Evaluation of Bioactive Compounds in *Moringa oleifera* Flower Using Gas Chromatography Mass Spectrometry/Fourier Transform Infrared Spectroscopy: The Need for Good Postharvest Handling

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Abstract

Moringa oleifera flower is consumed as an edible flower by some human populations. The effect of postharvest handling was evaluated by identifying hydrophilic and hydrophobic bioactive compounds in flowers kept at ambient temperature in a cellophane paper bag and in freshly harvested flowers. Bioactive compounds were extracted using methanol and dichloromethane/methanol and the extracts were subjected to gas chromatography/mass spectrometry to separate and identify bioactive compounds. Fourier transform infrared spectroscopy was used to show functional groups of bioactive compounds in the various extracts. The most abundant compounds from the methanol extract of the flowers kept at ambient temperature were 1,3-Butadiene-1-carboxylic acid, D-Allose and 1,12-Tridecadiene while the most abundant compounds from the dichloromethane/methanol extract were hydrazine-1,1-dimethyl, acetic acid, urea and isobutylamine. The most abundant compounds from dichloromethane/methanol extract of freshly harvested flowers were hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid methyl ester and 10,13-Octadecadienoic acid methyl ester. Fourier transform infrared spectroscopy confirmed the presence of amine, aldehyde, alkane, hydroxyl, methoxy, methyl ether, carboxylic acids, amide, organic nitrates and organic siloxane compounds. Compounds from the various extracts have been reported to have beneficial attributes for wellness which provides support for the use of *Moringa oleifera* flower as food. Postharvest handling in cellophane bag at ambient temperature resulted to the formation of compounds reported to be toxic such as being carcinogenic. Therefore, prior to processing such as drying, there is need for efficient postharvest handling.

Keywords: *Moringa oleifera* Flowers; Bioactive Compounds; Postharvest Handling; Gas Chromatography; Mass Spectrometry

Introduction

Edible flowers are commonly used in human nutrition and their consumption have increased in recent years [1]. For some human populations around the world, they form an integral part of human diet for various reasons which range from sensory impact, health benefits and culinary purposes. Phytochemicals are natural bioactive compounds found in plants. The benefits of plants are based on the fact that they contain bioactive compounds which have positive impact on health and longevity. Edible flowers are consumed fresh or processed using different drying methods. The safety of

flowers consumed as food depends much on postharvest handling and storage. Changes may occur in the bioactive compounds in the harvested plant part between the point of harvest and prior to further processing such as drying. Flowers which are incorporated in human diets must be toxic free and have good nutritional composition [2]. *Moringa oleifera* (Lam) plant belongs to the family Morinaceae [3]. It is considered one of the world's most useful trees as almost every plant part which include the leaves, seeds, roots and flowers are used as food for human and animal consumption, medication and industrial purposes [4,5]. The flowers are fragrant yel-

lowish white, bisexual borne in 10 to 25cm long axillary, compound inflorescence known as the panicles. [6-8]. Amino acids evaluation indicated that the flowers have higher amino acids content than the leaves and seeds [9]. Water accounts for more than 80% as the main ingredient in flowers [10]. The high moisture content of fresh flowers can lead to quick deterioration either by microbial activities or biochemical changes induced by enzymes following post-harvest handling at ambient temperature.

Analytical tools such as the use of gas-chromatography/mass spectrometry, high pressure liquid chromatography-diode array detector electrospray, high pressure liquid chromatography-diode array detector- ESI/MS/MS of bioactive compounds in various solvent extracts of *Moringa oleifera* flower has been reported [11]. GC-MS has found usefulness as a tool which qualifies for the effective separation of phytochemicals [12]. Fourier Transform Infrared (FTIR) determines the vibrations of bonds within chemical functional groups and generates a spectrum that can be considered as a biochemical or metabolic "fingerprint" of the sample [13]. FTIR and GC-MS techniques have become firmly established as key technological metabolic profiling of medicinal plants [14]. The main research interest in plants is aimed at unveiling the presence of some active components in them [15]. Methanol solvent has best extractive ability for different primary and secondary metabolites from plants and pave a way for functional groups analysis by FTIR and separation studies by GC-MS analyses [16]. It is in view of this that GC/MS analysis was adopted to separate, identify and evaluate the effect of postharvest handling by identifying hydrophilic and hydrophobic bioactive compounds in freshly harvested flowers kept at ambient temperature in a cellophane paper bag and in harvested flowers that were processed immediately in the fresh state. Fourier transform infrared spectroscopy (FTIR) was used to identify functional groups to which the bioactive compounds belong to.

