Analysis and identification of the volatile compounds in melon-bitter leaf soup

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Melon-bitter leaf soup is one of the most widely consumed soup delicacies across West Africa, owing to its unique and distinct flavour. The aim of this study therefore was to identify volatile compounds in this soup, thus establishing a critical baseline for further work. Melon-bitter leaf soup was prepared according to standard method. Functional groups of chemical moieties in the soup were obtained using Fourier Transform Infra-red Spectroscopy (FT-IR). Analysis and identification of the volatile compounds in the soup was by Headspace micro-extraction at 40°C coupled with gas chromatography/mass-spectroscopy (GC/MS) using helium as carrier gas at a constant flow rate of 1 ml/min and an injection volume of 0.5 µl (split ratio of 10:1). Amines, esters, acids, benzenes, alkanes, pyrazines and terpenes were established and authenticated. Major aroma contributors to the soup flavour were found to be acetic acid (22.31%), 3-methylbutanoic acid (13.38%), 2-methylbutanoic acid (6.38%), 2-methylpropanoic acid (4.69%), tetramethylpyrazine (4.45%), butanoic acid (2.51%), propanoic acid (2.09%), trimethylpyrazine (1.55%), methylpyrazine (1.10%), furfural (1.10%), hexanoic acid (0.64%), linalool (0.38%), 1,8-cineole (eucalyptol) (0.35%) and 2,5 dimethylpyrazine (0.29%), all characterized with distinct peaks on the chromatogram. Volatile flavor compounds associated with melon bitter leaf soup were isolated and identified. Acetic acid was the major flavorant.

Key words: Aroma, volatile compounds, melon- bitter- leaf soup, flavorant, Fourier transform infra-red spectroscopy (FT-IR), gas chromatography/mass-spectroscopy (GC/MS).

INTRODUCTION

Consumer and marketing studies invariably have shown that taste, as opposed to perceived nutrition or health value, is the key influence on food selection (Drewnowski, 1996; Glanz et al., 1998). More than ever before, consumers are demanding better tasty foods made from natural sources and usually at a reasonable price. This has necessitated research into the development of natural and nature identical food flavours (Lawless and Heymann, 2010). A determining factor in the acceptance or rejection of a foodstuff is its flavor, as this plays a very important role in palatability and is one of the key parameters determining the overall quality of a

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food product (Carterette and Friedman, 1989). Flavour is defined as the combined perception of odor, taste and mouthfeel (texture) (Ney, 1988). The flavour of a food is created by aromatic substances that are biosynthesized during normal metabolic processes in plants and animal possibly further modified by processing (Reineccius, 1999). A number of these flavours have been used in food industries as part of their key inputs. Understanding the mechanisms by which flavour compounds are formed can lead to optimized methods of food processing, allowing targeted formation and retention of flavor. Fundamental flavor chemistry information is also essential in genetic engineering of plants and animals for improving flavor in the raw materials of food products (Carterette and Friedman, 1989). Advances in analytic methodology have enabled the identification of numerous compounds with known flavor properties. As more compounds are correlated with characteristic flavors, there is a trend to study flavor precursors and to explain how flavor is developed and released. Understanding the chemical reactions involved in the processing and storage of foods helps in achieving optimum consumer acceptability (Teranishi, 1989). Besides flavor, other sensory properties with a great impact on the consumer are color and appearance. Flavor is generally thought to consist of the volatile components sensed in the nose, both through the nostrils (orthonasally) and from inside the mouth (retronasally), nonvolatile compounds sensed on the tongue and compounds that are perceived in the mouth as texture or mouthfeel. Flavor analysis has typically focused on measuring volatile compounds, for example by gas chromatography-mass spectroscopy (GC-MS) and GC-olfactory methods. Chicken flavor, a commonly used food flavour was evaluated by Gasser and Grosch (1990) who identified 2-methyl-3-furanthiol, methionol, 2,4,5-trimethylthiazole, 2-trans-nonenal and other compounds as significant components, but concluded that 2-methyl-3-furanthiol was the dominant flavor contributor. Many of these key aroma compounds in chicken soup were authenticated by Farmer (1999) to further include 3-methylthiopropanal. However, aldehydes, ketones and alcohols were identified as flavor compounds associated with fresh fish soup (Ölafsdóttir and Fleurence, 1998). The intensity of the aroma seems to be related more with chemical factors such as volatility and hydrophobic nature, and the stereo-chemical structure such as type and position of the functional groups of the aromatic compounds than with their concentration. It has been demonstrated that the size, shape, conformational structure and type and position of the functional groups of the aromatic compounds are important elements in establishing the appropriate bonds to the olfactory receptor proteins present in the olfactory epithelium, thereby giving rise to the perception (Pelosi, 1994). The flavour components of button mushroom soup was analyzed by Qin et al. (2011) who found 1-octen-3-ol (mushroom-like flavor), 1-octen-3-one (mushroom-like flavor), benzaldehyde (floral flavor), 2,5-dimethylpyrazine (popcorn-flavour), 2,6-dimethylpyrazine (roasted nut flavor), 3-methylbutanal (fruity flavor) and 2-acetylthiazole (meat flavor) to be the main components. On the other hand, duck soup exhibited significant amounts of pentanal, 3-ethyl-2-methyl-1,3-hexadiene and 2-pentyl furan amongst others as flavor components (Zhang et al., 2012). Takakura et al. (2014) identified the main active compounds contributing to the aroma in pork soup to be hexanoic, decanoic and octanoic acids.

