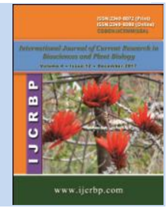
EXCELLENT PUBLISHERS

International Journal of Current Research in
Biosciences and Plant Biology

Volume 4 • Number 12 (December-2017) • ISSN: 2349-8080 (Online)

Journal homepage: www.ijcrbp.com



**Original Research Article** **doi:** https://doi.org/10.20546/ijcrbp.2017.412.005

# Volatile Compounds Analysis in Chestnuts and Chocolate-Coated Chestnuts Using GC-MS

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## Abstract

Chestnut (*Castanea sp.*) is an important nut crop of the world whose nut is consumed by a large proportion of population and used for different purposes. The objective in this work was to study the flavoring components in fresh roasted chestnut (FRC), roasted freeze-dried chestnut (RFDC), dark chocolate-coated chestnut (DCC) and milk chocolate-coated chestnut (MCC). A method based on headspace solid-phase micro-extraction (HS-SPME) has been applied for the analysis of volatile components in samples. As a result, 55 volatile organic major compounds were identified in FRC, 44 in RFDC while the processed chestnut with chocolate showed 49 and 43 in MCC and DCC respectively after 180 days storage. Styrene (11.19%), Furfural (10.15%), methyllaurate (9.05%), propanone (6.06%) and butanone (5.08%) were found to be dominant in FRC. However, ethanol, hexanal and butanediol, which were not found in the row material, were identified as important aroma impact compounds in the processed chestnut. These results demonstrate that there is variability in fresh roasted chestnut and the freeze dried one for odorants. The coating process modified strongly the volatile composition of the different types of product, particularly the levels of trans-.beta.-Ionon-5,6-Epoxide, 2-propanone, 2-butanone, acetic acid, furfural, hexanal, ethanol, 2,3- Butanediol and vanillin.

## Article Info

*Accepted: 25 November 2017*
*Available Online: 06 December 2017*

## Keywords

Chestnut,
Chocolate coating,
GC MS,
HS-SPME,
Volatile compounds

## Introduction

Unlike the majority of tree nuts, chestnuts (*Castanea sp.*,Mill) are characterized by low fat and protein but high carbohydrate content (McCarthy and Meredith, 1980; Senter, Payne, Millerand Anagnostakis, 1994; Morini and Maga, 1995a; Künsch et al., 2001; Silvanini et al., 2014; Moronne et al., 2015; Li et al., 2016). Nevertheless, this fruit is an important source of essential fatty acids (Moronne et al., 2015), mainly linoleic and linolenic acid, which playan important role in preventing cardiovascular diseases and in promoting cerebral and retinal development in infants (Conner, 1997).

Today, besides classical preparations of chestnuts (roasted or candied chestnuts, as puree or creams), new commercial products have been launched on the market (liqueur, aperitif, beer, pasta, dairy products). Even vacuum-packed or frozen chestnuts are available

(Gloaguen, Nourani, and Morvan, 1998, Carocho et al., 2016; Li et al., 2016).

The current chestnut market in producing countries is considerable and offers new perspectives to growers and industrialists. The market is mainly focused on high quality variety, which is mostly consumed as roasted products (Gounga, Xu, and Wang, 2007).

In our previous studies, roasted Chinese chestnuts (*Castanea mollissima*) were freeze-dried and coated with whey protein isolate-pullulan edible film (Gounga et al., 2008), then doubly coated with chocolate. Physical characteristics (color, decay incidence) as well as nutritional values of chocolate-coated chestnuts were investigated (Gounga, Xu, and Wang, 2008). The changes in protein and carbohydrate contents and in color reported would indicate that these components are quite reactive during processing and responsible for the formation of the characteristic pleasant aroma associated with processed chestnuts. More recently, the decay incidence of the product and its acceptability has been studied (Gounga et al., 2017).

As pointed out in a review (Maga, 1991), extensive literature has been published on the volatile constituents of different nut varieties (Li et al., 2016; Munekata, 2016). Little studies have been reported on the flavor properties of chestnuts (Krist, Unterweger, Bandion, and Buchbauer, 2004).Morini and Maga (1995b) studied the volatile compounds in roasted andboiled Chinese chestnuts.The volatile compounds extracted from the chestnut are very important, since they are related to some flavor properties of the fruit.

