

# Identification of odour-active compounds in a paste of roasted hazelnut from Piedmont Tonda Gentile Trilobata

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## Abstract

Application of gas chromatography-olfactometry on the volatiles isolated from roasted Tonda Gentile Trilobata (TGT) hazelnut paste by means of solvent extraction and solid phase extraction revealed 129 odour-active areas. Besides known hazelnut odorants, 24 components have been identified for the first time as odour-active compounds in roasted hazelnuts, namely 3-methylbutyl acetate, ethyl hexanoate, methyl phenylacetate, 3-methyl-2,4-nonandione,  $\alpha$ -phellandrene, isoeugenol, 2-vinyl-3,5-dimethylpyrazine, 2-acetyl-2-thiazoline, 3-methyl-2-buten-1-thiol, 3-mercapto-3-methylbutyl formate, methyl-3-(methylthio)furan, furfuryl methyl sulphide,  $\delta$ -nonalactone,  $\gamma$ -decalactone,  $\delta$ -decalactone, massoia lactone,  $\gamma$ -dodecalactone,  $\delta$ -dodecalactone, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, 3-hydroxy-2-methylpyran-4-one, methyl cyclopentenolone, 2-aminoacetophenone, 3- and/or 4-ethylphenol and 3-methylindole.

**Keywords:** roasted hazelnut paste, solid phase extraction, gas chromatography-olfactometry, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, TGT/TGL

## Introduction

The principal countries producing hazelnut are Turkey, Italy, Spain, USA and Greece (<http://www.fao.org/docrep/>) [1]. Italy is the second worldwide hazelnut producer with 132,700 t (<http://dati.istat.it/>) [2]. The 98% of Italian producing surface is located in four regions: Campania, Latium, Piedmont and Sicily.

Nut production in Piedmont is very variable among the years and, in 2018, was 36 477 tons (<http://dati.istat.it/>). It is based on a single cultivar: Tonda Gentile delle Langhe (TGL) or also known, since the end of XIX century, as “Tonda gentile del Piemonte” or “Tonda gentile trilobata (TGT)” that has been investigated since the 1950s [3, 4]. TGL was selected directly by farmers for its good environmental adaptability to Piedmont climate and for the excellent quality of the kernel [5]. Another important characteristic of this hazelnut is the high degree of peelability after roasting.

Roasting is the key technological process in hazelnut industrial transformation. The main purpose of roasting is to improve colour, crispiness and crunchiness of the product but beside all these aspects, the principal reason of roasting is to improve the flavour.

The unique pleasant aroma of roasted hazelnuts has been the topic of several investigations in recent years. In 1972, Kinlin et al. [6] were the first to investigate the volatile fraction of roasted hazelnuts and identified over 200 volatile compounds using various extraction methods and packed column gas chromatography.

However, only few groups undertook efforts to distinguish the odour-active compounds from the bulk of odourless volatile compounds identified in raw or roasted hazelnuts by application of gas chromatography-olfactometry on the volatile fraction of hazelnuts so far [7-13].

Several different extraction methods have been studied on different hazelnut samples in the past to identify new aroma compounds. During the present study, a combination of solvent extraction followed by Solid Phase Extraction (SPE) was applied as sample preparation method the first time.

SPE as sample preparation procedure offers the advantage of being proceeded at low temperatures. Moreover, the aqueous conditions often used resemble the conditions during consumption of food and thus mimic the aroma release from the hazelnut paste matrix during natural consumption conditions. Additionally, using SPE as sample preparation approach, we expected to find further odour-active compounds particularly among the low volatile compounds as reported by Engel et al. [10]. In their study, quantitative data obtained by mixtures of n-alkanes (C-10 to C-26) distillates by means of the SAFE technique at 35 °C showed that the yields for n-alkanes up to a chain length of 14 (n-tetradecane, boiling point: 252 °C) were 100%, while the yield of n-hexadecane (boiling point: 284 °C) and n-octadecane (boiling point: 317 °C) was only 59 and 12 %, respectively [10].

