International Food Research Journal 23(3): 1212-1216 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of cooking lamb using maguey leaves *(Agave salmiana)* on meat volatile composition

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Article history

Received: 21 January 2015
Received in revised form: 18 September 2015
Accepted: 2 October 2015

Keywords

Barbacoa
Volatile compounds
Maguey
Traditional food

Abstract

The preparation and consumption of meat cooked inside a hole in the ground, previously covered with leaves from several plants, is a traditional culinary practice in Latin America. Among those typical dishes is the Mexican steamed sheep meat known as "barbacoa". The meat has to be covered with maguey leaves *(Agave salmiana)* and steamed. The use of maguey leaves could affect to the "barbacoa" edible quality. In the present study, the volatile compounds of maguey leaves, raw and after being steamed were determined. The volatile compounds of steamed lamb either with or without direct contact with the maguey leaves were also determined. Volatiles in lamb were analyzed immediately after cooking, and after 5 days of storage under refrigeration. A total of 18 volatile compounds, mainly aldehydes, benzene compounds and terpene compounds, were detected in the headspace of the maguey leaves. The contact of maguey leaves with the meat during the steaming showed a significant protective effect on the oxidative stability of the subcutaneous fat, which was in direct contact with the maguey leaves. However, no significant effect on the volatile composition of the steamed lamb meat was found when the subcutaneous fat was removed.

Introduction

Cooking meat in a hole in the ground covered with different plants has been a traditional culinary practice used by Mesoamerican cultures to prepare meat from different animals (Ávila *et al*., 1988). Many dishes are still prepared around the world using this culinary technique, such as the tapao in Guatemala, the pachamanca, wathia and phampaku in Bolivia and Peru, the calapurca in Argentina and Chile, and the paparuto in Brazil (Montecino, 2003; Castillo, 2010; Grados-Silverio, 2010). The barbacoa is produced in Mexico as a traditional dish obtained by steaming sheep meat in the ground with the addition of specific vegetal ingredients and been covered with maguey leaves *(Agave salmiana)* (Gay, 2001). The "barbacoa" has a special relevance because most of the sheep meat consumed in Mexico (ca. 1 kg per capita) is consumed as such (Rubio *et al.*, 2004; Cuellar, 2007; Sanchez, 2010).

The preparation of traditional barbacoa is carried out outdoors, usually in a courtyard with ground floor in which there is a hole of between 0.8 to 1 m of depth and 0.60 to 0.80 m of diameter (Ávila *et al*., 1988; Gay, 2001). As described by Peña-Ramos (2008), the process starts by filling the hole with stones about one-third full and adding wood (about 40 kg), which is fired until it is turned into charcoal. Secondly, the walls of the hole are coated with maguey leaves, which have been previously grilled by direct exposure to flames, and a metal pot (approximately 40 cm of diameter) containing water and some vegetables (i.e., rice, potatoes, carrots, garlic, onion, chili peppers and chickpeas) is introduced in the hole and fixed in the charcoal. In the third step, a metal grid is then placed on top of the pan with meat cuts (leg, shoulder, loin, ribs) and the animal's stomach stuffed with other edible viscera seasoned with salt, chili pepper and local herbs. Finally, the meat is completely covered with maguey leaves, the hole is covered with earth, and the meat is steamed into the hole overnight (6-12 h). After this time, the earth and the maguey leaves are removed and first the steamed meat and then the pot with the broth (called consomé) are obtained. Meat is then seasoned with dry salt.

The plants traditionally used to prepare steamed meat in a hole, such as banana-tree leaves or maguey leaves, could have a distinctive effect on the quality of the meat cooked in contact with them. In fact, agave plants are characterized by high contents

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of polyphenols with antioxidant capacity, such as kaempherol and quercetin (Rice *et al*., 1997; Duke, 2000; Almaraz-Abarca *et al*., 2009; Yokosuka and Mimaki, 2009; Ben Hamissa *et al*., 2012), and terpenes (Peña-Alvarez *et al*., 2004). The objective of this study was to evaluate effects of cooking sheep meat in direct contact with maguey leaves on the volatile compounds of the steamed meat.

Materials and Methods

Experimental design and samples

Two experiments were carried out: the determination of the volatile compounds of grilled maguey leaves, and the effect of maguey leaves on steamed lamb meat volatile composition. Fifteen maguey leaves (*Agave salmiana* Otto ex. Salm-Dyck) were collected in San Lorenzo Sayula, Cuautepec de Hinojosa, Hidalgo State, Mexico. The edges of the leaves were removed, their thickness was uniformized to 2 cm with the help of a knife, and they were grilled by direct exposure to an open fire for approximately 5 min each side. Finally, the charred parts were removed. After sampling, the grilled leaves were transported to the lab and freeze at -20ºC until further use.

