixture of isooctane and HCl was considered as blank and the absorbance at 275 nm was measured. The bitterness value was calculated by multiplying the absorbance value by a factor of 50 (Jaskula et al. 2007).

Sensory evaluation

The SE was added to a malt extract beverage (devoid of foaming additives) to achieve target final levels of 20 and 30 mg/kg total saponins (safe level for consumption). Samples were evaluated for visual acceptability (color, opacity, foaming), taste attributes (bitterness, oral sensation) and overall acceptance. Prepared samples and commercial product of the same malt extract beverage (in the presence of hops and foaming ingredient), were presented to 35 semi-trained panelists to score each attribute following the 5-point hedonic adaptation. Scoring scale followed as: very good (5), good (4), moderate (3), bad (2), very bad (1). These scores were considered as parametric data and ANOVA was performed to evaluate the significancy of difference due to the effectiveness of plant extract on defined parameters.

Statistical analysis

Statistical analysis was performed using SPSS Statistical Software version 21 (SPSS Inc., Chicago, IL, USA). All

descriptive data from the experiments were expressed as mean and standard deviation. The t test ($p < 0.05$) was used to compare AE and SE treatments (adjusted to similar value of Brix as AE). One-way ANOVA ($p < 0.05$) was applied to determine the significant difference among the means of NWSs, CWSs, NMSs and CMSs.

Results and discussion

Proximate analysis of *A. laxiusculum*

Table 1 depicts compositional analysis of *A. laxiusculum* root, its aqueous (AE) and spray-dried (SE) derivatives. Separation of woody part of the root by boiling approach in this study resulted in almost 60% transfer of soluble total saponins and total phenolics to AE, while most of the fat, protein and reducing sugars were still associated with the root residues.

Total saponin content

The total saponin contents of *A. laxiusculum* roots and AE were 159.28 and 304.83 mg/g dry matter, respectively. These values were higher compared to some other plant sources such as *Allium nigrum* root (20 mg/g), aqueous bitter melon (43.3 mg/g) and lower than *Paramignya trimera* root (520.5 mg/g) (Mostafa et al. 2013; Nguyen et al. 2016; Tan et al. 2014).

Table 1 shows that the extraction method was efficient to extract total saponins in the form of AE, meanwhile, spray drying of the same AE increased the percentage of total saponins in SE dry matter. Percent decrease of other compounds such as total phenolic compounds could account for this difference.

Emulsification index

Emulsion index of AE was 46.6%, highlighting its effective emulsifying properties. The emulsion index of SE was not significantly different from that of AE, which indicates that the spray drying process did not significantly affect this index of the *A. laxiusculum* extract (Table 1). However, spray drying as a thermal process may result in some modifications in the structure of total saponins, which needs to be investigated by other methods.

Antifungal activity

By increasing the total saponin level with the addition of SE, the yeast colony growth rate decreased within 48 h and a significant 56.09% inhibitory activity at 50 mg/kg total saponin concentration was observed (Fig. 1a). One of the saponins antifungal mechanisms is due to their affinity for

Table 1 Proximate analysis of *A. Laxiusculum* root and its aqueous and spray-dried extract

