Notes

Efficiency of Foam Fractionation for the Enrichment of Nonpolar Compounds from Aqueous Extracts of Plant Materials

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Biologically active compounds from several useful plants were enriched using foam fractionation, a separatory method belonging to the adsorptive bubble separation (ABS). Nonpolar humulones (**1**−**6**) from Pilsener beer, curcuminoids (**7**−**9**) from turmeric, and carotenoids (**16** and **17**) from carrot juice were enriched fast and quantitatively, depending on the process parameters, whereas more polar compounds such as catechins from green tea (**11**, **12**, **14**, and **15**) and naringin (**18**) and hesperidin (**19**) from orange and grapefruit juices could not be enriched.

Developing biologically active natural products from medicinal herbs or useful plants is of great interest to the pharmaceutical and food industries. For their isolation, methods such as solvent extraction (SE) or supercritical fluid extraction (SFE) are usually employed. However, these methods tend to burden the ecosystem by using organic solvents extensively or demand considerable attention and, hence, greater investment and maintenance costs. During SE and SFE, fat and chlorophyll are co-extracted and, thereafter, have to be separated from the active substance by column chromatographic methods. An alternative method of general interest is the so-called "foam fractionation", a method based on adsorptive bubble separation (ABS). For the enrichment of surface-active substances, gases (e.g., nitrogen, oxygen, air, carbon dioxide, etc.) are introduced, whereupon the substances tend to adsorb on the hydrophobic surface of gas bubbles and, therefore, enrich in the formed foam.[1] As aqueous extracts are used for foam fractionation, the problem that fat and chlorophyll have to be additionally separated does not occur. Furthermore, the residues after SE and SFE usually contain quite high amounts of extractable substances, either valuable or undesirable, such as pesticides. As foam fractionation is more effective, especially at low initial concentrations of substances,[2] it could thus be employed as a supporting tool. Furthermore, it uses mostly an inert gas (e.g., nitrogen) at room temperature, which makes it a mild method for extracting substances that are sensitive to oxidation and heat. The avoidance of organic solvents assists with sustainability and eco-friendliness. The equipment used is simple, and the demands on energy and running costs are low.[3] Other advantages are a greater extent of enrichment from highly diluted solutions and continuous feasibility according to process parameters.[4]

Lemlich placed the beginning of foam fractionation on a laboratory scale as early as 1900 with the elimination of sodium oleate from aqueous solutions to verify the Gibbs adsorption theorem. In separate reviews, Lemlich[5a] and Karger and DeVivo[5b] outlined different operational modes of foam fractionation. Ostwald and Siehr ingeniously investigated the fractionation of proteins from sap of plants, such as the amniotic fluids of potato and sugar beet, and proposed steps for improving their separation.[6] More recently, Crofcheck et al. recovered proteins from tobacco extract through tagging them with histidine, and Joeng et al. have enriched proteins from complex *Mimosa pudica* L. seed extracts.[7] The latter researchers demonstrated the importance of matrix and process parameters, such as ionic strength, surfactant choice, and pH value, to achieve the highest enrichment possible.

Foam fractionation has also been applied for effluent treatment. Hussenot et al. worked on intensive wastewater systems from costal wetlands by combining foam fractionation with other methods to achieve cleaning.[8] Doyle investigated the metallurgical potential of ion flotation (a part of foam fractionation) and recovered metal cations from dilute solutions using collectors.[9] This investigator found that the correct design of equipment is imperative for the improvement of the technique, and that it is preferred to solvent extraction for highly diluted solutions due to greater reagent requirement. Foam fractionation can well be applied for trace analysis and the elimination of undesired byproducts, such as the flavokavines A and B from Kava−Kava (*Piper methysticum* Forster) or α-solanine and α-chaconine from potatoes (*Solanum tuberosum* L.).[10]

As has been demonstrated well by research in the past, the events during foaming in dependence of physicochemical parameters are complicated and have not yet been sufficiently investigated for optimization of this technique. Herein, the foam fractionation of natural compounds from aqueous extracts of useful plants and herbs was investigated, focusing on the optimization of enrichment by varying the most important process parameters.