Materials and Methods

Sourcing of flower samples and extraction of bioactive compounds

Fresh *Moringa oleifera* flowers were harvested from a farmyard around Abia State University in Umudike Location (5°28'19.79"N; 7°32'33.59"E). Two portions of the freshly harvested samples were put in black cellophane bags and kept at room temperature (25 ± 2°C) for 6h. The third portion of the flower sample was processed in its fresh state immediately after harvest. Bioactive compounds

in the various samples were extracted according to the method described by [17]. Each sample was pulverized and 10g of each sample was put into labeled conical flasks respectively. Two hundred (200) ml methanol and 200ml dichloromethane/methanol (1:1, v/v) were added to conical flasks containing the respective samples, shaken vigorously and covered using aluminum foil. They were allowed to stand for 24h at room temperature after which each sample mixture was filtered through No1 Whatman filter paper respectively. The extracts were concentrated by heating over a boiling water bath to remove excess solvent. The extracts were subjected to Gas Chromatography - Mass Spectrometry (GCMS) and Fourier Transform Infrared Spectroscopy (FTIR) analysis for the separation, identification and characterization of bioactive compounds.

Gas chromatography mass spectrometry analysis (GCMS)

GCMS analysis of each flower extract was done using Gas Chromatography-Mass Spectrometry (Agilent GG 7890B-MSD 5977A, Agilent Technology, USA). The gas chromatograph was equipped with capillary column of 30mm length, 0.25mm diameter and 20 m film thickness. Helium (99.99%) was used as carrier gas. The temperature programming was set with initial column oven temperature of 50°C, hold time of 3 min by 200°C to a final temperature of 325°C with hold time of 2 min. 3µl of each *Moringa oleifera* flower extract was injected respectively using a Hamilton syringe into the gas chromatograph for total ion chromatographic analysis with split injection technique (5:1). The injector temperature was 25°C while for the mass spectrometer, the ion source temperature was 23°C with an interface temperature of 30°C and recorded over a scan range of 46 to 800m/z with electron impact ionization energy of 70 ev. Total run time of the gas chromatography-mass spectrometry analysis for the methanol and dichloromethane methanol extracts of the stored *Moringa oleifera* flowers was 64 and 69 min respectively while the total run time for dichloromethane/methanol extract of the freshly harvested and processed flowers was 69min.

Fourier transform infrared spectroscopy (FTIR)

FTIR of each extract was analyzed by the method described by [17]. A small quantity of each flower extract was dropped between two plates of sodium chloride and placed in the FTIR spectroscopy chamber (Cary 630, Agilent Technologies, USA) respectively. Analysis was done in a scan range from 650-4000 cm with a resolution of 8cm.

Results and Discussion

Gas chromatography-Mass Spectrometry (GCMS) analysis of *Moringa oleifera* flower revealed the presence of 23 compounds in the methanol extract of fresh flowers stored at ambient temperature, 43 compounds in dichloromethane/methanol extract of freshly plucked and processed flower samples without storing at ambient temperature and 13 compounds in dichloromethane/methanol extract of fresh flower sample stored at ambient temperature. Table 1 shows results of compounds identified in the methanol extract of freshly plucked *Moringa oleifera* flowers kept at ambient temperature in a cellophane bag. The most abundant compounds found in this extract were 1,3-Butadiene-1-carboxylic acid, D-allose and 1,12-Tridecadiene. These had relative abundance of 13.43, 19.93 and 29.78% respectively and representing 63.14% of the hydrophilic bioactive compounds. [18] reported the pres-

ence of 26 compounds in methanol extract of shade dried *Moringa oleifera* flower grown in Tamilnadu, India for which oleate, quinic acid and Cis-9-hexadecenal were the major constituents. 1,3-Butadiene is an unsaturated fatty acid has been reported to have anti-tumorigenic, Beta 2-receptor agonist and β -glucuronidase inhibitory activity [19]. D-allose is an aldohexose sugar and a C-3-epimer of glucose. D-allose has been reported to have antioxidative activity [20], it suppresses renal inflammation by attenuating cisplatin-induced nephrotoxicity [21] and has anti-proliferative effects on prostate cancer cell lines [22]. 1,12-Tridecadiene is an alkadiene which is an unsaturated aliphatic hydrocarbon. It has been reported in *Curcubita maxima* seed [23] and as one of the major components found in cuticular abdominal glands of *Zonocerus variegatus* [24]. No clear specific biological activity has been reported for 1,12-Tridecadiene, however similar analogues have been reported to have medicinal properties.

