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Enhanced production of xylooligosaccharides from vine shoots and their impact on the nutritional and technological properties of spreadable goat cheese

Graziana Difonzo [a], Claudia Antonino [a], Giusy Rita Caponio [b], Mirco Vacca [a], Federico Liuzzi [c], Isabella De Bari [c], Vito Valerio [c], Michele Faccia [a,*], Maria De Angelis [a]

[a] Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Via Amendola 165/a, 70126, Bari, Italy
[b] Department of Bioscience, Biotechnology and Environment, University of Bari Aldo Moro, Via Orabona 4, 70125, Bari, Italy
[c] ENEA, Italian National Agency for New Technologies, Energy and Sustainable Economic Development, Trisaia Research Centre, S.S. 106 Jonica, 75026, Rotondella, (MT), Italy

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ABSTRACT

In order to improve the daily intake of prebiotic fiber in the staple foods of the Mediterranean diet, the potential of including prebiotic xylooligosaccharides (XOS) in spreadable goat cheese was investigated. Preliminarily, this study aimed to obtain XOS extract from vine shoots by steam explosion. Thus, xylooligosaccharide extract (XE), also used as purified extract (XEP) were added in the goat cheese. The application of XE and XEP in the cheese, significantly changed the proximal composition, providing more than 3 g of fiber per 100 g allowing the claim "source of fiber" according with the EU Regulation 1924/2006, reducing further the predicted glycemic index. The purification treatment of the extract highlighted the potential of XEP in promoting a healthier gut microbiota by stimulating the growth of *Bifidobacterium* and lactic acid bacteria. Structure analysis and volatile profile showed a great similarity between the samples added with XEP and the control cheese.

1. Introduction

The consumers' lifestyle is increasingly as the result of the combination of a healthy diet with physical activity, two habits that can limit the risk of chronic disease onset such as obesity, hypertension, cardiovascular diseases, osteoporosis, type II diabetes, and cancers (Li et al., 2020). Even functional foods have been shown to have beneficial effects on consumers (Plasek, Lakner, Kasza, & Temesi, 2020) making them optimal dietary choice for promoting a healthy lifestyle. Therefore, food companies are also geared on developing functional products to meet the growing global demand for health-promoting foods. Dairy products are among the most consumed foods in daily life and are a valuable source of proteins, lipids, vitamins (vitamin A, B_2, and B_{12}), and minerals (Comerford et al., 2021). In the dairy sector, cow milk is the most used in cheese making (Bittante et al., 2022) but, in the last decades, thanks to the proven ability to produce functional foods compared to cow's milk, goat's milk has received increasing attention. This milk has shown some nutritional advantages, such as a greater presence of taurine, short and medium fatty acids chain, minerals such as iron and potassium, and greater digestibility (Lad, Aparnathi, Mehta, & Velpula, 2017). No less important is the technological potential in cheese production, because with goat's milk have been obtained cheeses with excellent characteristics of consistency, color, viscosity, and melting properties (Bittante et al., 2022; Boukria et al., 2020). The ongoing challenge to achieve health by diet is to increase daily fiber consumption, because it widely demonstrated the effectiveness in reducing the risk of diseases, such as obesity, coronary heart disease, diabetes, gastrointestinal disorders, inflammatory bowel diseases, and colon cancer (Barber, Kabisch, Pfei, & Weickert, 2020). Among the non-digestible oligosaccharides are xylooligosaccharides (XOS), which are derived from lignocellulosic materials and contain two to ten xylose molecules bound by β 1–4 bonds (Corim Marim & Gabardo, 2021). These compounds present prebiotic activity, since it is known as they selectively support the homeostasis of the intestinal microbiota stimulating the growth of *Bifidobacterium* and *Lactobacillus*, as well as generating benefits for human health and well-being (Samanta et al., 2015). The positive effects of ingesting XOS also include reducing blood cholesterol and the incidence of colorectal cancer; reducing disorders such as Crohn's disease; increasing the

* Corresponding author. Università degli Studi di Bari Aldo Moro, Via Amendola 165/a, I-70126, Bari, Italy.
E-mail address: michele.faccia@uniba.it (M. Faccia).

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concentration of short-chain fatty acids (SCFAs); and stimulating the absorption of calcium and minerals. They also can reduce glycated hemoglobin levels, as well as low-density lipoprotein (LDL) levels and apolipoprotein B, thus reflecting a positive effect on obesity and diabetes mellitus type 2. Moreover, the sweetening power and low caloric value of XOS are worthy of note (Corim Marim & Gabardo, 2021). Numerous studies have shown the richness of functional compounds of the winery by-product industry. Vine shoots are waste of the wine industry that are generated as a result of the winter pruning of the vine (Guerrero et al., 2016). From a circular economy perspective, using by-products into novel food formulations represents the culmination of a design circle that aims to boost the financial value and lessen the environmental effect of supply chains (Difonzo, Grassi, & Paciulli, 2022). The use of vine shoots for XOS extraction means to add more value to the raw material through a valorization process, precisely because the vine shoots are currently not reused in any way but disposed of in compost or green waste collection centers (Aschemann-Witzel et al., 2023).

Being made of lignocellulosic material, this biomass can be used for the extraction of prebiotic oligomers as XOS. A low-cost and environmentally more acceptable treatment process to obtain prebiotic oligomers is the steam explosion technique. The biomass is treated with hot steam (160–260 °C) and pressure (0.69–4.83 MPa), followed by explosive decompression at atmospheric pressure, which causes the rigid structure of the biomass fibers to break down. The pressure generates a shearing force that breaks the glycosidic bond and hydrogen bonds, resulting in two fractions, one rich in sugars derived from hemicellulose and the other rich in cellulose and lignin (Hoang et al., 2023). At the same time, the hydrolysis of the lignocellulosic fraction is accompanied by the release of compounds potentially inhibiting microbial growth under a concentration-dependent manner. From the degradation of furans and deacetylation of the biomass, aliphatic acids such as acetic and formic acid are obtained; furfural and 5-hydroxymethylfurfural (HMF) are derived from the dehydration of pentose and hexose sugars; phenolic and aromatic compounds, on the other hand, are derived from lignin (Fernández-Sandoval et al., 2023). The low molecular weight of phenolic compounds allows them to penetrate the cell membrane and cause internal damage by altering cell replication, sugar metabolism, enzyme activity and cell integrity (Kim, 2018). Similarly, furan compounds penetrate the bacterial cell by inhibiting enzymes, reducing NADH/NADPH, and creating DNA damage. Moreover, the action of aliphatic acids on the reduction of the intracellular pH allows the accumulation of anions in the cell and causes the reduction of ATP production (Fernández-Sandoval et al., 2023). Therefore, the use of anionic and cationic resins is an effective strategy for the purification of lignocellulosic extracts that based its effectiveness onto a selective retention of furan and phenolic compounds while allowing XOS to be recovered (Cebreiros et al., 2021; Li et al., 2018).

Thus, by starting with vine shoots and combining steam explosion and purification with resins, it is possible to obtain a pure XOS extract with prebiotic power. With this strategy, the growing global demand for prebiotics and functional ingredients is followed by research into different efficient production methods in the food industry. The available literature highlights the stimulation of the growth of healthy gut bacteria by XOS increases the production of SCFA, which in turn reduces luminal and faecal pH, helping to inhibit the growth of pathogenic bacteria (Palaniappan, Antony, & Emmambux, 2021). In particular, compared to other prebiotics, XOS showed high resistance to digestion in the upper gastrointestinal tract, better ability to stimulate the growth of *Bifidobacteria* and *Lactobacillus,* and the production of SCFA and lactate to a greater extent. XOS has also been shown to inhibit pathogenic microorganisms such as *Enterococcus* spp., *Escherichia coli*, *Clostridium difficile,* and *Clostridium perfringens* (Crittenden et al., 2002), and prevent the adhesion of *Listeria monocytogenes* to the intestinal epithelium (Preechakun, Pongchaiphol, Raita, Champreda, & Laosiripojana, 2022). Furthermore, the inclusion of prebiotic components in dairy products has been shown to enhance the products' anti-diabetic and anti-hypertensive features, improve immunity, and increase the blood lipid profile and gut flora (Rosa et al., 2021). Studies on the application of XOS in cheese are currently few, even though the effects of prebiotics in dairy products have been extensively investigated. Particularly, the available literature does not currently address the application of XOS, derived from vine shoots, and obtained using the steam explosion, in goat cheese production. Ferrão et al. (2018) tested the addition of commercial XOS (3.3 g/100 g), derived by enzymatic hydrolysis, in requeijão cremoso, obtained from cow's milk. The sample showed better physicochemical and sensory characteristics, an increase in sweet and acid flavors, greater elasticity, consistency, homogeneity of the product, and a decrease in bitterness. However, Li, Ding, and Zhao (2019) produced yogurt from milk fortified with 3–5 g/kg of commercial XOS. Results indicated that the addition of XOS had no effect on physico-chemical characteristics and fermentation of the yogurt, but that it could enhance consumers' health by supplying dietary fiber and increasing the populations of lactic acid bacteria strains.