Headspace solid-phase micro-extraction (HS-SPME) has been applied for the analysis of volatile components in food. It is a relatively new methodology, providing many advantages over conventional sample preparation techniques (Zhao, Tang, and Ding, 2007).It is a solvent-free, rapid and versatile method for the extraction of organic compounds. It consists of a fused-silica fiber, coated with a polymeric stationary phase introduced into a liquid or gas sample. The method involves two processes: the partitioning of the analytes between the coating and the sample and the thermal desorption of the analytes into gas chromatograph. This technique has been first utilized successfully for evaluation of environmental materials (Arthur, Killam, Motlagh, Lim, Potter, and Pawliszyn, 1992; Potter and Pawliszyn, 1992).Since then, the interest in using SPME and relative methods for food flavor analysis has been increased during the past few years (Krist et al., 2004; Sanchez-Silva, Lopez-Hernandez, and Paseiro-Losada, 2005; Munekata et al., 2016; Servillo et al., 2016).

The objective in this work was to study the flavoring components in fresh roasted Chinese chestnut (FRC), roasted freeze-dried chestnut (RFDC), dark chocolate-coated chestnut (DCC) and milk chocolate-coated chestnut (MCC).

## Materials and Methods

### Material

FRC, RFDC, DCC, and MCC were similar to those used in our previous studies (Gounga, Xu, and Wang, 2007; Gounga, Xu, and Wang, 2008, Gounga et al., 2008; Gounga et al., 2017).

### Methods

#### Headspace solid-phase micro extraction (HP-SPME)

A SPME holder (Supelco, Shanghai, China) for manual sampling was used to perform the experiments. 5.0 g of ground sample was transferred to the SPME vial (15 mL). An SPME fiber (75 μm carboxen-polydimethylsiloxane CAR/PDMS coating) was inserted through the septum and exposed to HP-SPME vial. Extraction was carried out at 50 °C for 40 min while stirring in a water bath. The fiber was then taken out and inserted into the GC injector for desorption at 250 °C for 3 min, while start the instrument for getting the data.

#### Determination of volatile compounds

GC-MS (Finnigan TRACE MS 2000, USA) was used to perform the analysis. The volatile compounds were separated using a 30 m x 0.25 mm (i.d.) DB-WAX capillary column, film thickness was 1.0 mm. The ionization voltage was 70 eV. The initial temperature of the column, 40 °C, was held for 4 min and then increased first at a rate of 6 °C $min^{-1}$ to a temperature of 80 °C. From this point, the temperature was increased at 8 °C $min^{-1}$ to a temperature of 180 °C, then to 220 °C, at 10 °C $min^{-1}$ rate, which was held for 6 min. The injector port transfer line temperature was 250 °C. The carrier gas was ultra-purified helium at a constant flow rate of 1.0 ml/min. The spectrometer was operated in electron-impact (EI+) mode; emission current was 200 μA and detector voltage 350 V. The ion-source temperature was 200 °C. Volatile compounds were identified by use of mass spectrometry database libraries (WILLEY, REPLIB, NISTDEMO and MAINLIB).

## Results and Discussion

### Freshly roasted chestnut (FRC)

Fifty five volatile compounds were identified in FRC (Table 1; Fig. 1). The main compounds were Styrene; Furfural; Methyllaurate; 2-Propanone; 2-Butanone; Acetic acid; 1-Hexanol, 2-ethyl-; 2-Furanmethanol; Nonanal; and Hexanal. Some other minor components were also identified. Most of these aroma compounds were identified in previous studies which focused on the volatile compounds in roasted and boiled Chinese chestnuts (Morini and Maga, 1995b; Li et al., 2016) and other nut products (Lasekan, Alfiand Abbas, 2012; Aponte et al., 2013). Hexanal and heptanal, for example, also identified in roasted Italian chestnut (Krist et al., 2004), were responsible for the fatty-nutty aroma impression of the investigated FRC. Furfural and Styrene did provide the sweet aroma as seen in sensory evaluation (Gounga, Xu and Wang, 2007). FRC revealed also some fruity odor notes compared to the freeze-dried nuts. This could be attributed to 2,2,4-Trimethyl-3-pentanone and 6-Methyl-5-hepten-2-one (Table 1).

