Chemical Characterization of Three Accessions of *Brassica juncea* L. Extracts from Different Plant Tissues

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Abstract: Indian mustard or *Brassica juncea* (*B. juncea*) is an oilseed plant used in many types of food (as mustard or IV range salad). It also has non-food uses (e.g., as green manure), and is a good model for phytoremediation of metals and pesticides. In recent years, it gained special attention due to its biological compounds and potential beneficial effects on human health. In this study, different tissues, namely leaves, stems, roots, and flowers of three accessions of *B. juncea*: ISCI 99 (Sample A), ISCI Top (Sample B), and “Broad-leaf” (Sample C) were analyzed by HPLC-PDA/ESI-MS/MS. Most polyphenols identified were bound to sugars and phenolic acids. Among the three cultivars, Sample A flowers turned were the richest ones, and the most abundant bioactive identified was represented by isorhamnetin 3,7-diglucoside (683.62 µg/100 mg dry weight (DW) in Sample A, 433.65 µg/100 mg DW in Sample B, and 644.43 µg/100 mg DW in Sample C). In addition, the most complex samples, viz. leaves were analyzed by GC-FID/MS. The major volatile constituents of *B. juncea* L. leaves extract in the three cultivars were benzenepropanenitrile (34.94% in Sample B, 8.16% in Sample A, 6.24% in Sample C), followed by benzofuranone (8.54% in Sample A, 6.32% in Sample C, 3.64% in Sample B), and phytone (3.77% in Sample B, 2.85% in Sample A, 1.01% in Sample C). The overall evaluation of different tissues from three *B. juncea* accessions, through chemical analysis of the volatile and non-volatile compounds, can be advantageously taken into consideration for future use as dietary supplements and nutraceuticals in food matrices.

Keywords: *Brassica juncea* spp.; metabolites; foods; volatile; non-volatile; nutraceuticals; HPLC; GC
1. Introduction

Brassicaceae vegetables, consumed worldwide, represent an important part of the human diet, due to their remarkable supply of health-promoting substances that can potentially reduce the risk of diseases. These vegetables are potential sources of glucosinolates, carotenoids, amino acids, vitamins (C and E), and polyphenolic compounds [1–13]. The most consumed vegetable species is *B. oleracea* L., which include vegetable and forage forms, such as kale, cabbage, broccoli, Brussels sprouts, cauliflower, and others. *Brassica juncea* (L.) Czern. (*B. juncea*), also known as Indian mustard or brown mustard, is well-known for its green vegetable. It is also used as a root and leaf vegetable in China and as a condiment in Europe and America.

In general, it has been reported that a high intake of *Brassicaceae* is associated with the prevention of several cancers, such as colon and lung cancers [14]. Specifically, *B. juncea* leaves extract has shown in vitro anticancer-activity against colon and lung cancers [15], antioxidant activities [16], decrease of lipid peroxidation under diabetic oxidative stress [17], and inhibition of body fat accumulation [18]. In addition, *B. juncea* polyphenols have also shown beneficial effects concerning treatment for the cognitive disorder associated with diabetes [19].

Among bioactive molecules, flavonoids and phenolic acids are the most characterized groups in *Brassica* species. Flavonoids contain a basic structure of two aromatic benzene rings separated by an oxygenated heterocyclic ring with variation in the number and distribution of phenolic hydroxyl groups across the molecules and differences in substitution [20]. Flavonoids protect plants against UV radiation, pathogenic microorganisms, insects, and plant-feeding animals.

The most ubiquitous subclass of flavonoids in plant foods and *Brassica* crops, in general, is flavonols, with the main aglycones, quercetin, kaempferol, and isorhamnetin, are most commonly found as O-glycosides, whereas myricetin is less common. The flavonols occur in plant tissues as complex conjugates, with one to five sugar moieties bound to the aglycone, and they are often acylated with hydroxycinnamic acids. Variation in the polyphenolic content is related to the biosynthesis in the plant, which is influenced by many factors, such as cultivar, climate, postharvest treatments, and agricultural and environmental factors [1].

The nutritional interest of *Brassica* crops is partly related to their polyphenolic compound contents, which can be quite different among species and even among crops from the same species. The polyphenol composition of different *Brassica* species has been described, revealing distinct qualitative and quantitative profiles [21]. For example, total polyphenol content in edible parts of *B. oleracea* L. has been reported to be 2-fold higher than in Brussels sprouts, cauliflower, and broccoli [22]. In other papers, the content of flavonol aglycones in *B. oleracea* has been reported [23–25].

Moreover, it has already been reported that volatile compounds occurring in plant foods, besides being responsible for organoleptic properties, do present favorable properties for human health [26]. Concerning *B. juncea*, some of its volatile compounds, e.g., alkanes, ketones, and isothiocyanates, have been already described [27–29].

The objective of this study was to carry out the determination of the metabolite content by HPLC-PDA/ESI-MS/MS of different portions (leaves, stems, roots, flowers and seeds) of three accessions of *B. juncea*, ISCI 99 (sample A), ISCI Top (sample B), and ISCI “Broad-leaf” (sample C), in order to be implemented in the food matrices as nutraceuticals. The volatile content of the most complex samples, *viz.* leaves, was also analyzed by GC-FID/MS, along with the chemical characterization of defatted seed meals (DSM).

Sample A, one of the first selections at CREA-CI (Bologna, Italy) [30,31], was selected for its so-called biofumigation technique, especially applied in greenhouse contexts. For this reason, its tissues present a high content of glucosinolates. Furthermore, this plant has a brief cycle and an early flowering time during summer, with a good plasticity and adaptation to different pedoclimatic conditions. As the other Brassicaceae, with small seeds, Sample A can be sown during both fall and springtime, and prefers refined ground even though it can afford sod seeding. Sample B was recently registered at the Plant Variety Protection Office (PVPO) through a USDA PVP certificate.
As for the older Sample A, this variety was bred and selected at CREA-CI (Bologna, Italy) for biofumigation purposes, and applied as green manure through soil incorporation. Sample C line has some interesting peculiarities with respect to the other varieties, such as a high biomass production, with a very characteristic broadleaf, even though it is more sensitive to low temperatures, pests, and diseases.

So far, only scattered information is currently present in literature concerning both volatile and non-volatile (metabolic) compositions of the *B. juncea* species. Recently, the determination of the metabolite content of the *B. juncea* accessions presented in this study was reported by the authors using comprehensive two-dimensional liquid chromatography coupled with a photodiode array and mass spectrometry detection [32].

2. Results and Discussion

2.1. Identification and Semi-Quantification of the Metabolite Content in *B. juncea* Cultivars by HPLC-PDA/ESI-MS/MS

So far, Brassicaceae metabolite composition has been widely examined [2–8,25,32]. The main flavonols in Brassica vegetables are reported to be O-glycosides of quercetin, kaempferol, and isorhamnetin. The sugar moiety found in Brassica vegetables is glucose, occurring as mono-, di-, tri-, tetra-, and pentaglucosides, also commonly found acylated by different hydroxycinnamic acids. The latter are phenolic acids occurring in Brassica vegetables, with the most common ones represented by p-coumaric, caffeic, sinapic, and ferulic acids. Figure 1 and Table 1 report the metabolite characterization of the flower extracts of the three *B. juncea* accessions described in Materials and Methods, which turned out to be the most complex ones among the samples investigated.

Compound identification was carried out, based on retention time, UV, ESI-MS/MS spectra, and literature information. For example, Km 3-diglucoside-7-glucoside (peak 3) and Km glucoside (peak 30) had characteristic UV $\lambda_{\text{max}}$ around 265 and 345 nm, whereas Qn-3-diglucoside (Peak 14) or Is-3,7-diglucoside/Is-glucoside (peak 23/peak 29) had UV absorption maxima around 256 (or plus a shoulder around 266) and 354 nm. The attachment of a hydroxycinnamoyl group to the glycosyl function leads to a shift of UV absorption maxima to 326–340 nm, while the molecular ion was increased by 162, 176, 192, and 206 Da (or the sum of two acyl groups when they occur in the glucoside) for caffeoyl, feruloyl, hydroferuloyl, and sinapoyl groups, respectively [6].

Among the identified compounds, sinapic acid and ferulic acid derivatives were the major phenolic acids, both occurring in 13 of them. Concerning flavonoids, kaempferol derivatives were the most representative ones (11), followed by quercetin (5) and isorhamnetin (2). In exception for the two isorhamnetin glucosides and quercetin 3-diglucoside, all of the other flavonoids occurred as acylated by different hydroxycinnamic acids. The sugar moiety was represented by glucose or sophorose in the form of mono-, di-, tri-, and tetra-glucosides [2–8,25,32]. Furthermore, the early eluting compounds were represented by malic acid and citric acid as earlier reported [32]. Compounds not detected in any of the plant tissues analyzed are labeled as Nd: not detected.