Thus, studying the potential of steam explosion as a green technique to obtain XOS from vine shoots, and its subsequent purification, could stimulate the use of alternative food-grade techniques in a sustainable and circular economy. Furthermore, considering the absence of studies on the application of XOS in goat cheese, our study could provide useful results to use the goat milk in alternative was.

Based on these considerations, the objectives included the extraction of XOS from vine shoots using steam explosion and the purification of the extract, by anionic and cationic resins, from microbial growth-inhibiting compounds. Therefore, the use of extract rich in xylooligosaccharides (XE), and the same extract purified (XEP), in the formulation of spreadable goat cheese has been evaluated. In particular, the aim was to use a comprehensive approach to analyze the different effects of raw and purified XOS extract on the chemical, physical, structural, and prebiotic properties of the spreadable cheese. The study also included the possibility of applying the claim "source of fiber" (at least 3 g of fiber per 100 g of product) in accordance with the EC Regulation No. 1924/2006.

2. Material and methods

2.1. Preparation of the xylooligosaccharide extract and purification

2.1.1. Extraction by steam explosion technique

The xylooligosaccharide extract (XE) used in this study was prepared from vine shoots of different varieties grown in Locorotondo (Puglia, Italy) pretreated by the ENEA Research Center of Trisaia (Rotondella, Basilicata, Italy). The vine shoots biomass was reduced to particle sizes ranging from 1.7 to 5.6 mm and soaked in demineralized water for 10 min at a 40 g/100 mL suspension. 0.75 kg of wet biomass was pretreated in steam explosion with ENEA 10 L Staketech batch digestor at 210 °C for 5 min under a pressure of 19 bar. The steam explosion process was carried out in duplicate. After 5 min of each pre-treatment with saturated steam, the biomass was rapidly transitioned to atmospheric pressure by opening an electronic valve. This decompression promotes further breakdown and the recovery of xylan in the liquid fraction of hemicellulose as xylose and xilooligomers. The resulting product was filtered to separate residue solids from the soluble fraction, which contained soluble hemicellulose, including xilooligomers. To obtain XE, the syrup was directly freeze-dried with Lyophiliser Buchi (Switzerland, LyovaporTM L-200) at −53 °C and 1.5 mBar, and stored at −20 °C. While XEP was first purified and then freeze-dried and stored at −20 °C (Fig. 1).

2.1.2. Purification of xylooligosaccharide extract

The purification process became necessary following data from preliminary experiments (not shown) that revealed microbial growth inhibition, probably due to the presence of inhibitory compounds in the extract. To obtain purified xylooligosaccharide extract (XEP), the

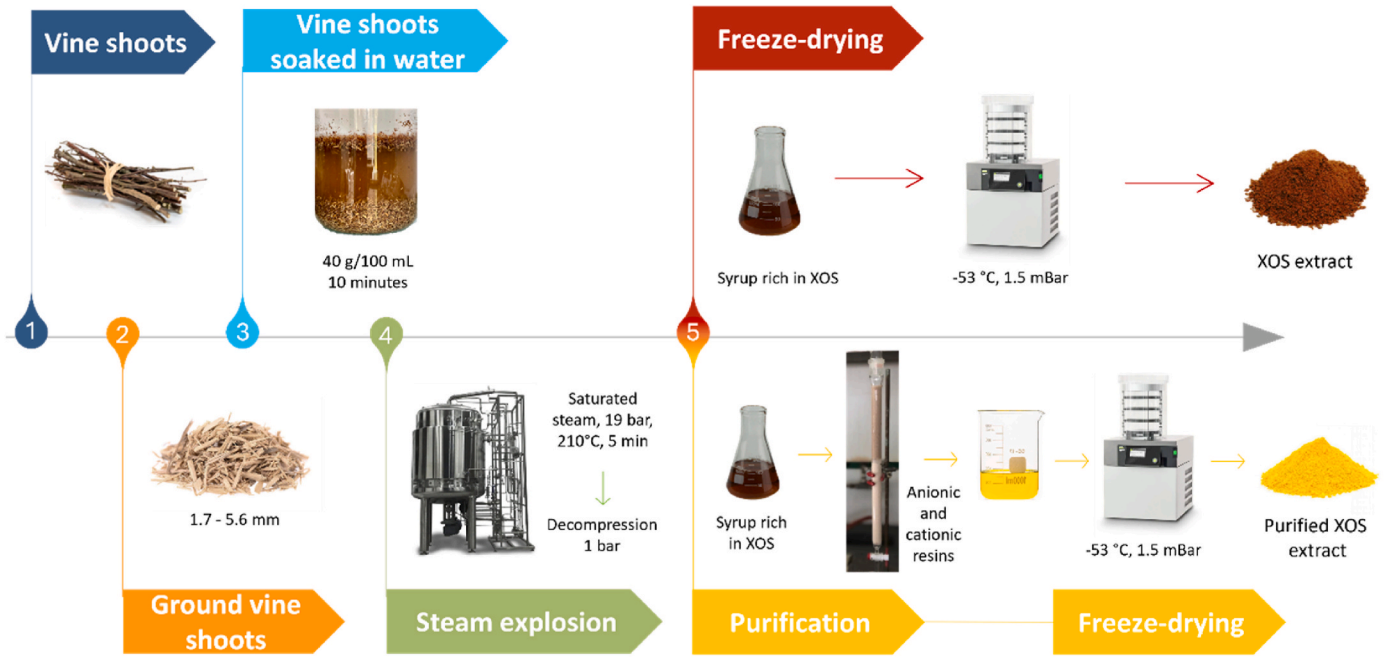

Fig. 1. Diagram of extraction by steam explosion and purification of xylooligosaccharides (XOS) from vine shoots.

purification process involved the passage of the extract through a column with both anionic and cationic resins. The sugar-containing extract underwent gravimetric elution through a burette (500 mm long, 40 mm diameter) filled with 200 mm of anionic resin Purolite® A103S plus (spherical beads of crosslinked macroporous polystyrene with divinylbenzene, containing tertiary amine functional group) and 200 mm of cationic resin Purolite® c150s (spherical beads of crosslinked macroporous polystyrene with divinylbenzene, containing sulfonic acid functional groups), separated by glass wool. The elution through cation resin is mainly aimed at the deashing. A second elution on anionic resin alone (400 mm) was performed to enhance purification from pigments.

2.1.3. Oligomers quantification

The concentrated sugar syrup components were analyzed using High-Performance Ion Chromatography (HPIC). The system employed was the DIONEX ICS2500 with a Nucleogel Ion 300 OA column operated at 60 °C. The mobile phase consisted of a 10 mL H_2SO_4 solution (0.4 mL/min). Detection was performed with a Shodex RI101 refractive index. Duplicate analyses were conducted. Carbohydrates, including glucose and xylose, were determined using the HPIC DX 300 chromatographic system (Dionex, Sunnyvale, CA, USA) equipped with a Nucleogel Ion 300 OA column (Macherey-Nagel, Düren, Germany) operating at 60 °C. The mobile phase was a 10 mL H_2SO_4 solution (0.4 mL/min). Before injection, the sample was pre-filtered with a 0.45 μm membrane, and the injected volume was 25 μL. Detection was achieved using a Shodex RI101 refractive index. The glucooligomers and the xylooligomers content were quantified (g/L) by measuring the difference after sugar acid hydrolysis in an autoclave at 120 °C and 1 bar for 45 min, utilizing 3 g/100g H_2SO_4. The glucooligomers were 28.04 g/L for XE, and 25.20 g/L for XEP, instead, xylooligomers were 34.72 g/L for XE, and 29.80 g/L for XEP.

