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Analytical Methods

Gas chromatographic–olfactometric characterisation of headspace and mouthspace key aroma compounds in fresh and frozen lamb meat

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ABSTRACT

Two different techniques for obtaining extracts representing the most relevant aroma chemicals from grilled lamb have been developed and applied to the elucidation of the gas chromatographic–olfactometric (GC–O) profiles of grilled lamb from fresh or frozen samples. The first technique attempted to collect the aroma released during grilling; and the second one, the aroma released during eating. The GC–O work was complemented with a thorough isolation and identification study comprising liquid chromatography and dual gas chromatography with simultaneous MS and olfactometric detection. Eight different aroma compounds with meaty odours were detected of which (*Z*)-2-heptenal, 2,5-dimethylpyrazine, (*Z*)-2-decenal, 2-butyl-2-octenal, 2-acetyl-2-thiazoline and (*E,E*)-2,4-decadienal could be identified. Moreover, 2-isopropyl-3-methoxypyrazine, 2-methylbenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde (vanillin) were described for the first time in lamb. Frozen samples contained higher levels of ethyl hexanoate and butanoic and 3-methylbutanoic acids, while samples obtained directly from the grill were richer in pyrazines. Furaneol was only important in samples directly collected from the mouth.

1. Introduction

Meat flavour, rather than its taste and texture, is the most important sensory attribute with which animal species could be identified (Matsuishi, Igeta, Takeda, & Okitani, 2004) and it has also been reported that consumers consider flavour one of the main sensory properties decisive in their selection, acceptance, and ingestion of a particular food (Fisher & Scott, 1997). In this particular context flavour refers mainly to the taste and aroma perceptions linked to the meat consumption. It should be noted that while the characteristic taste of a food is normally related to a single class of compounds (Fisher & Scott, 1997), an odour can be elicited by a combination of volatile compounds, each of which imparts its own aroma. In addition, aroma is the main determinant of meat flavour (Mottram, 1998), which suggests that the knowledge of the chemicals behind the different aromatic perceptions linked to meat consumption is essential for understanding meat quality.

Lamb meat is widely consumed in Mediterranean countries, Spain being one of the most important producers and consumers of this type of meat (Sañudo, Sanchez, & Alfonso, 1998). The seasonal supply of lambs is a fact in the ovine market (Chemineau et al., 1995), with a high supply during spring and a high demand at Christmas time, which results in fluctuations in its price (Hansen et al., 2004). Meat producers try to freeze meat attempting to stabilise its price (Pietrasik & Janz, 2009; Wheeler, Miller, Savell, & Cross, 1990). Freezing procedures should ensure not only the nutritional quality, which has already been demonstrated, but also the sensory quality of frozen meat. The latter is closely linked to meat aroma and this can only be controlled if the chemical compounds responsible for the most relevant sensory properties of fresh and frozen (Campo et al., 2006; Muela, Sañudo, Campo, Medel, & Beltrán, 2010) lamb meat are known.

During storage unwanted oxidation processes may occur, especially lipid oxidation processes (Zhang, Farouk, Young, Wieliczko, & Podmore, 2005). It has been shown recently that oxygen is a direct catalyst of lipid oxidation reactions that occur in muscle. Lipid oxidation can produce undesirable rancidity causing rejection by the consumer (Campo et al., 2006). This deterioration of meat odour and taste is directly related to changes in fatty acids (Mottram, 1998).

Generally, meat is consumed processed instead of raw. This treatment or cooking has to ensure the microbiological safety, enzyme inactivation, destruction of toxic substances, preservation and of course the development of new aromas, colours and flavours (Friedman, 1996). Heat treatment and meat composition (carbohydrates and proteins) enable the Maillard reaction. Intermediate moisture products, temperatures above 50 °C and

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pH (Madruga & Mottram, 1995) between 5 and 7 favour this reaction. One consequence of this reaction is the formation of aromatic compounds, such as pyrazines and thiazoles (roasted meat odour), thiols (meat or stew odour), and 5-hydroxymethylfurfural (candy-butter odour) and furfural (wood, nuts and raisins, sweet odour). Furthermore, aroma compounds that finally reach the olfactory receptors can be the result of complex reactions between enzymes and meat aroma precursors and transport phenomena from the meat matrix to the olfactory region.

Considering the importance of aroma in lamb meat quality, several papers have examined its volatile profile (Elmore, Mottram, Enser, & Wood, 2000; Osorio, Zumalacarregui, Cabeza, Figueira, & Mateo, 2008; Priolo et al., 2004; Rota & Schieberle, 2006; Young, Lane, Priolo, & Fraser, 2003). These papers have studied the important volatile compounds in cooked and raw meat, or the effect of variations in diet, age and castration, but none of them studied the effect of freezing on the aroma profile and none of them studies compares the headspace and mouthspace in meat.

To analyse the mouthspace, several *in vivo* methodologies have been used. Buccal Odour Screening System (BOSS) is an intra-oral extraction based on stir bar sorptive extraction (SBSE), which has only been carried out with liquids, such as milk (Buettner & Mestres, 2005), wine (Buettner, 2004) or other aqueous solutions (David & Sandra, 2007). The highly lipophilic character of SBSE polymers limits the type of aroma compounds that can be extracted. Proton-transfer reaction mass spectrometry (PTR-MS) could be used with more viscous matrices, such as gels (Buettner et al., 2008; Mestres, Kieffer, & Buettner, 2006) to study the influence of texture on different parameters, for example *in vivo* aroma release. Such continuous MS monitoring techniques are excellent for studying the release of major volatiles in real time, but they lack the required sensitivity for detecting many aroma compounds present at trace levels.

