International Journal of Agriculture Innovations and Research
Volume 7, Issue 3, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 04/12/2018 | Accepted on: 13/12/2018 | Published: 28/12/2018

Functional Properties and Volatile Compounds of Baishouwu Flowers (Cynanchum Auriculatum)

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Abstract – **The well-known Chinese medical herb, namely Baishouwu owns an increasing interest, since its root extracts as well as fractions have considerable range of important pharmacological values. This work aims at studying the functional properties and volatile compounds of Baishouwu flowers. The study uses a Solid Phase Microextraction (SPME) with a Gas Chromatography coupled with a Mass Spectro--metry (GC/ MS). The results of our studies can be summarized as follows: the Water Absorption Index (WAI) of Baishouwu flowers equals 348.94 ± 0.5%, while the Oil Absorption Index (OAI) is 121.43 ± 0.2%. A good foaming capacity and stability value of Baishouwu flowers is reached, 11.7 ± 0.5 % after one hour. The bulk density of Baishouwu flowers is 0.54 ± 0.01 g/ml, which is essential for packaging as well as transportation. The swelling power of Baishouwu flowers reaches 9.74 ± 0.22 g/g, while the water solubility is 42.8 ± 0.7 %. Furthermore, total of 98 volatile compounds are detected. These include acids, aldehydes, alcohols, esters, hydrocarbons, ketones, furans, halogens, phenols, bases, lactones, flavors list, pyrans, coumarins in addition to other compounds.**

Keywords – **Baishouwu Flowers, Cynanchum Auriculatum, Functional Properties, Volatile Compounds.**

I. Introduction

Natural products such as plants, animals, etc. were main sources applied to produce medical instances aiming at flavoring and conserving food, eliminating or dealing with diseases and health disorders, etc. Based on [1], humans used medical plants since more than 60,000 years. These plants present even an important aspect of the daily life of a wide range of people, especially in rural areas [2].

Herbalism, a synonym of medical plants and the backbone of traditional medicine, is considered a resource rich in ingredients utilized in producing drugs and also synthesis. So, medical plants are crucial for the development of human cultures as well as eco-systems worldwide, especially in developing countries [3]. The World Health Organization (WHO) has a considerable contribution in reporting the utilization of medicinal plants by local people groups from various regions of the world [4].

It is worth noting that the use of medicinal plants as a mainstay for health care has widespread in China. The Chinese herbal plants (CHP) are considered nowadays an intrinsically tied part to the Chinese public health system. The use of medical plants also appears in the western countries as complementary or even alternative medicine. This has motivated the merge between traditional and western medical systems to form a medical syncretism characterized by both health concepts [5]. In recent years, many studies have been accomplished on further using Chinese herbs due to their advantages of being natural, easy to prepare, inexpensive, etc. Note also that they have few side effects for the environment [6].

The well-known Chinese herb, Baishouwu (cynanchum auriculatum) belongs to Asclepiadaceae family. It owns an increasing interest, since the root of this herb contains many crucial nutrients like starch, sugars, protein, lipid, vitamins, etc. [7]. It can be applied for instance for the treatment of gastric disorders [8], immunity enhancement [9], antitumor [10], antioxidant activity [11], antidepressant activities [12]. Furthermore, health reports show that C-12 steroidal glycosides present in the root of Baishouwu posse an efficacy of antineoplastic on human tumor cell lines [13].

It is known that plants synthesize and emit a wide range of volatile organic compounds that have important physiological as well as ecological functions [14]. These volatiles can also be used as components for aroma quality in plant-derived foods as well as cosmetics [15]. It is worth noting that functional characteristics of food materials are crucial factors that should be considered in this context, especially in the formulation of new food products with certain aroma quality.

To the best of our knowledge, there exist no studies until now that comprehensively focuses on the functional properties and volatile compounds of Baishouwu flowers. In this concern, a comprehensive analysis of the functional properties and volatile compounds of Baishouwu flowers will be considered an important contribution. This is the main focus of this paper, which is structured as follows: the material and the method applied are discussed in section (2). This section discusses in detail how the plant material and sample are prepared and how different characters of Baishouwu flowers are extracted. Section (3) presents the results we obtained from our study and discusses them in detail. Finally, the main results are concluded in section (4).