2.2. Manufacturing the spreadable goat cheese

Raw goat milk was taken from a local farm and pasteurized at 65 °C for 30 min in a thermostatic bath. After lowering the temperature to 30 °C, a starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) and a small amount of rennet was added (75 μL/L). Coagulation occurred at 37 °C overnight, then the curd obtained was cut into fragments of about 3 cm to allow a slight whey draining and transferred into a plastic vessel. The cheese was then added with lyophilized XE or XEP (5.5 g/100 g of cheese) and homogenized; the added dose was based on the richness in XOS of the extracts, in order to obtain a cheese with 3 g of fiber per 100 g of cheese. After even distribution of XE or XEP, the cheeses were placed in hermetically sealed glass containers, pasteurized at 65 °C for 30 min, and stored at 4 °C. Three formulations were made, as follows: control spreadable goat cheese (CC), spreadable goat cheese with XE (CX) and spreadable goat cheese with XEP (CXP).

2.3. Total phenolic content (TPC) and antioxidant activity

For the TPC and antioxidant activity the samples were prepared as follows: XE and XEP (0.05 g) was resuspended in 10 mL of H_2O Milli-Q. The resultant was used for the analysis. The phenolic extraction from CC, CX, and CXP was carried out following the procedure reported by Caponio, Difonzo, Calasso, Cosmai, and De Angelis (2019) with some modifications. To remove lipid, an amount of 4 mL of *n*-hexane was added to 5 g of sample and 10 mL of methanol:H_2O (80:20 v/v). After stirring at 800 rpm for 15 min (AREX heating magnetic stirrer, Velp Scientifica SRL), samples were sonicated (Ultrasonic cleaner CP104, EIA) for 15 min and then stirred again for 15 min. Finally, the samples were centrifuged at 10,000×*g*/min for 10 min at 24 °C (Thermo Fisher Scientific, Osterodeam Harz, Germany). One further extraction was performed on the particle, and the supernatant was collected. The two hydroalcoholic extracts were mixed and centrifugated at 10,000×*g*/min for 2 min at 24 °C. Then, the hydroalcoholic phases were filtered through a nylon filter (pore diameter 0.45 μm, Sigma, Ireland) within an amber vial.

The Folin-Ciocalteu technique was used to calculate the TPC. 980 μL of H_2O Milli-Q and 20 μL of the sample were added to 100 μL of Folin-Ciocalteu reagent. After 3 min, 800 μL of Na_2CO_3 were added, and the sample was left in the dark for 60 min. Using the Cary 60 UV–Vis from Agilent Technologies (Mulgrave, Australia), the absorbance was measured at 720 nm. Gallic acid equivalents (GAE) per gram of dry weight sample (mg GAE/g dw) were used to express the results. Each sample was analyzed in triplicate.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed on

the 50 μL of each sample that was combined with 950 μL DPPH solution (Difonzo et al., 2018). The decrease in absorbance was read at 517 nm using a Cary 60 UV–Vis spectrophotometer (Agilent, Cernusco, Milan, Italy). The results were expressed in μmol Trolox equivalents/grams of dry weight for all samples (μmol TE/g dw). All determinations were carried out in triplicate. Antioxidant activity was also determined by the ABTS-TEAC assay. 950 μL of the ABTS solution was mixed with 50 μL of each sample. After 8 min, the decrease in absorbance was measured at 734 nm, using a Cary 60 UV–Vis spectrophotometer (Agilent, Cernusco, Milan, Italy). The results were expressed in μmol TE/g of dry weight sample (μmol TE/g dw). Each sample was analyzed in triplicate.

2.4. Proximate composition

Protein, ash, lipids, and total fiber content were determined using the AOAC methods 979.09, 923.03, 945.38, and 991.43, respectively. All reagents were from Carlo Erba (Milan, Italy), and the equipment was from VELP Scientifica (Usmate, Italy). Protein was determined by the Kjeldahl method. In brief, the method involved an initial digestion step of 0.5 g of sample with a Kjeldahl catalyst tablet, copper oxide, and 25 ml of H_2SO_4 for 4 h until reaching 400 °C. The nitrogen in the form of ammonia (NH_3) present in the samples was then distilled and fixed with 50 ml of boric acid solution (4 g/L). The sample was then titrated with an aqueous solution of HCl (8.33 g/L) until the Kjeldahl indicator was neutralized. The total protein content (g/100g) was calculated by multiplying the nitrogen content by a conversion factor of 6.25.

Cheese fat content was determined by the Soxhlet equipment. Once 5 g of sample were weighed, diethyl ether (85 mL) was used as the fat extraction solvent. The extracted fats were weighed and expressed in g per 100 g cheese. The official method 991.43 (AOAC, 2006) was used to quantify total dietary fiber. As defined in the method, the sample was first degrassed using the Soxhlet method described above, and then 2 g of sample was subjected to the digestion step using 50 μL of α-amylase at 100 °C for 15 min. Next, 100 μL of protease was added and left to act at 60 °C for 30 min. Finally, 300 μL of amyloglucosidase (60 °C for 30 min) was used to finish the digestion step. The digestate was then filtered using the filtration system. The last step in quantifying fiber was to use a part of the filtrate to determine protein using the Kjeldahl method mentioned above, and a part incinerated. Ash was determined by placing the sample in a muffle furnace at 550° for 5 h.

A moisture analyzer (Model MAC 110/NP, Radwag Wagi Elektroniczne, Poland) was used to measure the moisture content in 1 g of sample, at 105 °C until the weight remained constant. The pH was measured with a pH-meter (HANNA Instruments, Woonsocket, RI, USA). In accordance with the manufacturer's instructions, the water activity (a_w) of samples was measured using the Aqua Lab 4 TE water activity meter (Meter Group Inc., Pullman, WA, USA). The analyses were carried out in triplicate.

2.5. Color evaluation

The CM-600d colorimeter (Konica Minolta, Tokyo, Japan) and SpectraMagic NX software were used for the color analysis. The sample (20 g) was placed in a transparent quartz container for analysis. This container was placed on the colorimeter light source, allowing the entire light rays to pass through the sample and determine the parameters. Brightness (L*), red index (a*), and yellow index (b*) are considered in accordance with the International Commission on Illumination (CIE). The samples were analyzed in triplicate.

2.6. Texture analysis

Back extrusion was determined according to De Angelis, Squeo, Pasqualone, and Summo (2022) with some modifications. Was used Z1.0 TN texture analyzer (Zwick Roell, Ulm, Germany) equipped with a 40 mm diameter compression disc and a 50 mm diameter standard rear extrusion container, filled with 80 g of sample. The analysis is based on a double compression cycle at 1 mm/s up to 50% compression, was achieved with 50 N load cell. Analyses were performed in triplicate and the samples had constant temperature at 5 °C. The indices were: the "Firmness" (N), which is the maximum positive force registered during the first compression; the "Cohesiveness" (N), which is the maximum negative force recorded between the two compression cycles; the "Consistency" (mJ), which is the positive area under the curve registered during the first compression; the "Viscosity Index" (mJ), which is the negative area of the graph, represented by the sample remaining on the surface of the compression disc when the probe returns to its initial position, synonymous with the resistance of the cheese to flow.

