Comparison of Odorants in Beef and Chicken Broth—Focus on Thiazoles and Thiazolines

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Abstract: The shift in consumer landscape towards vegan, vegetarian and flexitarian diets has created an unprecedented challenge in creating meat aroma from plant-based alternatives. The search for potential vegan solutions has thus led to a renewed interest in authentic meat flavour profiles. To gain a better understanding of the qualitative odour differences between boiled beef and boiled chicken, aroma extracts were isolated using Likens-Nickerson simultaneous distillation-extraction (SDE), selected expressly because the in-situ heating of the sample facilitates the capture of aroma intermediates during the cooking process, thereby mimicking the cooking of meat in stocks and stews. The extracts were then analysed by Gas Chromatography-Mass Spectrometry (GC-MS) and GC-Olfactometry (GC-O). Most of the volatiles identified in this study were sulfur-containing compounds, such as sulfides, thiols, mercaptoaldehydes and mercaptoketones, which are derived from the Maillard reaction. Meanwhile, lipid oxidation results in the formation of unsaturated aldehydes, such as alkenals and alkadienals. Families of thiazoles and 3-thiazolines were found in the extracts. Two novel 3-thiazolines (5-ethyl-2,4-dimethyl-3-thiazoline and 2-ethyl-4,5-dimethyl-3-thiazoline) which may also contribute to the meaty aroma were identified in this work and synthesised from their respective aldehyde and mercaptoketone precursors.

Keywords: beef; chicken; GC-O; thiazole; thiazoline

1. Introduction

The rapid growth in vegan, vegetarian and flexitarian diets has led to renewed interest in the development of process flavourings with authentic flavour profiles of cooked meat, which is an important determinant of consumer acceptance [1]. Despite the advances in analytical techniques, recent studies were more interested in specific animal breeds [2–5], distinct animal parts [5,6] or special cooking techniques [7–9] rather than a comparison between species using the same cooking and extraction technique.

The characteristic aroma of cooked meat is generated from thermally induced reactions, principally the Maillard reaction and lipid oxidation, between non-volatile components of lean and adipose tissues during heating. These complex reaction pathways lead to the formation of a diverse range of products, accounting for the large number of volatile compounds found in cooked meat [2–9]. Aroma compounds produced during the Maillard reaction are typically responsible for the savoury, meaty (e.g., S-containing compounds such as 2-methyl-3-furanthiol), roast (e.g., pyrazines such as 2-ethyl-3,5/6-dimethylpyrazine) and boiled characters. Meanwhile, those derived from lipid oxidation (e.g., saturated and unsaturated aldehydes such as 2-alkenals and 2,4-alkadienals) impart fatty aromas to cooked meat and can contribute to species-specific notes [10].

In this study, the volatiles present in boiled chicken (BC) and boiled beef (BB) were isolated using Likens-Nickerson simultaneous distillation-extraction (LN–SDE), a technique which offers the advantage of in situ heating and extraction. This mimics the cooking of meat in stocks and stews in a kitchen setting and avoids the loss of aroma during sample
transfer inevitable in other methods. Equipped with more sensitive analytical techniques as compared to decades ago, the aim of this work is to gain a better understanding of the qualitative odour differences (i.e., odour activity, quality and intensity) between these species based on a fair comparison (i.e., same cooking and extraction technique), which will be useful in the creation and modification of process flavourings in products such as soups, stocks, and bouillons.

2. Results and Discussion
2.1. Similarities between Boiled Beef (BB) and Boiled Chicken (BC)

The aroma extracts of both BB and BC had an intense aroma characteristic of their respective species and the volatiles identified by GC-O and GC-MS for BB and BC extracts are listed in Table 1. A total of 58 and 64 odour-active volatiles were identified in BB and BC, respectively. The major classes of compounds in both extracts comprised S- and/or N-containing compounds, including heterocycles such as thiazoles, thiazolines and pyrazines, as well as straight chain saturated and unsaturated aldehydes, alcohols and ketones.

Many of the early-eluting odorants (LRI < 500 on DB-5 column) were highly volatile and potent S-containing compounds, namely hydrogen sulfide, methanethiol, ethanethiol and 2-propanethiol. Thus, some were not detected by GC-MS and only by GC-O as the human nose can be a more sensitive detector. Nevertheless, the majority of the compounds with the highest total odour intensity scores and detection frequencies in both the extracts were S-containing compounds, such as 2-methyl-3-furanthiol (cooked meat, roasted), 2-furylmethanethiol (roasted, burnt) and 3-(methylthio)propanal (potato), which were among the primary odorants identified in chicken broth [11]. As observed for these compounds, not all S-containing compounds impart meaty odours. Meanwhile, the N-containing compounds, tetramethylpyrazine and 2-ethyl-3,5-dimethylpyrazine, imparted carboard, earthy and medicinal notes. As for the N- and S-containing heterocycles, 3-thiazolines and thiazoles, odours ranging from savoury (meaty, fatty and onion) to sweet (popcorn, nutty and roasted rice) were detected and these will be discussed in greater detail in Section 2.3. The Strecker degradation products also contributed to the aroma of both the extracts: 3-methylbutanal (malty, cocoa, nutty), 2-methylbutanal (chocolate liquor, aldehydic) and phenylacetaldehyde (floral, rose, perfume, green) are derived from leucine, isoleucine [12] and phenylalanine [13], respectively.