### Roasted freeze-dried chestnut (RFDC) and chocolate-coated chestnuts (DCC and MCC)

A total of 44 headspace volatile compounds isolated by SPME were tentatively identified in RFDC by GC–MS. The variation in the volatile compound of FRC (Fig.1) and RFDC (Fig. 2) may be due to moisture differences in the 2 samples or due to the more severe loss of volatiles associated with the long drying treatment. The fact that Styrene was not detected in RFDC could explain the low sweetness while Furfural was detected in less proportion compared to FRC.

**Fig.1** Chromatogram profiles of volatile compounds in FRC

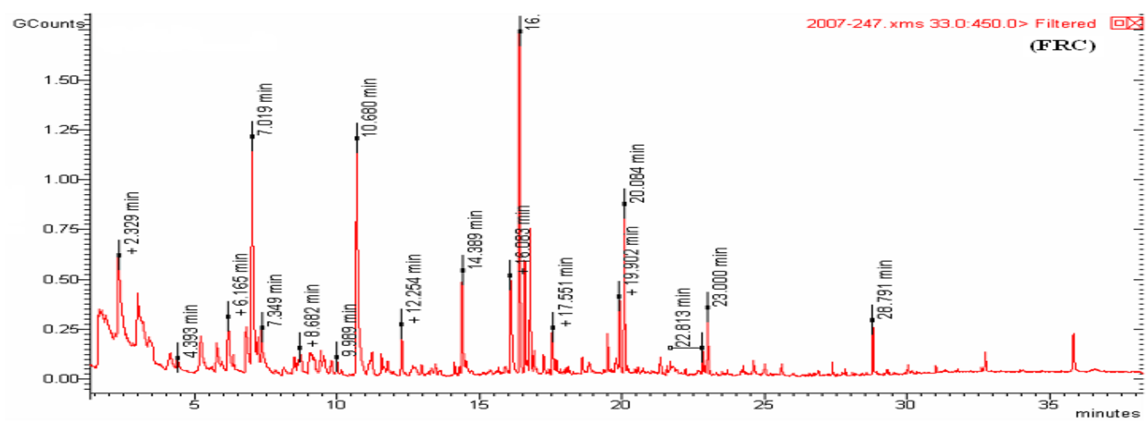



**Fig.2** Chromatogram profiles of volatile compounds extracted from RFDC

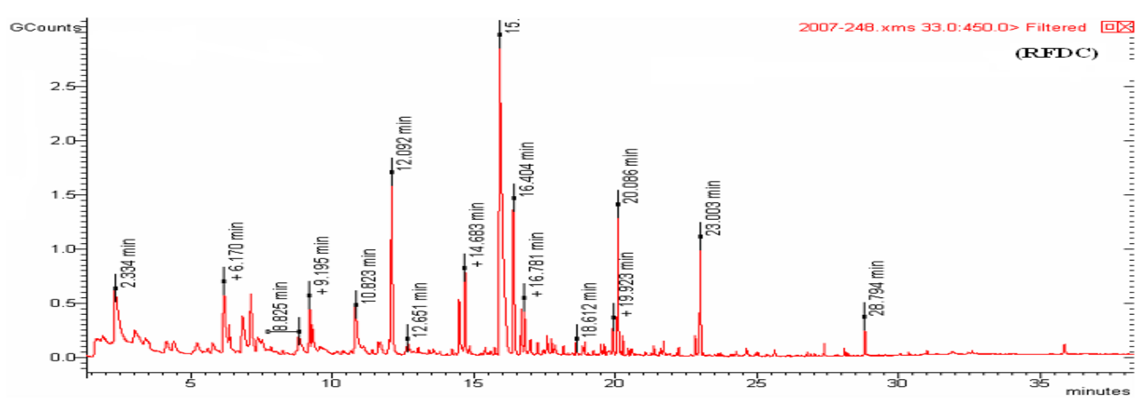
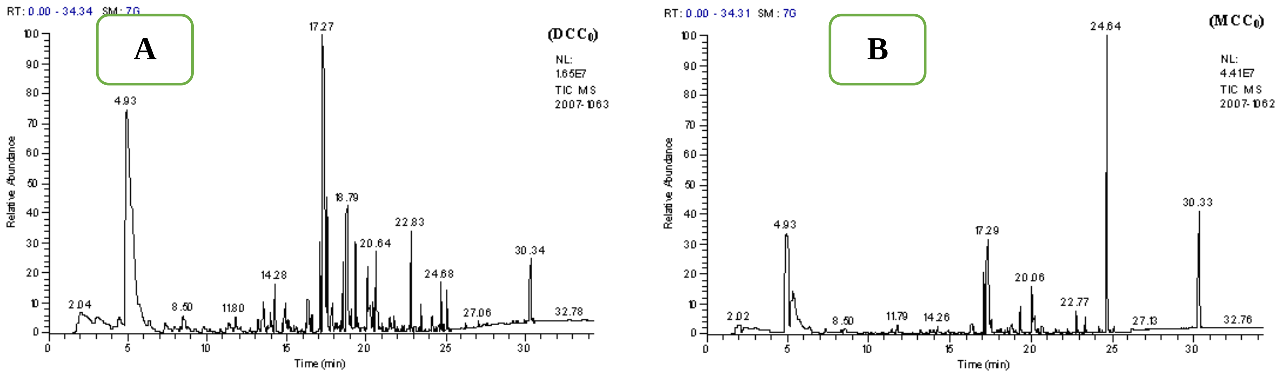


