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Comparison of Volatile Profile of *Moringa oleifera* Leaves from Rwanda and China Using HS-SPME

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Abstract: *Moringa oleifera* leaf samples from China and Rwanda were used for volatile profile analysis. Volatile compounds were analyzed using the Headspace-Solid Phase Microextraction (HS-SPME)-Gas Chromatography-Mass Spectrometry (GC-MS). A total of 93 volatiles consisting of aldehydes, alcohols, ketones, hydrocarbons, esters, terpenoids and acids were identified. While a total of 61 compounds were contained in the sample from China with acetic acid (12.54% of total volatiles) as the most abundant volatile compound, Rwandan sample contained 59 compounds having hexanoic acid (19.81% of total volatiles) as the most abundant one. Chinese sample showed a higher amount of fat (3.56%) and protein (29.54%) than Rwandan sample fat (3.48%) and protein (25.26%). The ash content was found to be higher in Rwandan sample than in Chinese sample 17.26% and 13.44% respectively. The total polyphenol and total flavonoid contents of Chinese and Rwandan samples were respectively as follow Chinese sample (24.65 mg GAE/g dry weight basis and 39.08 mg Rutin Eq/g dry weight basis) and Rwandan sample (30.01 mg GAE/g dry weight basis; 52.78 mg Rutin Eq/g dry weight basis).

Key words: *Moringa oleifera* leaves, HS-SPME, volatiles, phenolics, proximate analysis

INTRODUCTION

Moringa oleifera, Lam is one of the most widely cultivated species of the monogenic family Moringaceae in recent times. This so called "Miracle Tree" is believed to originate from sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. *Moringa oleifera* tree was first introduced in Eastern Africa from India at the beginning of 20th century; it is widely distributed in different parts of the continent including Rwanda and Uganda in East Africa, Ghana in West Africa and South Africa. It is a perennial softwood tree with timber of low quality, due to its rapid growing nature. It has become one of the world's most useful plants for human nutrition, traditional medicine, nutraceutical purposes, water purifying and industrial uses (Fahey, 2005; Anwar *et al*., 2007).

In developing nations, *Moringa* is used as an alternative to imported food supplements to treat and combat malnutrition, especially among infants and nursing mothers; by virtue of its chemical constituents coupled with their ubiquitous distribution in the tree, all parts of the plant are edible. The leaves are cooked and eaten like spinach or used to make soups and salads. The fresh leaves have been reported to contain vitamin C (Mahmood *et al*., 2010) and vitamin A, more than those reported in carrots and oranges. The Calcium content in *Moringa oleifera* is more than that in milk and also has more potassium than in bananas, more iron than spinach and its protein quality vies that of milk and egg (Jongrungruangchok *et al*., 2010). In addition, it has been reported that the micro-nutrient content is more concentrated in dried leaves: ten times (10) the vitamin A of carrots, seventeen (17) times the calcium of milk, fifteen (15) times the potassium of bananas, twenty five (25) times the iron of spinach and nine (9) times the protein of yogurt but the vitamin C drops to a half of that of oranges (Mahmood *et al*., 2010).

Recently, researchers have become convinced that the compounds derived from plants for instance, phenolic, flavonoid and antioxidant compounds, do more in preventing different diseases. *Moringa* has been found to be a good source of polyphenols and antioxidants; the leaves of *M. oleifera* have various biological activities including, anticancer activities, prevention of cardiovascular diseases, liver diseases (Kumar and Pari, 2003) antitumor, nervous disorders, inflammation, digestive disorders, skin disorders and regulation of thyroid status (Cajuday and Pocsidio, 2010; Khalafalla *et al*., 2010). *Moringa* leaves are extensively utilized to improve the nutritional, nutraceutical and organoleptic properties by incorporating its extract into food products like sauces, juices, spices, milk, bread and instant noodles.