Parameters	AE (15° Bx)	SE (adjusted to 15° Bx)	*A. laxiusculum* Root
pH	4.34[a]	4.34[a]	–
Ash (w/w %)	0.63 ± 0.10[a]	0.54 ± 0.01[a]	13.29 ± 0.50
Fat (w/w %)	0.04 ± 0.01[a]	0.05 ± 0.01[a]	0.58 ± 0.01
Protein (w/w %)	0.04 ± 0.01[a]	0.06 ± 0.01[a]	1.38 ± 0.22
Reduced Sugar (w/w %)	6.23 ± 0.05[a]	6.50 ± 1.54[a]	33.65 ± 1.41
Total Saponin (mg/g dry matter)	304.83 ± 0.14[b]	345.65 ± 0.05[a]	159.28 ± 0.34
Total phenols (mg/g dry matter)	4.50 ± 0.26[a]	3.51 ± 0.14[b]	2.35 ± 0.10
Antioxidant Activity (%)	60.56 ± 0.76[a]	39.44 ± 0.81[b]	–
Surface Tension (mN/m)	42.41 ± 0.38[a]	41.20 ± 0.17[b]	–
Emulsion Index (%)	46.60 ± 2.21[a]	47.53 ± 1.38[a]	–
Foam Formation (%)	38.43 ± 0.60[b]	43.32 ± 1.93[a]	–
Foam Stability (%)	58.66 ± 5.78[b]	67.39 ± 1.75[a]	–
L*	23.42 ± 1.50[a]	15.31 ± 0.10[b]	–
a*	21.64 ± 1.56[a]	17.32 ± 0.29[b]	–
b*	32.68 ± 3.77[a]	19.88 ± 0.50[b]	–
ΔE*	79.90 ± 0.78[b]	81.79 ± 0.29[a]	–
Bitterness	3.70 ± 0.05[a]	3.46 ± 0.02[b]	–

Values are mean ± standard deviation in triplicate exept Emulsion Index (n = 6)

Mean values with different letters at the same row are statistically different ($p < 0.05$)