In order to include also the polar compounds among the low volatile components in the work-up procedure, solvent extraction prior to SPE was performed using water as a polar solvent. A portion of 10 % ethanol was added to provide a reasonable efficient extraction of nonpolar compounds as well. The use of this water/ethanol mixture

(90/10; w/w) should on one hand guarantee an extraction of the low volatile compounds including the polar compounds, and on the other hand prevent the extraction of fat.

The aim of the present study was, therefore, to use a combination of solvent extraction followed by solid phase extraction as sample preparation method in order to identify the odour-active compounds of a roasted TGT hazelnut paste and characterise them by means of gas chromatography-olfactometry (GC-O), gas chromatography-mass spectrometry (GC-MS) and heart-cut gas chromatography-gas chromatography-olfactometry-mass spectrometry (GC/GC-O/MS).

## Experimental

### *Roasted hazelnut paste*

The roasted hazelnut paste under investigation was provided by Soremartec Italy Srl. Commercial sample of hazelnut *Corylus avellana* L. TGT, harvested in 2017 in Piedmont area. In order to get a representative sample, 2 tons of hazelnuts (calibre ranging from 12 to 14 mm) have been roasted following industrial roasting process protocol. After roasting the hazelnuts have been grinded to obtain a paste that was stored in fridge (4°C, controlled humidity) until the time of extraction.

### *Isolation of volatiles*

A mixture of 180 g tap water (40 °C) and 20 g ethanol (96 vol%) was added to the hazelnut paste under investigation (40 g) and stirred vigorously with an Ultraturrax T18 (IKA GmbH, Staufen, Germany) for 2 minutes. The mixture was centrifuged for 30 min at 4 °C and 13000 RPM (UniCen MR, Herolab GmbH, Wiesloch, Germany). Then the aqueous phase was decanted cautiously and filtered through a coffee filter. Another portion of water/ethanol as described above was added to the centrifuge tube containing the remaining sediment and fat and the mixture was again stirred, ultrafiltrated and the aqueous phase decanted, filtered and combined with the first aqueous phase (extraction cycle 1). This procedure was repeated for five times taking portions of fresh hazelnut paste (40 g) each time (extraction cycles 2 to 6). Finally, all six portions of aqueous filtrate were combined. The combined aqueous phases now contained the ingredients of 240 g hazelnut paste and each portion of hazelnut paste (40 g) was extracted twice.

### *Solid Phase Extraction (SPE)*

An SPE cartridge containing 2 g of Chromabond HR-P (Macherey-Nagel GmbH, Düren, Germany) was activated with ethanol (96 vol%, 12 mL) and rinsed with tap water (12 mL). The combined aqueous phases obtained as described above were adsorbed on the SPE cartridge with minimum pressure using a SPE vacuum manifold and rinsed with tap water (12 mL). Then the SPE cartridge was turned around and desorbed with ethanol in the opposite direction of the adsorption one. The aqueous phase (approximately 1.5 mL) was discarded and the ethanolic phase (4 mL) was collected and dried over sodium sulphate.