Peak Number	Retention Time (Min)	Compound Name	Relative Abundance (%)	Molecular Formula	Molecular Weight
1	6.018	1,2-Dichlororethylhydroperoxide	2.65	C ₂ H ₄ Cl ₂ O ₂	130.952
2	6.367	1H-1,3-benzimidazole-2-carboxylic acid, 1-methyl	0.98	C ₉ H ₈ N ₂ O ₂	176.17
3	6.678	4-Ethynyl-1-methyl pyrazole	1.48	C ₆ H ₆ N ₂	106.128
4	7.143	Pent-2-ynal,4,4-dimethyl	2.06	C ₇ H ₁₀ O	110.156
5	7.996	1,3-Butadiene-1-carboxylic acid	13.43	C ₅ H ₆ O ₂	98.099
6	8.655	1-Methoxy-1-buten-3-yne	1.67	C ₅ H ₆ O	82.102
7	9.779	2-Pentyn-1-ol	4.87	C ₅ H ₈ O	84.118
8	11.407	1,5-Heptadiene-3-yne	2.23	C ₇ H ₈	92.141
9	12.764	2-Propenenitrile	1.04	C ₃ H ₃ N	53.064
10	13.191	4-Cyclopentene-1,3-diol, trans	0.96	C ₅ H ₈ O ₂	100.12
11	13.888	2-Propenenitrile	1.19	C ₃ H ₃ N	53.064
12	14.974	Carbonic acid, but-3-yn-1-yl-undecyl ester.	2.16	C ₁₆ H ₂₈ O ₃	268.391
13	16.719	N'-(Diaminomethylidene)butane-hydrazide	3.95	C ₉ H ₂₀ N ₄ O	200.28
14	39.863	6-Methyl-triazolo (4,3-b))1,2,4)-triazine	1.11	C ₅ H ₅ N ₅	135.13
15	42.344	1,5-Hexadiyne	1.43	C ₆ H ₆	78.114
16	46.299	3-Dimethyl(Phenyl) Silyloxytridecan	2.92	-	-
17	50.214	D-Allose	19.93	C ₆ H ₁₂ O ₆	180.156
18	50.873	6-(1-Adamantylamino)-2,4,5-trichloronicotinonitrile	1.50	C ₆ H ₁₆ O ₂	356.672

