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Development of standard fingerprints of naked oats using chromatography combined with principal component analysis and cluster analysis

XiGuang Qi, Ling Zhu, Chaoqun Wang, Hui Zhang*, Li Wang, Haifeng Qian

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi, 214122, China

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ABSTRACT

Oat samples of different varieties were collected from various habitats for the determination of avenacoside, β-glucan and fatty contents. The variation coefficients of the three components were 12.13%, 20.79% and 22.46%, respectively. Thus, those three indicators cannot represent the information of all samples, and are not suitable for evaluating the quality of oat raw materials and products. Fatty acid profiles were analyzed using gas chromatography combined with principal component analysis (PCA) and cluster analysis. Fourteen leather oat varieties were distinguished through a PCA scores scatterplot. Forty-six naked oat varieties were selected by cluster analysis and eleven characteristic peaks in these naked oats were identified. Finally, accurate fatty acid standard fingerprints of naked oats were constructed. The results of methodological indicated high precision, reproducibility and stability, in line with fingerprint testing requirements. This study fills the knowledge gap for naked oat fingerprint information, expands the grain fatty acid database, and lays the foundations of a grain nutritional liposome identification technology system.

1. Introduction

Oats are widely planted, rich in variety and rank as the sixth most important cereal crop of the world (Arendt and Zannini, 2013). Particular chemical composition of oats (including a larger amount of soluble fiber, essential acids, total protein, unsaturated fatty acid, minerals, vitamins) provide nutritive and health effects (e.g. β-glucan has ability to reduce total blood cholesterol and increase viscosity in the GI tract, has hypocholesterolaemic and anticancer properties) (Biel et al., 2009). Oats have value in medical, health protection and cosmetic applications (Lin et al., 2011; Runyon et al., 2015). Saponins are extensively distributed in plants (including Leguminosae, Rosaceae, Cucurbitaceae and Amaranthaceae, ginseng, capsicum peppers, tea, yam and oats) and lower marine animals (such as sea cucumbers and starfish) (Pecio et al., 2013). As the only saponin containing cereal, oats have been used in many functional food or non-food applications. The main saponins in oats are avenacosides detected in the grain (Onning and Asp, 1995). Furthermore, oats are a natural functional food (Givens et al., 2004). Oats flour plays an important role in breakfast cereals, biscuits, infant food, cookies and flour blends. The flour products contain oat flour as an ingredient, and the most common ones are oat-wheat flours (Holguín-Acuña et al., 2008; Ryan et al., 2011). The addition of oats brings more nutritious components to the food (Wang et al., 2007). Although widely used, there are also some major problems associated with oats. Driven by economic interests, eventually leading to food adulteration problems in the market, especially adulteration of raw materials (Alvarez et al., 2013). Generally, the addition of low cost cereal flours, or only adding a small amount of oats to oat products are common commercial fraud problems in food manufacturing (Alary et al., 2007). These food adulteration issues not only affect health and economic interests, but also have negative influences on the whole food industry chain (Rai et al., 2014). However, researches on detecting the adulteration in flour products and monitoring of the flour production process are very rare (Wang et al., 2014). Therefore, it is urgency to establish a standard for quality control of cereal products. In the long term, it is a large oversight in cereal food quality control management.

Generally, methods of detecting food adulteration have relied on targeted analysis. In other words, the amount or types of

* Corresponding author.
E-mail address: zhanghui@jiangnan.edu.cn (H. Zhang).

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adulterated component are known and the components of authentic product are relative simple. However, some adulterations continue to be ignored. In such cases, fingerprinting techniques bywhich multiple compounds can be determined represent a promise approach and =considered to be potential discriminators (Jahandideh et al., 2016). Many chromatographic fingerprints of herbal medicines, oil crops and oil products have been widely accepted and applied. Fan et al. reported the quality control of herbal medicines by multiple chromatographic fingerprinting (Fan et al., 2006). Tian et al. developed the fingerprint of white apricot almond oil by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) (Tian et al., 2014). Tres et al. aimed to evaluate and develop a method to verify the organic identity of feed by fatty acid fingerprinting and chemometric classification techniques (Tres and van Ruth, 2011).

This study aims to develop a standard fingerprint of oats by GC combined with cluster analysis and principal component analysis (PCA). Experiments were undertaken to compare the chemical composition (β-glucan, saponin, fatty acid content and composition) of multiple oat samples of different varieties and from different habitats.

2. Materials and methods

2.1. Materials

2.1.1. Oat cultivars

Sixty-two samples of oats grains come from different producing areas were collected as widely as possible from 2014 harvests. Those samples were collected from major areas in China, which include Hebei (No. 1–15), Shanxi (No. 16–27), Gansu (No. 28–42), Qinghai (No. 43–50), Xinjiang (No. 51, 52), Yunnan (No. 53, 54), Jilin (No. 55–57), Ningxia (No. 58, 59), Neimeng (No. 60, 61)as well as Sichuan (No. 62). Most samples were naked oats, while no. 1–7, 15, 17, 18, 45, 47–49 were hulled oats with an asterisk (*) mark. Put all collected grains into bags exhausted the air. Then, grains were sealed and stored at −18 °C for chemical composition test.

