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ORIGINAL ARTICLE

Volatile profile of fermented sausages with commercial probiotic strains and fructooligosaccharides

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Abstract The effect of the partial substitution of pork back fat by fructooligosaccharides (FOS) and the probiotic strains *Lactobacillus paracasei* and *Lactobacillus rhmanosus* on the generation of volatile organic compounds in fermented sausages was investigated. The results obtained showed that these factors significantly affected the total content of organic volatile compounds (7484, 8114, 8372 and 10,737 AU × 10^4/g for FOS.GG, CON, FOS.BGP1 and FOS samples, respectively). A total of 59 volatile components, mainly hydrocarbons, ketones and esters were isolated. The reduction of fat content by including FOS in the formulation results in positive effects and a greater stability of the volatile profile of the fermented sausages, increasing ester compounds and reducing the undesirable notes of hexanal (probiotic samples showed values < 2 AU × 10^4/g). Moreover, there was a symbiotic effect when the aforementioned prebiotic fiber was combined with probiotic *Lactobacillus* strains.

Keywords Meat product · Prebiotic · FOS · Lactic acid bacteria · Aroma · Volatile compounds

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Introduction

Both lifestyle and the consumption of some foods have been associated with the development of several diseases increasing the concern of consumers for health. Recently, different strategies have been tested to develop healthy meat products (Heck et al. 2017). Functional foods are one of these research areas, resulting in beneficial effects on human health in addition to their nutritious function (Zhang et al. 2010).

Prebiotics are non-digestible substances that serve as substrate to the microorganisms of the human gastrointestinal tract improving human health (De Vrese and Schrezenmeir 2008). Fructooligosaccharides (FOS) are prebiotic foods and, recently, they have been used in functional food research (Salazar et al. 2009; Felisberto et al. 2015; Bis-Souza et al. 2019a). FOS are oligosaccharides that occur naturally in traditional medicinal plants (Sridevi et al. 2014) and are classified as Generally Recognize as Safe (GRAS), which allows their use in food products as safe additives (FDA 2017). These compounds present a high resistance to digestion and absorption by the gastrointestinal tract resulting in a reduced caloric content (Ruiz-Aceituno et al. 2018). They have a beneficial effect on specific bacteria, stimulating the growth of non-pathogenic intestinal microflora, decreasing the growth of potentially pathogenic strains and enhancing the immune system (Gibson et al. 2017). Besides these effects, it is also worth mentioning the reduction of cholesterol levels and blood pressure, as well as their potential anti-cancer properties (Jiménez-Colmenero et al. 2001). When this prebiotic carbohydrate is administered in combination with strains of probiotic microorganisms, they can act synergically in the human intestines where they ensure the

viability of these beneficial microorganisms (Öztürk and Serdaroğlu 2017).

Consequently, the partial substitution of pork fat by functional ingredients in meat products, such as prebiotic dietary fiber, has been the objective of several studies. In fact, the positive effect of partial substitution of pork back fat by prebiotic carbohydrates has been reported in previous studies where FOS is shown to improve the technological parameters, texture properties and sensory characteristics (Salazar et al. 2009; Bis-Souza et al. 2018). In this regard, Bis-Souza et al. (2019b) reported that the addition of FOS as fat replacer resulted in higher hardness values, probably due to the more pronounced moisture loss that occurred in these samples during the dry-curing process. Moreover, FOS did not show a significant effect on sensory attributes such as appearance, texture, flavor and overall acceptability (Bis-Souza et al. 2019b).

A large number of research projects have been carried out in order to develop functional meat products, including the use of potential probiotic strains in fermented sausages (Ba et al. 2018). Traditional fermented sausages, made exclusively of pork lean and back fat, salt and spice (Fonseca et al. 2015), could be a good matrix in which to develop these healthy products. This type of dry-fermented sausage is traditionally made without adding a starter culture. However, the use of a starter culture could guarantee a more hygienic process, which standardizes sensorial and technological characteristics in a shorter ripening time (Lorenzo et al. 2014a). Lactic acid bacteria (LAB) are responsible for the acidification of the product during fermentation, resulting in improvements of flavor, taste and the biological safety of the products (Lorenzo et al. 2016a).