AE aqueous extract, *SE* spray-dried extract

Foam stability reported at 10 h

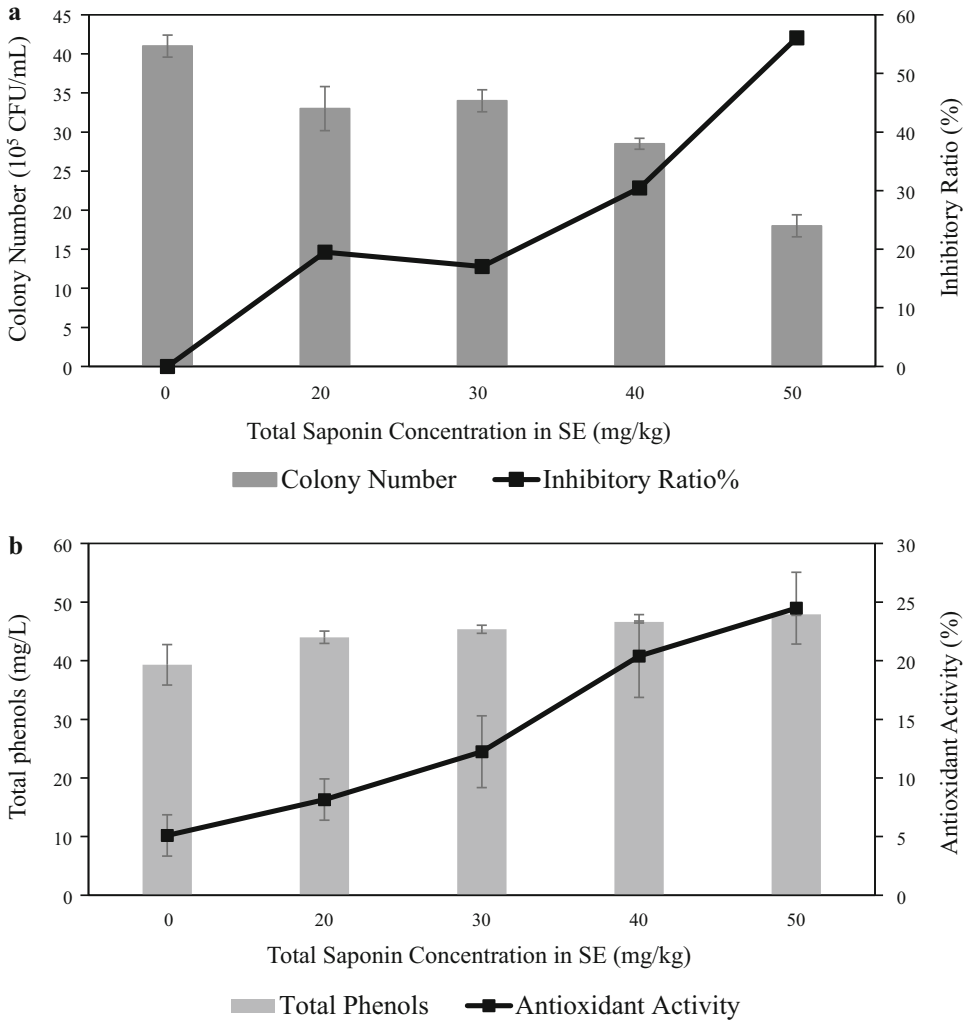

Fig. 1 Antifungal activity, total phenolic content and antioxidant activities of SE of *A. laxiusculum* (0–50 mg/kg total saponin concentration). **a** Antifungal activity of Non-carbonated Water Saponin solution (NWS) samples against *saccharomyces cerevisiae*. **b** Total phenolic content and Antioxidant Activity of Carbonated Malt Saponin beverages (CMS). Values are mean ± standard deviation, in triplicate

cholesterols in the yeast cell membrane. This formed stable complex lead to cell membrane destruction, leakage of cytoplasm compounds and eventual yeast cell death (Ayres and Loike 2005). The results showed that *A. laxiusculum* Spray-dried Extract (SE) had the ability to inhibit the growth of *Saccharomyces cerevisiae* as an alcohol-producing yeast in non-alcoholic malt beverages.

Total phenolic content

Table 1 presents *A. laxiusculum* root, AE and SE as rich sources of phenolic compounds. Comparatively, Nasri et al (2012) reported less content of these compounds in *Quillaja saponaria* (72.14 mg/g) and Lee et al (2011) evaluated higher content in soy bean (1.13 mg/g) and mung bean (2.03 mg/g).

Most of these phytochemicals were extracted into AE of *A. laxiusculum*, but the spray drying conditions of inlet/outlet temperature at 140 °C/80 °C reduced total phenolic compounds content, significantly. Similarly, Georgetti et al (2008) reported total phenolic reduction of soybean extract in the presence of colloidal silicon, maltodextrin and starch after spray drying at inlet temperature of 150 °C. Also, Caliskan and Dirim (2013) showed that an increase in the inlet/outlet temperature from 160/80 °C to 180/90 °C resulted in a 15% decrease in phenols content of sumac extract powder.

Expectedly, addition of SE of *A. laxiusculum* to carbonated malt extracts (CMSs) raised the initial total phenolic content, depending on the level of addition (Fig. 1b).

Antioxidant activity

Antioxidant activity of *A. laxiusculum* aqueous extract (AE) (Table 1) was near equal or even higher than some rich sources of antioxidant compounds including soy bean (59.5%) and sumac aqueous extracts (23.2%) (Caliskan and Dirim 2013; Georgetti et al. 2008). This characteristic is attributed to the simultaneous presence of total phenolic compounds and total saponins in AE of *A. laxiusculum*.

Hydroxyl groups in the structure of phenols and saponins can act as hydrogen donors and change the stable radicals of DPPH to non-radical form of DPPH-H (Lee et al. 2011). However, as shown in Table 1, spray drying of AE reduced antioxidant activity in SE of *A. laxiusculum*. According to the studies by Caliskan and Dirim (2013) and Georgetti et al (2008), an increase in inlet/outlet temperature of spray dryer reduced total phenolic compounds and antioxidant activity of sumac and soybean extract which are in line with our results. Expectedly, by the addition of SE of *A. laxiusculum*, the antioxidant activity of CMS was enhanced depending on the level of total phenolics and total saponins contained in SE (Fig. 1b).

