Potential probiotic salami with dietary fiber modulates antioxidant capacity, short chain fatty acid production and gut microbiota community structure

Sergio Pérez-Burillo, Trupthi Mehta, Silvia Pastoriza, Denise Lynette Kramer, Oleg Paliy, José Ángel Rufián-Henares

Departamento de Nutrición y Bromatología, Instituto de Nutrición Y Tecnología de los Alimentos, Centro de Investigación Biomédica, Universidad de Granada, Granada, Spain

Department of Biochemistry and Molecular Biology, Boonshoft School of Medicine, Wright State University, Dayton, OH, USA

Instituto de Investigación Biosanitaria ibs.GRANADA, Universidad de Granada, Granada, Spain

ARTICLE INFO

Keywords:
Fermented sausage
Prebiotic
Antioxidant
Short chain fatty acids
Gut microbiome

ABSTRACT

Dry-fermented sausages are an important and abundant component of the diet of many people. Improving their composition and thus their potential health effects is therefore important. Here we quantified the antioxidant capacity, short chain fatty acids (SCFAs) production, and gut microbiota structure of different salami formulations after in vitro digestion and subsequent fermentation with human gut microbiota. The addition of different types of fiber (citrus fiber, arabinogalactan, and inulin), a probiotic Lactobacillus rhamnosus, and an herbal extract to the salami formulation was tested. Incorporating any dietary fiber into salami formulation increased sausage antioxidant capacity and the amount of SCFAs produced during microbiota fermentation. These effects were highest for salami with citrus fiber and citrus fiber with herbal extract. Presence of fiber in salami also led to an altered gut microbiota structure. Citrus fiber and arabinogalactan but not inulin promoted an increase in the abundance of several known polysaccharide degrading genera and resulted in a reduction in the abundance of Escherichia, a bacterial genus known to contain many human intestinal pathogens. Overall, the addition of dietary fiber to salami formulation prior to curing improved beneficial health markers of this food product.

1. Introduction

Salami is a dry-fermented sausage consisting of mixtures of lean meats and fatty tissues combined with salts, nitrate (curing agent), sugars, spices, and other non-meat ingredients filled into casings. Salamis acquire their properties (flavor, texture, color, etc.) through a fermentation process, in which mainly lactic acid bacteria and coagulase-negative staphylococci are involved (FAO, 2017).

Salami is generally considered as a food with unbalanced nutritional value due to the high fat and salt content and the lack of bioactive molecules, such as phenolic compounds and other phytochemicals (Martínez, Nieto, & Ros, 2014), but they are highly consumed in many countries around the world (Blaiotta, Murr, Cerbo, Romano, & Aponte, 2018). Thus, there is a significant interest to improve nutritional properties of salami, for example, by using probiotic bacteria as a starter for the fermentation process (Giello, La Storia, De Filippis, Ercolini, & Villani, 2018), or by adding different kinds of fibers to the sausage composition (dos Santos, Campagnol, Pacheco, & Pollonio, 2012). Addition of dietary fiber to salami can lead to an increased production of short chain fatty acids (SCFAs) either during salami preparation/curing, or when it is fermented in the colon by resident microbes after ingestion. SCFAs have been associated with many health benefits: they help maintain proper function of the colon (Ríos-Covián et al., 2016), have a protective role on the diet-induced obesity (Lin et al., 2012), protect against colorectal cancer (Ríos-Covián et al., 2016), and regulate intestinal inflammation (Ríos-Covián et al., 2016). Nevertheless, the previous efforts are centered on the SCFAs production during fermentation and ripening process, because SCFAs in the salami influence its taste (Iacumin, Comi, Cantoni, & Cocolin, 2006). Very few in vitro studies have focused on SCFAs production by the gut microbiota after meat intake (Shen, Chen, & Tuohy, 2010). To our knowledge, only Thøgersen et al. (2018) studied the addition of inulin on Frankfurt salami.
salami, and in the amounts that they were going to be added to salami.