The characteristic flavor of these meats is one of the most appreciated attributes for the consumer and one which really affects their acceptance (Gómez et al. 2015; Pateiro et al. 2015; Bosse et al. 2017). Although the main volatile compounds influencing the aroma of the final product belong to different chemical families, not all these compounds have the same importance on the overall aroma perception (Domínguez et al. 2019). The concentration and the olfactory threshold are the main factors that determine the final aroma of the meat product (Rivas-Cañedo et al. 2012). The characteristic aroma of these products is conferred by many different non-volatile and volatile compounds, resulting from numerous and complex reactions. In most cases, the volatile profile is the result of the reactions that take place during the ripening time, mainly from carbohydrate fermentation and lipolytic and proteolytic processes (Lorenzo et al. 2013; Montanari et al. 2018). Moreover, spices and the activity of endogenous meat enzymes are other sources of volatile compounds (Gómez and Lorenzo 2013). Most commercial fermented sausages use a combination of starter cultures to guarantee the typical flavors and aromas of these meat products. *Lactobacillus*, *Staphylococci* and/or *Micrococci* are the genus strains commonly used (Cheng et al. 2018).

However, there is scarcely any information about FOS and the symbiotic effect when a meat product contains both probiotics and prebiotics in their formulation, especially regarding their volatile profile, which determines the typical aroma of this fermented product. Therefore, the present study aims to evaluate the influence of the addition of fructooligosaccharides and two different probiotic commercial strains on the volatile profile of low-fat fermented sausages.

Materials and methods

Prebiotic and probiotic materials

The prebiotic fiber used as fat substitute was NutraFlora® P95 soluble prebiotic fiber (short-chain fructooligosaccharide—moisture 4%; total dietary fiber 95% dry basis) (Ingredion, Westchester, USA).

The commercial *starter* culture used was Bactoferm T-SPX (*Pediococcus pentosaceus* + *Staphylococcus xylosus*, (Chr.Hansen, Hørsholm, Denmark). The commercial probiotics strains used were the Lyofast BGP 1 composed of *Lactobacillus paracasei* (Sacco System, Cadorago, Italy) and *Lactobacillus rhamnosus* GG (Chr.Hansen, Hørsholm, Denmark).

Fermented sausage manufacture

Four different batches (CON, FOS, FOS.BGP1 and FOS.GG) of low-fat fermented sausages were manufactured in the pilot plant of the Meat Technology Center of Galicia (San Cibrao das Viñas, Ourense, Spain).

A control formulation (denominated CON) without any probiotic strains or fructooligosaccharides added was prepared using lean pork (80 g/100 g), pork back fat (15 g/100 g) both from Celta pigs, the "542 Salchichón" supplement (Laboratorios Ceylamix, Valencia, Spain), [5 g/100 g, composed, in unknown proportions, of sugar, salt, dextrin, spices (black and white pepper and nutmeg), milk protein, monosodium glutamate (E621), phosphates (E450i and E451i), sodium erythorbate (E316), potassium nitrate (E252) and coloring (E120)] and a commercial starter culture Bactoferm T-SPX (0.025 g/100 g). Three other batches of low-fat fermented sausages were produced using lean pork (80 g/100 g), pork back fat (10 g/100 g^{-1}), cold water to homogenize (3 g/100 g^{-1}), FOS (2 g/100 g) "542 Salchichón" (5 g/100 g) supplement, and commercial *starter* culture Bactoferm T-SPX (0.025 g/100 g). The probiotic strains (0.01 g/kg) *L. paracasei* and *Lactobacillus*

rhmanosus GG were added to the two formulations denominated FOS.BGP1 and FOS.GG, respectively. The other formulation with no strain added was denominated FOS.

