

## Changes in valerenic acids content of valerian root (*Valeriana officinalis* L. s.l.) during long-term storage

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### ABSTRACT

Dried valerian (*Valeriana officinalis*) root powder was stored at 5, 14 and 30 °C under low, moderate and high humidity conditions for 6 months, and the level of the valerenic acids monitored on a monthly basis. From an initial moisture content of 5 g/kg, the moisture level during storage decreased in root powder stored at low humidity and increased during storage at high humidity, with the effect greatest at 30 °C.

The concentration of valerenic acid significantly decreased over time and was affected by temperature and humidity with the loss greatest at 30 °C in low humidity. Acetoxyvalerenic acid also decreased over time but with greatest loss at 30 °C and high humidity. Hydroxyvalerenic acid was not detected in the fresh sample but was present in all stored samples and tended to reflect the decrease in acetoxyvalerenic acid. The concentration of total valerenic acids essentially mirrored the change in valerenic acid.

It is concluded that maximum retention of valerenic acids would be achieved by storing valerian at a temperature lower than about 14 °C with humidity not a great concern. If storage is to occur at higher temperatures, retention of valerenic acids is favoured by storage under a high humidity but such a recommendation is not considered practical due to the risk of microbial growth and increasing difficulty of postharvest handling of moist material.

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### 1. Introduction

The root and rhizome of the valerian plant (*Valeriana officinalis* L. s.l.) is used medicinally for its sedative properties with indications including nervous tension, insomnia, anxiety and stress (Houghton, 1998). The chemical constituents responsible for these effects have not been fully elucidated, but sedative activity has been reported for the valerenic acids (Hendriks, Bos, Allersma, Malingfe, & Koster, 1981) and they are often used as an indicator of medicinal quality. In addition, the valerenic acids can be used to ensure that the correct species is used, as they are unique to *V. officinalis* and several closely-related species (Bos, 1997; Bos et al., 1996; Hänsel & Schulz, 1982). The valerenic acids are sesquiterpenes based on the dual ring valerane structure with the main representatives being valerenic acid ((*E*)-3-[(4*S*,7*R*,7*aR*)-3,7-dimethyl-2,4,5,6,7,7*a*-hexahydro-1*H*-inden-4-yl]-2-methylprop-2-enoic acid; C<sub>15</sub>H<sub>22</sub>O<sub>2</sub>), acetoxyvalerenic acid and hydroxyvalerenic acid (Bos et al., 1996).

After harvest, fresh valerian roots are washed to remove occluding soil and other debris, then usually dried on-farm and chopped into smaller pieces. At this point, they are often subjected to a period of storage, either on-farm awaiting sale, during transport, or

prior to processing by the manufacturer. Traditionally, storage recommendations for valerian have been a closed container protected from light, air and moisture (Chapelle & Denoel, 1972; Upton, 1999). Perry, Burgess, Lorimer, and van Klink (1996) compared the levels of valerenic acid in freshly dried roots (5.8 mg/g) to those reported by Hänsel and Schulz (1982) in commercial samples of valerian root (0.49 mg/g). They suggested that the substantial differences might be due to deterioration during long-term storage.

Acetoxyvalerenic acid is believed to undergo hydrolysis to form hydroxyvalerenic acid when roots are stored under high humidity (Bos, 1997; Hänsel & Schulz, 1985; Schimmer & Röder, 1992) but little else is known of the fate of the valerenic acids under different storage conditions. Thus, long-term storage of dried valerian roots was investigated under different conditions of temperature and humidity.

### 2. Materials and methods

The roots of 24, 12 months old valerian plants cv. 'Anthos' (Richters, Ontario) were washed by hand using high-pressure water from a garden hose and dried in a fan-forced hot air dryer (GTD, Sydney) at 40 °C until commercially dry (moisture content of 50–100 g/kg). The dried root was broken into sections of <1 cm<sup>2</sup> by grinding in an ice-crusher (Catering Equipment, Tuggerah, Australia) then powdered in a laboratory mill (Perten

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Instruments, Huddlinge, Sweden) to a particle size of <250 µm, in order to produce a homogeneous powdered sample. The powdered root material was separated into 18 plastic containers (500 ml) with holes pierced in the lid and two containers were allocated to each of nine treatments. The design of the experiment was orthogonal with three temperatures (5, 14 and 30 °C) and three levels of humidity (designated as low, moderate and high). A low, moderate and high humidity was respectively achieved by placing silica gel (8–13% RH), a saturated salt solution of MgCl<sub>2</sub> (33% RH) and a saturated salt solution of K<sub>2</sub>SO<sub>4</sub> (97% RH) in open glass petri dishes into a large airtight plastic chamber (4 l) into which the sample containers were placed (Lide & Frederikse, 1995). The relative humidity was monitored over the course of the experiment using a humidity meter (MeterMate, Vaisala, Finland).