On the contrary, there has also been a rise in the studies aiming to extend the shelf life of salami. Most advancements in this area make use of natural antioxidants or plant extracts to avoid or delay lipid oxidation while preserving taste and smell (Cullere, Hoffman, & Dalle Zotte, 2013). Antioxidants can also reduce oxidative damage that is one of the major causes of aging and various chronic diseases including cancer, Alzheimer’s disease, inflammation, diabetes, atherosclerosis, and Parkinson’s disease (Pastoriza, Delgado-Andrade, Haro, & Rufián-Henares, 2011). However, very little has been done to examine the antioxidant capacity that such improved salami products could provide, even though there is evidence for the presence of strong antioxidant compounds such as carnosine in meat products (Martínez et al., 2014).

The aims of this paper were to evaluate the antioxidant capacity of salami, to evaluate SCFAs production during salami digestion and fermentation by intestinal microbiota, and to study the alterations in the gut microbial community structure after in vitro digestion-fermentation process. To accomplish these aims, antioxidant assays, SCFA measurements, and microbial community structure characterization were performed on traditional salami (control) and several salami samples enriched with different types of fiber and probiotic bacteria.

2. Materials and methods

2.1. Chemicals

Trolox (±)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), carmine indigo, hydrogen peroxide, gallic acid, 2,2′-Azobis(2-methylypropionamidine) dihydrochloride (AAPH), potassium persulphate, sodium hydroxide, iron (III) chloride hexahydrate, sodium acetate, potassium chloride, potassium di-hydrogen phosphate, sodium mono-hydrogen carbonate, sodium chloride, magnesium chloride hexahydrate, ammonium carbonate, calcium chloride dihydrate, sodium di-hydrogen phosphate, tryptone, cysteine, sodium sulphide, resazurin, salivary alpha-amylase, pepsin from porcine, bile acids (porcine bile extract), ethanol, hydrochloric acid, acetonitrile, and acetate, propionate, and butyrate standards were from Sigma-Aldrich (Darmstadt, Germany). Pancreatin from porcine pancreas was purchased from Alpha Aesar (United Kingdom).

2.2. Materials

Salamis samples were manufactured and provided by a local Spanish company (Elpozo Alimentación, S.A., Alhama de Murcia, Murcia). Samples consisted of a salami control and four salamis with modified formulation. Each formulation contained probiotic Lactobacillus rhamnosus, and (i) citrus fiber (commercial name “Citri-Fi”) obtained from orange pulp dehydration and composed of 42% pectin, 25% cellulose and hemicellulose, and acquired from Fiberstars (USA), (ii) citrus fiber with additional herbal extract, (iii) inulin Orafti HPX, which was acquired from Beneo (Belgium), a long-chain chitory inulin product containing 99.5% inulin, or (iv) arabinogalactan (commercial name “Acacia-fiber” acquired from Nexira (France) and characterized in Dague, Pinheiro, Verheij, Poissemiers, and Marzorati (2016)). Fibers and herbal extract were chosen from a previous experiment in which we tested antioxidant capacity and SCFA production. These experiments were carried out with fibers and herbal extracts alone, without salami, and in the amounts that they were going to be added to salami. Results are shown in Supplemental Tables 1 and 2.

2.3. Salami preparation and formulation

Each salami sample was formulated according to the following traditional recipe: a mixture of pork meat and fatty tissues was combined with salt, nitrate (curing agent), sugars, black pepper, starch/fiber, and herbal extract.

For the salami control, 2% w/w of starch was added, and a standard starter composed of non-probiotic strains of lactic acid bacteria and catalase-negative streptococci was applied. Fiber-added salamis were supplemented with a probiotic Lactobacillus rhamnosus as a starter culture, and appropriate fiber instead of starch was added. Fibers were added in a 2% w/w ratio. An antioxidant herbal extract, composed of equal proportions of lemongrass and rosemary extracts (acquired at a local supermarket), was also incorporated as an ingredient in one salami sample (0.2% w/w), which is the usual proportion for seasoning in sausages and the one used by the company who provided the samples in their other commercial products.

Each formulated salami mix was put into casings and subjected to a ripening-drying process for 40 days. Sampling was done at the end of the ripening process. Each formulation was manufactured in triplicate. Once in the laboratory, and after the analysis of composition (to make sure the replicates had the same composition), the replicates were mixed together. Samples were homogenized and stored at −80 °C until further analysis. All the analyses were carried out in triplicate.

