Effects of Expression Technology on Physical Parameters and Phytochemical Profile of *Echinacea purpurea* Juice

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Abstract. The objective of this study was to compare the quality of *E. purpurea* juice obtained using two types of press, a hydraulic bag press (BP) and a mechanical screw press (SP). Cichoric acid concentration was about twice as high in the SP juice compared to the BP juice. Qualitative and quantitative differences in the alkylamides were observed, in favor of the SP. BP juice had a significantly higher pH, density and viscosity. No differences were observed in levels of soluble or total solids. The results of this study suggest that SP is more suitable for *Echinacea* juice expression than BP. Engineering and juice quality considerations in the selection of expression technology for medicinal plants are discussed in the context of therapeutic value.

Immunoenhancing properties have long been associated with different species of the genus *Echinacea*. The use of these plants is well established in traditional and alternative medicine for the treatment of a wide variety of ailments (Hobbs, 1994). *Echinacea* holds a dominant place among products in today's herbal market, where *Echinacea* preparations account for more than 10% of total herbal product sales on the North American market (Brevoort, 1998). Over 800 different herbal products sold on the European market contained at least one component from *Echinacea* plants (Bauer, 1998). In 1998, global cultivation of *Echinacea* spp. was estimated at 16,000 hectares (Brevoort, 1998).

The medicinal properties of *Echinacea* have been attributed to a range of secondary compounds found in varying amounts depending on plant part and species. These compounds include caffeic acid derivatives, alkylamides, and certain high molecular weight polysaccharides. Cichoric acid, a major caffeic acid derivative of *E. purpurea*, has been associated with increased phagocytosis (Bauer, 1998), antihyaluronidase activity (Facino et al., 1993) and free radical scavenging (Facino et al., 1995). Other studies have demonstrated an effect of cichoric acid against viruses (Cheminat et al., 1988) including HIV-1 (Lin et al., 1999; Robinson et al., 1996). The anti-inflammatory activity of

alkylamides isolated from *Echinacea* is suggested by the inhibition of cyclooxygenases and 5-lipoxygenase (Muller-Jakic et al., 1994). In addition, polysaccharides from *Echinacea* have been shown to increase cytokine production (Proksch and Wagner, 1987) as well as leucocyte phagocytosis (Roesler et al., 1991) in different animal and human models.

Extraction of active compounds from fresh plant material is done through a range of methods, including solvent extraction and mechanical expression. Mechanical expression is of particular importance for the production of *Echinacea* juice, one of the most important formulations available on the herbal market today. Variations in phytochemical content of *Echinacea* preparations due to different processing methods has been observed (Bauer, 1999). Studies have demonstrated the susceptibility of phenolic compounds found in the *Echinacea* genus such as echinacoside to hydrolytic and redox reactions affected by factors such as temperature and pH (Baugh and Ignelzi, 2000). Although the medicinal value of echinacoside is still debated, it remains a very important marker compound to the nutraceutical industry as a quality indicator of preparations containing *E. angustifolia* and *E. pallida*.

These considerations highlight the importance of evaluating the products obtained from the different expression technologies commonly utilized for commercial preparations. As the consistency of medicinal benefits

becomes an increasingly important concern in the herbal market (Awang, 2000), the focus of processing technology evaluations should be placed on the stability of marker and active compounds thereby conserving therapeutic value, all the while keeping in mind production efficiency and engineering considerations. Very little to date has been published to address the question of quality differences in *Echinacea* preparations due to postharvest factors. Most of the presently available information deals with effects of drying on the important constituents of *Echinacea* preparations (Kim et al., 2000; Perry et al., 1999) and does not specifically describe the impact of the different technologies used in processing.

The following experiment was conducted as a comparative assessment between two commonly used juice extraction technologies, a hydraulic bag press and a Reitz screw press. This assessment was based on physical and phytochemical parameters measured from the *E. purpurea* juices extracted by the two types of press.