Surface tension and optical images of foam bubbles

Spray drying caused a slight decrease in the surface tension of SE in comparison with AE (Table 1). The presence of lesser total saponins in AE caused a higher surface tension, when compared to SE with a higher total saponin concentration and lower surface tension. Saponins are one of the most effective surface-active compounds with hydrophilic (sugar chains) and hydrophobic (sapogenin) heads which tend to allocate between the aqueous and non-aqueous layers of the two phases. The hydrophobic part of saponin is set parallel to the air–water interface and the hydrophilic part is immersed in the aqueous solution (Stanimirova et al. 2011). Due to a minor structural deformation of saponins, the bubble wall shows greater elasticity and these occurrences lead to reduced surface tension, thereby increasing foam formation and stability (Maeda et al. 1991; Stanimirova et al. 2011; Treter et al. 2010). Reichert et al (2018) points to the role of saponins contained in *Quillaja* in reduction of surface tension by affecting the tertiary structure of proteins and their interactions in mixing saponin–protein emulsions; which improve adsorption of these saponins at low concentrations.

Total saponins contained in SE of *A. laxiusculum* reduced the surface tension in NWS and NMS, as shown in Fig. 2a.

The comparison of NWS and NMS indicates that presence of malt enhanced the effect of total saponins on surface tension reduction delivered by SE of *A. laxiusculum*. The initial surface tension of malt could have been caused by phenolic compounds (Di Mattia et al. 2010) and this effect was possibly intensified by the presence of saponins containing powder samples.

As shown in Fig. 2b, reduction in surface tension occurred concurrently with the appearance of smaller but greater number of bubbles, which in turn was induced by an increase in the total saponin concentration in NWSs and NMSs.

Foam formation

Various factors affect foam formation, including total phenols and total saponins in plant extract. Association between concentration of polyphenols and degree of foam formation has been recognized by Bishop et al (1974). Table 1 shows there was greater foam formation in SE than AE, possibly due to the higher total saponin content of solution reconstituted by addition of SE at similar Brix.

The effect of the saponin content in plants on the foam formation has been reported by Treter et al (2010) who examined the effect of the extract from *Ilex paraguariensis)Aquifoliaceae*) family on foam formation and compared it with synthetics such as sodium dodecyl sulfate and polysorbate 80. They concluded that this kind of saponin extract increased the formation and stability of surfactant foam in solution.

In our experiments, an increase in added SE to NWSs caused an elevated total saponin content, therefore increased foam formation (Fig. 2c). Furthermore, replacement of water in NWSs with malt in NMSs enhanced foam formation significantly, especially at higher total saponin concentrations of 40 and 50 mg/kg (Fig. 2c). Also, addition of carbon dioxide to NWSs and NMSs had a positive impact on the foam formation, as observed in CWSs and CMSs.

Notably, commercial beverages and CMS-0 samples, both loaded with malt extract free of saponins, exhibited the lowest foam formation among malt containing samples, and were altered by the addition of saponin containing SE of *A. laxiusculum*. Apparently, the two factors of malt matrix and the presence of carbon dioxide influence foam formation. In addition, according to the observations in this study, saponin concentration has been found to be more influential on foam formation.

Foam stability

Table 1 reports foam stability of different treatments after 10 h (25 °C), which indicates excellent resistance to collapse of the foam formed by SE of *A. laxiusculum*. Foam stability of SE of *A. laxiusculum* was recorded as 98.55%, 87.15% and 67.39% after 1, 5 and 10 h, respectively, and higher than AE of *A. laxiusculum* (96.66%, 82.27% and 58.66%, after 1, 5, 10 h, respectively) due to the elevated total saponin concentration in SE.