The lean pork was ground using a 10 mm diameter mincing plate while the pork back fat was ground through an 8 mm diameter mincing plate. The batches were mixed in a vacuum mincer (Fuerpla, Valencia, Spain) for 2 min and kept at 3–5 °C for 24 h. Then, the batch was stuffed into natural casing 35 cm long and 60 mm in diameter, so that the final weight of each sausage was around 400 g. The sausages were kept in a fermentation chamber for 2 days at 20 °C and 80% relative humidity and then transferred into a drying-ripening chamber where they were kept for 43 days at 8–12 °C and 75–60% of relative humidity.

Six replicates from each batch were taken after 45 days of ripening. The four aforementioned formulations were manufactured in two batches with the same ingredients, formulation and technology, first in March and then in April of 2018.

Chemical composition

In order to characterize the final products, the proximate composition was determined. Moisture, protein, fat and ash contents were quantified according to Lorenzo et al. (2016b).

Volatile compounds

The extraction of the volatile compounds was performed using solid-phase microextraction (SPME), following the method described by Domínguez et al. (2019) with modifications. For headspace SPME (HS-SPME) extraction, 1 g of each sample, after being ground using a commercial grinder, was placed in a 20 mL vial. The conditioning, extraction and injection of the samples were carried out with a PAL RTC 120 auto sampler (CTC Analytics AG, Zwingen, Switzerland). The extractions were carried out at 37 °C for 30 min, after equilibration of the samples for 15 min at the temperature used for extraction, ensuring a homogeneous temperature for sample and headspace. Once sampling was finished, the fibre was transferred to the injection port of the gas chromatograph–mass spectrometer (GC–MS) system. A 7890B gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a 5977B MSD mass selective detector (Agilent Technologies) and a DB-624 capillary column (30 m, 0.25 mm i.d., 1.4 µm film thickness) (J&W Scientific, Folsom, USA) was used for volatile analysis. Compounds were identified by comparing their mass spectra with those contained in the NIST14 (National Institute of Standards and Technology, Gaithersburg, USA) library, and/or by comparing their mass spectra and retention time with authentic standards (Supelco, Bellefonte, USA) and/or by calculation of the retention index relative to a series of standard alkanes (C5-C14), Supelco 44585-U, (for calculating Linear Retention Index, (Supelco, Bellefonte, USA) and matching them with data reported in the literature. The results are expressed as area units (AU) $\times$ 10^4/g of sample.

Statistical analysis

A total of 48 fermented sausages: six fermented sausage samples for each batch $\times$ four batches (CON, FOS, FOS.BGP, FOS.GG) $\times$ one ripening time (45 days) $\times$ two different manufactured batches (March and April 2018) were analyzed for different dependent variables. After that, normal distribution and variance homogeneity were tested (Shapiro and Wilk 1965).

For the statistical analysis of the results of low-fat fermented sausages, an analysis of variance (ANOVA) with the mixed-model was performed for all variables considered in the study. These parameters were set as dependent variables, while formulation was included in the model as fixed effect and the different manufacture and replicates were considered as random effects. Duncan's method was used to assess the pairwise differences between least square means wherein such differences were considered significant if $P \leq 0.05$. The values were expressed as mean values and standard error of the mean (SEM). All statistical analysis was performed using Statistica 7.0 software.

Results and discussion

Chemical composition of low-fat fermented sausage

As expected, the proximate composition of the different low-fat fermented sausages at the end of ripening showed significant differences for fat content ($P < 0.05$), which is the one of the main objectives of this research. A decrease in the value was observed when low-fat treatments were compared with CON (19.70 g/100 g vs mean values of 13.59 g/100 g for CON and low-fat fermented sausages, respectively). This difference is explained by the partial substitution of pork back fat in the low-fat formulations by fructooligosaccharides (FOS). The addition of probiotic strains showed no effect on the fat composition of the different treatments with FOS since the same amount of pork back fat was used in their formulation. Regarding moisture, protein and ash contents, the results obtained show no significant differences between the treatments (mean values of 31.54 g/100 g, 16.65 g/100 g and 2.86 g/100 g for moisture, protein and ash contents, respectively). In general, these results were similar to those reported by

other authors in dry-cured sausages (Domínguez et al. 2016).

