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Pretreatment of milk thistle seed to increase the silymarin yield: An alternative to petroleum ether defatting

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Abstract

Milk thistle (*Silybum marianum* L.) seed meal is extracted for the flavonolignans, silychristin, silydianin, silybinin A, silybinin B, isosilybinin A and isosilybinin B, which are collectively known as the silymarin complex. To obtain the flavonolignans, the meal is usually treated with successive washes of petroleum ether to remove the lipids, followed by extraction of the flavonolignans with ethanol. This work examines the possible replacement of petroleum ether and ethanol by water or other aqueous solutions in these processes. To replace petroleum ether, pretreatments with 1.2% NaOH (w/w), 1.5% H_2SO_4 (w/w), 2% $NaHCO_3$ (w/w), 0.14% cellulase and water were investigated. Of these pretreatments, 1.5% H_2SO_4 and water produced similar flavonolignan yields as petroleum ether. Results established that pretreating the milk thistle seed meal with 1.5% H_2SO_4 (w/w) at 50 °C for 18 h could replace the petroleum ether pretreatment. In addition, it was shown that similar amounts of flavonolignan could be recovered with a 1.5% H_2SO_4/water (100 °C) extraction as with a petroleum ether/ethanol extraction. Although cellulase pretreatment was not examined extensively, significant advances in cellulase effectiveness and cost have occurred in the past few years by companies such as Genencor International and Novozymes. These advances should help to make enzyme use for cellulose conversion, as well as extraction pretreatment, technically and economically feasible.

Keywords: Milk thistle; Pretreatment; Silymarin; Flavonolignans; Water extraction

1. Introduction

Phytochemicals are extracted from plant materials to service the pharmaceutical and the dietary supplement industries. Processes such as supercritical fluid extraction (SFE) (Femenia et al., 2001), pressurized fluid extraction (PFE) with enhanced solvent diffusivities (Benthin et al., 1999) or leaching with generally regarded as safe (GRAS) solvents provide efficient extraction processes for these compounds (Tanko et al., 2005). To increase the extraction yield, the biomass can undergo a physical, chemical or biological pretreatment (Lynd et al., 1999), which breaks down the rigid cell wall matrices, thereby resulting in a more efficient extraction. Physical pretreatment, such as grinding or freeze explosion, can be used as the sole pretreatment step or as a treatment prior to additional chemical or biological pretreatment. Chemical pretreatments with dilute sulfuric acid (Allen et al., 2001), ammonia (Belkacemi et al., 1998), dilute sodium hydroxide (Li et al., 2001) and water (Allen et al., 2001) have been used in the conversion of cellulose to sugars, particularly when enzymatic hydrolysis is used. In addition to chemical or physical pretreatment, hydrolytic enzymes have been used as pretreating agents, acting on cell walls and breaking down structural integrity, thereby increasing the surface area of the material. As an example, cellulose pretreatment was found to be effective in releasing lutein from marigold flowers (Barzana et al., 2002).

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Extracts of milk thistle (*Silybum marianum* L.) seed have a long tradition for treating liver ailments (Flora et al., 1998). In 2005, milk thistle seed products placed in the top ten of dietary supplements, at about $8.3 million (Herbalgram, 2006). The seeds contain the silymarin complex, which is composed of the six flavonolignans silychristin (SC), silydianin (SD), silybinin A (SA), silybinin B (SB), isosilybinin A (ISA) and isosilybinin B (ISB) (Wallace et al., 2003a). Because milk thistle seed contains 20–25% (w/w) lipid (Hamid et al., 1983; Carrier et al., 2002), the seeds are usually extracted with petroleum ether to defat the seed prior to flavonolignan extraction (Benthin et al., 1999). Petroleum ether is regulated as a volatile organic compound (VOC), is expensive and, after its use, requires recovery or disposal. Additionally, the trace quantities of petroleum ether still present in the final extract need to be removed to meet consumer acceptability. Thus, alternative pretreatment techniques are sought. Kahol et al. (2001) ground and froze milk thistle seed prior to flavonoligan extraction, but cryogenic treatment is an expensive process. Because flavonolignan extraction with water has been successful on both defatted seed and seed that had not been defatted (Wallace et al., 2003b; Duan et al., 2003), an attempt was made to interface novel pretreatment techniques with water extraction. Thus, the objectives of this paper were to perform pretreatment comparison studies with cellulase enzymes and dilute solutions of H_2SO_4, NaOH and $NaHCO_3$ as alternatives to traditional petroleum ether pretreatment, and to interface pretreatment with water extraction.