2.4. In vitro gastrointestinal digestion

All samples were subjected to an in vitro digestion process followed by an in vitro fermentation to mimic physiological processes in the human gut. The in vitro digestion method was carried out according to the protocol described by Pérez-Burillo, Rufián-Henares, and Pastoriza (2018b). The solid residue obtained after gastro-intestinal digestion was subjected to in vitro fermentation.

2.5. In vitro gut microbial fermentation

The in vitro fermentation was carried out according to the protocol described by Pérez-Burillo et al. (2018b). In vitro fermentation was carried out using faecal samples from three healthy donors (not taking antibiotics and with mean Body Mass Index = 21.3) that were pooled together to reduce inter-individual variability. As a control, a separate fermentation was performed using only the fecal fermentation mixture without any salami digests or products (called fermentation fluid or FF). In vitro fermentation was carried out at 37 °C for 24 h. The fermentation medium was composed of peptone, cysteine, and resazurin.

After in vitro gastrointestinal digestion and in vitro fermentation, three different fractions were obtained: digestion supernatant (fraction available for absorption at the small intestine), fermentation supernatant (fraction available for absorption at the large intestine) and fermentation solid residue (fraction not available for absorption and excreted with feces).

2.6. Antioxidant assays

Antioxidant capacity was measured in three fractions of each salami sample: (i) supernatant obtained after in vitro gastrointestinal digestion representing the fraction available for absorption in the small intestine; (ii) soluble phase (supernatant) obtained after in vitro microbial fermentation representing the fraction available for absorption in the large intestine; and (iii) solid residue that is left after in vitro fermentation representing the non-absorbable fraction that is excreted. The sum of the three fractions represents the total antioxidant capacity of each sample.

Antioxidant capacity was determined using five different methods:

- TEAC<sub>ABTS</sub> assay (Trolox equivalent antioxidant capacity against
ABTS \(^+\) radicals). It measures the scavenging capacity of samples against the artificial radical ABTS \(^+\). The antioxidant capacity was estimated in terms of radical scavenging activity following the procedure described by Re et al. (1999).

- **TEAC\(_{FRAP}\) assay (Troxol equivalent antioxidant capacity referred to reducing capacity).** It measures the reducing capacity of samples. The ferric reducing ability of each sample was estimated according to the procedure described by Benzie and Strain (1996) and adapted to a microplate reader (FLUOStar Omega, BMG Labtech, Germany).

- **TEAC\(_{OH}\) method (Troxol equivalent antioxidant capacity against hydroxyl radicals).** The principle underlying this method is to determine the scavenging activity against hydroxyl (OH) radicals using carmine indigo as indicator, at physiological pH (7.4) (Pérez-Burillo, Rufián-Henares, & Pastoriza, 2018a).

- **TEAC\(_{AAPH}\) method (Troxol equivalent antioxidant capacity against AAPH radicals).** The method was performed to analyze scavenging activity against AAPH radicals by using indigo carmine as indicator, at physiological pH (7.24) (Pérez-Burillo et al., 2018a).

- **GEAC\(_{RGP}\) method (Gallic acid equivalents antioxidant capacity referred to reducing capacity).** This method allowed the analysis of the global reducing capacity of the samples at physiological pH (7.24) (Pérez-Burillo et al., 2018a).

- **Solid residue antioxidant capacity.** Antioxidant capacity of the solid residue was determined following the “QUENCHER” procedure described by Gökmen, Serpen, and Fogliano (2009). It was carried out using the five antioxidant methods described above.

2.7. Short chain fatty acids determination

The production of SCFAs as a measure of the gut microbiota functionality was assessed according to the procedure described in Panzella et al. (2017). The analysis of SCFAs was carried out on Accela 600 HPLC (Thermo Scientific).