Volatile compounds of low-fat fermented sausage

Table 1 shows the effect of FOS and probiotic strains on the volatile profile of low-fat fermented sausages at the end of ripening (expressed as UA × 10^4/g of dry matter). Statistical analysis showed that total volatile compounds contents were significantly affected ($P < 0.05$) by FOS and the *Lactobacillus* strains (7484, 8114, 8372 and 10,737 AU × 10^4/g for FOS.GG, CON, FOS.BGP1 and FOS samples, respectively).

Hydrocarbons are the major volatile group identified in the volatile profile of the fermented sausages. Their contents represented between 75 and 85% of total volatile compounds. Then, in order of importance, came ketones, esters, and alcohols and organic acids that presented similar content percentages, while aldehydes were the last group.

Regarding the hydrocarbons, 25 have been identified in fermented sausage, terpenes being predominant in the volatile fraction of this product. This could be related to the relatively large amount of spices included in its formulation (Rivas-Cañedo et al. 2009; Domínguez et al. 2016). The addition of FOS and probiotic strains did not have a significant ($P > 0.05$) effect on the total content of these groups of volatile compounds. Despite not being significantly different, the FOS treatment displayed the highest amount (7999 AU × 10^4/g vs 7110, 6170 and 6054 AU × 10^4/g for FOS vs FOS.BGP1, CON and FOS.GG samples, respectively), which could be due to the great affinity of the starter cultures for FOS, selectively favoring its development. While in the samples that contain the starter cultures, FOS and one or another probiotic species, competition for this substrate could occur which would condition the enzymatic reactions that contribute to the development of aroma and flavor (Hierro et al. 1997).

In addition, the contribution of lactic acid bacteria to flavor could be limited by their carbohydrate catabolism, whereas gram-positive strains such as *Staphylococcus* might be more appropriate in the generation of aromatic compounds specific to fermented sausages (Leroy et al. 2006).

Terpenes have their origin in the spices used in manufacture of low-fat fermented sausages, in particular black and white pepper (Montanari et al. 2018). α-Thujene (1252 AU × 10^4/g), β-Terpinene (1024 AU × 10^4/g), (-)-β-Pinene (985 AU × 10^4/g), *o*-Cymene (1292 AU × 10^4/g) and D-Limonene (777 AU × 10^4/g) were the major individual hydrocarbons identified. Significant contents of γ-Terpinene, α-Phellandrene, 3-Carene, β-Myrcene and α-Terpinene were also found. These results were also seen with other dry-cured meat products (Rivas-Cañedo et al. 2009). Among the terpenes isolated, only α-Thujene, β-Myrcene, β-Phellandrene and δ-Carene show significant ($P < 0.05$) differences between the different treatments. The incorporation of FOS in the formulation of fermented sausages as fat replacer results in the release of a greater amount of volatile terpenes, while α-Thujene showed similar values in FOS and FOS.BGP1 samples. In contrast, volatile compounds that originate from biochemical changes (microbial metabolism and endogenous reactions) were less common. This is confirmed by the fact that the addition of FOS and *Lactobacillus* strains scarcely affected these compounds.

Ketones were the second largest group isolated from the fermented sausages. The total contents were significantly ($P < 0.001$) affected by the compositions of this product. FOS samples showed significantly higher values than those obtained in the other treatments. The contents found in FOS samples (1700 AU × 10^4/g) was almost double the CON contents (977 AU × 10^4/g) and triple and fivefold the amounts detected in FOS.GG (520 AU × 10^4/g) and FOS.BGP1 (348 AU × 10^4/g), respectively. The lower contents observed in the samples that contain the probiotic strains could be related to the greater presence of microbial strains (Sánchez-Peña et al. 2005).