II. Materials and Methods

A. *Plants Material, SPME and Chemicals*

We have bought dried Baishouwu flowers in 2017 from Jinfang Shouwu Technology Development Co. Ltd. (Yancheng, China). The separation of volatile compounds has been achieved using the SCION Single Quadrupole mass spectrometer (SCION SQ 456–GC). Gas

Chromatography/ Mass Spectrometry (GC/MS) used was fitted with a DB-wax (Agilent Technologies). The Solid Phase Microextraction (SPME) fiber (Triphasic DVB/ Carboxen/ PDMS (50/30 µm Divinylbenzene /Carboxen/ Polydi- methyl-siloxane) was utilized. All standards were provided from Sigma Aldrich (St. Louis, Mo., U.S.A.).

B. *Plants Preparation*

We have crowded Baishouwu flowers into flour by an electric grinder (platinum European hardware 40 Y, 220 v, 50 - 60 Hz, 32000 r/min, 2080 W). Thereafter, we have passed them through a mesh sieve (400 µm), and packed them then in polyethylene bags. Following that, we have kept them at -18 ℃ for further work.

C. *Water and Oil Absorption Index*

First, one gram of the powder was weighted into a centrifuge tube, which is pre-weighed. 10 ml of distilled water were then added. The samples were moved vortex-like for 1 min, and permitted to stand for 30 min at 25 ± 2 ◦C before being centrifuged at 4000 rpm for 25 min. The tubes were then inverted over an absorbent paper, which results in decanting the excess water. The samples were allowed to drain. With respect to the oil absorption, 10 ml refined sunflower oil were utilized. The weights of both water and bound oil samples were estimated by the difference [16].

D. *Foaming Capacity and Stability*

So as to determine the foaming capacity of Baishouwu flowers, 2 g of the material was weighed and, then, transferred into a standard electric blender. Thereafter, an addition of 100 ml sample of distilled water has been done. The suspension was blended, then, at 12,000 rpm for 6 min at 27 ◦C. An immediate and also quantitative transferring of the contents has been achieved to a 250 ml measuring cylinder. Thereafter, the volume of the foam was recorded. The foaming capacity was expressed as a percentage increase in the volume. In order to determine the foam stability, the fall in the foaming capacity value was monitored as a function of time after 1 h [17].

E. *Bulk Density*

The bulk density is defined as the amount of powder by weight present in a pre-defined volume. This value is expressed as g/ml. It results from measuring the volume of a fixed weight of powder after being tapped for a certain number of times. It is worth noting, that the bulk density is a crucial parameter for both process development and solid dosage manufacturing.

The bulk density was determined in this study according to [18]. The sample used (10 g) was measured in a graduated cylinder (25 mL) after this cylinder being tapped from a height of 5 – 8 cm on a laboratory bench certain amounts of times, i.e. until no more visible decrease in volume was noticeable. The final test flour volume was measured and expressed as follows:

$$\text{Bulk density}\left(\frac{\text{g}}{\text{ml}}\right)=\frac{\text{Weight of sample}}{\text{Voluem of sample after tapping}} \quad (1)$$

F. *Swelling Power and Water Solubility Index*

The determination of swelling power and solubility has been done according to the method described in [19] with slight modifications. First, we added 15.0 mL of distilled water to 0.5 g of sample in a centrifuge tube. Thereafter, we heated the tube in a water bath for 30 min at a temperature of 95°C with constant stirring. We cooled, then, the slurry resulting to the room temperature and centrifuged for 15 min at 4000 rpm. The supernatant was decanted, thereafter, into an evaporating dish, and then dried in an oven for 4 h at 105 °C. Finally, we weighted the supernatant dried and the sediment, and calculated the percentage of solubility (S %) as well as swelling power (SP g/g) on dry basis as follows:

$$Soluability\ (S\ \%)=\frac{Dry\ sediment\ weight}{Dry\ sample\ weight}\times 100 \quad (2)$$

$$Swelling\ power\left(SP\,\frac{g}{g}\right)=\frac{Sediment\ weight\times 100}{Dry\ sample\ weight}\times(100-S) \quad (3)$$

G. *Volatile Compounds Using SPME/GC/MS*

It is known that SPME is a well-accepted methodology for the preparation of samples and the isolation of volatile compounds [20]. The parameters applied for this extraction include: equilibrium time, extraction time and extraction temperature. The SPME/ GC/ MS we apply combines between an advanced pre-method, namely SPME, together with gas chromatography coupled with mass spectrometry. The SPME method deploys a fiber capable of equilibrating with the headspace of interest. The Volatile Organic Compounds (VOCs) are adsorbed on the fiber. They are desorbed, then, from the fiber onto the GC column using thermal desorption [21].