2.8. High-throughput amplicon sequencing

Bacterial genomic DNA was isolated from each fermented sample using ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research) as we did previously (Rigsbee, Agans, Foy, & Palay, 2011). Genomic DNA was amplified using two pairs of primers, one targeting 16S rDNA V1–V2 region [forward primer 16S gene complementary sequence AGRTGYATMYTGTCAG TACG and reverse primer 16S gene complementary sequence GCCWGCCWCCGTAGGGWT, and another targeting V4 region [forward GCCAGCMCGCGGG and reverse GGACTACHVGGGTWTCTA AAT complementary sequences, respectively]. Two different regions were interrogated to reduce biases in community composition estimates associated with the use of any one region of 16S rDNA gene (Liu, DeSantis, Andersen, & Knight, 2008). Forward primers also contained Ion Torrent P1 adapter sequence and 6-nucleotide barcode. PCR amplification was performed with 25 ng of starting DNA material and included 10 cycles of linear elongation with only the forward primers used, followed by 25 cycles of traditional exponential PCR (Palay & Foy, 2011). Inclusion of linear PCR step decreased the stochasticity of the first few PCR reaction steps and allowed the use of a single PCR amplification reaction per sample (Rigsbee et al., 2011). Purified amplimers were pooled equimolarly and sequencing libraries were prepared with Ion PGM Template OT2 400 kit (Life Technologies, Inc.) according to the manufacturer’s protocol. High throughput sequencing was performed on Ion Torrent PGM using Ion PGM Sequencing 400 kit and Ion 316 chip. We obtained an average of 12,889 sequence reads per sample. Sequence reads were processed in QIME (Caporaso et al., 2010). Sequence read counts for each OTU were adjusted by dividing them by known or predicted number of 16S rDNA gene copies in that organism’s genome following a previously described approach (Rigsbee et al., 2011). Thus derived cell counts were sub-sampled (rarefied) to the lowest value among all samples. The cell counts obtained independently for each sample based on the sequencing of V1–V2 and V4 16S rRNA gene regions were merged together into a single taxon abundance estimate via \(A_{\text{CUM}} = \sqrt{(A_{\text{V1-V2}} + A_{\text{V4}})^2} \) calculation, where \(A\) is an abundance value for each taxon.

To compare microbial community structures, unconstrained principal coordinates analysis (PCoA) utilizing phylogenetic weighted UniFrac distance as a measure of sample dissimilarity was performed on the genus-level microbial abundance dataset (Palyi & Shankar, 2016).

2.9. Statistical analyses

Statistical significance of the data and differences among samples were tested by Student’s t-test at \(\alpha = 0.05\) significance level. Evaluation of the relationship among different assays was carried out by computing the Pearson correlation coefficient. These statistical analyses were performed using Statgraphics Plus software (Statpoint Technologies, Inc., The plains, USA, version 5.1, 2001).

3. Results and discussion

3.1. Antioxidant capacity

3.1.1. Antioxidant capacity of the gastrointestinal digestion supernatant

Antioxidant capacity values are shown in Table 1. Adding fiber increased the antioxidant capacity, and addition of citrus fiber plus herbal extract had the highest effect. Overall, antioxidant capacity increased 30% on average due to the addition of fiber. However, this increase was not always statistically significant. Only in the case of citrus fiber plus herbal extract values were, for all five methods, significantly higher than that for the control sample. Citrus fiber salami was also significantly higher than the control in TEAC\(_{FRAP}\), TEAC\(_{RGP}\), and TEAC\(_{OH}\). Salami with inulin and arabinoxylan was only significantly higher in antioxidant capacity based on the TEAC\(_{FRAP}\) method.

Most of the antioxidant capacity generated during gastrointestinal digestion could come from peptides released by protein hydrolysis such as the dipeptides carnosine and anserine, which have been reported as effective hydrophilic antioxidants (Martinez et al., 2014). Gullon et al. (2015) also described an increase in the antioxidant capacity of fiber rich samples after gastrointestinal digestion. As explained by those authors, low pH could prompt structural changes in components such as fiber, but can also help release other compounds trapped in the fiber matrix. Moreover, structural changes during digestion could lead to the increased exposure of functional groups able to scavenge for oxidant species (Panzella et al., 2011).

Citrus fiber is mainly composed of pectin, which is a highly branched polysaccharide rich in galacturonic acid and other sugars. Our results are in accordance with previous literature, in which strong antioxidant capacity has been revealed for polysaccharides containing galacturonic acid (H. Wang et al., 2018; Yao et al., 2018).

