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APPLICATION OF FOAM COLUMN AS GREEN TECHNOLOGY FOR CONCENTRATION OF SAPONINS FROM SISAL (*Agave sisalana*) AND JUÁ (*Ziziphus joazeiro*)

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Abstract - Saponins, molecules classified as triterpenic or steroidal glycosides, are metabolites distributed in all the plant kingdom that can be used for the production of foods, cosmetics, and pharmaceuticals, as well as in soil bioremediation. Saponins are normally extracted from natural resources with water, ethanol and/or methanol, and then concentrated by liquid-liquid partitioning with n-butanol. An alternative concentration method is with a foam column, by which the saponins can be concentrated via preferential adsorption at a gas-liquid interface. Therefore, the objective of this work was the use of a foam column for the concentration of saponins from juá and sisal, evaluating parameters such as: initial working volume in the column, saponin concentration in the extracts from juá and sisal, air flow rate, pH, Raschig rings loading and operation time. When a gradient air flow rate and 25 g of Raschig rings were used, 82.6% of the jua saponins loaded onto the system were recovered in a 3.46-fold concentrated solution after 9 h of operation. Regarding sisal saponins, a concentration factor of 1.98 was observed with 90.5% of saponin recovery during 4.5 h of operation.
Keywords: Foam Column; Saponins; Sisal; Juá.

INTRODUCTION

Saponins are widely distributed in the plant kingdom and are constituted by a steroidal or triterpenic aglycone linked to one or more sugar molecules (Figure 1). Their main physiological functions in the plants are as a resistance factor against pathogens and alellopathic interactions (Oleszek, 2000; Kalinowska *et al.*, 2005; Güçlü-Üstündag and Mazza, 2007). The structural diversity of saponins is reflected in their physicochemical (foaming, emulsification, solubilization, sweetness, bitterness) and biological (haemolytic, antimicrobial, molluscacide, insecticide, ichthyocide) properties, which are exploited in many industrial applications, as ingredients for foods, cosmetics and pharmaceuticals (intermediates in steroidal drugs synthesis), and also in soil bioremediation (Sparg *et al.*, 2004; Vincken *et al.*, 2007).

In Brazil, two plants present great potential as sources of saponins: juá (*Ziziphus joazeiro*) and sisal (*Agave sisalana*). Due to its saponins content (2-10% m/m), jua bark has been used in the formulation of detergents, dentifrices and phytotherapic products (Higuchi *et al.*, 1984; Barbosa-Filho *et al.*, 1985; Schühly *et al.*, 2000), whereas sisal waste, a mucilaginous liquid from the sisal defibring process, for which Brazil is the largest producer worldwide, has been used as a synthetic intermediate for the production of steroidal drugs by the pharmaceutical industry (Ding *et al.*, 1989, 1993; Oashi, 1999; Zou *et al.*, 2006).

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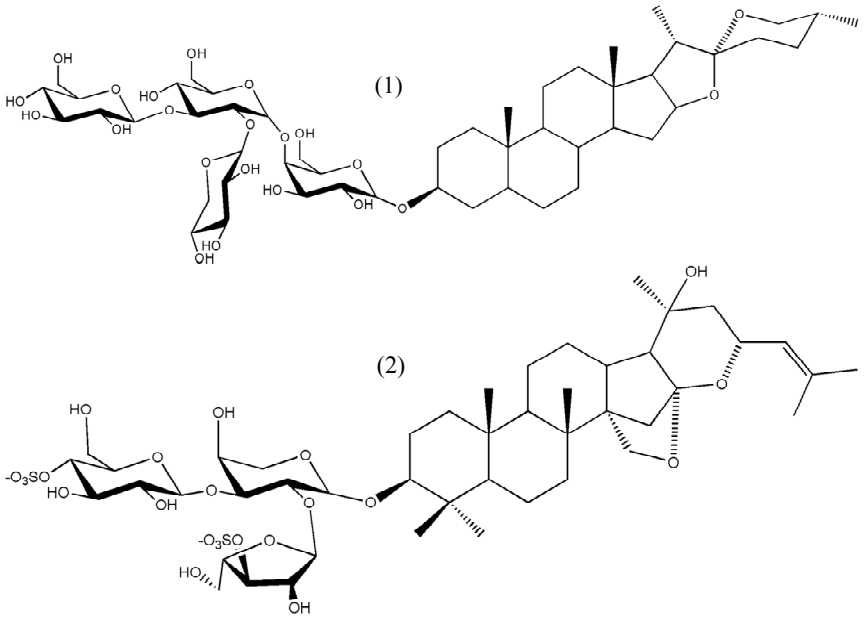

Figure 1: Saponins from sisal (1) Dongnoside E, Ding *et al.*, 1989) and juá (2) Jujuboside, Higuchi *et al.*, 1984).