### *Gas chromatography-Olfactometry (GC-O)*

Gas chromatography-olfactometry was performed by means of a gas chromatograph GC 2010 (Shimadzu, Duisburg, Germany) with Helium serving as carrier gas at a pressure of 70 kPa. Samples were injected by the split/splitless-injection onto capillaries DB-5 or DB-FFAP (both 30 m, 0.25 mm i.d., 0.25 µm film thickness; J&W, Agilent Technologies, Waldbronn, Germany). The end of the capillary was connected to a deactivated Y-shaped glass splitter dividing the effluent of the column into two equal parts, which were then transferred via two deactivated but uncoated fused silica capillaries (30 cm x 0.25 mm i.d.) to a sniffing port and a FID, respectively. The sniffing port which was mounted on a detector base of the GC was heated to 230 °C, the FID was operated at 240°C. Split/splitless-injection of the samples (1.0 µL) was performed at an oven temperature of 30°C. After 2 min, the temperature was raised by 10°C per min to 60°C and then by 6°C per min to 240°C (DB-FFAP) or 250°C (DB-5). The final temperature was held for 10 min. During a GC run, the panellist placed his/her nose closely above the top of the sniffing port and evaluated the odour of the chromatographic effluent. Sniffing was performed by three trained sniffers. Linear retention indices (RI) of the compounds were calculated from the retention times of n-alkanes.

### *Gas chromatography-Mass Spectrometry (GC-MS)*

Gas chromatography-mass spectrometry was performed by means of a Shimadzu GC-MS QP2010 (Shimadzu, Duisburg, Germany) with the quadrupole mass spectrometer running in the electron ionization mode (EI) at 70 eV. Samples were injected by the split/splitless-injection onto capillaries DB-5 or DB-FFAP (both 30 m, 0.25 mm I.D., 0.25 µm film thickness; J&W, Agilent Technologies, Waldbronn, Germany) with helium serving as carrier gas at a pressure of 70 kPa. Temperature programs were the same as used for GC-O.

### *Heart Cut Gas chromatography- Gas chromatography-Olfactometry-Mass spectrometry (GC/GC-O/MS)*

Heart-cut GC/GC-O/MS was performed using a GC2010 (Shimadzu, Duisburg, Germany) connected to a GC-MS QP2020 (Shimadzu, Duisburg, Germany). In the first dimension, the separation of the extract was achieved on a DB-FFAP column (30 m x 0.25 mm fused silica capillary DB-FFAP, 0.25  $\mu$ m, J&W, Agilent Technologies, Waldbronn, Germany). The elution range containing the selected odorants was transferred to a second capillary column by means of a Multi-Deans Switch system. The second capillary column was a DB-5 (30 m x 0.25 mm fused silica capillary, 0.25  $\mu$ m, J&W, Agilent Technologies, Waldbronn, Germany). For mass chromatography, the second column was connected to a quadrupole mass spectrometer QP 2020 (Shimadzu, Duisburg, Germany) running in the electron ionization mode (EI) at 70 eV.

For simultaneous GC/GC-O/MS, the effluent of the first column was divided by means of the Multi-Deans Switch system and was then transferred to the second capillary column. Furthermore, the end of the second capillary column was connected to a deactivated Y-shaped glass splitter dividing the effluent of the column into two equal parts, which were then transferred via two deactivated but uncoated fused silica capillaries (30 cm x 0.25 mm i.d.) to a sniffing port and the mass spectrometer, respectively. The sniffing port which was mounted on a detector base of the second GC was also heated to 230 °C.

## **Results and discussion**

Application of gas chromatography-olfactometry on the ethanolic roasted hazelnut extract revealed 129 odour-active areas showing a high variety of odour qualities such as malty, fruity, roasted, hazelnut-like, earthy, popcorn-like, sweet and fatty. The flavour extract was evaluated by sniffing the effluent eluting from two capillary columns of different polarity. While most of the odour impressions were instrumentally detected on both capillary columns, some of them were perceived by sniffing on only one of them – likely due to either coelution of aroma impressions or varying elution characteristics resulting of the different polarities of the compounds.

The calculation of retention indices and their comparison with data of an in-house database and literature data was used to determine compounds for the odour-active areas.

These structures were finally confirmed by comparing the mass spectra of the analytes as well as their odour qualities and odour potencies with those of the respective authentic reference compounds.

However, for several odour-active areas, no unequivocal mass spectra could be obtained by one-dimensional GC-MS. Using a two-dimensional GC/GC-MS system, the interesting elution range was transferred from the first column (DB-FFAP) to a second capillary column (DB-5) by means of a heart-cut Deans Switch System. The compounds eluting from DB-5 were also evaluated by means of simultaneous GC-O and GC-MS.

