Analysis of aroma compounds of Roselle by Dynamic Headspace Sampling using different preparation methods

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The influence of different methods of sample preparation on the aroma profiles of dried Roselle (Hibiscus sabdariffa) was studied. Least amounts of aroma compounds were recovered by analysis of whole dry calyxes (WD) followed by ground dry (GD), blended together with water (BTW), and ground and then mixed with water (GMW). The highest number of aroma compounds was found in Roselle treated in water bath (2 h/40°C) (GMWKB). GMW was chosen as the preparation method because it was shown to be an efficient extraction method without the possibility of excessive chemical changes of the sample.

Introduction:

The role of Roselle's (*Hibiscus sabdariffa* L.) aroma and flavour in food industry processing is given special attention in the support of the growing Roselle industry in Malaysia. Utilization of Roselle as a food product is still considered meagre but has financial potential. Generally, Roselle is cultivated to utilize the calyx of the flower to produce soft drinks due to the calyx being a rich source of vitamin C, phytochemicals, natural food coloring, outstanding processed fruit quality and its flavour, which contributes to commercial interest. Roselle tastes like berries, and has a sweet and tart flavor [1,2]. Hence, its flavour is appealing to many consumers. Although Roselle has been widely planted and consumed in many countries, the study of Roselle flavour is still very limited. Different sample preparation methods may lead to differences in the flavour profile, thus care must be taken to avoid sampling procedures which may alter the substances being studied. However, sample preparation has received limited attention. Therefore, this study addresses the influence of different methods of sample preparation on the aroma profiles of dried Roselle flower.

Experimental:

Material:

Oven dried Roselle (*Hibiscus sabdariffa* L.) of the UMKL cultivar (obtained from HERBagus Sdn. Bhd., Penang, Malaysia) was chosen to study aroma profiles.

Sample preparation:

Samples were prepared by five different procedures and analysed in duplicate:
Whole, dry (WD): 10g of whole Roselle was sampled by DHS.
Ground, dry (GD): Whole Roselle was ground for 2 min using a blender (KRUPS Speedy PRO) and 10g was sampled by Dynamic Headspace Sampling (DHS).

Blended together with water (BTW): 20 g of whole Roselle was blended with 40 ml water using a blender (KRUPS Speedy PRO). Internal standard (1 ml of a 5 ppm 4-methyl-1-pentanol solution was added to an amount of the mixture corresponding to 10 g of Roselle and sampled by DHS.

Ground, mixed with water (GMW): Whole Roselle was ground for 2 min using a blender (KRUPS Speedy PRO) then 10g of ground dried Roselle was mixed with 40 mL of tap water, ratio (1:4). Again, 1 mL of internal standard was added to an amount corresponding to 10g Roselle and sampled by DHS.

Ground, mixed with water, kept in water bath (2 h/40°C) (GMWKB): Whole Roselle was ground for 2 min using a blender (KRUPS Speedy PRO). Ground dried Roselle (10 g) was mixed with 40 ml of tap water, internal standard (1 ml of 5 ppm 4-methyl-1-pentanol) was added and kept in water bath (2 h/40°C) before sampling by DHS.

*Dynamic Headspace Sampling (DHS):*

Each sample was placed in a glass flask (300 ml, 7.5 cm diameter). 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 before purging with nitrogen (100 ml/min) for 30 min. The traps were dry-purged with nitrogen (100 ml/min) for 10 min to remove water.

Gas Chromatography-Mass Spectrometry and Multivariate Data Analysis:

The collected volatiles and multivariate data analysis were determined as previously described by [3]. Volatile compounds were identified by probability based matching of their mass 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. Retention Indices were calculated after analysis under the same conditions of an n-alkane series (C9–C24).

Results:

A total of 125 compounds were identified including terpenes (32), aldehydes (20), esters (16), ketones (14), alcohols and furans (13), acids (9), sulphurs (3), lactones (2) and others (3). The total numbers of volatile compounds for each class found are presented in Figure 1.
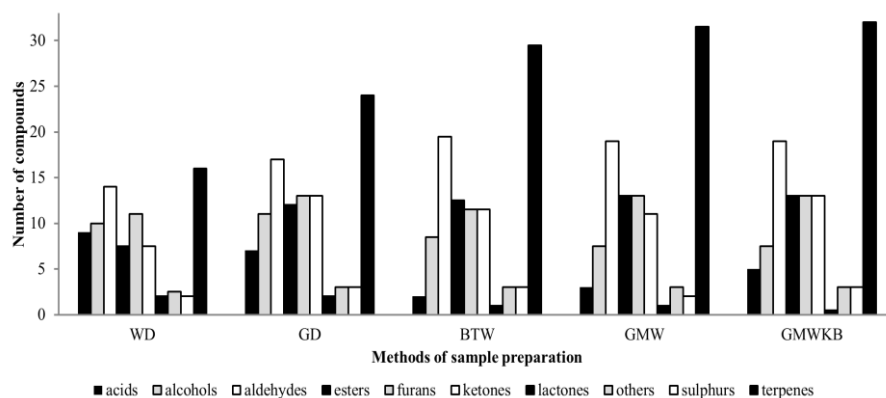
Figure 1. The total numbers of volatile compounds for each class in Roselle determined using GC-MS in combination with different sample preparation techniques.