The most common methods for the extraction of saponins are those which use solvents such as water, methanol, ethanol or hydroalcoholic mixtures, generally in a Soxhlet extractor or an orbital shaker. One of the concentration methods most used is based on the partitioning between aqueous extracts and n-butanol (Hostettmann and Marston, 2005; Güçlü-Üstündag and Mazza, 2007). In this context, concentration with a foam column can be considered to be an alternative green concentration method, substituting the solvents cited above with air, which is safer and readily available.

Foam consists of a large number of little bubbles dispersed in a continuous liquid phase. Its stability depends mainly on the resistance to solvent loss (drainage) from the thin liquid film that involves the gas (Costa, 1999). Foam columns concentrate the solution by preferential adsorption of the solute at a gas-liquid interface created by air or inert gas injection into the solution and the formation of a stable surfactant-rich foam (Grieves, 1975; Singh and Rizvi, 1995). Some authors reported the use of this method to concentrate saponins from *Quillaja saponaria* in a 175 cm high and 2.5 cm internal diameter column with a gas flow rate of 22.60 mL/min through a solution with 80 mg/L of saponins, at pH 3, for 3.5 h, obtaining a concentration factor (β_{SAP}) of 10 (Costa, 1999). In another work, Yan *et al.* (2011) reported the concentration of tea saponins by a two-step method in a foam column: first, using an air flow rate of 150 mL/min at 65 °C, they obtained a high β_{SAP} (3.47); then increasing the air flow rate to 200 mL/min and decreasing the temperature to 30 °C, they achieved a high saponin recovery (65.2%). Thompson (2004) and Backleh-Sohrt *et al.* (2005) showed that a foam column aided by saponins can be also interesting for the concentration of other molecules such as catechins, faradiol esters, carotenoids and alkaloids.

In the present work, the objective was the use of a foam column measuring 130 cm in height x 1.85 cm diameter with type III frits, for the concentration of saponins from hydroalcoholic extracts obtained from juá and sisal, exploring variables such as the air flow rate, the initial volume of the extract in the column, the concentration of saponins in the extract, pH, loading of Raschig rings and operation time.

MATERIALS AND METHODS

Materials

Sisal wastes were donated by APAEB (Association for Sustainable and Solidary Development of the Sisal Region) from Valente, Brazil, and juá bark was purchased from the company Santos Flora (São Paulo, Brazil). All chemical compounds were obtained from VETEC Química Fina (Duque de Caxias, Brazil).

Production of Extracts from Juá and Sisal

Juá bark powders and sisal mucilaginous waste were incubated with ethanol 30% v/v (ratio raw material/solvent of 0.2) for 4 h at 50 °C and 200 rpm in a simple orbital extractor. After centrifugation at 5000 rpm for 10 min, the extract was stored, and the solid residue was subjected to two more re-extractions, using the same previous conditions.

Optimization of the Foam Column Operation

A central composite rotatable design (CCRD) was employed in order to explore the effect of several parameters on saponins recovery and concentration: initial extract volume, air flow rate and relative saponin concentration in the extract (Table 1). The concentration was varied by diluting the extracts in a 30% v/v ethanol solution, where 100% represents the crude extract without any dilution. All runs were operated in a batch mode for 15 min. Based on the results from this first set of experiments, variable ranges were altered and two more CCRD were performed, each one for a different raw material (Table 2). The response variables of the foam column operation were: concentration factor (β), which is the ratio between the saponin or phenolic compound concentrations in the foam and in the extract (initial solution); recovery (%R), which is the mass ratio of saponins or phenolic compounds obtained in the foam and in the extract; and volume ratio (V), the relation between foam volume and residual volume of the extract in the column. Results with $\beta = 0$, represent unstable foam (which was only slightly formed and not collected), while $\beta = 1$, mean that all solution was collected as foam, without any concentration.

Table 1: Exploratory central composite rotatable design for saponin concentration by a foam column.

Assays	Volume (mL)	Air flow (mL/min)	Relative saponins concentration (%)
1	70	600	25
2	70	600	75
3	70	1000	25
4	70	1000	75
5	100	600	25
6	100	600	75
7	100	1000	25
8	100	1000	75
9	60	800	50
10	110	800	50
11	85	460	50
12	85	1140	50
13	85	800	8
14	85	800	92
15 (C)	85	800	50
16 (C)	85	800	50
17 (C)	85	800	50

Once the conditions were defined, other parameters and ranges were tested aiming to increase the concentration factor of saponins: other values of air flow rate (400, 600 and 800 mL/min for juá, and 1000, 1200 and 1400 mL/min for sisal); pH (3, 4, 5, 6, 7, 8, 9, 10 and 11 adjusted with either 6M NaOH or 8N HCl), and the presence of Raschig rings (5, 15

Table 2: Central composite designs for saponin concentration by a foam column.