Fish flavors are mainly characterized by the volatile compounds in fish. Ölafsdóttir and Fleurence (1998) presented a good review on the main groups of fish odors. These are species related fresh fish odor, microbial spoilage odor, oxidized odor, environmentally derived odor, and processing odor. The type of functional group is related more with the intensity of the aroma than with its type. Katanaka and Kajiwara (1992) found that the intensity of aroma of the unsaturated C6 - aldehydes is between 10 - and 1000-fold stronger than that of their corresponding hexenols, which have the double bond at the same position and with identical geometry. While a number of scientific investigations have been conducted on the flavor profiles of these foreign soups, there is paucity of data on some soup delicacy such as melon bitter leaf soup widely consumed in Nigeria and indeed in West Africa. Omah et al. (2015) compared two Nigerian soups (Egusi soup and bitter leaf soup) and concluded that the instant bitter leaf soup was most preferred as compared to instant Egusi soup.

Solid phase microextraction (SPME) is the most recently applied technique in the analysis of volatile compounds. Pawliszyn’s (2001) group was the first to develop the SPME method, and they applied it to environmental analysis. Since then, it has become a widely used technique for the analysis of volatiles in foods. The technique employs a fiber of adsorbent material placed inside a modified chromatographic needle to isolate and concentrate the compounds. The fiber is positioned in the headspace of the sample for a specific time. The volatile compounds are diffused and distributed on the polymer coating as a function of their coefficients of distribution. The fiber is removed from the sample and placed in the GC injector, where the compounds are thermally desorbed.

The objective of this study was to isolate and identify the volatile compounds associated with melon-bitter leaf soup.

MATERIALS AND METHODS

Collection of raw materials

The following material inputs: dried melon seed (Citrullus colocynthis), dried catfish (Clarias gariepinus), stockfish (Brosme
Heating of palm oil (for 2 min)

Addition of onions and locust beans (frying for about 5 min)

Inclusion of grinded pepper (cooking for about 15 min)

Addition of milled melon seed (further cooking for 10 min)

Progressive addition of boiled stockfish, dried fish and crayfish (cooking for 5 min)

Addition of salt (mixing)

Mixing with shredded melon bitter leaf

Steaming (5 min)

Melon bitter leaf soup

**Figure 1.** Flowchart of melon bitter leaf soup preparation.

*brosme*, dried crayfish (*Cambarus robustus*) and table salt were used. Other materials included, chili pepper (*Capsicum annuum*), onion (*Allium cepa*), tomatoes (*Solanum lycopersicum*), fresh bitter leaf (*Vernonia amygdalina*), fermented locust beans (*Parkia biglobosa*) and palm oil. All materials used were obtained from retail outlets in Bodija Ibadan, Nigeria.