### *Corroboration of formerly identified odour-active compounds in hazelnuts*

Several of the odour-active compounds detected in this study have already been reported as potent odorant in roasted hazelnut paste before. 3-Methylbutanal with its malty odour has already been reported by Burdack-Freitag and Schieberle as odorant in roasted hazelnut paste [9]. The same was true for the fruity smelling ethyl 2-methylbutanoate, the fruity, hazelnut-like smelling 3-methyl-4-heptanone, the also fruity, hazelnut-like smelling 5-methyl-(E)-2-hepten-4-one and 5-methyl-(Z)-2-hepten-4-one, octanal (fatty, green), 2-acetyl-1-pyrroline (popcorn), dimethyl trisulphide (onion, sulphury), (Z)-2-octenal (green, fatty), 2-propionyl-1-pyrroline (popcorn), 3-(methylthio)propanal (cooked potato),  $\gamma$ -heptalactone (fruity, honey, floral), (E,E)-2,4-decadienal (fatty, fishy, metallic), 2-methoxyphenol (smoky, vanilla, phenolic), and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel, cotton candy). Ethyl 2-methylbutanoate, (E,E)-2,4-decadienal, and (E)- $\beta$ -damascenone had already been reported as potent odorants in hazelnut oil by Matsui et al. before [8].

Additionally, multiple compounds which had already been identified or identified tentatively before as volatile compounds in hazelnuts, but without evaluation of their odour quality, have now been characterised as odour-active compounds by means of GC-O.

### *Newly identified odour-active compounds in hazelnut paste*

In this study, 24 components have been identified for the first time as odour-active compounds in roasted TGT hazelnut paste, namely 3-methylbutyl acetate, ethyl hexanoate, methyl phenylacetate, 3-methyl-2,4-nonanedione,  $\alpha$ -phellandrene, isoeugenol, 2-vinyl-3,5-dimethylpyrazine, 2-acetyl-2-thiazoline, 3-methyl-2-buten-1-thiol, 3-mercapto-3-methylbutyl formate, methyl-3-(methylthio)furan, furfuryl methyl sulphide,  $\delta$ -nonalactone,  $\gamma$ -decalactone,  $\delta$ -decalactone, massoia lactone,  $\gamma$ -dodecalactone,  $\delta$ -dodecalactone, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, 3-hydroxy-2-methylpyran-4-one (maltol), methyl cyclopentenolone, 2-aminoacetophenone, 3- and/or 4-ethylphenol and 3-methylindole. Table 1 summarises the newly identified odour-active compounds, their retention indices, odour description as well as their mode of identification.

### Unknown compounds

Various compounds which were detected sensorially during sniffing remained unidentified. Many of these still unknown compounds showed relatively high retention indices on either or both capillary columns and, according to their odour qualities, some of them might significantly contribute to the overall aroma of the roasted hazelnut paste. The retention indices of the unknowns are given in Table 1. The identification of these compounds will be the task for further studies.

