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Effect of the Leavening Agent on the Compositional and Sensorial Characteristics of Bread Fortified with Flaxseed Cake

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Featured Application: Different fortified bread formulations were developed combining the use of flaxseed cake as an extra ingredient and different leavening agents (sourdough versus baker's yeast). By the analysis of physicochemical, nutritional, and sensorial data, the best recipe in terms of percentage of flaxseed cake as a function of the leavening agent utilized was defined. This research approach allowed achieving the desired quality level of the flaxseed cake fortified bread regardless of the leavening agent utilized. Therefore, the innovative approach proposed could represent a tool for the bakery industry to select the breadmaking process that better fits with their production needs.

Abstract: Health and well-being improvement is currently driving innovation in bread, using a wide variety of value-added compounds as extra ingredients, including food industry by-products in a circular economy concept. In this context, this research aimed at evaluating the effect of the fortification of bread with different percentages of flaxseed cake, comparing two leavening agents: sourdough and baker's yeast. Sensorial, physicochemical, and nutritional properties, including pH, the main fermentative metabolites, fatty acids, total phenols, antioxidant capacity, and volatile organic compounds were determined for fortified bread. The results showed a significant improvement of nutraceutical profile of the bread fortified with flaxseed cake in a dose-dependent manner. Regardless of the leavening agent, the fortification determined a decrease of n-6:n-3 ratio, reaching the recommended value (<3) already at the 7.5% level. Furthermore, under the same fortification level, sourdough breads showed a higher level of total phenols and antiradical activity than baker's yeast breads. Sensory profiles were instead deeply influenced by both the fortification percentage and the leavening agents. In conclusion, considering both nutritional and sensory results, the best formulation as a function of leavening agent utilized was defined as 5% and 7.5% when sourdough and baker's yeast were used, respectively.

Keywords: sourdough; baker's yeast; bread; flaxseed; fortification; by-products; nutraceuticals; sensory analysis

1. Introduction

Bread is a baked dietary item that is obtained from the fermentation of wheat flour sugars liberated from starch by the action of natural flour enzymes [1]. The nutritional composition of bread as well as its sensory, texture, and shelf life features depend on the bread formulation (type of flour used to produce the bread, addition of fortifying ingredients, choice of the leavening agent) [1–7].

Basically, bread is produced from flour of cereal grains (i.e., wheat, rye, oats) and/or the flour of pseudo-cereals and/or legumes, water, and a leavening agent. Commonly, two categories of leavening agents are used in breadmaking: chemical (mainly used at industrial levels) and biological (i.e., baker's yeast or sourdough).

Between biological ones, baker's yeast (the trade name of the yeast strain from the species *Saccharomyces cerevisiae*) is the primary leavening agent in breadmaking, especially for industrial bakeries due to its technological properties [8]. Yeast commercial strains were industrially selected on the basis of their performance, in terms of fermentation power, development of flavor, and increase of nutritional value [9]. Baker's yeast has the advantages of simplifying the manufacturing process, getting the highest yields possible and reducing costs [10].

Sourdough, instead, is an old biological starter that is traditionally used in regional bakery products. With respect to baker's yeast, it is associated with many advantages, such as an improved bread profile, in terms of nutritional value, sensory profile, rheological features, and shelf life [11,12]. It is basically a dough constituted by flour and water and spontaneously fermented by yeasts and lactic acid bacteria (LAB) [3,13], the metabolic activity of which, such as lactic acid fermentation, proteolysis, synthesis of flavors, and antimicrobial compounds, is fundamental in affecting the sourdough bread quality [14].

The performance of biological leavening agents during bread dough fermentation is deeply influenced by their genetic background, the ingredients of dough, and the process parameters [8].

For all these reasons, any modifications of the breadmaking process or recipe therefore lead to changes in the quality of the final product. This is particularly important, especially since several researches have been recently performed about the development of new bread formulations that are suitable for nutritional and health improvement using bread as a vehicle for nutraceutical delivery considering its worldwide consumption [6,15–18]. Nowadays, a notable economic growth of the nutraceutical products has been observed due to the rapidly changing lifestyle of people, which is focusing more and more on food sustainability and healthy eating.

In this context, oilseeds, pulses, and fibers as well as food by-products are frequently used to improve the technological properties (water absorption, viscoelastic properties, shelf life, rheological features, etc.) and the nutritional profile of bakery products, as they are good sources of amino acids, vitamins, minerals, and fats but also bioactive compounds, such as antioxidants and fibers, which are useful to boost their nutraceutical profile [5,6,19–27]. Moreover, also, the new trend of the food industry in exploring the potential use of edible insects' flour as protein-rich ingredient for bakery products should be mentioned, although Western consumers consider insect as an unusual food ingredient, and most of them are repelled by the idea of eating insect-based food [28]. Finally, in relation to animal-based protein powder, although animal-derived proteins constitute the majority of the protein consumed in the human diet, plant-derived proteins can satisfy the same requirement with less environmental impact [29].

Many researchers are focusing their attention on the seeds of *Linum usitatissimum* (flaxseed) as a tool to enrich the nutritional profile of food, due to their richness of beneficial components [30]. In particular, many studies are available in the literature about the use of flaxseed and its by-products, mainly seed cake, to improve bakery product compositional attributes, especially fatty acid profiles (α-linolenic acid, n-6 to n-3 ratio, etc.), soluble and insoluble dietary fiber (mucilage gums, cellulose and lignans, i.e., secoisolariciresinol diglucoside), plant protein and micronutrients (vitamins, i.e., tocopherols, and minerals as calcium, magnesium, and phosphorus) content [5,31–35].

Namely, flax lignans, which are known for their antioxidant and healthy properties [34], are proved to be stable to baking conditions without losing their functionality [35]. Moreover, the fortification with ground flaxseed hulls at levels lower than 5% was sufficient to significantly boost the antioxidant power in breads [23].

Furthermore, although the addition of oilseeds can deeply affect the organoleptic profile as well as the technological features of the product [36], it has been demonstrated that if compared with other oilseed used for bread fortification such as chia, sunflower, sesame, etc., the incorporation of flaxseed does not significantly affect the consumption preferences [6].