The use of Headspace Solid Phase Microextraction (HS-SPME) in the preparation of analyte has become much popular for, it is a rapid, simple, sensitive, reliable,

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solvent-free method for volatile analysis. HS-SPME has been applied to analyze volatiles compounds of different kinds of foods such as fruit juices, (Lee *et al*., 2007) orange juice (Beltran *et al*., 2005) and dry beans (Oomah and Liang, 2007). Presently, the nutritional and medicinal information about *M. oleifera* leaves is much available in the literature, but there is rare information about volatile components therein.

The aim of this study was, therefore, to investigate the volatile profile of *Moringa oleifera* leaves using Headspace Solid Phase Microextraction (HS-SPME) with Gas Chromatography - Mass Spectrometry (GC-MS). And to analyze the chemical compositional and polyphenol content of *M. oleifera* leaves obtained from two different countries (China and Rwanda).

MATERIALS AND METHODS

Materials and Reagents: The dried leaves of *Moringa oleifera* lam, were separately collected from China (Hunan Province, *Moringa* growing farmer) and Rwanda (obtained from The Institute of Scientific Research (IRST), South Province). The chemicals used were; standard of rutin hydrate, gallic acid, sodium nitrite, aluminum chloride. The other chemical/solvents used were of analytical grade.

Plant material preparation: The leaves were carefully separated from the stalk and then ground into fine powder in electric mill (DFY-500 samples mill, Da De Zhong Yao Ji Xie Company, Wenling, China). The material was passed through a 60 mesh sieve before conducting analysis methods.

Proximate analysis: Chemical analysis to determine proximate composition of samples was carried out using standard procedure. Moisture content was estimated by using the air drying method, fat by Soxhlet extraction, Ash by incineration (ICC, 1990). Protein by the Kjedahl method (AOAC, 1990), using a nitrogen to protein conversion factor of 6.25.

Extraction of phenolic compounds: The powdered leaves of *Moringa* (4 g) was extracted in 250 ml beaker with 80 ml of 80% Methanol solution (Methanol, Water; 80:20 v/v) using a laboratory scale microwave extraction apparatus (WP800SL 23-2 MZG 1500S-Microwave). The microwave power was set to 500 W for 3 min and the temperature was controlled at $\leq 50^{\circ}C$. After extraction the crude extract of each sample was filtered with filter paper. The filtrate obtained was collected into a graduated cylinder and the volume adjusted up to 100 ml with the same extracting solvent in order to estimate the total polyphenol content and total flavonoid content. Each sample Chinese sample (CN sample) and Rwandan sample (RW sample) was triplicated.

Estimation of Total Polyphenol Content (TPC): The amount of TP was calculated using the Folin-Ciocalteu reagent (Sultana *et al*., 2009) with little modification. An aliquot of crude extract 40 µl was mixed with 0.2 mL Folin-Ciocalteu reagent and 2 ml distilled water into 10 ml test tube. The mixture was vortexed for 10 sec and then rested for 5 min before adding 20% sodium carbonate (0.5 ml). The mixture was incubated in a water bath at $40^{\circ}C$ for 30 min. After cooling to room temperature, the absorbance was read at 760 nm using a spectrophotometer (MAPADA UV-1600 Spectro Photometer). Amounts of TP were expressed as Gallic Acid Equivalents (GAE) mg/g of dry weight basis. The range of a calibration curve was from 0.2-0.8 mg/ml with $R^2 = 0.9991$. All samples were analyzed three times and the results were averaged. The results were reported on dry weight basis.

Estimation of Total Flavonoid Content (TFC): The total flavonoids content was measured by the aluminum chloride colorimetric assay (Sultana *et al*., 2009). An aliquot (1 ml) of extract or standard solution of Rutin (20, 40, 60, 80 and 100 mg/l) was added to 10 ml test tube containing 4 ml of distilled water. Immediately, 0.15 ml 5% $NaNO_2$ solution was added to test tube. After 5 min, 0.15 ml 10% $AlCl_3$ was added and allowed to stay for 6 min before addition of 2 ml 1.0 M NaOH and the total volume adjusted to 10 ml with distilled water and then vortex for 10 Sec. The absorbance was measured against prepared reagent blank at 510 nm and the total flavonoid content was expressed as Rutin Equivalent (mg/g of dry weight basis) with a calibration curve ranging from 0.1-1.0 mg/ml and $R^2 = 0.9993$. Three measurements were taken for each sample and the results were averaged.