As far as quantification is concerned, normally, the determination of Brassica spp. content is carried out after acidic and/or alkaline hydrolysis, due to the lack of commercial standards, [2,4,5]. Following the approach employed in our previous work, limited to only three samples of the different cultivars [31], semi-quantification of the native flavonoid composition of all thirty-six samples analyzed, the three cultivars of *B. juncea* was carried out by the RP-HPLC system coupled to PDA detection. Notably, due to the unavailability of corresponding reference materials, three selected standards, representatives of the distinct chemical classes, namely, Km 3-O-glucoside, Is 3-O-glucoside, and Qn 3-O-glucoside, were adopted, and corresponding calibration curves were prepared. Table 2 reports calibration curves, correlation coefficients ($R^2$), limits of detection (LoDs), limits of quantification (LoQs), and relative standard deviations (RSDs) of the peak areas for each standard selected. $R^2$ values ranged from 0.9939 to 0.9963, LoQ and LoD values ranged from 13 to 48 ppb and from 43 to 159 ppb, respectively, whereas RSD values were lower than 0.41%.
Figure 1. HPLC-PDA chromatograms of the metabolite content of flowers of the three Brassica accessions investigated. (A) B. juncea ISCI 99; (B) B. juncea ISCI Top; (C) B. juncea ISCI “Broad-leaf”.
### Table 1. Metabolite determination of the flowers extracts of the three *B. juncea* accessions using HPLC-PDA-ESI-MS/MS.

| No | Tentative ID | λ max (nm) | t_R (min) | [M - H]^− | MS^2 ions | Sample A (µg/100 mg DW) | Sample B (µg/100 mg DW) | Sample C (µg/100 mg DW) |
|----|-------------|------------|-----------|-----------|------------|--------------------------|--------------------------|--------------------------|
| 1  | Malic Acid  | 215; 260   | 1.34      | 133.0     | -          | *                        | *                        | *                        |
| 2  | Citric acid | 215; 260   | 1.53      | 191.0     | -          | *                        | *                        | *                        |
| 3  | Km 3-diglucoside-7-glucoside | 265; 345 | 4.20      | 771.2     | 609        | *                        | *                        | *                        |
| 4  | Feruloylglycoside | 236; 285 | 13.40     | 355.2     | 193        | Nd                      | Nd                      | Nd                       |
| 5  | Qn 3-sophoroside-7-glucoside | 257; 352 | 13.90     | 787.2     | 625        | 9.73 ± 0.31              | 2.85 ± 0.64              | 3.63 ± 0.07              |
| 6  | Rhamnose-ellagic acid | 283; 313 | 14.28     | 447.0     | -          | Nd                      | Nd                      | Nd                       |
| 7  | Rhamnose-ellagic acid | 283; 313 | 14.55     | 447.0     | -          | *                        | *                        | *                        |
| 8  | Qn 3-hydroxyferuloylsophoroside-7-glucoside | 247; 335 | 15.07     | 979.2     | 625        | 65.60 ± 0.50             | 11.17 ± 2.12             | 6.60 ± 0.92              |
| 9  | Km 3-sophoroside-7-glucoside | 266; 343 | 15.33     | 771.2     | 609        | 5.25 ± 0.06              | 6.60 ± 0.86              | 19.05 ± 0.55             |
| 10 | Qn 3-cafeoylsophoroside-7-glucoside | 242; 330 | 15.77     | 949.2     | 625        | 22.04 ± 0.75             | 9.69 ± 2.32              | 5.53 ± 0.17              |
| 11 | Km 3-hydroxyferuloylsophoroside-7-glucoside | 232; 330 | 16.65     | 963.2     | 609        | 35.52 ± 0.07             | 8.16 ± 2.22              | 6.58 ± 0.00              |
| 12 | Feruloylglycoside | 236; 326   | 16.88     | 355.2     | 193        | *                        | *                        | *                        |
| 13 | Km 3-cafeoylglucoside-7-glucoside | 233; 330 | 17.57     | 933.2     | -          | 5.20 ± 0.10              | 4.91 ± 1.21              | 8.57 ± 0.04              |
| 14 | Qn 3-diglucoside | 256; 360 | 17.76     | 625.1     | 463; 301   | 71.75 ± 3.11             | 31.12 ± 8.80             | 40.05 ± 0.60             |
| 15 | Qn 3-sinapoyltriglucoside-7-glucoside | 238; 330 | 17.90     | 1155.3    | 993        | Nd                      | Nd                      | 21.86 ± 0.34             |
| 16 | Qn 3-sinapoyltriglucoside-7-glucoside | 245; 340 | 18.03     | 1155.3    | 993        | 49.06 ± 0.89             | 18.83 ± 3.89             | Nd                       |
| 17 | Km 3-hydroxyferuloylsophoroside-7-glucoside | 254; 338 | 18.51     | 963.2     | 625        | 35.62 ± 0.08             | 9.87 ± 2.66              | 5.93 ± 0.06              |
| 18 | Km 3-hydroxyferuloylsophorotrioside-7-glucoside | 268; 330 | 18.53     | 1125.3    | 963        | Nd                      | Nd                      | Nd                       |
| 19 | Km 3-sinapoylsophorotrioside-7-glucoside | 268; 330 | 18.78     | 1139.3    | 771        | 1.80 ± 0.06              | 7.85 ± 2.02              | 10.72 ± 0.11             |
| 20 | Km 3-sinapoylsophorotrioside-7-glucoside | 268; 330 | 19.29     | 1139.3    | 771        | 1.80 ± 0.06              | 7.85 ± 2.02              | 10.72 ± 0.11             |
| 21 | Km 3-sinapoylsophorotrioside-7-glucoside | 268; 333 | 19.50     | 977.2     | 609; 815   | 18.69 ± 0.10             | 5.36 ± 1.30              | 5.29 ± 0.00              |
| 22 | Km 3-feruloylsophorotrioside-7-glucoside | 266; 341 | 20.26     | 947.2     | 609        | 72.18 ± 0.08             | 29.23 ± 8.00             | 25.17 ± 0.50             |
| 23 | Is 3,7-diglucoside | 252; 352 | 21.20     | 639.1     | 477; 315   | 683.62 ± 1.14            | 433.65 ± 2.94            | 644.43 ± 0.63            |
| 24 | Feruloyl malte | 242; 323 | 24.23     | 309.1     | -          | *                        | *                        | *                        |
| 25 | Sinapic acid | 270; 326 | 24.27     | 223.1     | 179        | Nd                      | Nd                      | Nd                       |
| 26 | Sinapoyl malic acid | 240; 326 | 25.05     | 339.1     | 223        | *                        | *                        | *                        |
| 27 | Sinapoyl-feruloyl-triglucoside | 280; 325 | 25.21     | 885.3     | 499        | Nd                      | Nd                      | Nd                       |
| 28 | Sinapoyl-hydroxyferuloyl-diglucoside | 244; 330 | 29.46     | 739.2     | 515        | *                        | *                        | *                        |
| 29 | Isorhamnetin glucoside | 256; 351 | 31.94     | 477.1     | -          | 48.63 ± 0.10             | 30.37 ± 8.49             | 49.87 ± 0.07             |
| 30 | Km glucoside | 269; 330 | 31.49     | 447.0     | -          | Nd                      | Nd                      | Nd                       |
| 31 | Disapoyl-gentiobiose | 240; 330 | 33.32     | 753.2     | 529; 499   | *                        | *                        | *                        |
| 32 | Sinapoyl-feruloyl-gentiobiose | 240; 330 | 34.20     | 723.2     | 529; 499   | *                        | *                        | *                        |
| 33 | Diferuloyldiglucoside | 240; 326 | 34.82     | 693.1     | 499        | *                        | *                        | *                        |
| 34 | Trisapoyl-gentiobiose | 240; 326 | 36.53     | 959.3     | 735; 529   | *                        | *                        | *                        |
| 35 | Feruoyl-disapoyl-gentiobiose | 240; 326 | 37.28     | 929.3     | 705; 511   | *                        | *                        | *                        |

Nd: not detected. * Not quantified in absence of standard. DW: Dry weight.
Table 2. Quantitative performance of the flavonoidic reference materials used in this study.

| Reference Material | Standard Curve | $R^2$ | LoD (µg/mL) | LoQ (µg/mL) | Precision (RSD, %) |
|--------------------|---------------|------|------------|------------|-------------------|
| Qn 3-O-glucoside   | $y = 13,424x + 898.59$ | 0.9939 | 0.013 | 0.043 | 0.41 |
| Is 3-O-glucoside   | $y = 14,948x - 2966.9$ | 0.9963 | 0.048 | 0.159 | 0.34 |
| Km 3-O-glucoside   | $y = 17,660x - 10,681$ | 0.9963 | 0.021 | 0.072 | 0.36 |

