INTERNATIONAL JOURNAL OF FOOD SCIENCES AND NUTRITION
2021, VOL. 72, NO. 3, 367–374
https://doi.org/10.1080/09637486.2020.1799956

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STUDIES IN HUMANS

Effect of consumption of ancient grain bread leavened with sourdough or with baker's yeast on cardio-metabolic risk parameters: a dietary intervention trial

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ABSTRACT

The aim of this study was to compare the effect of consumption of ancient grain "Verna" bread obtained by two different leavening agents, sourdough (SD) and baker's yeast (BY), on inflammatory parameters and cardiometabolic risk factors. Seventeen clinically healthy subjects were included to consume SD or BY bread for 4 weeks each, and blood analyses were carried out. The consumption of "Verna" bread obtained with both leavening agents led to a significant improvement of LDL cholesterol. A reduction of −10.6% and −8.53% was observed after replacement with SD and BY bread, respectively. A significant increase in fasting blood glucose (+6%) was observed only after the intervention with BY bread. A 10.7% decrease of vascular endothelial growth factor was found after the SD bread replacement period. The consumption of "Verna" bread resulted significantly associated with an improvement in the cardiometabolic and inflammatory profile. However, only consumption of BY bread determined a significant increase in blood glucose levels.

ARTICLE HISTORY
Received 23 June 2020
Revised 20 July 2020
Accepted 20 July 2020

KEYWORDS
Sourdough; baker's yeast; cereals; ancient grain; bread; clinical trial

Introduction

In industrialised countries, cardiovascular diseases (CVD) is the leading cause of death and disability. Nutrition is one of the main factors that can profoundly change the health status of individuals. Several studies in recent decades have clearly demonstrated the beneficial effect of the Mediterranean diet on the onset of chronic-degenerative diseases and on the modulation of CVD risk factors (Dinu et al. 2018). Moreover, several epidemiological studies have shown that the usual consumption of cereals – especially whole grains – is strongly correlated with a reduced incidence of CVD and cancer (Aune et al. 2016).

One of the key elements of the Mediterranean diet is certainly bread, a staple food consisting of water, flour and leavening agent. Human studies conducted so far have shown several positive effects on human health due to the consumption of bread made from ancient grain flour, such as the "Verna" variety (Sofi et al. 2010; Sereni et al. 2017). Ancient grains are reported as a potential source of healthy food products, due to their higher content of beneficial nutrients such as antioxidant molecules, vitamins and minerals (Dinu et al. 2018). However, the quality of bread does not only depend on the type of wheat used, but is also influenced by the baking process, including the type of leavening agents. Two types of leavening agent are commonly used: the sourdough and the baker's yeast.

The sourdough is a mixture of water and flour fermented by lactic acid bacteria and yeasts, responsible for acidification and leavening, respectively. The "backslopping" technique – which consists in keeping the sourdough active by using the sourdough of the previous fermentation cycle as a starter to ferment a new mixture of flour and water (Ercolini et al. 2013)– allows to select a stable and characteristic microbiota able to give to bakery products some specific sensorial, structural and nutritional characteristics. In particular, growing evidence suggests that sourdough fermentation is able to modulate the glycemic response to bread intake (Stamataki et al. 2017), to

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promote better post-prandial gastrointestinal function in healthy adults and to be more acceptable than those prepared with baker's yeast (Polese et al. 2018).

To date, only a few studies have investigated the effect of the type of leavening on the beneficial effects attributed to grain on health status, and none of these has taken into consideration the same bread made with ancient grain and different types of leavening. Therefore, the aim of this project is to compare the effect of consumption of ancient grain "Verna" bread with two different leavening agents, sourdough (SD) and baker's yeast (BY), on inflammatory parameters and cardiometabolic risk factors.