GC/MS analysis of volatile compounds: The GC-MS analysis was performed according to the method reported by (Song *et al*., 2010) with slight modifications. The volatile compounds of *Moringa* powdered leaves were sampled with an SPME-fibre (75 µm, carboxen/ poly-dimethylsiloxane) and assayed with a Gas Chromatography-Mass Spectrometer (Finnigan Trace GC/MS, Finnigan, USA). For each sample, 3.0 g was placed into a 15-ml glass vial and then sealed with PTFE/BYTL septum. The sealed veal was left at $55^{\circ}C$ for 30 min in the presence of SPME fiber to equilibrate the volatiles in the headspace. When equilibration process was completed, the injection was conducted in a splitless mode for 3 min at $250^{\circ}C$. Separation of volatile compounds was carried out on a DB-WAX (30 m x 0.25 mm x 0.25 µm, J and W Scientific, Folsom, CA, USA) capillary column. The column flow rate was 1.8 ml/min, using helium as a carrier gas. The column temperature program was set at $40^{\circ}C$ for 3 min, 40-$80^{\circ}C$ at $5^{\circ}C$/min, 80-$160^{\circ}C$ at $10^{\circ}C$/min for 0.5 min, 160-$175^{\circ}C$ at $2^{\circ}C$/min, 175-$230^{\circ}C$ at $10^{\circ}C$/min and $230^{\circ}C$ for 7 min. The GC was equipped with a mass spectrometric detector, operating in the electron impact mode with an energy voltage of 70 eV and emission current of 35 µA. The

detector scanning range was set at 35 to 450 m/z at a rate of 4.45 Scans/s.

Volatile compound identification: The Identification of volatiles was done by comparing obtained mass spectral data of samples with those of the NIST 98 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and the Wiley 6.0 (Wiley, New York, NY, USA) library.

Statistical analysis: The data were subjected to statistical analysis to verify and evaluate the difference between chemical compositions of studied samples. The data were expressed as mean±SD (n = 3) where 'n' represents the no. of samples. Results were analyzed statistically using a two sample t-test, assuming equal variances and One-way Analysis of Variance (ANOVA) on a statistical program SPSS ver.16.0 for Windows. The difference was considered significant if $p<0.05$.

RESULTS AND DISCUSSION

Proximate analysis: The proximate analysis of both samples is presented in Table 1. The moisture contents of RW and CN samples were 8.44 and 5.92% respectively. The result showed that CN sample has the higher amounts of fat (7.56%) and protein (29.54) than that of RW (6.48% and 25.26% for fat and protein respectively). However the ash content of RW sample was higher (17.26%) compared with CN samples (13.42%). The comparison of chemical composition between CN and RW samples was significance ($p<0.05$). The moisture, protein and ash content values of both samples of the present study were in good agreement with those of obtained from Ghana (Oduro *et al*., 2008), Thailand (Jongrungruangchok *et al*., 2010), India (Joshi and Mehta, 2010) and Burkina Faso (Yameogo *et al*., 2011). Fat and ash contents were merely in a range with data reported by (Joshi and Mehta, 2010; Yameogo *et al*., 2011). The variation in proximate contents between the samples may depend on the season variations, the stage of development and the techniques employed to collect plant's leaves before the experimental analysis. Protein content was nearly the same in both samples suggesting that production locations may not has an effect on protein content of *Moringa oleifera* leaves. Therefore *Moringa* leaves appeared to be a good source of protein.

