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The impact of sourdough fermentation of spelt (*Triticum dicoccum*) from Garfagnana on gut microbiota composition and *in vitro* activity

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ABSTRACT

The aim of this study has been to evaluate the impact of the fermentation process of the spelt from Garfagnana on its chemical composition and short-chain carbohydrates (SCCs) levels, and on the *in vitro* microbial growth and metabolism.

The fermentation process of spelt significantly increases its protein and mannitol content, and decreases its dietary fiber content and fructose, glucose, sucrose, maltose, and raffinose concentration. Fermented spelt modulates the *in vitro* intestinal microbiota, promoting a stimulation of *Lactobacillus* and *Bifidobacterium* spp. growth accompanied by a high production of lactate, acetate, and propionate, both in human gut microbiota from normal weight and obese subjects. The multivariate approach (PCA) combining viable counts and metabolite concentration values has suggested that spelt fermentation could beneficially modulate the gut microbiota from normal weight and obese individuals, stimulating bacteria eliciting anti-inflammatory responses. Further, *in vivo* studies are recommended for the impact that fermented spelt could have in human nutrition in health and disease.

1. Introduction

Cereal grains are one of the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fiber for people all over the world (O'Neil, Nicklas, Zanovec, & Cho, 2010).

Spelt (*Triticum dicoccum*) is one of the oldest cereals that contains high levels of proteins, carotenoids, and bioactive compounds (Serpen, Gökmen, Karagöz, & Köksel, 2008). Spelt has a long tradition in Garfagnana, a small mountain area in Tuscany, and it has obtained the European Protected Geographical Indication (PGI) in 1996. It can be considered a food speciality with a peculiar regional identity.

The fermentation of cereals is an ancient and widespread technique that can be defined as a desirable process of biochemical modification of primary food operated by microorganisms and their enzymes. It may improve the shelf life, palatability, texture and flavour, digestibility and significantly reduce the antinutrient content of cereal products (Kohajdova & Karovicova, 2007). Moreover, it lead to the production of a larger quantity of vitamins (group B and K), aminoacids, micronutrients, polyunsaturated fatty and phenolic compounds (Colosimo, Gabriele, Cifelli, Longo, Domenici, & Pucci, 2020; Ferri, Serrazanetti, Tassoni, Baldissarri, & Gianotti, 2016; La Marca, Beffy, Pugliese, & Longo, 2013).

Fermented cereal products are suitable for people with lactose intolerance, milk allergies, or people following a low lipid or vegan dietary pattern (Aydar, Tutuncu, & Ozcelik, 2020). They are also considered novel sources of probiotics, prebiotics, or both, as well as potential functional foods (Wuyts, Van Beeck, Allonsius, van den Broek, & Lebeer, 2020) because of their ability to promote the growth of beneficial bacteria (*Bifidobacterium* and *Lactobacillus* spp.). Thus, they may contribute to the maintenance of intestinal homeostasis and to prevent the risk of disease. The consumption of fermented food cereals promotes healthy status both through the interactions between the host and the live microorganisms (probiotics), and the metabolites and health-promoting compounds produced by microbes themselves (biogenic), such as microbiota-accessible carbohydrates converted into Short Chain Fatty

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Acids (SCFAs) (Wuyts, Van Beeck, Allonsius, van den Broek, & Lebeer, 2020).

Although there are many studies in the literature related to traditional and modern fermented cereal products (Petrova & Petrov, 2020), few data are reported on fermented spelt (Colosimo, Gabriele, Cifelli, Longo, Domenici, & Pucci, 2020).

The aim of this study has been to determine the potential antimicrobial activity and prebiotic effect of fermented spelt at different fermentation times (0, 24, 48, 72 and 96 h) on the gut microbiota composition through two procedures. First, we have assessed the effect on *in vitro* growth kinetics of specific microbial strains selected based on their potential pathogenic or beneficial impact on the host. Second, we have investigated the modulatory effect of spelt on gut human microbiota simulating normal conditions, i.e. studying the effect of spelt on microbiota from "normal weight individuals" (NGM), and simulating dysbiotic conditions, i.e. studying the effect of spelt on gut microbiota from "obese individuals" (OGM). The experiments were performed in a pH-controlled, stirred, batch-culture fermentation system reflecting the distal region of the human large intestine, focusing on the viable count of *Lactobacillus* and *Bifidobacterium* groups and on thee quantitative determination of SCFAs in various conditions.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, acetone, sucrose, maltose, mannitol, raffinose, NaCl, KH_2P0_4, K_2HP0_4, $MgS0_4{\cdot}7H_20$, $CaCl_2{\cdot}6H_20$, $NaHC0_3$, $NaCl_2$, $CaCl_2$, HCl, $NaHCO_3$, NaOH, Tween-80, were purchased from PanReac AppliChem (ITW Reagents, Spain). Fructose, glucose and kestose, lactic, acetic, and propionic acids standards, porcine pancreatin (EC 232.468.9), porcine pepsin (EC 3.4.23.1), porcine bile salts, haemin, Vitamin K1, L-cysteine-HCl, resazurin were purchased from Sigma-Aldrich Co. (St Louis, MO, U. S.A.).

Difco^{TM}Lactobacilli MRS Broth (MRS) from BD (MD, USA), Brain Heart Medium (BHM) and yeast extract were purchased from Condalab (Madrid, Spain). Nycodenz® was purchased from PROGEN (Biotechnik GmbH, Heidelberg, Denmark). Peptone was purchased from BD (Becton Dickinson GmbH, Heidelberg, Germany).

2.2. Spelt fermentation and sampling

Spelt (*Triticum dicoccum*) from Garfagnana was supplied by "Unione dei Comuni della Garfagnana" (Lucca, Italy) and flour samples were prepared from ground spelt. The fermentation of the spelt flour was performed as previously described (Colosimo, Gabriele, Cifelli, Longo, Domenici & Pucci, 2020). Briefly, spelt flour was mixed with water with $[Cl^-] \leq 0.8$ mg/L and sourdough starter (Lievitamente SNC, Viareggio, Lucca, Italy) in a steel container for food (KitchenAid, Whirlpool Corporation, Benton Harbor, MI) adapted to guarantee a continuous air flow and constant temperature (38 °C) for 96 h. The fermentation pH ranged between 5.8 (at the beginning) and 4 (at the end). Before fermentation and after 0, 24, 48, 72 and 96 h of fermentation, samples were collected and lyophilized in a freeze dryer (Lio 5P, 5 Pascal, Tezzano Sul Naviglio, Milan, Italy). Samples are referred as spelt flour (nominally called "spelt"), T0, T24, T48, T72 and T96. T0 sample contains all the components of the dough before starting the fermentation process.

2.3. Chemical composition

The composition of spelt, T0, T24, T48, T72 and T96 flour samples in term of energy, carbohydrate, dietary fiber, protein, fat, and ash was determined according to AOAC Official Methods by Neotron (Modena, Italy).

The energy was calculated by the calorimetric method, which determines the amount of heat produced when a sample is burned. The carbohydrate content was calculated by subtracting the percentage of moisture, protein, fat, and ash contents from the total (method 954.11). The fiber content was determined by the enzymatic–gravimetric method (method 985.29). The protein content was determined by using Dumas combustion method (N × correction factor, namely 6.25) (method 990.03). The Soxhlet extraction method was used for the determination of fat content (method 920.39). The ash content was determined by incinerating the samples in a muffle furnace at 550 °C (method 930–05). All results were corrected for the moisture content and expressed as % of dry matter (d.m.)

2.4. Short-chain carbohydrates quantification (SCCs)

One g of spelt, T0, T24, T48, T72 and T96 flour samples was extracted with 3 ml methanol:water (80:30, v/v) by shaking 5 h at 60 °C with a rotating shaker (Hybaid, Thermo Fisher Scientific, Waltham, MA). The samples were centrifuged for 10 min at 3500 rpm and the supernatants were evaporated in a Speed Vac (Eppendorf, Life Sciences, Hamburg, Germany) at 45 °C. The pellets were resuspended in 6 ml acetone–water (50:50, v/v), filtered through 0.22 μm-pore-size nitrocellulose filters (Millipore, United States) and stored at 4 °C until analysis. After dilution (1:20, v/v), SCCs of spelt, T0, T24, T48, T72 and T96 extracts were analyzed using a specific high resolution anion exchange column to separate oligosaccharides (CarboPac PA100 column). Detection after chromatographic separation was performed by pulsed amperometric detection in a ICS3000 chromatographic system (Dionex, Sunnyvale, CA).

For the separation a gradient from 10 to 100 mM NaOH was applied at 27 °C for 15 min at a flow rate of 1 ml/min. Calibration curves were prepared injecting standard solutions of fructose and glucose (500, 250, 125 and 62.5 μM), sucrose, maltose, mannitol and raffinose (200, 100, 50 and 25 μM), kestose and nystose (100, 25, 75, 50 and 25 μM). SCCs concentrations are reported as mg/g of fresh weight sample.

2.5. Effect of fermented spelt extracts on bacterial growth

The impact of the samples extracts on bacterial growth was investigated both in potential pathogens such as *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli*, and *Listeria innocua*, as substitute for the pathogen *Listeria monocytogenes*, and potential beneficial bacteria such as *Lactobacillus* members *(L. casei*, *L. plantarum* and *L. plantarum*) and *Bifidobacterium animalis* subsp. *lactis*. Table 1 shows the list of bacteria, culture medium, temperature and growth conditions used in this study. One g of spelt, T0, T24, T48, T72 and T96 flour samples was extracted with 3 ml methanol–water (80:30, v/v) by shaking for 5 h at 60 °C with a rotating shaker (Hybaid, Thermo Fisher Scientific, Waltham, MA). The sample was centrifuged for 10 min at 3500 rpm and the supernatant was diluted (1:4, v/v) with distilled water.

The growth pattern was monitored in the presence/absence of 10% v/v (20 μL) of all diluted extracts. Cultures for each strain were inoculated in 200 μL of the specific medium (Table 1) to obtain a final optical density at 595 nm of 0.05 absorbance unit, and incubated for 20 h at 37 °C. Afterwards, the maximal optical density (OD max) at 595 nm was determined using a POLARStar reader (BMG LABTECH, Ortengerg, Germany). The results are reported as difference of O.D. max compared to control (O.D. max = 0).

2.6. In vitro colonic fermentation

We analyzed the effect of digested spelt, T0, T24, T48, T72 and T96 flour samples in the population of *Lactobacillus* and *Bifidobacterium* and SCFAs production of the gut microbiota from "Normal weight Gut Microbiota" (NGM) and "Obese Gut Microbiota" (OGM) volunteers using a specific *in vitro* colonic fermentation system. *Lactobacillus* and *Bifidobacterium* were quantified by qPCR and SCFAs were analyzed by reversed phase chromatography with UV detection.

Table 4
Pearson's correlations among lactic acid, acetic acid, propionic acid, Total SCFAs concentration, and levels of *Lactobacillus* and *Bifidobacterium* genus.