Assays	SISAL			JUÁ		
	Volume (mL)	Air flow (mL/min)	Relative saponins concentration (%)	Volume (mL)	Air flow (mL/min)	Relative saponins concentration (%)
1	85	800	70	85	400	20
2	85	800	90	85	400	40
3	85	1200	70	85	800	20
4	85	1200	90	85	800	40
5	115	800	70	115	400	20
6	115	800	90	115	400	40
7	115	1200	70	115	800	20
8	115	1200	90	115	800	40
9	75	1000	80	75	600	30
10	125	1000	80	125	600	30
11	100	660	80	100	260	30
12	100	1340	80	100	940	30
13	100	1000	63.2	100	600	13.2
14	100	1000	96.8	100	600	46.8
15 (C)	100	1000	80	100	600	30
16 (C)	100	1000	80	100	600	30
17 (C)	100	1000	80	100	600	30

and 25 g), which are cylindrical borosilicate glass tubes 0.5 cm in height and 0.5 cm in diameter. With these data, a kinetic concentration study was done to investigate the most proper time for the recovery of the foam concentrated in saponin. In the case of sisal, operation time was 4.5 h, replacing the hydroalcoholic extract of mucilaginous waste by a liquid waste that had a higher saponin content. For jua the operation time was extended to 9h with preconcentrated jua extract using 20% w/v sodium carbonate instead of hydroalcoholic extract. Furthermore, air flow was decreased to the minimum value that produced bubbles in the column and then the air flow was increased in a gradient system, ranging from 150 to 330 mL/min using jua extract, and 55 to 300 mL/min using liquid sisal waste.

Analytical Methods

Total saponins content was determined by the vanillin-sulfuric acid method (Makkar *et al.*, 2007). For this, 165 μL of sample (solution diluted 50-fold in 80% v/v methanol) were added to 165 μL of an ethanolic solution of vanillin (8% w/v) and then 1.65 mL of a fresh aqueous solution of sulfuric acid (72% v/v) was added. The resulting acid solution was maintained at 60 °C for 10 minutes. The standard curve was done using diosgenin (0.5 g/L) as standard, and the final absorbances of all solutions were detected in a spectrophotometer at 544 nm.

Total phenolic compounds content was measured by the Folin-Denis method (Waterman and Mole, 1994), by mixing 10 μL of sample with 1.69 mL deionized water and 0.2 mL of a saturated solution of sodium carbonate and then adding 0.1 mL of Folin-Denis reagent (10% w/v sodium tungstate, 2% w/v phosphomolybdic acid and 5% v/v orthophosphoric acid, previously prepared). The mixture was kept in the absence of light for 30 minutes. The standard curve was done using tannic acid (0.1 g/L) as standard, and the final absorbances were detected in a spectrophotometer at 760 nm.

RESULTS AND DISCUSSION

The foam column method is based on the adsorption of a surfactant at the hydrophobic surface of an air bubble, and presents some advantages for saponin concentration such as simplicity (the system is comprised of a column coupled to an air compressor), low cost and low energy consumption (Backleh-Sohrt *et al.*, 2005; Yan *et al.*, 2011). Initially, in this method, juá and sisal extracts (obtained by simple orbital extraction with two additional re-extractions) were used in an exploratory CCRD. However in the case of sisal, no conditions promoted enough foam formation for it to be collected. Thus, for sisal, a simple extract without re-extractions was used. Table 3 shows that the relative saponin concentration was an important factor for obtaining higher β_{SAP}. Probably, in the case of jua extract (6.97 g/L of saponins at 100% relative concentration), as a result of the high saponin content and air flow rate, all the initial volume was washed out and resulted in foaming without any concentration (that is, $\beta_{SAP} = 1$, $V = \infty$). The best working range was between 8 and 50% relative saponin concentration. When sisal extract (4.58 g/L of saponins at 100% relative concentration) was used, on the other hand, in several runs it did not foam enough to collect samples ($\beta_{SAP} = 0$, $V = 0$). It was due to the lower saponin content in this latter extract when compared to jua extract, indicating that the ideal relative concentration range would be above 75%.

Therefore, experimental designs were redefined and optimal conditions were obtained by the desirability approach (geometric mean of all response variables: Calado and Montgomery, 2003) with the objective of maximizing β_{SAP}, and obtaining intermediate values of saponin recovery. High values indicate low saponin concentration and minimize volume ratio, without however reaching zero. The influence of the terms on the response variables was quite relative, obtaining $R^2 = 0.731$, 0.944 and 0.832 for β_{SAP}, $\%R_{SAP}$ and V from juá saponins, and 0.871, 0.752 and 0.701, for sisal saponins.