**Fig.3** Chromatogram profiles of volatile compounds extracted using HS-SPME from the samples of chocolate coated chestnuts before storage




A: DCC 0 day storage; B: MCC 0 day storage

**Fig.3** Chromatogram profiles of volatile compounds extracted using HS-SPME from the samples of chocolate coated chestnuts after 180 d storage

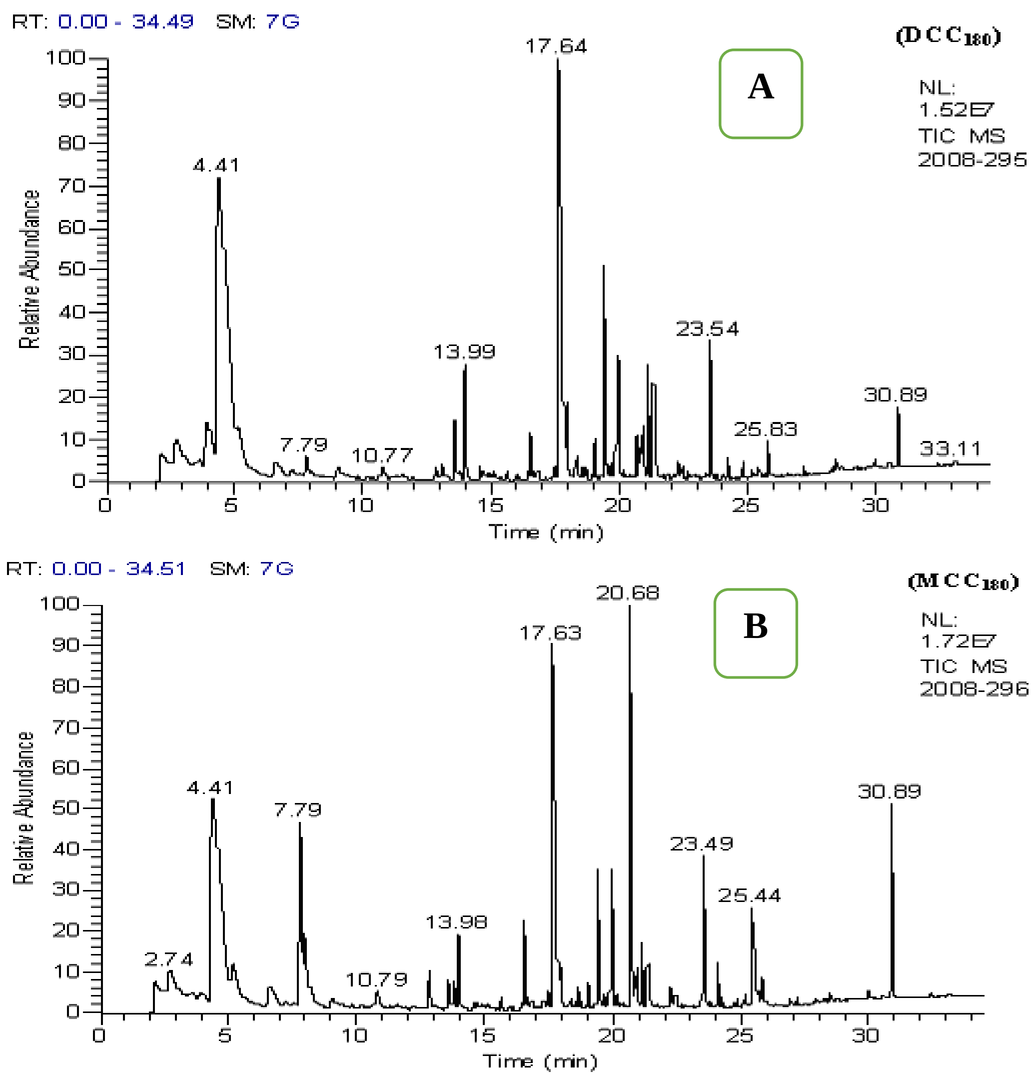


**Table 1.** SPME headspace volatiles of FRC, RFDC, DCC and MCC

| No | Compounds | Peak area abundant (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | FRC | RFDC | DCC | | MCC | |
| | | | | 0 d | 180d | 0d | 180d |
| 1 | Trans-.beta.-Ionon-5,6-Epoxide | 4.981 | 0.082 | – | – | – | – |
| 2 | Butethamate | 0.464 | – | – | – | – | – |
| 3 | Ethyne, fluoro- (CAS) | 0.137 | – | – | – | – | – |
| 4 | Ethyne, fluoro- (CAS) | 0.137 | – | – | – | – | – |
| 5 | Octane (CAS) | 0.06 | – | – | – | – | – |
| 6 | Hydroperoxide, 1-methylhexyl | 0.052 | – | – | – | – | – |
| 7 | 2-Propanone (CAS) | 6.056 | 5.272 | – | – | – | – |
| 8 | Acetic acid, ethyl ester (CAS) | 0.048 | – | – | – | – | – |
| 9 | 2-Butanone (CAS) | 5.084 | 1.296 | – | – | 0.14 | – |
| 10 | Dihydroxyacetic acid | 0.272 | – | – | – | – | – |
| 11 | 2-Butanone, 3-methyl- (CAS) | 0.538 | – | – | – | – | – |
| 12 | Molybdenum, di-.mu.-chlorobis[(1,2,3,4,5 | 1.7 | – | – | – | – | – |
| 13 | 2,3-Pentanedione (CAS) | 1.637 | 1.139 | 0.20 | – | – | – |
| 14 | Acetic acid, 2-propenyl ester (CAS) | 0.445 | – | – | – | – | – |
| 15 | Hexanal (CAS) | 2.568 | 5.121 | 0.19 | – | 0.42 | – |
| 16 | Undecane (CAS) | 0.382 | 1.296 | – | – | – | – |
| 17 | Methyl Crotonate | 2.544 | – | – | – | – | – |
| 18 | Methyllaurate | 9.054 | – | – | – | – | – |
| 19 | O-Xylene | 1.538 | – | – | – | – | – |
| 20 | Benzene, (1-methylethyl)- | 0.168 | – | – | – | – | – |
| 21 | Benzene, 1,2-dimethyl- (CAS) | 0.695 | – | – | – | – | – |
| 22 | Cyclohexene, 1-methyl-4-(1-methylethenyl | 0.33 | – | – | – | – | – |
| 23 | Heptanal (CAS) | 0.833 | – | – | – | – | 11.11 |
| 24 | Hydroxylamine, O-(2-methylpropyl)- | 0.321 | – | – | – | – | – |