Flavonoid determination in the three *B. juncea* breeding lines is reported in Figure 2, and Table 1 and Tables S1–S3. Among all samples tested, the flowers presented the highest flavonoid content (Sample A, 1124.69 µg/100 mg dry weight (DW); Sample B, 623.78 µg/100 mg DW; Sample C, 876.45 µg/100 mg DW). Considering the three different flavonoid classes, Is derivatives were the most abundant in all the three cultivars: Sample A (732.24 µg/100 mg), Sample C (694.30 µg/100 mg), and Sample B (464.02 µg/100 mg). Notably, Is 3,7 diglucoside turned out to be the most abundant flavonoid in each cultivar investigated (683.62 µg/100 mg DW in Sample A, 433.65 µg/100 mg DW in Sample B, and 644.43 µg/100 mg DW in Sample C), followed by Km 3-feruloylsophoroside-7-glucoside (72.18 µg/100 mg DW) in Sample A, Qn 3-diglucoside (31.12 µg/100 mg DW) in Sample B, and Is glycoside (49.87 µg/100 mg DW) in Sample C.

2.2. Determination of the Volatile Content of *B. juncea* Accessions Using GC-FID/MS

Recently, there has been more interest in the determination of organic compounds from plants and plant material, in order to evaluate their potential biological activity [29]. GC–MS is the most ideal technique for qualitative analysis of volatile and semi volatile bioactive compounds [33]. As a general rule, when using an MS detector, operating in scan mode, % abundance (% area) should not be employed, since the linear dynamic range of this detector is narrow. On the other hand, quantification is possible in Selected Ion Monitoring (SIM) mode, with selected ions calibrated with external standards. In this work, all samples were analyzed by GC-MS for compound identification, and FID for reliable peak quantification [34,35]. Figure 3 reports the GC-MS chromatograms of the volatile content of leaves of the three *Brassica* cultivars investigated, collected at the edible salad phase, when plants are about 15–20 cm high, while Table 3 reports the correspondent quantitative determination by GC-FID. It can be appreciated how all of the extracts from the three *B. juncea* accessions are characterized by mixtures of different types of organic compounds. The identified compounds include alcohols, aldehydes, esters, fatty acids, ketones, sulfur compounds, and other compounds. The major volatile constituents of *B. juncea* L. leaf extracts in the three cultivars were benzenepropanenitrile (34.94% in Sample B, 8.16% in Sample A, 6.24% in Sample C), followed by benzofuranone (8.54% in Sample A, 6.32% in Sample C, 3.64% in Sample B), and phytone (3.77% in Sample B, 2.85% in Sample A, 1.01% in Sample C) [29,36]. It is worth mentioning that the concentrations and profiles of different compounds in *Brassica* genus vary according to cultivar vegetable parts and the development stage of the plant [37]. Among alcohols, in all cultivars, the most abundant one was represented by phenethyl alcohol (4.16% in Sample A, 2.68% in Sample C, 2.39% in Sample B). Methyl benzoate (0.42% in Sample B, 0.27% in Sample C, 0.22% in Sample A) was the ester with the highest content, whereas the most abundant aldehydes, responsible for characteristic aromas [38], were represented by *n*-Nonanal in Sample B (1.24%) and Sample A (1.13%), and safranal in Sample C (1.80%). Among fatty acids (Z,Z,Z)-9,12,15-Octadecatrienoic acid was the one with major content and was detected only in Sample C (0.51%) and Sample A (0.11%).
Figure 2. Polyphenol total content in (µg/100 mg DW) of different portions of three accessions of B. juncea. (A) B. juncea ISCI 99; (B) B. juncea ISCI Top; (C) B. juncea ISCI “Broad-leaf”.

- Quercetin derivative total content (µg/100mg)
- Kaempferol derivative total content (µg/100mg)
- Isoflavonoid derivative total content (µg/100mg)
2.2. Determination of the Volatile Content of B. Juncea Accessions Using GC-FID/MS

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Interestingly, selected sulfur compounds (isothiocyanates, ITC) were also detected as they belong to major secondary metabolites of the Brassicaceae family. Among them, 2-propenyl-isothiocyanate was the major ITC derived from aliphatic glucosinolates, and was detected in all cultivars (2.07% in Sample B, 0.74% in Sample A, 0.61% in Sample C). The content of the ITCs may vary, depending on the plant species studied, side-chain substitutions, cellular pH, and iron concentration [39,40].

**Figure 3.** GC-MS analysis of the chemical components from extracts of the leaves of the three *Brassica* cultivars investigated, collected at the edible salad phase. (A) *B. juncea* ISCI 99; (B) *B. juncea* ISCI Top; (C) *B. juncea* ISCI “Broad-leaf”.
Table 3. Quantitative determination by GC-FID of the chemical components of the leaves of the three Brassica accessions investigated. FFNSC: Flavor and Fragrance Natural and Synthetic Compounds; LRI: Linear Retention Indices.

| N.  | Compounds Name              | Lib. Name | Id. Method | Similarity | LRI Lib | LRI Exp | Sample A | Sample B | Sample C |
|-----|-----------------------------|-----------|------------|------------|---------|---------|----------|----------|----------|
| 1   | Ethanoic acid               | FFNSC 4.0 | MS, LRI    | 98         | 661     | 665     | 4.83     | 4.18     | 4.66     |
| 2   | 2-Butenenitrile             | W11N17    | MS, LRI    | 90         | 664     | 675     | 0.27     |          | 0.44     |
| 3   | Hydroxyacetone              | FFNSC 4.0 | MS, LRI    | 94         | 682     | 684     | 0.07     | Nd       | 0.15     |
| 4   | 3-hydroxy-Pentene           | FFNSC 4.0 | MS, LRI    | 92         | 691     | 691     | 0.06     | Nd       | 0.20     |
| 5   | 3-Pentenone                 | FFNSC 4.0 | MS, LRI    | 93         | 677     | 693     | Nd       | 0.45     | Nd       |
| 6   | n-Pentanal                  | FFNSC 4.0 | MS, LRI    | 94         | 696     | 701     | 0.52     | 0.41     | 0.40     |
| 7   | methyl-Thiocyanate          | FFNSC 4.0 | MS, LRI    | 91         | 710     | 711     | Nd       | 0.54     | Nd       |
| 8   | Propionic acid              | FFNSC 4.0 | MS, LRI    | 91         | 698     | 711     | 0.53     | 0.21     | 0.38     |
| 9   | Acetoin                     | FFNSC 4.0 | MS, LRI    | 90         | 716     | 716     | Nd       | Nd       | 0.05     |
| 10  | Methyl propenyl ketone      | FFNSC 4.0 | MS, LRI    | 91         | 733     | 737     | 0.01     | 0.13     | 0.01     |
| 11  | dimethyl-Disulfide          | FFNSC 4.0 | MS, LRI    | 93         | 722     | 741     | 0.08     | Nd       | 0.01     |
| 12  | (E)-2-Pentenal              | FFNSC 4.0 | MS, LRI    | 92         | 751     | 753     | 0.17     | 0.31     | 0.17     |
| 13  | Isobutyric acid             | FFNSC 4.0 | MS, LRI    | 91         | 774     | 758     | 0.07     | 0.06     | Nd       |
| 14  | Senecionitrile              | FFNSC 4.0 | MS, LRI    | 93         | 756     | 760     | Nd       | Nd       | 0.05     |
| 15  | Pentyl alcohol              | FFNSC 4.0 | MS, LRI    | 96         | 763     | 767     | 0.13     | 0.07     | 0.05     |
| 16  | 2,3-Butadienol              | FFNSC 4.0 | MS, LRI    | 96         | 788     | 788     | 0.58     | 0.20     | 1.80     |
| 17  | n-Hexanal                   | FFNSC 4.0 | MS, LRI    | 98         | 801     | 802     | 0.36     | 0.37     | 0.34     |
| 18  | 3-Butenoic acid             | W11N17    | MS         | 91         | -       | 806     | Nd       | 0.30     | Nd       |
| 19  | Butyric acid                | FFNSC 4.0 | MS, LRI    | 93         | 818     | 808     | 0.10     | Nd       | Nd       |
| 20  | 2-methyl-Pyrazine           | FFNSC 4.0 | MS, LRI    | 95         | 820     | 828     | 0.07     | 0.07     | Nd       |
Table 3. Cont.