In the experimental design related to juá saponins, the interaction term between air flow rate and relative saponins concentration had a positive impact on all variables, while the other terms showed reverse action on $\beta_{SAP,}$ $\%R_{SAP}$ and V. The linear terms of relative saponin concentration and air flow had a positive effect on $\%R_{SAP}$ and V, but negative in relation to β_{SAP}. The linear and quadratic terms of volume, the quadratic term of air flow and the interaction term between volume and relative saponins concentration acted negatively on $\%R_{SAP}$ and V, but positively on β_{SAP}. The interaction term between volume and air flow had a negative effect on β_{SAP} and V, but positive on $\%R_{SAP}$. The reverse action was found for the quadratic term of relative saponins concentration. In Figure 2, the desirability function behavior can be seen against the variations in air flow rate, volume and relative concentration of saponins obtaining as optimum working condition for juá: 125 mL, 940 mL/min and 30% concentration.

Table 3: Exploratory central composite rotatable design results for saponin concentration by a foam column.

Assays	Volume (mL)	Air flow (mL/min)	Relative saponins concentration (%)	JUÁ					SISAL				
				β_{SAP}	$\%R_{SAP}$	β_{FEN}	$\%R_{FEN}$	V	β_{SAP}	$\%R_{SAP}$	β_{FEN}	$\%R_{FEN}$	V
1	70	600	25	1.02	52.66	1.03	53.11	1.059	0	0	0	0	0
2	70	600	75	1	100	1	100	∞	0	0	0	0	0
3	70	1000	25	1.01	78.71	1.02	79.90	3.667	0	0	0	0	0
4	70	1000	75	1	100	1	100	∞	1.69	0.36	1.17	0.25	0.0021
5	100	600	25	1.49	20.87	1.20	16.81	0.163	0	0	0	0	0
6	100	600	75	1	100	1	100	∞	1.91	0.86	1.26	0.57	0.0045
7	100	1000	25	0.98	73.75	1.04	78.85	3.082	0	0	0	0	0
8	100	1000	75	1	100	1	100	∞	1.63	0.41	1.42	0.36	0.0025
9	60	800	50	1	100	1	100	∞	0	0	0	0	0
10	110	800	50	1	100	1	100	∞	0	0	0	0	0
11	85	460	50	1	100	1	100	∞	0	0	0	0	0
12	85	1140	50	1	100	1	100	∞	0	0	0	0	0
13	85	800	8	0	0	0	0	0	0	0	0	0	0
14	85	800	92	1	100	1	100	∞	1.32	4.33	1.42	4.67	0.0338
15 (C)	85	800	50	1	100	1	100	∞	0	0	0	0	0
16 (C)	85	800	50	1	100	1	100	∞	0	0	0	0	0
17 (C)	85	800	50	1	100	1	100	∞	0	0	0	0	0

β_{SAP} ands β_{FEN} are the concentration factors of saponins and phenolic compounds, respectively;
$\%R_{SAP}$ and $\%R_{FEN}$ are recoveries in mass of saponins and phenolic compounds, respectively;
V is the volumar ratio (volume in the foam phase divided by the residual volume in the column)