Acetoin, 2-butanone and 2,3-octanedione were the main compounds identified. Acetoin was the major ketone detected. As occurred with the total contents, FOS showed significantly ($P < 0.001$) higher contents than the other batches (1685 AU × 10^4/g vs 960, 486 and 327 AU × 10^4/g for FOS vs CON, FOS.GG and FOS.BGP1 samples, respectively). There are two possible origins of this compound, (i) Maillard reactions (Pérez-Santaescolástica et al. 2018) or microbial carbohydrate metabolism (Petričević et al. 2018). This volatile compound has a very low odor threshold, so it contributes to the typical flavor of dry-cured meat products (Sidira et al. 2016). Another important ketone detected was 2-butanone. Although there was no significant difference, the contents of FOS.GG were higher than the other three treatments. The oxidation of free fatty acids (FFA) is related to the origin of this compound (Narváez-Rivas et al. 2012), and gives important aroma notes to associated meat products because of its peculiar and intense odor (Pastorelli et al. 2003).

Regarding esters, the *Lactobacillus* strains had a significant ($P < 0.05$) effect on the total content of this group of volatile compounds (497 and 345 AU × 10^4/g for FOS.BGP1 and FOS.GG vs 87 and 105 AU × 10^4/g for FOS vs CON samples, respectively). The composition of the identified esters also differed for the formulations under study. Butanoic and hexanoic acid ethyl esters were the most abundant in the FOS.BGP1 and FOS.GG samples (representing 52% and 60% of the total esters,

Table 1 Volatile compounds of low-fat fermented sausage (expressed as UA × 10^4/g dry matter)