**Preparation of melon-bitter leaf soup**

Two hundred and fifty grams each of clean dry cat and stock fishes were boiled in a liter of water for about 20 min. Melon seed (600 g), crayfish (150 g), tomatoes (100 g), chili pepper (25 g) and onions (100 g) were sorted and separately milled. The bitter leaf was wet cleaned and thinly shredded. These inputs were next used in the soup preparation as described by FIIRO (2006) (Figure 1). The resulting soup sample was allowed to cool, freeze dried (-45°C) and stored until further analysis.

**Evaluation of some chemical properties of the soup mix**

Moisture, protein, fat and carbohydrate contents as well as pH and titratable acidity of the soup mix were evaluated according to standard methods (AOAC, 2005).

**Extraction and analysis of volatiles in the key soup ingredients and soup using HS-SPME-GC/MS**

One gram each of sample stock (locust bean, onion, melon seeds, crayfish, stockfish, bitter leaf and the soup) was extracted at 40°C by automated headspace solid-phase micro-extraction with the agitation of the sample. The SPME fiber (Sigma Aldrich, Dorset, UK) was coated with DVB-Carboxen on PDMS 50/30 μm (1 cm in length) (Sigma Aldrich, Dorset, UK), according to the method of Rocha et al. (2001). The compound absorbed by the fiber was desorbed in the injection port. Analysis was carried out on a GC
Table 1. Proximate composition of the freeze dried soup mix.

| Parameter       | Quantity       |
|-----------------|----------------|
| Moisture (%)    | 4.36 ± 0.02    |
| Protein (%)     | 40.43 ± 0.05   |
| Fat (%)         | 7.32 ± 0.03    |
| Carbohydrate (%)| 30.28 ± 0.05   |
| pH              | 5.36 ± 0.06    |
| Titratable acidity (mg/100 ml) | 18.00 ± 0.03 |

Each value is mean ± SD of triplicates.

Determination of functional groups with FTIR spectroscopy

Eight milligrams of the sample was thoroughly blended with potassium bromide powder and pelletized (13 mm diameter and 1 mm thickness). The sample was next introduced to FTIR (Spectro-BX, Perkin Elmer, UK) scanned at a range of 350-4000 nm to determine the functional groups inherent in the soup.

Statistical analysis

Data of proximate analysis were expressed as means ± standard deviation (SD).

RESULTS AND DISCUSSIONS

Some chemical properties of the soup mix

The physicochemical properties of soup (freeze dried) are presented in Table 1. The moisture content of the freeze dried sample (4.36%) was lower than that reported by Omah et al. (2015). Moisture content closely linked with low water activity has been implicated as a key factor in flavor developments in foods (Tamanna and Mahmood, 2015). Fat content (7.32%) was lower than the values reported for Ogbono soup mix (34.62%) (Bamidele et al., 2015). Fat plays an essential and direct role in flavor perception of flavorant, the most important flavor compound being free fatty acids produced by lipolysis (Engels, 2014). The carbohydrate content (30.28%) was higher in values than that reported for Ogbono soup mix (27.18%) (Bamidele et al., 2015).

High content of protein was expected since most ingredients that made up the soup are highly proteinaceous. Hydrolytic reaction resulted in amines and amino acids which ostensibly led to the formation of some volatile aroma compounds (Reineccius, 1999). The soup’s titratable acidity (18 mg/100 ml) and pH (5.36) reflects the acidic nature of the soup which may have enhanced hydrolytic reactions, yielding various flavor precursors (Belitz et al., 2009). The pH values have been reported to have significant influence on volatile compounds formed in Maillard type reaction as envisaged here (Shaoping et al., 2013). The formations of several volatile compounds including heterocyclic compounds have been a function of prevailing pH (Madruga and Mottram, 1998). Belitz et al. (2009) remarked that acidic medium also promotes the formation of furan and its derivatives in soups.