Materials and Methods

Plant material and pressing. *E. purpurea* was grown from seed at an Access Business Group certified organic farm located in Lakeview, Calif. During Spring 2000, the aerial parts of *E. purpurea* were field-mowed and chopped to $\approx 1/4$ inch using an Urschel mill. Six lots of material were segregated and blanched independently in a steam blancher at 100 °C for 3 min. The material reached a minimum of 82.2 °C before being discharged from the blancher via a screw conveyor. After blanching, three lots of the material were pressed using a hydraulic bag press (Good Nature Products, Buffalo, N.Y.) and three lots were pressed using a Reitz screw press (Bepex, Minneapolis). Samples were collected for the following determinations: cichoric acid concentration, alkylamide fraction profile, total solids, soluble solids, color, viscosity, and pH. Each sample was analyzed for its chemical composition immediately following pressing.

Cichoric acid HPLC analyses. Twelve samples were prepared for each press type by mixing 1 mL of juice and 1 mL of methanol. After shaking, samples were filtered using a 1- μ m glass fiber filter and 0.2- μ m polyvinylidene filter. Ten microliters of the sample were injected in a Hewlett Packard 1100 model HPLC (Palo Alto, Calif.). Separation was done on a Nova-Pack C18 3.9-mm $\times$ 300-mm column at room temperature with a constant flow rate of 1 mL/min. The initial mobile phase consisted of 20% Acetonitrile (solvent A) and 80% 0.4% $\text{H}_3\text{PO}_4/\text{H}_2\text{O}$ (solvent B). A linear gradient was applied over 10 min, increasing solvent A to 35%. Cichoric acid was detected with a Photodiode Array Detector set at a wavelength of 320 nm. An HPLC grade cichoric acid standard, purchased from Apin Chemicals, product #15486C (Abingdon, Oxon, U.K.) was used for identification and quantification of samples.

Alkylamide thin-layer chromatography (TLC) profile. Pressed juice samples of 5

mL were extracted with 4mL of CHCl_3 and centrifuged. The CHCl_3 layer was separated and the sample was dried over anhydrous Na_2SO_4 . Alltech (Nicholasville, Ky.) HPTLC silica gel 60 GF254 plates were used with a 7 hexane : 3 ethyl acetate mobile phase. Volumes of 80 μL were spotted along an 8-mm-wide line. Alkylamides were detected by spraying anisaldehyde reagent (0.5% anisaldehyde with 10 mL of glacial acetic acid in methanol, heated at 105 $^\circ\text{C}$). The alkylamides appear as purple in color. The major alkylamides found in the aerial parts of *E. purpurea* belong to 2,4-diene structure type. The most abundant are 2E,4E,8Z,10E-dodeca-tetraenoic acid isobutylamide and 2E,4E,8Z,10Z-dodeca-tetraenoic acid isobutylamide (Fig. 1). Both of these compounds have an R_f value of 0.33 under the TLC conditions described above. In order to confirm the identification of alkylamides in the separated bands, these were collected individually from TLC plates, washed with MeOH and analyzed with HPLC/MS to determine molecular mass and separation patterns. Due to the unavailability of commercial standards at the time of this study, quantitative evaluation was not possible. In light of this, TLC analysis was chosen as a reliable, semi-quantitative and faster alternative to HPLC analysis (Wagner and Bladt, 1996).

Physical parameters. Total solids were assessed by measuring loss of mass after drying liquids for 4 h at 80 $^\circ\text{C}$. Soluble solids were determined by a PR-101 Atago digital refractometer (Atago U.S.A., Kirkland, Wash.). Color (spectral reflectance) was determined using a top-port Minolta CM 3500-d spectrophotometer (Minolta, Osaka, Japan), which was calibrated using as references the black and white tiles provided by the manufacturer. The juice viscosity was evaluated with a Brookfield Digital Viscometer, model LVTDV-II (Brookfield Engineering Laboratories, Stoughton, Mass.). This provides an objective measure of juice consistency by measuring the torque required to rotate a specified cylinder immersed in the juice at specified rotational rates. All of the above physical parameters were measured on twelve samples for each of the press types.

