Effect of handling and storage on alkylamides and cichoric acid in *Echinacea purpurea*

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Abstract: Changes in alkylamide and cichoric acid concentrations during the handling and storage of freshly harvested and dried *Echinacea purpurea* plants were investigated. Plants subjected to varying degrees of physical damage to simulate rough handling were found to show no change in the concentrations of alkylamides and cichoric acid when subsequently dried within 24 h. Storage of undamaged fresh plant material at 20 °C and 60% RH for 30 days also showed no significant loss of either group of constituents. Storage of dried crushed plant material showed that alkylamides were degraded at 20 and 30 °C, especially when held in light, but no loss occurred when stored at 5 °C in the dark. Cichoric acid was found to be stable at 5, 20 and 30 °C provided that the moisture content remained low or enzymic activity was eliminated by blanching. The findings have implications for the handling and storage of echinacea to optimise retention of alkylamides and cichoric acid.
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Keywords: *Echinacea purpurea*; alkylamides; cichoric acid; post-harvest handling; storage

INTRODUCTION

Echinacea purpurea (L) Moench is a traditional North American perennial medicinal herb that has gained popularity internationally in recent years through claims that it beneficially stimulates the human immune system.[1] Extensive qualitative research has established that the chemical composition of *Echinacea* spp is complex, and of the identified constituents, the alkylamides, caffeoyl-phenols and polysaccharides have attracted the most interest in terms of having beneficial pharmacological activity.[2] As medicinal herbs attract greater consumer acceptance, there is increasing pressure to provide a consistent high-quality product that contains expected levels of active constituents.[3] However, surveys of retail products show that the levels of alkylamides and cichoric acid present vary greatly, with many products containing very low levels.[4,5] A survey of dried *E purpurea* grown and marketed in Australia also showed a large range in the levels of both constituents.[6] However, it was not possible in the study to determine whether this variation was due to differences in genetics, growing conditions or post-harvest handling operations.

Echinacea fields are often some distance from a drying facility, and a range of handling operations are inflicted on fresh plants during harvesting and transport from the farm. This handling invariably results in some physical damage to plant cells, and it is generally considered that physical damage of freshly harvested horticultural commodities induces enzymatic and/or chemical reactions that promote water loss and enhance metabolism.[7] In addition, it is logistically difficult to dry a large crop in a relatively short period, as the industrial drying of echinacea is a lengthy process.[8] This often results in freshly harvested material being stored at ambient conditions for some time before being dried. There are no published data on the stability of the active constituents of echinacea during post-harvest handling and storage, hence this study examined the effect of a range of handling conditions and subsequent storage of freshly harvested plant material on the levels of alkylamides and cichoric acid and of changes in dried crushed plant material stored under various environmental conditions. The structures of cichoric acid and one of the abundant alkylamides are shown in Fig 1.

EXPERIMENTAL

Mature *E purpurea* plants were obtained from a commercial farm on the Central Coast of New South Wales, Australia. Alkylamide and cichoric acid levels were determined by HPLC using the methods described by Stuart and Wills,[9] which in summary involved separations that were performed on an RPC_{18} 150 mm × 4.6 mm, 5 μm spherical column (Alltech, Deerfield, USA) fitted with a C_{18} guard column, at 40 °C. The alkylamides examined were detected at 254 nm and are listed in Table 1. The gradient utilised acetonitrile/water at 1 ml min^{-1}, commencing at 40% acetonitrile for 10 min and followed by a linear gradient ramp to 53% acetonitrile at 35 min. Cichoric

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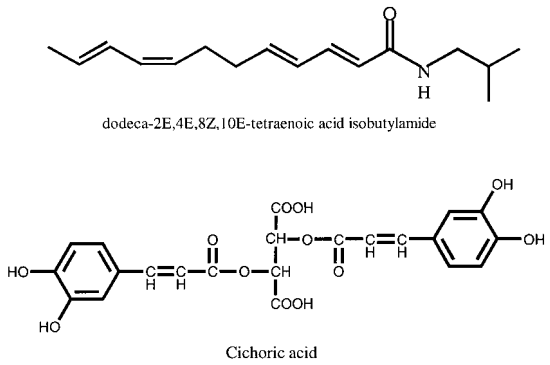

Figure 1. Structures of cichoric acid and the most abundant alkylamide.

acid was detected at 330 nm. The mobile phase was acidified (1% of 0.1 M phosphoric acid) methanol/water at 1 ml min^{-1}, commencing at 10% methanol and followed by a linear gradient ramp to 50% methanol at 20 min.