Peak Number	Retention Time (Min)	Compound Name	Relative Abundance (%)	Molecular Formula	Molecular Weight
1	8.383	2-Pentene,3-Methyl, E-	0.01	C ₆ H ₁₂	84.16
2	15.594	Octanoic acid, methyl ester	0.15	C ₉ H ₁₈ O ₂	158.23
3	23.735	2,4-Decadienal	0.11	C ₁₀ H ₁₆ O	152.23
5	30.093	Nonanoic acid, 9-oxo-methyl ester	0.03	C ₁₀ H ₁₈ O ₃	186.25
6	33.738	Undecanoic acid, 10-methyl-, methyl-1-ester	0.09	C ₁₃ H ₂₆ O ₂	214.34
7	37.537	Dodecanoic	0.13	C ₁₂ H ₂₄ O ₂	200.32
8	41.801	Methyl tetradecanoate	0.05	C ₁₅ H ₃₀ O ₂	242.40
9	44.942	Acetamide, N-(3-oxo-4-isi-xazolidinyl-, (R)	0.02	C ₅ H ₈ N ₂ O ₃	144.13
10	48.392	Bicycle[4.1.0]heptane,-3-cyclopropyl,-7-carbomethoxy, trans	0.01	C ₁₂ H ₁₈ O ₂	194.27
11	49.594	Hexadecanoic acid, methyl ester	24.44	C ₁₇ H ₃₄ O ₂	270.50
12	51.067	Bicyclopentylidene	0.02	C ₁₀ H ₁₆	136.23
13	52.424	3,4-Heptadiene	0.04	C ₇ H ₁₂	96.17
14	54.595	9,12-Octadecadienoic acid, methyl ester	20.28	C ₁₉ H ₃₄ O ₂	294.47
15	56.882	10,13-Octadecadienoic acid, methyl ester	20.28	C ₁₉ H ₃₄ O ₂	294.47
16	57.309	trans-13-Octadecenoic acid	2.93	C ₁₈ H ₃₄ O ₂	282.50
17	57.464	Methyl-9-Cis-,11-trans.t,13-trans.- Octadecadienoate	0.72	C ₁₉ H ₃₄ O ₂	294.47
18	57.658	10-Nonadecenoic acid, methyl ester	0.04	C ₂₀ H ₃₈ O ₂	310.50
19	57.890	13-Octadecenal (Z)-	0.02	C ₁₈ H ₃₄ O	266.468
20	58.821	9,17-Octadecadienal, (Z) -	0.77	C ₁₈ H ₃₂ O	264.40
21	59.170	Cis-Methyl 11-eicosenoate	4.36	C ₂₁ H ₄₀ O ₂	324.50
22	59.674	Methyl 18-methylnonadecanoate	4.92	C ₂₁ H ₄₀ O ₂	326.60
23	59.945	Dichloroacetic acid, undec-2-enyl ester	0.16	C ₁₃ H ₂₂ Cl ₂ O ₂	281.22
24	60.061	6-Nitroundec-5-ene	0.25	C ₁₁ H ₂₁ NO ₂	199.29
25	60.294	2,4-Hexadiene-1,6-dimethoxy, (E,E)	0.09	C ₈ H ₁₄ O ₂	142.20
26	60.410	Z-2-Dodecenol	0.05	C ₁₂ H ₂₄ O	184.32
27	60.565	Oxirane undecanoic acid, 3-pentyl 1-methyl ester, cis-	0.05	C ₁₉ H ₃₆ O ₃	312.49
28	60.682	α-d-,Glucose-4,6-0-isopropylidene-1-0-methyl-6-0 [4-bromobenzenesulfonate]	0.09	C ₁₆ H ₂₁ BrO ₈	453.30
29	61.031	Heneicosanoic acid, methyl ester	0.25	C ₂₂ H ₄₄ O ₂	340.60
30	61.535	Methyl-2-octylcyclopropene-1-octanoate	0.76	C ₂₀ H ₃₆	308.49
31	61.806	2H-Azepin-2-one,hexahydro-7-methyl	0.13	C ₇ H ₁₃ NO	127.18
32	62.271	Cyclododecanol,1-aminomethyl	0.20	C ₁₃ H ₂₇ NO	213.36
33	62.659	Docosanoic acid, methyl ester	4.83	C ₂₃ H ₄₆ O ₂	354.61

34	63.046	8-(2-Octanoylcyclopropyl)Octanoic acid, methyl ester	0.61		
35	63.318	1,3,5-Triazin-2(1H)-one-4,6-bis-(ethylamino)	2.19	$C_7H_{13}N_5O$	183.21
36	63.938	Tricosanoic acid, methyl ester	0.65	$C_{24}H_{48}O_2$	368.6
37	64.093	Pentanoic acid, 2-methyl-,1-methyl propyl ester	0.25	$C_{10}H_{20}O_2$	172.26
38	64.869	2-Ethylbutyric acid, 2-hexyl ester	1.29	$C_{12}H_{24}O_2$	200.32
39	65.411	Tetracosanoic acid, methyl ester	7.24	$C_{25}H_{50}O_2$	382.70
40	65.915	Fumaric acid, isohexyl-4-methyl pent-2-yl ester	0.61	$C_{16}H_{28}O_4$	284.31
41	66.032	Octanoic acid, 2-pentadecyl ester	0.11	$C_{23}H_{46}O_2$	354.6
42	66.574	Pentacosanoic acid, methyl ester	0.16	$C_{26}H_{52}O_2$	316.7
43	67.233	Cyclohexanol,1R-4-acetamido-2,3-cis epoxy	0.03	-	-

Table 2: Gas Chromatography Mass Spectrometry analysis of bioactive compounds in dichloromethane/methanol extract of freshly harvested and processed *Moringa oleifera* without storage at ambient temperature.