Variables	Lactic acid	Acetic acid	Propionic acid	Total SCFAs	*Lactobacillus*	*Bifidobacterium*
Lactic acid	**1**					
Acetic acid	**0.822**	**1**				
Propionic acid	**0.386**	**0.473**	**1**			
Total SCFAs	**0.896**	**0.989**	**0.485**	**1**		
Lactobacillus	0.086	0.130	**0.288**	0.128	**1**	
Bifidobacterium	**0.614**	**0.650**	**0.525**	**0.669**	−0.035	**1**

SCFAs: short chain fatty acids. Values in bold are different from 0 with a $p < 0.05$.

Table 1
Culture medium, temperature and conditions used for the growth of bacteria evaluated in this study.

Bacterial species	Collection number	Culture medium	Temp-Time	Conditions
Staphylococcus aureus	CECT 86	BHM	37 °C-20 h	Aerobic
Salmonella enterica subsp. *Enterica* Serovar. *Thyphi*	CECT 4138	BHM	37 °C-20 h	Aerobic
Escherichia coli	ATCC 25922	BHM	37 °C-20 h	Aerobic
Listeria innocua Serotype 6a	CECT 910	BHM	37 °C-20 h	Aerobic
Lacticaseibacillus *casei*	CECT 5275	MRS	37 °C-20 h	Aerobic
Lactoplantibacillus plantarum	CECT748	MRS	37 °C-20 h	Aerobic
Lacticaseibacillus *rhamnosus*	ATCC 53103	MRS	37 °C-20 h	Aerobic
Bifidobacterium animalis subsp. *lactis*	DSM 15954	MRS + 0.05% L-cys	37 °C-20 h	Anaerobic

BHM: Brain Heart Medium. MRS: Difco™Lactobacilli MRS Broth. L-Cys: cysteine. CECT: Spanish National Culture Collection (www.cect.org/). ATTC: American Type Culture Collection (www.atcc.org). DSM: German Collection of Microorganisms and Cell Cultures (www.dsmz.de/). A single colony of each strain was grown overnight before the experiment. Culture strains were stored in cryovial with 20% of glycerol at –80 °C.

2.7. Gut microbiota samples

Faecal samples were obtained from a pool of 3 normal weight adults (BMI < 25 kg/m^2) and a pool of 3 obese adults (BMI ≥ 30 kg/m^2) participating in a previous study (Garcia-Mantrana, Selma-Royo, Alcantara, & Collado, 2018) to test whether the spelt would have a differential effect depending on gut microbiota composition from lean *vs* obese subjects (normal *vs* dysbiotic microbiota). Clinical, anthropometric, and nutritional characteristics were recorded. All participants have followed an unrestricted diet and have not taken antibiotics or probiotics during the previous three months. Written informed consent was obtained from all volunteers, and the study protocol was approved by the local ethics committee of the Atencion Primaria-Generalitat Valenciana (CEIC-APCV).

2.8. In vitro gastrointestinal simulated digestion of samples

In vitro enzymatic digestion, was performed according to the protocol previously described (Jadán-Piedra, Alcántara, Monedero, Zúñiga, Vélez, & Devesa, 2017), slightly modified. Fresh human saliva (pH 7) collected from three normal weight donors who did not take antibiotics in the previous three months and filtered using a 0.22 μm regenerate cellulose filter (Millipore, Milan, Italy). One hundred mg of spelt, T0, T24, T48, T72 and T96 flours was added to 1 ml of H_2O (w:v), 54 μL of saliva, 0.04 mM $CaCl_2$, and mixed for 30 min. Then, 2 μL of simulated gastric juice (pH 2), containing porcine pepsin (200 μg/ml of digesta) and 0.1 M HCl, were added to the samples and mixed for 90 min. Subsequently, 12.5 μL of simulated intestinal juice (pH 6.5) containing pancreatin (50 μg/ml of digesta) and bile salts (312.5 μg/ml of digesta) and 0.1 M $NaHCO_3$ were added and incubated for 120 min. The oral, gastric and intestinal steps were performed at 37 °C under constant gentle mixing on a horizontal shaker at 120 rpm. The pH of the mixtures was adjusted to pH 7, 2 or 6.5, depending on the digestion step, with 1 M NaOH or 6 M HCl.

2.8.1. Gut microbiota in vitro fermentation

2.8.1.1. Inoculum preparation and in vitro batch fermentation. For the microbiota separation we adopted the method of Hevia and colleagues (2015), slightly modified (Rubio-del-Campo, Alcántara, Collado, Rodríguez-Díaz, & Yebra, 2020). A pooled faecal sample (1.5 g) was diluted 1:10 (w/v) in 0.9% NaCl and homogenized by rotation for 20 min (Roller Mix, OVAN, Barcelona, Spain). The mixture was gently decanted, and 10.5 ml of cell suspension was added to 3.5 ml of Nycodenz® 80% solution (w/v) and centrifuged at 4 °C for 60 min at 4000 rpm (Hermle Z383K, Hermle LaborTechnik GmbH, Wehingen, Germany). The bacterial cells were collected from the interface and washed twice with 0.9% NaCl, resuspended in 80% glycerol and stored at –80 °C until the experiment.

The experiments with both gut microbiota samples from normal weigh (NGM) and obese subjects (OGM) were performed in triplicate with pooled faecal samples as previously described by Rubio-del-Campo et al. 2020. Ten μl of faecal samples (NGM and OGM) were aseptically added to 1.86 ml of sterile basal medium (BM) containing 2 g/l peptone, 2 g/l yeast extract, 0.1 g/l NaCl, 0.04 g/l KH_2P0_4, 0.04 g/l K_2HP04, 0.01 g/l MgS04·7H20, 0.01 g/l $CaCl_2{\cdot}6H_20$, 2 g/l $NaHC0_3$, 2 ml Tween-80, 0.05 g/l haemin solution 10 μL Vitamin K1, 0.5 g/l L- cysteine-HCl, 0.5 g/L bile salts, and 4 ml of 0.025% resazurin as an anaerobic indicator (Salazar, Ruas-Madiedo, Kolida, Collins, Rastall, Gibson, & de los Reyes-Gavilán, 2009). The tubes were stabilized overnight in anaerobic conditions using an anaerobic atmosphere generation system (AnaeroGen™, Oxoid™, Thermo Fisher Scientific, Waltham, MA). After that, 5 mg of digested spelt, T0, T24, T48, T72 and T96 flour samples or 10% glucose (non-selective control) were added to each tube. A negative control was also included. The batch cultures were run for a period of 48 h at 37 °C in anaerobic conditions and samples obtained from each tube were centrifuged for 10 min at 13000 rpm. From batch cultures at selected times, pellets were used for the targeted microbial qPCR (*Bifidobacterium* and *Lactobacillus*) and supernatants were used for the determination of SCFAs as detailed below.

2.8.1.2. Total DNA extraction and targeted quantification of bacterial genera by qPCR. Total DNA was extracted from the pellet of each sample using the Master-Pure DNA extraction kit (Epicentre, Madison, WI, United States) following the manufacturer's instructions with the following modifications: samples were treated with lysozyme (20 mg/ml) and mutanolysin (10 U/ml) for 60 min at 37 °C and a preliminary step of cell disruption was performed with 0.1 mm diameter glass beads followed by 1 min at 2000 oscillations by disruptor (FastPrep-24™ 5G MP), according to manufacturer's instructions. DNA concentration was measured using Qubit 2.0 Fluorometer (Life Technology, Carlsbad, CA, United States) for further analysis. Targeted qPCR assays were

performed to quantify *Bifidobacterium* (Gueimonde, Tölkkö, Korpimäki, & Salminen, 2004) and *Lactobacillus* (Walter, Hertel, Tannock, Lis, Munro, & Hammes, 2001) groups and total bacteria (Farhana et al., 2018) as described previously (Collado, Isolauri, Laitinen, & Salminen, 2008; Mira-Pascual et al., 2015). The qPCR amplification and detection was performed by duplicate on Real-Time PCR System (Light Cycler 480, Roche, Basel, Switzerland). Ten μL of each reaction mixture were composed of SYBR Green PCR Master Mix (Roche, Basel, Switzerland), 0.5 μL of each primer (10 mmol/L), and 1 μL of DNA template. The bacterial concentration in each sample was calculated by the comparison with the Ct values obtained from standard curves that were obtained by serial tenfold dilution of specific DNA fragments with each set of primers.

2.8.1.3. Microbial activity measured by short chain fatty acids (SCFAs) analysis. One ml of the batch supernatant of each sample was diluted in 2 ml 0.1% phosphoric acid, as described elsewhere (Sarmiento-Rubiano, Zúñiga, Pérez-Martínez, & Yebra, 2007). Samples were vortexed and then centrifuged at 9600 rpm for 5 min at 4 °C. Supernatants were filtered through 0.45 μm-pore-size nitrocellulose filters (Millipore, United States) and stored at − 80 °C until HPLC analysis.

An Agilent 1260 Infinity HPLC system (G1311B quaternary pump) equipped with 1260 Infinity High Performance Degasser, a TCC G1316A thermostat, 1260ALS autosampler (G1329B) and UV/vis diode array (1260 DAD G4212B) was employed. The identification of SCFAs was based on the comparison of the retention time and UV spectra of standard compounds. The 220 nm detection was selected to control the interference of high absorbing compounds. The chromatographic separation was carried out by Zorbax Phenyl-Hexyl RP C18 (Agilent Technologies, Santa Clara, CA) 250 × 4.6 mm (silica particle size 4 μm) at 45 °C using the following elution profile: 15 min isocratic elution with 0.1% phosphoric acid (pH 2.2), followed by 10 min gradient to 80% methanol and 10 min isocratic elution in 80% methanol (flow 0.8 ml/min). The column was rinsed with 100% methanol for 15 min and the re-equilibration step was performed. All the solutions were filtered using a 0.22 μm regenerate cellulose filter (Millipore, Milan, Italy) (Campanella, Lomonaco, Benedetti, Onor, Nieri, & Bramanti, 2020).

2.9. Statistical analysis

All statistical analyses were performed by the XLSTAT® for Microsoft Excel® version 2016 (Microsoft Corp., Redmond, Washington DC). Results were expressed as mean values ± standard deviation. An unpaired Student's *t*-test was used to compare chemical composition results of spelt flour and T96 flour. One-way ANOVA was performed by F-test followed by LSD post-hoc for non-significant Levene's test for homogeneity of variances to compare spelt, T0, T24, T48, T72 and T96 flours for results of SCCs, effects of fermented spelt extracts on bacterial growth, quantification of *Bifidobacterium* and *Lactobacillus* by qPCR and SCFAs. The *in vitro* colonic fermentation results were analysed by Pearson's correlation. A Principal Component Analysis (PCA) was performed to visualize the differences among the samples and the measurements performed in the *in vitro* colonic fermentation experiment.

3. Results

3.1. Chemical composition

Table 2 shows the chemical composition of spelt and fermented spelt T96. Spelt fermentation for 96 h did not change energy, carbohydrate, fat, and ash content, but significantly reduced dietary fiber content ($p < 0.001$) and increased protein content ($p < 0.01$). No significant differences were found in the chemical composition of T0, T24, T48 and T72 samples and spelt sample (data not shown for brevity).