| N. | Compounds Name               | Lib. Name | Id. Method | Similarity | LRI Lib | LRI Exp | Sample A | Sample B | Sample C |
|----|------------------------------|-----------|------------|------------|---------|---------|----------|----------|----------|
| 21 | Furfural                     | FFNSC 4.0 | MS, LRI    | 92         | 845     | 831     | 0.10     | 0.29     | 0.15     |
| 22 | Sclerosol                    | FFNSC 4.0 | MS, LRI    | 96         | 827     | 841     | 0.50     | 0.04     | 0.15     |
| 23 | (E)-2-Hexenal                | FFNSC 4.0 | MS, LRI    | 95         | 850     | 852     | 0.50     | 1.76     | 0.57     |
| 24 | (Z)-3-Hexenol                | FFNSC 4.0 | MS, LRI    | 95         | 853     | 854     | 0.61     | 0.51     | 0.33     |
| 25 | Isovaleric acid              | FFNSC 4.0 | MS, LRI    | 94         | 842     | 860     | 1.02     | 0.07     | 0.17     |
| 26 | 2-methyl-Butyric acid        | FFNSC 4.0 | MS, LRI    | 89         | 881     | 867     | 0.73     | 0.21     | 0.29     |
| 27 | allyl-Thiocyanate            | FFNSC 4.0 | MS, LRI    | 94         | 865     | 869     | 0.17     | 0.24     | 0.10     |
| 28 | n-Hexanol                    | FFNSC 4.0 | MS, LRI    | 91         | 867     | 869     | 0.26     | 0.20     | 0.03     |
| 29 | 2-propenyl-Isothiocyanate    | FFNSC 4.0 | MS, LRI    | 96         | 876     | 880     | 0.74     | 2.07     | 0.61     |
| 30 | 1-(3-methylenecyclopentyl)-Ethanone | W11N17 | MS | 91 | - | 884 | 0.47 | 0.23 | 0.34 |
| 31 | n-Heptanal                   | FFNSC 4.0 | MS, LRI    | 97         | 906     | 902     | Nd       | 0.17     | 0.16     |
| 32 | n-Pentanoic acid             | FFNSC 4.0 | MS, LRI    | 92         | 911     | 903     | 0.90     | 0.20     | 1.27     |
| 33 | 3-methyl-Crotonic acid       | FFNSC 4.0 | MS, LRI    | 91         | 907     | 905     | 0.12     | Nd       | 0.02     |
| 34 | 2-butoxy-Ethanol             | W11N17   | MS, LRI    | 95         | 906     | 907     | 0.08     | 0.06     | Nd       |
| 35 | 2-acetyl-Furan               | FFNSC 4.0 | MS, LRI    | 94         | 913     | 910     | 0.10     | 0.14     | Nd       |
| 36 | 2(SH)-Furanone               | FFNSC 4.0 | MS, LRI    | 91         | 907     | 911     | Nd       | Nd       | 0.03     |
| 37 | γ-Butyrolactone              | FFNSC 4.0 | MS, LRI    | 95         | 910     | 912     | 0.89     | 0.45     | 0.30     |
| 38 | 2,5-dimethyl-Pyrazine        | FFNSC 4.0 | MS, LRI    | 92         | 912     | 916     | 0.33     | 0.41     | 0.09     |
| 39 | 1,1'-sulfonylbis-Methane     | W11N17   | MS, LRI    | 95         | 922     | 916     | 0.15     | 0.10     | Nd       |
| 40 | methyl-Hexanoate             | FFNSC 4.0 | MS, LRI    | 92         | 922     | 924     | 0.07     | 0.03     | 0.02     |
| 41 | sec-butyl-Isothiocyanate     | FFNSC 4.0 | MS, LRI    | 96         | 929     | 929     | 0.10     | 0.03     | 0.05     |
| 42 | 2,7-dimethyl-Oxepine         | W11N17   | MS, LRI    | 88         | 944     | 931     | 0.03     | 0.02     | 0.01     |
Table 3. Cont.

| N. | Compounds Name                        | Lib. Name | Id. Method | Similarity | LRI Lib | LRI Exp | Area % | Area % | Area % |
|----|--------------------------------------|-----------|------------|------------|---------|---------|--------|--------|--------|
| 43 | 1-butoxy-2-Propanol                  | W11N17    | MS, LRI    | 91         | 945     | 938     | 0.02   | 0.01   | Nd     |
| 44 | dihydro-3-methyl-2(3H)-Furanone       | W11N17    | MS, LRI    | 94         | 941     | 948     | 0.05   | 0.02   | Nd     |
| 45 | γ-Pentalactone                        | FFNSC 4.0 | MS, LRI    | 91         | 954     | 953     | 0.16   | 0.02   | 0.06   |
| 46 | (E)-2-Heptenal                        | FFNSC 4.0 | MS, LRI    | 95         | 956     | 956     | 0.05   | 0.11   | 0.41   |
| 47 | Benzaldehyde                          | FFNSC 4.0 | MS, LRI    | 98         | 960     | 963     | 0.54   | 0.66   | 0.23   |
| 48 | N-2-propenyl-Acetamide                | W11N17    | MS         | 94         | -       | 964     | 0.11   | Nd     | 0.07   |
| 49 | Dimethyl trisulfide                   | FFNSC 4.0 | MS, LRI    | 97         | 969     | 970     | 0.12   | 0.12   | 0.04   |
| 50 | n-Heptanol                            | FFNSC 4.0 | MS, LRI    | 92         | 970     | 970     | 0.04   | 0.02   | 0.02   |
| 51 | 3,5,5-trimethyl-2-Hexene              | W11N17    | MS, LRI    | 92         | 985     | 974     | 0.33   | 0.14   | 0.38   |
| 52 | 1-Octen-3-one                         | FFNSC 4.0 | MS, LRI    | 91         | 973     | 977     | 0.03   | 0.03   | 0.12   |
| 53 | Vinyl amyl carbinol                   | FFNSC 4.0 | MS, LRI    | 94         | 978     | 979     | Nd     | 0.10   | Nd     |
| 54 | 3-butenyl-Isothiocyanate              | FFNSC 4.0 | MS, LRI    | 91         | 978     | 980     | Nd     | Nd     | 0.70   |
| 55 | 6-methyl-Hept-5-en-2-one              | FFNSC 4.0 | MS, LRI    | 95         | 986     | 985     | 0.32   | 0.29   | 0.31   |
| 56 | 2-pentyl-Furan                        | FFNSC 4.0 | MS, LRI    | 92         | 991     | 989     | 0.34   | 0.15   | Nd     |
| 57 | 2,3,5-trimethyl-Pyrazine              | FFNSC 4.0 | MS, LRI    | 90         | 1002    | 1001    | 1.08   | 0.30   | Nd     |
| 58 | 2-ethyl-5,5-methyl-Pyrazine           | FFNSC 4.0 | MS, LRI    | 88         | 1005    | 1001    | Nd     | 0.19   | Nd     |
| 59 | 2-ethyl-6-methyl-Pyrazine             | FFNSC 4.0 | MS, LRI    | 91         | 1000    | 1002    | 0.47   | Nd     | Nd     |
| 60 | n-Octanal                             | FFNSC 4.0 | MS, LRI    | 91         | 1006    | 1004    | 0.45   | 0.14   | 1.06   |
| 61 | (E,E)-2,4-Heptadienial                | FFNSC 4.0 | MS, LRI    | 95         | 1013    | 1011    | 0.04   | 0.59   | 0.82   |
| 62 | n-Hexanoic acid                      | FFNSC 4.0 | MS, LRI    | 97         | 997     | 1013    | 2.45   | 0.64   | 3.09   |
| 63 | 2-ethenyl-6-methyl-pyrazine           | W11N17    | MS, LRI    | 82         | 1031    | 1020    | 0.36   | 0.54   | Nd     |
| 64 | (Z)-3-Hexenoic acid                  | FFNSC 4.0 | MS, LRI    | 94         | 996     | 1022    | 0.47   | 0.74   | 1.07   |
| 65 | 2-ethyl-Hexanol                      | FFNSC 4.0 | MS, LRI    | 88         | 1030    | 1030    | 0.33   | 0.20   | Nd     |
## Table 3. Cont.