Table 3 shows results of dichloromethane/methanol extract of fresh *Moringa oleifera* flowers sample stored at ambient temperature. The most abundant compounds were hydrazine-1,1-dimethyl, acetic acid, urea and isobutylamine and they had relative abundance of 19.87%, 7.45%, 10.42% and 10.69% respectively. These account for 58.5% of bioactive compounds in this hydrophobic extract. Results showed the presence of degradation products of amino acids and nitrogenous compounds. [9] reported *M. oleifera* flower to have higher amino acid content than the leaves or seeds. Some of the compounds from this extract such as hydrazine-1,1-dimethyl and its analogue hydrazine-1,2-dimethyl have deleterious biological activities and were found in appreciable quantities. They are derived from hydrazine. These two compounds are classified as alkylating agents and are able to introduce alkyl radicals into biologically active molecules thereby prevent their proper functioning and so can be carcinogenic, mutagenic, teratogenic and possess immunosuppressant actions [29,30]. Acetic acid has been reported to have acidulant, antibacterial, mucolytic, osteolytic, perfumery, pesticide, protisticide, spermicide, ulcerogenic and verrucolytic activities [31]. Isobutylamine is a monoalkylamine containing a primary aliphatic amine group. There are applications for isobutylamine as it can be used as food additive such as flavoring agent or flavor adjuvant [32]. Urea is used as food additive in some sugar free chewing gum to adjust the texture and its addition up to 3% cannot be of toxicological concern [33]. However, postharvest handling in a

cellophane bag at ambient temperature resulted to a 'build up' of urea to a high concentration (10.42%). This could have occurred due to activities of endogenous enzymes on amino acids present in the *Moringa oleifera* flowers. The amount of urea formed could be of toxicological concern if ingested. Another compound found in significant quantity was thiirane and it has been reported to have anticancer activity [34].

Peak Number	Retention Time (Min)	Compound Name	Relative Abundance (%)	Molecular Formula	Molecular Weight
1	49.671	Acetic acid	7.45	C ₂ H ₄ O ₂	60.052
2	54.789	Hydrazine-1,1-dimethyl	19.87	C ₂ H ₈ N ₂	60.10
3	55.254	Thiirane	8.76	C ₂ H ₄ S	60.114
4	55.603	Urea	10.42	CH ₄ N ₂ O	60.056
5	57.386	N-Ethylformamide	2.25	C ₃ H ₇ NO	73.095
6	58.976	Acetic acid	10.07	C ₂ H ₄ O ₂	60.052
7	60.992	Carbonyl sulfide	2.39	COS	60.07
8	61.612	Hydrazine-1,2-dimethyl	7.79	C ₂ H ₈ N ₂	60.10
9	61.961	Propanamide	9.20	C ₃ H ₇ NO	73.095
10	62.349	Isobutyl amine	10.69	C ₄ H ₁₁ N	73.139
11	63.977	2-Butanamine	4.95	C ₄ H ₁₁ N	73.139
12	64.636	O-n-Propylhydroylamine	3.44	C ₃ H ₉ NO	75.11
13	67.233	Silamamine,N-Silyl	2.72	HNSi ₂	71.185

Table 3: Gas Chromatography Mass Spectrometry analysis of bioactive compounds in dichloromethane/methanol extract of freshly harvested *Moringa oleifera* flower and kept in cellophane bag at ambient temperature.

Functional groups enumerated by Fourier Transform Infra Red (FTIR) studies correlate to compounds elucidated by GCMS. It is used as a supportive tool in qualitative bioactive compound screening. Table 4-6 shows FTIR spectroscopy results of the various extracts. The polarity of the respective solvents played a major role in extracting more specific bioactive compounds with methanol extracting more of the carboxylic acid compounds, alcohols, aldehydes, alkanes, alkenes, alkynes and nitrogen containing compounds, Dichloromethane/methanol extracted more of the fatty acids, fatty acid methyl esters, amine, alkane, alkene and cyclic compounds.

