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Suitable Drying Temperature for Preserving Cucurbitacins in Fruit of Wild Cucumber and Wild Watermelon

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Summary. **The thermostable cucurbitacin A and B from mature fruit of wild cucumber (*Cucumis myriocarpus*) and wild watermelon (*Cucumis africanus*), respectively, are used in product development for various industries. Mature fruit from wild cucumber and wild watermelon suffer from high incidents of postharvest decays. Drying fruit at the recommended temperatures of 30 to 40 °C for medicinal plants resulted in molds developing on the material, with optimum temperature to prevent decays being at 52 °C. The influence of 52 °C and higher temperatures on active ingredients in the two fruit had not been documented. The objective of this study, therefore, was to determine the relative effects of increasing drying temperatures above the 52 °C standard on concentrations of cucurbitacin A and B in fruit of wild cucumber and wild watermelon. Fruit pieces were oven-dried at 52, 60, 70, 80, 90, and 100 °C for 72 hours. Relative to 52 °C, higher temperatures resulted in 25% to 92% less cucurbitacin compared with the maximum produced at 60 °C. In contrast, relative to 52 °C, higher temperatures reduced concentrations of cucurbitacin B by 47% to 86%. In conclusion, the compromise temperature of 52 °C for preserving fruit pieces in wild cucumber and wild watermelon from decay should also be viewed as the optimum temperature for preserving cucurbitacin A and B.**

Fruit of wild cucumber and wild watermelon are used in medicinal systems, nutrition, pharmaceutical, cosmetic, and pesticidal industries (Lee et al., 2010; Mashela et al., 2011; Thies et al., 2010; Van Wyk and Wink, 2012; Van Wyk et al., 2002). Fruit of wild cucumber and wild watermelon contain cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{46}O_8$), respectively (Chen et al., 2005; Jeffrey, 1978). The two thermostable chemical compounds (Krieger, 2001) are classified as triterpenoids (Chen et al., 2005; Van Wyk and Wink, 2012). Cucurbitacin A, which is soluble in water (Jeffrey, 1978), is unstable and readily oxidises to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$), whereas the insoluble cucurbitacin B is stable (Jeffrey, 1978). Fruit of wild cucumber and wild watermelon are seasonal, but cannot be stored in fresh form due to the high incidents of postharvest decays. Mphahlele et al. (2012) identified the causal agent as the acid-loving fungus *Penicillium simplicissimum*. Usually, fungal decay promotes losses of constituents of the affected organs. Fungi digest food outside its cells by secreting acids and powerful hydrolytic enzymes that decompose complex molecules into simpler compounds that the fungus can absorb and metabolize (Campbell, 1990). Fungal decays have been ameliorated through drying, which had been successfully used to preserve active ingredients in most organs used in various medicinal systems (Danso-Boateng, 2013; Diaz-Maroto et al., 2002; Mudau and Ngezimana, 2014; Rocha et al., 2011).

The recommended drying temperature range for various organs in medicinal plants is 30 to 40 °C (Müller and Heindl, 2006). However, when fruit pieces of the wild cucumber and wild watermelon were dried within the recommended range, most of the cucurbitacin materials were lost to decay, with a blue, bluish-green, or olive green colors, surrounded by white mycelium and a band of water-soaked tissues that characterize *P. simplicissimum* infection. A preliminary optimum drying temperature to prevent growth of mycelia and, therefore, subsequent decay, was established at 52 °C (Mashela,

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1	micron(s)	µm	1
1	mmho/cm	$dS \cdot m^{-1}$	1
0.1333	mm Hg	kPa	7.5006
28.3495	oz	g	0.0353
1	ppm	$\mu g \cdot mL^{-1}$	1
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

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2002). However, there was no information on the impact of this and higher temperatures on cucurbitacin A or B concentrations in fruit of wild cucumber and wild watermelon. The objective of this study was to determine the relative effects of increasing drying temperatures above 52 °C on concentrations of cucurbitacin A and B in fruit of wild cucumber and wild watermelon.

Materials and methods

Study site and raising wild cucumber and wild watermelon. The study was conducted at the Green Technologies Research Center, University of Limpopo, South Africa (lat. 23°53′10″S, long. 29°44′15″E). Soil at the site comprised Hutton sandy loam (65% sand, 30% clay, 5% silt) containing 1.6% organic carbon, with electrical conductivity of 0.148 $dS \cdot m^{-1}$ and pH of 6.5. The hot and dry summers usually have day maximum temperatures ranging from 28 to 38 °C, with mean annual rainfall below 500 mm. Seedlings of wild cucumber and wild watermelon were raised in adjacent separate fields, containing five plots (1 × 1 m), each plot with four plants. One experiment evaluated cucurbitacin A from fruit of wild cucumber, whereas the second experiment only evaluated cucurbitacin B from wild watermelon fruit. Each plant was fertilized once using 3 g of 6.3N–9.4P–6.3K fertilizer (Omnia, Bryanston, South Africa) and plots irrigated weekly using sprinklers.