For the analysis of volatile compounds, six of the sampled leaves were randomly chosen and thawed at 4ºC overnight. Two rectangular prism-shaped samples of approximately 50 g were obtained from each leaf. One sample was immediately analyzed for volatile compounds (raw state), whereas the other was steamed before analysis with the following procedure: 6 h in a stainless steel pan with boiling water.

Eighteen legs from Suffolk x Dorset 6-months old lambs were obtained from the slaughterhouse. Meat pieces from the muscles Biceps gluteus and Semitendinosus and the adjacent subcutaneous fat layer (approximately 200 g) were obtained and divided into two groups of 9 pieces each (experimental group and control group). Firstly, nine prism-shaped portions of grilled maguey leaves (ca. 0.5 cm in thickness and 12 cm^2 of surface) were placed in contact with the meat pieces (one leaf portion per meat piece): leaf external surfaces against meat external surfaces. Each maguey-and-meat set was wrapped in kraft paper and aluminum foil, and steamed for 6 h. The same procedure was followed with the control group (meat portions alone). Both sets were cooled for 1 h at room temperature (20ºC). Afterwards, the subcutaneous fat layer was separated with the help of a knife, frozen at -50ºC, and stored for up to 2 months until volatile analysis; the resulting meat pieces (without subcutaneous fat) were homogenized in a food processor and divided in two sub-sets: one was vacuum packaged and frozen (-50ºC), and the other was wrapped in oxygen-permeable polyethylene film and stored in darkness for 5 days at -4ºC before being frozen (-50ºC).

Analysis of volatile compounds

After thawing the samples (maguey leaves, subcutaneous fat, and meat without subcutaneous fat) at 4ºC for 24 h, they were blended in a domestic food processor and a 5 g aliquot was used for analysis. The extraction of volatile compounds was carried out into a 20 ml vial containing 0.073 g of common salt and 4.67 ml of water. The vial was sealed with a silicone/polytetrafluoroethylene septa cap (Agilent Technologies, Zwingen, Switzerland) and placed in an automatic injector (Combi PAL autosampler; CTC Analytics AG, Zwingen, Switzerland). Samples were conditioned for 40 min at 60ºC with shaking (750 rpm) at 5-s intervals. Volatile compounds were analyzed using a GC 7890A coupled to a MS 5975C mass detector (Agilent Technologies). Injection was made using a syringe preheated at 100ºC, operating with a filling and injection speeds of 100 µl/s and 250 µl/s, respectively; the injection volume was 1 ml and the injector port operated at a temperature of 260°C in split mode (2:1 split rate). Compounds were separated using a DB-5MS column (60 m x 250 µm x 0.25 µm; J&W Scientific, Zwingen, Switzerland). Helium was used as carrier gas at a constant flow rate of 1.5 ml/min. The oven temperature program consisted of an initial step at 37°C for 1 min followed by linear increases from 37 to 50°C at 10°C/min, to 140ºC at 4ºC/min, to 200°C at 20°C/min, and to 250°C at 50°C/min, and this temperature was held for 11 min. The mass spectrometer operated in electron-impact mode with an electron energy of 70 eV and an emission current of 50 µA. Relative voltage was maintained in 1235 V. Line transfer temperature was 280ºC and ion source was operated at 250ºC. Data was obtained using a scan mode and mass range of m/z 40-400 and a speed of 3.94 scan/s.

Compounds were identified by first comparing their mass spectra with those contained in the NIST/EPA/NIH Mass Spectral Database together with personal interpretation. Moreover, a series of n-alkanes (Hydrocarbons/C5-C20; Sigma-Aldrich, Saint Louis, MO, USA) was used for calculating the experimental linear retention index (LRI) values for each volatile, and compound identities were confirmed wherever possible by comparison of their experimental LRI to those from the literature. LRI was calculated as described David *et al.* (2002).