Materials and methods

Study population

The study population included 17 clinically healthy volunteers (7 women, 10 men) with a mean age of 34.6 ± 9.1 years and a mean body mass index (BMI) of 22.4 ± 2.5 kg/m^2. All subjects were recruited within the University of Florence staff and their family/friends. The inclusion criteria for participation in the study were: to be between 18 and 65 years of age, to be in good general health, not to be pregnant nor lactating, not to have gluten allergy or gastrointestinal disorders (e.g. chronic constipation, diarrhoea, inflammatory bowel disease, irritable bowel syndrome or other chronic gastrointestinal disorders) and gallbladder problems. Subjects were to be free of symptoms of vascular and inflammatory diseases. All participants were selected on the basis of their liking of wheat products and to be prepared to adhere to the diet as prescribed by the study period. This was ascertained prior to the experiment through a detailed interview, aimed at addressing both personal and familial history. Written informed consent was obtained from each participant before the start of the trial. Study procedures were approved by the Ethics Committee of the Tuscany Region, Careggi University Hospital (SPE 12.630), and adhered to the principles of the Declaration of Helsinki and the Data Protection Act.

Data collection and measurements

The participants were interviewed and examined at the University Hospital of Careggi (Florence, Italy) using standardised methods. At the time of the visit information was obtained regarding personal medical history, demography, family history of coronary or other atherosclerotic diseases, drugs, body weight, blood pressure, blood lipids, diabetes and lifestyle habits in relation to physical activity and smoking habit. Smokers were defined as those who smoked at the time of the physical examination. Diabetic subjects were classified according to the American Diabetes Association on the basis of self-reported data (if confirmed by medication or chart review). Dyslipidemia was defined, either according to the Third report of the National Cholesterol Education Programme (NCEP- III), or if the participant reported taking anti-dyslipidemic drugs, as verified by the physician. Hypertension (raised blood pressure) was defined as systolic blood pressure 140 mm Hg or more and/or diastolic blood pressure 90 mm Hg, in accordance with the guidelines of the European Society of Cardiology. Physical activity was assessed as either absent (sedentary lifestyle), mild or moderate, on the basis of the duration and intensity over the preceding 6 months. Weight was determined to the nearest 100 g by using a digital balance, and height was measured to the nearest 0.5 cm by using a wall-mounted stadiometer. BMI was calculated as weight (kg)/height (m)^2. Subjects were classified as overweight if their BMI was more than 25 kg/m^2 but less than 30 kg/m^2, and obese if their BMI was 30 kg/m^2 or more.

Study design

The present study was a randomised, double-blinded cross-over trial, with two intervention phases and a wash-out period. Before starting, a 2-week run-in period was performed for all subjects. After the run-in period, the eligible participants were randomly divided into 2 groups (Group A and Group B). For 4 weeks, 9 subjects (Group A) consumed bread made with ancient grain "Verna" and sourdough (SD), while 8 subjects (Group B) consumed control bread, made with ancient grain "Verna" and baker's yeast (BY). At the end of the first intervention phase, a wash-out period of 4 weeks was carried out, during which the participants were allowed to eat all the foods according to their usual eating habits. After the wash-out period, the second intervention phase was implemented, with Group A assigned to consume the control bread, made with ancient grain "Verna" and BY and Group B assigned to consume bread made with ancient grain "Verna" and SD.

Participants were provided 150 g/day of bread prepared using ancient grain (*Triticum aestivum*), "Verna" cultivar, at the bakery Forno Garbo s.r.l (Florence, Italy). The quantity of bread to be consumed was supplied twice a week to each participant, in weighed portions, at no cost. All the procedures for

the preparation of the transformation were identical both for the intervention breads and for the control breads, with the exception of the type of leavening agent used. The two types of breads seemed to be similar, making it impossible to distinguish them. Throughout the study period, all participants were instructed to maintain their usual eating and lifestyle habits and were not allowed to eat other types of bread. At the baseline and the end of each phase, blood samples were taken from all subjects between 07:00 and 09:30 after a 12 h fasting period. In addition, subjects were asked not to engage in intense physical activity during the day before the test.

Sourdough sampling and processing

SD was sampled once a week until the end of the study period for microbiological and chemical analysis. After sampling at the bakery, the microorganisms were enumerated. Ten grams of SD was transferred into 90 mL of sterile physiological saline solution, then homogenised for 2 min in a Stomacher Lab Blender 400 (Seward Ltd, Worthing, West Sussex, UK). After decimal dilutions, 100 µL of these suspensions were plated for cell enumeration in specific media using the pour plate method. For lactic acid bacteria (LAB) enumeration MR3I agar (Galli et al. 2019) was used. After 24–48 h of incubation at 30 °C under microaerophilic conditions, LAB colonies were counted. Yeasts, plated on MYPG agar containing sodium propionate (2 g/L), were counted after incubation for 48 hours at 30 °C under aerobic conditions. Plate counts were performed in duplicate. At least 10 colonies of presumed LAB and yeasts for each sampling point were randomly selected from the plates and then analysed by molecular methods.