2.6.1. Volatile organic compounds (VOCs)

The volatile compounds of samples were analyzed by solid-phase headspace microextraction (HS-SPME), coupled with a gas chromatography/mass spectrometry (GC-MS) as reported by Difonzo et al. (2018). In detail, 0.5 g of sample was weighed in 200 ml vials, and 100 μL of 1-propanol (Sigma-Aldrich, Milano, Italy) was added as an internal standard. 4 mL of NaCl aqueous solution (200 g/L) was added to each vial. The vials were closed with aluminum caps with a butyl rubber septum. The separation of volatile compounds was carried out using an Agilent 6850 gas chromatograph fitted with an Agilent 5975 mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) and an HP-Innowax polar capillary column (60 m length x 0.25 mm i.d. x 0.25 μm film thickness). The volatile substances were extracted by exposing a 75 μm SPME fiber of carboxen/polydimethylsiloxane (CAR/PDMS) (Supelco, Bellefonte, PA, USA) in the headspace of the vials at 40 °C for 50 min, then, they were desorbed for 6 min into the injection port of the gas chromatograph, operating in split-less mode at 230 °C for 3.5 min. At the injector temperature of 250 °C, the volatile compounds were separated using helium as the carrier gas at a flow rate of 1.5 mL/min. The oven temperature was held for 5 min at 35 °C, then increased by 5 °C min until it reached 50 °C. This temperature was maintained at this level for 5 min, then raised to 210 °C at a rate of 5.5 °C per minute, and then maintained at this level for an additional 5 min. The mass detector was configured with the following parameters: scanning range 33–260 Amu, ionization energy 70 eV, interface temperature 230 °C, and source temperature 230 °C. Volatile compounds were quantified by standardizing the peak area of 1-propanol the internal standard with the peak areas of the compounds of interest. The analyses were performed in triplicate.

2.7. Predicted glycemic index

The predicted glycemic index (pGI) of cheeses was determined according to simulating the *in vivo* starch digestion (Pontonio et al., 2022) with some modifications. The analysis of pGI was carried out by the *in vitro* analysis, consisting in salivary amylase, pepsin, and α-amylase enzymatic treatments, mimicking the *in vivo* digestion of starch (de Angelis et al., 2009). Glucose content resulting from the hydrolysis of starch and lactose was quantified using the D-Glucose Assay Kit (GOPOD Format) from Megazyme, located in Wicklow, Ireland. Subsequently, these samples underwent *in vitro* gastrointestinal digestion via an enzymatic process involving pancreatic amylase and pepsin-HCl. The released glucose content was then measured again using the same D-Glucose Assay Kit. Simulated digests were dialyzed using a membrane with a cutoff of 12,400 Da for 180 min. Samples of the dialysate, containing free glucose and partially hydrolyzed starch, were collected at 30-min intervals and treated with amyloglucosidase. Free glucose levels were determined using the afore-mentioned enzyme-based kit, and the resulting data were used to calculate the extent of starch hydrolysis in cheeses. The results were expressed as hydrolysis index (HI) equivalent considering starch contained in wheat bread as the reference product (HI = 100). The pGI was calculated using the equation: pGI = 0.549 * HI +39.71 as described by Caponio et al. (2022). Each sample was analyzed

in triplicate.

2.8. Cheese simulated colonic fermentation in vitro

To obtain the fecal medium, a NaCl solution (9 g/L) was added with specific supplements (K_2HPO_4 2 g/L, $C_2H_3NaO_2$ 5 g/L, $C_6H_{17}N_3O_7$ 2 g/L, $MgSO_4$ 0.2 g/L, $MnSO_4$ 0.05 g/L, glucose 2 g/L, inulin 4 g/L, fructo-oligosaccharides 4 g/L, Tween 80 polysorbate 1 mL/L, bacteriological peptone 5 g/L, yeast extract 5 g/L). After adjusting the pH to 7.0 with a NaOH (6 mol/L) solution, the medium was sterilized (121 °C for 20 min). Except for inulin and fructo-oligosaccharides, which were provided by Farmalabor Srl (Canosa di Puglia, Italy), all reagents were purchased by Sigma-Aldrich (St. Louis, MO, USA). As suggested by Pérez-Burillo et al. (2021), before to proceed with the viable cell microbiota inoculum, the fecal medium accounted for the addition of 0.5 g/100 ml of experimental and control cheeses. In the present study the experimental dietary component was tested as permeate and retentate sample resulting from *in vitro* starch hydrolysis assay.

The inoculum (fecal slurry) was obtained as previously detailed (Vacca et al., 2023). Hence, fresh feces were provided by one healthy volunteer who had no antibiotics or probiotics treatments in the last 3 months before sample delivery. After collection in sterile tubes, which were filled until 4/5 of the total volume, feces were processed within 1 h from sampling in bags with filter (250 μm) added with distilled water (in a final ratio of 32g/100 mL), homogenized in a lab stomacher for 3 min, centrifuged (8000 g/min, 20 min, 4 °C). Pellets from 2.5 mL of solution containing viable cells were recovered while 7 mL of fecal medium and 0.5 mL of permeate or retentate cheese-sample were added to constitute the fecal batch. Fecal batches were incubated anaerobically at 37 °C, for 24 h and 48 h, under gentle stirring conditions (150 rpm).

2.9. Viable fecal microbiota profiling

Viable fecal microbiota was profiled by plate-counts based on serial 10-fold dilutions in sterile saline (9 g/L) solution. Different culturing media were used, which were: Plate Count Agar (PCA); Wilkins-Chalgren anaerobe agar (WCAn); de Man, Rogosa and Sharpe (MRS) agar; M17 agar; Violet Red Bile Glucose Agar (VRBGA); and modified *Bifidobacterium* agar (mBifA) that allowed to assess cell densities (log_{10} CFU/mL) of total aerobes (TAMC), total anaerobes (TANMC), lactic acid bacteria (LAB – bacilli); LAB (cocci); *Enterobacteriaceae*; and fecal *Bifidobacterium*, respectively. Except for mBifA, which was purchased by Becton Dickinson GmbH (Heidelberg; Germany), all other media were purchased by Oxoid Ltd. (Basingstoke, Hampshire, England). WCAn and mBifA were incubated anaerobically, whereas others were incubated aerobically at 37 °C. The incubation time respected that defined by the related medium manufacturer. To validate data from MRS and M17 media, cells were randomly visualized with an optical microscopy.

2.10. Statistical analysis

Minitab19 (Minitab Inc., State College, PA, USA) was used for the statistical analysis of all results, reported as mean ± standard deviation (SD) of three replications. To evaluate the differences between samples, one-way ANOVA followed by Tukey's HSD test was applied.

3. Results and discussion

3.1. Total phenolic content (TPC) and antioxidant activity

Table 1 shows the results of TPC and antioxidant activity measured in XE and XEP. It is possible to highlight a correlation between antioxidant activity and the TPC in all extracts. Furthermore, the good performance of resins used for detoxification in retaining polyphenolic molecules was observed. The TPC went from 70.74 mg GAE/g dw in XE to 0.57 mg GAE/g dw in XEP. In particular, the resins retained 99.19% of total polyphenols, and reduced antioxidant activity by 99.79% considering the DPPH assay and 97.48% for the ABTS assay. Similar results were performed to Preechakun et al. (2022) who purified XOS derived from lignocellulosic biomass with macroporous adsorption resin.

Table 1
Total Phenolic Content (TPC), antioxidant activity (determined by DPPH and ABTS assay) of xylooligosaccharides extract, and purified xylooligosaccharides extract.

Sample	TPC	DPPH	ABTS
	(mg GAE/g dw)	(μmol TE/g dw)	(μmol TE/g dw)
XE	70.74 ± 0.12^a	315.50 ± 0.81^a	558.02 ± 0.72^a
XEP	0.57 ± 0.14^b	0.64 ± 0.51^b	14.01 ± 0.34^b

Results are expressed as mean ± standard deviation. Different letters in the same column indicate statistical differences according to the Tukey test ($p < 0.05$). Analysis performed in triplicate. Abbreviation: XE, xylooligosaccharides extract; XEP, purified xylooligosaccharides extract.

The incorporation of XE in the spreadable goat cheese formulation significantly affected the TPC and antioxidant activity (Table 2). Specifically, TPC value exhibited an increase in CX (2.87 mg GAE/g) compared to a minimum quantity observed in CC (0.34 mg GAE/g), which directly derived from milk. Similarly, lower values were identified in CXP (0.40 mg GAE/g), with no significant differences from the control cheese. The same trend was observed for the antioxidant activity, which was found to be significantly higher in CX than in the control cheese and CXP. A greater similarity was shown between CC and CXP, confirming the limited presence of polyphenols and antioxidant compounds in the purified extract added to the formulation.