Besides the Strecker aldehydes, carbonyl compounds derived from the oxidative degradation of unsaturated fatty acids were also identified in both extracts. (E,E)-2,4-Decadienal (fatty, fried), (E)-2-nonenal (fatty, aldehydic, waxy), 1-octen-3-one (cooked mushroom) and (E,E)-2,4-nonadienal (fatty) are formed from the autoxidation of n-6 fatty acids such as linoleate [14] and arachidonate [15,16]. Meanwhile, aldehydes such as octanal (fruity, sweet) and nonanal (fatty, citrus, leafy) originate from the autoxidation of n-9 fatty acids such as oleate. Although none of these carbonyl compounds identified possessed aroma characteristics resembling the complete spectrum of cooked chicken [17], it was reported that the removal of carbonyl compounds from the volatile fraction resulted in a loss of chicken odour and an intensification of meaty odour [18]. However, it was also demonstrated that the omission of (E,E)-2,4-decadienal alone did not result in a significant aroma change [19]. Thus, it is likely that it is the delicate balance of the group of carbonyl compounds which contribute to the overall aroma rather than a single aroma compound alone.
Table 1. Odour-active compounds in the boiled beef (BB) and boiled chicken (BC) extracts.

| Compound                  | Description | BB     | GC-O | GC-MS | LC-MS-MS | BC     | GC-O | GC-MS | LC-MS-MS | ID      | Ref      |
|----------------------------|-------------|--------|------|-------|----------|--------|------|-------|----------|---------|----------|
| Hydrogen sulfide           | Faecal, rotten-egg, sulfur | 31 6  <500 <500 <800 n.d. 28 6 <500 <500 <800 n.d. O, ms, lri | [20,21] |
| Acetaldehyde               | Sweet solvent | 9 3  <500 <500 <800 n.d. 15 3 <500 <500 <800 695 O, MS, LRI | [22,23] |
| Methanethiol               | Leftover chicken, faecal, rotten cabbage | 35 6  <500 <500 <800 n.d. 24 5 <500 <500 <800 n.d. O, ms, lri | [22,23] |
| Ethanol                    | Town gas, sulfur | 18 4  <600 n.d. <800 n.d. 12 2 <500 n.d. <800 n.d. O, lri | [24] |
| 2-Propanethiol             | Raw onion    | 27 4  <600 n.d. n.d. n.d. 43 6 <500 <500 <800 n.d. O, lri | [22,24] |
| Formaldehyde               | Mustard, brassica, | 36 5  <500 n.d. n.d. n.d. n.d. n.d. | O, MS, LRI |
| 2,3-Butanedione            | Buttery, cheesy, sweaty, creamy | 35 6  574 n.d. 987 987 21 5 586 n.d. 985 980 O, MS, LRI | |
| 1-Propanethiol             | Raw onion, musty, meaty | 13 4  603 608 n.d. n.d. 19 6 <600 n.d. <800 n.d. O, MS, LRI |
| 3-Methylbutanal            | Malty, cocoa, nutty | 36 6  646 644 921 919 29 6 644 646 925 931 O, MS, LRI |
| 2-Methylbutanal            | Chocolate liquor, aldehydic | 7 3  655 654 914 915 13 3 <800 n.d. O, MS, LRI | |
| Formic acid                | Mustard, brassica, | 36 5  574 n.d. 987 987 21 5 586 n.d. 985 980 O, MS, LRI | |
| 2,3-Pentanedione           | Creamy, buttery, cheesy, sweaty | 31 6  696 696 1061 1068 16 5 695 697 1058 1080 O, MS, LRI | |
| 1,3-Pentanediol            | Unidentified | - - - - - - - - - | O, MS, LRI | |
| Methanethiol               | Raw/rotting onion, catty, petroleum | 38 6  737 n.d. n.d. n.d. n.d. n.d. n.d. O, lri | [25] |
| Dimethyl disulfide         | Raw/rotting onion, sulfur, bad egg | 23 6  745 744 1080 1072 18 4 744 744 1081 1083 O, MS, LRI | |
| 1-(Methylthio)propane      | Onion, pungent, paint, petal | 36 6  766 763 n.d. n.d. 43 6 765 764 1256 1259 O, MS |
| Hexanal                    | Green, fatty, grassy | 36 6  799 801 1097 1082 32 6 799 801 1070 1101 O, MS, LRI |
| Mercaptopropanoic acid     | Vegetable, sulfur, garlic, beer headspace | 28 6  805 n.d. 1352 1352 22 4 805 n.d. 1353 1351 O, lri | [26,27] |
| Unknown                    | Brothy, meaty | 10 2  813 - - - n.d. n.d. n.d. O, MS, LRI |
| Unknown                    | Vegetable, sulfur, pyrazine, cardboard | 28 6  817 818 1270 n.d. 17 4 <800 n.d. O, MS, LRI |
| 3-Methyl-2-butanethiol     | Sweded onion, garlic, meaty, beer headspace | 26 6  823 n.d. n.d. n.d. n.d. n.d. O, MS, LRI |
| 1-(Methylthio)-ethanethiol | Rotting onions, sulfur, catty, petroleum | 38 6  845 847 1221 n.d. 40 6 846 847 1222 1231 O, MS, LRI |
| Ethyl methyl disulfide     | 2-Methyl-3-uranylan | 41 6  866 872 1303 n.d. 32 5 869 874 1330 1320 O, MS, LRI |
| Trimethylxazolone          | Popcorn, nutty, vegetable | 20 5  869 851 1150 n.d. n.d. n.d. O, MS, LRI |
| 2-Methyl-2-thiazoline      | Boiled/rotting onion, catty, sulfur, medicinal | 22 5  876 873 1275 n.d. 45 6 877 884 1278 1284 O, MS, LRI |
| 2,4-Dimethylxazolone       | Meaty, brassica | 11 3  890 889 1270 1283 14 4 883 889 1260 1276 O, MS, LRI |
| (Z)-4-Heptenal             | Lamb fat, potato, meaty, buttery | 49 6  895 900 1234 n.d. 38 6 <800 n.d. 900 1240 O, MS, LRI |
| Heptanal                   | Fruity | n.d. n.d. n.d. n.d. n.d. n.d. n.d. O, MS, LRI |
| 3-Methylcap-2-panone       | Onion, catty | 20 4  898 903 1346 n.d. 25 4 900 903 1353 1358 O, MS, LRI |
| 3-(Methylthio)propanal     | Potato | 46 6  901 909 1451 1467 33 5 905 909 1443 1448 O, MS, LRI |
Table 1. Cont.