Genotypic identification of lactic acid bacteria and yeasts

LAB isolates were identified by Amplified Ribosomal DNA Restriction Analysis (ARDRA). DNA was extracted and 16S rDNA gene was amplified in a thermocycler (Techne LTD, Cambridge, UK) using the primers FD1 (5′-CAACAGAGTTTGATCCTGGCT CAG-3′) and RD1 (5′-GCTTAAGGAGGTGATCCAG CC-3′). The 16S rDNA PCR products were purified using the Nucleo Spin Extract II (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and sent to BMR Genomics (Padua, Italy) for sequencing. The sequences obtained in FASTA format were compared with those stored in the GenBank DNA database using the basic BLAST search tools. Yeast isolates were identified by PCR-RFLP analysis of the rDNA ITS (Internal Transcribed Spacer) region as described by Granchi et al. (1999) using *Hae*III and *Hinf*I (Fermentas Inc, Burlington, Ontario, Canada) as restriction endonucleases. The restriction fragments were separated (at 100 volt for 2.5 h) on 2% (w/v) agarose gel (Lonza Group Ltd, Basel, Switzerland), containing ethidium bromide (Sigma Aldrich, St Louis, Missouri, US) and TEB buffer (1 M Tris, 10 mM EDTA, 0.9 M boric acid, pH 8.3). The profiles were observed by UV transillumination and compared with those reported in the literature (Granchi et al. 1999; Pulvirenti et al. 2004). The profiles were captured on a UV transilluminator.

Sourdough chemical analyses

The pH values were determined by a pH-meter (Metrohm Italiana Srl, Varese, Italy). The total titratable acidity (TTA) was measured using 10 g of dough samples, homogenised with 90 mL of distilled water for 3 minutes and expressed as a quantity (mL) of 0.1 N NaOH to reach a pH of 8.5. For organic acid determination, the SD samples were diluted ten times with distilled water and then filtered by Amicon® Ultra-4 Centrifugal Filters (3.000 Da NMWL) (Merck Millipore, Burlington, MA, US) before the injection. Organic acids were determined by High-Performance Liquid Chromatography (HPLC) analysis (Varian Inc, Palo Alto, CA, US) according to Venturi et al. (2012). The fermentation quotient (FQ), defined as the molar ratio between lactic and acetic acids, was calculated.

Blood measurements

Venous blood samples were taken by the study physician after an overnight fasting, into evacuated plastic tubes (Vacutainer, BD, Oxford, UK). Samples, obtained by centrifuging at 3,000 xg for 15 min at 4 °C, were stored in aliquots at −80 °C until analysis. Lipid variables, blood glucose, serum electrolytes and serum minerals were assessed by conventional methods. Pro- and anti-inflammatory cytokines were determined by using the Bio-Plex cytokine assay (Bio-Rad Laboratories Inc., Hercules, CA, US), according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the statistical package PASW 20.0 for Macintosh (SPSS, Inc.). All variables were checked for a normal distribution

Table 1. Microbiological and chemical characterisation of sourdough used for the manufactured of experimental breads.

Microbiological parameters			Acidification parameters		
LAB (CFU/g)	Yeasts (CFU/g)	LAB/YS	pH	TTA(mL)	FQ
$(2.64 \pm 0.78) \times 10^8$	$(5.75 \pm 1.97) \times 10^6$	46	4.01 ± 0.25	10.77 ± 2.38	3.61

Results are expressed as mean ± standard deviation. CFU: colony forming units; FQ: fermentation quotient; LAB: lactic acid bacteria; LAB/YS: LAB/Yeasts ratio; TTA: total titratable acidity.

before data analysis. Data that were not normally distributed were logarithmically transformed. Data were expressed as arithmetic means and standard deviations or geometric mean with 95% confidence intervals, as appropriate. Categorical variables were presented in terms of frequencies and percentages. The $\chi 2$ test was used for dichotomous variables. Absolute change (mean baseline value subtracted from mean value after intervention) was estimated by an independent sample t test. All data were treated as paired samples from a crossover study. The two interventions were analysed by taking into account both periods in the two groups of subjects at different stages. A general linear model for repeated measurements, with adjustments for age and gender, was used to compare the effect of the two different treatments. We evaluated the sequence effect to confirm whether the impacts of the intervention and control breads were different when the order of administration changed. This effect was estimated by comparing the geometric mean change difference between the treatments in the intervention and control groups after adjustment for the order of treatment. A p value $<.05$ was considered to indicate statistical significance.