Table 2
Chemical composition of spelt flour and T96 flour.

	Spelt	Fermented spelt T96	p
Energy (Kcal/100 g)	396.4 ± 2.0	393.5 ± 3.1	n.s.
Energy (KJ/100 g)	1672.3 ± 22.0	1682.1 ± 10.0	n.s.
Carbohydrate (% d.m.)	74.9 ± 0.6	74.8 ± 0.3	n.s.
Dietary fiber (% d.m.)	7.1 ± 0.1	5.3 ± 0.0	<0.001
Protein (% d.m.)	13.0 ± 0.3	14.9 ± 0.4	<0.01
Fat (% d.m.)	3.3 ± 0.1	3.1 ± 0.1	n.s.
Ash (% d.m.)	1.7 ± 0.1	1.7 ± 0.1	n.s.

Results are reported as means values ± SD (n = 3).
Significantly different samples are indicated following Student's *t*-test ($p < 0.05$).

3.2. Short-chain carbohydrates profile

Table 3 shows the results of the poorly absorbed SCCs. Fructose, glucose, and maltose content of T0 flour was significantly higher than spelt flour. Throughout the fermentation period, fructose, glucose, and maltose content increased significantly after 24 h and decreased by 48 and 96 h and sucrose content decreased significantly after 24 h of fermentation. The raffinose content began to decrease significantly after 24 h of fermentation and remained significantly lower throughout the fermentation. The kestose content was slightly lower in spelt flour with sourdough (T0) compared to spelt flour, but it started to decrease significantly after 24 h of fermentation and remained significantly lower throughout the fermentation. The nystose content did not vary with the addition of sourdough (T0), it began to decrease significantly after 24 h of fermentation, and it remained low throughout the fermentation. Unlike the others SCCs, the mannitol content increased significantly after 24 h (T24) until the end of fermentation.

3.3. Effect of fermented spelt extracts on specific bacterial growth

The results of the antimicrobial activity showed that the extract from 96 h fermented spelt (T96) inhibited the *Salmonella enterica* and *Listeria innocua* growth ($p < 0.05$) compared to control (Fig. 1). No significant effects were found after incubation of spelt and fermented spelt extracts with *Staphylococcus aureus* and *Escherichia coli* and with potential beneficial bacteria, such as *Lacticaseibacillus casei*, *Lactoplantibacillus plantarum*, *Lacticaseibacillus rhamnosus GG*, and *Bifidobacterium animalis* (data not shown for brevity).

3.4. In vitro microbiota colonic fermentation

Changes in the SCFA (C and D panels) levels after incubation of spelt, T0, T24, T48, T72 and T96 samples without and with NGM/OGM across 48 h were observed (Fig. 2). The results of *in vitro* colonic fermentation showed a significant increase of the levels of *Lactobacillus* genus after incubation of T0, T24, T48 and T96 spelt samples with NGM and of T24, T48 and T96 without NGM compared to spelt flour ($p < 0.05$) (Fig. 2A). A significant increase of the levels of *Lactobacillus* genus was observed after incubation of T72 and T96 with OGM and of T0, T24, T48, T72 and T96 without OGM compared to spelt flour ($p < 0.05$) (Fig. 2B). We observed a significant increase of *Bifidobacterium* after incubation of T48, T72 and T96 with NGM compared to spelt flour (Fig. 2C), and after incubation of T24, T48, and T96 with OGM compared to spelt flour, but not without NGM and OGM (Fig. 2D). However, no significant differences of the total bacteria were found between the different treatments in the batch culture (data not shown for brevity).

Fig. 3 shows changes on the concentrations of lactic acid (A and B panels), acetic acid (C and D panles), propionic acid (E and F panels), and total SCFAs (G and H panels) after incubation of spelt, T0, T24, T48, T72 and T96 samples with and without NGM/OGM across 48 h.

The incubation of T72 and T96 flours with NGM and of T48, T72 and T96 flours with OGM significantly increased the concentration of lactic

Table 3
SCCs (mg/g fresh weight sample) of spelt, T0, T24, T48, T72 and T96 samples.

	Mono- and disaccharides				Sugar polyols	GOS	FOS	
	Fructose	Glucose	Sucrose	Maltose	Mannitol	Raffinose	Kestose	Nystose
Spelt	$0.79_{c} \pm 0.13$	$0.42_{b} \pm 0.08$	$4.19_{a} \pm 0.23$	$0.89_{c} \pm 0.09$	$0.15_{c} \pm 0.06$	$3.49_{a} \pm 0.15$	$8.40_{a} \pm 0.51$	$8.49_{a} \pm 1.40$
T0	$3.17_{b} \pm 0.19$	$1.74_{b} \pm 0.13$	$4.04_{a} \pm 0.11$	$7.64_{b} \pm 0.26$	$0.19_{c} \pm 0.07$	$2.80_{b} \pm 0.14$	$7.02_{b} \pm 0.39$	$6.79_{a} \pm 1.72$
T24	$22.16_{a} \pm 0.93$	$15.37_{a} \pm 2.73$	$0.65_{b} \pm 0.06$	$12.66_{a} \pm 0.08$	$7.87_{a} \pm 0.65$	$0.29_{c} \pm 0.05$	$0.07_{c} \pm 0.01$	$1.59_{b} \pm 0.54$
T48	$0.05_{c} \pm 0.01$	$0.03_{b} \pm 0.00$	$0.42_{bc} \pm 0.09$	$7.77_{b} \pm 3.26$	$6.57_{b} \pm 0.10$	$0.18_{c} \pm 0.04$	$0.10_{c} \pm 0.05$	$1.44_{b} \pm 0.77$
T72	$0.08_{c} \pm 0.02$	$0.06_{b} \pm 0.02$	$0.39_{bc} \pm 0.10$	$2.37_{c} \pm 0.34$	$7.24_{ab} \pm 0.18$	$0.17_{c} \pm 0.03$	$0.15_{c} \pm 0.00$	$0.80_{b} \pm 0.34$
T96	$0.04_{c} \pm 0.02$	$0.02_{b} \pm 0.01$	$0.23_{c} \pm 0.06$	$0.58_{c} \pm 0.25$	$7.42_{ab} \pm 0.50$	$0.16_{c} \pm 0.01$	$0.12_{c} \pm 0.00$	$0.19_{b} \pm 0.14$

GOS: galacto-oligosaccharides; FOS: fructooligosaccharides. Mean values ± SD (n = 3). Different superscript letters within rows indicate differences following ANOVA post hoc LSD ($p < 0.05$).

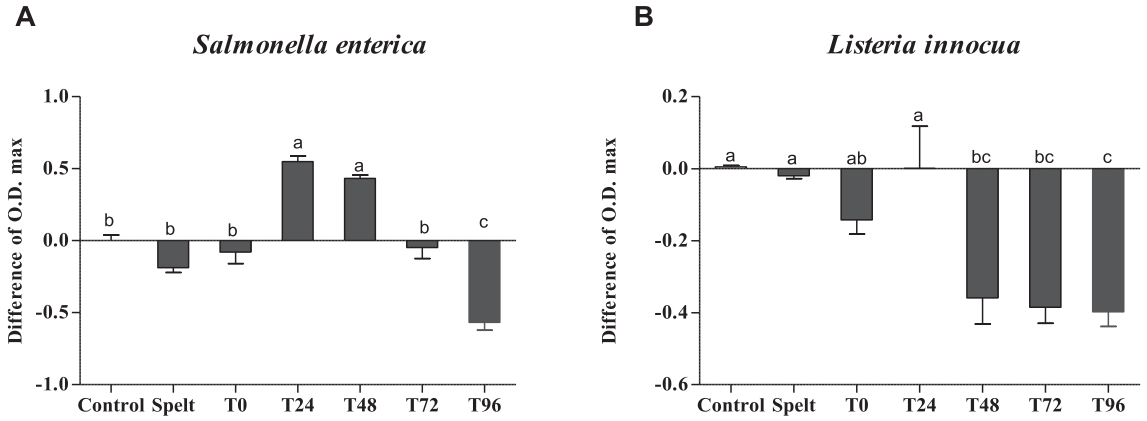

Fig. 1. Effect of spelt (Spelt) and spelt fermented at different times (T0, T24, T48, T72 and T96) on growth of *Salmonella enterica* and *Listeria innocua* (difference of O.D. max). The letters above the columns indicate significant difference between the groups ($p < 0.05$) (ANOVA post hoc LSD). Results are reported as means values ± SD (n = 3).

acid compared to spelt flour (Fig. 3A and 3B). The incubation of T72 and T96 flours with NGM and with OGM significantly increased the content of acetic acid compared to spelt flour (Fig. 3C and 3D). The incubation of T96 flour with NGM and of T72 and T96 flours with OGM significantly increased the synthesis of propionic acid compared to spelt flour (Fig. 3E and 3F). Thus, also the total SCFAs concentration significantly increased following incubation of T72 and T96 with NGM and OGM (Fig. 3G and 3H). No differences were found in the concentration of lactic, acetic, and propionic acids and total SCFAs between flours incubated without NGM or OGM.

In order to identify possible correlations between the different parameters studied and to highlight the effect of fermentation time, a principal component analysis (PCA) was performed (Fig. 4). Table 4 reports the Pearson's correlations among lactic acid, acetic acid, propionic acid, Total SCFAs concentration, and levels of *Lactobacillus* and *Bifidobacterium* genera.

Lactobacillus spp. growth and propionic acid content show a significant positive correlation ($R = 0.288$, $p < 0.05$); *Bifidobacterium* growth correlates with lactic acid ($R = 0.614$, $p < 0.05$), acetic acid ($R = 0.650$, $p < 0.05$), propionic acid ($R = 0.525$, $p < 0.05$) and total SCFAs ($R = 0.669$, $p < 0.05$).

Fig. 4 shows the score plot of PCA, corresponding to a projection of the variables investigated on a two-dimensional basis (Fig. 4). The score plot on F1-F2 explained 79.73% of the total variance. F1 accounting for 61.45% of the existing variability is responsible for separation of samples based on higher spelt fermentation times (T72 and T96) with the samples with more fermented spelt on the right side. F2 accounting for 18.28% of the existing variability is responsible for separation of samples based on the presence of NGM or OGM. Our results demonstrate that longer fermentation times (T72 and T96) increase SCFAs and *Lactobacillaceae* and *Bifidobacterium* content in microbiota from normal weight and obese subjects.

However, the microbiota from normal weight volunteers behaves differently with respect to the microbiota from obese patients. Fermented spelt increases the growth of *Bifidobacterium* and acetic and lactic acids and total SCFAs content of the gut microbiota from normal volunteers and increases the synthesis of propionic acid in the gut microbiota from obese individuals (F2 axis).

4. Discussion

This study evaluated the chemical composition, SCCs content and the potential antimicrobial activity and potential prebiotic effect in spelt flour from Garfagnana, during 96 h of controlled sourdough fermentation. Several studies have demonstrated the beneficial effects obtained by fermentation on different cereal matrices (Verni, Rizzello, & Coda, 2019). This is the first study on this typical Italian cereal.