The difference between sample preparations is considerable: The lowest number of aroma compounds was recovered in WD followed by GD, BTW and GMW. Roselle treated as GMWKB showed the highest number of aroma compounds compared to the other sample preparations. Terpenes and aldehydes were the most represented classes by number, followed by esters, furans, ketones, alcohols, acids, sulphurs, lactones and others. Roselle treated as WD showed chromatograms with fewest peaks (chromatogram not shown), still having terpenes and aldehydes as predominant and lactones, sulphurs and others present in traces. The high number of aroma compounds found in Roselle treated as GMWKB was probably due to release of glycosidically bound volatiles.

To give an overview of the effect of the different treatments on the peak sizes obtained, a Principal Component Analysis was carried out (Figure 2).
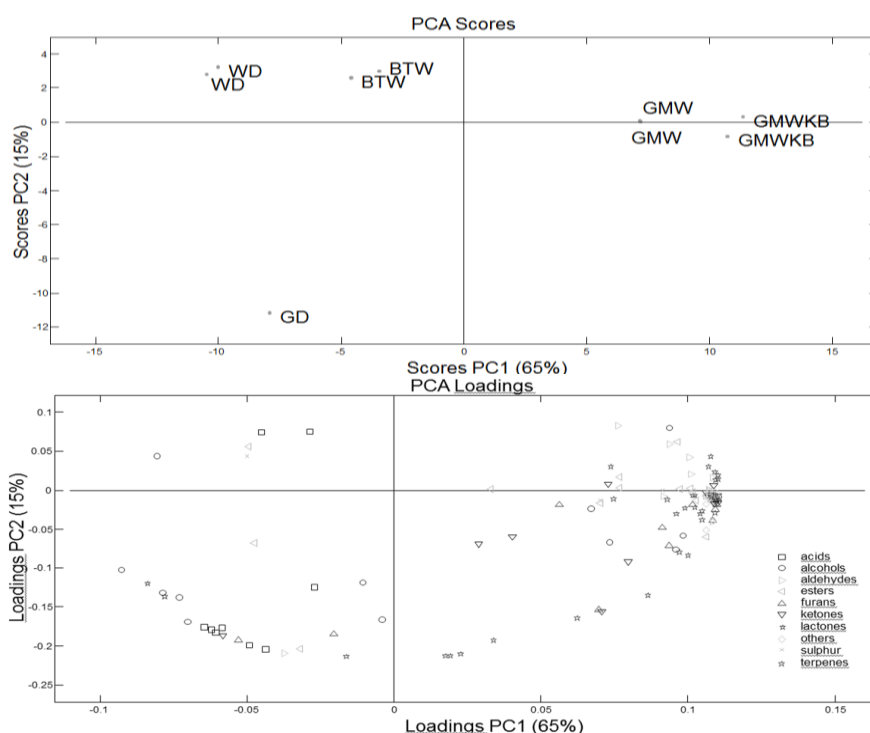
Figure 2. Principal component analysis (PCA) scores and loadings plot of Roselle volatiles.

The first principal component (PC1) explained 65% of the variance, i.e. the main variation was described by this component, while PC2 only explained 15% of the variance. Since GD is the only treatment yielding deviating values of PC2, and since one replicate of sample GD was lost, it was decided only to discuss differences relating to PC1. Going from WD through GD, BTW, and GMW to GMWKB the

position in the score plot moves to the right. Since most compounds, and among them all the terpenes and most of the esters and aldehydes, were placed to the right in the loadings plot, it means that this sequence represents increasing levels of almost all compounds. A smaller number of compounds are decreasing (mainly alcohols and acids, placed to the left in the loadings plot).

So from a sensitivity point-of-view, GMKWB would be the preferred sample preparation technique. It must, however, be observed that under the GMKWB conditions (grinding, mixing with water, keeping at 40°C for 2 h), both enzymatic and thermal degradation reactions may occur. The GMWKB treatment facilitates swelling and hydration of the plant material, which improves the rates of mass transfer and breaks the cell walls, resulting in increased extraction efficiency [4], but also increases enzymatic and chemical reactions, for example leading to facilitated release of terpenes. On the other hand, if grinding and addition of water is omitted, the release of volatiles is strongly decreased, and an incomplete volatile profile is obtained. It was therefore decided to use the GMW preparation method in future experiments. It is found to be a good compromise since it resembles the realistic consumption conditions, it is sensitive, it allows for the addition of an internal standard, and excessive enzymatic and chemical changes of the sample is avoided.

Limonene, α-terpineol and 1,8-cineole are three of the most abundant aroma compounds from the terpene group in Roselle calyx. These results were in agreement with research done by Jung and coworkers [5]. A large amount of furfural was found. It is suggested that furfural was formed mainly during the drying process. This is supported by [6] who found that only a small amount of furfural is present in fresh Roselle. Thermal processing through air drying has been demonstrated to produce a caramel-like aroma [6] which might be related to furfural and 5-methyl-2-furfural. Both compounds could be formed by sugar degradation [7].

As reported, furans (2-pentylfuran, 2-acetylfuran, and furfural) may be produced from drying process mainly the thermal processing and thermal decomposition of hydroperoxides or cyclic peroxides of linoleate [8-9]. Eugenol, one of the phenolic derivatives, was also found in all five different methods of sample preparation (data not shown). Eugenol is one of the major volatiles in Roselle. It is synthesized from phenylalanine in plants [9] and is known to be thermally stable during the drying treatment.

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