Results

Microbiological and chemical characterisation of the sourdough

The results of the microbiological and chemical parameters, commonly used to characterise sourdough, are reported in Table 1.

Data were collected during the study period by SD sampling at the bakery. SD was characterised by a stability of the considered parameters, although they showed some differences during the study period. LAB were found in higher concentration compared to yeasts, (2.64 ± 0.78) × 108 and (5.75 ± 1.97) × 106 UFC/g, respectively. The chemical analyses pointed out an adequate acidification in terms of pH and total titratable acidity. Lactic and acetic acid content led to a fermentation quotient of 3.61. Molecular analysis showed the stable presence of two LAB species, *Lactobacillus sanfranciscensis* and *Lactobacillus plantarum*, with a slight prevalence of *Lactobacillus sanfranciscensis*, 60% of the isolates; the dominant yeast population was *Saccharomyces cerevisiae*.

Study population

The mean age of the participants was 34.6 ± 9.1 years, with a statistically significant difference ($p = .015$) among the two groups (SD: 38.4 ± 8.1 vs. BY: 30.3 ± 8.5 years). No significant differences were reported for body weight, BMI, cardiovascular risk factors, family history for cardiovascular disease, smoking habit or physical activity.

Variations in the biochemical profile

A general linear model for repeated measurement, adjusted for age and gender, showed a statistically significant reduction of LDL cholesterol after both SD and BY (Table 2).

In particular, a reduction of −10.6% ($p = .025$) and −8.53% ($p = .047$) was observed after SD and BY, respectively. Furthermore, a statistically significant increase of fasting blood glucose (+6%; $p = .012$) was observed only after BY, while no significant variations were observed after SD. No significant changes were reported for triglycerides, total cholesterol, HDL cholesterol, and uric acid.

With regard to the mineral profile, a significant increase in potassium (+8.3%; $p < .001$) was reported only after BY, with an opposite and statistically significant trend of variation between the two interventions ($p = .003$). On the other hand, a significant decrease (–3.6%; $p = .028$) in calcium was reported only after SD, with an opposite and statistically significant trend of variation between the two interventions ($p = .034$). An opposite trend of variation was also observed for sodium levels, which increased of +0.9% after BY and decreased of −0.9% after SD. No significant changes were reported for iron and magnesium levels.

Variations in the inflammatory profile

The inflammatory profile was also tested in the study population through an evaluation of various pro- and anti-inflammatory cytokines (Table 3).

A general linear model for repeated measurement, adjusted for age and gender, showed a statistically significant ($p = .039$) reduction in the circulating levels of pro-inflammatory Vascular Endothelial Growth Factor (VEGF) (−10.7%) after SD period. No

Table 2. Modifications in the biochemical profile.