Based on the results on chemical composition of spelt and fermented spelt T96 obtained on this work, 96 h fermentation did not change the energy, carbohydrate, fat and ash content profile but significantly decreased dietary fiber content and increased protein content. The partial degradation of dietary fiber is in accordance with other studies (Ihediohanma, 2011; Ojokoh & Eromosele, 2015). Ihediohanma (2011) showed that dietary fiber content gradually decreases during 72 h of fermentation of the cassava granules. Ojokoh and Eromosele (2015) revealed that protein and fat content increased, while carbohydrate, crude fiber and anti-nutrient content decreased after 72 h of fermentation of sorghum and sorghum and pumpkin bland. The partial degradation of dietary fiber can be explained by the contribution of endogenous and bacterial enzymes, especially xylanases (Crittenden et al., 2002). Literature data are conflicting about the effect of fermentation on protein content. Several authors reported an increase of protein and/or some amino acids content upon fermentation (El Hag et al., 2002; Doudu, Taylor, Belton, & Hamaker, 2003; Pranoto, Anggrahini, &

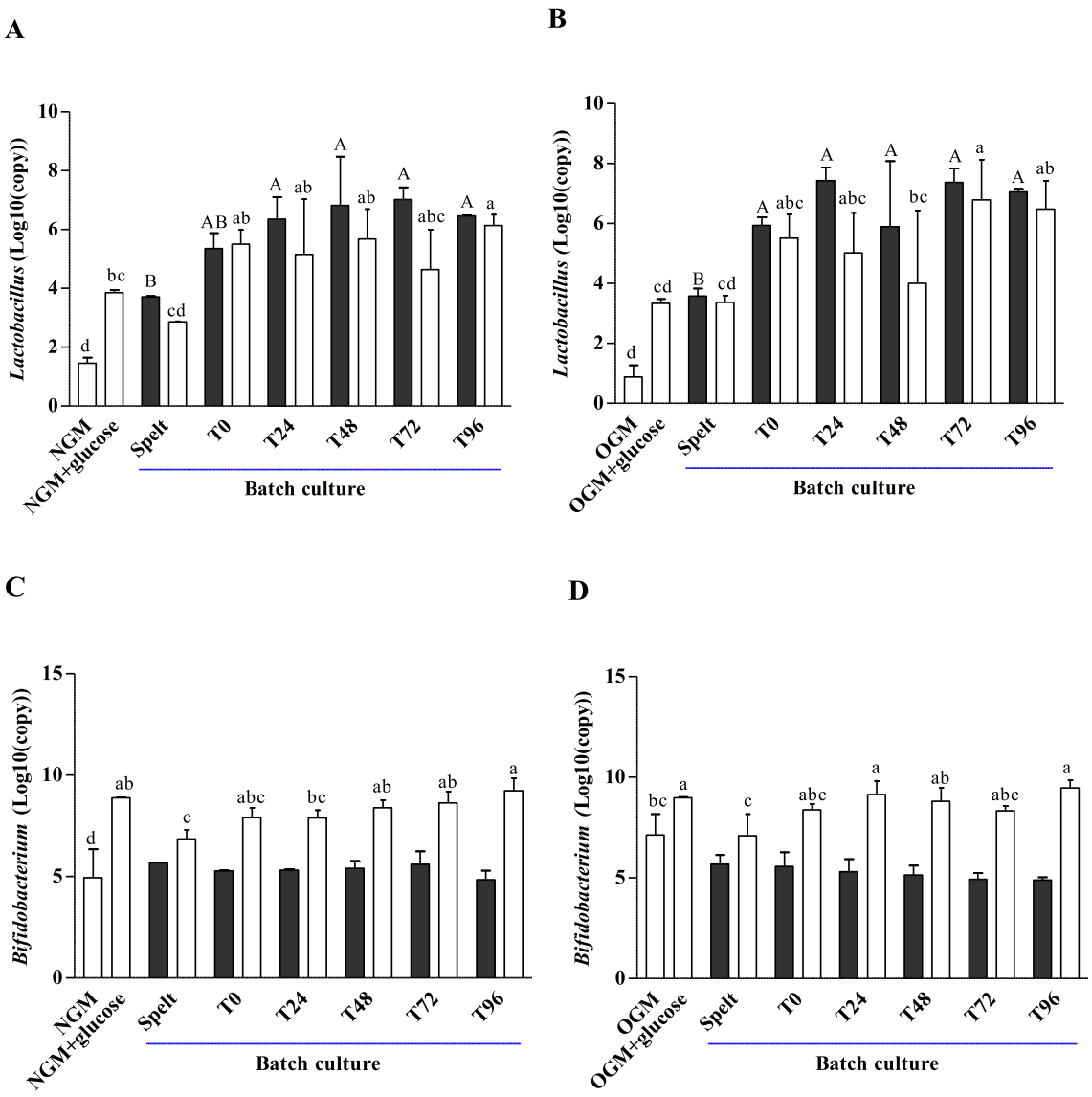

Fig. 2. Changes in the levels of *Lactobacillus* (A and B) and *Bifidobacterium* (C and D) genus after incubation of spelt, T0, T24, T48, T72 and T96 samples without gut microbiota (■) and with gut microbiota (□) from NGM and OGM across 48 h. The letters above the columns indicate significant difference between the groups ($p < 0.05$) (ANOVA post hoc LSD). Results are reported as mean values ± SD (n = 3).

Efendi, 2013). Xu et al. (2019) reported an increase of protein content in fermented quinoa by three medicinal mushrooms. Protein content increased as well as after fermentation with yeasts such as *S. cerevisiae* of rice-black gram mixed flour (Rani et al., 2018). Furthermore, Pranoto, Anggrahini and Efendi (2013) demonstrated that sorghum flour with *Lactoplantibacillus plantarum* led to a protein increase of 12.39% in sorghum. Contradictory reports on protein content in fermented pearl millet are also available. Osman (2011) found no substantial changes in protein content for 24 h fermentation of pearl millet flour, while El Hag et al. (2002) observed a decrease in protein in fermented pearl millet.

It seems that most of these effects may reflect changes due to loss of dry matter because of microorganisms hydrolyzing and metabolizing carbohydrates and fats as source of energy.

In this study we analyzed how the content of most of the SCCs normally present in spelt flour (Biesiekierski et al., 2011; Zörb, Betsche, Langenkämper, Zapp, & Seifert, 2007) changes during 0, 24, 48, 72 and 96 h of sourdough fermentation and we demonstrated that fructose, glucose and maltose content of T0 flour was significantly higher than spelt flour, probably due to their presence in the sourdough starter necessary for the survival of lactic acid bacteria (LAB) and yeast during the storage (Leroy, De Winter, Adriany, Neysens & De Vuyst, 2006; Carvalho et al., 2004). In fact, sourdough is a nutrient-rich ecosystem that contains complex carbohydrates, but their partial hydrolysis to di-saccharides (mainly maltose) and mono-saccharides (fructose and glucose), by flour and microbial amylases, rapidly occurs (Minervini, De Angelis, Di Cagno & Gobetti, 2014).

Moreover, throughout the fermentation period, fructose, glucose, and maltose content increased significantly after 24 h and decreased by 48 and 96 h. We hypothesized that the sourdough microorganisms hydrolyze raffinose into fructose, glucose, and maltose using them for their sustenance. Similar results related to the increase and decrease of glucose and fructose during 24 h of lactic acid fermentation were reported for pearl millet, while the maltose content immediately dropped below the detection limit (Osman, 2011).

Sucrose content decreased significantly after 24 h of fermentation possibly because it was hydrolyzed into glucose and fructose by microorganisms.

During sourdough fermentation the metabolism of carbohydrates depends on the available substrates, on the microbial and endogenous enzymes of the flour and on the interactions between microbial

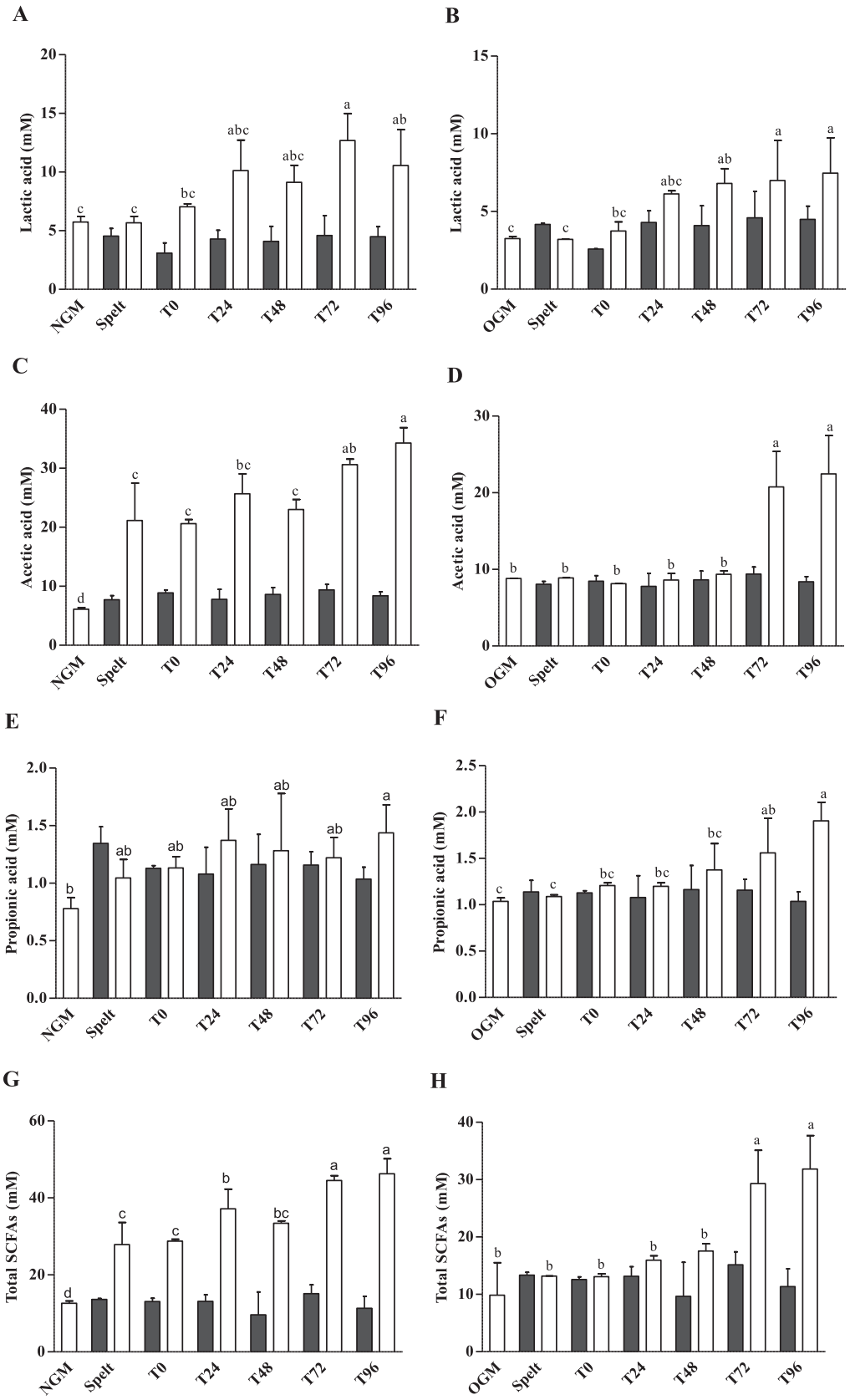

Fig. 3. Alterations in the concentrations of lactic acid (A and B), acetic acid (C and D), propionic acid (E and F), and total short chain fatty acids (SCFAs) (G and H) after incubation of spelt, T0, T24, T48, T72 and T96 samples without gut microbiota (■) and with gut microbiota (□) from NGM and OGM across 48 h. The letters above the columns indicate significant difference between the groups ($p < 0.05$) (ANOVA post hoc LSD). Results are reported as mean values ± SD ($n = 3$).