The material in each sample container was analysed by HPLC for valerenic, acetoxyvalerenic and hydroxyvalerenic acids monthly for 6 months. At each sampling, an accurately weighed sub-sample of approximately 5 g was taken from a sample container and extracted three times by sonication (Ney Dental International, Yucaipa, CA) in methanol (30 ml), with 5 min for each extraction. The extract was filtered through paper (Whatman #1, Maidstone, Kent) into a 100 ml volumetric flask and made up to volume with methanol. Two aliquots (20 µl) were injected into an HPLC using the method of Shohet, Wills, and Stuart (2001). This involved separation on a reversed-phase column (Alltima C18, 250 × 4.6 mm; Alltech, Sydney) with gradient elution of a mobile phase of acetonitrile and aqueous phosphoric acid with peak detection at 225 nm. The mobile phase consisted of two solutions: solution **A**, comprising 20% v/v acetonitrile in water and solution **B** 80% acetonitrile in water, each with the addition of 0.12% v/v 85% phosphoric acid solution. The initial solution comprised 45% **B** for 5 min followed by a linear gradient up to 100% **B** in 24 min. The limit of detection for all compounds was determined as 0.01 µg per 20 µl aliquot which, based on the amounts used in the method, equated to 0.01 mg/g dried root. The moisture content of root powder was determined on samples (1 g) of stored dried material at the time of each HPLC analysis, by drying in a vacuum oven (Thermoline, Sydney) at 60 °C and –70 kPa until constant weight was achieved. All analytical data were corrected for moisture content and expressed on a dry weight basis. The experiment was repeated with another batch of fresh valerian roots. Data from the two experiments were combined and subjected to ANOVA using NCSS (Number Cruncher Statistical Systems, Kaysville, UT). Protected LSD's were calculated in the case of significant *F*-tests at  $\alpha = 0.05$ .

The findings at 30 °C were confirmed with six freshly harvested plants that were dried and crushed as above. A sample was assayed for valerenic acids and the remaining crushed root placed into four plastic petri dishes, with two stored at 30 °C in air of high humidity and the other two in air at low humidity. A duplicate sample from each treatment was assayed for valerenic acids after 3 and 6 months.

### 3. Results

#### 3.1. Moisture content

The moisture content of valerian root was affected by storage under different temperature and humidity conditions (Table 1). From an initial moisture content of 50 g/kg, the samples stored under moderate and high humidity conditions gained moisture whilst the level declined in samples stored under low humidity. Over the 6 months storage period, the average moisture content across the three temperatures for valerian stored under low, moderate and high humidity was 31, 85 and 185 g/kg, respectively. The overall effect of temperature on moisture content was much smaller, ranging from 97 g/kg in valerian stored at 30 °C to 103 g/kg when stored at 14 °C. There was, however, an interaction between humidity and temperature with valerian stored at 30 °C showing the greatest decrease in moisture when stored in low humidity and the greatest increase when stored in high humidity. Thus humidity has a greater impact on moisture absorption and release as the storage temperature increases.

#### 3.2. Valerenic acids

The levels of valerenic acid, acetoxyvalerenic acid, hydroxyvalerenic acid and total valerenic acids in valerian root during storage under different temperature and humidity conditions are given in Table 2 with the average effect of temperature and humidity over the storage period given in Table 3.

##### 3.2.1. Valerenic acid

The initial concentration of valerenic acid was about 1 mg/g of valerian powder but significantly decreased over time (Table 2) and decreased faster at 30 °C than at 5 and 14 °C, and under low humidity compared to high humidity (Table 3). The effect of temperature and humidity, however, was due mainly to storage at 30 °C where there was a decrease in valerenic acid with decreasing

**Table 1**  
Moisture content of valerian root powder stored for 6 months under different humidity and temperature conditions.