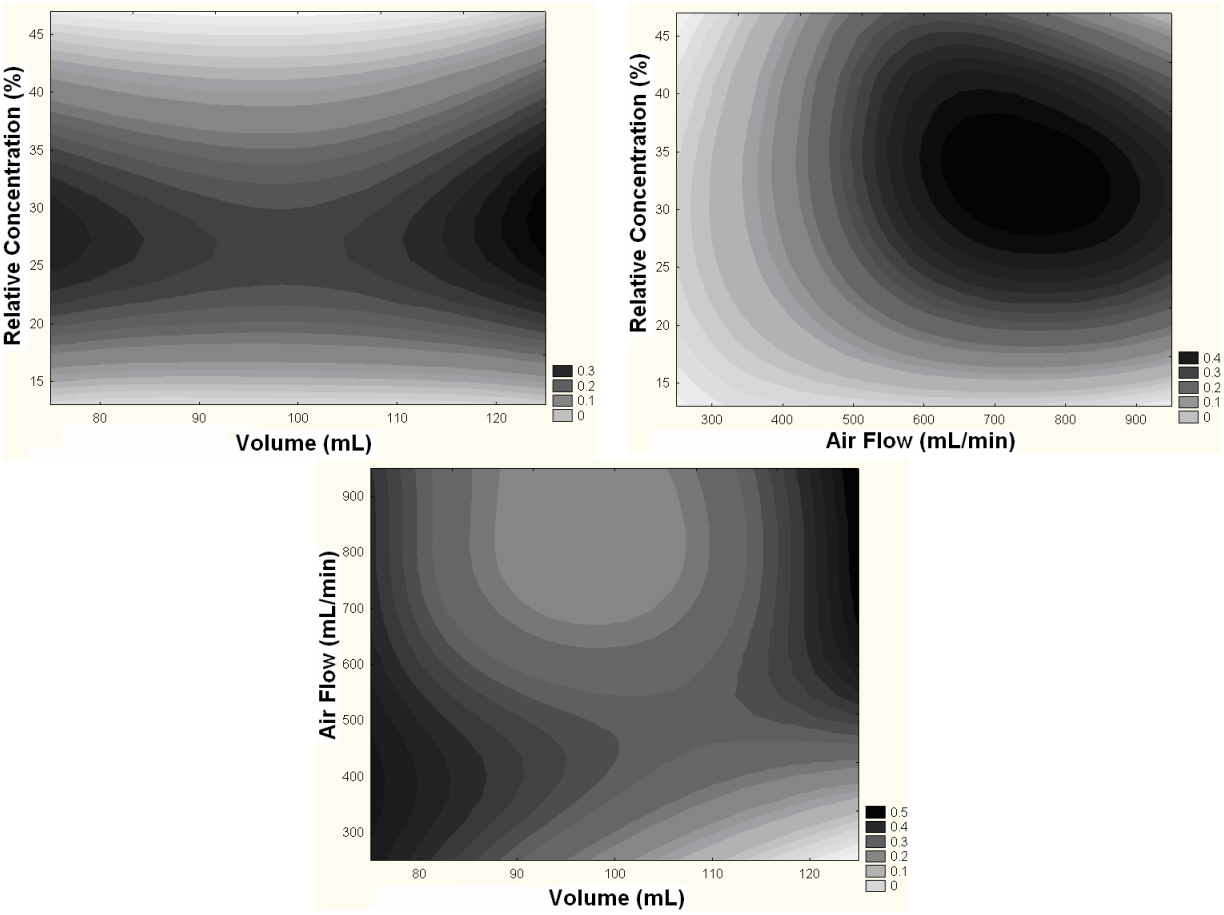

Figure 2: Optimization of parameters for juá saponin concentration by a foam column.

In the experimental design related to sisal saponins, the interaction terms between air flow and relative saponin concentration and between volume and air flow rate resulted in a negative impact. The quadratic term of air flow rate had a positive effect on all variables, while the other terms presented the reverse action on β_{SAP}, $\%R_{SAP}$ and V. The linear term of air flow rate and the interaction term between volume and relative saponin concentration presented a positive effect on $\%R_{SAP}$ and V, but negative on β_{SAP}. The linear and quadratic terms of relative concentration and the linear term of volume acted negatively on $\%R_{SAP}$ and V, but positively on β_{SAP}. The quadratic term of volume had a positive effect on β_{SAP} and $\%R_{SAP}$, but negative on V. In Figure 3, the desirability function behavior can be seen with variations in air flow, volume and relative concentration of saponins, obtaining as optimum working conditions for sisal: 75 mL, 1300 mL/min and 90% concentration.

The results obtained in the present work are slightly different from the literature. Backleh-Sohrt *et al.* (2005) utilized a N_2 flow of 15 mL/min, an initial concentration of quillaja saponins between 0.4 and 0.5 g/L, volumes of 70-100 mL and an operation time of 60-100 min. Similarly, Costa (1999) also worked with a N_2 flow of 13.6 mL, whereas Yan *et al.* (2011) used an air flow of 150 mL/min, initial concentration of tea saponins of 3.56 g/L and a volume of 200 mL (with a 3.3 cm internal diameter column). To compare data for different gas flows requires a standardization, based on data about gas temperature and pressure. For example, a commercial N_2 tank usually presents 145- 190 atm of pressure, which is equivalent at ambient temperature to a minimum air flow of 2000 mL/min, considering 15 mL/min N_2. Therefore, 150-2000 mL/min was the range which was employed.