| 25 | Pyridine (CAS) | 1.951 | 0.846 | 0.47 | 0.78 | 0.16 | – |
| 26 | 2,2,4-Trimethyl-3-pentanone | 0.672 | – | – | – | – | – |
| 27 | Butanoic acid, butyl ester (CAS) | 0.782 | – | – | – | – | – |
| 28 | Hexanoicacid, ethyl ester (CAS) | 0.505 | – | – | – | – | – |
| 29 | Styrene | 11.19 | – | – | – | – | – |
| 30 | 4-Ethylphenethylamine | 1.641 | – | – | – | – | – |
| 31 | Octanal (CAS) | 0.451 | 0.878 | 0.28 | 0.28 | – | 0.82 |
| 32 | 2-Propanone, 1-hydroxy- | 1.473 | 7.541 | 1.61 | 3.53 | 0.46 | 1.86 |
| 33 | 6-Methyl-5-hepten-2-one | 0.297 | – | – | – | – | – |
| 34 | 3-Methylheptyl acetate | 0.377 | – | – | – | – | – |
| 35 | 2-Nonanone (CAS) | 0.181 | – | – | 0.18 | – | 0.11 |
| 36 | Nonanal (CAS) | 2.769 | 2.276 | 0.81 | 1.09 | 0.4 | 1.89 |
| 37 | Acetic acid (CAS) | 3.866 | 25.8 | – | 22.44 | 10.32 | 18.89 |
| 38 | Furfural | 10.15 | 6.155 | 3.56 | – | – | 1.11 |
| 39 | 2-Propenoic acid, 2-ethylhexyl ester (CA | 2.086 | 1.291 | – | – | – | – |
| 40 | 1-Hexanol, 2-ethyl- (CAS) | 3.493 | 1.641 | 0.16 | – | 0.11 | – |
| 41 | Decanal (CAS) | 0.747 | – | – | 0.19 | – | 0.39 |
| 42 | Ethanone, 1-(2-furanyl)- | 0.606 | 0.38 | 0.15 | – | – | – |
| 43 | Benzaldehyde (CAS) | 1.552 | 0.672 | 1.55 | 0.73 | 0.21 | 0.44 |
| 44 | 2-Nonenal, (E)- | 0.258 | 0.412 | – | – | – | – |
| 45 | 1-Nonanol (CAS) | 0.122 | – | – | – | – | – |
| 46 | 2-Furancarboxaldehyde, 5-methyl- (CAS) | 0.456 | 0.484 | – | – | – | – |
| 47 | Butanoic acid (CAS) | 0.857 | – | – | 0.60 | – | 8.57 |
| 48 | Ethanone, 1-phenyl- (CAS) | 1.936 | 1.042 | – | – | – | – |
| 49 | 2-Furanmethanol (CAS) | 3.357 | 3.899 | 0.70 | – | 0.19 | – |
| 50 | 1H-Indene, 1-methylene- (CAS) | 0.445 | – | – | – | – | – |
| 51 | Hexanoic acid (CAS) | 0.595 | 0.595 | – | – | 1.00 | 2.10 |
| 52 | 3,4-Dimethyl-2-butenoic acid gamalacton | 1.598 | 3.008 | – | – | – | – |
| 53 | Triethylene glycol | 1.211 | 0.97 | – | – | – | – |
| 54 | Dibutyl phthalate | 0.397 | – | – | – | – | – |
| 55 | Hexadecanoic acid (CAS) | 1.197 | – | – | – | – | – |
| 56 | Dimethylamine-D1 | – | 1.048 | – | – | – | – |
