Artigo

VOLATILE COMPOUNDS IN THE THERMOPLASTIC EXTRUSION OF BOVINE RUMEN

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The volatile compounds of raw and extruded bovine rumen, extracted by dynamic headspace, were separated by gas chromatography and analyzed by GC-MS. Raw and extruded materials presented thirty-two volatile compounds. The following compounds were identified in raw bovine rumen: heptane, 1-heptene, 4-methyl-2-pentanone, toluene, hexanal, ethyl butyrate, *o*-xylene, *m*-xylene, *p*-xylene, heptanal, limonene, nonanal, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane and octadecane. The following compounds were identified in the extruded material: 1-heptene, 2,4-dimethylhexane, toluene, limonene, undecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane and nonadecane. Mass spectra of some unidentified compounds indicated the presence of hydrocarbons with branched chains or cyclic structure.

Keywords: bovine rumen; extrusion; volatile compounds.

INTRODUCTION

Slaughterhouse by-products are parts of animals that are not consumed either for cultural reasons or because they are unappealing to one of the senses. These products have been the targets of attempts at using textural improvement through thermoplastic extrusion.[1] Among these by-products, the first stomach of bovines, or bovine rumen, is underused when compared with other meat cuts, although it is used in small scale as tripe stew or "buchada" (a traditional Brazilian dish).

Taking into account that rumen represents about 0.6% of the bovine weight, i.e. *ca* 2.7 kg,[2] and that about 30 million animals were slaughtered in Brazil in 2006,[3] around 81 million kg of nutritionally sound tissue were produced last year.

Bovine rumen protein has high digestibility (97%) and can provide all of the essential amino acids recommended by FAO/WHO[4] to preschool children.[5] Bovine rumen flour, obtained after its mincing, lyophilization and milling, contains 85% of protein and 17% of lipid on a dry basis.[6]

One way to use the bovine rumen is through thermoplastic extrusion, provided it is previously defatted to produce enough barrel resistance in the process. The extrusion is a feasible process used to add value to products by texturizing proteins that would not be used in human nutrition otherwise. The process of extrusion employs high temperature and shear rate for a short time, and yields textured and expanded products such as breakfast cereals, snacks, textured vegetable proteins, pet foods, pasta, and confectionery: toffee, chewing gum, wine gums and jellies, taffy, hard candy and caramels, licorice and chocolate.[7-10]

Rumen protein after extrusion can be used as a food ingredient, adding value to a series of comminuted products, especially meat products. Acceptability of this potential ingredient is dependant on several factors like texture, color and aroma.

It is known that aroma is affected by any heat processing such as extrusion with carbohydrates, proteins, lipids and water acting as aroma precursors.[11] Caramelization, Maillard reaction, oxidation, degradation, fragmentation and polymerization are the main chemical reactions that produce aromatic compounds during thermal processing.[10] In rumen extrusion the substrates for aroma development are mainly the residual lipid after extraction and protein. Rumen flour is usually bland without any characteristic aroma and flavor,[12] and this can help the use of this material without any further processing as a food ingredient. However, as extrusion creates desirable texture, stabilizes the final product and usually improves some functional properties, it is likely to be used in the recovery and upgrade of this raw material. Therefore, any contribution of extrusion for developing new aroma compounds in the extruded product needs to be investigated.

The objective of this work was therefore to detect formation of volatile compounds, mainly from fatty acids, during rumen extrusion and to contribute for the understanding of its flavor and aroma, which may affect the extruded product acceptability by the consumers.

EXPERIMENTAL

Material

Bovine rumen, provided by Sadia S/A (Toledo-PR, Brazil), was removed from healthy animals in proper condition for human consumption, inspected by SIF (Federal Inspection Service, Brazilian Agriculture Ministry), minced and frozen immediately after slaughter (kept at –30 ºC until use). It was then lyophilized by Nutribrás S/A (São Paulo-SP, Brazil).

The dried rumen was defatted with a mixture of hexane/ethanol in a 2:1 ratio. About 1 kg of rumen was defatted with 6 L of the solvent at room temperature for 17 h and then for another 3 h with the use of a mechanical shaker (2,800 rpm). This process was repeated once with pure solvents. The defatted rumen was further dried in an air oven (65 °C) for solvent evaporation.