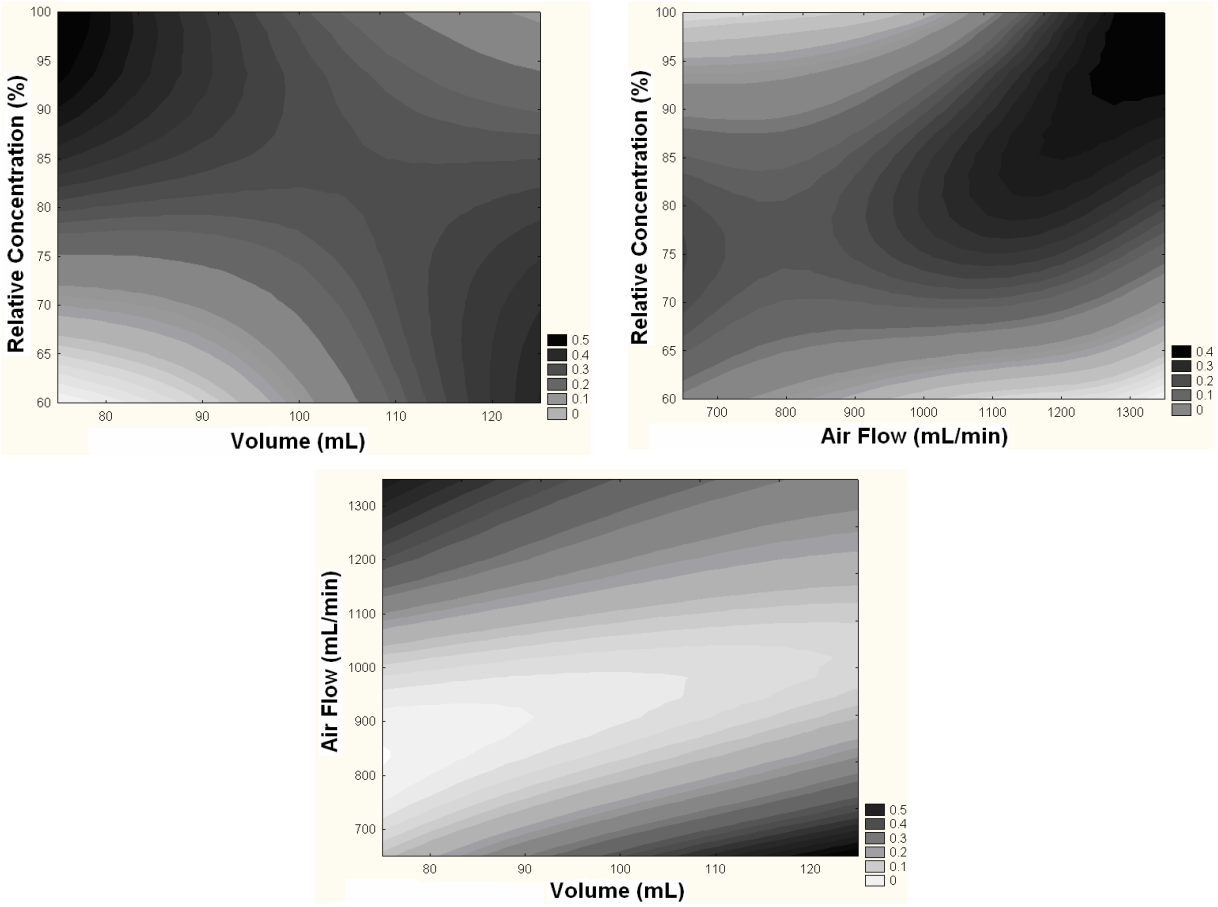

Figure 3: Optimization of parameters of sisal saponins concentration by a foam column.

The literature indicates that the initial volume of extracts and the critical micellar concentration (CMC) of saponins also influence the foam column operation. Costa (1999) tested quillaja saponin solutions at different concentrations and volumes, and verified that, in concentrations above the CMC (0.8 g/L), β_{SAP} was only 1.18 in 7 h of operation; when performed exactly at the CMC (0.2 g/L), after 10 h of operation, the author obtained β_{SAP} = 9.63; below the CMC (0.08 g/L), this value was 10 times more concentrated in 2 h, with the same volume in all conditions, representing 85% total column volume. However using 50% volume and concentrations below 0.08 g/L, the foam generated was not stable and could not be collected. This explains the impossibility of working with sisal extract with two more re-extractions.

Thus, a lower air flow rate could enable a higher concentration factor for saponins, due to a longer time to drain the liquid from the formed foam. In the present work, other values of air flow were tested based on the optimal conditions obtained in the experimental design, as can be seen in Figure 4. In this case, the focus was mainly on the increase of β_{SAP}, leaving as a secondary factor the saponin recovery. The best air flow rates for saponin concentration from juá and sisal extracts were 400 and 800 mL/min, respectively.

Other parameters were also tested for saponin recovery, such as pH (Figure 5) and the addition of Raschig rings (Figure 6). As the experiments were carried out simultaneously, the air flow rates for the concentration of juá and sisal saponins were 600 and 1000 mL/min, respectively. Regarding the pH effect, it was observed that the profiles of variation of saponin and phenolic compound recoveries and the volume ratio were similar for both saponins. Nevertheless the concentration factor of saponins from juá and sisal were maximum at pH 4 and 5, respectively, while β_{FEN} was the highest at pH 10 and 11 for juá, and at pH 4, 5 and 8 for sisal. These results could be explained by a pH influence increasing the CMC of saponins, meaning higher electrostatic repulsion, in the case of juá saponins aided by the presence of sulfated groups, and forming more stable foams (Mitra and Dungan, 1997). Alkaline pHs cause ionization of phenolic compounds, enabling their concentration by saponin foam (Backleh-Sohrt *et al.*, 2005). When 25 g of Raschig rings were used, the highest β_{SAP} was observed. It is believed that their effect is to increase the interfacial area and mass transfer between the gas phase (air) and liquid phase (extract), besides increasing the bubble residence time in the column, thereby facilitating the adsorption of saponins on the bubbles.