Together with the scientific evidence supporting the consumption of flaxseed for its peculiar nutraceutical profile [37], the recovery of its by-products, such as the flaxseed cake obtained after the industrial oil extraction process, is a topic of growing importance. After screw-pressed oil extraction, a huge amount of bioactive compounds remains in the pressed flaxseed cake, making it a valuable by-product. Thus, flaxseed cake, can be proficiently valorized through novel food product development, in accordance with a circular economy perspective [38].

Thereafter, recently our research group investigated the effect of fortification by means of flaxseed cake in combination with the use of sourdough as a leavening agent [39].

The choice of the leavening agent actually affects a number of technological factors (e.g., costs and time of processing) as well as the bread sensory, texture, nutritional, and shelf life features [12,40–43].

Nevertheless, to the best of our knowledge, there are no studies available in the literature aimed at evaluating the effect of flaxseed cake fortification as a function of the leavening agents adopted.

Thus, we investigated how and if the choice between sourdough fermentation and the use of baker's yeast could exploit the potential of flaxseed cake to fortify bread, focusing on its compositional and sensory attributes.

So, in the present study, fortified bread at increasing percentages of flaxseed cake were produced using baker's yeast or sourdough fermentation to assess the effect of leavening strategy on bread fortification efficacy as well as on bread quality. Breads obtained by baker's yeast or sourdough fermentation were subjected to a compositional and sensory characterization and compared to assess the effect of fermentation on bread fortification by flaxseed cake. Finally, the best formulation as a function of both the leavening agent and the percentage of flaxseed cake used will be individuated to produce new products with nutritional advantages and precise sensory characterization.

2. Materials and Methods

2.1. Chemicals

All chemicals used for the analysis, including standard, methanol (HPLC grade), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). A Milli-Q water purification system from Millipore (Bedford, MA, USA) was used for water purification.

2.2. Raw Material

The sourdough was supplied by Dolcezze Savini Srl (Florence, Italy), while fresh compressed baker's yeast was produced by Zeus Iba S.r.l. (Florence, Italy).

The wheat flours (hard wheat flour type 0) used for the back-slopping and the breadmaking (weak wheat flour type 0) were provided by Molino F.lli Giambastiani Srl (Lucca, Italy).

Brown seeded flax seeds, belonging to Sideral variety, were organically produced in the 2017–2018 growing season in the lowland area of the Pisa Province (Northern Tuscany, Italy, latitude 43°40'48'' N, longitude 10°30'1'' E). After harvest, accomplished at the beginning of July, the seeds were cleaned and cold-pressed in an edible oils manufacturing industry, through a screw press. The process is able to provide both virgin oil and seed cake; the latter represents an excellent co-product with great potential.

Flaxseed cake was ground and stored at −20 °C in a sealed vacuum container until analysis and processing. Chemical characterization (dry matter, water activity, protein percentage, fat percentage, total phenols, total flavonoids, antioxidant capacity, most representative fatty acids) of both flour type 0 and flaxseed cake was determined as previously reported [39].

2.3. Bread Preparation

Sourdough was maintained through consecutive back-slopping procedures to provide constant and repeatable leavening and acidifying performances, which were periodically monitored [3]. The back-slopping procedure as well as baking protocol and operating conditions (time and temperature) adopted were performed as described in Figure 1.