Physical handling treatments

Immediately after harvest, 60 plants were divided into five groups each consisting of six replications of two plants. Each group of plants was subjected to different handling conditions designed to inflict a range of physical damage. The handling conditions, presented in order of severity, were: undamaged (designated as D0); leaves and stems compressed with a 20 kg weight for 5 s, resulting in bruising to the roots and flowers and compression of the leaves and stems (D1); leaves cut into four pieces, stems cut into 8 cm lengths and roots and flowers cut in half (D2); and leaves crushed by a roller applying 80 kg pressure, stems cut into 1 cm lengths and roots and flowers cut into 1 cm^2 pieces (D3). The treated samples were placed in a hot air dryer (GTD, Sydney, Australia) at 40 °C and dried to a moisture content of <12 g per 100 g fresh weight. A fourth treatment was the same as D3 but with a 24 h delay period at 25 °C before being placed in the drier (D4). The dried plant sections were then crushed to <200 μm particle size in a laboratory mill (Perten, Huddinge, Sweden) and immediately analysed for alkylamides and cichoric acid.

Storage trials

Fresh plants

Freshly harvested plants (40) were subdivided into four groups each containing five replications of two plants. One group was dried at 40 °C for 48 h and analysed for active constituents. The remaining groups were held in air at 20 °C and 60% RH and the moisture loss over time was recorded. After 10, 20 and 30 days storage a group of samples was dried at 40 °C for 24 h before the flowers and roots were analysed for alkylamides and cichoric acid.

Dried plant material

Dried *E purpurea* root and aerial sections and *E angustifolia* root material was obtained from suppliers throughout Australia and crushed to <200 μm. The material was combined to create one *E purpurea* aerial sample and two *E purpurea* and one *E angustifolia* root samples. Subsamples (20 g) from each sample were then placed in a Petri dish and covered with a lid before being placed in four environmental conditions: in the dark at 5 °C and >80% RH; in the dark at 20 °C and 60% RH; in the light at 20 °C and 50–60% RH; and in the dark at 30 °C and <60% RH. Analysis for moisture content, alkylamides and cichoric acid was conducted on each subsample at 0, 10, 20, 30 and 60 days.

Blanched dried roots

A follow-up storage trial was conducted on two fresh *E purpurea* root samples (four roots per sample) that were obtained from a local grower. One group of samples was blanched by submersing in boiling water for 3 min. The blanched and unblanched samples were then dried at 40 °C for 48 h and crushed to <200 μm. Each sample was then divided into two subsamples (20 g) onto a Petri dish with a lid. One subsample was placed in a desiccator in the dark at 5 °C and <20% RH and the other in the dark at 5 °C and >80% RH. Analysis for moisture content and cichoric acid was conducted on each subsample at 0, 10, 20, 30 and 60 days.

RESULTS AND DISCUSSION

Effect of physical damage to active constituents in fresh plants

Concentrations of alkylamides in echinacea root increased as the degree of damage increased (Table 2). It is unlikely that damaging plant tissue would activate the synthesis of alkylamides, hence the increase must

Alkylamide	*% of total alkylamides*
Undeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide	6
Undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide	18
Dodeca-2*E*,4*Z*-diene-8,10-diynoic acid isobutylamide	13
Undeca-2*E*,4*Z*-diene-8,10-diynoic acid-2-methylbutylamide	3
Dodeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide	5
Dodeca-2*E*,4*Z*-diene-8,10-diynoic acid-2-methylbutylamide	4
Dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide	15
Dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide	35

Table 1. Identification and relative proportion of alkylamides in *Echinacea purpurea* root extracts separated by HPLC

Table 2. Concentration of alkylamides and cichoric acid in *Echinacea purpurea* plant sections that had varying levels of damage inflicted on freshly harvested plants