| 57 | Decane (CAS) | – | 0.963 | – | – | 0.77 | – |
| 58 | Benzene, (2-methyloctyl)- | – | 0.799 | – | – | – | – |
| 59 | Tetradecane, 1-chloro- (CAS) | – | 0.147 | – | – | – | – |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 60 | 2-Butenoic acid, methyl ester, (E)- (CAS | – | 3.206 | – | – | – | – |
| 61 | Benzene, ethyl- (CAS) | – | 5.799 | – | – | – | 0.08 |
| 62 | 1-Butanol (CAS) | – | 0.129 | – | – | – | – |
| 63 | Dodecane (CAS) | – | 3.307 | 0.77 | – | 0.56 | – |
| 64 | Bicyclo[4.2.0]octa-1,3,5-triene | – | 3.257 | – | – | – | – |
| 65 | Benzene, 1,2,3-trimethyl- (CAS) | – | 0.232 | – | – | – | – |
| 66 | 2-Butanone, 3-hydroxy- (CAS) | – | 0.477 | 0.64 | 1.75 | 0.19 | 0.80 |
| 67 | Tridecane (CAS) | – | 0.52 | – | – | – | – |
| 68 | 2-Heptenal, (E)- (CAS) | – | 0.575 | – | – | 0.15 | – |
| 69 | Pentadecane | – | 2.709 | – | – | 0.26 | – |
| 70 | 3-Octen-2-one, (E)- | – | 0.259 | – | – | – | – |
| 71 | Decane (CAS) | – | 0.617 | – | – | 0.77 | – |
| 72 | Propanoic acid (CAS) | – | 0.345 | – | – | 0.34 | – |
| 73 | 2-Cyclopentene-1,4-dione | – | 0.314 | 0.13 | – | 0.07 | – |
| 74 | Pentadecane (CAS) | – | 0.325 | – | – | 0.26 | – |
| 75 | 2(5H)-furanone | – | 0.333 | 0.47 | 0.20 | – | 0.23 |
| 76 | 2-Methoxy-4-vinylphenol | – | 0.289 | – | – | – | |
| 77 | Butanal, 3-methyl- | – | – | – | 3.15 | – | 0.61 |
| 78 | Ethanol | – | – | – | 35.73 | – | 23.32 |
| 79 | Benzene, methyl- | – | – | – | 1.26 | – | 1.64 |
| 80 | 3-Pentanone, 2-methyl- | – | – | – | 0.41 | – | – |
| 81 | Hexanal | – | – | – | 1.3 | – | 11.11 |
| 82 | 3-Penten-2-one | – | – | – | 0.89 | – | – |
| 83 | Ethylbenzene | – | – | – | – | – | 0.76 |
| 84 | 1-Pentanol | – | – | – | 0.38 | – | 1.14 |
| 85 | Pyrazine, methyl- | – | – | – | 0.73 | – | – |
| 86 | Propanoic acid, 2-hydroxy-, methyl ester | – | – | – | 0.30 | – | 0.14 |
| 87 | 1-Pentanol, 3-methyl- | – | – | – | – | – | 0.25 |
| 88 | Pyrazine, 2,5-dimethyl- | – | – | – | 0.38 | – | – |
| 89 | Pyrazine, 2,6-dimethyl- | – | – | – | 0.20 | – | – |
| 90 | Tetradecane | – | – | – | 0.16 | – | 0.21 |