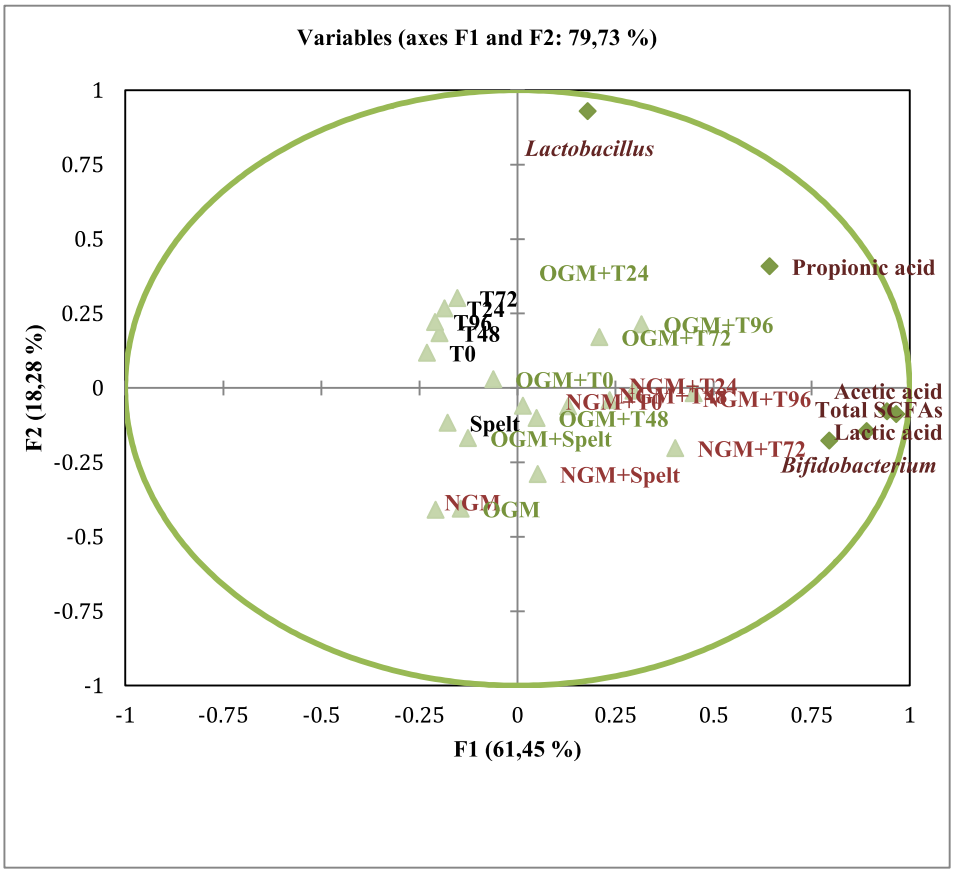

Fig. 4. Principal component analysis (PCA) of the effect of spelt fermentation on acetic acid, lactic acid, propionic acid and total short chain fatty acids (SCFAs) concentration, and levels of *Lactobacillus* and *Bifidobacterium*.

populations. Other studies have shown that during typical sourdough fermentation, flour α-amylase hydrolyzes starch to maltodextrins, which are converted by flour β-amylase into maltose (Colosimo, Gabriele, Cifelli, Longo, Domenici & Pucci, 2020; Osman, 2011). At dough stage, microbial invertase rapidly cleaves flour sucrose into glucose and fructose (Gänzle, 2014). Glucose is used as energy source (Osman, 2011), whereas fructose may be reduced by heterofermentative LAB to mannitol (Sahin et al., 2019; Wisselink, Weusthuis, Eggink, Hugenholtz & Grobben, 2002). Overall, all the fermentable carbohydrates (sucrose, maltose, glucose, and fructose) are quickly depleted during the first hours of fermentation.

Raffinose, that begins to significantly decrease after 24 h of fermentation and remains significantly lower throughout the rest of the fermentation period, belongs to the galacto-oligosaccharides (GOS) and is non-digestible in the human gut due to the absence of α-galactosidase in the intestinal mucosa. Consequently, intact oligosaccharides pass directly into the lower intestine where they are metabolized by bacteria which possess this enzyme, resulting in the production of gases. Lactic acid bacteria possess α-galactosidase activity which can hydrolyze the raffinose and stachyose during fermentation (Sumarna, 2008; Wang, Yu, Yang & Chou, 2003).

Kestose and nystose belong to fructooligosaccharides (FOS) and are recognized as prebiotics because they are non-digestible compounds, and can stimulate the growth of colon bacteria (Rao, 2001).

Prebiotics can be defined as 'food materials composed by fibers of natural origin that are not digested in the upper gastrointestinal tract and improve the health of the host by selectively supporting the development and activity of particular genera of microorganisms in the colon, mostly lactobacilli and *Bifidobacterium*' (Patel, Singh, Panaich & Cardozo, 2014; Pandey, Naik & Vakil, 2015). Examples of prebiotics are GOS, FOS, inulin, pectin, mannitol, and maltodextrin. Recently, the prebiotic definition was expanded to include non-carbohydrate substances and alternative application sites, such as polyphenols (Rodríguez-Costa, Cobas, Saavedra, Arias, Miranda & Cepeda, 2018; Cardona, Andrés-Lacueva, Tulipani, Tinahones & Queipo-Ortuño, 2013).

Our results show that the kestose content is slightly lower in spelt flour with sourdough (T0) compared to the only spelt flour, but the nystose content does not vary with the addition of sourdough (T0). During the sourdough fermentation of spelt both the kestose content and the nystose content begin to decrease significantly after 24 h of fermentation and remain low throughout the fermentation duration.

Differently from the other SCCs, mannitol content increases significantly after 24 h of fermentation and remains significantly higher during the fermentation.

Mannitol is a sugar alcohol which results from the metabolism of fructose and may be inhibited by the presence of glucose (Gänzle, 2015). It has indigestible properties and for this reason it has been grouped as a prebiotic (Yong, Lai, Ghazali, Chang & Pui, 2020; Maekawa, Ushida, Hoshi, Kashima, Ajisaka & Yajima, 2005; Liong & Shan, 2005).

Therefore, we can assert that during sourdough spelt fermentation the content of most SCCs normally found in spelt flour decreases (Menezes et al., 2018; Struyf et al., 2017), except for mannitol. Despite the clear evidence of health benefits for some SCCs, such as prebiotic effects (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004), there is a proportion of the general population who are 'intolerant' to the malabsorption of SCCs in the small intestine, also called FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) (Gibson and Shepherd, 2005). A recent study demonstrated that the addition of sourdough during the production of marketable bread alters the FODMAP composition, by reducing the fructan content and increasing the mannitol content (Schmidt and Sciurba, 2021). Pejcz et al. (2021) demonstrated that the spontaneous fermentation and the

inoculation of both *L. plantarum* and *L. casei* contributes similarly to reduce FODMAP and to increase mannitol in wheat sourdough after 72 h of fermentation.

Several gastrointestinal disorders, such as upper gastrointestinal disorders and bowel disorders, are considered as complications of obesity (Camilleri, Malhi, & Acosta, 2017). Dietary interventions are part of the treatment for both IBS (Irritable Bowel Syndrome) and obesity. A reduced intake of FODMAPs will often relieve IBS symptoms, and a variety of low-energy diets can lead to weight reduction in subjects with obesity (Paduano, Cingolani, Tanda, & Usai, 2019; Aasbrenn, Lydersen, & Farup, 2018).

In summary, the extract of fermented spelt after 96 h (T96) has a selective antimicrobial activity against *Salmonella enterica* and *Listeria innocua*, probably due to the presence of compounds synthesized during fermentation. Some studies demonstrated that, during the fermentation process, LAB produce antimicrobial substances, such as acid lactic, nisin or other bacteriocins, that inhibit pathogenic bacteria (Barbosa et al., 2014; Jiang et al., 2012). A study conducted by Onipede and Fawole (2017) evaluated the *in vitro* antibacterial activities of LAB isolated from two fermented cereal products, *ogi* and *kunun-zaki*, against organisms implicated in gastrointestinal tract infections, and that *E. coli*, *Shigella* and *S. typhi* were all susceptible to all the isolates used against them, while *Klebsiella* sp. and *E. faecalis* varied in their susceptibility. Dejene et al. (2021) demonstrated that cell-free supernatant produced by isolated lactic acid bacteria from fermented beverage (Borde) and finfish showed antimicrobial activity against a wide range of Gram-positive and Gram-negative foodborne bacteria, suggesting its potential application as a natural antimicrobial agent against foodborne pathogens.

Moreover, during fermentation different types of hydrolysis reactions may be induced by the inherent enzymes of microorganisms, which can bioactivate and increase the bioaccessibility and bioavailability of molecules such as phenolic compounds and peptides (Colosimo, Gabriele, Cifelli, Longo, Domenici & Pucci, 2020; Ripari, Bai, & Gänzle, 2019; Koistinen, Katina, Nordlund, Poutanen, & Hanhineva, 2016; Arte, Rizzello, Verni, Nordlund, Katina, & Coda, 2015). These molecules play an important role in the antibacterial activity. The antimicrobial activity of polyphenols occurring in vegetable foods has been extensively investigated against a wide range of microorganisms (Daglia, 2012). Many mechanisms of antimicrobial action have been suggested such as damage to the bacterial membrane, and suppression of some virulence factors, including enzymes and toxins (Barbieri et al., 2017). Moreover, it has been demonstrated that the increase of peptides during cereal fermentation may be associated with the antimicrobial activity against foodborne pathogens (Singh, Vij, Hati, Singh, Kumari & Minj, 2015; Amadou, Le & Shi, 2013).

In the presence of T96 fermented spelt no significant effects were observed on the growth of probiotic bacteria tested (data not shown). The prebiotic effect of fermented products appears to be strain specific. Coman and colleagues showed the effect of buckwheat flour and oat bran as prebiotics to improve the growth, activity and viability of L. rhamnosus IMC 501®, L. paracasei IMC 502® and their combination SYNBIO® on whole milk (Coman et al., 2013). However, with *Bifidobacterium animalis* ssp. *lactis* BB-12 and *Lactobacillus acidophilus* La-5 no effects were observed by the addition of up to 3% quinoa flour (Casarotti, Carneiro & Penna, 2014).