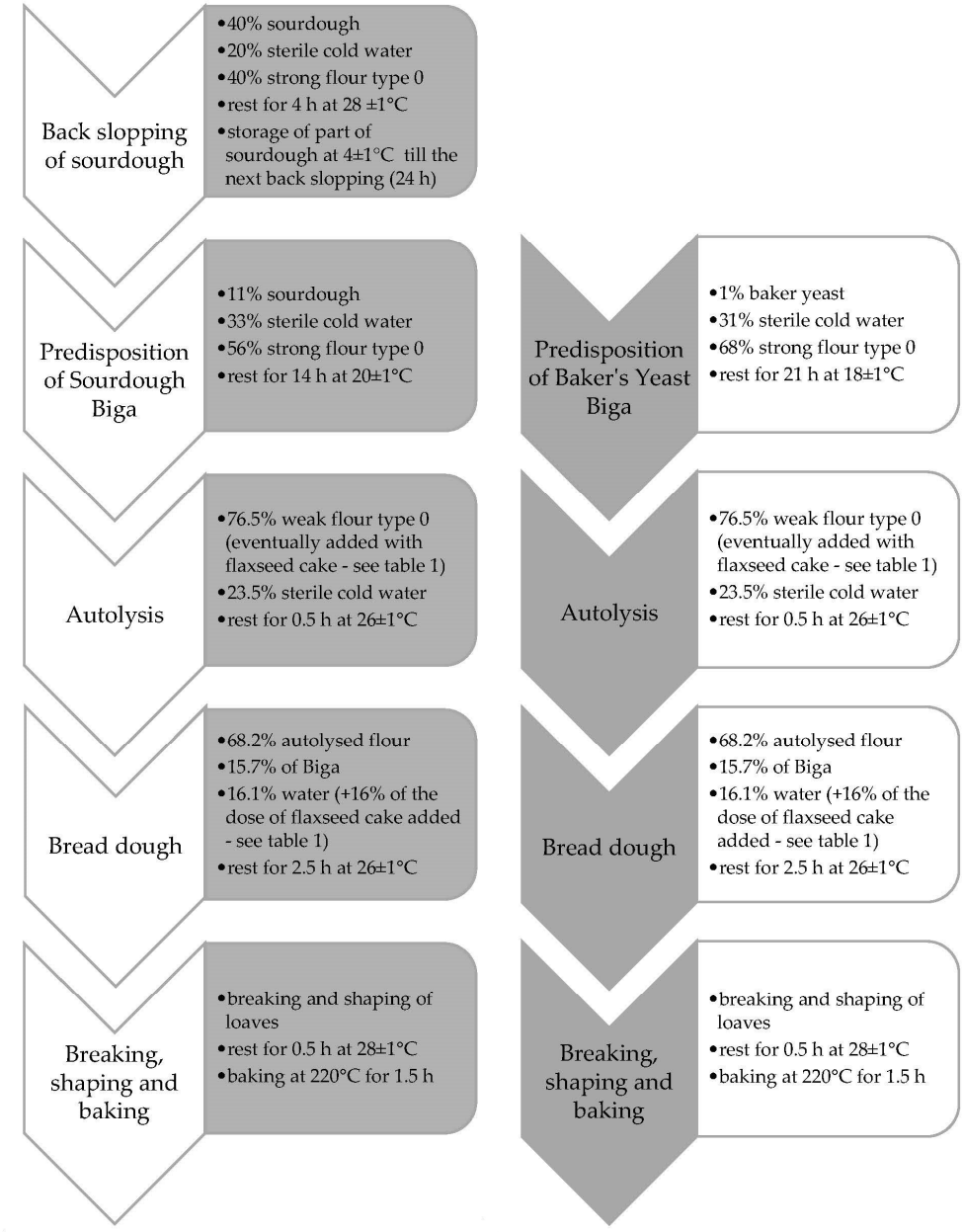


Figure 1. Back-slopping procedure, baking protocol, and operating conditions (time and temperature) adopted during the experimental research for sourdough bread (on the left) and baker's yeast bread (on the right).

In particular, the bread-making procedure was performed with the two-step method of "biga", a pre-ferment prepared with sourdough (SB) or baker's yeast (YB). Sourdough biga was obtained as reported in Figure 1, while baker's yeast biga was made by mixing a strong wheat flour type 0 (68% *w*/*w*), sterile water (31% *w*/*w*), and 1% (*w*/*w*) of baker's yeast with a following fermentation time of 21 h at 18 °C.

Four formulations of sourdough bread (SB) and four formulation of baker's yeast bread (YB) were produced with water 32%, leavening agent (biga) 16%, and flour 52%, using flaxseed cake flour at 0%, 5%, 7.5%, and 10%, as described in Table 1.

Table 1. Sample codes and different formulations of bread objects of the research. Data reported in *italics* were already published in Sanmartin et al., 2020 [39]. SB: sourdough, YB: baker's yeast.

Sample Code	Formulation
Sourdough biga	*Water 33%; sourdough 11%, strong wheat flour 56%*
SB0	*Water 32%; sourdough biga16%, weak wheat flour 52%*
SB5	*Water 32%; sourdough biga 16%, weak wheat flour 47%, flaxseed cake flour 5%*
SB7.5	*Water 32%; sourdough biga 16%, weak wheat flour 44.5%, flaxseed cake flour 7.5%*
SB10	*Water 32%; sourdough biga 16%, weak wheat flour 42%, flaxseed cake flour 10%*
Baker's Yeast biga	Water 31%; baker's yeast 1%, strong wheat flour 68%
YB0	Water 32%; baker's yeast biga 16%, weak wheat flour 52%
YB5	Water 32%; baker's yeast biga 16%, weak wheat flour 47%, flaxseed cake flour 5%
YB7.5	Water 32%; baker's yeast biga 16%, weak wheat flour 44.5%, flaxseed cake flour 7.5%
YB10	Water 32%; baker's yeast biga 16%, weak wheat flour 42%, flaxseed cake flour 10%

Bread-making tests were carried out at the Food Technology laboratory of the Department of Agriculture Food and Environment of Pisa University, and each test formulation was performed in triplicate.

2.4. Physicochemical Characterization and Volatile Organic Compounds Profile

The value of moisture, pH, and free acidity of bread samples were determined according AACC (American Association of Cereal Chemists) standard methods [44–46]. Water activity was measured by a HygroPalm HP23-AW-A (Rotronic AG, Bassersdorf, Switzerland). Total titratable acidity (TTA) was carried out following Gélinas et al. [47]. The concentration of the main fermentative metabolites was monitored by using specific enzymatic kits (Megazyme Ltd., Wicklow, Ireland), as described elsewhere [48–51]. The aromatic profile of the bread samples (whole and sliced) was analyzed by gas chromatography–electron impact mass spectrometry (GC–EIMS) Agilent 7890 B gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an Agilent HP-5MS (Agilent Technologies Inc., Santa Clara, CA, USA) capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) coupled with an Agilent 5977 B single quadrupole mass detector (Agilent Technologies Inc., Santa Clara, CA, USA), using a Supelco divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) assembly (50/30 µm coating thickness, St. Louis, MO, USA) for the adsorption of the volatile analytes according to the protocol previously described by Sanmartin et al. [39].

2.5. Color Determination

The chromatic coordinates were determined on cooled baked crumb samples on the basis of the CIE *L*a*b** color System, using a benchtop tristimulus colorimeter (Eoptis, Mod. CLM-196 Benchtop, Trento, Italy) supplied with its own white reference standard. Two center slices of the loaf were taken for the analysis made on about 24 cm^2 of the crumb for each determination. The results were expressed as color difference (Δ*Eab**):

$$\Delta E^*_{ab} = \sqrt{\Delta L*^2 + \Delta a*^2 + \Delta b*^2} \quad (1)$$

where L^* is the lightness, and a^* and b^* are the red–greenness and blue–yellowness components, respectively.

2.6. Nutraceutical Characterization of Breads

The nutraceutical evaluation was carried out with respect to the total phenols and total flavonoids content, anti-radical activity, and fatty acid profile for each bread formulation.

2.6.1. Extract Preparation

Solid/liquid extraction (ratio 1/20 *w*/*v*) was performed with 80% methanol starting from 0.5 g of bread, sonicating the mixture for 30 min. All the extracts were centrifuged (15 min, 3500 rpm); then, the supernatant was filtered on a syringe filter (0.45 µm), recovered at 4 °C, and immediately analyzed.

2.6.2. Total Phenol Evaluation

Total phenol content was spectrofotometrically determined (Absorbance = 765 nm) through the Folin–Ciocalteu colorimetric method according to Tavarini et al. [30], and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample (dry matter, dm).