3.2. Proximate composition

The result of the proximate composition is shown in Table 3. The addition of XOS caused a slight increase of pH (4.12 for the CC, 4.22 for CX, and 4.16 for CXP), even though the pH values are mostly attributable to the acidification by lactic acid bacteria for obtaining isoelectric coagulation. In particular, the increase in pH is due to the addition of XE and XEP as its pH value corresponds to 4.40 and 4.38, respectively. A higher pH value of the extract, therefore, influences the slight increase in the pH of the cheese. The same trend was observed by Ferrão et al. (2018) in requeijão cremoso fortified with 3.3g/100g XOS extract, with pH 6.23 in control cheese and 6.29, 6.32, and 6.36 in fortified cheeses with different XOS formulations. The pH of the cheese is very important as it directly influences the structural and rheological characteristics of the finished product. In fact, with the change in pH, protein-water interactions can be modified (Monteiro, Tavares, Kindstedt, & Gigante, 2009), making the cheese more or less compact. No significant differences in water activity were found. The addition of XOS led to about 4.59% decrease in moisture in CX, and 4.31% decrease in moisture in CXP, compared to CC, probably due to a higher syneresis during the homogenization process. In addition, non-digestible oligosaccharides such as XOS contribute to an increase in soluble solids in cheese

Table 2
Total Phenolic Content (TPC), antioxidant activity (determined by DPPH and ABTS assays) of spreadable goat cheese and functional spreadable goat cheese.

Sample	TPC	DPPH	ABTS
	(mg GAE/g dw)	(μmol TE/g dw)	(μmol TE/g dw)
CC	0.34 ± 0.03^b	0.12 ± 0.03^b	0.12 ± 0.03^c
CX	2.87 ± 0.04^a	6.82 ± 0.36^a	5.86 ± 0.44^a
CXP	0.40 ± 0.01^b	0.19 ± 0.02^b	1.06 ± 0.08^b

Results are expressed as mean ± standard deviation. Different letters in the same column indicate statistical difference according to the Tukey test ($p < 0.05$). Abbreviation: CC, spreadable goat cheese; CX, spreadable goat cheese with xylooligosaccharides extract; CXP, spreadable goat cheese with purified xylooligosaccharides extract.

Table 3
Proximate composition (g/100g) of spreadable goat cheese and functional spreadable goat cheese.

Parameters	*CC*	*CX*	*CXP*
pH	4.12 ± 0.01^{b}	4.22 ± 0.01^{a}	4.16 ± 0.01^{a}
Water activity	0.99 ± 0.01^{a}	0.99 ± 0.01^{a}	0.99 ± 0.02^{a}
Moisture	74.6 ± 0.84^{a}	71.17 ± 0.91^{b}	71.38 ± 0.78^{b}
Lipids	11.89 ± 0.26^{a}	11.40 ± 0.10^{a}	11.65 ± 0.28^{a}
Total dietary fiber	nd	3.56 ± 0.09^{a}	3.41 ± 0.11^{b}
Proteins	8.88 ± 0.04^{b}	9.42 ± 0.10^{a}	9.39 ± 0.08^{ab}
Carbohydrates	4.00 ± 0.11^{a}	3.46 ± 0.09^{b}	3.26 ± 0.20^{bc}
Ash	0.63 ± 0.03^{c}	0.99 ± 0.05^{a}	0.91 ± 0.08^{b}

Results are expressed as mean ± standard deviation. Different letters in the same line indicate statistical differences according to the Tukey test ($p < 0.05$). Analysis performed in triplicate. Abbreviation: CC, Spreadable goat cheese; CX, spreadable goat cheese with xylooligosaccharides extract; CXP, spreadable goat cheese with detoxified xylooligosaccharides extract; nd, not detected.

formulation with a consequence on product moisture. This result has also been demonstrated by the addition of numerous extracts and flours obtained from by-products which have led to an increase in soluble solids and a consequent decrease in moisture in cow spread cheeses (Lucera et al., 2018). For the lipid content, no significant differences were detected. An opposite trend occurred for proteins that were found to be greater in CX and CXP, in accord with Ferrão et al. (2018). The results of the total dietary fibers highlight the high concentration of fibers in CX and CXP, on the contrary, an absence in CC. One of the objectives of this study was the enrichment of spreadable goat cheese with XOS for the attribution of the claim "source of fiber" (at least 3 g of fiber per 100 g of product in accordance with EC Regulation No. 1924/2006). The results showed that the amount of fiber found in functional cheese was major of 3 g per 100 g of cheese. Particularly, CXP had a content of 3.41 g per 100 g of cheese. It is possible to attribute the claim "source of fiber" to the functional spreadable goat cheese. Also, Ferrão et al. (2018), adding 3.3 g of XOS in the formulation of creamy processed cheese, obtained a functional and prebiotic cheese with 3.3 g of fiber per 100 g of product. Similarly, Souza et al. (2019) added 1.25 g/100 mL of XOS in a whey beverage, thus obtaining a beverage with fibers having prebiotic effects. Thus, as dairy products are consumed daily, new formulations enriched with XOS create a scenario of innovative and functional products with constituents not naturally present in matrices of animal origin that provide numerous health benefits to the consumer. These benefits include how dietary fiber is fermented by the gut microbiota to produce SCFAs. The latter promotes the increase of peptides and glucagon, which stimulate satiety, limiting diseases such as obesity. Besides, peptides and glucagon increase insulin sensitivity and glucose homeostasis, reducing diabetes. In addition, due to the production of SCFAs from fiber, cancer cell apoptosis, and increased intestinal integrity also benefit the consumer, limiting intestinal diseases and cancer (He, Wang &Wen, 2022).

3.3. Color and texture evaluation

Table 4 shows the results obtained from the color analysis. The addition of XOS extract determined a decrease in brightness for CX and CXP. Differently, a* and b* were higher in fortified samples compared to CC. Furthermore, for all parameters and appearance (Fig. 2), the CXP sample was the closest to the control cheese. The red and yellow parameters in cheese formulation depends on the type of extract added (El-Messery, El-Said, & Farahat, 2019; Parafati et al., 2021). In this case, the purification phase enables the removal of polyphenolic, acidic and furanic fractions as well as shoot residues with dark pigments.

As shown in Table 4, the textural parameters of the different cheese formulations were influenced by the presence of XOS extracts. The sample with the lowest firmness was CX, probably in connection with increase of pH, mentioned above. The pH of the cheese directly influences the structural and rheological characteristics since the change in pH influence the protein-water interactions, making the cheese more or less compact. This trend was also confirmed by Ong et al. (2020) who showed that, as the pH of cream cheese increased, there was a decrease in firmness. In addition, the presence of lignocellulosic residues and other compounds retained during the detoxification process can influence the formation of a more or less stable protein network. Indeed, CXP has shown firmness values similar to the control cheese. This explains easier solubilization of pure XOS in the matrix and a limited impact on structural characteristics. The addition of XOS extracts also caused a decrease in cheese viscosity, consistency, and cohesiveness, in accordance with the results of Ferrão et al. (2018). Opposite results were reported by Leddomado et al. (2021), with an increase of the textural parameters in dulce de leche fortified with XOS. The protein gel that is created as a result of milk coagulation is modified by the addition of other ingredients. According to the literature, the viscosity of the diary product can be affected by both the percentage of added fibers and the size and type of particles. The decrease in particle size added to the formulation may lead to an increase in viscosity (Xue et al., 2020).

3.4. Volatile profile of CC, CX, and CXP

The analysis of the volatile compounds of CC, CX, and CXP showed the presence of 35 molecules between alcohols, ketones, aldehydes, esters, hydrocarbons, free fatty acids, and furans (Table 5). Alcohols can be formed through lactose metabolism, ketone reduction, amino acid metabolism, and fatty acid degradation (Zhang et al., 2023). 3-Methyl-1-butanol, 3-octanol, and 2-heptanol are volatile compounds typical of goat cheese with citrus, fruit, and nut flavors (Bezerra et al., 2017; Kondyli, Pappa, & Svarnas, 2016). In CX there was a tendency to decrease in alcohols compared to CC and CXP. On the contrary, phenols, derived from XE are identified only in CX. These are reflected in a greater presence of herbaceous odor in the cheese. Differently, in CXP these compounds were not found, thanks to the efficiency of the resins in retaining the phenolic compounds. Aldehydes have the same odorous notes and affect the aroma as they have a low threshold of detection. CX has a higher concentration of these compounds which are generated by reactions with amino acids or by β-oxidation of unsaturated fatty acids (Zhang et al., 2023). 2-Methyl-butanal, 3-methyl-butanal, hexanal, and nonanal are synonymous of lipid oxidation. Their concentration increases significantly in CX, but with purified extract the concentration decreases in CXP. This may indicate that the presence of XE in cheese accelerates lipid oxidation. 1H-Pyrrole-2-carboxaldehyde has been found exclusively in CX. This compound is commonly found in tea and coffee products (Dadalı, 2022; Zhan, Liu, Su, Lin, & Ni, 2023), and its

Table 4
Colorimetric properties and texture parameters of control cheese and experimental cheeses.