After this preliminary evaluation in strictly controlled conditions, we moved to a complex ecosystem to investigate whether spelt and sourdough fermented spelt can affect the evolution of microbiota. More variables were assessed: sourdough fermentation time (spelt, T0, T24, T48, T72 and T96) and the microbiota (from normal weight and obese volunteers).

In the present study we showed the increase of *Lactobacillaceae* levels during the *in vitro* colonic microbiota fermentation (in both normal and dysbiotic microbiota from lean and obese volunteers) with the addition of fermented spelt and during the *in vitro* colonic fermentation without the microbiota. This means that lactobacilli derive from sourdough and that fermented spelt could exert a probiotic action.

In contrast to *Lactobacillus* group, the *Bifidobacterium* group increased only after the *in vitro* colonic fermentation of the microbiota from normal weight volunteers and obese patients after the addition of fermented spelt, but not after *in vitro* colonic fermentation of the fermented spelt alone without the microbiota. This means that fermented spelt exerted a prebiotic action on *Bifidobacterium* which may be due to the increase in the mannitol and polyphenol content during fermentation (Colosimo, Gabriele, Cifelli, Longo, Domenici & Pucci, 2020).

Fermented spelt exerted a different positive effect on microbiota from normal weight and obese donors: spelt samples fermented for 72 h and 96 h increased *Bifidobacterium* numbers of normal weight donors and *Lactobacillaceae* numbers of obese donors. This result suggests that the effect of the fermented spelt on the microbiota composition depends on the complex initial ecosystem, that is different in normal weight and obese subjects.

Some researchers demonstrated that traditional African fermented cereal-based beverages are potential probiotic carriers because of the probiotic *Lactobacillus* spp. and yeast that are employed for the fermentation of such products (Setta, Matemu & Mbega, 2020).

Bifidobacterium and *Lactobacillus* spp. are considered potential beneficial bacteria for humans. These bacteria produce bacteriocins and other antimicrobials and may interact with the immune system. For this reason they are the predominant bacteria used as probiotics (Mantziari, Tölkkö, Ouwehand, Löyttyniemi, Isolauri, Salminen & Rautava, 2020). Probiotics are live microbial food supplements that are consumed in order to provide a health benefit to the host by contributing to the maintenance of a favorable intestinal microbial balance (Hill, Guarner, Reid, Gibson, Merenstein & Pot, 2014) and these microorganisms are demonstrated to have beneficial effects on obesity and related metabolic complications including hypertension, distorted lipid profiles and offer higher immune-protection (Maldonando Galdeano, Cazorla, Lemme Dumit, Vélez & Perdigón, 2019; Qi, Nie & Zhang, 2020).

Lactic, acetic, and propionic acids are SCFAs synthetized by *Lactobacillus* and *Bifidobacterium*. SCFAs are produced by enteric microbes as end products of anaerobic fermentation of undigested, microbial accessible dietary carbohydrates, and have a variety of important roles in the gut, e.g. the regulation of host energy metabolism and immunity (Li, Shimizu, & Kimura, 2017).

In this work we also showed that the *in vitro* colonic fermentation of the microbiota from both normal weight and obese volunteers with fermented spelt gave an increase of lactic, acetic and propionic acids synthesis. The increase was not observed during *in vitro* colonic fermentation without the microbiota mentioned. This means that lactic, acetic, and propionic acids did not derive from the fermented spelt, but they were synthetized during the *in vitro* colonic fermentation from lactobacilli and bifidobacteria, and their increase was due to the presence of fermented spelt which exerted a function of carrier of *Lactobacillaceae* and a potential prebiotic effect.

SCFAs are beneficial to the host for multiple biological activities: anti-inflammatory activity and regulation of satiety, normalization of adipogenesis in the metabolic syndrome alterations, regulation of insulin secretion, fat accumulation, energy homeostasis, and plasma cholesterol levels (Canfora, Jocken & Blaak, 2015; González Hernández, Canfora, Jocken & Blaak, 2019; Marchesi et al., 2015). Therefore, the gut microbiota and the SCFAs may serve as a potential therapeutic target for the metabolic syndrome and the increased consumption of whole grains and their derived food products has been associated with protection against this pathology (Chambers, Preston, Frost & Morrison, 2018; Lu, Fan, Li, Lu, Chang & Qi, 2016; Masisi, Beta & Moghadasian, 2016; Lin et al., 2012).

In this work we found that the supplementation of fermented spelt had different positive effects on gut microbiota. The acetic and lactic acids synthesis increased with the gut microbiota of normal weight donors with spelt fermented for 72 and 96 h; the increase of the propionic acid synthesis occurred with the gut microbiota from obese donors

with spelt fermented for 72 h.

The statistical analysis (PCA) pinpointed a change in the ecosystem, and this effect was clearly distinguishable with fermented spelt at 72 h and 96 h, which increased *Bifidobacterium* growth and acetic and lactic acid synthesis in gut microbiota from normal weight donors, and *Lactobacillus* growth and propionic acid synthesis in gut microbiota from obese subjects. Thus, sourdough fermented spelt may change the whole ecosystem composition and exert a key role in fighting the dysbiosis.

The data presented in this paper are fundamental and preliminary to the design of *in vivo* animal intervention study and clinical trials to confirm the promising trends herein observed.

The limitations and strenghts of the *in vitro* batch fermentation model have been extensively reviewed (Pharm & Mohajeri, 2018). *In vitro* models, if compared to human or animal experiments, are cheaper, not invasive, can be operated under standardized conditions, they can be controlled and repeated. The *in vitro* batch fermentation model in particular is a valuable tool for studying and uncovering the impact of nutrition and dietary supplementation on the gut microbiota. Although *in vitro* models and batch fermentation model cannot include complete host factors, such as intestinal secretions, immunology, or absorption, they offer an inexpensive and reliable tool for modeling the microbial ecology and activity of the colon.

5. Conclusion

In this work we have demonstrated that sourdough fermentation of spelt decreased its SCCs content (fructose, glucose, sucrose, maltose, and raffinose), except for mannitol, and this makes it a suitable food for the intolerant population.

Sourdough fermented spelt extracts had no impact on the *in vitro* growth kinetics of *Staphylococcus aureus* and *Escherichia coli* and of probiotic bacteria, but the 96 h fermented spelt has an important role in the antibacterial effect towards *SalmonellaTyphimurium* and *Listeria*spp., potential pathogens.

The final multivariate approach combining both the viable count and SCFA profile applied to a complex ecosystem, such as gut microbiota, showed that sourdough fermented spelt has a beneficial modulation, promoting the growth of probiotic bacteria, demonstrating a bifidogenic and lactogenic effect in microbiota from both groups of normal weight and obese individuals, and increasing lactic, propionic and acetic acids synthesis.

Our findings suggest that the sourdough fermented spelt is suitable even for microbiota of normal weight and obese people through stimulation of bacteria eliciting anti-inflammatory responses. Further studies will be necessary to investigated how sourdough fermented spelt influences gut microbiota populations and how this may impact physiology of the host during *in vivo* studies and clinical trials.

Ethics statement

We confirm that the manuscript entitled: "The impact of sourdough fermentation of spelt (*Triticum dicoccum*) from Garfagnana on gut microbiota composition and *in vitro* activity" has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

(1) This material has not been published in whole or in part elsewhere;

(2) The manuscript is not currently being considered for publication in another journal;

(3) All authors have been personally and actively involved in substantive work leading to the manuscript, and will hold themselves jointly and individually responsible for its content.

CRediT authorship contribution statement

Luisa Pozzo: Conceptualization, Data curation, Formal analysis, Investigation, Funding acquisition, Writing – original draft. **Cristina Alcántara Baena:** Conceptualization, Investigation, Writing – original draft. **Marta Selma-Royo:** Data curation, Formal analysis, Investigation. **Izaskun Garcia Mantrana:** Data curation, Formal analysis, Investigation. **Emilia Bramanti:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Vincenzo Longo:** Supervision. **Maria Carmen Collado:** Conceptualization, Funding acquisition, Supervision. **Laura Pucci:** Conceptualization, 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.

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References

Aasbrenn, M., Lydersen, S., & Farup, P. G. (2018). A conservative weight loss intervention relieves bowel symptoms in morbidly obese subjects with irritable bowel syndrome: A prospective cohort study. *Journal of Obesity, 3732753*. https://doi.org/10.1155/2018/3732753

Amadou, I., Le, G. W., & Shi, Y. H. (2013). Evaluation of antimicrobial, antioxidant activities, and nutritional values of fermented foxtail millet extracts by *Lactobacillus paracasei* Fn032. *International Journal of Food Properties, 16*(6), 1179–1190. https://doi.org/10.1080/10942912.2011.579673

Arte, E., Rizzello, C. G., Verni, M., Nordlund, E., Katina, K., & Coda, R. (2015). Impact of enzymatic and microbial bioprocessing on protein modification and nutritional properties of wheat bran. *Journal of Agricultural and Food Chemistry, 63*, 8685–8693. https://doi.org/10.1021/acs.jafc.5b03495

Aydar, E. F., Tutuncu, S., & Ozcelik, B. (2020). Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. *Journal of Functional Foods, 9*, 734–758. https://doi.org/10.1016/j.jff.2020.103975

Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F., & Nabavi, S. M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbial Research, 196*, 44–68. https://doi.org/10.1016/j.micres.2016.12.003

Barbosa, M. S., Todorov, S. D., Belguesmia, Y., Choiset, Y., Rabesona, H., Ivanova, I. V., … Franco, B. D. G. M. (2014). Purification and characterization of the bacteriocin produced by *Lactobacillus sakei*MBSa1 isolated from Brazilian salami. *Journal of Applied Microbiology, 116*, 1195–1208. https://doi.org/10.1111/jam.12438

Biesiekierski, J. R., Rosella, O., Rose, R., Liels, K., Barrett, J. S., Shepherd, S. J., & Muir, J. G. (2011). Quantification of fructans, galacto-oligosaccharides and other short-chain carbohydrates in processed grains and cereals. *Journal of Human Nutrition and Dietetics, 24*, 154–176. https://doi.org/10.1111/j.1365-277X.2010.01139.x

Camilleri, M., Malhi, H., & Acosta, A. (2017). Gastrointestinal complications of obesity. *Gastroenterology, 7*, 1656–1670. https://doi.org/10.1053/j.gastro.2016.12.052

Campanella, B., Lomonaco, T., Benedetti, E., Onor, M., Nieri, R., & Bramanti, E. (2020). Validation and application of a derivatization-free RP-HPLC-DAD method for the determination of low molecular weight salivary metabolites. *International Journal of Environmental Research and Public Health, 17*(17), 6158–6175. https://doi.org/10.3390/ijerph17176158

Canfora, E. E., Jocken, J. W., & Blaak, E. E. (2015). Short-chain fatty acids in control of body weight and insulin sensitivity. *Nature Reviews Endocrinology, 11*, 577–591. https://doi.org/10.1038/nrendo.2015.128

Cardona, F., Andrés-Lacueva, C., Tulipani, S., J. Tinahones, F., & Queipo-Ortuño, M.I. (2013). Benefits of polyphenols on gut microbiota and implications in human health. *Journal of Nutritional Biochemistry*, 24: 1415–1422. https://doi.org/10.1016/j.jnutbio.2013.05.001.

Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2004). Effects of various sugars added to growth and drying media upon thermotolerance and survival throughout storage of freeze-dried *Lactobacillus delbrueckii* ssp. bulgaricus. *Biotechnology Progress, 20*, 248–254. https://doi.org/10.1021/bp034165y

Casarotti, S.N., Carneiro, B.M., & Penna, A.C.B. (2014). Evaluation of the effect of supplementing fermented milk with quinoa flour on probiotic activity. *Journal of Dairy Science,* 97(10): 6027-6035.https://doi.org/10.3168/jds.2014-8197.

Chambers, E. S., Preston, T., Frost, G., & Morrison, D. J. (2018). Role of Gut Microbiota-Generated Short-Chain Fatty Acids in Metabolic and Cardiovascular Health. *Current Nutrition Reports, 7*, 198–206. https://doi.org/10.1007/s13668-018-0248-8

Collado, M. C., Isolauri, E., Laitinen, K., & Salminen, S. (2008). Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *American Journal of Clinical Nutrition, 88*, 894–899. https://doi.org/10.1093/ajcn/88.4.894

Colosimo, R., Gabriele, M., Cifelli, M., Longo, V., Domenici, V., & Pucci, L. (2020). The effect of sourdough fermentation on *Triticum dicoccum* from Garfagnana: 1H NMR characterization and analysis of the antioxidant activity. *Food Chemistry, 305*, Article 125510. https://doi.org/10.1016/j.foodchem.2019.125510

Coman, M. M., Verdenelli, M. C., Cecchini, C., Silvi, S., Vasile, A., Bahrim, G. A., & Cresci, A. (2013). Effect of buckwheat flour and oat bran on growth and cell viability of the probiotic strains *Lactobacillus rhamnosus* IMC 501®, *Lactobacillus paracasei* IMC 502® and their combination SYNBIO®, in symbiotic fermented milk». *International Journal of Food Microbiology, 167*(2), 261–268. https://doi.org/10.1016/j.ijfoodmicro.2013.09.015

Crittenden, R., Karppinen, S., Ojanen, S., Tenkanen, M., Fagerström, R., Mättö, J., … Poutanen, K. (2002). *In vitro* fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. *Journal of Science of Food and Agriculture, 82*, 781–789. https://doi.org/10.1002/jsfa.1095

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology, 23*(2), 174–181. https://doi.org/10.1016/j.copbio.2011.08.007

Dejene, F., Regasa Dadi, B., & Tadesse, D. (2021). *In Vitro* Antagonistic Effect of Lactic Acid Bacteria Isolated from Fermented Beverage and Finfish on Pathogenic and Foodborne Pathogenic Microorganism in Ethiopia. *International journal of microbiology, 2021*, 5370556. https://doi.org/10.1155/2021/5370556

Doudu, K. G., Taylor, J. R. N., Belton, P. S., & Hamaker, B. R. (2003). Factors affecting sorghum protein digestibility. *Journal of Cereal Science, 38*, 117–131. https://doi.org/10.1016/S0733-5210(03)00016-X

El Hag, M. E., El Tinay, A. H., & Yousif, N. E. (2002). Effect of fermentation and dehulling on 531 starch, total polyphenols, phytic acid content and in vitro protein digestibility of pearl millet. 532. *Food Chemistry, 77*(2), 193–196. https://doi.org/10.1016/S0308-8146(01)00336-3

Farhana, L., Antaki, F., Murshed, F., Mahmud, H., Judd, S.L., Nangia-Makker, P., Levi, E., Yu, Y., & Majumdar, A.P. (2918), Gut microbiome profiling and colorectal cancer in African Americans and Caucasian Americans. *World Journal of Gastrointestinal Pathophysiology*, 9(2): 47-58. https://doi.org/10.4291/wjgp.v9.i2.47.

Ferri, M., Serrazanetti, D. I., Tassoni, A., Baldissarri, M., & Gianotti, A. (2016). Improving the functional and sensorial profile of cereal-based fermented foods by selecting *Lactobacillus plantarum* strains via a metabolomics approach. *Food Research International, 89*, 1095–1105. https://doi.org/10.1016/j.foodres.2016.08.044

Garcia-Mantrana, I., Selma-Royo, M., Alcantara, C., & Collado, M. C. (2018). Shifts on gut microbiota associated to mediterranean diet adherence and specific dietary intakes on general adult population. *Frontiers in Microbiology, 9*, 890. https://doi.org/10.3389/fmicb.2018.00890

Gänzle, M. G. (2014). Enzymatic and bacterial conversions during sourdough fermentation. *Food Microbiology, 37*, 2–10. https://doi.org/10.1016/j.fm.2013.04.007

Gänzle, M. G. (2015). Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food biotechnology. *Current Opinion in Food Science, 2*, 106–117. https://doi.org/10.1016/j.cofs.2015.03.001

Gibson, P. R., & Shepherd, S. J. (2005). Personal view: Food for thought – western lifestyle and susceptibility to Crohn's disease. The FODMAP hypothesis. *Alimentary Pharmacology and Therapeutics, 21*, 1399–1409. https://doi.org/10.1111/j.1365-2036.2005.02506.x

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews, 17*, 259–275. https://doi.org/10.1079/NRR200479

González Hernández, M. A., Canfora, A. A., Jocken, J. W. E., & Blaak, E. E. (2019). The short-chain fatty acid acetate in body weight control and insulin sensitivity. *Nutrients, 11*, 1943–1975. https://doi.org/10.3390/nu11081943

Gueimonde, M., Tölkkö, S., Korpimäki, T., & Salminen, S. (2004). New real-time quantitative PCR procedure for quantification of bifidobacteria in human fecal samples. *Applied and Environmental Microbiology, 70*(7), 4165–4169. https://doi.org/10.1128/AEM.70.7.4165-4169.2004

Hevia, A., Delgado, S., Margolles, A., & Sánchez, B. (2015). Application of density gradient for the isolation of the fecal microbial stool component and the potential use thereof. *Scientific Reports, 5*, 16807. https://doi.org/10.1038/srep16807

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., & Pot, B. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*, 506–514. https://doi.org/10.1038/nrgastro.2014.66

Ihediohanma, N. C. (2011). Determination of the glycemic indices of three different cassava granules (garri) and the effect of fermentation period on their glycemic responses. *Pakistan Journal of Nutrition*, 10: 6–9.https://doi.org/10.3923/pjn.2011.6.9.

Jadán-Piedra, C., Alcántara, C., Monedero, V., Zúñiga, M., Vélez, D., & Devesa, V. (2017). The use of lactic acid bacteria to reduce mercury bioaccessibility. *Food Chemistry, 228*, 158–166. https://doi.org/10.1016/j.foodchem.2017.01.157

Jiang, J., Shi, B., Zhu, D., Cai, Q., Chen, Y., Li, J., … Zhang, M. (2012). Characterization of a novel bacteriocin produced by *Lactobacillus sakei*LSJ618 isolated from traditional Chinese fermented radish. *Food Control, 23*, 338–344. https://doi.org/10.1016/j.foodcont.2011.07.027

Kohajdova, Z., & Karovicova, J. (2007). Fermentation of cereals for specific purpose. *Journal of Food & Nutrition Research, 46*(2), 51–57.

Koistinen, V. M., Katina, K., Nordlund, E., Poutanen, K., & Hanhineva, K. (2016). Changes in the phytochemical profile of rye bran induced by enzymatic bioprocessing and sourdough fermentation. *Food Research International, 89*, 1106–1115. https://doi.org/10.1016/j.foodres.2016.06.027

La Marca, M., Beffy, P., Pugliese, A., & Longo, V. (2013). Fermented wheat powder induces the antioxidant and detoxifying system in primary rat hepatocytes. *PLoS ONE, 8*(12), 1–9. https://doi.org/10.1371/journal.pone.0083538

Leroy, F., De Winter, T., Adriany, T., Neysens, P., & De Vuyst, L. (2006). Sugars relevant for sourdough fermentation stimulate growth of and bacteriocin production by *Lactobacillus amylovorus* DCE 471. *International Journal of Food Microbiology, 112*, 102–111. https://doi.org/10.1016/j.ijfoodmicro.2006.05.016

Li, X., Shimizu, Y., & Kimura, I. (2017). Gut microbial metabolite short-chain fatty acids and obesity. *Bioscience Microbiota Food Health, 36*, 135–140. 10.12938/bmfh.17-010.

Lin, H. V., Frassetto, A., Kowalik, E. J., Jr., Nawrocki, A. R., Lu, M. M., Kosinski, J. R., … Marsh, D. J. (2012). Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS ONE, 7*, Article e35240. https://doi.org/10.1371/journal.pone.0035240

Liong, M. T., & Shah, N. P. (2005). Production of organic acids from fermentation of mannitol, fructooligosaccharide and inulin by a cholesterol removing *Lactobacillus acidophilus* strain. *Journal of Applied Microbiology, 99*, 783–793. https://doi.org/10.1111/j.1365-2672.2005.02677.x

Lu, Y., Fan, C., Li, P., Lu, Y., Chang, X., & Qi, K. (2016). Short chain fatty acids prevent high-fat-diet-induced obesity in mice by regulating G protein-coupled receptors and gut microbiota. *Scientific Reports, 6*, 37589. https://doi.org/10.1038/srep37589

Maekawa, M., Ushida, K., Hoshi, S., Kashima, N., Ajisaka, K., & Yajima, T. (2005). Butyrate and propionate production from D-mannitol in the large intestine of pig and rat. *Microbial Ecology in Health and Disease, 17*, 169–176. https://doi.org/10.1080/08910600500430730

Maldonando Galdeano, C., Cazorla, S. I., Lemme Dumit, J. M., Vélez, E., & Perdigón, E. (2019). Beneficial effects of probiotic consumption on the immune system. *Annals of Nutrition and Metabolism, 74*, 115–124. https://doi.org/10.1159/000496426

Mantziari, A., Tölkkö, S., Ouwehand, A.C., Löyttyniemi, E., Isolauri, E., Salminen, S., & Rautava, S. (2020). The effect of donor human milk fortification on the adhesion of probiotics *in vitro*. *Nutrients*, *12*: 182-195. https://doi.org/10.3390/nu12010182.