Table 1. Volatile compounds in grilled maguey leaves before and after steaming (peak area x 10^{-6})

Compound	LRI	Before steaming (n = 6)	After steaming (n = 6)	SEM
Aldehydes				
2-Methyl-butanal	710	0.43	ND	
Pentanal	704	1.56	1.07	0.50
2-Methyl-pentanal	732	1.44	0.43	0.55
Hexanal	766	0.20	ND	0.21
Heptanal	894	0.09	ND	0.07
Octanal	966	0.18	ND	0.11
Nonanal	1089	0.34	0.52	0.15
Furfural	802	0.58	ND	0.34
Benzene compounds				
Benzene	680	0.42	ND	0.69
Toluene	770	1.07	1.83	0.54
Xilene	877	0.17	ND	0.18
Benzaldehyde	903	0.29	ND	0.06
Sulphur compounds				
Carbon Disulfide	544	144.37	35.00	0.64
Terpene compounds				
Cymene or isomer (C10H14)	1004	0.07	0.12	0.13
Limonene or isomer (C10H16)	1030	0.20	ND	0.16
1,2-Dihydro-1,1,6-naphthalene	1106	0.09	ND	0.16
Trimethyl-naphthalene or isomer (C13H16)	1355	ND	0.38	0.26
Terpenoid	1390	3.46	0.37	3.7

LRI: Experimental linear retention index.
SEM: Standard error of the mean.
ND: Not-detected in any sample, detection limit lower to 0.1 x 10^{-6} area units.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the volatile compound levels between raw and steamed maguey leaves (with steaming as the fixed factor) and the volatile composition of lamb subcutaneous fat between treatment and control samples (with the use of maguey as fixed factor). Moreover, a two-way ANOVA was used to compare the volatile compounds of lamb meat without subcutaneous fat (where the use of maguey and storage time were the fixed factors).

Results and Discussion

Volatile compounds in maguey leaves

Eighteen volatile compounds were identified in the maguey leaves (Table 1). Compounds are grouped in four classes according to the chemical family to which they belong, i.e., aldehydes, benzene compounds, sulfur compounds, and terpene compounds. All the 18 compounds were found in the raw leaves (uncooked), with the most abundant being carbon disulfide, pentanal, 2-methyl-pentanal, toluene, and an unidentified terpenoid. On the other hand, 10 volatile compounds were identified in the cooked leaves, being the most abundant carbon disulfide, pentanal, and toluene. Cooked leaves showed lower content of volatile compounds; this can be attributed to a loss of volatiles by evaporation, leaching or thermal degradation. Toluene and cymene were the only volatile compounds that did not show decrements after cooking.

Table 2. Volatile aldehydes in subcutaneous fat from lamb leg meat steamed in contact with (maguey) or without (control) maguey leaves

	LRI	Treatment		SEM
		Control (n = 9)	Maguey (n = 9)	
Pentanal	732	0.15	0.15	0.08
Hexanal	802	1.27^{a}	0.80^{b}	0.09
Heptanal	903	0.40^{a}	0.24^{b}	0.02
Octanal	1004	0.26^{a}	0.17^{b}	0.01
Nonanal	1106	0.29^{a}	0.21^{b}	0.02

LRI: Experimental linear retention index.
SEM: Standard error of the mean.
^{abc} Concentrations with different letter in the row are significantly different (P<0.05).

Peña-Alvarez *et al.* (2004) determined volatile compounds of maguey pine and, although the presence of aldehydes, esters, acids, and terpene compounds was detected, only terpenes were enumerated. In agreement with the results from this study, the identified terpenes were limonene, p-cymene, and naphthalene. The benzene compounds in maguey leaves could have been originated from the smoke in contact with the leaves during the previous grilling (pretreatment), because smoke is rich in those compounds (Wittkowski *et al.*, 1990).

Effect of the use of maguey on the volatile compounds of steamed lamb meat

Five aldehydes were by far the compounds detected more abundantly in the lamb subcutaneous fat regardless the type of treatment (control or in contact with maguey leaves) (Table 2). The presence of those aldehydes in meat has been attributed to lipid oxidation, being hexanal a relevant indicator of lipid oxidation in cooked meat (Frankel, 1982; Elmore *et al*., 1999; Pegg and Shahidi, 2012; Resconi *et al*., 2013). Significant differences were found in aldehyde concentrations among treatments, except for pentanal. Thus, when the maguey leaves were used in the steaming process, aldehyde levels were lower. This finding indicates that the contact of maguey with the lamb subcutaneous fat exerted an inhibitory effect on lipid oxidation during the cooking of the meat. This effect might be related to the presence of antioxidant polyphenols in the maguey (Almaraz-Abarca *et al*., 2009).