| Compound | Description | BB | Odour | LRI<sub>DB-5</sub> | DC<sub>-(Z)-1,5-Octadien-3-one</sub> | BC | Odour | LRI<sub>DB-5</sub> | DC<sub>-(E)-2-Heptenal</sub> | ID | Ref |
|-----------|-------------|-----|-------|-----------------|-----------------|-----|-------|-----------------|-----------------|----|-----|
| 2-Furfurylmethanethiol + 2-Mercapto-3-pentanone | Stewed potato, meaty, beef, gravy | 39 | 5 | 905 | 912 | 1340 | 1342 | 34 | 5 | 909 | 912 | 1438 | 1431 | O, MS, LRI |
| 2,4-Dimethyl-3-thiazoline | Meaty, brothly | n.d. | n.d. | n.d. | n.d. | n.d. | 24 | 5 | 928 | 928 | n.d. | n.d. | O, MS, LRI |
| 2-Acetyl-1-pyrroline | Basmati rice, pandan | 42 | 6 | 918 | 924 | 1322 | 1327 | 39 | 6 | 920 | 924 | 1321 | 1323 | O, MS, LRI |
| 2-Acetylthiazole | Toasted, biscuit, basmati rice | 20 | 5 | 1021 | 1024 | 1648 | 1657 | 24 | 5 | 1020 | 1023 | n.d. | 1637 | O, MS, LRI |
| 2,4-Dimethyl-5-ethyl-oxazole | Roasted/grilled meat | n.d. | n.d. | n.d. | n.d. | n.d. | 24 | 5 | 928 | 928 | n.d. | n.d. | O, ms, lri |
| 4-Mercapto-4-methyl-2-pentanone | Boiled chicken | n.d. | n.d. | n.d. | n.d. | n.d. | 7 | 2 | 938 | 936 | 1385 | n.d. | O, ms, lri |
| (E)-2-Heptenal | Green, citrus, waxy | 17 | 4 | 961 | 961 | 1318 | 1311 | n.d. | n.d. | 973 | 973 | 1455 | 1459 | O, MS, LRI |
| Unknown | Fruity, cat’s pee, blackcurrant | 9 | 2 | 963 | - | - | - | - | - | - | - | - | - |
| 1-Heptanol | Mushroom, fuel | n.d. | n.d. | n.d. | n.d. | n.d. | 17 | 3 | 973 | 973 | 1455 | 1459 | O, MS, LRI |
| Dimethyl trisulfide | Meaty, (picked) onion | 11 | 2 | 967 | 976 | 1362 | 1360 | 9 | 2 | 977 | 976 | 1332 | 1351 | O, MS, LRI |
| 1-Octen-3-one + 1-Octen-3-ol | Raw mushroom, green, flower | 15 | 6 | 976 | 982 | 1294 | 1292 | 11 | 2 | 976 | 983 | 1298 | 1295 | O, MS, LRI |
| (Z)-1,5-Octadien-3-one | Geranium | 21 | 4 | 978 | n.d. | n.d. | 19 | 4 | 981 | n.d. | 1361 | n.d. | O, lri |
| 2-Methyl-3-octanone | Vegetable, earth, plastic, garlic | 19 | 3 | 987 | 986 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | O, ms, lri |
| Trimethyl-3-thiazoline (I) | Pickled onion, cat’s pee | 31 | 5 | 990 | 990 | 1367 | 1363 | 17 | 3 | 990 | 990 | 1364 | 1351 | O, MS, LRI |
| Trimethyl-3-thiazoline (II) | Meaty, fried onion | 16 | 3 | 994 | 992 | 1373 | 1380 | 27 | 5 | 993 | 999 | 1376 | 1367 | O, MS, LRI |
| Octanal | Fruity, candy | 25 | 4 | 999 | 1005 | 1279 | 1265 | 11 | 2 | 1003 | 1005 | 1286 | 1288 | O, MS, LRI |
| Trimethylisothiazole | Odd sulfur, cardboard, earthy, green, savoury | 32 | 6 | 1010 | 1008 | 1395 | n.d. | n.d. | n.d. | 993 | 999 | 1376 | 1367 | O, MS, LRI |
| (E,E)-2,4-Heptadienal | Earthy, plastic | n.d. | n.d. | n.d. | n.d. | n.d. | 5 | 2 | 1016 | 1016 | 1477 | 1482 | O, MS, LRI |
| 2-Acetylthiazole | Toasted, biscuit, basmati rice | 20 | 5 | 1021 | 1024 | 1648 | 1657 | 24 | 5 | 1020 | 1023 | n.d. | 1637 | O, MS, LRI |
| 3-Octen-2-one | Earthy, plastic | 11 | 3 | 1039 | 1041 | 1376 | n.d. | n.d. | n.d. | 1047 | 1046 | 1429 | 1427 | O, MS, LRI |
| 4-Ethyl-2-methyl-thiazole | Popcorn, basmati, barley, biscuit, roasting skin | n.d. | n.d. | n.d. | n.d. | n.d. | 16 | 3 | 1047 | 1046 | n.d. | n.d. | O, MS, LRI |
| Phenylacetaldehyde | Floral, rose, green, perfume | 26 | 6 | 1047 | 1052 | 1644 | 1656 | 20 | 4 | 1051 | 1052 | 1650 | 1636 | O, MS, LRI |
| 2-Acetyl-1,4,5,6-tetrahydropyridine | Popcorn, roasted skin, fried rice, bread | 13 | 3 | 1056 | 1053 | n.d. | n.d. | 17 | 3 | 1053 | 1053 | n.d. | O, ms, lri |
| Trimethyl-5-hydroxy-3-thiazoline | Roasted, fatty chicken skin | n.d. | n.d. | n.d. | n.d. | n.d. | 14 | 2 | 1055 | 1060 | n.d. | n.d. | ms |
| 2-Acetylpyrrole | Rice, popcorn, toasted | n.d. | n.d. | n.d. | n.d. | n.d. | 17 | 3 | 1069 | 1069 | 1969 | 1970 | O, MS, LRI |
| 2-Ethyl-3,5-dimethyl-pyrazine | Cardboard, pyrazine, cocoa, soil | 24 | 5 | 1075 | 1076 | n.d. | n.d. | 19 | 4 | 1078 | 1081 | 1439 | 1446 | O, MS, LRI |
| 5-Ethyl-2,4-dimethyl-thiazole | Popcorn, nutty, sweet, roasted | n.d. | n.d. | n.d. | 1080 | 1425 | 1442 | 11 | 2 | 1080 | 1079 | 1425 | 1424 | O, MS, LRI |
Table 1. Cont.