Variable	SD pre	SD post	*p*-value	BY pre	BY post	*p*-value	Change SD	Change BY	*p*-value°
Triglycerides, mg/dL	97.82 (78.95; 116.69)	101.53 (84.01; 119.05)	.769	93.29 (80.13; 106.46)	96.82 (80.86; 112.79)	.545	3.71 (–22.86; 30.28)	3.53 (–8.67; 15.73)	.518
Total Cholesterol, mg/dL	186.29 (164.37; 208.22)	177.88 (158.24; 197.53)	.087	180.47 (162.87; 198.07)	172.53 (156.53; 188.53)	.087	–8.41 (–18.23; 1.41)	–7.94 (–17.20; 1.32)	.999
HDL-cholesterol, mg/dL	56.71 (51.10; 62.31)	59.24 (54.04; 64.44)	.136	61.12 (55.35; 66.88)	61.06 (55.83; 66.29)	.973	2.52 (–0.90; 5.96)	–0.06 (–3.77; 3.65)	.290
LDL-cholesterol, mg/dL	110.02 (90.95; 129.10)	98.34 (81.02; 115.66)	.025	100.69 (84.84; 116.55)	92.11 (78.82; 105.39)	.047	−11.68 (–21.66; −1.71)	–8.59 (–17.03; −0.15)	.786
Uric Acid, mg/dL	4.40 (3.92; 4.88)	4.48 (3.94; 5.02)	.741	4.14 (3.51; 4.78)	4.22 (3.76; 4.69)	.639	0.08 (–0.44; 0.61)	0.08 (–0.29; 0.45)	.734
Glucose, g/L	82.71 (78.85; 86.56)	83.29 (79.01; 87.58)	.759	79.82 (76.29; 83.36)	84.65 (81.14; 88.16)	.012	0.59 (–3.45; 4.62)	4.82 (1.25; 8.39)	.150
Iron, µg/dL	83.59 (70.22; 96.96)	87.94 (73.34; 102.55)	.281	99.76 (84.35; 115.18)	97.35 (84.23; 110.47)	.767	4.35 (–3.97; 12.67)	–2.41 (–19.56; 14.73)	.290
Sodium, mEq/L	141.96 (140.42; 141.70)	139.94 (138.63; 141.26)	.143	139.82 (138.99; 140.66)	141.06 (139.93; 142.19)	.062	−1.12 (–2.66; 0.43)	1.24 (–0.07; 2.54)	.011
Potassium, mEq/L	4.36 (4.21; 4.51)	4.27 (4.07; 4.47)	.354	4.13 (3.98; 4.28)	4.47 (4.38; 4.55)	<.001	–0.09 (–0.29; 0.11)	0.34 (0.21; 0.46)	.003
Calcium, mg/dL	9.32 (9.06; 9.58)	8.98 (8.74; 9.21)	.028	9.08 (8.86; 9.30)	9.15 (8.91; 9.40)	.442	–0.34 (–0.64; −0.04)	0.07 (–0.12; 0.26)	.034
Magnesium, mg/dL	2.26 (2.18; 2.34)	2.14 (2.06; 2.22)	.058	2.34 (2.21; 2.46)	2.19 (2.11; 2.28)	.106	–0.12 (–0.24; 0.00)	–0.14 (–0.32; 0.03)	.683

SD: Sourdough; BY: Baker's yeast General linear model for repeated measurements, adjusted for age and gender. Data are reported as geometric mean and 95% confidence interval ° independent *t*-test (for changes between the intervention and control groups).

Table 3. Modifications in the inflammatory profile.

Variable	SD pre	SD post	*p*-value	BY pre	BY post	*p*-value	Change SD	Change BY	*p*-value°
Interleukin-1ra, pg/mL	21.58 (12.60; 30.56)	26.01 (17.79; 34.22)	.079	23.72 (13.32; 34.12)	26.98 (11.92; 42.03)	.548	4.43 (–0.59; 9.44)	3.33 (–8.08; 14.59)	.433
Interleukin-4, pg/mL	0.27 (0.17; 0.37)	0.27 (0.17; 0.37)	.865	0.28 (0.19; 0.37)	0.23 (0.15; 0.31)	.178	–0.00 (–0.04; 0.03)	–0.06 (–0.15; 0.03)	.540
Interleukin-6, pg/mL	4.90 (1.49; 8.31)	6.84 (0.74; 12.93)	.423	6.56 (1.21; 11.92)	5.87 (0.91; 10.82)	.682	1.93 (–3.10; 6.96)	–0.69 (–4.24; 2.86)	.170
Interleukin-8, pg/mL	49.55 (37.77; 61.33)	44.85 (30.83; 58.87)	.416	57.75 (37.36; 78.14)	42.52 (33.87; 51.16)	.086	–4.70 (–16.71; 7.32)	–15.23 (–32.93; 2.47)	.760
Interleukin-10, pg/mL	12.82 (10.31; 15.33)	10.73 (8.53; 12.92)	.124	12.70 (9.74; 15.65)	9.90 (7.37; 12.42)	.131	–2.09 (–4.84; 0.65)	–2.80 (–6.54; 0.94)	.586
Interleukin-12, pg/mL	10.47 (6.58; 14.36)	9.68 (7.08; 12.27)	.449	10.84 (6.94; 14.74)	9.25 (5.92; 12.57)	.237	–0.80 (–2.99; 1.40)	–1.60 (–4.37; 1.17)	.734
Interleukin-17, pg/mL	2.65 (1.95; 3.34)	2.33 (1.54; 3.11)	.253	2.66 (2.03; 3.29)	2.18 (1.29; 3.08)	.301	–0.32 (–0.90; 0.26)	–0.47 (–1.42; 0.47)	.496
MCP-1, pg/mL	37.76 (32.80; 42.73)	37.26 (30.60; 43.93)	.827	35.33 (29.71; 40.94)	36.13 (27.50; 44.75)	.859	–0.50 (–5.31; 4.31)	0.80 (–8.74; 10.34)	.708
VEGF, pg/mL	34.51 (23.27; 45.75)	30.81 (20.25; 41.36)	.039	36.56 (23.27; 49.85)	35.62 (21.20; 50.04)	.750	–3.70 (–7.19; −0.213)	–0.94 (–7.14; 5.26)	.760
TNF-alpha, pg/mL	6.30 (4.57; 8.04)	4.89 (3.29; 6.48)	.151	6.15 (4.51; 7.79)	7.48 (3.51; 11.44)	.429	–1.42 (–3.42; 0.583)	1.33 (–2.16; 4.82)	.290