The protein content and the lipid content of raw and extruded rumen were determined by the micro-Kjeldahl method (N x 6.25) and by Soxhlet extraction with ethanol, respectively.

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Determining fatty acid composition by gas chromatography

The lipids were extracted from the samples according to the dry column method[13,14] using dichloromethane and methanol in a ratio of 9:1. About 100-150 mg of lipids were converted to fatty acid methyl esters using BF_3-methanol as the esterifying reagent.[15] After derivation, the methyl esters dissolved in hexane were analyzed by gas chromatography using a chromatographer (Chrompack CP 9002) with a flame ionization detector (FID) and a capillary column (CP-SIL 88 50 m x 0.25 mm). The detector temperature was set at 300 °C, the injector temperature was set at 270 °C and the oven temperature was programmed to range from 100 to 240 °C, at 5 °C/min. The carrier gas was hydrogen with a flow rate of 1.5 mL/min. The identification of fatty acids was made by comparing the retention time of the sample compounds with the authentic standards of fatty acid esters injected under the same conditions. The quantification of fatty acids was performed through the normalization of the affected areas by a Chromato-Integrator and is expressed in relative percentage.

Extrusion of the bovine rumen

A single-screw extruder (INBRAMAQ - Indústria de Máquinas Ltda., Ribeirão Preto-SP, Brazil) with a length/diameter ratio of 15.5:1 and a compression ratio of 3.6:1 was used. The extrusion conditions were: die diameter with 5 mm; feeding speed at 90 g/min; screw speed at 263 rpm; temperatures of the five zones of the barrel (from zone 1 –feeding zone– to 5 –die– respectively) at 30, 60, 80, 120 and 140 °C; moisture content of feeding material of 35% on a dry solids basis.

Extraction of volatile compounds

The volatile compounds from raw and extruded rumen were extracted by dynamic headspace as described by Franco and Rodriguez-Amaya.[16] The volatile compounds were vacuum swept (480 mmHg) to a Porapak Q trap (80-100 mesh from Water Associates - USA) for 1 h and then eluted with 300 μL of hexane (chromatographic grade, Merck, Germany). The polymer was previously conditioned under a stream of nitrogen.

A trained panel composed of four selected examiners certified that the headspace sample had the characteristic aroma of extruded rumen. This panel was selected by the ability to detect and accurately quantify, in a non structured scale,[17] the aroma in samples with distinct extruded rumen concentration. Examiners that showed $p_{sample} \leq 0.30$, $p_{repetition} \geq 0.05$ and coherence with the group were selected.[17]

Gas chromatographic analysis

A Chrompack CP 9002 equipped with Maestro I software version 2.3 and an FID detector was used. Volatile compounds were separated in a fused-silica capillary column (length: 30 m, internal diameter: 0.25 mm, film thickness: 0.50 μm – Varian CP-SIL 8 CB, J&W Scientific, Folsom, CA, USA). Chromatographic conditions were: split/splitless injector used in the splitless mode; hydrogen as the carrier gas with a flow rate of 1.7 mL/min; injector and detector temperatures of 230 and 250 °C, respectively. The column temperature was maintained at 40 °C for 6 min, raised to 75 °C (3°C/min), then to 150 °C (4 °C/min), and finally to 210 °C (6 °C/min), at which it was maintained for ten minutes. Two μL of the samples was injected for the analysis.

Kovats indices

First of all, raw and extruded extracts and a solution of alkanes (C_7–C_{19}, Polyscience) were separately injected under the same conditions described for the chromatographic analysis. Then, the solution of alkanes was co-injected with raw extract to verify elution order of volatile compounds and Kovats index for all peaks was calculated according to Ettre.[18]

Gas chromatography–mass spectrometry

A GC 17A gas chromatograph coupled to a Shimadzu GC-MS QP 5000 mass spectrometer was used. The interface temperature was set at 240 °C, the ionization voltage was set at 70 eV and scanning speed ranged between 35 and 350 amu/sec. The conditions of analysis were the same as those of the gas chromatographic analysis, with the exception that the oven temperature was raised from 40 to 240 °C at 3 °C/min. Helium was used as the carrier gas, with a flow rate of 1.0 mL/min.