		Concentration ($g\,kg^{-1}$)	
Plant section	*Damage level*[a]	*Alkylamides*	*Cichoric acid*
Root	D0	6.0	22.9
	D1	6.0	18.5
	D2	9.6	20.4
	D3	9.2	18.3
	D4	9.3	20.9
	$LSD_{(P=0.05)}$	±2.8	±5.2
Aerial	D0	0.74	21.0
	D1	0.46	18.4
	D2	0.62	18.7
	D3	0.82	19.3
	D4	0.52	22.0
	$LSD_{(P=0.05)}$	±0.28	±5.9

[a] D0–D3 refer to the levels of damage inflicted on fresh plants, ranging from undamaged (D0) to most damaged (D3) fresh plant material dried 2 h after harvest; D4 is the highest level of damage, with a 24 h delay before drying. Each value is the mean of five replicates.

be due to a reduced loss of alkylamides during drying of damaged plants. With the increased level of tissue damage, drying time was found to be substantially reduced. All material was dried to moisture contents of <12 g per 100 g, which required 48 h for D0 and D1 but only 12 h for D2, D3 and D4. The faster drying time was probably due to the increased exposed surface of cut tissue and easier leakage of moisture from damaged cells. Stuart[10] has found that increased heat exposure during drying enhances the loss of alkylamides, and it is postulated that the decreased heat load due to decreased drying time is causing the reduced loss of alkylamides.

The data in Table 2 also show that inflicting physical damage on fresh echinacea did not result in any significant change in the concentration of cichoric acid in the subsequently dried root or aerial sections. The effect is somewhat unexpected in view of Nublein *et al*[11] reporting that endogenous enzymes of *E purpurea* degrade caffeic acid derivatives and Bauer[12] observing a 20% loss of cichoric acid in flowers within 2 h of harvesting. The effect is further surprising given the appearance of brown discolouration on the plant material indicative that enzymic degradation was occurring. One possible explanation is that the method of damage (clean cutting creating local cross-sectional damage) only inflicted disruption to a small percentage of total cells throughout the plant matrix, and that a grinding or pressing action would produce a higher cell disruption. However, this does not explain the losses observed by Bauer.[12]

Change in active constituents during storage of fresh plants at 20 °C

Changes in the levels of alkylamides, cichoric acid and moisture in freshly harvested echinacea were followed during storage for 30 days at a common ambient condition of 20 °C and 60% RH. The data in Table 3 show that there was no significant change in the concentrations of alkylamides and cichoric acid in root and flower samples over the 30 day storage period. There was, however, a rapid rate of moisture loss, with root and flower samples attaining a moisture content of about 10% after 6 and 14 days respectively (Table 4). Thus the holding of freshly harvested echinacea plants under ambient conditions can result in obtaining a dried plant without loss of active constituents. The ability to hold plants under ambient conditions after harvest to partially or even totally dry the crop while maintaining medicinal quality should have certain attractions to industry, particularly as substantial energy savings are involved. Critical to the successful use of such natural drying is the restriction of mould growth. While no mould growth was observed during storage at 60% RH, the effects of a range of humidity conditions need to be assessed.

Table 3. Concentration of alkylamides and cichoric acid in *Echinacea purpurea* stored for up to 30 days at 20 °C and 60% RH

		Concentration ($g\,kg^{-1}$)	
Plant section	*Storage (days)*	*Alkylamides*	*Cichoric acid*
Root	0	7.0	22.1
	10	8.6	21.4
	20	7.7	18.6
	30	6.6	18.6
	$LSD_{(P=0.05)}$	±2.2	±3.9
Flower	0	1.7	26.1
	10	1.6	30.1
	20	1.0	30.0
	30	1.2	30.0
	$LSD_{(P=0.05)}$	±0.5	±3.8

Each value is the mean of six replicates.