Sample	L*	a*	b*	Firmness	Consistency	Cohesiveness	Viscosity
CC	82.17 ± 0.04^{a}	−1.51 ± 0.01^{c}	10.47 ± 0.06^{c}	3.92 ± 0.81^{a}	28.05 ± 1.09^{a}	−1.84 ± 0.10^{c}	11.69 ± 0.91^{a}
CX	50.78 ± 0.44^{c}	7.93 ± 0.22^{a}	26.11 ± 0.51^{a}	1.74 ± 0.07^{b}	12.34 ± 1.06^{c}	−0.66 ± 0.22^{a}	5.53 ± 0.63^{b}
CXP	78.43 ± 0.03^{b}	0.18 ± 0.03^{b}	15.20 ± 0.11^{b}	3.27 ± 0.45^{a}	18.64 ± 1.20^{b}	−1.34 ± 0.24^{b}	6.67 ± 1.58^{b}

Results are expressed as mean ± standard deviation. Analysis performed in triplicate. Different letters in the same column indicate statistical difference according to the Tukey test ($p < 0.05$). Abbreviation: CC, spreadable goat cheese; CX, spreadable goat cheese with xylooligosaccharides extract; CXP, spreadable goat cheese with purified xylooligosaccharides extract.

Fig. 2. Appearance of control cheese (**A**) and cheese fortified with xylooligosaccharide extract (**B**) and purified xylooligosaccharide extract (**C**).

presence in XE may result from compounds generated during the high-temperature extraction process. It can therefore be said that the increased presence of alcohols and aldehydes may be due to lipid oxidation, a common phenomenon that leads to the formation of aldehydes in fresh cheeses, with subsequent formation of the respective alcohols by dehydrogenation, and microbial metabolism. The presence of increased microbial activity in cheese, in the presence of other ingredients like XOS extract, is possible. A different trend was found for esters where, acetic acid-ethyl ester increased in CX (8.07 μg/g in CC and 17.75 μg/g in CX) and significantly decreased in CXP (2.63 μg/g). The same trend was shown by Natrella et al. (2020) with the addition of olive leaf extract to preserve stracciatella cheese. The ketone compounds found in cheeses are generated by non-enzymatic thermal processes (Lomelí-martín, Martínez, Welti-chanes & Escobedo-avellaneda, 2021). Their presence is due to the pasteurization process to which the milk and the finished product have been subjected. 7 ketones have been identified: 2,3-Butanedione, 2,3-pentanedione, 3-hydroxy-2-butanone, 2-heptanone, 2-butanone, and 2-nonanone are increased significantly in CX. While a higher concentration of 2-pentanone was found in CC. A major presence in CX may be due to XOS e other chemical compounds present in XE, which have been extracted from the vine shoots during steam explosion. These will contribute to the flavors of hazelnut, toasted and cooked milk (Su et al., 2020). With the purification process of the extract applied in the CXP sample it was possible to highlight a significant reduction of this class of compounds. The contribution of hydrocarbons to the aroma is not high as they have high threshold values (Kondyli et al., 2016) but can contribute to the formation of other aromatic compounds by acting as precursors. They are present in the plants and their extracts (Pinto et al., 2021). These can derive from the plants that the goats eat, and be transferred into their milk used to cheesemaking, or can be linked to vine shoots from which xylooligosaccharides have been extracted. Alpha-pinene was the most abundant hydrocarbon compound in all samples. Furanic compounds have been identified exclusively in CX. These compounds are obtained as a result of heat treatments such as roasting, and cooking processes, in which non-enzymatic reactions such as caramelization and Maillard reactions take place (Seok et al., 2015). It is evident how their presence in cheese is attributed to XE. These compounds can result in the presence of toasted, caramel, and coffee odor (Angeloni et al., 2021). Free fatty acids have a great impact on the cheese's aroma and, in particular, some fatty acids such as hexanoic acid and octanoic acid have been identified as being typical of goat cheese (Bezerra et al., 2017; Kondyli et al., 2016) and confer goaty and sweaty flavor. A significant increase in acetic acid in CX is related to its high concentration in XE (1308.47 ± 1.83 μg/g), and was the most abundant volatile compound, followed by alpha-pinene. An increase in acetic acid in stracciatella enriched with olive leaf extract was found by Natrella et al. (2020).

3.5. Predicted glycemic index

The significance of the findings of the present study presented herein is also attributed to the scientific evidence related to the predicted glycemic index (pGI). As explained below, because first experiments simulating CX digestion resulted in the inhibition of microbial growth during plating, pGI analysis was conducted on CC and CXP cheeses. Hydrolysis index (HI) calculated from the rate of starch hydrolysis over time and the respective pGI are shown in Fig. 3. Compared to CXP, CC achieved the highest value of HI and pGI, 89.28 and 88.73, respectively. In contrast, CXP recorded a significant reduction in pGI and HI values, about 71.69 and 58.26, respectively. This reduction in pGI could be attributed to the enriched fiber of cheese, specifically the XOS fraction.

The pGI is a parameter used to classify foods according to their postprandial glycemic response (Goñi, Garcia-Alonso, & Saura-Calixto, 1997). Dietary fiber content is recognized as one of the most important factors in reducing pGI. Notably, the rate of starch digestion significantly influences the release and absorption of glucose during digestion (Graça, Raymundo, & Sousa, 2021). Foods with high pGI are rapidly digested and absorbed, resulting in a spike in blood glucose (Brennan, 2005). Scientific evidence confirms the positive effect of fiber-enriched foods on reducing the glycemic index, as seen in studies on low degree of polymerization-inulin pasta and bread (Difonzo, de Gennaro, et al., 2022; Scazzina, Siebenhandl-Ehn, & Pellegrini, 2013). The postprandial glycemic response following the ingestion of inulin and resistant starch is well-established. However, other soluble dietary fibers may also contribute to glycemic control, such as arabinoxylan, β-glucan, gum, and psyllium (Giuntini, Sardá, & de Menezes, 2022). As observed, the addition of XOS in cheese leads to a significant reduction in the glycemic index, resulting in a gradual increase in blood glucose and insulin levels. These findings align with existing literature demonstrating a significant reduction in the glycemic index of fiber-rich foods, particularly those containing xylans and arabinoxylans (Palaniappan et al., 2021; Reis & Abu-Ghannam, 2014). Based on the results presented, it is evident that the impact of XOS replacement in cheese is reflected in glycemic index and its hydrolysis. Therefore, a possible correlation between XOS addition and pGI was shown.

From a health perspective, reducing the glycemic index of foods is a desirable factor not only for people with diabetes but also for a wider population, as it helps prevent the incidence of chronic metabolic diseases (Augustin et al., 2015).

3.6. Viable fecal microbiota profiling after simulated colonic fermentation

The first experiments simulating the digestion of CX resulted in microbial growth inhibition upon plating supporting the potential of polyphenols to reduce microbial growth in a concentration-dependent manner (Makarewicz, Drożdż, Tarko, & Duda-Chodak, 2021) and, therefore, the subsequent purification process that XEP underwent proved useful in mitigating this risk. Contextually, the purification process may facilitate an easier utilization of XOS by the fecal microbiota when the purified extract was added to the goat cheese formulation. To confirm this, retentate (R) solution from CC and CXP was used to compare its effects on viable cells of fecal microbiota *in vitro*. Although the permeate (P)-solution was also tested (Fig. 4), we did not discuss the resulting data considering how the P consists in the adsorbable fraction

Table 5
Volatile compounds (μg/g) found in control and experimental spreadable goat cheeses.