| 91 | Pyrazine,2-ethyl-3methyl | – | – | – | – | – | 0.18 |
| 92 | Pyrazine, trimethyl- | – | – | – | 0.21 | – | – |
| 93 | 2-Octenal, (E)- | – | – | – | – | – | 0.14 |
| 94 | Octanoic acid, ethyl ester | – | – | – | 0.22 | – | 0.27 |
| 95 | 3-Furaldehyde | – | – | – | 1.89 | – | – |
| 96 | Butanoic acid, 3-hydroxy-, methyl ester, (S)- | – | – | – | 0.32 | – | 0.11 |
| 97 | 1-Methyl-3(3,4dimetyhoxyphenyl)-6,7-dimethoxyisochromene | – | – | – | – | – | 0.11 |
| 98 | 2,3- Butanediol | – | – | – | 6.79 | – | 5.36 |
| 99 | Propanoic acid, 2-methyl- | – | – | – | 0.80 | – | 0.31 |
| 100 | 1-Octanol | – | – | – | – | – | 0.19 |
| 101 | 1,2-Propanediol | – | – | – | 0.24 | – | 0.18 |
| 102 | Butanoic acid, 4-hydroxy- | – | – | – | 0.66 | – | – |
| 103 | Decanoic acid ethyl ester | – | – | – | 0.46 | – | 0.45 |
| 104 | 3-Furanmethanol | – | – | – | 1.59 | – | 0.97 |
| 105 | 4-(Benzoyloxy)-2H-Pyran-3-one | – | – | – | – | – | 0.68 |
| 106 | Butanoic acid, 3-methyl- | – | – | – | 2.42 | – | 1.06 |
| 107 | Pentanoic acid | – | – | – | – | – | 0.27 |
| 108 | Oxime-, methoxy-phenyl-_ | – | – | – | 0.34 | – | 0.30 |
| 109 | Acetamide(CAS) | – | – | – | 0.18 | – | 0.18 |
| 110 | 2-Furanone, 2,5-dihydro-3,5-dimethyl | – | – | – | 2.23 | – | 1.91 |
| 111 | Phenylethyl Alcohol | – | – | – | 0.28 | – | 0.12 |
| 112 | Dimethyl sulfone | – | – | – | – | – | 0.59 |
| 113 | Ethanone, 1-(1H-pyrrol-2-yl)- | – | – | – | 0.23 | – | – |
| 114 | 5-Bromo-3-Methylidene-1-Methoxycyclohexane | – | – | – | – | – | 0.13 |
| 115 | Phenol | – | – | – | 0.14 | – | 0.20 |
| 116 | 4H-Pyran-4-one, 2-ethyl-3-hydroxy- | – | – | – | – | – | 2.94 |
| 117 | 1H-Pyrrole-2-carboxaldehyde | – | – | – | 0.15 | – | – |
| 118 | 2-Pyrrolidinone (CAS) | – | – | – | – | – | 0.34 |
| 119 | 1,2,3-Propanetriol, diacetate | – | – | – | 0.42 | – | – |
| 120 | Octanoic acid | – | – | – | – | – | 0.71 |
| 121 | 3-Hydroxy-4-methoxymandelic acid | – | – | – | 1.23 | – | – |
| 122 | Nonanoic acid | – | – | – | – | – | 0.12 |
| 123 | Vanillin | – | – | – | – | – | 3.72 |