Storage time (months)	Moisture content (g/kg)									Mean ( $\pm 1.9^b$ )
	5L <sup>a</sup>	5M	5H	14L	14M	14H	30L	30M	30H	
0	(50 <sup>c</sup> )									
1	45	67	114	38	68	131	23	49	155	<b>76</b>
2	43	73	145	31	74	169	16	54	176	<b>87</b>
3	43	81	173	38	84	186	21	69	201	<b>99</b>
4	44	92	183	33	100	197	16	95	212	<b>108</b>
5	37	101	216	30	112	207	16	92	220	<b>114</b>
6	36	109	223	30	114	220	26	95	206	<b>117</b>
Mean ( $\pm 3.7^b$ )	<b>41</b>	<b>87</b>	<b>175</b>	<b>33</b>	<b>92</b>	<b>185</b>	<b>20</b>	<b>76</b>	<b>195</b>	
<i>Overall means</i>										
Temperature ( $\pm 3.7^b$ )				5	14	30				
				<b>101</b>	<b>103</b>	<b>97</b>				
Humidity ( $\pm 8.3^b$ )				L	M	H				
				<b>31</b>	<b>85</b>	<b>185</b>				

<sup>a</sup> In this and subsequent tables, 5, 14 and 30 refer to the storage temperature (°C), and L, M and H refer to low, moderate and high RH.

<sup>b</sup> Indicates the LSD<sub>(5%)</sub> of the respective mean values.

<sup>c</sup> Values are the mean of two replicate samples of dried valerian powder.

**Table 2**  
Valerenic acid, acetoxyvalerenic acid, hydroxyvalerenic acid and total valerenic acids content of valerian root powder stored for 6 months under different humidity and temperature conditions.

Time (months)	5L	5M	5H	14L	14M	14H	30L	30M	30H	Mean
<i>Valerenic acid concentration (mg/g)</i>										
0	(1.07 <sup>b</sup> )									(±0.05 <sup>a</sup> )
1	1.00	1.02	1.01	1.03	1.08	1.08	0.85	0.92	1.00	<b>1.00</b>
2	1.00	1.00	1.01	1.00	0.99	0.97	0.74	0.86	0.93	<b>0.94</b>
3	0.99	0.99	0.98	0.94	0.92	0.92	0.62	0.81	0.93	<b>0.90</b>
4	0.97	0.97	0.98	0.91	0.92	0.93	0.60	0.81	0.92	<b>0.89</b>
5	0.94	0.96	0.99	0.87	0.87	0.94	0.54	0.80	0.88	<b>0.87</b>
6	0.91	0.96	1.00	0.84	0.89	0.92	0.53	0.75	0.86	<b>0.85</b>
Mean (±0.10 <sup>a</sup> )	<b>0.97</b>	<b>0.98</b>	<b>1.00</b>	<b>0.93</b>	<b>0.94</b>	<b>0.96</b>	<b>0.64</b>	<b>0.82</b>	<b>0.92</b>	
<i>Acetoxyvalerenic acid concentration (mg/g)</i>										
0	(1.09)									(±0.06 <sup>a</sup> )
1	1.03	1.07	1.06	1.06	1.09	1.09	0.80	0.87	0.77	<b>0.98</b>
2	1.02	1.04	1.06	1.03	1.03	0.90	0.66	0.79	0.47	<b>0.89</b>
3	1.00	1.04	1.00	0.95	0.95	0.81	0.57	0.74	0.38	<b>0.83</b>
4	0.97	0.99	0.93	0.90	0.90	0.73	0.53	0.66	0.28	<b>0.77</b>
5	0.92	0.95	0.88	0.85	0.84	0.64	0.46	0.59	0.16	<b>0.70</b>
6	0.90	0.93	0.84	0.84	0.81	0.55	0.45	0.52	0.13	<b>0.66</b>
Mean (±0.10 <sup>a</sup> )	<b>0.97</b>	<b>1.00</b>	<b>0.96</b>	<b>0.94</b>	<b>0.94</b>	<b>0.79</b>	<b>0.58</b>	<b>0.70</b>	<b>0.37</b>	
<i>Hydroxyvalerenic acid concentration (mg/g)</i>										
0	(<0.01)									(±0.03 <sup>a</sup> )
1	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.03	0.18	<b>0.04</b>
2	0.02	0.02	0.03	0.02	0.02	0.08	0.02	0.03	0.38	<b>0.07</b>
3	0.02	0.02	0.03	0.02	0.02	0.12	0.02	0.03	0.46	<b>0.08</b>
4	0.01	0.02	0.06	0.01	0.03	0.18	0.01	0.11	0.54	<b>0.11</b>
5	0.01	0.01	0.11	0.01	0.04	0.26	0.01	0.18	0.58	<b>0.13</b>
6	0.01	0.02	0.14	0.01	0.06	0.34	0.01	0.22	0.60	<b>0.16</b>
Mean (±0.10 <sup>a</sup> )	<b>0.02</b>	<b>0.02</b>	<b>0.07</b>	<b>0.02</b>	<b>0.03</b>	<b>0.17</b>	<b>0.02</b>	<b>0.10</b>	<b>0.46</b>	<b>0.10</b>
<i>Total valerenic acids concentration (mg/g)</i>										
0	(2.14)									(±0.10 <sup>a</sup> )
1	2.05	2.11	2.09	2.10	2.19	2.20	1.67	1.81	1.95	<b>2.02</b>
2	2.03	2.06	2.10	2.05	2.04	1.94	1.42	1.69	1.79	<b>1.90</b>
3	2.01	2.05	2.02	1.91	1.89	1.86	1.21	1.58	1.78	<b>1.81</b>
4	1.95	1.98	1.97	1.83	1.85	1.84	1.14	1.58	1.74	<b>1.77</b>
5	1.88	1.92	2.01	1.74	1.74	1.85	1.01	1.52	1.61	<b>1.70</b>
6	1.81	1.91	1.99	1.69	1.75	1.80	0.99	1.47	1.59	<b>1.67</b>
Mean (±0.13 <sup>a</sup> )	<b>1.96</b>	<b>2.00</b>	<b>2.03</b>	<b>1.89</b>	<b>1.91</b>	<b>1.92</b>	<b>1.24</b>	<b>1.61</b>	<b>1.74</b>	