H. *Sample Preparation and Extraction of Volatile*

So as to prepare the sample, we putted 2 g of Baishouwu flowers powder in a headspace bottle at 60 °C, which in turn was putted in water bath for equilibrium. The fiber was applied to absorb the VOCs for 30 min. The SPME fiber used in this study was a triphasic DVB/ Carboxen/PDMS (50/30 µm Divinylbenzene/ Carboxen/ Polydi-methyl-siloxane). The used extraction temperature was 60°C, while the time was 30 min. The desorption temperature applied was 250 °C for 5 min.

I. *GC/MS Conditions*

The chromatographic analysis was achieved using a GC coupled with an MS (SCION SQ 456–GC/MS) as mentioned before. The GC/MS used was fitted with a DB-wax (Agilent Technologies). We operated the GC under following conditions: the column was 30 m × 0.25 mm, the film thickness was 0.25 µm. The temperature of the oven was ranged between 40 and 230 °C, with a temperature ramp being programmed as 40 °C for 3 min. The temperature was increased 5 °C/min until 120 °C, then 0.8 °C/min until 230 °C. The final hold time equaled 10 min. The carrier gas was helium (99.999% purity) at 0.8 mL/min constant flow rate and splitless injection. The applied transfer line and source temperatures were 250 °C and 200 °C, respectively. The GC we used is interfaced with a quadrupole mass spectrometer, for which the ionization energy was 70 eV, EI+ mode, mass scan ranges from 33 to 450 u. The identification of volatile compounds were conducted out by matching their mass spectra of found standards compounds and national institute of standards and technology (NIST) 98 library of MS spectra. The matching was done based on their retention indices.

J. *Statistical Analysis*

Results were expressed as mean values and Standard Deviations (mean ± SD). All measurements and determinations accomplished were carried out in triplicate.

III. Results and Discussion

A. *Water and Oil Absorption Index*

As known, the food industry has to consider the functional characteristics of food materials, which are crucial especially in relation to the formulation of new food products. Table I provides the Water Absorption Index (WAI) and the Oil Absorption Index (OAI) of Baishouwu flowers.

Table. I WAI and OAI properties of Baishouwu flowers

Functional properties	**Mean ± SD**
Water Absorption Index (WAI)	384.94 ± 0.5%
Oil Absorption Index (OAI)	121.43 ± 0.2%

WAI is a protein–water interaction that appears in many food systems. WAI expresses the capability of a protein matrix to absorb and also retain bound, capillary, hydrodynamic and physically entrapped water against gravity [22]. The table show that the WAI value of Baishouwu flowers equals 384.94 ± 0.5%. This value is higher than in that of some Mucuna species, see [23]. The reason standing behind the high value of WAI could be the presence of high amount of carbohydrate in the sample. Concerning OAI, it is applied to evaluate the hydrophobic nature of particles. It is important, since oil functions as a flavor retainer. So, it increases the mouth feel of foods. Based on our results, the OAI of Baishouwu flowers is 121.43 ± 0.2 %, which is higher than that of moringa oleifera leaf as described in a previous study [24].

B. *Foaming Capacity and Stability*

Foaming capacity and stability depend on the interfacial film structured by proteins. This keeps the air bubbles in suspension and also slows down the coalescence rate. The foaming capacity and stability was calculated by the loss of liquid, which results from the destabilization 'leakage' measured as a volume decrease. Based on the results obtained, the foaming capacity and stability value of Baishouwu flowers after one hour is 11.7 ± 0.5 %, which is considered a good value. This relates to the high surface activities of soluble proteins seen in the continuous water phase. Based on [25], this high value might be attributed to the protein contents of the flowers as well as some other components, like carbohydrates. The foaming capacity and stability of Baishouwu flowers is lower than that of sunflower, which is 14.6% as provided in [26]. A very good value of foaming capacity and stability is an indicator that can be utilized as a substitute to foam forming food proteins.