Analysis of volatiles in the soup and its ingredients

Volatile compounds present in the key ingredients

The ingredient that made up the soup showed: compounds in ground melon seed (52), fresh bitter leaf soup (32), onions (33), locust beans (56), crayfish (23), stockfish (25) and dried catfish (62). All volatiles identified were compounds of esters, amides, alcohols, benzenes, alkenes and furans which may have reacted together during cooking process to yield the corresponding volatile compounds in the final soup.

FT-IR analysis of the soup

FT-IR analysis revealed the presence of various functional groups present in the soup samples (freeze dried). The frequency range and functional group obtained from absorption spectra were noteworthy (Table 2).

In this study, the band at 3404.00 cm⁻¹ was due to the presence of amines, the sharp peak at 2920.00 cm⁻¹ was attributed to alkyl group while the band at 1740.67 cm⁻¹ is
Table 2. FTIR functional group frequencies of melon-bitter leaf soup (freeze-dried).

| S/N | Wave number (cm\(^{-1}\)) | Types of vibration | Functional groups           | Compound                      |
|-----|-----------------------------|---------------------|-----------------------------|-------------------------------|
| 1   | 3404.00                     | Stretch             | N-H                         | Amines                        |
| 2   | 2920.00                     | Stretch             | C-H                         | Alkyl group                   |
| 3   | 1740.67                     | Stretch             | C=O                         | Ester                         |
| 4   | 1648.20                     | Stretch             | C=C                         | Alkenes                       |
| 5   | 1544.04                     | Stretch             | C=C                         | Alkenes                       |
| 6   | 1156.47                     | Stretch             | C-O                         | Acids, ester, anhydride       |
| 7   | 711.00                      | Bending             | C-H                         | Mono-substituted benzene      |

Figure 2. Chromatogram of volatile compounds in soup mix (freeze-dried)

an indication of presence of esters. The band at 1544.04 cm\(^{-1}\) was attributed to alkenes while the band at 1156.47 and 711.00 cm\(^{-1}\) showed the presence of acids, anhydrides and mono-substituted benzene respectively. A similar observation was made on button mushroom soup (Qin et al., 2011). These functional groups were found to be precursors of volatile compounds associated with melon-bitter leaf soup. Esters are traditionally formed from the esterification of alcohol with fatty acids (Van Der Sluis et al., 2002). The significance of ester contribution to development of aroma is well known. Short chain esters are highly volatile at ambient temperatures with perception threshold ten times lower than their alcohol precursors (Izco and Torre, 2000). Amines have also been found to be flavor precursors in meat and meat products (Smith, 1982).

Volatile compounds present in the soup

The GC-MS chromatogram of the volatile constituents in soup mix samples are displayed in Figure 2. The types of compounds identified in the soup, were pyrazines (5), benzene (11), esters (3), alcohols (6), aldehyde (5), organic acids (9), alkanes (7), furan (5) and some others (Table 3). Few volatile compounds (Table 3) are found to
### Table 3. Volatile compounds in melon bitter leaf soup (freeze-dried).