Foam stability results of NMSs and NWSs showed positive effect of total saponins on bubble durability (Fig. 2c). Moreover, at high total saponin concentrations (40 and 50 mg/kg), foam stability of NWSs was greater than NMSs, which is opposite of their foam formation behavior. This behavior is supported by the presence of total phenolic compounds in NMSs and their interaction

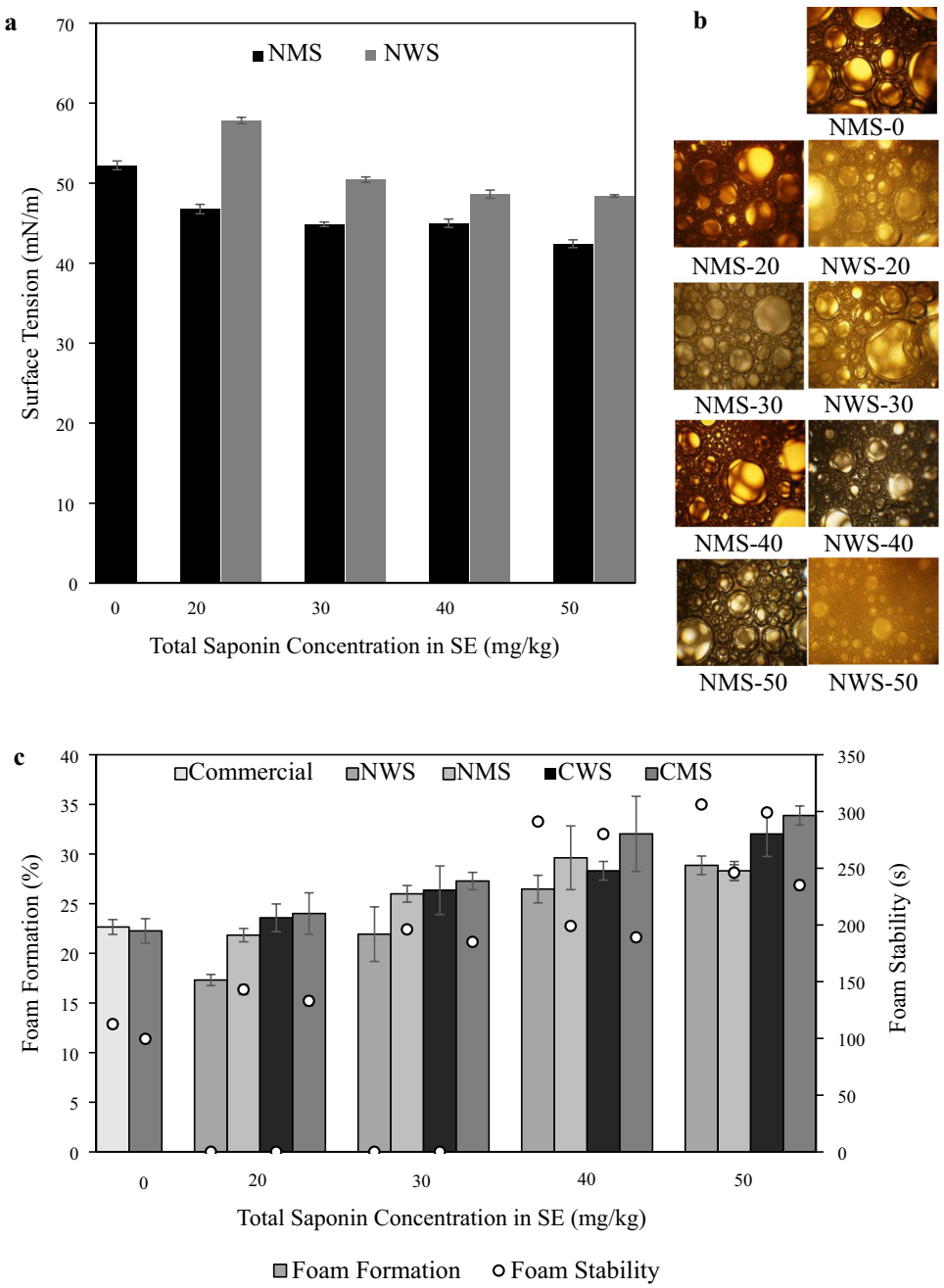

Fig. 2 Foaming attributes of SE of *A. laxiusculum* (0–50 mg/kg total saponin concentration). **a** Surface tension, **b** digital camera images, **c** foam formation and stability. NWS, non-carbonated Water Saponin solutions; NMS, non-carbonated Malt Saponin beverages; CWS, Carbonated Water Saponin solutions; CMS, Carbonated Malt Saponin beverages. Values are mean ± standard deviation, in triplicate