Experimental design and treatments. Sixty fruit from each plot were harvested at fruit maturity (Shadung et al., 2015), chopped into pieces, and divided equally into six portions. Each portion per plot was randomly assigned to one of the six forced-air drying ovens (EcoTherm; Labotech, Cape Town, South Africa). The drying treatment ovens were set at 52, 60, 70, 80, 90, or 100 °C and arranged in a complete randomized design, with five replications. Each drying treatment ran for 72 h and afterward the samples were ground in a Wiley mill to pass through a 1-mm sieve. Before extraction, samples were stored in hermetically sealed plastic bottles at room temperature.

Extraction and cucurbitacins quantification. A representative subsample of 4 g dried crude extracts of fruit from each treatment were extracted in closed conical flasks containing 100 mL methanol and dichloromethane at 1:1 (v/v) solution inside a rotary evaporator (Rotavapor model R-205; Buchi Labortechnik, Essen, Germany) set at 60 rpm at 40 °C for 4 h. After extraction, subsamples were concentrated by reducing the volume to 30 mL under reduced pressure on a rotary evaporator and then 1 mL aliquot centrifuged at 2422 g_n for 10 min before filtering through 0.22-µm filter (Miller; Sigma-Aldrich, Johannesburg, South Africa). Concentrations of cucurbitacin were quantified using the isocratic elution high-performance liquid chromatography (Prominence model LC-10 AD VP; Shimadzu, Kyoto, Japan) with detection using a diode array detector (CTO-20A; Shimadzu). Quantification was performed in a wide pore reverse phase C18 (25 cm × 4.0 mm, 5 µm) column (Sigma-Aldrich, Milan, Italy) using methanol and deionized water at 2:3 (v/v) solution that served as a mobile phase at a flow rate of 1.0 $mL \cdot min^{-1}$ in an oven at 35 °C, with wavelengths monitored at 230 nm for 43 min. Quantification of cucurbitacin A and B was accomplished by comparing the retention times and peak areas of subsamples to those of pure (about 98%) cucurbitacin A and B standards (Wuhan ChemFaces Biochemical Co., Wuhan, China). Standards were dissolved in methanol and prepared in serial dilutions of 0.02, 0.04, 0.06, 0.08, and 1.0 $\mu g \cdot mL^{-1}$.

Data analysis. Cucurbitacin A and B data were subjected to analysis of variance procedure using SAS software (version 9.2; SAS Institute, Carry, NC). When treatments were significant, the sums of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) in concentration of cucurbitacins. Mean separation was achieved using Waller–Duncan multiple range test. Unless otherwise stated, only treatment means significant at the probability level of 5% were discussed.

Results

Treatment effects. Increasing oven-drying temperatures had highly significant ($P \leq 0.01$) effects on concentrations of cucurbitacin A and B (Table 1). Increasing temperatures contributed 65% and 71% in TTV of cucurbitacin A and B concentrations, respectively.

Relative impact. The highest concentration of cucurbitacin A occurred in fruit dried at 60 °C. The higher temperatures reduced cucurbitacin A by 25% to 92%. In contrast, temperatures above 52 °C reduced cucurbitacin B by 28% to 86%.

Generated models. A quadratic relationship was observed between cucurbitacin A and B concentrations and drying temperature with an R^2 of 0.94 and 0.95, respectively (Fig. 1).

Discussion

Concentrations of cucurbitacin A and B triterpenoids (Chen et al., 2005) were inversely related to drying temperature. Others (Du et al., 2003; Hwang et al., 2014) observed that concentrations of ginsenoside (another triterpenoids) from ginseng roots decreased when dried at 40, 55, or 70 °C. Similar findings were noted with the pyrethrins—the monoterpenoids (Morris et al., 2006). Rosmarinic acid and sinenselin (phenolic compounds) from misai kucing (*Orthosiphon staminiues*), increased with increasing temperature below 40 °C, but decreased when dried at 40, 55, or 70 °C (Abdullah et al., 2011). Drying bush tea (*Athrixia phylicoides*) between 45 and 65 °C reduced total phenolic content when compared with freeze- and shade-drying (Mudau and Ngezimana, 2014).