Salami with added herbal extract also tended to possess high antioxidant capacity. The herbal extract we used was composed of lemon grass and rosemary. It has previously been reported that lemon grass has a high antioxidant capacity and is rich in phenolic compounds such as gallic acid, quercetin, isoquercetin, rutin or tannic acid (Somporn, Saenthaweewu, Naowaboot, Thaeomor, & Kukongviriyapan, 2018). Rosemary is also rich in polyphenols such as flavonoids or tannins possessing high antioxidant capacity (Y.-Z. Wang et al., 2018).

3.1.2. Antioxidant capacity of the fermentation supernatant

Antioxidant capacities of fermented samples are summarized in Table 1. In vitro fermentation also increased antioxidant capacity of salami samples with added fiber. However, in this case, differences with
Table 1
Antioxidant capacity values obtained for gastrointestinal digestion fraction, gut microbiota fermentation fraction, solid residue fraction, and global antioxidant capacity.

<table>
<thead>
<tr>
<th></th>
<th>TEAC&lt;sub&gt;GSH&lt;/sub&gt; mmol Trolox equivalents/kg</th>
<th>TEAC&lt;sub&gt;GABTS&lt;/sub&gt; mmol Trolox equivalents/kg</th>
<th>TEAC&lt;sub&gt;OH&lt;/sub&gt; mmol Trolox equivalents/kg</th>
<th>TEAC&lt;sub&gt;AAPH&lt;/sub&gt; mmol Trolox equivalents/kg</th>
<th>GEE&lt;sub&gt;ARED&lt;/sub&gt; mmol Gallic acid equivalents/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastro-intestinal supernatant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salami control</td>
<td>10.03 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.56 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.23 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.03 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.98 ± 1.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inulin salami</td>
<td>15.26 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.43 ± 3.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.89 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.59 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.23 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citrus fiber salami</td>
<td>16.23 ± 2.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.87 ± 1.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.01 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.56 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.26 ± 2.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citrus fiber + herbal extract salami</td>
<td>18.26 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.78 ± 3.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.13 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.56 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.56 ± 2.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acacia-fiber salami</td>
<td>17.89 ± 3.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.26 ± 2.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.56 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.16 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.23 ± 1.56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Microbial fermentation supernatant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salami control</td>
<td>35.69 ± 2.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>51.56 ± 2.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.37 ± 1.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.74 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.78 ± 2.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inulin salami</td>
<td>55.36 ± 3.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>44.78 ± 3.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.36 ± 0.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.29 ± 0.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>53.69 ± 4.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citrus fiber salami</td>
<td>81.23 ± 6.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>108.23 ± 6.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.68 ± 2.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.21 ± 2.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.95 ± 4.86&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citrus fiber + herbal extract salami</td>
<td>83.69 ± 7.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>115.45 ± 9.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.23 ± 3.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.26 ± 1.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.23 ± 7.66&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acacia-fiber salami</td>
<td>54.36 ± 2.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55.12 ± 2.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.56 ± 1.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.31 ± 1.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.24 ± 5.89&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Microbial fermentation solid residue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salami control</td>
<td>2.01 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.91 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inulin salami</td>
<td>3.05 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.69 ± 0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.32 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.25 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citrus fiber salami</td>
<td>3.25 ± 0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.97 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.51 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.05 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citrus fiber + herbal extract salami</td>
<td>3.65 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.36 ± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.91 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.91 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acacia-fiber salami</td>
<td>3.58 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.45 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Total antioxidant capacity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salami control</td>
<td>47.73 ± 2.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65.45 ± 4.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.85 ± 1.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.18 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.76 ± 3.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inulin salami</td>
<td>73.67 ± 4.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.51 ± 3.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.63 ± 3.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.20 ± 0.49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.17 ± 5.52&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citrus fiber salami</td>
<td>100.71 ± 8.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>124.33 ± 10.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.12 ± 2.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.28 ± 3.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.76 ± 6.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citrus fiber + herbal extract salami</td>
<td>105.60 ± 7.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>132.47 ± 9.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.79 ± 6.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.73 ± 2.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>108.70 ± 7.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acacia-fiber salami</td>
<td>75.83 ± 5.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>69.72 ± 5.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.43 ± 3.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.90 ± 3.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.92 ± 4.73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters within the same column and fraction indicate statistical significance ($p < 0.05$).
the salami control were noticeably larger than those observed after digestion step. TEACFRAP method showed significantly higher values in all samples with added fiber in comparison with salami control. The TEACOH differences were similar to those found for TEACABTS, with values significantly higher in both citrus fiber salamis. TEACAPH method also revealed significantly higher values in all salamis with fiber in comparison with the control. Finally, GEACRED showed the same tendency as that revealed by the TEACFRAP and TEACAPH methods (see Table 1).