C. *Bulk Density*

Based on the results, the bulk density of Baishouwu flower reaches 0.54 ± 0.01 g/ml, which considered a good value. Note that a high value of bulk density is essential for packaging as well as transportation. Furthermore, such high values are desirable, since they reduce costs significantly. Note also that many factors influence the bulk density. An important factor is the amount of air entrapped in the powder particles (occluded air) [27].

D. *Swelling Power and Water Solubility Index*

The swelling power of Baishouwu flowers reaches in this study 9.74 ± 0.22 (g/g), while the water solubility equals 42.8 ± 0.7%. The reasons standing behind the resulting high value of water solubility might be the presence of high values of protein and the high availability of polar amino acids in the sample studied.

The swelling power and the water solubility are influenced by a range of factors. These include the amylose and amylopectin characteristics, interaction between water molecules and starch chains in amorphous and crystalline areas, the existence of phosphorus and starch granules, etc. [28].

E. *Volatile Compounds*

The compounds present in Baishouwu flowers were extracted using SPME and analyzed by means of a GC/MS device. As shown in table II, a total of 98 compounds are detected. Acids, aldehydes, alcohols, esters, hydrocarbons, ketones, furans, halogens, phenols, bases, lactones, pyrans, in addition to other compounds have been identified and quantitated.

It is known that VOCs are one of the main issues in the environment. The wide distribution of them has raised major concerns, especially with environmental sciences. So, the analysis of VOCs receives increasing interest due to their impact on both global environmental conditions as well as human health. The table shows that 30 compounds of hydrocarbons have been identified. Note that hydrocarbons include any class of organic chemical compounds consisting only of the elements carbon (C) and hydrogen (H). Carbon atoms join together to form a framework of the compound, while hydrogen atoms attach to them in different configurations. The percentage of hydrocarbons in our results reaches 21.5%. Aldehydes group includes 16 compounds. Hexanal is the main aldehyde present in Baishouwu flowers with a percentage of 7.795%. This group has the highest concentration among other detected VOCs, namely 26.1 %. It is known that aldehydes are considered key functional groups in chemistry. This is due to the high reactivity they show, which enables a great range of useful reactions. Note also that aldehydes have very powerful aromas and are widespread used in various applications, e.g. agro--chemistry, manufacturing, etc. [29]. Concerning alcohols group, it contains 13 compounds in our study with a percentage value of 9.35 %. Unsaturated alcohol 1-octen-3-ol plays a crucial role in the formation of aroma of different foods due to the low threshold it has. Alcohols may be resulted from the oxidative degradation of fatty acids.

Table II. Volatile compounds present in Baishouwu flowers.