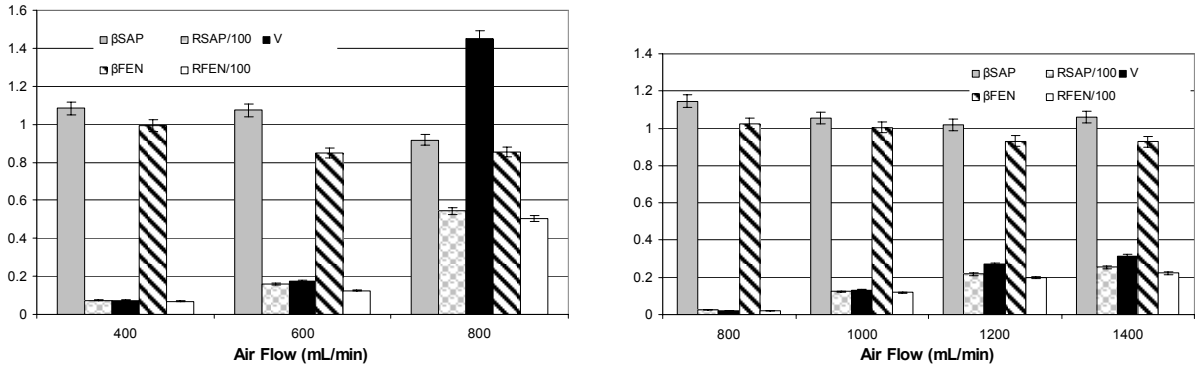

Figure 4: Effect of air flow rate on the concentration of juá (a) and sisal (b) saponins by a foam column.

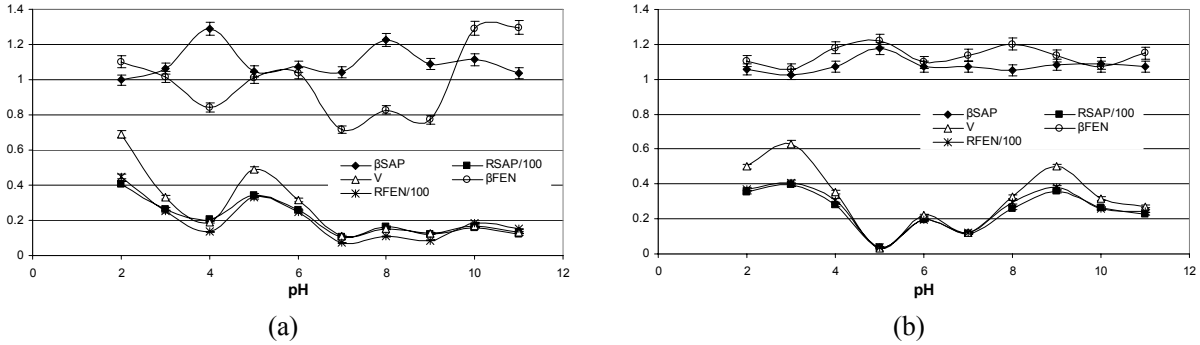

Figure 5: Effect of pH on the concentration of juá (a) and sisal (b) saponins by a foam column.

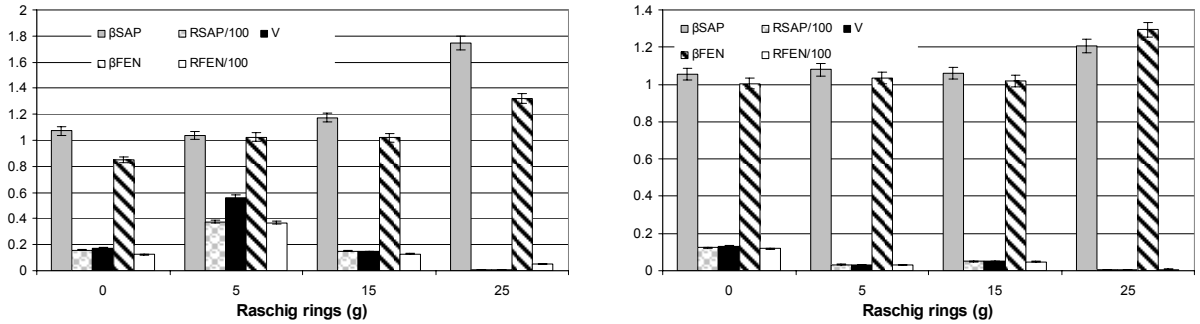

Figure 6: Effect of Raschig rings loading on the concentration of juá (a) and sisal (b) saponins by a foam column.