In vitro colonic fermentation seems to have a more marked effect on antioxidant capacity than in vitro gastrointestinal digestion due to microbial metabolism of available substrates. The higher values found in salami with added fibers in comparison to the standard formulation are likely the result of the microbial degradation of the added poly saccharides. This has previously been reported for other fiber-rich samples (Zamora-Gasga et al., 2015). Further, a common finding among the five antioxidant testing methods we used in this study was the higher antioxidant capacity found in salami with added citrus fiber. This finding could be due to fiber composition (pectin) yielding a different profile of metabolites with more antioxidant potential during microbial degradation. Another possible explanation is the source of citrus fiber. This fiber is extracted from citrus fruits, and other bioactive compounds could remain trapped inside the fiber structure and thus can be released upon digestion or fermentation (Pérez-Burillo et al., 2018b).

Finally, salami with added herbal extract had the highest antioxidant capacity measured by all five testing methods. Bioactive compounds coming from both lemon grass and rosemary could be the reason behind such high antioxidant capacity. Their metabolism by gut microbiota could yield simpler phenolic acids that contribute to the antioxidant activity (Selma, Espín, & Tomás-Barberán, 2009). According to our results, these new compounds would exert radical scavenging activity and reducing power.

3.1.3. Antioxidant capacity of the solid residue

The measurement of antioxidant capacity in the solid residue is important for several reasons (Gökmen et al., 2009; Pérez-Burillo et al., 2018b):

- Even after digestion and fermentation, there could be low molecular weight compounds still physically trapped within large macromolecules that resist digestion and fermentation.
- Other compounds that are chemically bound to macromolecules are also resistant to digestion and fermentation processes.
- The solid residue represents the antioxidant capacity exerted by large macromolecules that are not degraded by digestion or fermentation.

The antioxidant capacity values obtained for the solid residues are shown in Table 1. As previously stated for the other two fractions, the antioxidant capacity tended to be higher in the samples with added fiber in comparison with the control salami. Therefore, the solid residues retained some antioxidant power, although the values were significantly lower than the antioxidant capacity of the soluble fractions.

3.1.4. Total antioxidant capacity

Total antioxidant capacity of each salami sample is depicted in Fig. 1. The total antioxidant capacity was higher in salamis with fiber. In samples with citrus fiber alone or also supplemented with the herbal extract, the antioxidant capacity was significantly higher than in the rest of the samples. Overall, as result of adding fiber to salami formulation, the antioxidant capacity increased by 43% on average. The supernatant fraction obtained after sample fermentation was the main contributor to the global antioxidant capacity for all the samples (Fig. 2). Interestingly, contribution of the fermentation supernatant was higher in samples with added fiber, which reinforces the idea that fermentation of fiber by human gut microbiota can yield antioxidant compounds. Therefore, dietary fiber could be used as a good prebiotic supplement to increase the antioxidant capacity of a given food.

3.2. Analysis of short chain fatty acids

We analyzed SCFAs production after fermentation of salami samples (Fig. 3). Acetate production was significantly higher in all samples with added fiber. Salami with citrus fiber showed significantly higher acetate production than the other fiber-containing preparations (p < 0.05). Propionate production was also significantly higher in salamis containing fiber (p < 0.05), though the differences with the control sample were not as drastic as with acetate (see Fig. 3). Similarly, levels of butyrate were higher in all samples with added fiber. Overall, adding fiber to salami formulation increased SCFAs production: acetate, propionate and butyrate production increased on average by 66%, 20% and 58%, respectively. Citrus fiber showed higher potential for generating additional acetate compared with inulin and arabinogalactan, whereas propionate and butyrate levels were more similar among different fiber-supplemented salami samples (Fig. 3). Specifically, salami added with citrus fiber in addition to herbal extract (which showed the highest antioxidant potential) also had the highest potential for total SCFAs production. This sample produced significantly higher amounts of acetate, whereas in the case of propionate and butyrate, produced similar values than acacia-fiber salami.