2.6.3. Total Flavonoid Evaluation

The quantification of total flavonoids was performed by using aluminum chloride colorimetric method, according to the procedure reported by Kim et al. [52]. Absorbance was determined spectrophotometrically at 510 nm and the total flavonoid content was evaluated as mg of catechin equivalents (CAE) per gram of sample (dry matter, dm), using a standard curve of catechin.

2.6.4. Determination of Anti-Radical Activity

The anti-radical activity of all bread sample extracts was evaluated by both the DPPH free radical method [53] and ABTS [54]. The results were expressed as µmol Trolox equivalents (TE) per gram of sample, comparing the measured values with a standard curve of Trolox, in the range of 0–200 µmol L^{-1} for the DPPH assay and 0.2–1.5 mM range for ABTS.

2.6.5. Fatty Acid Profile Characterization

The fatty acids (FA) composition of bread samples was determined, after an acid trans-methylation, using a GC 2010 Shimadzu Gas Chromatograph (Shimadzu, Columbia, MD, USA), equipped with a flame-ionization detector and a high polar fused-silica capillary column (Chromepack CP-Sil88 Varian, Middleburg, Netherlands; 100m, i.d. 0.25 mm; film thickness 0.20 µm), as previously reported by Sanmartin et al. [39]. Individual FA methyl esters were identified by comparison with a standard mixture of 52 Component FAME Mix (Nu-Chek Prep Inc., Elysian, MN, USA).

2.7. Sensory Characterization (Crust and Crumb)

Sensory analysis of the bread samples was performed by a panel of trained assessors (10 assessors, 6 females and 4 males, aged between 23 and 60 years) who were members of the "expert panel" of the Department of Agriculture, Food and Environment (DAFE) of the University of Pisa, selected according to the internal procedure for assessor selection and training described by Venturi et al. [55]. The panel comprises permanent staff of the Food Technology research team specifically authorized for the sensory analysis of food. According to the "IFST Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods", all staff involved in the sensory analysis received appropriate training for their role and gave informed consent to tests on non-standard foods. Moreover, potential adverse effects related to health problems, allergies, and food safety were minimized: panel recruitment procedures were designed to identify known health problems and allergies, for example by seeking

information on the health status of the subject and any family history. The study was approved by the ethical committee of the University of Pisa (Comitato Bioetico dell'Università di Pisa).

After a training section based on the "Procedure for sensory evaluation of bread" [56], a method for the sensory evaluation of bread fortified with flaxseed cake was developed, after the definition and the introduction of specific descriptors through an especially devoted consensus panel. The list of the final set of 32 descriptive parameters for bread evaluation, including both quantitative and hedonic attributes, as well as the description of the specific tasting sections method were previously reported by Sanmartin et al. [39].

The panelists evaluated the intensity of each parameter from 0 (minimum scale) to 9 (maximum scale), for crust and crumb separately, by means of a technical evaluation sheet. Moreover, the hedonic behavior of the obtained breads was defined through the evaluation of some hedonic parameters related to the appearance, smell, and taste of both crumb and crust together with the visual attractiveness and the overall pleasantness of the whole bread.

2.8. Statistical Analysis

All the evaluations were performed in triplicate, and data are reported as mean values. Compositional data were subjected to two-way ANOVA (CoStat, Cohort 6.0), with leavening agent and percentage of flaxseed cake added as main factors, followed by the Tukey's HSD test at $p \leq 0.05$ of significance.

Statistical analysis of volatile organic compounds characterization and hierarchical cluster analysis (HCA) were performed according to [39] by JMP software package (SAS Institute, Cary, NC, USA). The principal component analysis (PCA) was performed selecting the two highest principal components (PCs) obtained by the linear regressions operated on mean-centered, unscaled data.

Sensory analysis results were processed by Big Sensory Soft 2.0 (ver. 2018), analyzing the data by two-way ANOVA, with panelists and samples as main factors [57].

3. Results

3.1. Physicochemical Parameters

Firstly, both sourdough and baker's yeast bigas used for breadmaking were chemically characterized in terms of dry matter percentage, pH, total titratable acidity (TTA), and main fermentative metabolites (Table 2). As expected, the differences identified were consistent with the microflora metabolic products of each different leavening agent adopted, with a significantly higher acidification and a drop in the pH detected in sourdough biga attributable to the LAB production of organic acids [58].

Table 2. Physical and chemical characterization of sourdough and baker's yeast biga: dry matter (dm %), pH, total titratable acidity (TTA), and main fermentative metabolites. Data presented are the mean of three replicates.

	p-Value [1]	Sourdough Biga	Baker's Yeast Biga
Dry matter (% dm)	*	52.90	59.50
pH	**	3.85	5.06
Total titratable acidity (meq lactic acid/g dm)	***	0.12	0.02
Acetic acid (mmol/g dm)	*	0.06	0.02
D-Lactic acid (mmol/g dm)	*	0.03	n.d. [2]
L-Lactic acid (mmol/g dm)	*	0.06	n.d.

Table 2. *Cont.*

	p-Value [1]	Sourdough Biga	Baker's Yeast Biga
Ethanol (mmol/g dm)	***	0.02	0.33

Notes: [1] Significance level *** $p < 0.001$, ** $p < 0.01$; * $p < 0.05$; ns: not significant ($p > 0.05$). [2] n.d: not detected.

Regarding cooked bread samples (Table 3), no significant effect was observed with regard to water activity and dry matter percentage. As expected [39], the free acidity increased together with the growing percentage of the flaxseed cake used for fortification. Furthermore, free acidity was significantly affected also by the leavening agent employed, with considerably higher values for sourdough breads (Table 3), showing—according to the two-way ANOVA analysis—a significant interaction between the two main factors ($p < 0.001$).