| S/N | Compound                                                                 | Retention time (min) | Linear retention index |
|-----|--------------------------------------------------------------------------|----------------------|------------------------|
|     | **Pyrazines**                                                            |                      |                        |
| 1   | Trimethylpyrazine                                                        | 15.07                | 821                    |
| 2   | 2, 5 (and/or 2, 6) Dimethylpyrazine                                      | 19.45                | 912                    |
| 3   | Trimethyl pyrazine + octanal (10:1)                                      | 23.57                | 1003                   |
| 4   | Tetramethylpyrazine                                                      | 27.20                | 1088                   |
| 5   | Ethyltrimethylpyrazine                                                   | 30.15                | 1162                   |
|     | **Benzenes**                                                             |                      |                        |
| 6   | Propylbenzene                                                            | 21.56                | 959                    |
| 7   | 1,2–Dimethylbenzene                                                      | 18.72                | 897                    |
| 8   | 1-Ethyl-3-methylbenzene                                                  | 21.90                | 966                    |
| 9   | 1 Ethyl-4-methylbenzene hexanoic acid (3:1)                              | 21.99                | 968                    |
| 10  | 1,3,5, -Trimethyl benzene                                                | 22.22                | 973                    |
| 11  | 1 Ethyl-2-methylbenzene                                                  | 22.77                | 985                    |
| 12  | 1,2,4-Trimethylbenzene                                                   | 23.38                | 999                    |
| 13  | 1,4 – Dichlorobenzene                                                    | 24.26                | 1019                   |
| 14  | 1,2,3–Trimethyl benzene                                                  | 24.71                | 1030                   |
| 15  | A methylvinylbenzene                                                     | 25.35                | 1045                   |
| 16  | MW 134 benzene                                                           | 27.05                | 1085                   |
|     | **Esters**                                                               |                      |                        |
| 17  | 1–Methoxy-2-propylacelate                                                | 17.33                | 868                    |
| 18  | 2-Ethylhexanoic methyl ester                                             | 25.22                | 1042                   |
| 19  | 2-Ethylhexylacrylate                                                     | 32.65                | 1227                   |
|     | **Alcohols**                                                             |                      |                        |
| 19  | 2,3-Butanediol enantiomer 2                                              | 13.39                | 786                    |
| 20  | Toluene + 1-pentanol (1:1)                                                | 12.52                | 768                    |
| 21  | 2-Methylphenol                                                           | 25.69                | 1053                   |
| 22  | 1-Octanol                                                               | 26.33                | 1068                   |
| 23  | Phenol                                                                  | 22.42                | 978                    |
| 24  | Menthol                                                                 | 30.86                | 1180                   |
|     | **Aldehydes**                                                            |                      |                        |
| 25  | Pentanal                                                                | 9.05                 | 696                    |
| 26  | (E)-2-Heptanal                                                           | 21.49                | 957                    |
| 27  | Octanal                                                                 | 23.57                | 1003                   |
| 28  | Nonanal                                                                  | 27.73                | 1101                   |
| 29  | Hexanal                                                                  | 14.02                | 799                    |
|     | **Acids**                                                                |                      |                        |
| 30  | Acetic acid + tri-diacetyl                                               | 5.48                 | 607                    |
| 31  | 2 Methyl propionic acid                                                  | 12.13                | 760                    |
| 32  | Butanoic acid                                                            | 12.95                | 777                    |
| 33  | 3-Methylbutanoic acid                                                    | 16.39                | 848                    |
| 34  | 2-Methylbutanoic acid                                                   | 16.85                | 858                    |
| 35  | Pentanoic acid                                                           | 17.72                | 876                    |
| 36  | Hexanoic acid                                                            | 21.59                | 968                    |
| 37  | Tri octanoic acid                                                        | 29.97                | 1157                   |
be predominant in the soup aroma and included acetic acid, propanoic acid, 2-methylpropanoic acid, butanoic acid, trimethyl pyrazine, 3-methylbutanoic acid, furfural, 2-methylbutanoic acid, 2,5-dimethylpyrazine, hexanoic acid, 1,8-cineole, tetra-methylpyrazine, linalool, camphor and menthol.

Apparently, quite a number of volatiles found in the soup ingredients were absent in the end product while several volatiles compounds were generated in situ. These developments may be a function of thermal processing culminating perhaps in either the release of volatiles already existing in the ingredients or degradation of amino acids, sugars, nucleotides and/or Maillard reactions occurring between amino acids and reducing sugars (Qin et al., 2011).