with positive foam stable proteins at the boiling stage of the wort leads to their precipitation and elimination via sedimentation (Evans and Bamforth 2011). Phenolic compounds increase liquid drainage of foam and decrease the surface viscoelasticity of bubble walls, leading to increased coalescence and decreased foam stability, however, the functional properties of total phenolic compounds in foam stability seems to be more complicated (Evans and Bamforth 2011; Rodríguez et al. 2015).

The same effect of increase in foam stability due to the presence of the malt can be seen in carbonated samples of malt and water solution in the presence of saponins. The extent of effect is reflected in the CWS-20 and CWS-30 samples where the foam stability is not measurable by instrument due to their low life time in these samples. Presence of the carbon dioxide either induced no change (mainly in malt samples) or reduced foam stability especially in SE samples containing 40 and 50 mg/kg total saponin.

Relation between reduced surface tension and consequent increased foam stability has been confirmed previously by Maeda et al (1991). Monodesmosidic saponins arrange side-on configurations by placing sapogenin in the hydrophobic area and the sugar chain in the hydrophilic

part. Bidesmosidic saponins arrange end-on configurations by placing sapogenin and one sugar chain in the hydrophobic area and another sugar chain in the hydrophilic part. Also, bidesmosidic saponins arrange lay-on configurations by placing sapogenin alone in the hydrophobic phase and other sugar chains in the hydrophilic part at air–water interface to contribute to foam stabilization. These saponins configurations cause various interactions such as van der Waals bonds between aglycones and hydrogen links between sugar chains, amongst themselves and with neighboring absorbed molecules like proteins that form a network to give the bubble wall viscoelastic properties (Stanimirova et al. 2011). The viscoelasticity of the bubble wall prevents Ostwald ripening, which means a decrease in coalescence of small bubbles with high surface energy to prevent creation of larger ones with lower surface energy (Stanimirova et al. 2011).

Apparent viscosity

The viscosity curves obtained using a rheometer indicated that the applied concentrations of total saponin in SE (20–50 mg/L) did not induce a difference in apparent viscosity. For the convenience of readers, only samples containing 30 mg/L total saponin are illustrated in Fig. 3. Linear regression of shear stress and rate, hence Newtonian behavior of all samples is evident. The AE and SE of *A. laxiusculum* with similar total reduced soluble sugar (15° Bx) showed similar apparent viscosity. The CWSs (up to 50 mg/kg total saponin) showed the lowest apparent viscosity probably due to their zero brix. Meanwhile, the CMSs showed higher values of apparent viscosity due to initial total sugar content, induced by the presence of malt (5.3°Bx). The CMS samples containing saponins had similar apparent viscosity compared to the commercial sample which was saponin-free, thus emphasizing the effect of reducing sugar content on viscosity, rather than that of total saponin (within the range of this study).

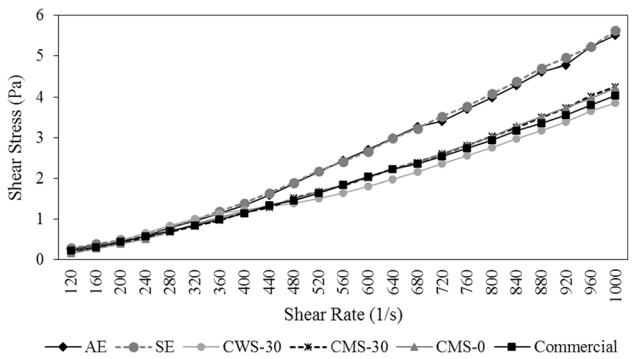

Fig. 3 Newtonian behavior of Aqueous Extract (AE), Spray-dried Extract (SE), Commercial beverage, Carbonated Water Saponin solutions at 30 mg/kg (CWS-30), Carbonated Malt Saponin beverages at 30 mg/kg (CMS-30), Carbonated Malt Saponin beverages at 0 mg/kg (CMS-0) total saponin concentration in SE of *A. laxiusculum*