The volatile profile of DCC (Fig. 3 A) and MCC (Fig. 3 B) consisted of only 15 and 19 compounds, respectively, in the beginning of storage (0 d) (Table 1), and 43 and 49 compounds were identified in DCC and MCC respectively, after 180 d of storage (Fig. 4). The variation in the volatile compounds may be related to their main fatty acid components (Gounga, Xu, and Wang, 2008).

Compounds derived from lipids (2-butanone; decane; hexanal; ethylbenzene; 1-penten-3-ol; 1-methylethylbenzene; heptanal; 1-pentanol; octanal; 2-heptenal and 1,2,3-trimethylbenzene) were identified in both DCC and MCC. Abundant representatives in alcohols including (ethanol; 2,3- Butanediol; 3-Furanmethanol) and acids (acetic acid; 3-Hydroxy-4-methoxymandelic acid; 3-methyl-Butanoic acid; 3-Hydroxy-4-methoxymandelic acid) were identified in DCC and MCC after 180 d of storage. The presence of alcohols and acids could be attributed to chemical degradation of hydroxyperoxydases of unsaturated fatty acids or may result from microbial activity (Sanchez-Silva, Lopez-Hernandez, and Paseiro-Losada, 2005). The presence of some alcohols and acids in FRC, RFDC in one side, and DCC and MCC in the other side (at 0 d storage was related to their native compounds.

The presence of several monoterpenes and numerous derivatives of butane, pentane, hexane, and heptane were found to be of essential importance for the characteristic aroma of the good-tasting new processed products as perceived in the sensory evaluation test (Gounga et al., 2017).

## Conclusion

The significant aroma of chestnut and chocolate coated-chestnuts is not the result of one single odor impression. Several monoterpenes and numerous derivatives of butane, pentane, hexane, and heptane were found to be of essential importance for the characteristic aroma of these digestible and good-tasting new processed products. Basic knowledge of volatile compounds constituting the unique chestnut on one side, and chocolate flavor on the other side can facilitate better quality control of final product and also help product developers meet flavor-delivery challenges.

## Conflict of interest statement

Authors declare that they have no conflict of interest.

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**How to cite this article:**

Mahamadou Elhadji Gounga, Khamis Ali Omar and Issoufou Amadou 2017. Volatile Compounds Analysis in Chestnuts and Chocolate-Coated Chestnuts Using GC-MS. Int. J. Curr. Res. Biosci. Plant Biol. 4(12), 60-67. **doi:** https://doi.org/10.20546/ijcrbp.2017.412.005