Journal of Food Composition and Analysis 59 (2017) 27–34

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original research article

Colorimetric assessment of kava (*Piper methysticum* Forst.) quality

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ARTICLE INFO

Article history:
Received 5 December 2016
Received in revised form 6 February 2017
Accepted 8 February 2017
Available online 10 February 2017

Keywords:
Chemotype
Kava
Flavokavain
Food analysis
Food composition
Food quality assessment
Kavalactone
Piper methysticum

ABSTRACT

The present study aimed at evaluating the potential of diethyl ether extracts UV/visible (UV/vis) absorbance for assessing the suitability of commercial lots of kava (*Piper methysticum*). The UV/vis absorption spectra of diethyl ether root extracts of 15 cultivars clustered them into three groups in parallel to their known genetic relatedness and their chemical composition determined by GC–MS and LC–MS analyses. Absorption peaks at 250 nm and 290 nm respectively corresponded to kavain, the most health-promoting kavalactone, and dihydromethysticin a non-desirable kavalactone. The absorbance peak at 340–350 nm reflected the yellow coloration of the extract, which was mainly due to the undesirable flavokavins, desmethoxyyangonin and yangonin. Ratios of absorbance values at 250 nm and 290 nm significantly differentiated all three groups of cultivars, namely 'noble' which provide health benefits from 'two-day' and 'wichmannii' that are health damaging. These results provide a robust and rapid colorimetric test for routine control of a critical aspect of the quality of kava batches.

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1. Introduction

Kava (*Piper methysticum* Forst.), a member of the piper family Piperaceae, is a small shrub that is endemic to South Pacific islands and Papua New Guinea. Cold water extraction of its underground organs generates the traditional beverage of Pacific island countries used for ceremonial and recreational purposes (Lebot et al., 1997). Over the past century, kava beverage has become popular worldwide and now represents one of the major cash crops in Fiji, Pohnpei, Samoa, Tonga, and Vanuatu. Upon ingestion, it promotes relaxation and a sense of well-being without compromising cognitive capacities and triggering addictive behaviors (Sarris et al., 2011). It is also claimed to bear medicinal properties, such as attenuation of menopausal symptoms, neuroprotection, anti-bacterial, anti-mycotic, anti-epileptic, spasmolytic, analgesic, locally anesthetic diuretic and soporific (Showman et al., 2015) and crop protective effects (Xuan et al., 2003). In sharp contrast, several cases of hepatotoxicity have been reported and prompted European authorities to temporarily ban kava imports (BfArM, 2003; Bilia et al., 2002; Clough et al., 2003). Reasons for these health problems stem from increased demand, which may have prompted traders to sell extracts from non-traditionally-used and inappropriate plant parts or varieties. Stem peelings and leaves, indeed, specifically accumulate pipermethysticin, a toxic alkaloid (Dragull et al., 2003; Jhoo et al., 2006).

The species *P. methysticum* also appeared to include three genetically and chemically distinct groups (Lebot et al., 1991; VandenBroucke et al., 2015). The most ancient one called 'wichmannii' comprises wild ancestors which are currently only found in Papua New Guinea, the Solomon Islands and Vanuatu and from which a group of cultivars collectively called 'two-day' was derived. This latter group of cultivars served as a reservoir for the selection of a third group of cultivars called 'noble' which are currently only multiplied vegetatively because of their sterility. Diversification among the 'noble' cultivars arose *via* the section of somaclonal variants (Lebot and Lévesque, 1989). According to local tradition, only these later cultivars are suitable for daily consumption. Though they may differ slightly in their phytochemical content because of genetic, ontogenic and cultivation differences (Wang et al., 2013; WHO (World Health Organization),

