Aroma profile and proximate composition of Roselle seeds: Effects of different origins and different sample preparation methods

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Abstract

The influence of different origin on proximate composition of Roselle seeds and different sample preparation methods on the aroma profiles of Roselle seeds were studied. It was seen that sample origin affected the proximate composition and volatile profiles. Ground dry (GD) was chosen as the preparation method because it was an efficient method with less chemical changes of the samples whereas, Roselle seeds of Malaysian origin was selected as potential food ingredient because it has high lipid, protein, and total dietary fiber content.

Introduction

Roselle (*Hibiscus sabdariffa* L.) is an important food and medicinal plant, among other things due to its high content of antioxidants, for example anthocyanins and vitamin C. It is also used as a natural food colorant. Normally, in food industry only Roselle calyces are used to produce various food products; the seeds are removed and disposed as a by-product. However, Roselle seeds are also edible [1]. To our knowledge, the study of Roselle seeds is limited and there are no aroma profiles of Roselle seeds being reported. Therefore, this study addresses the influence of different origin on proximate composition of Roselle seeds and tests different sample preparation methods to determine the aroma profiles of Roselle seeds.

Experimental

Materials

Two types of sun dried Roselle (*Hibiscus sabdariffa* L.) seeds commercially available were obtained to study aroma profiles and proximate analysis: 1) Roselle seeds of the UMKL cultivar (obtained from HERBagus Sdn. Bhd., Penang, Malaysia) 2) Roselle seeds of Chinese origin (obtained from Sichuan Keren Imp & Exp Trading Co. Ltd, Sichuan, China).

Sample preparation

For aroma analysis, samples were prepared by two different procedures and analyzed in triplicate: Ground, dry (GD): Whole Roselle seeds were ground for 90 sec using a laboratory blender Model 38BL41 (Waring, USA). Internal standard (1 mL of a 5 ppm 4-methyl-1-pentanol solution) was added to 25 g of Roselle seeds and volatiles were sampled by Dynamic Headspace Sampling (DHS).

Ground, mixed with water (GMW): Whole Roselle seeds were ground as mentioned above and then 25 g of ground Roselle seeds were mixed with 100 mL of tap water, ratio (1:4). Again, 1 mL of a 5 ppm 4-methyl-1-pentanol solution was added and DHS was carried out.

B. Siegmund & E. Leitner (Eds): Flavour Sci., 2018, Verlag der Technischen Universität Graz
DOI: 10.3217/978-3-85125-593-5-22,

Dynamic Headspace Sampling (DHS) and Gas Chromatography-Mass Spectrometry (GC-MS)

The DHS method was adopted and modified from Starr et al. [2]. Each sample was placed in a 500 mL glass flask. A trap containing Tenax-TA (200 mg) was attached to the sealed flask. The flasks containing the samples were immersed in a water bath held at 40 °C. Under magnetic stirring (200 rpm), the sample was tempered for 10 min and then purged with nitrogen (100 mL min^{-1}) for 40 min. The traps were purged with a flow of nitrogen (100 mL min^{-1}) for an extra 10 min to remove water.

In GC-MS analysis, the collected volatiles were determined as previously described by Starr et al. [2]. Volatile compounds were identified by probability based matching of their spectra with those of a commercial database (Wiley275.L, HP product no. G1035A). The software program, MSDChemstation (Version E.02.00, Agilent Technologies, Palo Alto, California), was used for data analysis. Amounts are presented as peak areas. Volatile compound identification was confirmed by comparison with retention indices (RI) of authentic reference compounds or retention indices reported in the literature.

Proximate composition

In proximate analysis, samples were treated according to the AOAC standard methods [3]. Moisture content (hot-air oven method), ash (dry ashing method), lipid (Soxhlet extraction), protein (Micro-Kjeldahl method) and total dietary fiber [4] were analyzed and calculated. All measurements were conducted in triplicate. The results were expressed as a percentage (wet weight).

Data analysis

Multivariate data analysis (principal component analysis (PCA)) using the Latentix software (LatentiXTM 2.0 Latent5, Copenhagen, Denmark, www.latentix.com) was applied to GC-MS data to evaluate the variation between the different samples from different countries and one-way analysis of variance (ANOVA) was performed using the software JMP (version 12.0, SAS Institute Inc.) to test for differences in proximate composition.

Results and discussion

A total of 61 volatile compounds were identified including alcohols (18), terpenes (15), aldehydes (13), ketones (9), furans (2), phenols (2), ester (1), and lactone (1). An equal number of aroma compounds was recovered in GD and GMW. Roselle seeds from China and Malaysia had different volatile profiles (chromatogram not shown). But both volatile profiles were dominated by alcohols, terpenes and aldehydes and had phenols, an ester and a lactone present in traces. A Principal Component Analysis was carried out using the peak areas obtained, in order to provide an overview of the influences of different sample preparation methods and different origins of Roselle seeds (Figure 1). The first principal component (PC1) explained 58 % of the variance while PC2 explained 22 % of the variance. The samples were clearly separated according to country and also by different sample preparation methods. The differences are probably due to different harvesting time, harvesting place, climate zone and varieties.