Volatile compounds	LRI	R	Bach				SEM	*P* value
			CON	FOS	FOS.BGP1	FOS.GG		
Butanoic acid	929	21.23	77.82	98.08	81.63	84.36	4.37	0.393
Butanoic acid, 3-methyl-	983	23.59	33.10	52.42	39.47	30.89	3.39	0.102
Hexanoic acid	1104	28.79	15.72	16.93	12.50	13.04	0.97	0.313
Acetic acid	684	10.68	0.00^{b}	0.00^{b}	52.77^{a}	66.76^{a}	7.24	0.001
Total organic acids			126.63	167.43	186.37	195.05	6.43	0.141
Benzyl alcohol	1149	30.73	57.32	64.83	55.33	68.85	4.04	0.619
4-Thujanol	1151	30.82	10.27^{b}	17.27^{a}	10.39^{b}	9.24^{b}	0.62	0.001
Linalool	1171	31.68	8.95	14.91	11.05	11.38	1.01	0.209
Terpinen-4-ol	1237	34.53	55.74^{b}	79.78^{a}	75.22^{a}	81.81^{a}	2.38	0.001
Total Alcohols			131.55^{b}	176.61^{a}	151.07^{ab}	175.40^{a}	5.41	0.004
Butanal, 3-methyl-	653	9.31	18.55^{b}	26.09^{a}	18.97^{b}	14.44^{b}	1.14	0.001
Hexanal	881	18.75	8.16^{a}	5.77^{b}	0.00^{c}	1.65^{c}	0.59	0.001
Benzaldehyde	1063	27.03	8.92^{bc}	8.20^{c}	13.18^{b}	22.80^{a}	1.15	0.001
Benzeneacetaldehyde	1142	30.44	34.63^{a}	22.47^{b}	7.22^{c}	7.80^{c}	1.89	0.001
(E)-Hexadec-2-enal	1156	31.04	4.47	4.76	5.05	4.83	0.88	0.997
Total aldehydes			74.73^{a}	63.23^{ab}	34.33^{c}	50.83^{bc}	3.13	0.001
Acetic acid ethenyl ester	576	6.01	39.62	52.21	31.36	34.02	3.25	0.094
Ethyl acetate	588	6.53	28.05	16.31	21.77	16.51	2.51	0.296
Propanoic acid, ethyl ester	736	12.91	1.82^{a}	0.00^{b}	5.77^{a}	5.31^{a}	0.87	0.049
Butanoic acid, ethyl ester	861	18.29	9.77^{b}	5.05^{b}	135.15^{a}	104.49^{a}	17.96	0.013
Butanoic acid, 2-methyl-, ethyl ester	918	20.75	7.17^{b}	4.65^{b}	65.72^{a}	46.93^{b}	7.16	0.001
Butanoic acid, 3-methyl-, ethyl ester	922	20.93	25.95	16.48	85.95	58.85	13.81	0.257
Hexanoic acid, ethyl ester	1069	27.29	6.17^{b}	2.69^{b}	123.62^{a}	101.75^{a}	15.71	0.004
Octanoic acid, ethyl ester	1233	34.34	2.69^{b}	0.00^{b}	40.18^{a}	29.18^{ab}	5.36	0.013
Total esters			104.68^{b}	86.82^{b}	497.13^{a}	345.33^{ab}	52.76	0.018
Octane	825	16.76	20.96	20.14	21.15	13.99	1.43	0.251
Heptane, 3-ethyl-	915	20.61	10.95	27.67	36.88	21.39	2.94	0.160
α-Thujene	984	23.61	992.25^{b}	1356.56^{a}	1454.25^{a}	1202.88^{ab}	57.47	0.015
β-Terpinene	992	23.96	957.45	1256.60	1002.93	878.66	72.54	0.280
Heptane, 3-ethyl-5-methylene-	999	24.24	12.92^{a}	11.21^{a}	5.97^{b}	4.63^{b}	0.88	0.001
Camphene	1011	24.76	21.61	29.63	24.10	23.05	1.88	0.465
β-Phellandrene	1037	25.89	138.25^{b}	271.09^{a}	196.58^{ab}	164.31^{b}	17.69	0.041
Nonane, 3-methylene-	1038	25.94	18.54	20.77	17.60	17.42	0.83	0.469
(-)-β-Pinene	1040	26.02	984.10	1162.44	870.61	923.43	59.89	0.357
Decane	1047	26.33	42.82^{a}	38.07^{a}	25.95^{b}	20.95^{b}	2.38	0.001
β-Myrcene	1049	26.41	201.80^{b}	299.34^{a}	235.76^{ab}	211.23^{b}	13.96	0.047
α-Phellandrene	1065	27.11	368.33	407.35	341.50	395.85	31.54	0.894
3-Carene	1069	27.26	356.13	365.14	282.72	285.89	18.33	0.228
α-Terpinene	1077	27.62	150.03	246.85	236.75	205.67	15.26	0.093
D-Limonene	1087	28.06	664.08	908.09	780.71	755.12	32.61	0.053
o-Cymene	1091	28.23	1048.78	1499.01	1399.78	1221.90	77.57	0.169
γ-Terpinene	1113	29.18	267.36	433.11	415.09	369.90	25.71	0.086
Undecane	1135	30.15	70.92^{a}	69.95^{a}	36.51^{b}	33.84^{b}	4.45	0.001
δ-Carene	1139	30.30	59.17^{b}	101.01^{a}	82.75^{ab}	74.46^{ab}	5.23	0.028
4-Vinyl-*o*-xylene	1153	30.89	47.06	68.32	59.89	50.80	3.73	0.177
Dodecane	1215	33.60	46.18^{a}	49.50^{a}	37.97^{ab}	28.09^{b}	2.59	0.007
1-Decene, 2,4-dimethyl-	1230	34.22	0.00^{b}	0.00^{b}	19.60^{a}	22.85^{a}	2.43	0.001