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http://dx.doi.org/10.1016/j.jfca.2017.02.005
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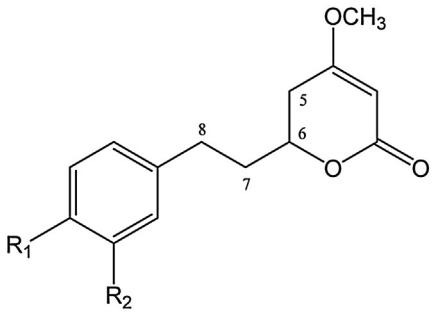
2007), they exhibit great chemical differences from two-day and wichmannii cultivars by having a much higher proportion of kavalactone (KL) kavain (K) and a near complete absence of a group of chalcones called flavokavains (FKs) (Fig. 1) (Lebot et al., 2014). This agrees with the suspected hepatotoxicity of FKs, and especially FKB, the major FK (DiSilvestro et al., 2007; Zhou et al., 2010). Though all three groups of kava cultivars can be distinguished by the morphology of their aerial parts, the slighter differences between two-day and noble cultivars only supports a separation of the species into two varieties, *P. methysticum* var. *methysticum* (noble and two-day) and *P. methysticum* var. *wichmannii* (Applequist and Lebot, 2006). This, and the fact that traded plant material consists of powdered, dry underground organs, make attribution of commercially sold material to a specific cultivar, or even a group of cultivars, impossible. This uncertainty and misidentification may have been the cause of the reported liver problems (Kuchta et al., 2015; Martin et al., 2014; Teschke et al., 2011).

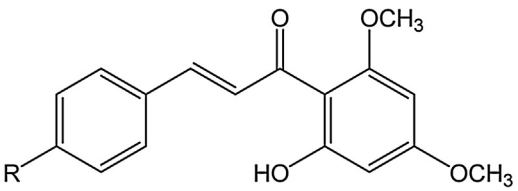
In an effort to support their kava industry, Pacific island countries are aiming to set up quality standards (FAO/WHO, 2013; IKEC, 2004; SPC, 2001; Teschke et al., 2011; Vanuatu Legislation, 2002). A variety of sturdy and reliable HPLC- and GC-based protocols have been developed (Bilia et al., 2004; Ganzera and Khan, 1999; Gaub et al., 2004a) for the quantitative analysis of the six major KLs (methysticin M, dihydromethysticin DHM, kavain K, 7,8-dihydrokavain DHK, desmethoxyyangonin DMY, yangonin Y) and the two major FKs (FKA, FKB) (Fig. 1). Detection is made through diode array and mass spectrometer detectors because these substances display characteristic UV/visible (UV/vis) absorption spectra and masses (Meissmer and Häberlein, 2005). The nature of the extraction solvent has some importance, as the highly non-polar solvent, hexane, and the very polar solvent, water, both have lower extraction capacities in agreement with the medium polarity of KLs and FKs. A comparison of the medium polarity solvents methanol, ethanol, dichloromethane, chloroform and acetone revealed only minor differences in extraction capacity (Xuan et al., 2008). The recent development of HP-TLC (Lebot and Legendre, 2014) and NIRS-based analytical protocols (Gaub et al., 2004b; Gautz et al., 2006; Wang et al., 2010) have respectively reduced solvent volumes and analytical time while still being able to distinguish the three major kava chemical groups. They nevertheless require equipment that is too costly for most of the Pacific island countries that produce kava. These methods are also too time-consuming for surprise inspections by control officers. For this reason, direct UV absorbance measurements have been applied to acetone extracts of kava root powder (Lebot and Legendre, 2016). Through the analysis of more than 200 plants, a positive correlation was found between the absorbance at 400 nm and total FKs content, in agreement with the fact that FKs and the KLs Y and DMY are

A



Kavalactone (KL)	R1	R2	C5-C6	C7-C8
Methysticin (M)	OCH_2O			=
Dihydromethysticin (DHM)	OCH_2O			
Kavain (K)				=
7,8-Dihydrokavain (DHK)				
Desmethoxyyangonin (DMY)			=	=
Yangonin (Y)	OCH_3		=	=

B



Flavokavain (FK)	R
Flavokavin A (FKA)	OCH_3
Flavokavin B (FKB)	

Fig. 1. Major constituents of kava. The structures of the 6 major kavalactones (A) and flavokavains (B) of kava roots are shown. Abbreviations used in the text are placed in parenthesis after each name.

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