Total Polyphenol Content (TPC) and Total Flavonoid Content (TFC): Phenolic compounds such as flavonoids, phenolic acids contribute more to the antioxidant properties of plant food product (Tai *et al*., 2010), necessitating the need to investigate TPC and TFC in plant material as well as in food products. Table 2 presents the analytical data for total phenolic and total flavonoid contents of the studied samples. The total phenolic compounds of the extracts were expressed as

Table 1: Proximate composition of Rwandan and Chinese *Moringa oleifera* leaves

	Sample	
Contents	CN	RW
Moisture (%/wt)[1]	5.92±0.08[b]	8.44±0.06[a]
Fat (%/wt)	7.56±0.21[a]	6.48±0.16[b]
Protein (%/wt)	29.54±0.26[a]	25.25±0.54[b]
Ash (%/wt)	13.42±0.53[b]	17.26±0.46[a]

[1]Percent weight. [a,b]Values with different letters in the same row are significantly different ($p\leq0.05$)

Table 2: Comparison of total polyphenol content and total flavonoid content

Sample	TPC	TFC
RW	30.02±0.20[a]	52.80±0.37[a]
CN	24.65±0.08[b]	39.08±0.87[b]

[a,b]Values with different letters in the same column are significantly different ($p\leq0.05$)

Gallic Acid Equivalent in mg/g dry weight basis and the flavonoids were expressed as rutin equivalent in mg/g dry weight basis. The higher total phenolic content and total flavonoid content were found in the sample obtained from Rwanda (30.01 mg GAE/g dry weight basis and (52.78 mg rutin/g dry weight basis, respectively). The sample obtained from China gave 24.65 mg GAE/g dry weight basis amount of total phenolic content and 39.08 mg Rutin Eq/g dry weight basis amount of total flavonoid content. A significant difference in total phenolic content and flavonoid content has been observed between both samples. The variation might be attributed to many factors, including climatic and conditions and geographical location. Geographically China and Rwanda are different with varying seasons and climatic conditions. Iqbal and Bhanger (2006) reported that an increase in unsaturated fatty acids is generally associated with cooler climates and the use of phenolic compounds as an antioxidants to create a self-defense system against environmental stress cannot be overlooked. Moreover the composition of phenolic compounds varies widely with other factors such as the stage of maturity, variety, part of the plant analyzed, post-harvest handling, processing and storage (Sreelatha and Padma, 2009). This supports our present findings, giving a rise of obvious effects of agro climatic difference of both countries China and Rwanda.

Content of volatile compounds: The volatile compounds of *Moringa oleifera* leaves from China and Rwanda were analyzed by GC-MS and the spectral data of identified compounds were compared with those of NIST 98 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and the Wiley 6.0 (Wiley, New York, NY, USA) library. A total of ninety-three (93) volatiles were identified with only 27 being common in both sample (Table 3). CN sample contained 61 volatile compounds of which Acetic acid was the most abundant

Table 3: Comparison of volatile compounds of Rwandan and Chinese *Moringa oleifera* leaves