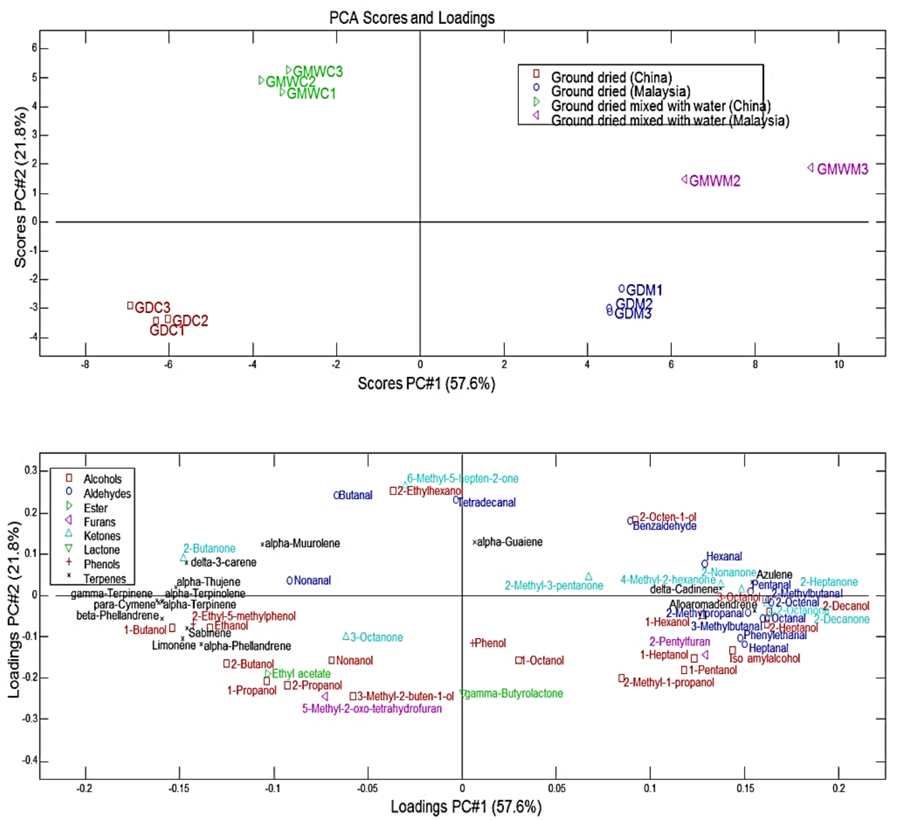

Figure 1: PCA scores and loadings plot of volatile compounds for Roselle seeds

For most volatiles the largest peak areas were obtained using the GD sampling technique: only some ketones had higher peak areas in the GMW samples. GD would therefore be the preferred sample preparation technique compared to GMW. It seems that addition of water during sample preparation does not improve the efficiency of sampling of Roselle seeds. Nevertheless, care must be taken to avoid sampling procedures which may alter the substances being studied. GD was assumed to cause less chemical changes of the samples, it was simple, and it was easy to handle. Therefore, it was decided to use the GD preparation method in further experiments.

The loadings plot shows that Roselle seeds from Malaysia were found to have a larger amount of many alcohols, aldehydes, and ketones, whereas samples from China were high in most terpenes (Figure 5). The major volatile compounds (by peak size; 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, α-phellandrene, hexanal, 2-methyl-1-propanol, sabinene, 4-methyl-2-hexanone, β-phellandrene, 3-methylbutanol, 2-pentylfuran, 1-pentanol, ρ-cymene, 1-hexanol, and 2-ethyl-5-methylphenol) were found in all samples in varying levels.

The composition of Roselle seeds is rarely studied compared to the calyces and studies on proximate composition of Roselle seeds are limited compared to studies on other seeds such as black cumin seed (*Nigella sativa* L.) and jojoba seed (*Simmondsia chinensis*) [5]. The results of proximate compositions of Roselle seeds are presented in

Table 1. The total dietary fiber, protein and lipid of Roselle seeds ranged between 47.1 and 47.3 %, 21.3 and 23.6 %, 11 and 16.2 %, respectively. The differences in proximate composition may be attributed to the different origins, agricultural practices, and varieties. Previous studies have also shown that Roselle seeds contain high protein, dietary fiber, and minerals such as phosphorus, magnesium and calcium. This contributes to the strength of the seed compared to other common sources of dietary fiber such as wheat and rice bran, oat, and fiber from fruits [5]. Furthermore, El-Adawy and Khalil [6] reported that the lipid from Roselle seeds contained more than 70 % of polyunsaturated fatty acids and Dhar et. al. [7] found high content of γ-tocopherol.

In conclusion, it was found that Roselle seeds of Malaysian origin had more volatile compounds recovered in both types of sample preparation, GD and GMW, and had higher lipid content. The effect of the volatile profile on the sensory quality remains to be elucidated, but a high lipid content in the seeds is considered an advantage due to its richness in polyunsaturated fatty acids (PUFAs) and γ-tocopherol which possess potential health benefits. Thus, Roselle seeds of Malaysian origin were selected instead of Roselle seeds of Chinese origin as a potential food ingredient for further exploration in development of bakery products using Roselle seeds.

Table 1: Proximate composition of Roselle (*Hibiscus sabdariffa* L.) seeds

Type of analysis	*Malaysia*	*China*	*Significance*
Moisture content (%)	8.4[a]	7.9[b]	***
Ash (%)	6.5[a]	4.8[b]	***
Lipid (%)	16.2[a]	11[b]	***
Protein (%)	21.3	23.6	ns
Total dietary fiber (%)	47.3	47.1	ns

Values in a column not marked with the same letters are significantly different, Student t-test ($p<0.05$).
*** Indicates significant at $p<0.001$; ns, no significant difference between the samples.

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