Change in active constituents during storage of dried crushed plants

Changes in the active constituents of dried crushed echinacea were examined during storage in unsealed containers at temperatures from 5 to 30 °C in the absence of light and at 20 °C under constant incandescent lighting. Crushed echinacea exposed to atmospheric conditions was used to simulate maximum rates of loss of active constituents. The data in Fig 2

Table 4. Moisture content of *Echinacea purpurea* stored at 20 °C and 60% RH

	Moisture content ($g\,kg^{-1}$)							
Plant section	*Day 0*	*3*	*6*	*7*	*10*	*14*	*20*	*30*
Root	880	250	190	170	190	150	180	190
Flower	900	460	290	210	190	130	100	100

Each value is the mean of six replicates.

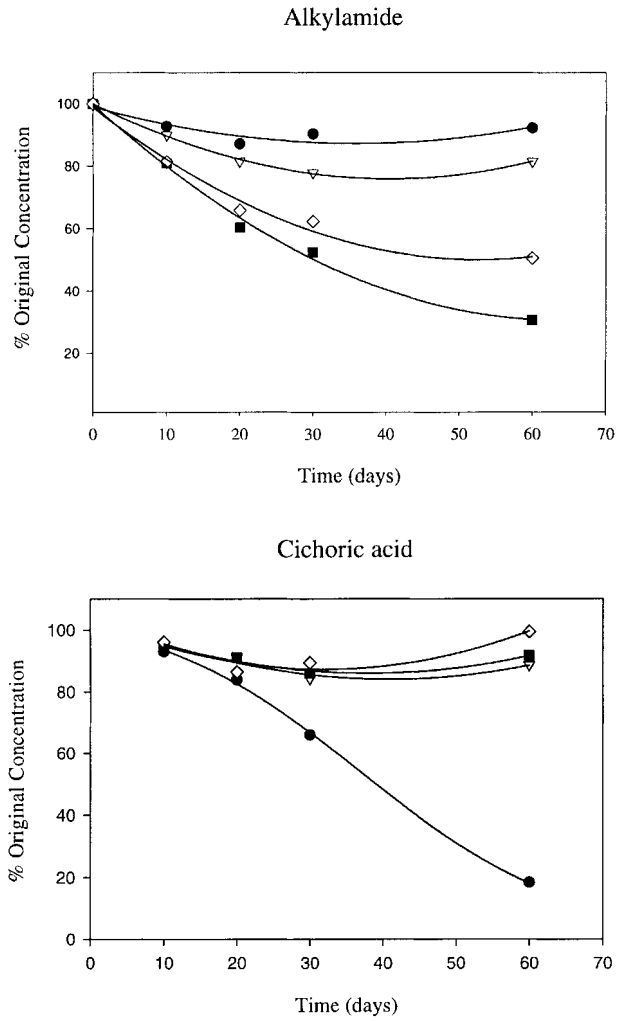

Figure 2. Change in alkylamide and cichoric acid concentrations of dried crushed *Echinacea purpurea* stored under different environmental conditions: ●, 5 °C, dark; ▽, 20 °C, dark; □, 20 °C, light; ◇, 30 °C, dark. Each value is the mean of three replications.

show that storage in the presence of light at 20 °C and in the dark at 30 °C resulted in a significant decrease in alkylamide concentration (the quadratic regression equation for 20 °C was $y = 0.01x^2 - 1.19x + 100$ ($P < 0.01$) and for 30 °C was $y = 0.02x^2 - 1.85x + 100$ ($P < 0.01$)). The findings are in agreement with previous research[13–15] which examined changes at ambient temperature in reduced or atmospheric pressure. However, additional data generated in this study showed that the presence of light strongly promoted the loss of alkylamides ($y = 0.02x^2 - 2.16x + 100$ ($P < 0.01$)) and that storage at 5 °C (dark) resulted in no significant loss of alkylamides over 60 days ($P = 0.05$).