Table 3. Water activity (aw), dry matter (dm %), free acidity, and most representative fatty acids (relative %) of cooked breads. Data presented are the mean of three replicates. Data reported in *italics* were already published in Sanmartin et al., 2020 [39].

	p-Value [1]	SEM	*SB0*	*SB5*	*SB7.5*	*SB10*	YB0	YB5	YB7.5	YB10
water activity (aw)	n.s./n.s./n.s.	0.01	*0.96*	*0.96*	*0.96*	*0.96*	0.95	0.97	0.96	0.96
% of dry matter (% dm)	n.s./n.s./n.s.	0.29	*56.90*	*55.70*	*55.40*	*57.90*	56.80	56.10	55.50	56.10
free acidity (acidity degrees)	***/§§§/¶¶¶	0.80	*9.12* ^{c}	*11.20* ^{b}	*12.30* ^{b}	*14.31* ^{a}	3.82 ^{e}	4.72 ^{d,e}	4.80 ^{d,e}	5.19 ^{d}
palmitic acid (C16:0)	**/§/¶	0.92	*15.22* ^{b}	*11.05* ^{c}	*15.26* ^{b}	*11.84* ^{c}	18.25 ^{a}	17.13 ^{a}	15.14 ^{b}	13.54 ^{b}
elaidic acid (C18:1t9)	***/§§§/¶¶¶	0.19	*0.48* ^{b}	*0.57* ^{b}	*0.82* ^{b}	*1.28* ^{a}	0.13 ^{c}	0.58 ^{b}	0.79 ^{b}	0.54 ^{b}
stearic acid (C18:0)	***/§§§/¶¶¶	0.14	*1.01* ^{b}	*0.54* ^{c}	*1.33* ^{a,b}	*1.12* ^{b}	0.68 ^{c}	1.04 ^{b}	1.41 ^{a}	1.56 ^{a}
oleic acid (C18:1c9)	n.s./§§§/¶¶	1.14	*8.80* ^{c}	*8.88* ^{c}	*13.37* ^{b}	*12.44* ^{b}	6.99 ^{c}	9.82 ^{c}	11.66 ^{b,c}	21.02 ^{a}
linoleic acid (C18:2n-6)	n.s./§§§/¶	1.58	*62.50* ^{a}	*56.44* ^{b}	*47.12* ^{c}	*44.32* ^{c}	64.93 ^{a}	56.22 ^{b}	49.24 ^{c}	37.00 ^{d}
linolenic acid (C18:3n-3)	***/§§§/¶¶¶	0.53	*3.67* ^{d}	*19.07* ^{b}	*17.35* ^{b}	*23.98* ^{a}	3.76 ^{d}	11.05 ^{c}	17.14 ^{b}	22.34 ^{a}

Notes: [1] Significance level—***/§§§/¶¶¶: $p < 0.001$; **/¶¶: $p < 0.01$; §/¶: $p < 0.05$; SEM: Standard error of the mean; ns: not significant ($p > 0.05$). In the same row, different letters indicate significant differences among samples; no letters were reported when differences were not significant. Legend for symbol's meaning: * = Leavening agent as main factor for ANOVA; § = Flaxseed cake percentage as main factor for ANOVA; ¶ = interaction among main factors (leavening agent)·(flaxseed cake percentage).

These results are related to the free fatty acids content, in particular unsaturated fatty acids, of flaxseed cake. Moreover, the detected difference between breads produced with the same percentage of flaxseed cake but with different leavening agents could be due to both the interference of organic acids produced by the LAB of sourdough together with the activity of lipase. Lipase, which is responsible for the hydrolysis of fatty acids, was differently influenced by the pH associated to the leavening agent systems considered. As reported in literature, indeed, lipase activity in flaxseed is promoted at a pH close to 4.5 [59]; this value is comparable to the pH of sourdough biga and far below that of brewer's yeast (Table 2).

Regarding FA composition, we observed a significant increase of α-linolenic acid with the rising percentage of the flaxseed cake (Table 3). On the contrary, the high level of flaxseed reduced significantly the percentage of C16:0 and C18:2n-6 (Table 3). We also observed the highest level of C18:1t9 for SB10 sample, while all the others did not show significant differences.

As shown in Table 4, while the main fermentative metabolites concentration was related to the different leavening agent used, it did not appear to be significantly influenced by the level of fortification, thus indicating that the fermentation activity seemed not to be significantly affected by the addition of flaxseed cake.

Table 4. Concentration of main fermentative metabolites in cooked bread. Data reported in *italics* were already published in Sanmartin et al., 2020 [39].

	p-Value [1]	SEM	*SB0*	*SB5*	*SB7.5*	*SB10*	YB0	YB5	YB7.5	YB10
Acetic acid (mmol/g dm)	n.s/n.s/n.s.	0.01	*0.08*	*0.08*	*0.08*	*0.07*	0.09	0.09	0.08	0.08
D-Lactic acid (mmol/g dm)	***/n.s./n.s.	0.001	*0.014*	*0.015*	*0.013*	*0.011*	n.d.	n.d.	n.d.	n.d.
L-Lactic acid (mmol/g dm)	***/§/¶	0.005	*0.040* ^{b}	*0.055* ^{a}	*0.054* ^{a}	*0.050* ^{a}	n.d.	n.d.	n.d.	n.d.
Ethanol (mmol/g dm)	***/§§§/¶¶¶	0.007	*0.050* ^{c}	*0.050* ^{c}	*0.060* ^{c}	*0.065* ^{c}	0.066 ^{c}	0.078 ^{b}	0.116 ^{a}	0.143 ^{a}

Notes: [1] Significance level—***/§§§/¶¶¶: $p < 0.001$; §/¶: $p < 0.05$; SEM: Standard error of the mean; n.d: not detected. In the same row, different letters indicate significant differences among samples; no letters were reported when differences were not significant. Legend for symbol's meaning: * = Leavening agent as main factor for ANOVA; § = Flaxseed cake percentage as main factor for ANOVA; ¶ = interaction among main factors (leavening agent)·(flaxseed cake percentage).

The only exception was ethanol concentration in baker's yeast bread, which significantly increased together with the percentage of flaxseed cake added and therefore seemed to promote yeast metabolism in the range of fortification considered, with a strong interaction ($p < 0.001$) between the two factors considered.

3.2. Color Determination

The color of the crumb of bread was significantly affected by both flaxseed cake percentage and leavening agent used (Figure 2), with a strong interaction between the two factors ($p < 0.001$) for all three-color coordinates (Table 5).