| N. | Compounds Name                  | Lib. Name | Id. Method | Similarity | LRI Lib | LRI Exp | Sample A | Sample B | Sample C |
|----|---------------------------------|-----------|------------|------------|---------|---------|----------|----------|----------|
| 66 | Benzyl alcohol                  | FFNSC 4.0 | MS, LRI    | 90         | 1040    | 1037    | 0.41     | 0.22     | 0.17     |
| 67 | (E)-2-Hexenoic acid             | FFNSC 4.0 | MS, LRI    | 90         | 1036    | 1039    | 0.34     | 0.32     | 0.05     |
| 68 | Oct-3-en-2-one                  | FFNSC 4.0 | MS, LRI    | 93         | 1036    | 1040    | 0.22     | 0.18     | 0.11     |
| 69 | Phenylacetaldehyde              | FFNSC 4.0 | MS, LRI    | 97         | 1045    | 1043    | Nd       | 0.51     | 0.12     |
| 70 | γ-Hexalactone                   | FFNSC 4.0 | MS, LRI    | 98         | 1060    | 1054    | 0.68     | 0.11     | 0.39     |
| 71 | (E)-2-Octenal                   | FFNSC 4.0 | MS, LRI    | 90         | 1058    | 1061    | 0.22     | 0.11     | 0.20     |
| 72 | α-Phenylethanol                 | FFNSC 4.0 | MS, LRI    | 94         | 1064    | 1063    | 0.10     | 0.05     | 0.07     |
| 73 | Acetophenone                    | FFNSC 4.0 | MS, LRI    | 91         | 1068    | 1065    | Nd       | 0.12     | 0.12     |
| 74 | 2-acetyl-Pyrrole                | FFNSC 4.0 | MS, LRI    | 94         | 1060    | 1068    | 0.30     | 0.15     | 0.46     |
| 75 | (E,E)-3,5-Octadien-2-one        | W11N17    | MS, LRI    | 90         | 1073    | 1071    | 1.07     | 0.53     | 1.16     |
| 76 | n-Octanol                       | FFNSC 4.0 | MS, LRI    | 92         | 1076    | 1073    | 0.35     | 0.19     | 0.44     |
| 77 | p-Cresol                        | FFNSC 4.0 | MS, LRI    | 89         | 1072    | 1077    | Nd       | Nd       | 0.12     |
| 78 | 2-Pyrrolidone                   | FFNSC 4.0 | MS, LRI    | 92         | 1070    | 1078    | 0.46     | 0.02     | Nd       |
| 79 | 2-ethyl-3,6-dimethyl-pyrazine   | FFNSC 4.0 | MS, LRI    | 87         | 1079    | 1080    | 0.15     | 0.05     | Nd       |
| 80 | n-Heptanoic acid                | FFNSC 4.0 | MS, LRI    | 88         | 1116    | 1092    | 0.90     | 0.28     | 1.06     |
| 81 | 3,5-Octadien-2-one              | W11N17    | MS, LRI    | 90         | 1091    | 1095    | 1.15     | 0.56     | 0.60     |
| 82 | n-Nonanal                       | FFNSC 4.0 | MS, LRI    | 95         | 1107    | 1104    | 1.13     | 1.24     | 0.93     |
| 83 | 2,6-dimethyl-Cyclohexanol       | W11N17    | MS, LRI    | 89         | 1112    | 1113    | 2.19     | 0.27     | 0.58     |
| 84 | Phenethyl alcohol               | FFNSC 4.0 | MS, LRI    | 95         | 1113    | 1117    | 4.16     | 2.39     | 2.68     |
| 85 | methyl-Octanoate                | FFNSC 4.0 | MS, LRI    | 92         | 1125    | 1124    | 0.20     | Nd       | 0.05     |
| 86 | Isophorone                      | FFNSC 4.0 | MS, LRI    | 88         | 1123    | 1126    | 0.41     | 0.08     | 0.11     |
| 87 | 2-Heptenoic acid                | W11N17    | MS          | 93         | -       | 1130    | Nd       | Nd       | 0.24     |
| 88 | 2-nitro-Phenol                  | W11N17    | MS, LRI    | 96         | 1135    | 1131    | Nd       | 0.16     | Nd       |
| N.  | Compounds Name                          | Lib. Name | Id. Method | Similarity | LRI Lib | LRI Exp | Area % | Area % | Area %  |
|-----|----------------------------------------|-----------|------------|------------|---------|---------|--------|--------|---------|
| 89  | Benzene acetonitrile                    | FFNSC 4.0 | MS, LRI   | 91         | 1138    | 1138    | Nd     | 0.09   | Nd      |
| 90  | Oxophorone                              | FFNSC 4.0 | MS, LRI   | 90         | 1148    | 1147    | 0.48   | 0.18   | 0.43    |
| 91  | 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one | W11N17    | MS, LRI   | 93         | 1151    | 1148    | 0.20   | 0.16   | 0.49    |
| 92  | (E,Z)-2,6-Nonadienal                    | FFNSC 4.0 | MS, LRI   | 93         | 1153    | 1152    | Nd     | 0.15   | 0.48    |
| 93  | γ-Heptalactone                          | FFNSC 4.0 | MS, LRI   | 91         | 1155    | 1153    | 0.05   | Nd     | Nd      |
| 94  | Menthone                                | FFNSC 4.0 | MS, LRI   | 90         | 1158    | 1156    | Nd     | 0.05   | Nd      |
| 95  | (E)-2-Nonenal                           | FFNSC 4.0 | MS, LRI   | 90         | 1163    | 1159    | Nd     | 0.11   | 0.26    |
| 96  | 2,2,6-trimethyl-1,4-Cyclohexanedione     | W11N17    | MS, LRI   | 89         | 1183    | 1172    | 0.22   | Nd     | Nd      |
| 97  | 2,4-dimethyl-Benzaldehyde               | FFNSC 4.0 | MS, LRI   | 91         | 1190    | 1175    | 0.42   | 0.16   | Nd      |
| 98  | Menthol                                 | FFNSC 4.0 | MS, LRI   | 97         | 1184    | 1178    | 0.47   | 0.22   | Nd      |
| 99  | n-Octanoic acid                         | FFNSC 4.0 | MS, LRI   | 95         | 1192    | 1192    | 2.33   | 1.25   | 6.63    |
| 100 | n-Dodecane                              | FFNSC 4.0 | MS, LRI   | 97         | 1200    | 1200    | 0.10   | 0.13   | Nd      |
| 101 | Safranal                                | FFNSC 4.0 | MS, LRI   | 97         | 1201    | 1203    | 0.61   | 1.01   | 1.15    |
| 102 | n-Decanal                               | FFNSC 4.0 | MS, LRI   | 96         | 1208    | 1207    | 0.29   | 0.07   | 1.80    |
| 103 | β-Cyclocitrinal                         | FFNSC 4.0 | MS, LRI   | 90         | 1223    | 1224    | 0.74   | 0.05   | 0.34    |
| 104 | 3-ethyl-4-methyl-1H-Pyrrole-2,5-dione   | W11N17    | MS, LRI   | 92         | 1239    | 1239    | 0.88   | Nd     | 1.00    |
| 105 | Benzenepropanenitrile                   | W11N17    | MS, LRI   | 98         | 1244    | 1244    | 8.16   | 34.94  | 6.24    |
| 106 | 2-Phenethyl acetate                     | FFNSC 4.0 | MS, LRI   | 95         | 1257    | 1257    | 0.20   | 0.10   | 0.24    |
| 107 | β-Cyclohomocitral                       | FFNSC 4.0 | MS, LRI   | 90         | 1256    | 1257    | Nd     | 0.12   | Nd      |
| 108 | γ-Octalactone                           | FFNSC 4.0 | MS, LRI   | 95         | 1263    | 1259    | 0.14   | Nd     | Nd      |
| 109 | Benzeneacetic acid                      | W11N17    | MS, LRI   | 88         | 1262    | 1259    | Nd     | Nd     | 0.09    |
| 110 | 2-phenyl-Crotonaldehyde                 | FFNSC 4.0 | MS, LRI   | 88         | 1272    | 1273    | 0.29   | 0.22   | Nd      |
Table 3. Cont.