<sup>a</sup> Indicates the LSD<sub>(5%)</sub> of the respective mean values.

<sup>b</sup> Values are the mean of two replicate samples of dried valerian powder.

humidity. There was no significant effect of humidity on valerenic acid levels when valerian was stored at 5 and 14 °C.

### 3.2.2. Acetoxyvalerenic acid and hydroxyvalerenic acid

Acetoxyvalerenic acid concentrations showed a significant loss over time (Table 2) and the loss increased in root powder stored under increasing humidity and increasing temperature (Table 3). Hydroxyvalerenic acid was not detected in the fresh sample but was present in all stored samples, although, the level was only <0.02 mg/g in all except high humidity treatments and moderate humidity treatments at 14 and 30 °C. The highest level of hydroxyvalerenic acid was 0.60 mg/g after 6 months storage at 30 °C and high humidity.

The 50% loss of acetoxyvalerenic acid under low humidity at 30 °C was similar to valerenic acid but no protection was conferred by high humidity. The greatest loss of 88% therefore occurred at 30 °C in high humidity. The results at high humidity support the belief that acetoxyvalerenic acid hydrolyses to hydroxyvalerenic acid (Bos et al., 1996; Hänsel & Schulz, 1985; Schimmer & Röder, 1992) as the decrease in acetoxyvalerenic acid content was paralleled by an increase in hydroxyvalerenic acid.

### 3.2.3. Total valerenic acids

The concentration of total valerenic acids (the sum of valerenic, acetoxyvalerenic and hydroxyvalerenic acids) decreased significantly over the 6 months storage period (Table 2) as well as with increasing temperature and decreasing humidity (Table 3). The

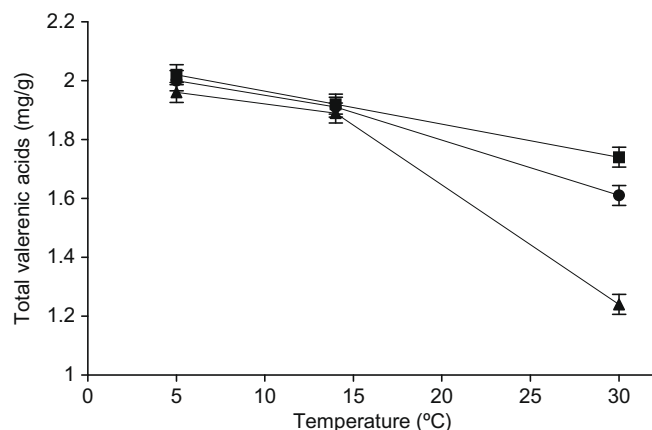
trend effect was similar to valerenic acid, since the changes in acetoxyvalerenic and hydroxyvalerenic acids were counter-related. The significant interaction between temperature and humidity is illustrated in Fig. 1 which shows that whilst there was no significant difference between humidity levels at 5 and 14 °C, at 30 °C the loss of total valerenic acids increased as the humidity decreased. The greatest loss of over 50% was thus at 30 °C in low humidity.