Because of the dynamic nature of the aromatic perception in meat, two different types of aroma isolation techniques have been studied in this paper. The first one will attempt to directly capture the aroma released during grilling the lamb, an idea previously considered by Schäfer, Meinert, and Aaslyng (2008), and the second one will attempt to capture the aroma that emerges while eating by sampling exhaled air from the panellists consuming the meat. This work attempts to study in depth the chemical basis of the aroma of grilled lamb by using gas chromatography–olfactometry (GC–O), complemented with extensive isolation and identification work. A second major objective is to determine the major aroma changes linked to meat freezing.

2. Material and methods

2.1. Animals and samples

This study used five 75 days-old Rasa Aragonesa male lambs with cold carcass weight of 11.4 ± 0.4 kg. Animals were fed in the same way under intensive husbandry conditions, with milk the first 40 days and fodder with cereals until slaughtering in an EU-licensed abattoir following standard protocols. Carcasses were aged at 2–4 °C for 24 h, before the muscle *Longissimus lumborum* was excised with the subcutaneous fat, vacuum packaged and kept and 2–4 °C until 4 days of ageing. Those samples used as fresh were then analysed. The samples from one animal were previously obtained, frozen and kept at −18 °C for 1 year until performing the analysis.

2.2. Reagents and standards

2.2.1. Solvents

Dichloromethane, methanol, pentane and diethyl ether (gas chromatography quality) were purchased from Merck (Darmstadt, Germany); water was purified in a Milli-Q system from Millipore (Bedford, MA).

2.2.2. SPE cartridges materials and classical liquid chromatographic column

Lichrolut EN® resins (styrene/divinylbenzene copolymer), 1 mL and 3 mL internal volume polypropylene cartridges and Silica 60 (230–400 mesh) were supplied by Merck.

2.2.3. Chemical standards

Anhydrous sodium sulphate 99%, 4-hydroxy-3-methoxybenzaldehyde (vanillin) *puriss.* and 2,6-di-*tert*-4-butyl-4-hydroxytoluene (BHT) were supplied by Panreac (Barcelona, Spain); (*E*,*E*)-2,4-decadienal and 3-methylbutanoic acid 98% by Lancaster Synthesis (Eastgate, UK); 2,6-dichlorophenol 99% and ethyl cyclohexanoate ⩾98% by Alfa Aesar (Karlsruhe, Germany); 2,6-dimethylpyrazine ⩾98%, 2-acetyl-2-thiazoline ⩾96% and isobutanol 99% by SAFC (Steinheim, Germany); *m*-cresol 99%, 2-phenoxyethanol 98%, 2-isopropyl-3-methoxypyrazine 97%, 2-isobutyl-3-methoxypyrazine 99%, 1-octen-3-ol 98%, nonanal 95%, (*E*)-2-octenal 94%, 3-(methylthio)propanal (methional), octanal 99%, (*E*)-2-nonenal 97%, benzyl alcohol 99%, 2-ethyl-3,(5 and 6)-dimethylpyrazine 95%, 2-methylbenzaldehyde 97%, 1-methoxy-4-(1-propenyl)benzene (trans-anethole) 99%, (*E*,*E*)-2,4-nonadienal ⩾85%, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (furaneol) 98% and 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (sotolon) 97% by Aldrich (Madrid, Spain); and *p*-cresol ⩾99%, octanoic acid 98%, butanoic acid 99.5%, ethyl hexanoate 99%, 2,5-dimethylpyrazine ⩾90%, benzenemethanethiol ⩾99%, β-phenylethanol 99%, heptanal ⩾95%, 1-octen-3-one >99%, eugenol 99%, linalool 98.5%, standard alkane solution (C8–C20), 40 mg L^{-1} in hexane, docosane ⩾98%, tetracosane ⩾99%, hexacosane ⩾99% and octacosane ⩾98% by Fluka (Madrid, Spain).

2.3. Loin grilling

Frozen sample was thawed at 4 °C for 24 h before analysis, reaching a total of 4 days of ageing. Fresh samples were analysed after reaching 4 days of ageing, without undergoing freezing. The loin (loin average weight = 176 ± 14 g) was wrapped in aluminium foil and roasted (with the fat side up) on an industrial double-plate grill (GRS-5 SAMMIC) at 200 °C until the internal temperature reached 70 °C (Resconi, Campo, Furnols, Montossi, & Sanudo, 2009), which was monitored by an internal thermocouple. Once grilled, the subcutaneous fat and external connective tissue were removed.

2.4. Location of important aromatic compounds

2.4.1. Grill extract

One hundred milligrams of LiChrolut EN® resins were put into cartridges of 1 mL enclosed by frits and glass wool. Cartridges were cut to the length of the pump, conditioned with 5 mL of dichloromethane, dried under vacuum and were introduced inside the gas-extraction pump PAS-500 (Supelco, Bellefonte, PA). The pump, previously calibrated at 500 mL min^{-1}, was placed on the 8 mm central hole of a 18 × 47 × 37 cm (high, length, width) temperature resistant methyl polymethacrylate gas collecting hood, with 10 cm methyl polymethacrylate legs. Thus volatiles were collected until the muscle internal temperature was 70 °C. Resin amount and pump flow were previously optimised (data not shown).

Glass wool was removed prior to the elution process by cutting the cartridge; compounds were then eluted with 1 mL of dichloromethane/methanol 1:19 (v/v) (Ferreira et al., 2009) and using a prefilter to prevent glass wool traces in the eluate. Extracts were dried with anhydrous Na_2SO_4 and were concentrated under a nitrogen stream to 200 μL.