Structure assignment

The identification of volatile compounds was carried out by comparison between the mass spectra of standard compounds found in the instrument library (US National Institute of Standards and Technology, NIST) and the mass spectra of unknown compounds. The retention indices obtained were also compared with values found in the literature.[19,20] Compounds were considered positively identified when the data were confirmed by the mass spectra of standard compounds.

RESULTS AND DISCUSSION

The lipid content and the fatty acid profiles of raw rumen before and after defatting are presented in Table 1.

About 80% of lipids were removed from rumen during defatting. Saturated fatty acids were removed during defatting; there was a reduction in the relative amount of polyunsaturated fatty acids and a consequent increase in monounsaturated one.

The volatile compounds extracted from rumen can be found in the supplementary material as Table 1S. Thirty-two compounds were detected in the headspace of both raw and extruded rumen (Figure 1), but only the identified compounds are presented in the table. The volatile compounds of the bovine rumen were predominantly hydrocarbons (> 50% of the total area; Table 2).

Hexane used to remove fat from rumen was analyzed by GC-MS in the same conditions, and it did not contain any other hydrocarbon, as it can be seen in Figure 1.

All of the compounds were already described in meats, except for ethyl butyrate and nonadecane. The following compounds were found in both raw and extruded rumen: 1-heptene, toluene, limonene, tetradecane, pentadecane, hexadecane, heptadecane and octadecane. All compounds, except toluene, showed an increase in relative percentage after the extrusion process.

The headspace volatile compounds of raw and extruded rumen might originate from lipid oxidation (Table 1). Aliphatic aldehydes, alcohols, hydrocarbons and ketones, all of them with five or more carbon atoms in the chain, are derived from triacylglycerols and phospholipids, after thermal oxidation of fatty acids.[21] These products may react with the Maillard reaction's products, such as pyrazines, thiophenes, thiazoles, furanones and furfurals, and produce other compounds.[22]

The characteristic aroma of different meats and meat products is the result of changes that occur in lipids during cooking and of the release of volatile compounds originally trapped in the fat.[21,23,24] The high concentration of hydrocarbons in the volatile fraction, as well as aldehydes, alcohol, ketone and ester, might be a result of the thermal oxidation of the unsaturated fatty acids present in the rumen.

Table 1. Lipids and fatty acids profile of bovine rumen before and after defatting

	Before defatting (%)	After defatting (%)
Lipids*	17.3	3.36
Fatty acid**		
Myristic (C14:0)	6.4	-
Pentadecanoic (C15:0)	4.2	-
Palmitic (C16:0)	2.9	-
Stearic (C18:0)	17.0	-
Oleic (C18:1n9)	21.6	14.6
Linoleic (C18:2n6)	8.6	9.1
Linolelaidic (C18:2n6)	11.5	-
Arachidic (C20:0)	12.5	-
Eicosenoic (C20:1n9)	3.3	-
Eicosapentenoic (C20:5n3)	2.8	6.0
Heneicosanoic (C21:0)	2.2	-
Docosahexenoic (C22:6n3)	5.0	7.1
Nervonic (C24:1n9)	2.1	63.2