Color

Colorimetric data of AE and SE obtained from *A. laxiusculum* root which were adjusted to the same total reduced soluble sugar (Bx) is presented in Table 1. Results showed that spray drying had a reducing effect on L* (darkness to lightness), a* (greenness to redness), b* (blueness to yellowness). The reduction in L* could be due to plant extract sugars that could participate in the non-enzymatic browning of *A. laxiusculum* root powder. Spray drying decrease total phenolic contents, which cause a reduction in the a* index, hence decreased redness (Gong et al. 2007).

The colorimetric properties did not differ significantly among the CMSs and CWSs at various SE total saponin levels (Table 2). Addition of plant powder extract SE in CMSs increased the ΔE index compared to CMS-0, possibly due to the bleaching properties of total saponins, which researchers have observed in other products (Çelik et al. 2007).

Bitterness

Spray drying reduced bitterness of AE in comparison with SE (Table 1). In brewing industry, bitterness is mainly attributed by phenolic compounds including iso-alpha-acids. However, our CWSs showed no detectable amount of iso-alpha-acids at applied concentrations of SE. While total phenols were measurable in the commercial beverage, they did not increase by the addition of SE (Table 2). Addition of SE to the CWSs in spite of their bitter taste did not significantly affect the measurable bitterness and this method is based on the measurement of iso-alpha-acids, an ingredient abundantly available in hops and malt beverages (Jaskula et al. 2007). Accordingly, bitter taste of AE and SE extracts are mainly attributed to total phenolic compounds or the presence of total saponins rather than iso-alpha-acids. In this case, sensory evaluation is a better approach to identify the effect of total saponins on the bitterness of the products.

Sensory evaluation

The sensory evaluation showed that adding SE of *A. laxiusculum* to the malt beverage at levels of 20–30 mg/kg total saponin content did not significantly affect product acceptance in terms of color, opacity and oral sensation (Fig. 4). Greater foaming preference was assessed by panelist for samples containing SE of *A. laxiusculum* in comparison to commercial beverages. Conversely, the high total saponin level in SE (30 mg/kg) decreased the

Table 2 Effect of different saponin concentrations on color, opacity and bitterness properties of Carbonated Water Saponin solutions (CWS), Carbonated Malt Saponin beverages (CMS) and Commercial beverage

Samples	Total saponin (mg/kg)	Color				Bitterness
		L*	a*	b*	ΔE*	
CWS-20	20	76.86 ± 0.12^{a}	-0.89 ± 01^{e}	-0.73 ± 0.10^{d}	17.09 ± 0.13^{e}	ND
CWS-30	30	76.80 ± 0.11^{a}	-0.91 ± 0.01^{e}	-0.67 ± 0.10^{d}	17.14 ± 0.11^{e}	ND
CWS-40	40	76.80 ± 0.02^{a}	-0.92 ± 0.01^{e}	-0.65 ± 0.10^{d}	17.15 ± 0.02^{e}	ND
CWS-50	50	76.83 ± 0.27^{a}	-0.92 ± 0.01^{e}	-0.67 ± 0.10^{d}	17.11 ± 0.27^{e}	ND
Commercial	0	56.23 ± 0.29^{c}	10.24 ± 0.07^{a}	58.19 ± 0.57^{b}	73.47 ± 1.11^{a}	15.15 ± 0.30^{a}
CMS-0	0	55.37 ± 0.37^{d}	9.13 ± 0.07^{b}	61.65 ± 1.04^{a}	69.98 ± 0.63^{b}	15.08 ± 0.07^{ab}
CMS-20	20	58.28 ± 0.25^{b}	7.65 ± 0.13^{d}	56.38 ± 0.88^{c}	67.17 ± 0.85^{d}	14.96 ± 0.05^{ab}
CMS-30	30	58.00 ± 0.08^{b}	7.60 ± 0.05^{d}	56.71 ± 0.52^{c}	67.59 ± 0.49^{d}	14.90 ± 0.10^{b}
CMS-40	40	57.89 ± 0.07^{b}	7.56 ± 0.04^{d}	56.74 ± 0.24^{c}	67.67 ± 0.24^{d}	14.96 ± 0.10^{ab}
CMS-50	50	56.41 ± 0.40^{c}	8.53 ± 0.27^{c}	57.01 ± 0.96^{c}	68.82 ± 0.89^{c}	14.95 ± 0.08^{ab}