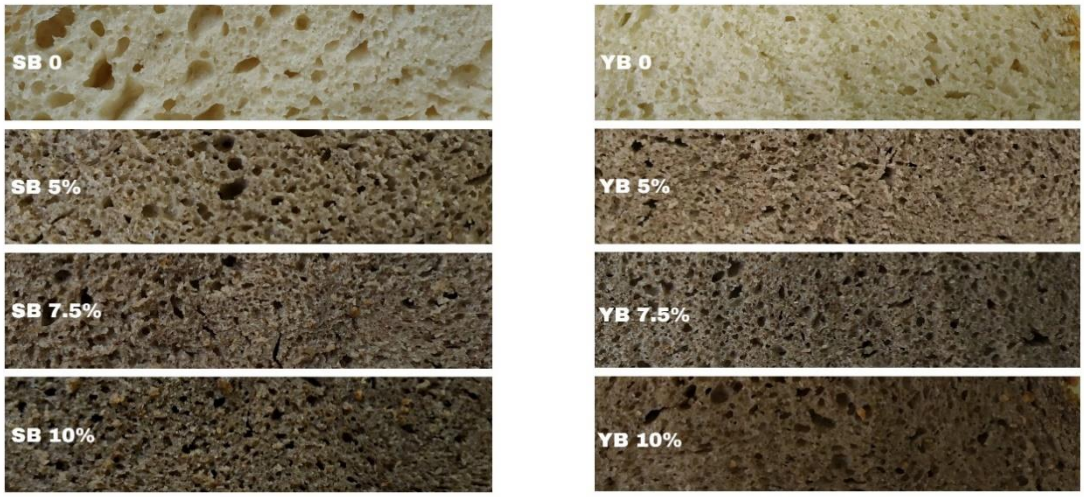


Figure 2. Pictures of the crumb of sourdough bread (**a**) and baker's yeast bread (**b**) produced with increasing flaxseed cake percentage (0, 5, 7.5, 10%).

Table 5. Color coordinates L*a*b* values of the cooked bread samples. Data presented are the mean of three replicates. Data reported in *italics* were already published in Sanmartin et al., 2020 [39].

	***p*-Value** [1]	**SEM**	***SB0***	***SB5***	***SB7.5***	***SB10***	**YB0**	**YB5**	**YB7.5**	**YB10**
L*	***/§§§/¶¶¶	1.0	*62.8* ^{b}	*49.4* ^{c}	*44.0* ^{e}	*40.8* ^{f}	66.5 ^{a}	46.8 ^{d}	42.6 ^{e,f}	43.4 ^{e,f}
a*	***/§§§/¶¶¶	0.2	*−0.7* ^{e}	*2.8* ^{d}	*3.2* ^{c,d}	*3.5* ^{b,c}	−0.7 ^{e}	3.2 ^{c,d}	3.9 ^{a,b}	4.1 ^{a}
b*	***/§§§/¶¶¶	0.2	*17.5* ^{a}	*13.4* ^{b,c}	*12.6* ^{c}	*13.1* ^{b,c}	17.4 ^{a}	14.0 ^{b}	13.4 ^{b,c}	13.4 ^{b,c}

Notes: [1] Significance level—***/§§§/¶¶¶: $p < 0.001$; SEM: Standard error of the mean; ns: not significant ($p > 0.05$). In the same row, different letters indicate significant differences among samples. Legend for symbol's meaning: * = Leavening agent as main factor for ANOVA; § = Flaxseed cake percentage as main factor for ANOVA; ¶ = interaction among main factors (leavening agent)·(flaxseed cake percentage).

Breads without flaxseed cake, for both leavening systems, showed higher values of lightness (L*), especially when baker's yeast was used, while the red–green components (a*) and the blue–yellow components (b*) significantly changed with the fortification, with higher values of both coordinates for baker's yeast breads compared to sourdough ones. Moreover, the distance between the chromatic coordinates (ΔE^*_{ab}) showed how all the fortified breads could be distinguishably discriminated in color if compared with each other, with the greatest difference between YB0 and SB10 (Table 6).

Table 6. Cie L*a*b* color differences (ΔE^*_{ab}) among cooked bread samples. Data reported in *italics* were already published in Sanmartin et al., 2020 [39].

ΔE^*_{ab}	***SB5***	***SB7.5***	***SB10***	**YB0**	**YB5**	**YB7.5**	**YB10**
SB0	*13.6*	*19.7*	*22.8*	3.9	16.6	21.0	21.9
SB5		*5.4*	*9.3*	16.7	3.9	8.1	9.0
SB7.5			*3.3*	23.3	3.3	1.8	4.7
SB10				26.4	6.2	1.9	2.4
YB0					20.3	24.6	25.5
YB5						4.3	4.9
YB7.5							5.5

3.3. Nutraceutical Characterization of Bread

From a nutraceutical point of view, total phenols, total flavonoids, and anti-radical activity showed noteworthy differences in the different breads, depending on the percentage of flaxseed cake used for the fortification (Table 7).

As general trends, these parameters showed a significant increase ($p < 0.001$) with the growing percentage of flaxseed cake added to bread, indicating that the nutraceutical properties were significantly enhanced by flaxseed cake fortification in a dose-dependent manner. These observations were in accordance with the relative high content of bioactive compounds that characterize the flaxseed cake.

When leavening agent is assumed as a main factor for ANOVA, sourdough breads showed increased levels of total phenols and anti-radical activity. On the contrary, the highest flavonoids were detected in baker's yeast breads.

As expected [39], with increasing flaxseed cake addition, saturated fatty acids (SFA) significantly decreased, while monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) n-3 significantly increased, regardless of the leavening agent considered.

This is consistent with the results of individual fatty acids that show also higher levels of n-3 alpha-linolenic acid, in comparison with the control, in the breads fortified with flaxseed cake. Accordingly, the n-6/n-3 ratio decreased, reaching the lowest value at the highest cake fortification amount (Table 7).