The volatiles compounds detected in the lamb meat without subcutaneous fat are shown in Table 3. Twenty two compounds were detected; 21 were

Table 3. Volatile compounds (peak area x 10^{-6}) in a lamb meat pieces, to which subcutaneous fat was removed, steamed in contact with (maguey) or without (control) maguey leaves and sampled at two refrigerated storage times

Compounds	LRI	With maguey (storage day)		Without maguey (storage day)		SEM	*P*-level		
		0 (n = 9)	5 (n = 9)	0 (n = 9)	5 (n = 9)		M	T	M*T
Alkanes									
Pentane	500	6.86	16.66	13.46	15.93	2.92	NS	#	NS
Hexane	600	1.49	0.98	1.23	0.90	0.51	NS	NS	NS
Heptene	690	0.03	0.04	0.12	0.10	0.03	NS	NS	NS
Heptane	700	0.20	0.48	0.28	0.48	0.10	NS	*	NS
Octane	800	0.60	0.90	0.55	0.79	0.15	NS	#	NS
Nonadecane	1900	0.68	0.16	0.41	1.63	0.31	NS	NS	#
Subtotal		9.87	19.22	16.05	19.73	3.31	NS	*	NS
Aldehydes									
Pentanal	732	3.71	5.44	4.30	4.66	0.67	NS	#	NS
Hexanal	802	20.49	31.07	26.26	27.19	4.65	NS	NS	NS
Heptanal	903	1.03[b]	1.64[a]	0.88	1.27	0.17	NS	*	NS
Octanal	1004	0.43	0.65	0.42[b]	0.65[a]	0.06	NS	**	NS
Nonanal	1106	0.67	0.82	0.58	1.07	0.12	NS	NS	NS
9-Octadecenal	<2000	0.05	0.01	0.14	0.39	0.12	NS	NS	NS
Octadecanal	<2000	0.78	0.31	0.84	0.75	0.24	NS	NS	#
Subtotal		27.16	39.93	33.41	36.00	5.52	NS	NS	NS
Ketones									
2.3-octanedione	985	0.19	0.42	0.32	0.39	0.08	NS	*	NS
Alcohols									
1-Pentanol	767	0.03	0.07	0.05	0.03	0.02	NS	NS	NS
1-Octen-3-ol	980	0.13	0.25	0.20	0.24	0.05	NS	NS	NS
Subtotal		0.24	0.31	0.25	0.33	0.07	NS	NS	NS
Furans									
2-Pentilfuran	990	0.15	0.23	0.14	0.21	0.05	NS	#	NS
Fatty-acid ester									
Ethyl-hexadecanoate	1780	ND	ND	0.27	5.48	1.71	-	NS	NS
Benzene compounds									
Toluene	766	1.87	1.77	1.24	1.16	0.30	NS	NS	NS
Benzaldehyde	966	0.26	0.21	0.25	0.34	0.03	NS	NS	*
Subtotal		2.22	1.98	1.50	1.65	0.30	NS	NS	NS
Sulfur compounds									
Benzothiazole	1235	0.95	0.36	0.33	1.64	0.52	NS	NS	NS
57, 71(92), 83(58), 43(54), 41(51), 69(44), 56(38), 55(35), 40(31)	1747	0.22	ND	ND	2.02	0.56	-	-	-
Total		40.99	62.15	51.98	67.45	8.55	NS	*	NS

LRI: Experimental linear retention index.
SEM: Standard error of the mean.
P-level: Level of significance found by analysis of variance: NS, no significant; #, $P < 0.1$; * $P < 0.05$; and ** $P < 0.01$
ND: not-detected (Pike area x 10^{-6} <0.05).
$ Charge/mass ratio of ions with higher abundance (the relative percentage of abundance is included between parenthesis).

identified and grouped in 8 chemical families. The most abundant were alkanes and straight chain aldehydes. The presence of most of the detected compounds (alkanes, pentanol, 1-octen-3-ol, 2,3-octanodione and penthyl furan) could be attributed to lipid oxidation [Frankel, 1982; Pegg and Shahidi, 2012; Resconi *et al.,* 2013]. Consequently, the levels of these compounds increased along the storage period, with some of them showing statistical difference ($P<0.05$) or trend ($P<0.1$). Moreover, the volatile compounds from steamed lamb meat without subcutaneous fat were not affected by the use of maguey leaves. Therefore, in contrast to the results found in the subcutaneous fat samples, the use of maguey did not exert a protective effect against oxidative degradation of the lipids from meat samples without subcutaneous fat.

Conclusions

The volatile compounds identified in the headspace of grilled maguey leaves were aldehyde, benzene compounds, and terpenes; and the levels of most of them decreased with the steaming treatment. The use of maguey showed a stabilizing effect against lipid oxidation in the subcutaneous fat in contact with the maguey leaves during the steaming treatment; however, this effect was not evidenced in the meat lipids from the intramuscular and intermuscular fat.

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