Hydrocarbons	Name	CAS number	%	Retention time (min)
1	Heptane, 2, 2, 4, 6, 6-Pentamethyl-	13475-82-6	2.822	4.888
2	2,6-Dimethyloctane	2051-30-1	0.051	6.846
3	Docosane	629-97-0	0.127	7.102
4	Pentacosane	629-99-2	0.136	9.584
5	Heneicosane	629-94-7	0.873	11.195
6	2-Methylundecane	7045-71-8	0.668	11.372
7	Cyclododecane	294-62-2	0.409	13.55
8	1-Tetradecene	1120-36-1	0.031	14.083
9	1-Nonadecene	18435-45-5	0.258	14.92
10	Heptadecane	629-78-7	0.547	15.004
11	Triacontane	638-68-6	1.017	15.068
12	3-Methyltridecane	6418-41-3	0.675	15.408
13	Tetradecane	629-59-4	2.141	16.046
14	Cyclotetradecane	295-17-0	1.001	17.687
15	Heptacosane	593-49-7	0.089	17.437
16	Undecane	1120-21-4	4.864	8.342
17	Nonadecane	629-92-5	0.446	19.154
18	2, 2, 4, 4-Tetramethyloctane	62183-79-3	1.03	6.036
19	Decane, 3,7-Dimethyl-	17312-54-8	0.085	7.199
20	Tetradecane, 2, 2-Dimethyl-	59222-86-5	0.07	7.286
21	Cyclohexane, 2-Propyl-1, 1, 3-Trimethyl-	81983-70-2	0.096	10.423
22	Cis-1, 2, Trans-1,3-; Cyclohexane	7667-55-2	0.522	11.783
23	Tridecane, 4-Methyl-	26730-12-1	0.273	12.946
24	11-Tricosene	52078-56-5	0.804	13.388
25	2-Butyl-1-decene	51655-65-3	0.879	14.196
26	7-Methylheptadecane	20959-33-5	0.224	14.561
27	5-Ethyl-1-nonene	19780-74-6	0.705	15.256
28	Cyclopentane, (2-Methylpropylidene)-	53366-58-8	0.224	16.336
29	Tridecane, 3-Methylene-	19780-34-8	0.332	16.71
30	10-Heneicosene (c,t)	95008-11-0	0.096	26.839
Carbonyls, Aldehydes'	**Name**	**CAS**	**%**	**Retention time (min)**
1	Pentanal	110-62-3	0.905	5.268
2	Butanal, 3-Methyl- (CAS)	590-86-3	1.224	4.068
3	Hexanal (CAS)	66-25-1	7.795	7.795
4	Heptanal (CAS)	111-71-7	1.414	10.696
5	2-Hexenal, (E)- (CAS)	6728-26-3	1.422	11.591
6	Octanal	124-13-0	0.618	13.618
7	2-Heptenal, (E)-	18829-55-5	2.806	14.433
8	Nonanal (CAS)	124-19-6	2.85	15.92
9	2 OCTENAL	2363-89-5	1.328	16.557
10	2, 4-Heptadienal, (E,E)- (CAS)	3/5/4313	0.604	17.62
11	Benzaldehyde (CAS)	100-52-7	1.631	18.103
12	Trans-2-CIS-6-Nonadienal	557-48-2	0.277	19.014
13	Benzeneacetaldehyde	122-78-1	0.484	19.782
15	2-Butenal	4170-30-3	1.883	6.659
16	2, 4-Hexadienal, (E,E)- (CAS)	4488-48-6	0.861	17.135
'Alcohols	**Name**	**CAS**	**%**	**Retention time (min)**
1	1-Hexanol, 2-ethyl- (CAS)	104-76-7	0.844	9.757
2	1-Octanol, 2-butyl-	2/8/3913	0.374	14.841
3	1 OCTEN 3 OL	3391-86-4	1.561	17.013
4	6-Methyl-hept-5-en-2-ol	1569-60-4	1.272	19.274
5	Benzenemethanol	100-51-6	2.463	22.707
6	Phenylethyl Alcohol	60-12-8	1.533	23.192
7	1-Penten-3-ol, 2-Methyl-	7/5/2088	0.138	24.219
8	4-Ethyl-1-octyn-3-ol	5877-42-9	0.229	13.205
9	1-Decanol, 2-Hexyl-	2425-77-6	0.234	13.73
10	2,3-Dimethylcyclohexanol	1502-24-5	0.258	15.721
11	11-Methyldodecanol	85763-57-1	0.254	16.629
12	2-Pentadecyn-1-ol (CAS)	2834-00-6	0.042	19.68
13	2,4,4-trimethylcyclohexa-5-en-1-ol	21592-95-0	0.149	24.672
Furans	**Name**	**CAS**	**%**	**Retention time (min)**
1	Furan, 2-pentyl- (CAS)	3777-69-3	0.334	12.051
2	Linalool Oxide Trans	34995-77-2	0.545	17.307
3	Ethanone, 1-(2-fFuranyl)- (CAS)	1192-62-7	0.484	17.801