SD: Sourdough; BY: Baker's yeast; MCP-1: Monocyte Chemotactic Protein-1; TNF-alpha: Tumour Necrosis Factor-alpha; VEGF: Vascular Endothelial Growth Factor General linear model for repeated measurements, adjusted for age and gender. Data are reported as geometric mean and 95% confidence interval ° independent *t*-test (for changes between the intervention and control groups).

significant changes were reported for the remaining pro- and anti-inflammatory cytokines, even if a decreasing trend was observed after both SD and BY.

Discussion

This study is the first evidence of dietary intervention that evaluated the effects of consumption of ancient grain bread with two different types of leavening agent, SD and BY, on cardiometabolic and inflammatory parameters in healthy adults. Regarding the biochemical profile, a statistically significant reduction in LDL cholesterol after both SD and BY periods, and an increase of fasting blood glucose only after BY period, were observed. Regarding the inflammatory profile, a significant reduction of VEGF was observed only after SD period, although both SD and BY led to a decreasing trend of almost all inflammatory cytokines.

Bread is one of the major staple food in human nutrition. It has been widely demonstrated that consumption of bread – and in particular whole-meal bread – is associated with positive health outcomes such as coronary heart disease, CVD, total cancer and mortality from natural causes (Aune et al. 2016). However, not all types of bread have the same positive effects on human health. The taste, texture and nutritional properties are profoundly influenced by the type of grain used, as well as the baking process and the type of leavening. We have recently reported beneficial effects of bread made with ancient grains on several cardiometabolic risk parameters (Dinu et al. 2018). However, there is still a certain degree of uncertainty on the possible effect of leavening on the contribution of these beneficial effects.

In the last decade, rapid leavening processes by chemicals and/or BY have almost replaced the use of SD. The polymeric components of cereals (i.e. proteins and starch) undergo a very slight or absent hydrolysis during processing with these leavening agents (Rizzello et al. 2019). On the contrary, the synthesis of organic acids, the activation of different enzymes and the synthesis of microbial metabolites occurring during SD fermentation have the ability to positively influence the sensory, consistency, nutritional and shelf-life characteristics of bread (Gobbetti et al. 2014). Among the main advantages related to the use of SD, the increase in the digestibility of proteins in vitro, and the amount of soluble fibres, the decrease in the glycaemic index, the phytate content, trypsin inhibitors, and other anti-nutritional factors, have been described (Montemurro et al. 2019). These properties relies on the stability of the microbial populations that are present in the sourdough.