2.1.2. Samples preparation

All naked oats grains were cleaned and milled using a TM05C laboratory mill (Japan zuozhu Machinery Co. Ltd), whereas hulled grains need a pretreatment (JLGJ2.5 laboratory huller) before milling. The obtained flour was sieved through 40-mesh. After determining moisture contents, flour samples were sealed into bags and stored in a −18 °C fridge until analyzed.

2.1.3. Chemicals

Analytical and chromatographic-grade chemicals were used for the analysis of β-glucan, avenacoside, oil and fatty acids. Standards (ginsenoside, 19 alkyl acid and Supelco 37 Component FAME mix) were purchased form Sigma-Aldrich. β-glucan kits were purchased from Nanjing Built Biological Science and Technology Co., Ltd.

2.2. Methods

2.2.1. Chemical composition

Avenacosides content in oats was determined by colorimetry with Ginseng saponins as standard sample (T6-UV spectrophotometer) (Cao et al., 2014). The standard curve was y = 4.4959x-0.0868 (R^2 = 0.9991) and the method was as follows: 0.1 mg/mL standard solution was prepared with anhydrous methanol. Then, 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mL standard solutions were transferred into test tubes and blew with nitrogen for 30min. 0.2 mL of 5% vanillin acetic acid solution and 0.8 mL perchloric acid were added into standard solutions. Sealed tubes were heated at 60 °C for 15min and cooled with ice water. Then, 5 mL acetic acid was added, placing at room temperature for 10min. The standard curve was drawn with the absorption values at 534 nm.

Oats flour samples were defatted by Hexane at 40 °C for 8 h with a ratio of 1:8 (w/v). Then, 2 g of defatted oat flour was weighed and extracted with 80% ethanol (1:12, w/v) for 1 h at 60 °C, following under ultrasonic irradiation for 30min and were extracted for 1 h. Repeated ultrasonic for 30min, extraction for 1.5 h and then samples were centrifuged at 3000r/min for 10min, using the supernatant. 1 mL of supernatant was dried at 70 °C water bath. 2 mL water and 2 mL n-butanol saturated solution were mixed, under ultrasonic for 2min. Finally, samples were centrifuged for 30 min at 3000r/min, reserving supernatant. The absorption values were determined according to the standard curve method, and the avenacosides contents in oats were observed.

The total β-glucan content was determined according to the streamlined enzymatic method (AOAC995.16, 2000). For each sample, 0.0800–0.1000 g oat flour samples were accurately weighed into test tubes. Then, 0.2 mL 50% ethanol was added and 4.0 mL phosphate buffer was mixed. Immediately, tubes were placed in boiling water bath and incubated 1min. After removing from water bath, tubes were mixed vigorously for seconds and transferred to boiling water bath for another 2min and shaken. On the following, tubes were incubated at 50 °C for 5min, 0.2 mL lichenase solution was added and mixed, another 60 min at 50 °C. During incubation, tubes were stirred for 3 to 4 times. Subsequently, tubes were removed, cooled to room temperature and centrifuged at 1000 g for 10min. 0.1 mL of each supernatant was accurately transferred to 3 tubes. 2 tubes were treated with β-glucosidase (0.1 mL) and the other one was added acetate buffer as blank value (0.1 mL, 50 mmol/L). 3.0 mL glucose oxidase-peroxidase-buffer mixture was added to each tube and incubated at 50 °C for 20min. Finally, β-glucan content was calculated according to the absorption values at 510 nm.

The total fat content was determined by Soxhlet method (AOAC930.39C, 2000).

All above determinations were acquired on a dry matter basis.

2.2.2. Fatty acid analysis

The preparation of fatty acid methyl esters for gas chromatographic analysis was rapidly accomplished by extracted fat. 2 mL of methanol sodium hydroxide was added to 20 mg of the extracted fat. All samples were immersed in water bath at 65 °C with magnetic stirring until they completely melted. Then 2 ml of BF3-CH3OH (25%) was added and the samples were immersed in water bath at 60 °C for a further 20min. Then 2 mL of hexane and 2 mL of saturated sodium chloride were added and the samples were centrifuged for 15 min at 3000 rpm. Each sample was stored at −4 °C for GC use (AOCS, 2005).

The fatty acid profiles were determined for each sample by the AOCS official method Ce 1b-89 with modification. Injections were performed in a silica capillary column (0.32 mm internal diameter, 30 m long and 0.25 μm thick). High purity nitrogen was used as carrier gas of 3 mL/min and a split ratio of 100:1. The injection port temperature was 250 °C and initially temperature was set at 80 °C for 3min and then increase to 215 °C at a rate of 15 °C/min. Fatty acids were identified using 37 fatty acid methyl esters (Sigma reference standards) and quantified using an internal standard (pentadecanoic acid, Sigma), according to the AOCS method (AOCS, 2005).

2.2.3. Methods validation

The precision degree of the GC method was determined by testing one fatty acid methyl ester sample six times during the day. Repeatability was determined by testing the same six oat samples