Fig 2 also shows that cichoric acid was stable in all the environmental conditions except at 5 °C, where 80% loss of the original concentration occurred over 60 days as described by a significant sigmoidal regression, $y = 107(1 + e^{-c})^{-1}$, where $c = (x - 37/-14)$ ($P < 0.001$). It was, however, considered unlikely that storage at low temperature would accelerate loss of cichoric acid. Examination of the moisture content

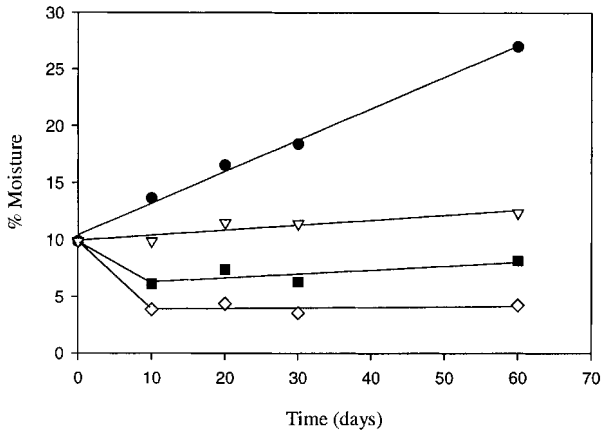

Figure 3. Change in moisture content of dried crushed echinacea stored under different environmental conditions: ●, 5 °C, dark; ▽ 20 °C, dark; ■, 20 °C, light; ◇, 30 °C, dark. Each value is the mean of three replications.

showed a marked increase in samples stored at 5 °C (Fig 3), and a highly significant correlation of moisture content and cichoric acid degradation was determined ($P < 0.001$). It was postulated that the degradation was an enzymic process caused by the increased moisture enhancing enzymic activity, and the degradation pattern therefore following classical sorption isotherms.[16] This was confirmed in a subsequent experiment involving the storage of blanched and unblanched echinacea roots at 5 °C under low- and high-humidity conditions. The data in Fig 4 show considerable degradation of cichoric acid in unblanched samples stored in high humidity where there was considerable uptake of water (rising from 10 g per 100 g to 25 g per 100 g after 60 days storage), and there was little change in any blanched sample or in unblanched samples held at low humidity (water content falling to 5 g per 100 g after 60 days). While the critical water content for inhibition of enzymic activity was not

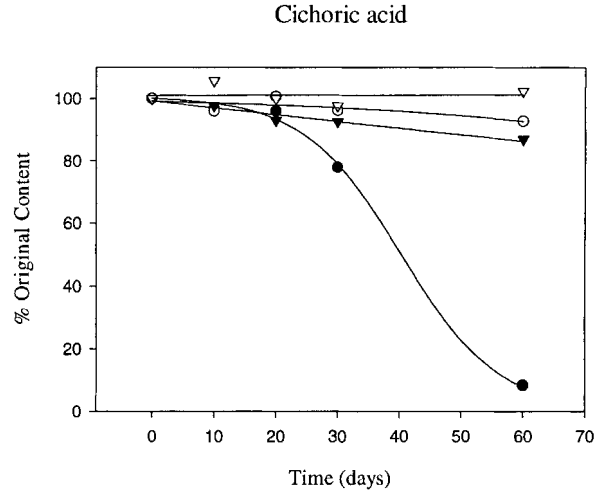

Figure 4. Loss of cichoric acid concentration in blanched and unblanched dried ground echinacea roots stored at 5 °C in dry and humid atmospheres: ▽, unblanched, dry; ○, blanched, dry; ▼, blanched, humid; ●, unblanched, humid.

determined, it is estimated to be of the order of 10–12%.

The simultaneous monitoring of alkylamides and caffeoyl-phenols enabled assessment of whether cichoric acid was providing a protective antioxidant mechanism for the alkylamides at its own expense as suggested by Lugasi *et al.* [17] This was found not to be occurring, as no correlation between cichoric acid concentration and alkylamide degradation was observed.

CONCLUSIONS

The major findings of this study have implications for the handling and storage of echinacea to ensure maximum retention of alkylamides and cichoric acid. It was found that freshly harvested plants subjected to some damage during harvesting show little loss of alkylamides and cichoric acid if plants are dried within 24 h. If drying facilities are not readily available, freshly harvested undamaged plants can be stored at ambient temperatures in air of relatively low humidity with minimal loss of these compounds. Under such conditions, partial or even complete drying can be achieved. The storage environment of dried echinacea has a major effect on retention of active constituents, with optimum retention of alkylamides and cichoric acid occurring during storage in the absence of light at low temperature and in a low-humidity atmosphere.

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