Values are mean ± standard deviation in triplicate. Mean values with different letters at the same column are statistically different ($p < 0.05$)
ND not detected

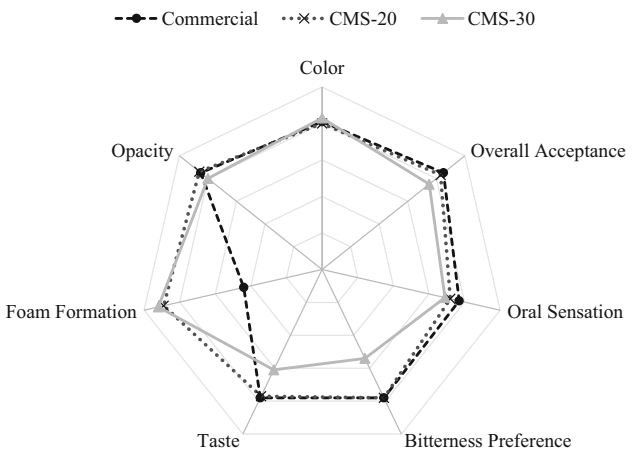

Fig. 4 Sensory evaluation of commercial beverage, Carbonated Malt Saponin beverages at 20 mg/kg (CMS-20) and Carbonated Malt Saponin beverages at 30 mg/kg (CMS-30) total saponin concentrations in SE of *A. laxiusculum*

bitterness scores by evaluators. Overall acceptance score of CMS-20 and CMS-30 samples were 4.15 and 3.75, respectively, which were not significantly different compared to the commercial beverage product with a bitterness score of 4.25. Sensory evaluation indicated the possibility of using the *A. laxiusculum* roots spray-dried extract (SE) to improve the product's foaming characteristics without having an adverse effect on its desirability.

Conclusion

Spray-dried extract (SE) of *A. laxiusculum* could be successfully used in malt beverages and brewing industry to confer several important techno-functional properties to the products without having adverse impacts on their sensory properties. The foam formation and stabilization in malt beverages increased to about 49.5% and 108.8%, respectively, by the addition of SE (50 mg/kg total saponin content) when compared to the commercial malt beverage. Addition of SE caused a significant increase in the antioxidant activity (up to about 380%), and the antifungal activity was considerable to prevent the growth of *Saccharomyces cerevisiae*. Overall, the application of spray dried extact of *A. laxiusculum* roots (SE) at 30 mg/kg total saponin content can be recommended for use in the malt beverage industry.

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Author contributions SJ and AM conceived the study concept and designed the experiments. SA, VM and NM performed the experiments. SA analyzed the data and wrote the original draft. SJ, AM and FM co-wrote the paper. All authors read and approved the final version.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical standards Procedures with participation of humans were approved by "Faculty of Nutrition Science and Food Technology Shahid Beheshti University of Medical Sciences Ethics Committee" with the approval number of IR.SBMU.NNFTRI.REC.1398.015.

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