| N.  | Compounds Name                  | Lib. Name | Id. Method | Similarity | LRI Lib | LRI Exp | Sample A | Sample B | Sample C |
|-----|--------------------------------|-----------|------------|------------|----------|---------|----------|----------|----------|
| 111 | 3,3-dimethyl-2,7-Octanedione    | W11N17    | MS, LRI   | 88         | 1290     | 1277    | 1.46     | 0.45     | 0.96     |
| 112 | n-Nonanoic acid                | FFNSC 4.0 | MS, LRI   | 96         | 1289     | 1280    | 0.48     | 0.31     | 1.18     |
| 113 | Menthyl acetate                | FFNSC 4.0 | MS, LRI   | 92         | 1290     | 1289    | Nd       | 0.05     | Nd       |
| 114 | Isobornyl acetate              | FFNSC 4.0 | MS, LRI   | 95         | 1287     | 1291    | 0.16     | Nd       | Nd       |
| 115 | (E)-Cinnaminitrile             | FFNSC 4.0 | MS, LRI   | 96         | 1294     | 1295    | 0.14     | 0.15     | 0.09     |
| 116 | n-Tridecane                    | FFNSC 4.0 | MS, LRI   | 94         | 1300     | 1298    | Nd       | 0.07     | Nd       |
| 117 | 4-vinyl-Guaiacol               | FFNSC 4.0 | MS, LRI   | 92         | 1309     | 1314    | 0.09     | 0.31     | 0.43     |
| 118 | γ-Nonalactone                  | FFNSC 4.0 | MS, LRI   | 94         | 1362     | 1364    | 0.36     | 0.10     | 0.41     |
| 119 | n-Decanoic acid                | FFNSC 4.0 | MS, LRI   | 94         | 1398     | 1368    | 0.45     | 0.24     | 2.12     |
| 120 | 2,6,10-trimethyl-Dodecane      | W11N17    | MS, LRI   | 91         | 1366     | 1376    | 0.09     | 0.37     | Nd       |
| 121 | α-Copaene                      | FFNSC 4.0 | MS, LRI   | 90         | 1375     | 1381    | 0.12     | Nd       | Nd       |
| 122 | 1-Tetradecene                  | FFNSC 4.0 | MS, LRI   | 94         | 1392     | 1390    | Nd       | 0.08     | Nd       |
| 123 | n-Tetradecane                  | FFNSC 4.0 | MS, LRI   | 95         | 1400     | 1400    | 0.42     | 0.42     | 0.70     |
| 124 | Vanillin                       | FFNSC 4.0 | MS, LRI   | 88         | 1394     | 1401    | 0.21     | 0.09     | 0.12     |
| 125 | 6,10-dimethyl-2-Undecanone     | W11N17    | MS, LRI   | 94         | 1408     | 1403    | 0.35     | 0.21     | 0.19     |
| 126 | (E)-α-Ionone                   | FFNSC 4.0 | MS, LRI   | 90         | 1421     | 1422    | Nd       | 0.13     | Nd       |
| 127 | (E)-Caryophyllene              | FFNSC 4.0 | MS, LRI   | 95         | 1424     | 1427    | 0.96     | Nd       | Nd       |
| 128 | β-Gurjunene                    | FFNSC 4.0 | MS, LRI   | 93         | 1437     | 1439    | 0.57     | Nd       | Nd       |
| 129 | (E)-Geranylacetone             | FFNSC 4.0 | MS, LRI   | 94         | 1450     | 1449    | 0.47     | 0.73     | 0.46     |
| 130 | 2,6,10-Trimethyltridecane       | W11N17    | MS, LRI   | 93         | 1449     | 1461    | 0.26     | 0.18     | Nd       |
| 131 | 2,6-bis(1,1-dimethylethyl)-2,5-| W11N17    | MS, LRI   | 90         | 1471     | 1461    | Nd       | 0.17     | Nd       |
Table 3. Cont.

| N.  | Compounds Name                                                                 | Lib. Name | Id. Method | Similarity | LRI Lib | LRI Exp | Sample A | Sample B | Sample C |
|-----|-------------------------------------------------------------------------------|-----------|------------|------------|---------|---------|----------|----------|----------|
| 132 | 2,6-Di-tert-butyl-4-hydroxy-4-methylcyclohexa-2,5-dien-1-one                   | W11N17    | MS, LRI    | 91         | 1475    | 1463    | 0.31     | Nd       | 0.63     |
| 133 | Phenylethyl isothiocyanate                                                   | FFNSC 4.0 | MS, LRI    | 95         | 1464    | 1470    | 0.32     | 0.25     | 0.79     |
| 134 | 1-chloro-Dodecane                                                            | W11N17    | MS, LRI    | 92         | 1469    | 1471    | Nd       | 0.10     | Nd       |
| 135 | Dodecanol                                                                    | FFNSC 4.0 | MS, LRI    | 94         | 1476    | 1477    | 0.23     | Nd       | 0.36     |
| 136 | 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl but-3-en-2-one)                        | W11N17    | MS, LRI    | 93         | 1485    | 1482    | 0.41     | 0.20     | 0.23     |
| 137 | (E)-,β-Ionone                                                                | FFNSC 4.0 | MS, LRI    | 93         | 1482    | 1485    | 2.53     | 2.44     | 0.89     |
| 138 | Ionone epoxide                                                               | FFNSC 4.0 | MS, LRI    | 90         | 1483    | 1488    | 1.59     | 1.16     | 0.65     |
| 139 | 1-Pentadecane                                                                | W11N17    | MS, LRI    | 96         | 1492    | 1493    | 0.91     | 0.19     | 0.56     |
| 140 | β-Selinene                                                                   | FFNSC 4.0 | MS, LRI    | 95         | 1492    | 1498    | 1.10     | Nd       | Nd       |
| 141 | n-Pentadecane                                                                | FFNSC 4.0 | MS, LRI    | 96         | 1500    | 1500    | 0.26     | 0.15     | 0.35     |
| 142 | Unknown                                                                      | -         | -          | -          | -       | -       | -        | -        | -        |
| 143 | 2,4-bis(1,1-dimethylethyl)-Phenol                                             | W11N17    | MS, LRI    | 92         | 1513    | 1507    | Nd       | 0.09     | Nd       |
| 144 | 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-Benzofuranone                     | W11N17    | MS, LRI    | 97         | 1532    | 1544    | 8.54     | 3.64     | 6.32     |
| 145 | n-Dodecanoic acid                                                            | FFNSC 4.0 | MS, LRI    | 92         | 1581    | 1564    | Nd       | Nd       | 0.29     |
| 146 | 3-methyl-Pentadecane                                                         | W11N17    | MS, LRI    | 90         | 1570    | 1571    | 0.07     | 0.10     | 0.19     |
| 147 | 2,2,4-Trimethyl-1,3-pentanediol disobutyrate                                 | W11N17    | MS, LRI    | 91         | 1588    | 1585    | Nd       | 0.17     | Nd       |
| 148 | 2-[[1-(4-hydroxy-4-methylpentyl), 3-cyclohexen-1-yl][methylene]amino], methyl-Benzoate | FFNSC 4.0 | MS, LRI    | 97         | 1589    | 1586    | 0.22     | 0.42     | 0.27     |
| 149 | n-Hexadecene                                                                | FFNSC 4.0 | MS, LRI    | 86         | 1593    | 1593    | 0.15     | 0.28     | 0.23     |
Table 3. Cont.