The pH was also reported in other works to be important for saponin concentration, due to its effect on net charge, on repulsive forces between molecules and on the CMC. Yan *et al.* (2011) reported that the increase of pH decreased the trends of tea saponins adsorbed on air bubbles, and increased the stability of the formed foam, due to the high viscosity generated by the negative charge of the carboxyl groups of saponins. At pH 2.06, there was a higher β_{SAP} and lower saponin recovery. Costa (1999) also verified that raising the pH with different quillaja saponin concentrations impacted β_{SAP}, and obtained β_{SAP} values of 10, 7.4 and 6.97 with 80 mg/L of saponins in the extract when the pH was varied from 3, 5 to 7; increasing saponin concentration at pH 5 from 80 to 350 to 800 mg/L, β_{SAP} varied from 7.4 to 1.93 to 1.24, respectively.

As a maximum concentration factor and recovery of saponins were not possible under the same conditions, a kinetic study of foam column operation was realized, focusing mainly on β_{SAP}, with minimum air flow to benefit the formation of stable and collectable foam, using a gradient system to maintain the formed foam flow and aid the drainage of the foam. Furthermore, extracts with higher saponin content were used, replacing the hydroalcoholic extract of mucilaginous sisal waste by liquid sisal waste, and the hydroalcoholic juá extract by a supramolecular phase formed by addition of 20% w/v Na_2CO_3, the jua extract being 1.57 times more concentrated. In the case of juá saponins (Figure 7), β_{SAP} reached 3.46, and saponin recovery reached 82.6% in 9 h of operation, while sisal saponins (Figure 8), in 4.5 h, gave a concentration factor of 1.98, and $\%R_{SAP}$ of 90.5%.

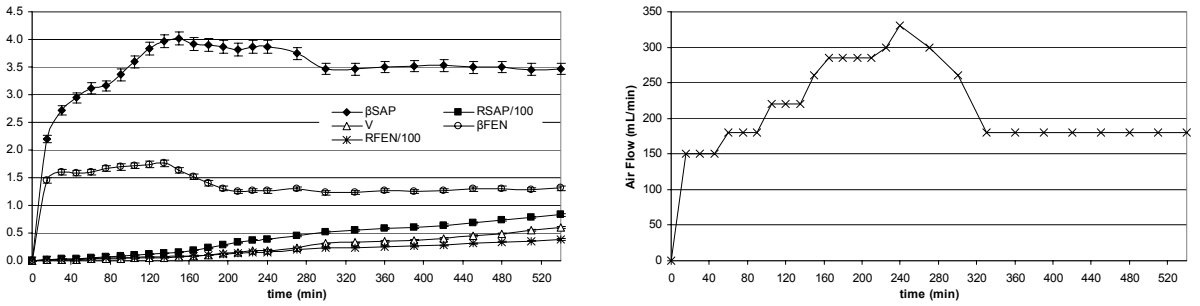

Figure 7: Concentration kinetics of juá saponins in a foam column using an air flow gradient.

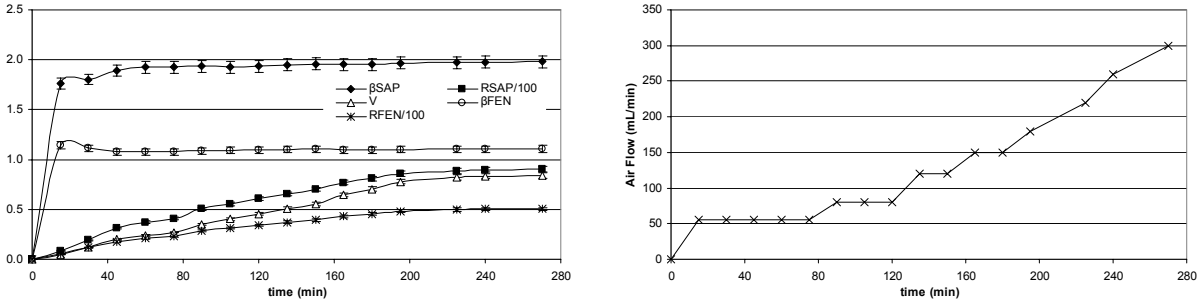

Figure 8: Concentration kinetics of sisal saponins in a foam column using an air flow gradient.

CONCLUSION

A foam column can be an interesting alternative for saponins concentration, since it promoted high values of saponin recovery (82.6% for juá saponins and 90.5% for sisal saponins) and concentration factors (3.46 and 1.98 for juá and sisal saponins, respectively). Some improvements and modifications such as the use of a column jacket to raise the temperature or columns with different geometries could enhance foam drainage, contributing to even better results.

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