* percent of lipids in the sample; ** relative percent among fatty acids

The oxidation of unsaturated fatty acids produces volatile compounds responsible for the aromatic characteristics of meats. Aldehydes, for instance, have a low threshold value, and can contribute to the aroma of cooked meat. Some furans and hydrocarbons, however, have high threshold values, and it is unlikely that these compounds significantly contribute to the aroma of meat.[22]
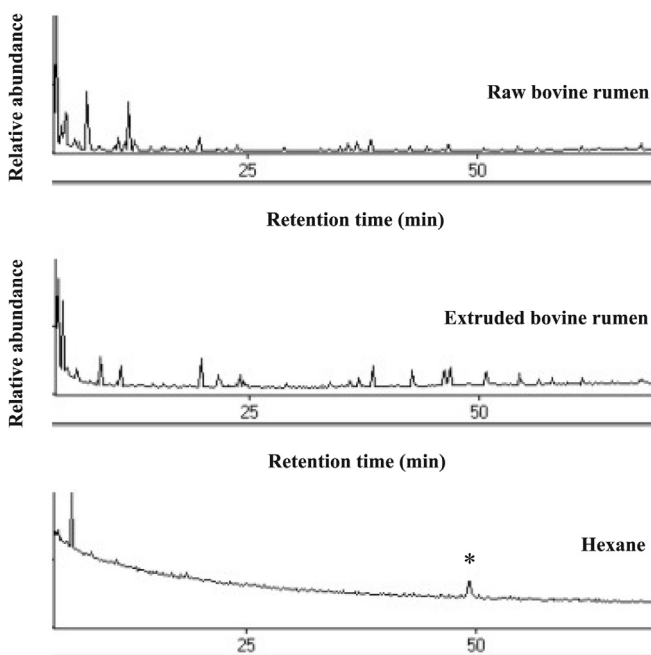
* Peak relative to a substance (butane, trichloroheptafluoro) present at the column, not at the solvent

Figure 1. *Chromatograms of raw and extruded bovine rumen and hexane used for extraction of volatile compounds*

Table 2. Chemical classes of volatile compounds of raw and extruded bovine rumen

Chemical class	Number of compounds found	
	Raw	Extruded
Hydrocarbon	20	23
Aldehyde	3	-
Alcohol	1	-
Ketone	1	-
Ester	1	-
Unidentified	6	9

The aroma of food depends on time and temperature.[23,25] The raw rumen was kept at 65 °C for 22 h after defatting, and it is most unlikely that the Maillard reaction would occur under these conditions. Extrusion was performed for a short period of time (around 2 min) and under high temperature (140 ºC) in this study, which is not adequate for the Maillard reaction to occur, although it can occur in a limited way.

Although sulphur and nitrogen compounds have been described in the aroma of cooked meat from degradation of sulphur amino acids, they were not found in the present work, probably because the temperatures and times used in extrusion (typically 150 ºC for 4 to 5 s) did not favor the formation of such compounds that require more drastic treatments.[23,25]

Extrusion changed the aroma of the bovine rumen. While raw rumen was practically odorless, the extruded product had a strong and characteristic aroma, resembling "visceral" flavor. It is well known that raw meat has very little odor; the desirable aroma of cooked meat results from chemical reactions that occur during the cooking process.[26] The same is true for rumen extrusion although the characteristic flavor produced may be not pleasant to everyone.

The impact of a volatile compound on food flavor depends on its threshold value, concentration in the food, solubility in water or fat and temperature.[23] It is unlikely that hydrocarbons present in the volatile fraction of both raw and extruded rumen be related to meat aroma, except for 1-heptene. This compound has been described as having a sulfurous aroma,[27] and in our study its relative percentage rose from 3.74 to 12.82 after extrusion. The non-identified compounds might account for the characteristic aroma of the extruded rumen.

CONCLUSIONS

Raw and extruded rumen showed different volatile compound profiles. The volatile fraction of the extruded rumen showed the characteristic aroma of a "visceral-type" product. The major volatile compounds extracted from both products were hydrocarbons. Other compounds, probably derived from the lipid fraction, were also identified and might account for the aroma of the product. Sulfur- and nitrogen- containing compounds were not identified. It seems that the time and temperature employed in this study were not adequate to originate such odoriferous substances.