RT	Volatile Compound	CC (μg/g)	CX (μg/g)	CXP (μg/g)
Alcohols				
20.44	3-Methyl-1-butanol	5.81 ± 0.13^{a}	4.39 ± 0.10^{c}	5.03 ± 0.07^{b}
24.21	3-Octanol	0.91 ± 0.46^{a}	1.16 ± 0.04^{a}	0.98 ± 0.24^{a}
24.69	2-Heptanol	1.40 ± 0.81^{a}	1.08 ± 0.04^{a}	1.17 ± 0.74^{a}
38.26	2,6-Dimethoxy-phenol	8.66 ± 0.33^{a}	8.03 ± 0.12^{b}	nd
41.76	Phenol	nd	4.17 ± 2.34^{a}	nd
Ketones				
7.61	2-Butanone	3.38 ± 0.16^{a}	3.98 ± 0.47^{a}	1.88 ± 0.11^{b}
9.85	2-Pentanone	8.82 ± 0.60^{a}	1.73 ± 0.25^{b}	0.19 ± 0.01^{c}
9.97	2,3-Butanedione	5.94 ± 1.03^{b}	18.4 ± 1.90^{a}	2.47 ± 0.55^{c}
12.88	2,3-Pentanedione	3.84 ± 0.12^{c}	5.96 ± 0.20^{a}	4.76 ± 0.22^{b}
17.48	2-Heptanone	6.26 ± 0.88^{a}	7.55 ± 1.42^{a}	3.74 ± 0.21^{b}
22.08	3-Hydroxy-2-butanone	14.45 ± 0.39^{c}	17.28 ± 0.35^{a}	16.29 ± 0.42^{b}
25.08	2-Nonanone	1.49 ± 0.12^{b}	2.93 ± 0.34^{a}	1.22 ± 0.08^{b}
Aldehydes				
7.92	2-Methyl-butanal	2.49 ± 0.13^{b}	5.95 ± 0.20^{a}	0.52 ± 0.03^{c}
8.05	3-Methyl-butanal	2.93 ± 0.23^{b}	13.47 ± 0.71^{a}	3.16 ± 0.47^{b}
13.62	Hexanal	6.00 ± 0.72^{b}	9.22 ± 0.72^{a}	2.83 ± 0.44^{c}
25.27	Nonanal	1.59 ± 0.10^{b}	3.94 ± 0.59^{a}	3.48 ± 0.98^{a}
42.15	1H-Pyrrole-2-carboxaldehyde	nd	9 ± 2.85^{a}	nd
Esters				
7.30	Acetic acid-ethyl ester	8.07 ± 0.27^{b}	17.75 ± 0.68^{a}	2.63 ± 0.33^{c}
16.72	3-Hydroxymandelic acid, ethyl ester, di-TMS	11.93 ± 0.76^{a}	11.31 ± 0.22^{a}	0.54 ± 0.03^{b}
Hydrocarbons				
6.53	Hexamethyl-cyclotrisiloxane	11.82 ± 1.36^{b}	19.16 ± 3.52^{a}	4.54 ± 0.49^{c}
11.22	Alpha-pinene	44.63 ± 0.48^{a}	45.24 ± 0.38^{a}	45.24 ± 0.76^{a}
11.76	Citronellol	1.53 ± 0.07^{a}	1.65 ± 0.19^{a}	1.52 ± 0.38^{a}
13.46	n-Undecane	1.70 ± 0.16^{ab}	1.89 ± 0.04^{a}	1.37 ± 0.28^{b}
14.15	Beta-pinene	5.94 ± 1.36^{a}	5.37 ± 1.55^{a}	5.40 ± 0.93^{a}
17.83	dl-Limonene	1.58 ± 0.35^{b}	2.84 ± 0.35^{a}	1.95 ± 0.06^{b}
Free fatty acids				
27.26	Acetic acid	11.58 ± 0.46^{b}	71.26 ± 14.69^{a}	12.03 ± 0.61^{b}
38.87	Hexanoic acid	22.86 ± 0.91^{b}	27.80 ± 1.01^{a}	11.33 ± 0.55^{c}
42.60	Octanoic acid	14.56 ± 1.59^{a}	16.86 ± 12.35^{a}	17.30 ± 1.06^{a}
Furans				
5.67	Furan	nd	2.63 ± 0.10	nd
6.95	2-Methyl-furan	nd	7.10 ± 0.44	nd
9.14	2,5-Dimethyl-furan	nd	2.59 ± 0.53	nd
19.11	2-Penthyl-furan	nd	0.46 ± 0.02	nd
27.83	2-Furancarboxaldehyde	nd	15.40 ± 1.33	nd
31.32	5-Methyl-2-furancarboxaldehyde	nd	3.48 ± 0.58	nd
34.86	2-Furanmethanol	nd	6.25 ± 0.62	nd

Results are expressed as mean ± standard deviation. Analysis performed in triplicate. Different letters in the same line indicate statistical difference according to the Tukey test ($p < 0.05$). Abbreviation: CC, spreadable goat cheese; CX, spreadable goat cheese with xylooligosaccharides extract; CXP, spreadable goat cheese with purified xylooligosaccharides extract, nd, not detected.

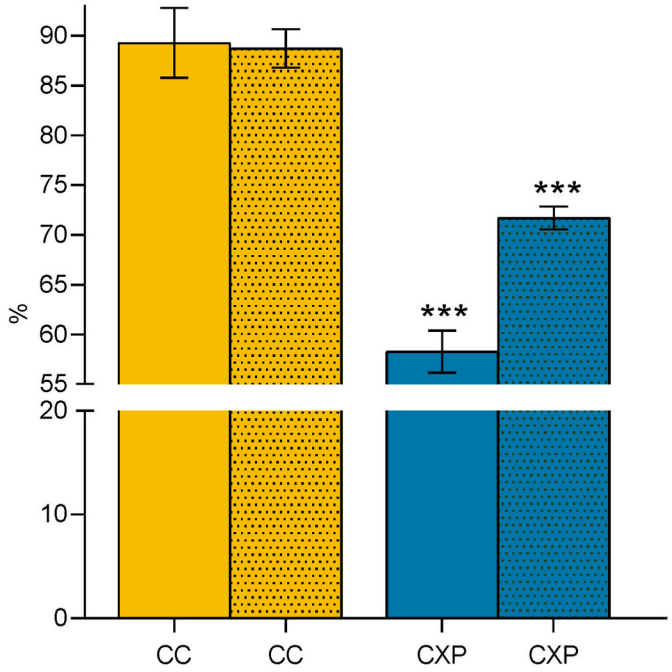

Fig. 3. Results of hydrolysis index (HI) (smoot bar) and predicted glycemic index (pGI) (textured bar) of control cheese (CC) and xylooligosaccharides enriched cheese (CXP). The values represent means of triplicates ± standard deviation; *** indicates significant differences ($p < 0.05$) vs. CC, according to Student's *t*-test.

of foods during the intestinal transit (Wojtunik-Kulesza et al., 2020).

During the first 24h of fecal microbiota simulated fermentation of R-samples (Fig. 5A), almost all plated bacterial groups increased compared to the inoculum. In detail, at 24h, all CC(R) reported a significant ($p < 0.05$) increase compared to the inoculum, with the only exception for Bifidobacterium, whose density reduced despite the absence of significance ($p > 0.05$). By contrast, while all CXP(R) reported intermediate values between the inoculum and CC(R), Bifidobacterium increased significantly in CXP(R).

Additional 24 h of simulated colonic fermentation, i.e., 48 h (Fig. B), reduced the variability, as the number of significant differences, between samples. In details, no differences were found between samples for TAMC, TANMC, and both morphologies of LAB. The significant differences found at 48h-fermentation involved *Bifidobacterium*, that were found to be higher in CXP(R), and *Clostridium* and *Enterobacteriaceae* density, which were higher in CC(R).