| Compound | Description * | BB | Odour | LRI<sub>DB-5</sub> d | LRI<sub>ZB-Wax</sub> e | BC | Odour | LRI<sub>DB-5</sub> d | LRI<sub>ZB-Wax</sub> e | ID f | Ref g |
|----------|---------------|----|-------|-------------------|-------------------|----|-------|-------------------|-------------------|-----|-------|
| 2-Ethyl-4,5-dimethyl-3-thiazoline (I) | Roasted onion, garlic | 5 | 1083 | n.d. | n.d. | 10 | 1085 | n.d. | n.d. | MS, LRI<sub>i</sub> | |
| Tetramethylpyrazine | Cardboard, pyrazine, medicinal | n.d. | n.d. | n.d. | n.d. | 26 | 5 | 1092 | 1088 | n.d. | 1458 | O, MS, LRI<sub>i</sub> |
| 5-Ethyl-2,4-dimethyl-3-thiazoline (II) | Fatty, beef fat, grilled meat, savoury | 33 | 6 | 1095 | 1094 | 1441 | 1443 | 20 | 3 | 1098 | 1095 | 1453 | 1451 | MS, LRI<sub>i</sub> |
| Nonanal | Floral, fruity, fatty, citrus, leafy | 12 | 3 | 1103 | 1107 | 1387 | 1375 | 14 | 3 | 1108 | 1107 | 1396 | 1391 | O, MS, LRI<sub>i</sub> |
| 2-Acetyl-2-thiazoline | Toasted, popcorn | 26 | 4 | 1109 | 1111 | n.d. | n.d. | 2 | 1 | 1110 | n.d. | n.d. | n.d. | MS, LRI |
| 4-Methyl-2-isopropylthiazole | Roasting tin bits, pyrazine, seasoning, onions | 22 | 4 | 1116 | 1120 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | O, MS | |
| Unknown | Earthy, soil | - | - | - | - | 16 | 3 | 1120 | - | - | - | - | - | [37] |
| 2-Acetyl-3,4,5,6-tetrahydropyridine | Popcorn, rice cracker, roasted | n.d. | n.d. | n.d. | n.d. | 20 | 4 | 1142 | 1142 | 1583 | n.d. | O, ms, lri | |
| (Z)-2-Nonenal | Fatty, waxy, fatty | 24 | 6 | 1149 | n.d. | 1498 | 1489 | n.d. | n.d. | n.d. | n.d. | n.d. | O, ms, lri | [33,38] |
| (E,E)-2,6-Nonadienal | Violets, floral, waxy, fatty | 29 | 6 | 1155 | n.d. | 1580 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | O, LRI | |
| 2-Methyl-3-furyl methyl disulfide | Casserole, meaty, beefy, peppery | n.d. | n.d. | n.d. | n.d. | 18 | 3 | 1158 | 1157 | 1582 | 1579 | O, ms, lri | [35,39] |
| Decanal | Orange | 29 | 6 | 1162 | 1165 | 1528 | 1529 | 15 | 3 | 1161 | 1165 | 1511 | 1524 | O, MS, LRI |
| 2,4,6-Trimethyl-1,3,5-trithiazoline | Black onion, soil | n.d. | n.d. | n.d. | n.d. | 4 | 1 | 1185 | 1181 | 1630 | 1649 | O, MS, LRI | |
| Furfuryl methyl disulfide | Chicken, fatty | n.d. | n.d. | n.d. | n.d. | 5 | 1 | 1228 | 1226 | n.d. | 1799 | O, MS, LRI | |
| Benzothiazole | Saffron, chicken fat, nutty | n.d. | n.d. | n.d. | n.d. | 18 | 4 | 1244 | 1249 | 1944 | 1937 | O, MS, LRI | |
| (E,Z)-3-Ethyl-5-methyl-1,2,4-trithiolane | Blackcurrant, cat’s pee, crispy chicken skin | n.d. | n.d. | n.d. | n.d. | 10 | 2 | 1262 | 1267 | 1699 | 1687 | ms, lri | [40] |
| Nonanoic acid | Sweaty | n.d. | n.d. | n.d. | n.d. | 9 | 2 | 1267 | 1268 | n.d. | 2179 | O, MS, LRI | |
| (E,E)-2,4-Decadienal | McCains, fried, fatty, meaty | 5 | 1 | 1337 | 1327 | 1812 | 1814 | 9 | 2 | 1331 | 1325 | 1814 | 1802 | O, MS, LRI | |
| (E)-2-Undecenal | Herby, fatty, meaty | 5 | 1 | 1547 | 1550 | 1715 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | O, MS, LRI | |