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Material Suplementar

Table 1S. Compounds extracted from raw and extruded bovine rumen

KI[a]	Compound[b]	Raw (%RA[c])	Extruded (%RA[c])	Odor[d]	Major ions (*m/z*) Relative abundance (%)						
708	Heptane[*24,28]	14.1	-	Cooked meat[27]	43	41	57	42	71	56	70
					100	*57*	*28*	*24*	*22*	*20*	*12*
714	1-Heptene[**28]	3.7	12.8	Sulfurous[27]	41	55	56	42	70	57	43
					100	*73*	*71*	*47*	*41*	*27*	*26*
724	2,4-Dimethyl-hexane[**26]	-	1.2	Not found	43	57	41	56	85	42	55
					100	*55*	*46*	*30*	*19*	*11*	*9*
728	4-Methyl-2-pentanone[**26]	6.0	-	Cooked cabbage[30]	43	41	58	57	42	44	55
					100	*31*	*21*	*16*	*13*	*10*	*5*
753	Toluene[**24,28,31]	1.3	1.2	Fruity,[27] bitter,[27] paint[29]	91	92	65	45	51	50	43
					100	*60*	*19*	*18*	*15*	*11*	*11*
792	Hexanal[*22,24,26,28,31,32]	12.4	-	Rancid,[27] grass,[29-30] tallow[29]	44	41	43	56	57	45	42
					100	*76*	*58*	*49*	*31*	*20*	*14*
800	Ethyl butyrate[*]	2.8	-	Apple[29]	43	41	71	42	88	45	44
					100	*46*	*35*	*22*	*16*	*13*	*12*
852	*m*-Xylene[**24,26,28] or *p*-Xylene[**28]	0.5	-	Fruity,[27] sickly,[27] fatty,[27] plastic[29]	91	106	51	43	65	77	50
					100	*30*	*26*	*18*	*14*	*12*	*10*
860	*m*-Xylene[**24,26,28] or *p*-Xylene[**28]	3.5	-	Fruity,[27] sickly,[27] fatty,[27] plastic[29]	91	106	51	105	77	52	65
					100	*45*	*25*	*21*	*14*	*13*	*10*
884	*o*-Xylene[**26,28]	10.9	-	Sweet[27]	91	106	51	105	77	52	50
					100	*40*	*27*	*16*	*15*	*12*	*11*
896	Heptanal[*22,26,28,31,32]	2.3	-	Green,[30] burnt,[30] fatty,[29] citric,[29] rancid[29]	44	43	41	42	55	70	57
					100	*89*	*82*	*62*	*43*	*39*	*33*
1023	Limonene[*26,28]	3.0	6.8	Lemon,[29] orange[29]	68	67	41	93	53	79	43
					100	*81*	*66*	*55*	*36*	*31*	*29*

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Table 1S. cont.

KI[a]	Compound[b]	Raw (%RA[c])	Extruded (%RA[c])	Odor[d]	Major ions (*m/z*) *Relative abundance (%)*						
1099	Nonanal[*22,24,26,31,32]	1.7	-	Green,[29,30] moldy,[30] fatty,[29] citric[29]	43	41	57	44	56	55	42
					100	98	82	75	45	39	30
1100	Undecane[*28]	-	1.3	Solvent[29]	43	41	57	71	57	85	55
					100	54	54	27	26	21	18
1200	Dodecane[*24,26,28,31]	0.8	-	Solvent[29]	43	57	41	71	55	42	85
					100	68	47	26	14	12	12
1327	Tridecane[*24, 26,28,31]	1.2	-	Solvent[29]	43	57	71	41	85	55	70
					100	64	46	30	23	14	10
1400	Tetradecane[*24,26,28,31]	2.4	4.3	Solvent[29]	43	57	41	71	85	55	42
					100	75	47	34	19	17	14
1542	Pentadecane[*24,26,28]	0.9	3.6	Solvent[29]	43	57	71	41	85	55	99
					100	69	54	37	36	14	11
1600	Hexadecane[*24,26,28]	1.2	3.7	Solvent[29]	43	57	41	44	71	40	85
					100	81	51	44	35	29	26
1700	Heptadecane[*24,28]	0.7	2.9	Solvent[29]	43	57	41	71	85	55	42
					100	95	58	52	28	20	12
1800	Octadecane[*24,26]	0.8	2.6	Solvent[29]	43	57	71	41	85	55	56
					100	83	49	46	27	23	16
1900	Nonadecane[*]	-	1.2	Solvent[29]	43	57	41	71	85	55	40
					100	90	57	44	29	21	15

[a] calculated Kovats Index; [b] references for volatile compounds previously reported in cooked beef; [c] relative area; [d] references for odor of volatile compounds. * compound identified by MS+KI; ** compound identified only by MS