Table 7. Effect of leavening agent and of flaxseed cake percentage on the total phenolic content, total flavonoids, anti-radical activity, and fatty acids in cooked bread samples. Data reported in *italics* were already published in Sanmartin et al., 2020 [39].

	p-Value [1]	SEM	*SB0*	*SB5*	*SB7.5*	*SB10*	YB0	YB5	YB7.5	YB10
Total phenols (mg GAE/g dm)	*/§§§/¶¶¶	0.073	*0.481* ^{g}	*0.671* ^{e}	*0.932* ^{c}	*1.041* ^{b}	0.301 ^{h}	0.586 ^{f}	0.833 ^{d}	1.212 ^{a}
Total flavonoids (mg CAE/g dm)	*/§§§/¶	0.039	*0.083* ^{d}	*0.165* ^{c}	*0.216* ^{b}	*0.241* ^{b}	0.075 ^{d}	0.183 ^{c}	0.226 ^{b}	0.303 ^{a}
DPPH (μmol TE/g dm)	*/§§§/n.s.	0.186	*0.505*	*1.734*	*2.329*	*2.826*	0.361	1.657	2.283	2.710
TEAC (μmoL TE/g dm)	n.s/§§§/¶	0.087	*0.259* ^{d}	*0.760* ^{c}	*1.258* ^{b}	*1.522* ^{a}	0.348 ^{d}	0.606 ^{c}	1.242 ^{b}	1.545 ^{a}
SFA (g/100 g of fatty acids)	**/§§§/¶	0.81	*20.46* ^{a}	*17.43* ^{b}	*17.74* ^{b}	*13.66* ^{d}	20.01 ^{a}	18.74 ^{a}	18.58 ^{a}	16.06 ^{c}
MUFA (g/100 g of fatty acids)	n.s./§§§/¶¶	1.00	*10.57* ^{c}	*10.40* ^{c}	*15.26* ^{b}	*16.41* ^{b}	8.43 ^{d}	11.76 ^{c}	13.61 ^{b,c}	23.24 ^{a}
PUFA n-6 (g/100 g of fatty acids)	n.s./§§§/n.s.	1.54	*63.32*	*57.63*	*48.27*	*45.23*	67.03	57.26	50.07	37.73
PUFA n-3 (g/100 g of fatty acids)	***/§§§/¶¶¶	0.50	*3.79* ^{d}	*19.18* ^{b}	*18.12* ^{b}	*23.98* ^{a}	3.80 ^{d}	11.33 ^{c}	17.21 ^{b}	22.34 ^{a}
PUFA/SFA	n.s./n.s./n.s.	0.56	*3.28*	*4.41*	*3.74*	*5.07*	3.54	3.66	3.62	3.74
n-6/n-3	n.s./§§§/n.s.	0.48	*16.81*	*3.01*	*2.66*	*1.89*	17.64	5.05	2.94	1.69

Notes: [1] Significance level ***/§§§/¶¶¶: $p < 0.001$; **/¶¶: $p < 0.01$; */¶: $p < 0.05$; SEM: Standard error of the mean; ns: not significant ($p > 0.05$). In the same row, different letters indicate significant differences among samples; no letters were reported when differences were not significant. Legend for symbol's meaning: * = Leavening agent as main factor for ANOVA; § = Flaxseed cake percentage as main factor for ANOVA; ¶ = interaction among main factors (leavening agent)·(flaxseed cake percentage). GAE = gallic acid equivalents; TE = Trolox equivalents; TEAC = Trolox equivalent antioxidant capacity; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

3.4. Volatiles Bouquet in the Headspace Emissions of the Cooked Breads

A total of 58 volatiles were identified among those spontaneously emitted by the bread samples, whole and sliced, accounting for 95.9–99.9% of total emission (data shown in the Supplementary Material, Table S1).

Volatiles were classified into four main chemical classes, among which non-terpene derivatives clearly prevailed (88.3–99.1%). Nitrogen derivatives, pyrroles, and pyrazines were mainly emitted when the highest percentage of flaxseed cake was added. As a result of their high percentages, non-terpene derivatives were further divided into chemical subclasses. Carboxylic acids, in particular acetic acid, characterized the emission of sourdough samples, mainly when flaxseed cake was absent or in small percentages. Conversely, non-terpene alcohol/ethers were emitted by brewer's yeast samples. Even if with more marked differences, a similar behavior was observed for non-terpene esters. Non-terpene aldehydes/ketones were detected in quite high percentages in all the samples, while non-terpene hydrocarbons were always present in smaller amounts.

For the volatile aroma compounds, it is clearly apparent from the HCA dendrogram (Figure 3) that the breads made only from wheat flour are placed in two different clusters on the basis of the adopted leavening agent used (brewer's yeast or sourdough), indicating that the type of leavening agent had a strong influence on the aroma. This is true for both the whole bread and the corresponding sliced samples.