2. Methods

2.1. Plant material

Milk thistle seed was purchased from Frontier (Norway, IA) and stored at 4 °C. The seed was ground in a household coffee grinder to a particle size of 0.4 mm, as determined by ASAE standard S319.1 (ASAE, 2002). Since size reduction prior to extraction is also a pretreatment technique, smaller particle sizes will likely enhance the rates or even possibly the yields during extraction. Abu Jadayil et al. (1999) presented a proximate analysis of milk thistle seed (three replicates with CV < 5%), and showed that the seed contained 5.8 g moisture, 19.1 g protein, 26.3 g fat, 25.4 g crude fiber, 4.8 g ash and 9.8 g iron per 100 g dry matter. The energy content was 410 kcal per 100 g dry matter.

2.2. Chemicals

Silybinin was purchased from Sigma (St. Louis, MO). Silychristin and silydianin were obtained from Phytolab (Hamburg, Germany). H_2SO_4 was secured from Fisher Scientific (Springfield, NJ), $NaHCO_3$ from Mallinckrodt (Phillipsburg, NJ) and cellulase (Trichoderma longibrachiatum, 0.61 U/mg) from Fluka (Milwaukee, WI). NaOH, methanol and petroleum ether were purchased from EM Science (Darmstadt, Germany), and ethanol was obtained from AAPER (Shelbyville, KY). At the time that this work was conducted, no standards were available for either isosilybinin A or for isosilybinin B.

2.3. Pretreatment studies

For each pretreatment, 8 g of ground milk thistle seed were added to 72 ml of either 1.2% NaOH (w/w), 1.5% H_2SO_4 (w/w), 2% $NaHCO_3$ (w/w), water or a solution of 0.14% (w/w) cellulase. The mixture was placed in 250 ml brown bottles and agitated at 60 rpm for 24 h in a shaking water bath (Precision, Winchester, VA). Depending on the experiment, the temperature was set at 40, 50, 60 or 70 °C. After the 24 h pretreatment, samples were centrifuged at 292*g* for 10 min. The supernatant was decanted and the seed residue was air-dried for 24 h at room temperature. For data presented in Tables 1 and 2, the control for pretreatment consisted of extracting 2 g of ground milk thistle seed in a Soxhlet apparatus with 200 ml of petroleum ether. During the petroleum ether or ethanol Soxhlet extractions,

Table 1
Effect of pretreatment on flavonolignan yields (mg/g seed) obtained from the extraction of *S. marianum* seed meal

Pretreatment	Yield (mg/g seed)				
	SC	SD	SA	SB	Total
Control[A] (petroleum ether)	2.77 ± 0.25^{a}	14.19 ± 1.13^{b}	2.12 ± 0.17^{b}	3.52 ± 0.29^{b}	24.24 (100%)
No pretreatment	2.61 ± 0.07^{a}	12.90 ± 0.06^{b}	2.00 ± 0.02^{b}	3.32 ± 0.04^{b}	20.70 (85%)
1.2% NaOH (w/w)	0.00 ± 0.00^{b}	0.19 ± 0.01^{c}	0.00 ± 0.00^{d}	0.00 ± 0.04^{d}	0.20 (1%)
1.5% H_2SO_4 (w/w)	3.12 ± 0.47^{a}	19.12 ± 3.26^{a}	3.15 ± 0.44^{a}	4.43 ± 0.65^{a}	28.93 (119%)
0.14% Cellulase (w/w)	2.80 ± 0.31^{a}	13.43 ± 1.55^{b}	2.26 ± 0.22^{b}	3.74 ± 0.36^{ab}	22.27 (92%)
2% NaHCO3 (w/w)	0.24 ± 0.07^{b}	0.46 ± 0.06^{c}	0.42 ± 0.11^{c}	0.89 ± 0.25^{c}	2.49 (10%)
Water	2.45 ± 0.09^{a}	11.40 ± 0.62^{b}	2.00 ± 0.09^{b}	3.30 ± 0.15^{b}	18.59 (77%)

Pretreatments were performed at 50 °C for 24 h, followed by extraction in boiling ethanol for 4 h in a Soxhlet apparatus. SC, silychristin; SD, silydianin; SA, silybinin A; and, SB, silybinin B.

Numbers in parentheses indicate the percentage recovered relative to the control.

The superscript letters following the calculated means and standard deviations are an indication of similarity in the data. Values sharing a letter within a column are not significantly different at $p < 0.05$.

[A] Control consisted of successive extractions in a Soxhlet apparatus with petroleum ether and with ethanol.