* Odour descriptors provided by 3 trained panellists; b Sum of odour intensities of duplicate samples recorded by panellists on DB-5 column (maximum score = 60); c Number of times the odour was detected by panellists (maximum n = 6); d Linear retention indices determined on DB-5 column (n.d. = not detected); e Linear retention indices determined on ZB-WAX column (n.d. = not detected); f Confirmation of identity where O = odour description agrees with literature; MS = mass spectrum agrees with that of authentic compounds; ms = mass spectrum agrees with reference spectrum in NIST 14/Inramass MS database or in literature; LRI = linear retention indices on DB-5 and/or ZB-WAX columns (where applicable) agree with that of authentic compounds; LRI<sub>S</sub> = linear retention indices on DB-5 and/or ZB-WAX columns (where applicable) agree with that of compounds synthesised in the laboratory; lri = linear retention indices on DB-5 and/or ZB-WAX columns (where applicable) agree with literature values; g Literature reference for LRI values.
2.2. Differences between BB and BC

Comparison of the two species showed that there were 21 volatiles identified in the BB extract which were not found in the BC extract and 14 volatiles vice versa. Although the majority of the volatiles were present in both the extracts, the odour intensities of these compounds varied, indicating their different levels of contribution to the aroma of each extract. Figure 1 depicts the difference in mean odour intensities between BB and BC, whereby positive values indicate that the compound was present at a stronger intensity in BB than BC and vice versa for the negative values. A few interesting observations could be made from this figure. The majority of the Maillard reaction products, such as the Strecker aldehydes and diketones, occurred at higher intensities in BB than BC. Meanwhile, among the lipid-derived aldehydes, the alkenals were more predominant in BC while alkenals were more prevalent in BB.

Some fatty odorants, such as \((E,E)-2,4\)-heptadienal and \((E,E)-2,4\)-nonadienal, were only found in the BC extract, while others such as \((E)-2\)-heptenal (green, citral, waxy), \((Z)-2\)-nonenal (fatty, plastic-like), \((E,Z)-2,6\)-nonadienal (violets, floral, waxy, fatty) were only found in the BB extract. However, the strength of fatty odours in the overall aroma is not a consequence of the quantity of identified aldehydes, and the odour activities of the volatiles ought to be considered (Table 2). Thus, it was possible that fatty odorants had a greater contribution to BC than BB aroma, as reported by Gasser and Grosch [11] who observed a stronger prevalence of fatty odorants such as \((E,E)-2,4\)-decadienal, \(\gamma\)-dodecalactone and \((E/Z)-2\)-undecenal in the aroma of BC as compared to BB as a result of the higher fat content in the former. Although \((E,E)-2,4\)-decadienal was present in both extracts, the total odour intensity was almost twice as strong in the BB extract as compared to the BC extract.

Table 2. Odour thresholds of selected alkenals and alkadienals.

| Alkenal/Alkadienal          | Odour Threshold (\(\mu g \text{ kg}^{-1}\) in Water) | Source     |
|-----------------------------|-----------------------------------------------|-----------|
| \((E)-2\)-Heptanal          | 13                                            | [41]      |
| \((E,E)-2,4\)-Heptadienal   | 0.032                                         | [42]      |
| \((E,E)-2,4\)-Nonadienal    | 0.06                                          | [43]      |
| \((E,Z)-2,6\)-Nonadienal    | 0.0045–0.02                                   | [42,43]   |
| \((E,E)-2,4\)-Decadienal    | 0.027–0.07                                    | [41,42]   |

As for the Strecker degradation products, 3-methylbutanal, phenylacetaldehyde and 3-(methylthio)-propanal were present at higher intensities in BB than BC as seen in Figure 1. More notably, bis(2-methyl-3-furyl) disulfide (beef fat, meaty, fatty) were only found in the BB extract. This latter compound, with an extremely low odour threshold value of \(2.4 \times 10^{-5} \text{ \(\mu g \text{ kg}^{-1}\) in water}\) [44], is a dimer of 2-methyl-3-furanthiol [45] and was also found in several other studies [7,46,47]. These results corroborated the findings of Gasser and Grosch [11] that bis(2-methyl-3-furyl) disulfide and the Strecker aldehydes were more predominant in the aroma of boiled beef. Furthermore, these authors also reported 12-methyltridecanal to be a beef-specific odorant with tallowy, beef-like notes, which was proposed to be liberated from plasmalogens in ruminants after a long heating period [48,49]. However, this branched aldehyde was not found in our study, which could be attributed to the milder heating conditions and shorter duration impeding the release of the aldehyde from plasmalogens, as it was also not reported in several other studies with similar experimental conditions [8,11,50–52].
Figure 1. Difference in mean odour intensities of duplicate samples recorded by three panellists on DB-5 column between BB and BC extracts (positive values indicate that the compound was present at a stronger intensity in BB than BC and vice versa for the negative values).
2.3. 3-Thiazolines and Thiazoles

For the first time, 5-ethyl-2,4-dimethyl-3-thiazoline (fatty, grilled meat, savoury) and 2-ethyl-4,5-dimethyl-3-thiazoline (roasted onion, garlic) were reported in BB and BC extracts. Not only are these 3-thiazolines structural isomers, but each also exists as geometric isomers represented by distinct peaks of similar ratios (based on percentage of total ion chromatogram peak area). The mass spectra of these isomers are provided in Table 3 while their formation mechanisms and mass fragmentation patterns are proposed in Figures 2 and 3, respectively.