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Table 1 shows yields and enrichment ratios representing optimum values, achieved due to systematic variation of process parameters. Experience obtained so far has shown that extracts from some plants contain already sufficient amounts of surface-active constitutes to form a stable foam, for example, ginger (*Zingiber officinale* L.) and rosemary (*Rosmarinus officinale* L.).[2,11] Pilsener beer containing bitter substances (*Humulus lupulus* L.) also belongs to this group, and the separation of the nonpolar compounds **1**−**6** is rapid (within 60 min) and almost quantitative (Figure S1, Supporting Information). When observing the course of enrichment via foam fractionation, it could be demonstrated that the enrichment takes place in a highly selective manner. Foam fractions were collected between 14 and 63 min of foaming, and already in the first fraction (after 14 min), the humulones **1**−**3** were completely enriched (100%). The isomers, however, enriched continuously over the course of foaming, and maximum concentrations for **5** and **6** were measured after 17 and 22 min, respectively, in the foam samples. The last foam fractions, collected between 42 and 62 min, contained a maximum concentration for **4** but only traces of **5** and **6**. Accordingly, the enrichment was most effective at pH 3, almost without any losses, which was not the case at higher pH values.

For the curcuminoids **7**−**9** (Figure S2, Supporting Information), the initial concentration as a parametric value requires discussion. The foaming of a diluted, yellow-colored sample, which contained ca. 5 mg L^{-1} of curcuminoids, led to a decolorized solution, but to a dark yellow colored foam after 10 min foaming time, showing visibly that enrichment took place (only traces remained in the initial solution). However, the foaming of a solution containing 50 mg L^{-1} showed the same effect after 80 min of foaming, demonstrating that foam fractionation is much useful for highly diluted solutions. As the initial concentration of curcuminoids in turmeric extracts is very low, methods have been developed for increasing their concentrations, which makes foam fractionation more practical for the enrichment of these substances.[12]

If the surface activity of an extract is not sufficient enough for the development of foam, or to keep the foam stable, then the addition of a foam building surfactant is possible. For this purpose, Quillaja saponin, obtained from the soapbark tree *Q. saponaria* Molina, is an useful agent.[10a] It is soluble in water and reduces the surface tension of polar fluids. Saponin, in turn, is contained in many plants, such as in green tea, investigated herein,[13] but in amounts too small to assist in foam development. The addition of saponin was not necessary for Pilsener beer, turmeric extract, and carrot juice, but was employed for grapefruit juice and green tea because these matrices showed only a weak capacity for foaming.

Although saponin helped to form stable and continuous foaming, the catechins **11**, **12**, **14**, and **15** could not be enriched, while **10** and **13** were present only in small amounts (Table 1) (Figure S3, Supporting Information). This might be ascribable to the high initial concentration, particularly of **11** and **12**, which were still in the range of the usual extent of concentration applicable for foam fractionation (within 10^{-3} to 10^{-7} mol for surface-active substances).[5b] Here, it can be assumed that the more polar nature of the catechin compounds is responsible for their low efficiency of enrichment and nonrecovery. However, such polyphenols can build up strong surface-active complexes with caffeine,[14] and thus, it may be possible to transfer them into the foam phase.

The polarity of a compound is an essential factor controlling the enrichment. In this respect, the log P_{ow} (*n*-octanol/water partition coefficient) of each substance can be considered, providing an indication about its hydrophobicity and solubility. The lower the value, the more hydrophilic a compound behaves in aqueous solution and thus is more difficult to be transferred in the foam phase (Table 1). The nonpolar carotenoids **16** and **17** (Figure S4, Supporting Information), showing high log P_{ow} values, could be enriched from carrot juice efficiently, whereas this was not the case for naringin (**18**) and hesperidin (**19**), either from standard solution or from juices (Figure S5 and S6, Supporting Information). They have glycosidic bonds and, thus, remained soluble in the aqueous phase. Therefore, it can be concluded that foam fractionation is not feasible for the enrichment of polar substances such as **18** and **19**, which tend to remain in the aqueous matrix.

To obtain as high yields as possible, it is imperative not only to correctly adjust the gas flow rate and, therefore, the foaming time but also to choose the correct initial solution volume. It is the aim during foaming that the foam should remain stable and ascend slowly through the column, which influences the drainage effect to obtain a foam that entrains high concentrations of the desired substances. If the foaming time is too fast, a wet foam is obtained, leading to a low enrichment factor. During our experiments, we found the initial solution volume of 100 mL and gas flow rates between 15 and 30 mL min^{-1} to be most appropriate for successfully running a foaming trial. The latter may be increased up to 100 mL min^{-1} at the end of the trial to boost foam development when necessary.