Undoubtedly, flavorants, especially the volatile compounds, are generated through chemical reactions between natural precursors present in raw ingredients during thermal processing. Limiting precursors or critical component leading to the formation of characteristic volatile aromas of cooked foods such as meat have been established (Farmer, 1999). Reactions leading to flavor development may include pyrolysis of amino acids and peptides, carbohydrate degradation, interaction of sugars with amino acids and peptides, breakdown of ribonucleotides and lipids (Shahidi, 1998). Evidently, most non-volatiles are odorless and extremely hydrophilic and includes compounds such as table salt, citric acid and sugar (Choudhury, 2008). These are known to impact significantly on the taste of substances and thus regarded as flavorants. A number of other non-volatiles such as amino acids, peptides, fats, carbohydrates and organic acids also provide and enhance tastes in food (Choudhury, 2008). Although, generally odorless, these also generate characteristic volatiles. Their chemical interaction such as during hydrolytic cleavages usually leads to the formation of specific aromas (Chen and Ho, 1998).

Understandably, the soup inputs such as locust beans, melon seed, bitter leaf, crayfish, stockfish, dried catfish and onion have their characteristic flavor owing to their inherent chemical compositions. Possible reaction routes involved in the formation of the key aroma compounds present in the soup are as follows:

### Table 3, Contd.

|   |   |   |
|---|---|---|
| 38 | Nonanoic acid | 33.72 | 1257 |
| 39 | Octane | 14.02 | 799 |
| 40 | 3-Methylundecane | 30.42 | 1169 |
| 41 | Pentadecane | 41.87 | 1499 |
| 42 | Hexadecane | 44.95 | 1599 |
| 43 | Undecane | 27.68 | 1099 |
| 44 | Heptadecane | 47.84 | 1699 |
| 45 | Tetradecane | 38.64 | 1398 |
| 46 | Tr-furfural | 16.39 | 848 |
| 47 | 2-Pentylfuran | 23.03 | 991 |
| 48 | Acetoin (3-hydroxy – 2 – butanone) | 9.55 | 707 |
| 49 | Trimethylamine |   |   |
| 50 | Limonene | 24.92 | 1035 |
| 51 | Linalool | 27.73 | 1101 |
| 52 | 1,6–Cineole (eucalyptol) | 25.08 | 1039 |
| 53 | Camphor | 29.97 | 1157 |
| 54 | MW 156 saturated hydrocarbon | 26.43 | 1070 |
| 55 | MW 170 saturated hydrocarbon | 27.49 | 1095 |
Table 4. Possible key contributors to flavour of melon bitter leaf soup (freeze-dried).

| Retention time (min) | Compound                        | Peak identification     | Formula                  | Relative peak area (%) |
|----------------------|---------------------------------|--------------------------|--------------------------|------------------------|
| 5.48                 | (Carboxylic acid)               | Acetic acid              | CH₂CO₂H                  | 22.31                  |
| 8.78                 | (Carboxylic acid)               | Propanoic acid           | CH₃CH₂CO₂H               | 2.097                  |
| 12.13                | (Carboxylic acid)               | 2-methylpropanoic acid   | CH₃(CH₂)CHCO₂H           | 4.69                   |
| 12.95                | (Carboxylic acid)               | Butanoic acid            | CH₃CH₂CH₂CO₂H            | 2.51                   |
| 15.07                | (Aromatic)                      | Methylpyrazine           | C₅H₆N₂                   | 1.20                   |
| 16.39                | (Carboxylic acid/aldehyde)     | 3-methylbutanoic acid+furfural | CH₃CH(CH₃)CH₂CO₂H ; C₅H₆N₂ | 13.38                  |
| 16.85                | (Carboxylic acid)               | 2-methylbutanoic acid    | CH₃CH₂CH(CH₃)CO₂H        | 6.37                   |
| 19.45                | (Carboxylic acid)               | 2,5-dimethylpyrazine     | CH₃CH₃N₂                 | 0.29                   |
| 21.99                | (carboxylic acid)               | Hexanoic acid            | CH₃CH₂CH₂CH₂CH₂CO₂H      | 0.64                   |
| 23.57                | (Aromatic)                      | Trimethylpyrazine        | C₇H₁₀N₂                  | 1.55                   |
| 25.08                | (Aromatic)                      | 1,8-Cineole (eucalyptol) | C₁₀H₁₈O                  | 0.35                   |
| 27.20                | (Aromatic)                      | Tetramethylpyrazine      | C₈H₁₂N₂                  | 4.45                   |
| 27.73                | (monoterpenoid)                 | Linalool                 | CH₃(2)₂C=CH(CH₂)₂C(CH₃)(OH) | 0.38                   |