3.3. Fermentation of salami preparations by human fecal microbiota promotes different community structures

The microbiota community structure was determined after fermentation of digested salami samples for 24 h. Overall, the community composition varied among different samples as determined by PCoA ordination analysis (Fig. 4A). Fermented salami samples containing citrus fiber (pectin is the primary digestible polysaccharide composed of galacturonic acid and other sugars) or acacia fiber (composed primarily of arabinogalactan polysaccharide) clustered together, indicating that these prebiotics promoted similar microbial community structure. Inulin, a polymer of fructose and glucose, gave rise to a noticeably different community, and all of these samples produced microbiota community organization different from the fermentation fluid control and unsupplemented salami (Fig. 4A). However, overall community diversity did not differ significantly among samples (data not shown). In most samples, Bacteroides, Bifidobacterium, Dorea, and Escherichia/Shigella were the most abundant genera (Fig. 4B). Compared to the fermentation fluid control, all salami samples had increased prevalence of Bacteroides, which is an efficient protein degrader (Macfarlane, Cummings, & Allison, 1986). Fermentation of inulin promoted Bacteroides and Parasutterella, consistent with previous findings (Sonnenburg et al., 2016; Zhang et al., 2018), whereas other fibers supported expansion of Clostridia members such as Dorea and Clostridium cluster XIVb (see Fig. 4B and C). Adding these fibers to salami also reduced the prevalence of Escherichia/Shigella genus (which is known to have many human gut pathogens) in the microbial communities.

4. Conclusions

In conclusion, in this project we studied the potential benefits of including dietary fiber and probiotic bacteria in salami dry sausage. Adding fiber to the salami increased both its antioxidant capacity and the amount of SCFAs produced during salami fermentation by human gut microbiota. While all tested fibers increased antioxidants and SCFAs
production, the effect was highest for citrus fiber. Incorporating herbal extract further elevated the beneficial properties of the salami. Majority of antioxidants were released during salami fermentation by human gut microbiota. In concert with this finding, adding fiber to salami also promoted different microbial structure compared to the traditional salami formulation. Especially noticeable was the ability of citrus fiber and acacia-fiber to reduce the prevalence of members of *Escherichia/Shigella*, a genus with many known pathogenic and toxin-producing species. We conclude that the addition of dietary fiber to dry-fermented meat products can improve their nutritional and health values, and these effects are achieved during fermentation of salami by human gut microbiota in the colon.

**Funding**

This study was supported by project AGL2014-53895-R from the Spanish Ministry of Economy and Competitiveness and by the European Regional Development Fund (FEDER) to JA Rufian-Henares, and by the National Science Foundation award DBI-1335772 to OP. It was also supported by a Spanish predoctoral fellowship F.P.U. (ref.: FPU14/01192) for S. Perez-Burillo from the Spanish Government.

**Conflicts of interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.
Fig. 4. Fermentation of salami preparations with fiber by human fecal microbiota promotes different community structures. Panel A displays the output of the unconstrained PCoA ordination analysis of microbial genus abundance dataset among all profiled samples. Phylogenetic weighted UniFrac distance was used to calculate the sample dissimilarity matrix. The percent of dataset variability explained by each principal coordinate is shown in parentheses in axis titles. Panel B shows relative abundances of the top nine most abundant microbial genera. Abundances of other genera were summed and are represented cumulatively as “other genera”. Each column represents a community derived from an independent microbiota-based fermentation of each salami preparation. Panel C displays the abundances of five select genera with varied prevalence among fermented samples. These genera were selected numerically as being at least 1.5-fold different between a sample with fiber and the control sample. Color codes and description of sample abbreviations are shown in the legends. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Acknowledgements

This paper will form part of the doctoral thesis by Sergio Pérez-Burillo, conducted within the context of the “Nutrition and Food Sciences Programme” at the University of Granada. The authors are grateful to EIPOzo Alimentación, S.A. for the test foods supplied.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jlt.2019.02.006.

References


Somporn, N., Saenaweeuk, S., Naowaboot, J., Thaeomor, A., & Rukamngiyayapan, V.