**Table 3.** Mass spectral data for 5-ethyl-2,4-dimethyl-3-thiazoline and 2-ethyl-4,5-dimethyl-3-thiazoline.

| Compound                              | LRI  | DB-5 a | Mass Spectral data, m/z (Relative Intensity) c |
|--------------------------------------|------|--------|-----------------------------------------------|
| 5-Ethyl-2,4-dimethyl-3-thiazoline (isomer I) | 1080 | 1419   | 69, 42 (72), 102 (64), 73 (44), 143 (43), 41 (38), 68 (36), 45 (33), 60 (32), 114 (27) |
| 5-Ethyl-2,4-dimethyl-3-thiazoline (isomer II)† | 1099 | 1446   | 69, 42 (70), 102 (58), 73 (40), 143 (40), 41 (39), 68 (34), 45 (34), 60 (30), 114 (25) |
| 2-Ethyl-4,5-dimethyl-3-thiazoline (isomer I)† | 1089 | 1443   | 114, 68 (67), 102 (44), 143 (55), 87 (27), 69 (20), 42 (20), 60 (20), 55 (15), 56 (14) |
| 2-Ethyl-4,5-dimethyl-3-thiazoline (isomer II) | 1100 | 1465   | 114, 68 (68), 102 (47), 143 (40), 87 (35), 42 (35), 69 (30), 45 (25), 41 (23), 39 (23) |

† Compound identified in boiled beef and boiled chicken aroma extracts; a Linear retention index on DB-5 column; b Linear retention index on ZB-Wax column; c First number is the base peak; molecular ion in bold type.

**Figure 2.** Proposed scheme for the formation of (a) 5-ethyl-2,4-dimethyl-3-thiazoline and (b) 2-ethyl-4,5-dimethyl-3-thiazoline.

**Figure 3.** Mass fragmentation pattern of 5-ethyl-2,4-dimethyl-3-thiazoline and 2-ethyl-4,5-dimethyl-3-thiazoline (Adapted with permission from Elmore et al. [53] and Bredie et al. [54]. Copyright 1997 and 2002 American Chemical Society).
Table 3. Mass spectral data for 5-ethyl-2,4-dimethyl-3-thiazoline and 2-ethyl-4,5-dimethyl-3-thiazoline.

| Compound                                      | LRI<sub>DB-5</sub><sup>a</sup> | LRI<sub>ZB-Wax</sub><sup>b</sup> | Mass Spectral data, m/z (Relative Intensity)<sup>c</sup> |
|-----------------------------------------------|-------------------------------|---------------------------------|--------------------------------------------------|
| 5-Ethyl-2,4-dimethyl-3-thiazoline (isomer I)  | 1080                          | 1419                            | 69, 42 (72), 102 (64), 73 (44), 143 (43), 41 (38), 68 (36), 45 (33), 60 (32), 114 (27) |
| 5-Ethyl-2,4-dimethyl-3-thiazoline (isomer II) | 1099                          | 1446                            | 69, 42 (70), 102 (58), 73 (40), 143 (40), 41 (39), 68 (34), 45 (34), 60 (30), 114 (25) |
| 2-Ethyl-4,5-dimethyl-3-thiazoline (isomer I)  | 1089                          | 1443                            | 69 (20), 42 (20), 60 (20), 55 (15), 56 (14) |
| 2-Ethyl-4,5-dimethyl-3-thiazoline (isomer II) | 1100                          | 1465                            | 114, 68 (67), 102 (44), 143 (55), 87 (27), 42 (35), 69 (30), 45 (25), 41 (23), 39 (23) |

† Compound identified in boiled beef and boiled chicken aroma extracts; <sup>a</sup> Linear retention index on DB-5 column; <sup>b</sup> Linear retention index on ZB-Wax column; <sup>c</sup> First number is the base peak; molecular ion in bold type.

The formation pathway was proposed to be the reaction of $\alpha$-hydroxyketones or $\alpha$-dicarboxyls and aliphatic aldehydes in the presence of hydrogen sulfide and ammonia, yielding 3-thiazolines which form thiazoles upon oxidation [53]. The nucleophilic addition of -SH to the carbonyl group of $\alpha$-dicarboxyls, followed by reduction, resulted in the formation of mercaptoketones, which could react with the imine intermediate, formed between ammonia and the aldehyde, in a nucleophilic attack. The subsequent ring closure and water elimination resulted in the formation of 3-thiazolines. Thus, in addition to the $\alpha$-hydroxyketones and $\alpha$-dicarboxyls, the presence of mercaptoketones, which are intermediates in the mechanism, could also result in the formation of 3-thiazolines and thiazoles. Since these precursors were identified in the GC-MS and GC-O, the formation of 3-thiazolines and thiazoles in the BB and BC extracts is very likely. Given the high odour intensity scores of these compounds, they could also be important contributors to boiled meat aromas.