Table 1 continued

Volatile compounds	LRI	R	Bach				SEM	*P* value
			CON	FOS	FOS.BGP1	FOS.GG		
Tridecane	1289	36.79	20.34	21.45	17.62	13.60	1.20	0.085
α-Copaene	1365	40.05	19.39	28.96	28.06	24.80	1.39	0.055
β-Caryophyllene	1404	41.75	91.93	106.62	91.14	97.73	4.49	0.599
Alkanes			128.49	142.90	105.23	84.77	10.57	0.223
Alkenes			6041.61	6335.80	5001.18	5969.63	410.08	0.696
Total hydrocarbons			6170.12	7998.70	7109.80	6054.40	397.01	0.265
2-Butanone	583	6.29	12.85	13.07	17.97	22.72	1.66	0.118
Acetoin	790	15.23	960.44^{b}	1684.77^{a}	327.25^{c}	485.60^{c}	103.33	0.001
2,3-Octanedione	1068	27.23	7.48^{b}	4.41^{b}	85.63^{a}	96.29^{a}	13.36	0.014
Total ketones			977.37^{b}	1700.04^{a}	348.07^{c}	520.44^{c}	99.09	0.001
Sulfide, allyl methyl	697	11.21	64.76	79.74	59.48	48.55	4.82	0.147
Disulfide, dimethyl	782	14.89	4.30^{b}	0.00^{b}	17.72^{a}	8.22^{ab}	2.11	0.018
Total sulphur compounds			69.06	79.74	77.20	56.77	5.94	0.399
Oxetane	510	3.16	22.53	31.06	25.17	29.63	2.12	0.463
Spiro[2,4]hepta-4,6-diene	807	15.99	21.31	28.16	32.61	32.93	3.66	0.658
Pyrazine, 2,6-dimethyl-	993	24.00	12.16	17.28	13.32	11.63	0.93	0.122
Pyrazine, trimethyl-	1079	27.71	12.70	22.80	20.94	18.97	1.42	0.062
Eucalyptol	1097	28.49	8.48^{b}	12.67^{a}	10.73^{ab}	10.17^{ab}	0.54	0.049
Safrole	1318	38.04	276.19^{c}	344.08^{a}	291.98^{bc}	330.04^{ab}	8.95	0.018
Methyleugenol	1396	41.38	7.71^{b}	13.63^{a}	11.56^{a}	13.90^{a}	0.58	0.001
Myristicin	1462	44.24	12.74^{b}	23.43^{a}	20.64^{a}	23.69^{a}	1.05	0.001
Total other compounds			373.82	493.11	426.95	470.96	11.49	0.168
Total volatile compounds			8113.90^{b}	10,737.02^{a}	8371.79^{ab}	7484.42^{b}	446.03	0.048

CON, control without probiotic strains and without FOS; FOS, low-fat with FOS 2 g/100 g^{-1} added; FOS.BGP1, low-fat with FOS 2 g/100 g^{-1} and *Lactobacillus paracasei* added; FOS.GG, low-fat with FOS 2 g/100 g^{-1} and *Lactobacillus rhmanosus* GG added; LRI, linear retention index; SEM, standard error of the mean

[a,b,c]Mean values in the same row with different letters presented significant differences

respectively), while acetic acid ethenyl ester and ethyl acetate were predominant in the CON and FOS samples (65% and 79% of the total esters, respectively). The esterase activity of lactic acid bacteria could be the answer to these differences by promoting the enzymatic esterification of fatty acids and alcohols (Narváez-Rivas et al. 2012). The ethyl esters, the main esters detected in the present study, are related to the flavor of fermented sausages as they can mask rancid odors (Andrade et al. 2010).

Four compounds were isolated in the group of alcohols. The total content of this group showed significant differences between the formulations under study (132, 151, 175 and 177 AU × 10^4/g for CON, FOS, FOS.BGP1 and FOS.GG, respectively; $P < 0.01$). Terpinen-4-ol was the most abundant, which coincides with previous studies that affirm that terpinen-4-ol, 4-Thujanol and linalool are the alcohols commonly detected in fermented sausage samples (Domínguez et al. 2019). FOS and the two probiotic-strain formulations showed similar values but significantly higher than those observed in CON. This could be related to the activity of *Lactobacillus*, which may favor the formation of the branched aldehydes associated with the origin of these alcohols (Narváez-Rivas et al. 2012).