Statistical analysis. Independent two group *t* tests were conducted for the following measured variables: pH, viscosity, density, solids (total and soluble), and cichoric acid concentration. The analyses were carried out using Systat (standard vers. 9.01) statistics software. Differences in means were considered statistically significant when *P* values were <0.05 .

Results and Discussion

Levels of soluble solids and of total solids was used to characterize the juices obtained with the different presses. In our study, no significant differences were observed in levels of either soluble or total solid between BP juice and SP juice (Table 1). There was a significant difference in pH, with SP juice (pH=5.4) being more acidic than BP juice (pH=5.6). Likewise, density and viscosity also differed significantly (Table 1), with BP juice being denser and more viscous than SP juice. SP juice was lighter and

greener than BP juice. Instrumentally, this was reflected by BP juice having a significantly lower L^* , hue, and chroma than SP juice as seen in (Fig. 2).

A significant difference was observed between cichoric acid concentrations. The mean cichoric acid concentration measured in the SP juice (0.85 $\text{mg}\cdot\text{mL}^{-1}$) was more than twice the concentration measured in the BP juice (0.38 $\text{mg}\cdot\text{mL}^{-1}$). This difference could be related to the significantly lower pH measured in the SP

juice compared to that of the BP juice. Nüsslein et al. (2000) observed increased stability of cichoric acid in *E. purpurea* preparations to which ascorbic acid was added for pH reduction. Furthermore, as seen in the TLC plates in Fig. 3, the relative concentration of the fat-soluble fraction and, more importantly, the major alkylamides at R_f 0.33 was much higher in SP juice than in BP juice. These results suggest that either the SP offers a more efficient extraction of secondary compounds

Fig. 1. Structures of the two major alkylamides found in aerial parts of *E. purpurea*: dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide and dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide.

Table 1. Measured physical parameters of *E. purpurea* juices obtained with a bag press (BP) or a screw press (SP).

Press type	Parameters				
	Soluble solids (%)	Total solids (%)	pH*	Density* ($\text{g}\cdot\text{mL}^{-1}$)	Viscosity* (cps)
BP	9.12	53.64	5.6	1.013	4.59
SP	9.37	53.73	5.4	1.008	4.26

*Parameters showing statistically significant differences ($P < 0.05$) between press types as determined by two-group *t* tests.

Fig. 2. Spectral reflectance profile of expressed *E. purpurea* juices from bag press (BP) and screw press (SP). *Significantly higher ($P = 0.00$) as determined by two-group *t* test ($n = 24$) between BP juice and SP juice.

Fig. 3. TLC plates of CHCl_3 extracted *E. purpurea* juices showing alkylamide profiles for three replicates of products from screw press (SPJ) and products from hydraulic bag-press (BPJ) using a 7 hexane : 3 ethyl acetate mobile phase. Alkylamides appear as purple.

from *E. purpurea* aerial parts or that the juice obtained from the SP is less susceptible to rapid degradation.

Despite that the feed : juice ratio is higher using the BP than the SP (50% vs. 34%), various engineering and processing efficiency considerations support the selection of SP over BP for *Echinacea* juice expression. SP design is more suited for easy cleaning and sanitization. Thorough cleaning of every part of the SP is simple as the press can be completely disassembled. Conversely, the actual bag for the BP requires tedious and time-consuming manual cleaning in order to maintain efficiency. Additionally, SP is more versatile in that the rotational speed can be varied. This provides flexibility with regard to throughput and shear forces. Finally, SP is of a more robust design and our experience has shown that little maintenance is needed to assure its reliability. On the other hand, BP requires that the bag be occasionally replaced during use. These engineering considerations are often related to the peculiarities of the material being pressed and should be interpreted with respect to *Echinacea* herb.

We have observed differences in both chemical and physical properties of the juices expressed with the two different presses. However, the observation of greatest importance

to the therapeutic value of the expressed *Echinacea* juice is that of the greater levels of cichoric acid and alkylamides, both of which are believed to contribute to the bioactivity of the plant. Therefore, based on these findings, SP appears to be a more suitable choice in the expression of *E. purpurea* aerial parts when compared to BP.

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