Other 3-thiazolines and several thiazoles were also identified in this study, among which 2,4-dimethyl-3-thiazoline (meaty, brothy, roasted), 2,4-dimethylthiazole (meaty, grilled chicken, roasted), trimethyl-3-thiazoline (pickled onion, cat’s pee; meaty, fried onion), and 5-ethyl-2,4-dimethylthiazole (popcorn, nutty, sweet and roasted) had been found in beef with odour thresholds of 0.02, 0.5, 0.1 and 0.002 mg kg$^{-1}$ in water, respectively [55]. The last two compounds were also found in fried chicken, along with 4-ethyl-2-methylthiazole (popcorn, basmati, biscuit, roasting skin) [56]. These authors also suggested that the lack of mention of 3-thiazolines in literature is due to their presence in trace quantities and susceptibility to oxidation. The presence of thiazolines also exists in other foods such as 2-ethyl-4,5-dimethyl-3-thiazoline in freeze-dried onion sprout [57], as well as trimethylisothiazole (odd sulfur, cardboard, earthy, green) in yeast extract paste [35] and sesame seed oil [58].

Among all the 3-thiazolines and thiazoles with confirmed identities (i.e., by odour description, GC-O and GC-MS LRIs), 2,4-dimethyl-3-thiazoline and 4-ethyl-2-methylthiazole were only identified in BC. These compounds share a common roast aroma, with the thiazoline possessing a meaty note and the thiazole bearing sweeter or more fragrant attributes such as popcorn, basmati and barley. The toasted and rice qualities could also be enhanced in BC by the higher odour intensity score of 2-acetylthiazole in BC than BB (24 vs. 20). Meanwhile, the 3-thiazolines present in BB were characterised by stronger savoury (onion and meaty) notes as manifested in the higher odour intensity scores for 5-ethyl-2,4-dimethyl-3-thiazoline (33 in BB vs. 20 in BC) and trimethyl-3-thiazoline isomer I (31 in BB vs. 17 in BC). Overall, 3-thiazolines and thiazoles contributed to the toast, nutty and rice notes in the BC aroma to create a more rounded profile, while the onion and meaty attributes could be enhanced in the BB aroma to produce a more intense savoury perception.
3. Materials and Methods

3.1. Reagents and Chemicals

Aroma chemicals were obtained from the following suppliers and were ≥95% in purity unless stated otherwise: acetaldehyde, hexanal, heptanal, octanal, nonanal, decanal, 3-(methylthio)propanal, 2-methylbutanal, (E)-2-nonenal, (E,E)-2,4-decadial (90%), (E,E)-2,4-nonadienal (≥89%), (E,E)-2,6-nonadienal, 1-heptanal, 1-octen-3-ol, Z)-4-heptenal, 1-octen-3-one, 2,3-butanediene, 2,3-pentanediene, 3-mercapto-2-butanalone, dimethyl disulfide, dimethyl trisulfide, benzothiazole, 2-methyl-3-furanyl methyl disulfide, 2-acetylpyrrole, 2-acetyl-2-thiazoline, tetramethylpyrazine and 2-isopropylpyrazine were from Sigma Aldrich (Gillingham, UK); 4-nonanone, 2-furylmethanethiol, 3-mercapto-2-pentanone, 2-methyl-2-thiazoline and 1-(methylthio)propane from TCI (Oxford, UK); (E,E)-2,4-decadial (90%) and trimethoxazole from Lancaster Synthesis (Heysham, UK); phenylacetaldehyde and 3-octen-2-one from Acros Organics (NJ, USA); 1-(methylthio)ethanethiol from Carbosynth (Newbury, UK); 1-propanethiol from Fisher Scientific (Loughborough, UK); furfuryl methyl disulfide, 2,4-dimethylthiazole and (E)-2-heptenal from Oxford Organics; 2-acetylthiazole from Fluorochem (Hadfield, UK); 3-methylbutanal from Alfa Aesar (Heysham, UK); 2-methyl-3-furanthiol from IFF (Haverhill, UK), 3-methyl-2-butene-1-thiol from AROXA (Surrey, UK), 2-acetyl-1-pyrroline (≥85%) from aromaLAB GmbH (Martinsried, Germany), nonanoic acid from Anitox (Wellingborough, UK). Pentane (≥98%), diethyl ether (≥99.5%), dichloromethane (≥99.8%), ammonium sulfide (20% wt. solution in H2O), propanal (97%) and 1-hydroxy-2-butanalone (Aldrich®; purity unknown) were from Sigma-Aldrich. 1-Hydroxy-2-propanone (97%) was from Fluka (Seelze, Germany). High purity water (18.2 MΩ) was obtained from a Select Fusion Ultrapure water deionisation unit (SUEZ, Peterborough, UK).

3.2. Likens-Nickerson Simultaneous Distillation-Extraction (LN-SDE)

Fresh beef silverside joint and fresh Class A chicken breast were purchased from a retail supermarket and used within the sell-by date. The meats were provided by one commercial supplier and from the same batch and farm. The chickens were a standard Ross 308 genotype and were reared, slaughtered and processed under commercial conditions. The meat was trimmed of extramuscular fat and minced using a food mincer with 4.5 mm screen (Kenwood, Hampshire, UK). The minced meat (500 g) and high purity water (500 g) were added to a round bottom flask. The sample was boiled at 100 °C in a heating mantle for 30 min. Likens-Nickerson SDE was performed using 30 mL of redistilled pentane/diethyl ether (9:1 v/v) for 2 h. The extract was concentrated to 0.5 mL using a Vigreux column and stored at −80 °C prior to analysis.