FOS along with the two added probiotic strains only had a significant effect on one of the organic acids isolated. However, acetic acid showed the lowest values in this group of volatiles. This result does not agree with the results found for other dry fermented sausages (Montanari et al. 2018). Only sausages inoculated with probiotic strains showed any values for this compound, which would explain its origin as being related to carbohydrate fermentation induced by microorganisms (Andrade et al. 2010). Despite not being significant, the other organic acids detected showed higher values in the FOS samples. The detected organic acids are described as potent odorants, so they contribute to the typical aroma of fermented sausage (Corral et al. 2013; Lorenzo et al. 2014b).

Aldehyde content represented < 1% of the total volatile compounds. This result does not agree with the results observed by other authors, who found that this group of

volatiles is one of the most important in fermented sausages (Domínguez et al. 2016). The aldehydes detected in the present study had three main origins. Branched aldehydes (butanal, 3-methyl) are related to proteolysis and amino acid degradation (Purriños et al. 2012): cycloaldehydes are derived from Streker degradation of amino acids (Lorenzo and Carballo 2015); and hexanal is related to the lipid oxidation of fatty acids (Montanari et al. 2018).

The CON samples showed significantly ($P < 0.001$) higher amounts of aldehydes than the probiotic and prebiotic batches (75, 63, 51 and 34 AU $\times$ 10^4/g for CON, FOS, FOS.GG and FOS.BGP1, respectively). In the present study, benzeneacetaldehyde, benzaldehyde and butanal, 3-methyl- were the main aldehydes isolated. Significant differences were found for the aforementioned compounds ($P < 0.001$). The first one is the most abundant in CON, benzaldehyde in FOS.GG and butanal, 3-methyl in FOS and FOS.BGP1 samples. Previous studies described that *Lactobacillus* strains has protein degradation mechanisms with the capability of producing useful amino acids (Kleerebezem et al. 2003), which are precursors of volatile compounds with a desirable impact on aroma (Toldrá and Flores 1998). This could justify the higher content of the cycloaldehydes derived from Strecker degradation of amino acids in samples that contain probiotic strains.

On the other hand, hexanal followed the trend of total aldehydes, with higher content in CON than in FOS and the inoculated samples. In fact, the inoculated samples showed very low values (< 2 AU $\times$ 10^4/g). This result contrasts with the higher content found for this volatile compound in other sausages inoculated with commercial starter cultures (75% of the total aldehydes) (Domínguez et al. 2016).

Finally, sulfur compounds were also detected in low-fat fermented sausages. Significant differences ($P < 0.05$) were found in dimethyl disulfide content, the samples inoculated with probiotic strains being the ones which obtained higher values for this volatile compound. The origin of these compounds is associated with the use of spices as ingredients in dry-cured sausages (Sunesen et al. 2001; Muriel et al. 2004).

Conclusion

The results found in the present study confirmed that the prebiotic fiber FOS and *Lactobacillus* strains have a significant and positive effect on fermented sausages, since they contribute to the aroma as well as the stability of the volatile profile of low-fat fermented sausages. The main groups of volatile compounds identified in low-fat fermented sausages are terpenes, related to spices used in the manufacture of this product, and some sulfur compounds. The second ones resulted from the dry-curing process. Hexanal, butanal, 3-methyl, acetoin, ethyl esters, acetic acid, butanoic acid and butanoic acid, 3-methyl- are included in this group. These compounds have a high influence in the aroma of fermented sausages.

The incorporation of FOS in the formulation of low-fat fermented sausages as fat replacer causes the release of a greater amount of volatile terpenes. The probiotic strains *L. paracasei* and *L. rhmanosus* showed high ester content, which would mask undesirable odors associated with fermented sausages. Moreover, the lower values for hexanal obtained in the FOS and the inoculated samples reflected a positive effect of this symbiotic combination against the rancid flavors usually associated with this compound.

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