**Formation of substituted compounds of pyrazine**

The related volatile compounds found in the melon bitter leaf soup in this study were the substituted pyrazine compounds which included trimethylpyrazine, 2,5-dimethylpyrazine, tetramethylpyrazine and ethylmethylpyrazine. These compounds showed high retention factors due to their high level of affinity for the stationary phase and their molecular weight (Owens et al., 1997). They may have been formed from amino acids and diketones present in melon seed, bitter leaf, onion, fish and locust beans, all of plant/animal origin as used in this study during processing temperatures (37-100°C). The condensation reaction between diketones and amino acids followed by Strecker degradation produced α-amino ketones and thereafter oxidation may have led to the final substituted pyrazine compounds detected in the final soup (Yaylayan, 2003). In heated foods, the main source of the aldehydes is Strecker degradation of amino acids, while in fat containing foods, it is lipid oxidation (Reineccius et al., 2003). A widely accepted mechanism of pyrazines formation is reportedly based on the Maillard reaction and Strecker degradation (Belitz et al., 2009). The reaction of α-amino acids and reducing sugars initially generated Amadori/Heyns compounds, a rearrangement reaction which led to the formation of reductones which included α-dicarboxyls and α-amino carboxyls condensed to pyrazines. Pyrazines can also be formed directly from the Strecker degradation (Whitfield, 1992).

The high protein (40.43%) and carbohydrate (30.28%) contents under acidic conditions (pH 5.36) and high temperature (100°C) promoted the formation of large concentration of aroma precursors, ostensibly through hydrolytic reactions. These aroma precursors such as amines, amino acids and reducing sugars are usually associated with carbonyl groups (Reineccius et al., 2003). Having condensed through pathways such as Strecker degradation and oxidation processes, these gave rise to unstable intermediate (dihydropyrazines) which culminated in the formation of pyrazines (Yaylayan, 2003). The greater possibility of forming 2,5-dimethylpyrazine rather than 2,6-dimethylpyrazine was reported in a model system of glycine peptides and glucose (Weenen, 1998). Alanine and phenylalanine are strong pyrazine producer. Acetaldehyde and formaldehydes are Strecker aldehydes and also could be degradation products from glucose and other amino acids (Qin et al., 2011).

Pyrazines are one of the most important groups among the identified volatiles in food systems as they are widely distributed in foods that are processed at high temperatures and low moisture conditions.

**Formation of furan and its derivatives**

The analysis carried out on melon bitter leaf soup indicated the presence of furfural and 2-pentyl furan (Table 4). These pathways have been identified by some related studies (Belitz et al., 2009).

The hydrolytic products arising from the thermal processing of the proteins and carbohydrates in the soup may have formed aldohexose derivatives which then undergo cyclization to form furan. The acidic medium of the soup may have enhanced the formation of mono and
di-unsaturated acids. These during cyclization may possibly have yielded furfural. Furfural has been reported to be the most important contributor to roasted meat flavours. It is also found in castor bean condiments (Ojinnaka and Ojimeku, 2013). This pathway may have led to the formation of furan and its derivatives in this study. The heating of monosaccharides gives rise to a large number of compounds of furan. As reported and demonstrated by Belitz et al. (2009), the formation can be explained by enolization and dehydration of carbohydrates.

**Formation of aldehydes**

Another group of products suggested to have been formed from Strecker degradation of α-amino acids were pentanal, 2-heptanal, octanal, nonanal and hexanal also with relatively high retention factors owing to their high affinity for the stationary phase and low affinity for the mobile phase. Hexanal has earlier been observed by Takakura et al. (2014), in pork soup characterized by fruity flavour. Aldehydes generated in this study were Strecker aldehydes and may have been formed from Strecker degradation of amino acids. Additionally, these aldehydes may also have emanated from lipid oxidation by hemolytic β-scission containing carbon atom of the fatty acid in the oil. This radical could then combine with a hydroxyl moiety to produce an alcohol, giving rise to an aldehyde (decanal) by tautomerization.