RT	Volatile compounds	CN sample Peak area (%)	RW sample Peak area (%)
	Aldehydes	(7.34)	(17.11)
6.77	(E)-2-Butenal	2.05	-
7.78	Hexanal	0.36	-
7.83	2,4-Pentadienal	0.57	-
8.84	(E)-2-Pentenal*	1.45	0.75
10.49	2-Hexenal*	1.02	3.30
12.13	(Z)-2-Heptenal*	0.61	0.30
13.04	Nonanal	-	2.13
13.16	(E,E)-2,4-Hexadienal*	0.67	0.44
14.26	(E,E)-2,4-Heptadienal	-	2.43
14.63	Benzaldehyde	-	5.12
14.73	(E)-2-Nonenal	-	0.66
15.87	2-Phenylacetaldehyde	-	1.65
16.19	2-Methyl furan	0.61	-
19.52	γ-Nonalactone	-	0.33
	Alcohols	(19.19)	(14.17)
8	2-Ethyl cyclobutanol	0.43	-
11.05	1-Pentanol	-	0.31
12.04	(Z)-2-Pentenol*	0.53	0.44
12.5	Hexanol	-	0.41
13.9	(Z)-3-Octenol	-	0.93
14.21	Trans-2-Ethyl-2-hexen-1-ol	2.58	-
14.9	Octanol	-	0.26
15.31	2,4-Dimethyl-Cyclohexanol	3.73	-
15.51	3,3-Dimethyl-Cyclohexanol*	10.19	0.64
15.73	(E) 2,6-Dimethyl-3,5,7-octatriene-2-ol	-	1.19
15.82	1-Pentadecanol	-	1.83
15.96	Nonanol	-	1.31
16.96	1-Undecanol	-	0.61
18.1	Benzyl alcohol*	0.42	2.25
18.43	Phenethyl alcohol	-	3.99
18.78	1-Dodecanol	0.31	-
	Ketones	17.63	8.20
12.29	Methyl heptenone*	0.50	0.42
14.33	1-(Furan-2-yl)ethanone	0.78	-
14.54	(E,E)-3,5-Octadien-2-one*	1.48	1.73
15.12	3,5-Octadien-2-one*	0.60	0.68
15.36	Methyl heptadienone	-	0.99
15.76	γ-Butyrolactone	0.43	-
16.88	4-Isopropyl-2-cyclohexenone	1.23	-
17.08	2-Hexen-4-olide*	0.97	0.39
18.96	2-Acetyl pyrrole*	1.01	0.38
20.81	7-Octen-2-one	0.70	-
21.39	3-Ethyl-4-methyl-1H-Pyrrole-2,5-dione*	3.67	0.93
22.35	Dihydroactinidolide*	6.22	2.68
	Hydrocarbons	9.21	1.45
11.76	Tridecane*	0.56	0.35
12.47	2-Methyl-Tridecane	0.56	-
13.52	Pentadecane*	0.53	0.38
13.7	2-Methyl tetradecane	2.14	-
13.86	2-Methyl-1-tetradecene	3.70	-
14.17	1-Tridecyne	-	0.32
14.95	3-Methyl Pentadecane	0.29	-
16.3	[R,R-(E)]-4,5-Dimethyl-, 2-Undecene	1.43	-
16.34	Heptadecane	-	0.40
	Esters	8.66	0.64
8.91	(Z)-Hexyl oleate	0.38	
9.98	Methyl hexanoate	-	0.28
12.69	Methyl acetate	0.36	-
13.05	Hexyl 3-methylbutanoate	2.23	-
14.11	(Z)-3-Hexen-1-yl valerate	0.44	-
14.74	Propyl 3-methylbutanoate	2.58	-

Table 3 Cont.:

RT	Volatile compounds	CN sample Peak area (%)	RW sample Peak area (%)
15.13	Octyl 2-methyl butyrate	0.25	-
15.8	Octyl 2-methyl butyrate	1.64	-
22.23	Diethyl phthalate	0.33	-
24.11	Dibutyl phthalate*	0.45	0.36
	Terpenoids	11.86	9.10
11.28	O-Cymene	0.46	-
16.54	alpha-Himachalene*	1.74	0.67
16.49	Longifolene	3.41	-
16.73	L-carvone	0.60	-
17.46	Citronellyl valerate	0.44	-
17.89	(E)-Geranyl acetone*	1.37	3.95
18.74	(E)-beta-Ionone*	2.20	2.20
19.21	beta-Ionone epoxide*	1.64	1.27
20.17	Hexahydrofarnesyl acetone	-	0.65
22.23	(E,E)-Farnesyl acetone	-	0.36
	Acids	21.65	41.00
13.64	Acetic acid*	12.54	6.95
14.59	Propionic acid	3.51	-
14.98	Dimethyl-Propanedioic acid	-	0.30
15.57	Butyric acid	0.30	-
15.61	Pentanoic acid*	0.98	0.31
16.04	3-Methylbutanoic acid	-	1.61
16.71	Pentanoic acid	-	0.63
17.71	Hexanoic acid*	1.63	19.81
18.38	4-Hexenoic acid	-	0.36
18.81	2-Hexenoic acid	-	2.11
19.6	Octanoic acid	-	1.05
20.46	Nonanoic acid	-	0.90
21.3	Decanoic acid	-	0.54
23.29	Dodecanoic acid	-	0.32
25.21	Octadecanoic acid*	0.26	0.45
26.3	Tetradecanoic acid	-	0.69
26.86	(E)-9-Octadecenoic acid*	0.93	2.33
31.24	Hexadecanoic acid*	1.50	2.64
	Others	1.40	0.80
7.02	Toluene	0.41	-
11.05	2,4-Lutidine	0.37	-
15.23	Dimethyl sulfoxide	0.62	-
18.61	Phenyl acetonitrile	-	0.80
	Total (93)	95.94	92.47