S/No	Band Position (cm ⁻¹)	Wave Intensity	Bond	Functional group assignment	Group frequency
1	3268.9	51.453	O-H broad	Hydroxy group, H-bonded	3600-3200
			N-H	Aliphatic primary amine	3330-3250
2	2929.7	81.688	O-H stretching	Intermolecular bonded alcohol	3200-2700

3	2855.1	87.952	C-H	Aldehyde	2860-2800
4	1640.0	70.354	C-N	Organic nitrates	1640-1620
5	1453.7	84.045	C=C	Aromatic ring stretch	1510-1450
6	1408.9	82.798	C=O	Carboxylate/ carboxylic acid salt	1420-1300
7	1013.8	57.085	>CH-	Methyne	1300-700
			>CH ₂	Cyclohexane ring vibrations	1055-1000
			G-F stretch	Aliphatic fluoro compound	1150-1000

Table 4: Fourier Transform Infrared Spectroscopy results on methanol extract of freshly harvested *Moringa oleifera* flowers stored at ambient temperature.

Figure 4: FTIR Spectrograph of methanol extract of freshly harvested *Moringa oleifera* flowers stored at ambient temperature.

Figure 5: FTIR Spectrograph of dichloromethane/methanol extract of freshly harvested *Moringa oleifera* flowers.

S/No	Band Position (cm ⁻¹)	Wave Intensity	Bond	Functional group assignment	Group frequency
1	3302.4	58.813	O-H broad	Hydrogen bonded alcohols	3600-3200
			N-H	Phenol, OH-group	
2	2948.3	78.385	O-H stretch N-H C-H stretch	Intermolecular bonded hydroxyl group, Amine Alkane	3200-2700 3000-2800 3000-2850
3	2836.5	81.937	C-H, O-CH ₃	Methoxy, methyl ether	2850-2815
4	2027.7	95.824	Unknown	Unknown	-
5	1654.9	83.739	C=O C=C	Saturated amide Alkene	1680-1620 1680-1620
6	1449.9	83.284	C=C-C Weak	Aromatic	1510-1450
7	1408.9	82.895	O-H bend	Carboxylic acid Phenol, tertiary alcohol	1440-1395 1410-1310
8	1114.5	85.165	C-O >CH ₂	Secondary alcohol Cyclohexane ring vibrations	1124-1087 1055-1000
9	1013.8	31.622	C-O Stretch	Esters, Alkoxy	1150-1000

Table 5: Fourier Transform Infrared Spectroscopy results of Dichloromethane/methanol extract of freshly harvested *Moringa oleifera* flowers.

S/No	Band Position (cm ⁻¹)	Wave Intensity	Bond	Functional group assignment	Group frequency
1	3268.9	51.453	O-H broad	Hydroxy group, H-bonded	3600-3200
			N-H	Aliphatic primary amine	3330-3250
2	2948.3	77.792	O-H stretch N-H C-H stretch	Intermolecular bonded hydroxyl group, Amine Alkane	3200-2700 3000-2800 3000-2850
3	2836.5	81.385	C-H stretch, O-CH ₃	Methoxy Methyl ether	2850-2815

4	1654.9	83.848	N-H	Amide	1680-1630
5	1449.9	82.900	C≡C	Aromatic ring stretch	1510-1450
6	1408.9	82.384	C=O	Carboxylate/Carboxylic acid salt	1420-1300
7	1110.7	84.466	Si-O-C	Organic siloxane/ Silicone	1110-1080
8	1013.8	30.196	>CH ₂	Cyclohexane ring vibrations	1055-1000
			C-O	Esters, Alkoxy	1150-1000

Table 6: Fourier Transform Infrared Spectroscopy results of Dichloromethane/methanol extract of fresh flowers of *Moringa oleifera* kept at ambient temperature in a cellophane bag.

Figure 6: FTIR Spectrograph of dichloromethane/methanol extract of freshly harvested *Moringa oleifera* flowers stored at ambient temperature.

Conclusion

The effect of postharvest handling of *Moringa oleifera* flower in cellophane paper bag and kept at ambient temperature resulted to the formation of deleterious compounds such as hydrazine-1,1-dimethyl and its analogue hydrazine-1,2-dimethyl which are carcinogenic. There was also a postharvest build-up of urea and acetic acid. Some compounds from the methanol extract as well as dichloromethane/methanol extract of the freshly harvested flowers have been reported to have beneficial effects for wellness and good health. This research work provides information on the need for efficient postharvest handling such as cold storage immediately after harvest prior to processing so as to avoid the build-up or formation of deleterious bioactive compounds.

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