| N.  | Compounds Name                | Lib. Name | Id. Method | Similarity | LRI Lib | LRI Exp | Sample A | Sample B | Sample C |
|-----|-------------------------------|-----------|------------|------------|---------|---------|----------|----------|----------|
| 150 | *n*-Hexadecane                | FFNSC 4.0 | MS, LRI   | 96         | 1600    | 1601    | 0.79     | 0.38     | 0.72     |
| 151 | 1-butylheptyl-Benzene         | W11N17   | MS, LRI   | 91         | 1632    | 1633    | 0.09     | 0.06     | 0.16     |
| 152 | Benzophenone                  | FFNSC 4.0 | MS, LRI   | 96         | 1627    | 1639    | 0.10     | 0.02     | 0.26     |
| 153 | 1-propyloctyl-Benzene         | W11N17   | MS, LRI   | 93         | 1643    | 1640    | 0.10     | 0.12     | 0.19     |
| 154 | 1,1′-oxybis-Octane            | W11N17   | MS, LRI   | 91         | 1659    | 1660    | Nd       | 0.27     | 0.19     |
| 155 | β-Eudesmol                    | FFNSC 4.0 | MS, LRI   | 89         | 1656    | 1666    | 0.58     | Nd       | Nd       |
| 156 | (Z,Z,Z)-1,8,11,14-Heptadecatetraene | W11N17 | MS, LRI   | 93         | 1664    | 1667    | 0.43     | 0.19     | 0.41     |
| 157 | *n*-Heptadecane               | FFNSC 4.0 | MS, LRI   | 94         | 1700    | 1700    | 0.10     | 0.08     | 0.32     |
| 158 | Tetradecanoic acid            | FFNSC 4.0 | MS, LRI   | 93         | 1773    | 1761    | Nd       | Nd       | 0.55     |
| 159 | 1-ethyldecyl-Benzene          | W11N17   | MS, LRI   | 90         | 1766    | 1763    | 0.02     | 0.04     | Nd       |
| 160 | 3-methyl-Heptadecane          | W11N17   | MS, LRI   | 92         | 1771    | 1769    | Nd       | 0.13     | 0.15     |
| 161 | 6-Hydroxy-4,4,7a-trimethyl-5,6,7a-tetrahydrobenzofuran-2(4H)-one | W11N17 | MS, LRI   | 90         | 1784    | 1778    | 0.02     | Nd       | 0.18     |
| 162 | 1-Octadecene                  | FFNSC 4.0 | MS, LRI   | 95         | 1793    | 1793    | 0.04     | 0.19     | 0.03     |
| 163 | *n*-Octadecane                | FFNSC 4.0 | MS, LRI   | 95         | 1800    | 1800    | 0.04     | 0.09     | 0.04     |
| 164 | Neophytadiene                 | FFNSC 4.0 | MS, LRI   | 90         | 1836    | 1837    | 0.03     | Nd       | 0.04     |
| 165 | Phytone                       | FFNSC 4.0 | MS, LRI   | 91         | 1841    | 1839    | 2.85     | 3.77     | 1.01     |
| 166 | *n*-Nonadecane                | FFNSC 4.0 | MS, LRI   | 90         | 1900    | 1897    | Nd       | 0.01     | Nd       |
| 167 | methyl-7,10,13-hexadecatrienoate | W11N17 | MS          | 91 -       | 1897    | 0.09     | Nd       | 0.27     |
| 168 | 3-Methyl-2-(3,7,11-trimethyldecyl) furan | W11N17 | MS          | 92 -       | 1913    | 0.05     | 0.72     | Nd       |
| 169 | methyl-Hexadecanoate          | FFNSC 4.0 | MS, LRI   | 94         | 1925    | 1926    | 0.09     | 0.05     | 0.06     |
| N. | Compounds Name                                | Lib. Name | Id. Method | Similarity | LRI Lib | LRI Exp | Area % | Area % | Area % |
|----|----------------------------------------------|-----------|------------|------------|---------|---------|--------|--------|--------|
| 170| (Z,Z,Z)-7,10,13-Hexadecatrienoic acid       | W11N17    | MS, LRI    | 92         | 1945    | 1938    | 0.11   | Nd     | Nd     |
| 171| n-Hexadecanoic acid                         | FFNSC 4.0 | MS, LRI    | 91         | 1977    | 1963    | 0.41   | 0.86   | 1.16   |
| 172| n-Eicosane                                   | FFNSC 4.0 | MS, LRI    | 94         | 2000    | 1997    | Nd     | 0.01   | Nd     |
| 173| methyl-Linoleate                             | FFNSC 4.0 | MS, LRI    | 91         | 2093    | 2094    | 0.02   | Nd     | 0.01   |
| 174| n-Heneicosane                                | FFNSC 4.0 | MS, LRI    | 90         | 2100    | 2097    | Nd     | 0.02   | Nd     |
| 175| methyl-Linolenate                            | FFNSC 4.0 | MS, LRI    | 93         | 2098    | 2100    | 0.07   | 0.00   | 0.05   |
| 176| (Z,Z)-9,12-Octadecadienoic acid             | W11N17    | MS, LRI    | 91         | 2133    | 2135    | 0.01   | Nd     | 0.10   |
| 177| (Z,Z,Z)-9,12,15-Octadecatrienoic acid       | W11N17    | MS, LRI    | 93         | 2139    | 2140    | 0.11   | Nd     | 0.51   |
| 178| n-Tetracosane                                | FFNSC 4.0 | MS, LRI    | 89         | 2400    | 2397    | Nd     | 0.00   | Nd     |
| 179| n-Pentacosane                                | FFNSC 4.0 | MS, LRI    | 92         | 2500    | 2496    | Nd     | 0.01   | 0.01   |
| 180| n-Heptacosane                                | FFNSC 4.0 | MS, LRI    | 92         | 2700    | 2700    | 0.01   | 0.02   | 0.03   |
|    | **Total Identified**                         |           |            |            |         |         | **83.15** | **86.37** | **75.53** |
Interestingly, selected sulfur compounds (isothiocyanates, ITC) were also detected as they belong to major secondary metabolites of the Brassicaceae family. Among them, 2-propenyl-isothiocyanate was the major ITC derived from aliphatic glucosinolates, and was detected in all cultivars (2.07% in Sample B, 0.74% in Sample A, 0.61% in Sample C). The content of the ITCs may vary, depending on the plant species studied, side-chain substitutions, cellular pH, and iron concentration [39,40].

2.3. Chemical Characterization of *B. juncea* DSMs

The chemical characterization of *B. juncea* DSMs is summarized in Table 4. Proteins were the main component of *B. juncea* DSMs; the results for Sample A are very similar to those of plants belonging to the Fabaceae family, such as soy [41]. The glucosinolate (GSL) analysis accounted for a maximum total of 205.4 µmol/g in Sample A and revealed a very similar profile in GSLs. Sample A and Sample B are characterized in particular by 97% of 2-propenyl GSL, 5% of but-3-enyl GSL, and only 2–3% of 4-hydroxy-3-indolymethyl GSL. The Sample C selection is characterized by a higher percentage (%) in but-3-enyl GSL, in comparison to the other two cultivars.

| DSM       | Moisture (% DW) | Oil Content (% DW) | Proteins (% DW) | Glucosinolates (µmol/g) | 4-hydroxy-3-indolymethylGSL (µmol/g) |
|-----------|-----------------|--------------------|----------------|-------------------------|-------------------------------------|
| Sample A  | 8.3 ± 0.1       | 11.1 ± 0.1         | 44.0 ± 0.5     | 2.4 ± 0.2               | 200 ± 3                             |
| Sample B  | 8.8 ± 0.5       | 15.7 ± 0.2         | 37.4 ± 0.2     | 2.1 ± 0.1               | 137 ± 3                             |
| Sample C  | 8.3 ± 0.3       | 16.9 ± 0.1         | 36.8 ± 0.2     | 4.9 ± 0.2               | 148 ± 2                             |

3. Materials and Methods

3.1. Chemical and Reagents

LC-MS grade water, methanol, acetonitrile, and acetic acid were obtained from Merck Life Science (Merck KGaA, Darmstadt, Germany). Km 3-O-glucoside, Is 3-O-glucoside and Qn 3-O-glucoside were obtained from Merck Life Science (Merck KGaA, Darmstadt, Germany). Stock solutions of 1000 mg/L were prepared for each standard by dissolving 10 mg in 10 mL of methanol.

3.2. Plant Material

*B. juncea* selections were provided by the *Brassica* seed collection of CREA-CI [42]. They were sown in autumn on 15 October 2017, each in a 30 m² plot, at the CREA experimental farm located at Budrio (Bologna) in the Po Valley area (Emilia Romagna region, 44°32’00’’ N; 11°29’33’’ E, altitude 28 m a.s.l.). The area was characterized by flat land with alluvial deep loamy soil, with a medium level of total nitrogen content and organic matter content. The cultivation was carried out without fertilization, and it did not require other agronomical input until harvest. Plant samples were collected at three different phenological phases: (i) the first phase, 12 ± 3 cm (Sample A) to 23 ± 3 cm (Sample B and Sample C) high, the edible salad phase; (ii) the second phase, 18 ± 2 cm (Sample A) to 33 ± 4 cm (Sample B and Sample C) high, the culmination edible salad phase, when stems and leaves started to have the same weight; and (iii) the third phase, when inflorescence was completely developed. For each sampling time, six different plants (randomly chosen) were manually harvested, brushed (to physically remove soil residue), and collected, distinguishing the different tissues (leaves, stems, roots, and flowers). Samples were immediately frozen and freeze-dried for storage in glass vacuum desiccators. Lyophilized tissues were finely powdered to 0.5 µm size for analysis.
3.3. Seed Cake Preparation and Main Characterization

Seed cake from *B. juncea* is the major by-product from this oilseed crop, and, to date, oil yield is its main economic value [43]. Seeds were extracted overnight at room temperature with n-hexane (1:10 w/v), in a rotary shaker. The aim was to preserve the largest number of bioactive molecules. Seed cake was pestled ground in a mortar and left to dry at 40 ºC until constant weight, and finally it was ground to 0.5 mm size. The *B. juncea* defatted seed meals (DSMs) were characterized for moisture, nitrogen, residual oil, and glucosinolate (GSL) content. Moisture content was determined by evaluating the difference between its weight before and after oven drying at 105 ºC for 12 h. Total nitrogen content was determined according to the American Society for Testing Materials (ASTM D5373 2016) [44], and the crude protein content was expressed as a percentage of dry matter and calculated from nitrogen using the conventional factor for soy proteins of 6.25 [45]. Residual oil was extracted by a standard automated continuous extraction, following the Twisselmann principle, by using an E-816 Economic Continuous Extraction (ECE) unit (BÜCHI Labortechnik AG, Flawil, Switzerland), and hexane as solvent. GSL content and profiles were determined by HPLC-UV analysis of desulfo-GSLs following the ISO 9167-1 method (ISO 9167-1:1992/Amd 1:2013) [46]. The desulfo-GSLs were detected monitoring their absorbance at λ = 229 nm and identified with respect to their UV spectra and retention times [47,48]; their amounts were estimated using sinigrin as the external standard. Each extraction and analysis was performed in triplicate.