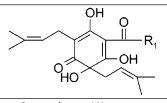
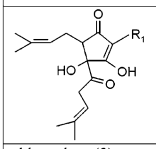
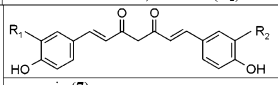
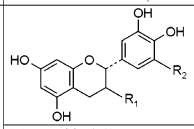
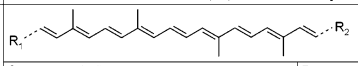
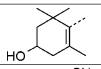
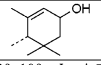
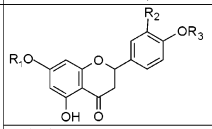
In conclusion, foam fractionation is useful for the selective separation of nonpolar compounds from plant materials, with or without saponin as a foam building agent and according to carefully chosen process parameters. The results obtained from this research have shown new possibilities as to how this method can be applied to isolate valuable compounds from useful plants and eliminate undesirable byproducts.

Experimental Section

General Experimental Procedures. Inert gas was led through the aqueous extract via a glass frit (porosity 3, pore size = 16−40 μm), whereupon foam was developed in the upper column part (Figure S7, Supporting Information). Thereafter, the foam disintegrated back to liquid, the so-called "foamate". Column geometry: 14.4 mm i.d. and 130 cm in length for 50 mL start volume; 18.5 mm i.d. and 130 cm in length for 100 mL start volume. Care was taken so that prior to each experiment, the column was free of lipid contamination and that, during the trials, the size of bubbles remained steadily the same. The batch mode was applied for foam fractionation, meaning that the whole amount of solution was foamed at once. Standard solutions were applied for foaming to ascertain whether all compounds can be enriched in pure form and to exclude matrix effects on the compounds, such as the formation of complexes. The pilot apparatus was designed in our institute with glass-blown framework and other equipment, such as for nitrogen supply and a flowmeter purchased from suppliers. A pilot plant is not commercially available yet, but the costs for the installation and maintenance are low.

Materials. All chemicals used were of analytical quality standard. In all experiments, double-distilled water was used. Quillaja saponin (CAS No: 8047-15-2, containing <20% sulfated ash, and 10% sapogenin) and the curcuminoids as a technical standard mixture were obtained from Sigma-Aldrich (Munich, Germany). The hop standards were purchased from the research department of the Swiss Brewery Association (Zurich, Switzerland), and the Pilsener beer in bottles from Weihenstephaner brewery (Freising, Germany; decoction No.

Table 1. Yield, Enrichment Ratios, and log P_{ow} Values of Compounds Obtained from Plant Aqueous Extracts via Foam Fractionation

Plant	Enrichment of compounds by foam fractionation at optimized conditions.[a]					
	Compound[15]	R	C [mg L^{-1}][b]	Yield[c] [%]	ER[d]	log Pow[e]
Humulus lupulus L. (Pilsener beer)						
	co-humulone (**1**)	R_1: 	0.9	92 ± 6	16.3 ± 1.1	2.92
	n-humulone (**2**)	R_1: 	0.7	93 ± 5	13.8 ± 0.7	3.01
						