Marchesi, J. R., Adams, D. H., Fava, F., Hermes, G. D. A., Hirschfield, G. M., Hold, G., & Hart, A. (2015). The gut microbiota and host health: A new clinical frontier. *Gut, 65*, 330–339. https://doi.org/10.1136/gutjnl-2015-309990

Masisi, K., Beta, T., & Moghadasian, M. H. (2016). Antioxidant properties of diverse cereal grains: A review on in vitro and *in vivo* studies. *Food Chemistry, 196*, 90–97. https://doi.org/10.1016/j.foodchem.2015.09.021

Menezes, L. A. A., Minervini, F., Filannino, P., Sardaro, M. L. S., Gatti, M., & Lindner, J. D. D. (2018). Effects of Sourdough on FODMAPs in Bread and Potential Outcomes on Irritable Bowel Syndrome Patients and Healthy Subjects. *Frontiers in Microbiology, 9*, 1972. https://doi.org/10.3389/fmicb.2018.01972

Minervini, F., De Angelis, M., Di Cagno, R., & Gobbetti, M. (2014). Ecological parameters influencing microbial diversity and stability of traditional sourdough. *International Journal of Food Microbiology, 171*, 136–146. https://doi.org/10.1016/j.ijfoodmicro.2013.11.021

Mira-Pascual, L., Cabrera-Rubio, R., Ocon, S., Costales, P., Parra, A., Suarez, A., … Collado, M. C. (2015). Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers. *Journal of Gastroenterology, 50*, 167–179. https://doi.org/10.1007/s00535-014-0963-x

O'Neil, C. E., Nicklas, T. A., Zanovec, M., & Cho, S. (2010). Whole-grain consumption is associated with diet quality and nutrient intake in adults: The National Health and Nutrition Examination Survey, 1999–2004. *Journal of the American Dietetic Association, 110*, 1461–1468. https://doi.org/10.1016/j.jada.2010.07.012

Ojokoh1, A.O. & Eromosele, O. (2015). Effect of Fermentation on Proximate Composition and Microbiological Changes of Sorghum and Pumpkin Blend. *British Microbiology Research Journal*, 10(6): 1-14. https://doi.org/10.9734/BMRJ/2015/19260.

Osman, M. A. (2011). Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. *Journal of the Saudi Society of Agricultural, 10*, 1–6. https://doi.org/10.1016/j.jssas.2010.06.001

Paduano, D., Cingolani, A., Tanda, E., & Usai, P. (2019). Effect of Three Diets (LowFODMAP, Gluten-free and Balanced) on Irritable Bowel Syndrome Symptoms and Health-Related Quality of Life. *Nutrients, 11*(7), 1566. https://doi.org/10.3390/nu11071566

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics and synbiotics – a review. *Journal of Food Science and Technology, 52*, 7577–7587. https://doi.org/10.1007/s13197-015-1921-1

Patel, P. J., Singh, S. K., Panaich, S., & Cardozo, L. (2014). The aging gut and the role of prebiotics, probiotics, and synbiotics: A review. *Journal of Clinical Gerontology and Geriatrics, 5*, 3–6. https://doi.org/10.1016/j.jcgg.2013.08.003

Pejcz, E., Lachowicz-Wiśniewska, S., Nowicka, P., Wojciechowicz-Budzisz, A., Spychaj, R., & Gil, Z. (2021). Effect of Inoculated Lactic Acid Fermentation on the Fermentable Saccharides and Polyols, Polyphenols and Antioxidant Activity Changes in Wheat Sourdough. *Molecules, 26*, 4193–4202. https://doi.org/10.3390/molecules26144193

Petrova, P., & Petrov, K. (2020). Lactic acid fermentation of cereals and pseudocereals: Ancient nutritional biotechnologies with modern applications. *Nutrients, 12*, 1118–1144. https://doi.org/10.3390/nu12041118

Pharm, V. T., & Mohajeri, M. H. (2018). The application of *in vitro* human intestinal models on the screening and development of pre- and probiotics. *Beneficial Microbes, 9*(5), 725–742. https://doi.org/10.3920/BM2017.0164

Pranoto, Y., Anggrahini, S., & Efendi, Z. (2013). Effect of natural and *Lactobacillus plantarum* fermentation on in-vitro protein and starch digestibilities of sorghum flour. *Food Bioscience, 2*, 46–52. https://doi.org/10.1016/j.fbio.2013.04.001

Qi, D., Nie, X.-L., & Zhang, J.-J. (2020). The effect of probiotics supplementation on blood pressure: A systemic review and meta-analysis. *Lipids in Health and Disease, 19*, 79–90. https://doi.org/10.1186/s12944-020-01259-x

Rani, P., Kumar, A., Purohit, S. R., & Rao, P. S. (2018). Impact of fermentation and extrusion processing on physicochemical, sensory and bioactive properties of rice-black gram mixed flour. *LWT - Food Science and Technology, 89*, 155–163. https://doi.org/10.1016/j.lwt.2017.10.050

Rao, V. A. (2001). The prebiotic properties of FOS at low intake levels. *Nutrition Research, 21*, 843–848. https://doi.org/10.1016/S0271-5317(01)00284-6

Ripari, V., Bai, Y., & Gänzle, M. G. (2019). Metabolism of phenolic acids in whole wheat and rye malt sourdoughs. *Food Microbiology, 77*, 43–51. https://doi.org/10.1016/j.fm.2018.08.009

Rodríguez-Costa, S., Cobas, A. C., Saavedra, P. R., Arias, J. J. P., Miranda, J. M., & Cepeda, A. (2018). *In vitro* evaluation of the prebiotic effect of red and white grape polyphenolic extracts. *Journal of Physiology and Biochemistry, 74*, 101–110. https://doi.org/10.1007/s13105-017-0573-1

Rubio-del-Campo, A., Alcántara, C., Collado, M. C., Rodríguez-Díaz, J., & Yebra, M. J. (2020). Human milk and mucosa-associated disaccharides impact on cultured infant fecal microbiota. *Scientific Reports, 10*(1), 11845.

Sahin, A. W., Rice, T., Zannini, E., Axel, C., Coffey, A., Lynch, K. M., & Arendt, E. K. (2019). *Leuconostoc citreum*, TR116: *In-situ* production of mannitol in sourdough and its application to reduce sugar in burger buns. *International Journal of Food Microbiology, 302*, 80–89. https://doi.org/10.1016/j.ijfoodmicro.2018.06.026

Salazar, N., Ruas-Madiedo, P., Kolida, S., Collins, M., Rastall, R., Gibson, G., & de los Reyes-Gavilán, C.G. (2009). Exopolysaccharides produced by *Bifidobacterium longum* IPLA E44 and *Bifidobacterium animalis* subsp. *lactis* IPLA R1 modify the composition and metabolic activity of human faecal microbiota in pH-controlled batch cultures. *International Journal of Food Microbiology*, 135: 260–267. https://doi.org/10.1016/j.ijfoodmicro.2009.08.017.

Sarmiento-Rubiano, L. A., Zúñiga, M., Pérez-Martínez, G., & Yebra, M. J. (2007). Dietary supplementation with sorbitol results in selective enrichment of lactobacilli in rat intestine. *Research in Microbiology, 158*, 694–701. https://doi.org/10.1016/j.resmic.2007.07.007

Schmidt, M., & Sciurba, E. (2021). Determination of FODMAP contents of common wheat and rye breads and the effects of processing on the final contents. *European Food Research and Technology, 247*, 395–410. https://doi.org/10.1007/s00217-020-03633-6

Serpen, A., Gökmen, V., Karagöz, A., & Köksel, H. (2008). Phytochemical quantification and total antioxidant capacities of emmer (*Triticum dicoccon* Schrank) and einkorn (*Triticum monococcum* L.) wheat landraces. *Journal of Agricultural and Food Chemistry, 56*(16), 7285–7292. https://doi.org/10.1021/jf8010855

Setta, A. C., Matemu, A., & Mbega, E. R. (2020). Potential of probiotics from fermented cereal-based beverages in improving health of poor people in Africa. *Journal of Food Science and Technology, 57*, 3935–3946. https://doi.org/10.1007/s13197-020-04432-3

Singh, B. P., Vij, S., Hati, S., Singh, D., Kumari, P., & Minj, J. (2015). Antimicrobial activity of bioactive peptides derived from fermentation of soy milk by *Lactobacillus plantarum* C_2 against common foodborne pathogens. *International Journal of Fermented Foods, 4*, 91–99. https://doi.org/10.5958/2321-712X.2015.00008.3

Struyf, N., Laurent, J., Verspreet, J., Verstrepen, K. J., & Courtin, C. M. (2017). *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* co-cultures allow reduction of fermentable oligo-, di-, and monosaccharides and polyols levels in whole wheat bread. *Journal of Agricultural and Food Chemistry, 65*, 8704–8713. https://doi.org/10.1021/acs.jafc.7b02793

Sumarna (2008). Changes of raffinose and stachyose in soy milk fermentation by lactic acid bacteria from local fermented foods of Indonesian. *Malaysian Journal of Microbiology*, 4(2): 26-34. https://doi.org/10.21161/mjm.12208.

Verni, M., Rizzello, C. G., & Coda, R. (2019). Fermentation biotechnology applied to cereal industry by-products: Nutritional and functional insights. *Frontiers in Nutrition, 6*, 42. https://doi.org/10.3389/fnut.2019.00042

Walter, J., Hertel, C., Tannock, G. W., Lis, C. M., Munro, K., & Hammes, W. P. (2001). Detection of *Lactobacillus, Pediococcus, Leuconostoc*, and *Weissella* Species in Human Feces by Using Group-Specific PCR Primers and Denaturing Gradient Gel Electrophoresis. *Applied and Environmental Microbiology, 67*(6), 2578–2585. https://doi.org/10.1128/AEM.67.6.2578-2585.2001

Wang, Y.-C., Yu, R.-C., Yang, H. Y., & Chou, C.-C. (2003). Sugar and acid contents in soymilk fermented with lactic acid bacteria alone or simultaneously with bifidobacterial. *Food Microbiology, 20*(3), 333–338. https://doi.org/10.1016/S0740-0020(02)00125-9

Wisselink, H. W., Weusthuis, R. A., Eggink, G., Hugenholtz, J., & Grobben, G. J. (2002). Mannitol production by lactic acid bacteria: A review. *International Dairy Journal, 12*, 151–161. https://doi.org/10.1016/S0958-6946(01)00153-4

Wuyts, S., Van Beeck, W., Allonsius, C. N., van den Broek, M. F. L., & Lebeer, S. (2020). Applications of plant-based fermented foods and their microbes. *Current Opinion in Biotechnology, 61*, 45–52. https://doi.org/10.1016/j.copbio.2019.09.023

Yong, A. K. L., Lai, K. W., Ghazali, H. M., Chang, L. S., & Pui, L. P. (2020). Microencapsulation of *Bifidobacterium animalis* subsp. *lactis* BB-12 with Mannitol. *Asia-Pacific Journal of Molecular Biology and Biotechnology, 28*(2), 32–42. 10.35118/apjmbb.2020.028.2.04.

Zörb, C., Betsche, T., Langenkämper, G., Zapp, J., & Seifert, M. (2007). Free sugars in spelt wholemeal and flour. *Journal of Applied Botany and Food Quality, 81*, 172–174.

Further reading

Onipede, G. O., & Fawole, A. O. (2017). Antibacterial Activity of Lactic Acid Bacteria Isolated From Fermented Cereal Products Against Organisms Implicated in Gastrointestinal Tract Infections. *Nigerian Journal of Microbiology, 31*(2), 3979–3985.

Xu, L. N., Guo, S., & Sheng Wan Zhang. (2019). Effects of solid-state fermentation on the nutritional components and antioxidant properties from quinoa. *Emirates Journal of Food and Agriculture, 31*(1), 39–45. https://doi.org/10.9755/EJFA.2019.V31.I1.1898