3.4. Sample Preparation

3.4.1. HPLC-PDA-MS

Extraction of the metabolite content was carried out based on the following protocol [7], with some modifications. All samples were spiked prior the extraction with 50 µL of apigenin (1000 ppm), which was evaporated with the use of nitrogen. The powder of different plant parts (seed, root, stem, leaf, and flower) of *B. juncea*, besides the DSM of the three different cultivars, were weighed into 100 mg. The samples were extracted twice with 5 mL of a mixture of methanol:water (60:40, v/v) for 30 min in a sonicator and centrifuged at 1000 x g for 15 min, followed by filtration of the supernatants through 0.45 µm filter paper; Merck Life Science (Merck KGaA, Darmstadt, Germany). The prepared organic extracts were subjected to evaporation in an EZ-2 evaporator, and then redissolved in 1 mL of the same solvent mixture of extraction methanol:water (60:40 v/v). A total of 10 µL was injected. The whole process is illustrated in Figure 4.
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Figure 4. Extraction of the metabolite content of *B. juncea* cultivars prior to HPLC-PDA-MS/MS analyses.

3.4.2. GC-FID/MS

Extraction of the volatile compounds was performed with mean of a DVB/CAR/PDMS (SPME fiber) of 50/30 µm (Merck Life Science, Merck KGaA, Darmstadt, Germany). The conditioning of the SPME was carried out according to Merck Life Science’s recommendations, through its insertion into the GC injector at 270 °C for 30 min. A total of 250 mg of each *B. juncea* sample was placed into a 20 mL sealed vial with a magnetic cap, with silicone/PTFE septa (Agilent Technologies, Santa Clara, CA, USA). The sample was stirred at 170 rpm at a temperature of 70 °C for 45 min. The SPME fiber was exposed to the GC injector at a temperature of 260 °C for 1 min, following by the exposition of the fibers to the headspace for 45 min, in the same above-mentioned conditions. The extracted volatile compounds that occurred in the fiber were introduced to the GC injector for thermal desorption.

3.5. Instrumentation

3.5.1. HPLC-PDA-MS

Analyses were performed on a Shimadzu system (Kyoto, Japan), consisting of a CBM-20A controller, two LC-30AD dual-plunger parallel-flow pumps, a DGU-20A5R degasser, a CTO-20AC column oven, a SIL-30AC autosampler, and an SPD-M30A PDA detector (1.0 µL detector flow cell volume). The LC system was hyphenated to an LCMS-8050 triple quadrupole mass spectrometer through an ESI source (Shimadzu, Kyoto, Japan). For data handling, the Shimadzu LabSolutions software (version 5.93) (Kyoto, Japan) was employed.
### 3.5.2. GC-FID/MS

Compound identification was carried out on a GCMS-QP2010 system (Shimadzu, Kyoto, Japan) equipped with a split–splitless injector. Data files were collected and elaborated by using Shimadzu “GCMS solution” software (version 4.45) (Kyoto, Japan).

Compound quantification was performed on a GC2010 system (Shimadzu, Kyoto, Japan) equipped with a split–splitless injector. Data files were collected and elaborated by using Shimadzu LabSolutions software (version 5.92) (Kyoto, Japan).

### 3.6. Analytical Conditions

#### 3.6.1. LC-PDA-MS

Analyses were performed on an Ascentis Express RP C18 column (150 × 4.6 mm, 2.7 µm I.D., Merck Life Science, Merck KGaA, Darmstadt, Germany).

The mobile phase consisted of water/acetic acid (99.85/0.15 v/v, solvent A) and acetonitrile (solvent B), with the following gradient elution: 0–5 min, 5% B, 5–15 min, 10% B, 15–30 min, 20% B, 30–60 min, 50% B, 60 min, 100% B.

Photodiode array detector was applied in the range of λ = 200–450 nm, where B. juncea polyphenols were detected at λ = 330 nm (sampling frequency: 12.5 Hz, time constant: 0.16 s). The entire LC flow was 1 mL/min and injection volume was 10 µL.

MS analysis was performed in negative and positive mode and scan range was set at m/z 100–1400; scan speed of 2727 amu/s. The conditions of ESI were as follows: event time 0.5 s; nebulizing gas (N₂) flow rate 3 L/min; drying gas (N₂) flow rate, 10 L/min; interface temperature: 300 °C; heat block temperature: 400 °C; DL (desolvation line) temperature: 250 °C; DL voltage: 1 V; interface voltage: −3 kV; Q3 pre-rod bias 15 V.

#### Construction of Calibration Curves

Due to the lack of commercial standards of native polyphenols, three standards, representative of the chemical classes under study were selected: Km 3-O-glucoside, Is 3-O-glucoside and Qn 3-O-glucoside. Standard calibration curves were prepared in a concentration range 0.1–100 mg/L with five different concentration levels. Triplicate injections were made for each level, and a linear regression was generated. The calibration curves with the external standards were obtained using concentration (mg/L) with respect to the area obtained from the integration of the PDA peaks at a wavelength of 330 nm. The amount of the compound was finally expressed in µg/100 mg DW.

#### 3.6.2. GC-FID/MS

Volatile compounds were analyzed on a GC-MS system using an SLB-5ms fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm df film thickness) (Merck Life Science, Merck KGaA, Darmstadt, Germany). The injection port was operated in splitless mode, at the temperature of 260 °C. Helium was kept at the linear velocity of 30.0 cm/sec corresponding to an inlet pressure of 24.2 KPa. The oven temperature program was set at 40 °C (held for 1 min); it was ramped up to 350 °C (held for 5 min) at a rate of 3 °C/min. The electron impact (EI) source temperature was maintained at 220 °C and the interface was set at the temperature of 250 °C. Mass range acquisition was made in full scan mode in the mass range of 40–660 m/z, with an event time of 0.2 s. Compounds were identified with the support of “FFNSC 4.0” (Shimadzu Europa GmbH, Duisburg, Germany), which consisted of a library of volatile compounds obtained and stored by GC-MS separation and “W11N17” (Wiley11-Nist17, Wiley, Hoboken, NJ, USA; Mass Finder 3). Identification was performed applying a spectral similarity filter (match over 85%) using also linear retention indices (LRI) that were calculated using a C7–C30 saturated n-alkane homologue series (1000 g/mL, 49451-U) supplied by Merck Life Science, Merck KGaA, Darmstadt, Germany.
The quantification of the volatile compounds was performed on a GC-FID system using the same capillary column and temperature program employed in the qualitative analysis. The carrier gas (helium) was kept at the linear velocity of 30.0 cm/s corresponding to an inlet pressure of 97.4 KPa and the split mode of the injector was set to splitless. The flame temperature was set at 350 °C (sampling rate 200 ms). Makeup flow was 30 mL/min and hydrogen and airflow was 40 mL/min and 400 mL/min, respectively.

4. Conclusions

A comprehensive characterization of the chemical profile of different tissues of *B. juncea* cultivars was reported. Specifically, leaves, stems, roots, and flowers of *B. juncea* were analyzed by HPLC-PDA/ESI-MS/MS. Moreover, the leaf extracts, which turned out to be the most complex ones in terms of volatile compounds, were analyzed by GC-FID/MS, along with a chemical characterization of defatted seed meals (DSM). As far as the volatile content was concerned, more than 179 chemical constituents were identified; on the other hand, for the non-volatile part, a total of 35 metabolites were positively identified, revealing a large number of highly glycosylated and acylated isorhamnetin, quercetin, and kaempferol derivatives. Among DSMs, interestingly, proteins were the main components accounting for 44.0% DW, 37.4% DW, and 36.8% DW for Sample A, Sample B, and Sample C, respectively. Based on the phytocomponents identified, this crop could have an important application in pharmaceutical and nutraceutical fields. At the same time, differences between varieties and plant tissues demonstrate the importance of cultivar selection and validation. To this regard, further studies are necessary to evaluate the bioactivity and toxicity profile through in vitro and in vivo models of materials from the most promising varieties.

Supplementary Materials: The following are available online. Table S1: Metabolite determination of the ISCI 99 cultivar extracts of *B. juncea* collected on different phenological stages by HPLC-PDA, Table S2: Metabolite determination of the ISCI Top cultivar extracts of *B. juncea* collected on different phenological stages by HPLC-PDA, Table S3: Metabolite determination of the ISCI “Broad-leaf” cultivar extracts of *B. juncea* collected on different phenological stages by HPLC-PDA.

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Sample Availability: Samples of the compounds are not available from the authors.

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