The reduction of chemical compounds with increasing drying temperature has been attributed to the accelerated degradation of the compounds (Phillips et al., 1960), which depends on the chemical bonds within the chemical compounds (Phillips et al., 1960). In essential oils, for example, drying temperature for oregano (*Origanum vulgare* ssp. *hirtum*) was optimized at 40 °C for 72 h (Novák et al., 2011), whereas at higher temperatures most essential oils were volatilized (Faridah et al., 2010; Radünz et al., 2003). Cucurbitacins are thermostable, with boiling temperatures of cucurbitacin A and B being at 731 and 699 °C, respectively, at 760 mm Hg (Krieger, 2001). The decrease in cucurbitacin with increasing temperature agreed with observations in other chemical compounds such as ginsenosides and pyrethrins

Table 1. Responses of sum of squares (SS) for cucurbitacin A and B concentrations from fruit of wild cucumber and wild watermelon, respectively, to different oven-drying temperatures (n = 30).

		Cucurbitacin A		Cucurbitacin B	
Source	df	SS	%	SS	%
Treatment	5	79.118	65**	141.362	71**
Error	24	42.039	35	58.368	29
Total	29	121.157	100	198.729	100

**Treatment effects were significant at $P \leq 0.01$.

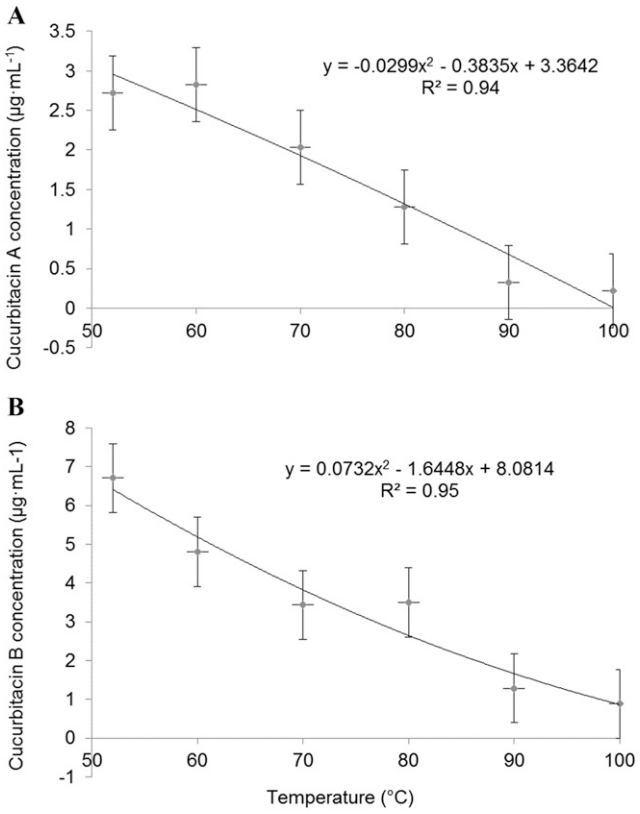

Fig. 1. Relationship between cucurbitacin A and B concentrations from fruit of wild cucumber and wild watermelon, respectively, over increasing drying temperatures at 72-h exposure time; (1.8 × °C) + 32 = °F, 1 µg·mL^{-1} = 1 ppm.

(Du et al., 2003; Hwang et al., 2014; Morris et al., 2006).

Drying fruit from wild cucumber and wild watermelon at 52 °C should be viewed as a compromise temperature for preserving cucurbitacins from *P. simplicissimum* postharvest decay losses. Optimizing temperature for the retention of cucurbitacins appeared to be above 52 °C for cucurbitacin A and below 52 °C for cucurbitacin B, where fruit are sensitive to decay. However, at 52 °C it is still necessary to establish the suitable exposure period since the drying periods are inversely proportional to the drying temperatures (Barbieri et al., 2004; Gregory et al., 2005; Hallström and Wimmerstedt, 1983). The proposed optimization of the drying period at 52 °C could reduce potential losses through degradation and volatilization.

The cucurbitacin concentrations vs. increasing drying temperatures had density-dependent growth (DDG) patterns (Liu et al., 2003; Salisbury and Ross, 1992). Apparently, should drying temperatures start from 25 to 100 °C, cucurbitacin concentrations would go through the three stages of DDG patterns, namely, stimulation, neutral, and inhibition (Mashela et al., 2015). In our study and those of others (Abdullah et al., 2011; Du et al., 2003; Morris et al., 2006), inhibition ranges were exhibited since the drying temperatures were already above the optimization temperatures for the variables. At temperature from 40 to 70 °C, Abdullah et al. (2011) observed that the phenolic compounds tested increased with increasing temperatures, which was a reflection of the stimulation stage (Liu et al., 2003).

Conclusion

The drying temperature for fruit of wild cucumber and wild watermelon should be retained at 52 °C as a compromise against decay at lower drying temperatures. However, it would be necessary to optimize the exposure period for drying fruit of wild cucumber and wild watermelon to ensure optimum retention of cucurbitacin A and B.

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