Peroxidation of polyunsaturated fatty acids was initiated by additional mixtures of aldehydes besides several shorter fatty acids, keto, hydroxy or epoxy compounds (Vistoli et al., 2013).

The heat induced formation of these compounds from various fatty acids has been investigated (Guillen and Goicoechea, 2008). Thus, peroxidation of (n-6) fatty acids (linoleic and arachidonic acids) produces well defined compounds, 2,4-decadienal and 3-nonenal from 9-hydroperoxy linoleate; hexanal and pentanal from 13-hydroperoxy linoleate, and 2-heptenal from 10-hydroperoxy linoleate. Other volatile decomposition compounds frequently encountered include: 2-hexenal, 2-octenal, 2,4-nonadienal, 4,5-dihydroxydecenal and especially 4-hydroxy-2, 3-trans-nonenal (4HNE).

**Formation of carboxylic acids and alcohols**

Acetic acid is a well-known degradation product of saccharides. The formation of carboxylic acids may be a result of oxidative α-dicarboxyl cleavage. Novotny et al. (2008) also reported the formation of carboxylic acids due to sugar degradation. Davidek et al. (2006), also observed the formation of acetic acid and other short chain carboxylic acid in Maillard model systems (90°C, pH 6-10) and concluded that thermal treatment of 1-deoxy-d-erythro-2,3-hexidiole (in the presence of oxygen enriched water under alkaline conditions) enhanced the formation of acetic acid. Acetic acid has been reportedly observed in pork soup and is characterized by sour flavour (Takakura et al., 2014). Melon bitter leaf soup also indicated the presence of butanoic, 3-methylbutanoic, 2-methylbutanoic, pentanoic, hexanoic, octanoic, nonanoic, 2-methylpropanoic and ethanoic acids (acetic acid) which may also have been formed through the hydrolysis of esters and amides in the soup ingredients (stockfish, melon seed and onion) at the cooking temperature and acidic pH of the soup (Choudhury, 2008).

In this study, it can be concluded that sugar degradation by carbonyl group is a major pathway for the formation of carboxylic acid. Propanoic and butanoic acids are generated as a result of heating and Maillard reaction (Mottram and Whitefield, 1995). Esters and amides are two of the carboxylic acid derivatives formed from carboxylic acids in reaction with alcohols and ammonia, respectively. These reactions are reversible implying that these derivatives can also undergo hydrolysis to form carboxylic acids, alcohol or ammonia.

**Formation of linalool, limonene and camphor**

Linalool may have been formed from geranyl acetate via hydrolysis into geraniol followed by isomerization into linalool under the cooking condition. Limonene (cyclic monoterpenes) possibly came from geranyl acetate by loss of acetate ion followed by cyclization. The camphor component which is a bicyclic monoterpenene may have emanated from geranyl acetate through isomerization and cyclization into intermediate products such as α-pinene via linalyl acetate. These reactions are usually favoured by high temperatures as obtained during the cooking process (Spracklen et al., 2008).

**Formation of alkenes and alkanes**

Octane, 3-methylundecane, pentadecane, hexadecane, undecane, heptadecane and tetradecane were identified to be present in the soup. These compounds may have been formed from peroxidation of oleic acid and some other fatty acids (Boonprab et al., 2003).

**Conclusion**

Several volatile compounds were identified as the key aroma contributor of the soup and included acetic, propanoic, 2-methylpropanoic and butanoic acids, and methylpyrazine, 2.5 and/or 2.6-dimethylpyrazine, hexanoic
acid, 1,8-cineole, tetramethylpyrazine, linalinol, camphor and menthol. Carboxylic acids were the most abundant class of compound representing over 50% of the total volatile compounds, followed by heterocyclics and monoterpenoids in that order.

Conflict of Interests
The authors declare that there is no conflict of interests.

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