	ad-humulone (**3**)	R_1: 	0.7	92 ± 5	13.8 ± 0.7	3.00
	co-isohumulone (**4**)	R_1: 	8.8	83 ± 5	14.0 ± 1.0	2.05
	n-isohumulone (**5**)	R_1: 	10.7	91 ± 6	16.8 ± 1.0	2.87
	ad-isohumulone (**6**)	R_1: 	4.1	90 ± 6	21.4 ± 1.4	2.53
	Start volume: 100 mL; flow rate (N_2): 15 mL min^{-1}; pH 3.0; foaming time: 60 min					
Curcuma longa L. (turmeric)						
	curcumin (**7**)	R_1: OCH_3 R_2: OCH_3	4.42	92 ± 7	19.5 ± 1.3	1.15
	demethoxycurcumin (**8**)	R_1: OCH_3 R_2: H	3.120	91 ± 5	17.6 ± 1.1	1.00
	bis-demethoxycurcumin (**9**)	R_1: H R_2: H	5.89	90 ± 5	15.4 ± 1.1	0.85
	Start volume: 100 mL; flow rate (N_2): 15 mL min^{-1}; pH 6.0; foaming time: 100 min					
Camellia sinensis (L.) O. Kuntze, Theaceae (green tea)						
	(+)-catechin (**10**)	R_1: exo-OH R_2: H	4.3	19 ± 2	34 ± 2.0	0.53
	(–)-gallocatechin gallate (**11**)	R_1: exo-gallate R_2: OH	63.4	n.d.[f]	1.0	0.12
	(–)-epigallocatechin gallate (**12**)	R_1: endo-gallate R_2: OH	96.5	n.d.[f]	1.0	0.09
	(–)-epicatechin (**13**)	R_1: endo-OH R_2: H	23.8	39 ± 4	14 ± 3.0	0.67
	(–)-epicatechin gallate (**14**)	R_1: endo-gallate R_2: H	23.7	n.d.[f]	1.0	0.07
	(–)-epigallocatechin (**15**)	R_1: endo-OH R_2: OH	13.0	n.d.[f]	1.0	0.13
	Start volume: 70 mL; flow rate (N_2): 15 mL min^{-1}; pH 6.5; addition of 30 mg saponin; foaming time: 100 min					
Daucus carota L. (carrot)						
	β-carotene (**16**)	R_1:  R_2: 	39.0	90 ± 7	16.3	4.19
	lutein (**17**)	R_1:  R_2: 	19.0	88 ± 7	16.3	3.89
	Start volume: 100 mL; flow rate (N_2): increased from 30–100 mL min^{-1}; pH 6.5; foaming time: 60 min					
Citrus sinensis (L.) Osbeck (orange); *Citrus paradisi* Macfad. (grapefruit)						
	naringin (**18**)	R_1: gluco-rhamnose R_2: H R_3: H	223.7	n.d.[f]	1.0	–0.15
	hesperidin (**19**)	R_1: rutinose R_2: OH R_3: CH_3	725.7	n.d.[f]	1.0	–0.21
	Start volume: 100 mL; flow rate (N_2): 15 mL min^{-1}; pH 4.5; addition of 50 mg saponin for grapefruit juice only; foaming time: 60 min					

[a] Three-fold replicate measurements. [b] Concentration of the compound in the initial solution. [c] Yield (recovery): absolute amount of the substance in the foam versus absolute amount of the substance in the initial solution × 100. [d] Enrichment ratio: concentration of the compound in the foam versus concentration of the compound in the initial solution. [e] Determined according to OECD guidelines.[16] [f] Not determined.

680-692, brewed in 2002). Turmeric powder (batch No. 9126024; 45% curcumin, 2001), catechin standards, and green tea leaves (Fannings, batch No. 9141025, 2001) were obtained from Adalbert-Raps research center (Freising, Germany). The fresh carrots, oranges, and grapefruits were obtained from the local market (Naturgarten Schoenegge, Nandlstadt, Germany). All other compounds were acquired from Merck (Darmstadt, Germany).

Sample Preparation before Foam Fractionation. Pilsener beer was freed of CO_2 in an ultrasound bath for 15 min. Turmeric powder (1 g) was extracted with 100 mL of water (pH 7) and boiled for 20 min under reflux. Dried green tea leaves (2 g) were extracted with 100 mL of water for 30 min under reflux; the pH was set to 5.6 with NaOH. Both extracts were filtered afterward. Carrot, grapefruit, and orange juices (100 mL) were freshly squeezed and filtered before foaming.

Sample Preparation after Foam Fractionation before HPLC. Prior to HPLC measurement, 100 mL of CO_2-free Pilsener samples were mixed with isooctane and HCl and shaken for 15 min. Turmeric and green tea extract were directly applied for HPLC. The carotenoids in the foamate and the remaining solution were extracted through precipitation using Carrez-I and Carrez-II solutions with 150 mL of acetone. Grapefruit and orange solution (5 mL of the initial, foamate, and residual) were prepared with 25 mL of 0.01 M acetic acid/dimethyl formamide (8:2) and then applied to HPLC.

HPLC/UV-DAD Parameters and Elution Solvents. Bitter substances from Pilsener beer; detection = 275 nm; flow rate = 1 mL min^{-1}; mobile phase = gradient; A, MeOH−H_2O−H_3PO_4, 725 mL:275 mL:17 g; B, MeOH. Curcuminoids; detection = K1, 254 nm; K2, 428 nm; 3D, 200−600 nm; flow rate = 0.8 mL min^{-1}; mobile phase = isocratic; A, CH_3CN−phosphate buffer, pH 4.4 (60:40). Carotenoids; detection = K1, 230 nm; K2, 450 nm; 3D, 340−550 nm; flow rate = 1 mL min^{-1}; mobile phase = isocratic; A, MeOH−tetrahydrofuran, 95:5. Polyphenols (green tea); detection = 280 nm; flow rate = 1 mL min^{-1}; mobile phase = gradient; A, acetic acid (2%); B, CH_3CN. HPLC column = Kromasil 100 C_{18}, 5 μm, 250 × 4.6 mm, from Knauer (Berlin, Germany); 25 °C column temperature; 20 μL sample loop.