3.3. Gas-Chromatography Olfactometry (GC-O)

GC-O analyses were performed on a HP 5890 Series II GC equipped with a flame ionisation detector (Hewlett Packard, Waldbronn, BaWü, Germany) and an ODO II odour port (SGE, Ringwood, Victoria, Australia). The instrument was controlled with ChemStation (version A.06.03). An aliquot of sample (2 µL) was injected in splitless mode, with the injector at 250 °C. Chromatographic separation was achieved on two columns of different polarities. For the non-polar Rxi-5 Sil MS column (30 m × 0.25 mm × 1 µm; Restek, Bellefonte, PA, USA), the oven temperature was programmed from 35 °C to 200 °C at 6 °C min⁻¹ and finally to 320 °C at 15 °C min⁻¹. For the polar ZB-Wax column (30 m × 0.25 mm × 0.25 µm; Phenomenex, Torrance, CA, USA), the oven temperature was programmed from 35 °C to 200 °C at 6 °C min⁻¹ and finally to 250 °C at 15 °C min⁻¹. All initial and final temperatures were held for 10 min. Helium was used as the carrier gas at a constant flow rate of 2.0 mL min⁻¹. The column effluent was split equally between the FID and odour port, where the odours of the eluting components were evaluated. The samples were analysed in duplicates by 3 trained assessors who were screened for olfactory performance in terms of threshold, discrimination and identification using a Sniffin’ Sticks test (Burghardt®, Wedel,
Germany). Each has an olfactory score of ≥38 (out of a maximum of 48) and at least one year of GC-O experience. Assesors recorded the descriptions and intensity scores on a scale of 1–10 (where 3 = weak, 5 = medium and 7 = strong) for the odours detected.

3.4. Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analyses were performed on an Agilent 7890A GC coupled to an Agilent 5975C inert XL EI/CI MSD triple axis MS (Agilent Technologies, Santa Clara, CA, USA) and controlled with Agilent MSD ChemStation (version E02.02). An aliquot of sample (2 µL) was injected in splitless mode, with the injector at 250 °C. Chromatographic separation was achieved on two columns of different polarities. For the non-polar HP-5 MS column (30 m × 0.25 mm × 1 µm; Agilent Technologies), the oven temperature was increased from 35 °C to 320 °C at 6 °C min⁻¹. For the polar ZB-Wax column (60 m × 0.25 mm × 0.25 µm; Phenomenex), the oven temperature was increased from 35 °C to 250 °C at 4 °C × min⁻¹. All initial and final temperatures were held for 10 min. The carrier gas was helium at a constant pressure of 124 kPa. The MS was operated in electron impact mode with a source temperature of 250 °C, ionisation energy of 70 eV and a scan range from m/z 29 to m/z 400.

3.5. Compound Identification and Quantification

The chromatograms were processed using Agilent MSD ChemStation (version F.01.03.2365; Agilent Technologies). A series of C₈–C₂₅ n-alkanes was analysed under the same conditions for the calculation of the linear retention index (LRI) of each compound. The identities of the compounds were confirmed based on a match of their mass spectra, LRI and odour descriptors with those of authentic compounds where available. Otherwise, a tentative identification was made by comparing their mass spectrum against the NIST 14 NIST/EPA/NIH EI MS Library (Gaithersburg, MD, USA) or INRAMASS MS library (INRA, France), as well as LRI and odour descriptors available in the literature.

3.6. 3-Thiazoline and Thiazole Synthesis

Synthesis was carried out using an adapted method from Elmore and Mottram [53]. Equimolar quantities (5 mmol) of an aldehyde and a mercaptoketone or α-hydroxyketone depending on availability (Table 4) were added to 50 mL of 0.1 M ammonium sulfide solution in a Duran bottle before heating in a 100 °C water bath for 30 min with constant stirring using a magnetic stirrer. The reaction mixtures were allowed to cool at room temperature before extraction with 30 mL dichloromethane. The extracts were flushed under nitrogen before storage at −20 °C until analysis by GC-MS as described in Section 3.4.

Table 4. Precursors for 3-thiazoline and thiazole synthesis.

| Reactants | Aldehyde | α-Hydroxyketone/Mercaptoketone | Products | 3-Thiazoline | Thiazole |
|-----------|----------|--------------------------------|----------|-------------|---------|
| Acetaldehyde | 1-Hydroxy-2-propanone | 2,4-Dimethyl- |
| Acetaldehyde | 1-Hydroxy-2-butanone | 4-Ethyl-2-methyl- |
Table 4. Cont.

| Reactants                  | Products                      |
|---------------------------|-------------------------------|
| Aldehyde                  | α-Hydroxyketone/ Mercaptoketone | 3-Thiazoline | Thiazole                |
| Acetaldehyde              | 3-Mercapto-2-pentanone        | 5-Ethyl-2,4-dimethyl- | |
| Propanal                  | 3-Mercapto-2-butanone         | 2-Ethyl-4,5-dimethyl-  | |
| Acetaldehyde              | 3-Mercapto-2-butanone         | Trimethyl-      |  |

4. Conclusions

This study has demonstrated the important contribution of 3-thiazolines in boiled meat aroma and their high aroma intensities could alter and/or enhance the meaty profile in BB and BC. The identification of 5-ethyl-2,4-dimethyl-3-thiazoline and 2-ethyl-4,5-dimethyl-3-thiazoline was also reported in BB and BC for the first time. Aroma extract dilution analysis and recombination studies could be carried out to further evaluate their importance. Nevertheless, the trends were that S-containing compounds and Strecker aldehydes were more prevalent in BB than BC, while fatty odorants, which were mainly lipid-derived aldehydes, were more predominant in BC than BB. This information would be useful for the creation of species-specific meat flavourings or those directed towards desired profiles.

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