Esters	Name	CAS	%	Retention time (min)
1	n-Octyl Acetate	112-14-1	1.87	18.295
2	Acetic Acid, 2-Phenylethyl Ester	103-45-7	0.195	21.908
Imported EU-Flavis list	**Name**	**CAS**	**%**	**Retention time (min)**
1	3,5-Octadien-2-one	30086-02-3	0.467	18.791
2	2-Buten-1-one, 1-(2, 6, 6-Trimethyl-1,3-cy	23696-85-7	0.122	22.012
3	2(4H)-Benzofuranone, 5, 6, 7, 7a-Tetrahydro	15356-74-8	0.315	27.347
Lactones	**Name**	**CAS**	**%**	**Retention time (min)**
1	2(3H)-Furanone, Dihydro- (CAS)	96-48-0	1.499	19.603
Acids	**Name**	**CAS**	**%**	**Retention time (min)**
1	Butanoic acid, 3-Methyl- (CAS)	503-74-2	3.473	20.109
2	Pentanoic acid (CAS)	109-52-4	0.432	20.984
3	3 Methyl Pentanoic acid	105-43-1	0.216	21.614
4	Pentanoic acid, 4-Methyl-	646-07-1	0.45	21.732
5	Hexanoic acid (CAS)	142-62-1	3.893	22.191
6	Heptanoic acid	111-14-8	2.125	23.36
7	Octanoic acid (CAS)	124-07-2	0.468	23.692
Carbonyls, Ketones	**Name**	**CAS**	**%**	**Retention time (min)**
1	3-Penten-2-one	625-33-2	0.565	8.978
2	4-Octen-3-one (CAS)	14129-48-7	1.545	13.93
3	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	1125-21-9	0.358	20.442
4	5-Hepten-2-one, 6-Methyl-	110-93-0	1.196	14.757
5	3-Octen-2-one	1669-44-9	0.883	16.17
6	3,5-Octadien-2-one	38284-27-4	0.962	18.037
7	3-Undecanone (CAS)	2216-87-7	0.275	18.714
8	.beta. Ionone	79-77-6	0.167	23.293
9	Trans-.beta.-ionon-5,6-epoxide	23267-57-4	0.333	23.864
10	2,15-Hexadecanedione	18650-13-0	0.086	25.055
11	2-Allyl-1,3-cyclohexanedione	42738-68-1	0.297	25.429
Pyrans	**Name**	**CAS**	**%**	**Retention time (min)**
1	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6	14049-11-7	14.585	21.117
Phenols	**Name**	**CAS**	**%**	**Retention time (min)**
1	2,6-Di-tert-butyl-4-methylphenol	128-37-0	0.096	22.911
2	Eugenol	97-53-0	0.118	25.506
Bases	**Name**	**CAS**	**%**	**Retention time (min)**
1	1H-Pyrrole-2-carboxaldehyde (CAS)	1003-29-8	0.077	24.137
2	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	20189-42-8	0.056	26.455
Others	**Name**	**CAS**	**%**	**Retention time (min)**
1	Cyclotetrasiloxane, Octamethyl-	556-67-2	0.234	5.842
2	Heptanal, 4-Methyl-4-nitro-5-oxo-	84996-36-1	0.33	10.084
3	Cyclopentasiloxane, Decamethyl-	541-02-6	0.716	10.269
4	Cytidine, N-acetyl- (CAS)	3768-18-1	4.047	16.839
Halogines	**Name**	**CAS**	**%**	**Retention time (min)**
1	1-Chlorododecane	112-52-7	0.026	9.378
2	3-Trifluoroacetoxy-6-ethyldecane	116436-59-0	0.612	15.568
3	Octadecyl chloride	3386-33-2	0.29	17.564

Furans compounds were synthesized from the interaction between amino and sugars trough Maillard reaction and Strecker degradation. In this study, three compounds of furans are detected, namely two of ester, three of imported EU-Flavis list and one of Lactones with a percentage of 5.83%. The percentage of acids group equals 11.06%.

Note that seven kinds of ester have been analyze. The most abundant acid is hexanoic acid followed by butanoic acid.

Pyrans is an oxygen-containing heterocyclic moiety. It is considered one of the important structural subunits found in natural products. Pyrans group contains in our results one compound, namely 2H-Pyran-3-ol, 6-ethenyltetrahydro-2, 2, 6 with 14.585 % percentage. The percentage of ketones group is 6.667% with eleven compounds detected. Finally, the percentage of phenols, bases, halogines and other compounds reaches 6.6 % in this study.

IV. Conclusion

This paper has provided a comprehensive analysis of the functional properties and volatile compounds of Baishouwu flowers. The results have shown that the Baishouwu flowers have good properties including WAI, OAI, bulk density, foaming capacity as well as stability, swelling power and water solubility which are good factors especially in the formulation of new food products. VOCS were extracted from Baishouwu flowers using SPME coupled with

GC/MS. A total of 98 compounds have been detected. The aldehydes group concentration was the highest among other groups followed by hydrocarbons group.

ACKNOWLEDGMENT

The work described in this article was supported by the National Key Technology R & D Program in the 13th Five Year Plan of China (2018YFC160015), the Forestry science and technology innovation and extension project of Jiangsu Province (No. LYKJ [2017] 26), National first-class discipline program of Food Science and Technology (JUFSTR20180509) and Science and Technology Plan of Changzhou City (CE20172002).

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