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Supporting Information Available: HPLC chromatograms of humulones, curcuminoids, catechins, carotenoids, naringin, and hesperidin, and details on running a foam fractionation trial. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Maas, K. In *Adsorptive Bubble Separation Methods, Methodicum Chimicum*; Korte, F., Ed.; Academic Press: New York, 1974; Vol. 1, pp 165−171.
(2) Backleh, M.; Ekici, P.; Leupold, G.; Coelhan, M.; Parlar, H. *Adv. Food Sci.* **2003**, *25*, 2−7.
(3) (a) Uraizee, F.; Narsimhan, G. *Enzyme Microb. Technol.* **1990**, *12*, 315−316. (b) Brown, K.; Kaul, A.; Varley, J. *Biotechnol. Bioeng.* **1999**, *62*, 278−290.
(4) (a) Timmons, M. B.; Weeks, N. C.; Chen, S. *Aquacult. Eng.* **1992**, *11*, 251−265. (b) Maas, K. *Sep. Sci.* **1969**, *4*, 457−465.
(5) (a) Lemlich, R. *Ind. Eng. Chem.* **1968**, *60*, 16−29. (b) Karger, B. L.; DeVivo, D. G. *Sep. Sci.* **1968**, *3*, 393−424.
(6) Ostwald, W.; Siehr, A. *Kolloid-Zeitschrift* **1937**, *79*, 11−15.
(7) (a) Crofcheck, C.; Maiti, I.; Pattanaik, S.; Jay, M. *Appl. Biochem. Biotechnol.* **2004**, *119*, 79−91. (b) Jeong, G. T.; Park, E. S.; Wahlig, V. L.; Burapatana, V.; Park, D. H.; Tanner, R. D. *Ind. Eng. Chem. Res.* **2004**, *43*, 422−427.
(8) Hussenot, J.; Lefebvre, S.; Brossard, N. *Aquat. Living Resour.* **1998**, *1*, 297−304.
(9) Doyle, F. M. *Int. J. Miner. Process.* **2003**, *72*, 387−399.
(10) (a) Backleh, M.; Ekici, P.; Leupold, G.; Parlar, H. *Naturwissenschaften* **2003**, *90*, 356−369. (b) Backleh, M.; Ekici, P.; Leupold, G.; Coelhan, M.; Parlar, H. *J. Sep. Sci.* **2004**, *27*, 1042−1044.
(11) Backleh, M.; Leupold, G.; Parlar, H. *J. Agric. Food Chem.* **2003**, *51*, 1297−1301.
(12) (a) Stranz, J. L. U.S. Patent 4,368,208, 1983. (b) Stransky, C. E. U.S. Patent 4,138,212, 1979.
(13) (a) Okubo, T.; Juneja, L. R. In *Chemistry and Application of Green Tea*; Yamamoto, T., Juneja, L. R., Chu, D.-C., Kim, M., Eds.; CRC Press: Boca Raton, FL, 1997; Chapter 10, pp 110−112. (b) Greentealovers.com; the ingredients in green tea, http://greentealovers.com/greenteahealthcatechin.htm#saponin (2005).
(14) (a) Chao, Y. C.; Chiang, B. H. *J. Sci. Food Agric.* **1999**, *79*, 1687−1690. (b) Martin, R.; Lilley, T. H.; Bailey, N. A.; Falshaw, C. P.; Haslam, E.; Magnolato, D.; Begley, M. J. *J. Chem. Soc., Chem. Commun.* **1986**, 105−106.
(15) Belitz, H. D.; Grosch, W.; Schieberle, P. In *Lehrbuch der Lebensmittelchemie*, 5th ed.; Springer: Berlin, Heidelberg, New York, 2001; pp 225, 226, 812, 814, 817, 883, 967−968.
(16) Organisation for Economic Cooperation and Development (OECD), Paris (France). Guidelines for Testing Chemicals; http://www.oecd.org.

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