The findings at 30 °C were confirmed in a follow up study of valerian root powder stored at high and low humidity (Table 4). Root material lost moisture during storage under low humidity

**Table 3**

Content of valerenic acid, acetoxyvalerenic acid, hydroxyvalerenic acid and total valerenic acids, averaged over 6 months (*n* = 12), in valerian root powder stored under different humidity and temperature conditions.

Compound	Concentration of valerenic acid (mg/g)							
	Temperature (°C)			LSD <sub>(5%)</sub>	Humidity			LSD <sub>(5%)</sub>
	5	14	30		L	M	H	
Valerenic acid	0.98	0.94	0.79	±0.08	0.85	0.91	0.96	±0.10
Acetoxyvalerenic acid	0.98	0.89	0.55	±0.28	0.83	0.88	0.70	±0.08
Hydroxyvalerenic acid	0.03	0.07	0.19	±0.18	0.01	0.05	0.23	±0.08
Total valerenic acids	2.00	1.90	1.53	±0.19	1.69	1.84	1.90	±0.14



**Fig. 1.** Interaction between temperature and humidity on the concentration of total valerianic acids in powdered valerian root during storage for 6 months at different temperature and humidity.  $\blacktriangle$  = low RH (8–12%),  $\blacklozenge$  = moderate RH (33%) and  $\blacksquare$  = high RH (97%).

**Table 4**

Moisture content and level of valerianic acids in valerian root powder stored for 6 months at 30 °C in high and low humidity.

Treatment	Time (month)	Concentration	
		Low	High
Moisture (g/kg)	0	45 <sup>a</sup>	
	3	10	116
	6	9	120
Valerianic acid (mg/g)	0	1.1	
	3	0.5	1.0
	6	0.4	1.0
Acetoxyvalerianic acid (mg/g)	0	0.7	
	3	0.3	0.2
	6	0.1	0.1
Hydroxyvalerianic acid (mg/g)	0	0.1	
	3	<0.01	0.3
	6	<0.01	0.4
Total valerianic acids (mg/g)	0	1.8	
	3	0.8	1.5
	6	0.5	1.5

<sup>a</sup> Values are the mean of two samples of dried valerian powder.

and gained moisture under high humidity, and root powder stored at low humidity showed a greater loss of valerianic acids than at high humidity. The loss in total valerianic acids during storage in high humidity after 6 months was about 15%, with the change mostly due to loss of acetoxyvalerianic acid, which was only partly replaced by a rise in hydroxyvalerianic acid. At low humidity, the loss in total valerianic acids was about 70% due to an enhanced loss of valerianic acid.

#### 4. Discussion

Thus valerianic acid is susceptible to degradation when stored at low humidity and, when combined with a high temperature of 30 °C, a 50–70% loss occurs over a 6 months storage period. This effect is of some concern as valerian is invariably handled and stored as a dried product and generally temperature control is not prac-

ticed in storage rooms. It was unexpected that the addition of water back into valerian through high humidity air would reduce the loss of valerianic acid, so that the concentration of valerianic acid at 30 °C and high humidity was similar to that at 5 °C. Reducing the moisture content of plant materials is undertaken to reduce metabolism but it seems that moisture uptake allows some system that is protective of valerianic acid to be activated, although, no suggestion is offered as to the nature of this system. The effect might, however, explain why Bos et al. (1996) reported that valerianic acid was stable in tinctures and why Shohet and Wills (2006) found that the valerianic acid concentration did not change significantly in fresh roots stored under ambient conditions for 10 days. In both cases, the appreciable water content may have prevented the loss of valerianic acids. It is not known why water had this effect but further investigation of the mechanism involved in this protection is warranted, as it could lead to development of a method for stabilising valerianic acid when storage at higher temperatures is anticipated.

It is concluded that for maximum retention of valerianic acids valerian root powder should be stored at a low to moderate temperature but determining the optimum temperature requires further storage studies in the range 14–30 °C. Whilst retention of valerianic acids at higher temperatures is favoured by storage under a high humidity, it would not be practical to recommend that dried material be stored at high humidity, firstly as the resultant uptake of water would negate the effort expended in drying the valerian and the moist material would make postharvest handling more difficult. There would also be a much enhanced risk of microbial growth on the moist valerian.

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