RT: Retention Time; CN: China, RW: Rwanda, *M. oleifera* leaves samples; *Common flavor volatile between rows

volatile accounting (12.54%) followed by 3,3-dimethyl-cyclohexanol (10.19%) and dihydroactinidolide (6.22%). Other notable compounds were 2,4-dimethyl-Cyclohexanol (3.73%) 2-methyl-1-tetradecene (3.7%) 3-ethyl-4-methyl-1H-pyrrole-2,5-dione 3.67, propionic acid (3.51%); longifolene (3.41%), trans-2-ethyl-2-hexen-1-ol 2.58%, propyl 3-methylbutanoate 2.58%, 2-Methyl tetradecane 2.14%, hexyl 3-methylbutanoate 2.23%, (E)-beta-ionone (2.20%), (E)-2-butenal (2.05%). In the case of RW sample, fifty nine (59) compounds were identified, of which hexanoic acid was the most abundant volatile compound accounting for 19.81% of total volatiles. Other major compounds noticed in RW sample were acetic acid (6.95%), benzaldehyde (5.12%), phenethyl alcohol (3.99%,) (E) -geranyl acetone 3.95%, 2-hexenal (3.30%), dihydroactinidolide (2.68%), hexadecanoic acid (2.64%), (E,E)-2,4-heptadienal 2.43%, (E)-9-octadecenoic acid 2.33%, benzyl alcohol 2.25%, (E)-beta-ionone (2.20%), nonanal (2.13%) 2-hexenoic acid (2.11%).

Toluene, hexanal, 2-hexenal, nonanal (E,E)-2,4-heptadienal, benzaldehyde, 1-pentanol, 1-hexanol, 1-octanol, 1-nonanol, benzyl alcohol were minor components also reported to be present in dry beans (*Phaseolus vulgaris* L.) (Oomah and Liang, 2007). However, the presence of 1-pentanol, 1-hexanol, acetic acid, 1-(2-furanyl) ethanone, benzaldehyde, 3-methylbutanoic acid, pentanoic acid, hexanoic acid, benzyl alcohol, octanoic acid, nonanoic acid, decanoic acid, methyl hexanoate, benzyl alcohol, nonanal, decanoic acid, 1 dodecanol, dodecanoic acid, tetradecanoic acid and hexadecanoic acid identified in the *Moringa oleifera* leaves, have been also reported to

be present in ethnic Agave alcoholic beverages and old plum brandes (Tesevic *et al*., 2006; Leon-Rodriguez *et al*., 2008).

The variation in the volatile compounds of two studied samples of *Moringa oleifera* may be related to their main fatty acid components as reported that hexanal, pentanal, are lipoxygenase catalyzed oxidation products of polyunsaturated fatty acids (Oomah and Liang, 2007). Ester-derived Compounds; [(z)-hexyl oleate, methey acetate, hexyl 3-methylbutanonate, (Z)-3-hexen-1-yl valerate, propyl 3-methylbutanoate, octyl 2-methyl butyrate, diethyl phthalate] were particularly found in Chinese *Moringa* whereas dimethyl-propanedioic acid, 3-Methylbutanoic acid, pentanoic acid, 4-hexenoic acid, 2-hexenoic acid, octanoic acid, nonanoic acid, decanoic acid, dodecanoic acid and tetradecanoic acid were conspicuously absent in CN sample. It is also possible that the chemical differentiation was correlated to the growth factors provoked by different climatic conditions between China and Rwanda.

Conclusion: This study can be considered as the first information on composition comparison of *Moringa* leaves from China and Rwanda, indicating that the cultivation area is an important factor that can influence plants' nutrient content and flavor profile. Finally it was found that, *Moringa oleifera* leaf is a good source of source of protein, antioxidant and mineral, making it a suitable functional ingredient for improving nutraceutical, nutritional and organoleptic properties of food products.

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