In the present paper, the SD made with "Verna" flour and used for the manufacture of breads consumed by the participants was characterised by a stability of the microbiological and chemical parameters. LAB and yeast concentrations and LAB/yeast ratio were typical of a mature and stable type I SD. Furthermore, the three identified dominant species are often associated with sourdough microbiota; in particular, *Lactobacillus sanfranciscensis* is considered the key species in this ecosystem. The values of acidification, expressed as pH and total titratable acidity, are consistent with those reported for stable SD (Venturi et al. 2012; Lattanzi et al. 2013), with a FQ value considered optimal for a positive influence on the aromatic profile and structure of the final product (Spicher 1983). Regarding the blood parameter analyses, we have observed a significant increase in fasting blood glucose only after the consumption of bread leavened with BY, while no variations were observed after the intervention with SD, so supporting the hypothesis that SD helps in reducing the glycemic index of the cereal consumed. To the best of our knowledge, no clinical intervention studies have investigated the effect of the consumption of products leavened with SD or BY on the blood glucose in a relatively long-term period since most of them have evaluated the "acute" effect of the ingestion of several cereal-based foods subjected to different types of leavening on the postprandial blood glucose (Maioli et al. 2008; Najjar et al. 2008; Scazzina et al. 2009). Evidences suggest that postprandial glucose response resulted to be improved after consumption of SD bread with respect to bread made with BY (Stamataki et al. 2017), regardless of the type of grain used (Scazzina et al. 2009). In fact, it has been previously demonstrated that SD fermentation reduces the availability of simple carbohydrates, increases the phenolic compounds and the fibre content of the bread (i.e. resistant starch) as well as delays gastric emptying, consequently improving the glycaemic response (Rizkalla et al. 2007).

With regard to cardiometabolic and inflammatory risk parameters, we have observed a significant decrease in LDL cholesterol and almost all pro-inflammatory cytokines after both intervention with the ancient "Verna" grain, regardless of the type of leavening. These results are in line with previous findings obtained in 2010 and 2017 by our group using the same ancient grain variety (Sofi et al. 2010; Sereni et al. 2017). These improvements could be related to

the higher amounts of dietary fibre, minerals and antioxidants present in the flour of this ancient variety (Sofi et al. 2010; Di Silvestro et al. 2012). Indeed, it has been widely demonstrated that an increase in fibre consumption is associated with a reduction in the absorption of dietary cholesterol (Brown et al. 1999). Similarly, adequate amounts of minerals (i.e. Cu, Zn, Mg, Mn) and antioxidant substances have been linked to reduced levels of free radicals which further leads to a reduced risk of inflammation (Arulselvan et al. 2016). On the other hand, it is interesting to note that a reduction of an important pro-inflammatory cytokine such as VEGF was reported only after the intervention with SD, while no significant changes emerged after the intervention with BY. Previous studies conducted by our University showed the production, by selected lactic acid bacteria, of peptides with antioxidant and anti-inflammatory activity in cultured cells during sourdough fermentation (Galli et al. 2018). Interestingly, recent findings indicated that these biological activities were also maintained after cooking, in the final product (Luti et al. 2020). This discovery may help to understand the reasons behind the anti-inflammatory properties of this ancient grain leavened with SD.

Finally, with regard to the mineral profile, we observed that SD led to a reduction in calcium and BY to an increase of potassium. These results seem to be controversial. In fact, it has been demonstrated that whole-meal flour contains considerable amounts of phytic acid which, by chelating the minerals, reduces their bioavailability, suggesting how the consumption of whole-meal bread can lead to a decrease in the absorption of minerals (Carbonetto et al. 2018). However, it has been shown that the phytate content is reduced in SD bread (Lopez et al. 2001). Previous studies of our group reported a significant increase of almost all minerals after the consumption of "Verna" bread, due to the higher amount of minerals typical of the ancient grain variety (Sofi et al. 2010; Sereni et al. 2017). Further intervention studies are needed to clarify these differences in results.

This study has several limitations. First, the limited sample size of the study. Although the results are promising, they should be interpreted with caution, unless validated in a larger population. Second, the lack of assessment of dietary habits in our study population. The possibility that changes in dietary habits may have had a significant influence on the parameters examined cannot be excluded, although participants received strict instructions to maintain their usual eating habits during the course of the study. Finally, the lack of a control condition with the intake of regular bread would ensure a better comparison of results.

Conclusion

In conclusion, the SD and BY breads made with ancient "Verna" grain seem to maintain their beneficial effects regardless of the type of leavening. However, the consumption of bread made with ancient "Verna" grain and leavened with SD has reported to have neutral effects on blood glucose compared to the consumption of bread made with the same ancient grain but leavened with BY that reported a significant increase in glucose levels. These results could be useful to recommend the consumption of bread in adequate quantities obtained in an optimal way in order to pursue the objectives functional to the improvement of the health status. However, larger and well-designed studies taking into account dietary habits of the participants are needed to confirm these promising results.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was funded by Fondazione Cassa di Risparmio, Florence, Italy.

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