The current study emphasizes more pronounced growth of LAB and *Bifidobacterium* in experimental samples containing purified XOS (CXP) compared to the control cheese (CC). From a dietary point of view, xylans are classified as non-digestible fibers. However, at the level of the large intestine, xylans, especially the soluble short-chain ones, are hydrolyzed and used as an energy source by the microbiota and by the

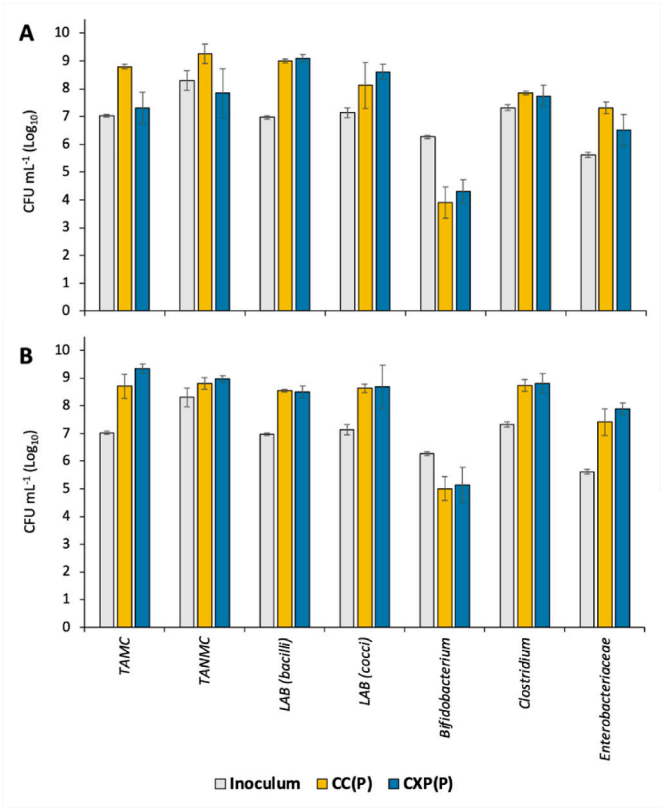

Fig. 4. Fecal microbiota viable bacterial counts (Log_{10} CFU mL^{-1}) after 24 h (panel **A**) and 48 h (panel **B**) of simulated colonic digestion of control cheese permeate (CC(P)) and added with the purified xylooligosaccharide extract (CXP (P)). Inoculum indicates the starting cell density of fecal microbes.

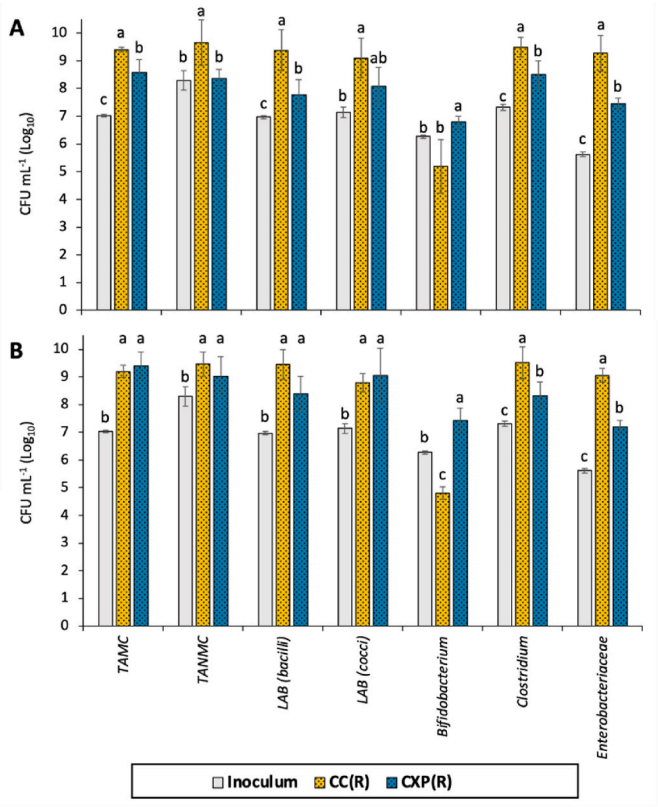

Fig. 5. Fecal microbiota viable bacterial counts (Log_{10} CFU mL^{-1}) after 24 h (panel **A**) and 48 h (panel **B**) of simulated colonic digestion of control cheese retentate (CC(R)) and added with purified xylooligosaccharide extract (CXP (R)). Inoculum indicates the starting cell density of fecal microbes. Different letters on the stacked bars indicate significant differences between cheese samples ($p < 0.05$; one-way ANOVA).

health promoting patterns, such as *Bifidobacterium*. Thus, short-chain xylans and arabinoxylans, called XOS and arabinoxyloligosaccharides (AXOS), can have prebiotic characteristics (An et al., 2022; Chen et al., 2020). Our results are in line with prior studies (Langa et al., 2019) which found that XOS stimulated the proliferation of *Bifidobacterium* and LAB in the intestine, consequently increasing SCFAs levels in these intestinal microbes. These findings have been correlated with improved intestinal barrier function. Some research suggests that XOS may have a positive impact on metabolic health, influencing blood sugar levels, improving insulin sensitivity, and even helping reduce the risk of diseases such as type 2 diabetes (Sheu, Lee, Chen, & Chan, 2008; Yang et al., 2015). The prebiotic effect of XOS shows promising potential in promoting a healthier gut microbiota by stimulating the selective growth of beneficial bacteria. Considering the potential of polyphenols to reduce the microbial growth under a concentration-dependent manner (Kim, 2018), these results suggest that the purification process in XEP may allow the microbial population of the intestinal microbiota to utilize more easily XOS when the purified extract is added to the goat cheese formulation.

4. Conclusions

Food innovation means also giving value to by-products from agriculture and the food industry by obtain novel food ingredients. In this context, many governments are moving towards more sustainable approaches, implementing the ambitious circular economy plan with the main objective of reducing and encouraging the appropriate use of unexploited sources containing bioactive compounds for a sustainable implementation of "zero policies" to recover organic matter for other technological applications (Caro et al., 2024). In this scenario, our study aims to obtain a XOS extract from vine shoots using the food-grade steam explosion technique. The results of the characterization of the XOS extract obtained from vine shoots revealed the presence of antimicrobial compounds, such as polyphenols and furans; this limitation was overcome thanks to the purification process with cationic and anionic resins, which allowed these compounds to be removed, making the extract an optimal substrate for the growth of beneficial microorganisms. The prebiotic XOS extract applied in the formulation of the goat cheese spread, provided a content of 3 g of fiber per 100 g of product. This would allow these products to comply with the claim "source of fiber". Color, texture and volatile profile analyses showed a different impact of the two different extracts in the formulation of CX and CXP. In particular, lower texture and brightness parameters were found in CX, with CXP being more similar to the control cheese. In fact, the CX cheese showed a different appearance, confirmed by the red and yellow parameters with higher values. The application of the resins to purify the extract was effective in retaining acetic acid, polyphenols, and furan compounds, potential inhibitors of microbial growth. This was evidenced by the lower concentration or absence of them in the CXP cheese volatile compounds. No less important were the microbiological findings that showed the ability of the added XOS to stimulate the proliferation of *Bifidobacterium* and LAB, consequently increasing SCFAs levels in these intestinal microbes. Finally, CXP showed a lower glycemic index than the control cheese. Therefore, the study helped provide both chemical-physical and prebiotic results of this innovative goat cheese formulation using a comprehensive approach. In this way, it was possible to study the technological influence of the XOS extract added to the cheese and its action in the nutritional improvement of the product. In future studies, the limitation of finding goat's milk, which is typical of certain geographical areas such as southern Europe, could be overcome

by studying the behavior of XOS in different cheeses, obtained from milk available in larger volumes worldwide, such as cow's milk. Moreover, further studies should focus on a sensory evaluation and subsequent acceptance of XOS-enriched goat cheese spread by consumers.

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CRediT authorship contribution statement

Graziana Difonzo: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Claudia Antonino:** Writing – original draft, Methodology, Investigation, Formal analysis. **Giusy Rita Caponio:** Writing – original draft, Methodology, Investigation, Formal analysis. **Mirco Vacca:** Writing – original draft, Methodology, Investigation, Formal analysis. **Federico Liuzzi:** Methodology, Investigation, Formal analysis. **Isabella De Bari:** Methodology, Investigation, Formal analysis. **Vito Valerio:** Methodology, Investigation, Formal analysis. **Michele Faccia:** Writing – review & editing, Supervision, Conceptualization. **Maria De Angelis:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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