**Table 1. Newly identified and unknown odour-active compounds in hazelnut paste.**

No.	Odorant <sup>a</sup>	Odour quality <sup>b</sup>	RI <sup>c</sup> DB-FFAP	RI <sup>c</sup> DB-5
1	3-methylbutyl acetate <sup>d, e</sup>	fruity, banana	1144	
2	3-methyl-2-buten-1-thiol <sup>d</sup>	beer, sulphur	1156	690
3	$\alpha$ -phellandrene <sup>d</sup>	fir needle, green, floral	1238	995
4	ethyl hexanoate <sup>d, e</sup>	fruity, floral, herbal	1251	
5	3-mercapto-3-methylbutyl formate <sup>d</sup>	cassis, roasted	1533	1030
6	2-vinyl-3,5-dimethylpyrazine <sup>d, e</sup>	earthy, roasted, green	1556	1121
7	unknown	bread, popcorn, roasted	1577	
8	3-methyl-2,4-nonandione <sup>d, e</sup>	floral, green	1723	
9	2-methyl-3-(methylthio)furan <sup>d</sup>	sulphury, coffee	1736	
10	methyl phenylacetate <sup>d</sup>	fruity, medical	1752	
11	2-acetyl-2-thiazoline <sup>d, e</sup>	nutty, roasted	1774	
12	unknown	citrus, smoky, fruity	1859	
13	methyl cyclopentenolone <sup>d, e</sup>	Sweet, maple, meaty, savoury	1936	
14	unknown	savoury, sulphury	1964	
15	unknown	violet, phenolic	1972	
16	3-hydroxy-2-methylpyran-4-one <sup>d, e</sup>	sweet	2040	
17	unknown	fir needles, fruity	2055	
18	isoeugenol <sup>d</sup>	clove	2098	
19	unknown	fruity, creamy, chocolate	2107	
20	unknown	goat shed, leather, animal	2175	
21	$\gamma$ -decalactone <sup>d, e</sup>	waxy, fruity	2182	1470
22	3-/ 4-ethylphenol <sup>d</sup>	animal, cow shed	2222	
23	$\delta$ -decalactone <sup>d, e</sup>	waxy, fruity	2229	1507
24	aminoacetophenone <sup>d, e</sup>	sweaty, floral	2238	
25	unknown	clove, faecal, flowery	2247	
26	massoia lactone <sup>d</sup>	peach, waxy	2269	
27	unknown	fruity, musty	2283	
28	unknown	herbal, clove	2299	
29	3-hydroxy-4,5-dimethyl-2(5H)-furanone <sup>d, e</sup>	savoury, Maggi	2302	1105
30	$\gamma$ -dodecalactone <sup>d</sup>	peach, waxy, fruity	2450	1670
31	unknown	nutty, earthy, musty	2467	
32	$\delta$ -dodecalactone <sup>d, e</sup>	coconut, creamy	2480	1712
33	3-methylindole <sup>d</sup>	faecal, bad breath	2568	
34	furfuryl methyl sulphide <sup>e</sup>	onion, meaty		1000
35	$\delta$ -nonalactone <sup>d</sup>	coconut		1436

<sup>a</sup> Structure assignment of each odorant was based on the comparison of the compound's retention indices on FFAP and/or DB-5. <sup>b</sup> Odour quality as perceived at the sniffing port during GC-O. <sup>c</sup> Retention index calculated from the retention time of the compound and the retention times of adjacent n-alkanes by linear interpolation. <sup>d</sup> Confirmation by odour quality of reference compound as perceived at the sniffing port during GC-O and retention index on FFAP and/or DB-5. <sup>e</sup> Confirmation by mass spectrum obtained by GC-MS or GC-GC-MS of sample extract.

### Conclusion

In summary, this study successfully characterised the odour-active compounds in hazelnut. Owing to its variety, geographical sourcing and controlled roasting conditions, Piedmont hazelnut (TGT) paste has a complex aroma, containing a high number of odorants. Twenty-four of them have been identified for the first time in roasted hazelnut paste. Some of these newly identified compounds are well known to be high impact odorants, which probably contribute to the characteristic profile of the TGT roasted hazelnuts.

Their presence in other hazelnut varieties/origins could be the objective of further studies.

Most of the newly identified compounds are polar and low volatile. This result meets the expectations of our study as the design of the work-up procedure including the application of aqueous extraction conditions, low temperatures and solid phase extraction was developed to include particularly the polar, low volatile compounds in the hazelnut paste aroma extract.

Therefore, extraction of aroma material with a polar solvent followed by solid phase extraction should be considered as versatile method for the identification of polar low volatile odour-active compounds.

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