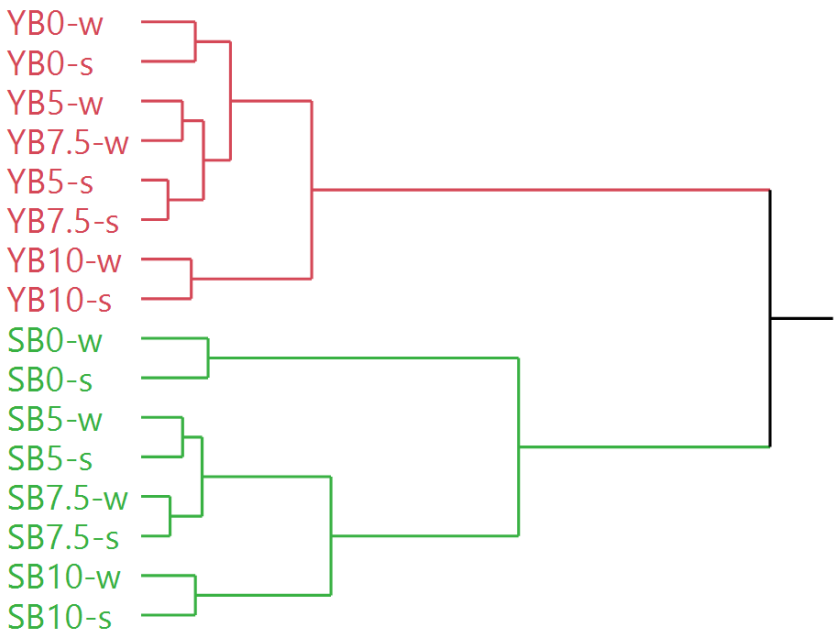


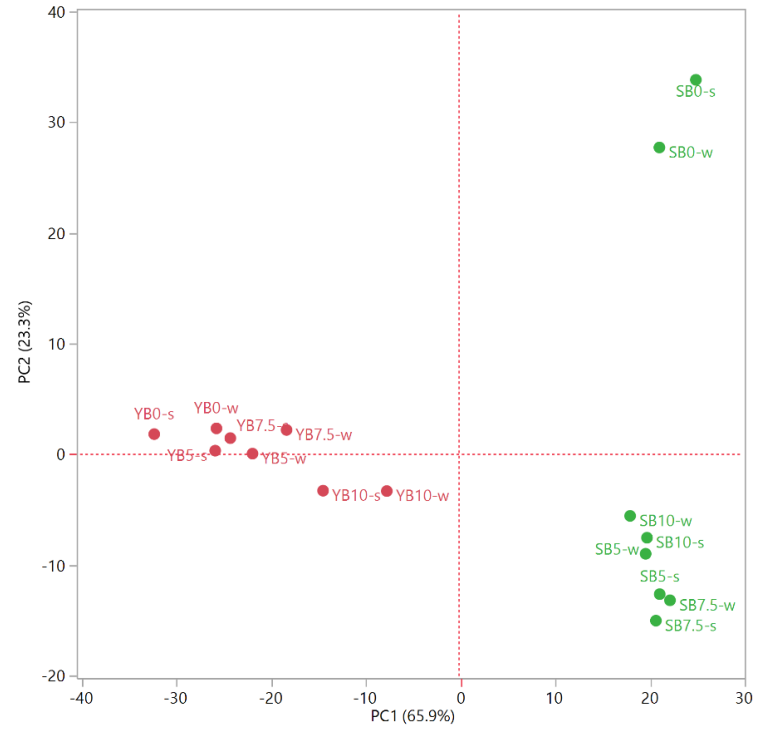
Figure 3. Hierarchical cluster analysis based on volatile compounds of breads (w = whole or s = sliced) as both a function of percentage of flaxseed flour and leavening agent used for fortification.

The adding of flaxseed cake to the dough permitted grouping the samples, within each subgroup, on the basis of the percentage adopted, regardless of whether whole or sliced bread was considered.

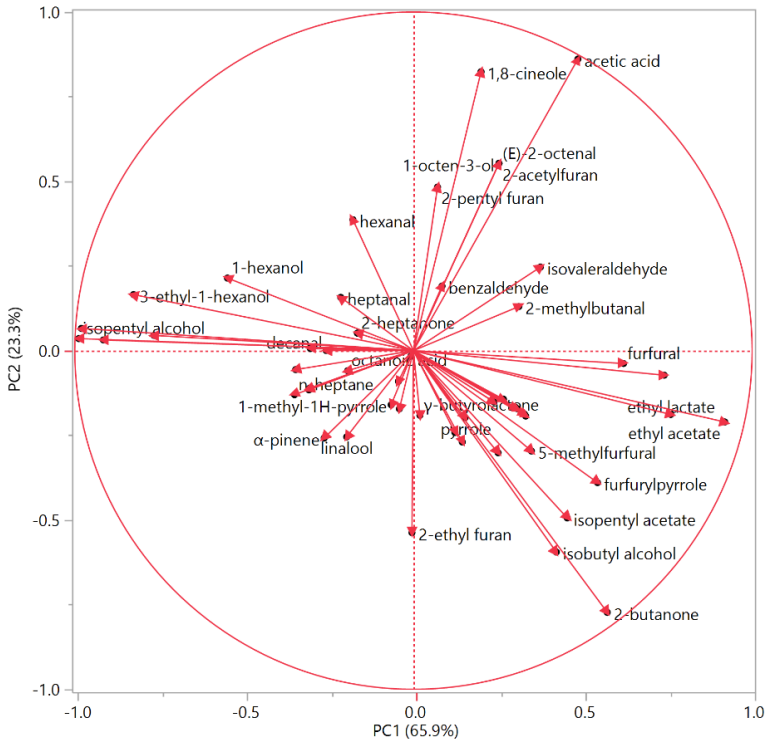
In this discussion, the only exceptions are represented by breads produced with baker's yeasts and 5% or 7.5% flaxseed cake in the recipe. In these conditions, indeed, the whole and the sliced samples cluster differently, regardless of the flaxseed cake percentage. These results can be probably explained if we consider that when the flavor's expression of the bread samples was very similar, the discrimination between the whole bread samples (higher crust percentage) and sliced ones (lower crust percentage) could be based mainly on the high aromatic impact of the flavor associated with the crust fraction as a consequence of cooking. In this context, the greater aromatic complexity in the case of sourdough, which is related to the proteolytic activity of LAB that results in the liberation of amino acids [60], probably made the impact of fortification dominant compared to the cooking effect, regardless of the flaxseed percentage.

At the highest percentage of flaxseed cake, the differences between the leavening agent type become less noticeable, and the samples clearly cluster separately also in the cases of brewer's yeast, which is probably because the volatile aroma compounds emitted by flax overcome those due to the leavening agent. Again, the slicing of the bread does not influence the clustering.

The PCA score plot (total explained variance 89.2%) confirms the clustering observed in HCA (Figure 4a,b). Here, all the sourdough samples show positive loadings on PC1. Among them, the adding of flaxseed cake is clearly evidenced by the negative loadings on PC2 with respect to the positive ones observed for the control samples.



(**a**)



(**b**)

Figure 4. (**a**) Score of the principal component analysis of volatile compounds of breads, (**b**) Loading plots of the principal component analysis of volatile compounds of breads (w = whole or s = sliced).

In the case of brewer's yeast samples, those having the highest content of flaxseed cake (10%) have both PC1 and PC2 negative loadings, while the addition of lesser amounts of flaxseed cake, together with controls, still have negative PC1 loadings but slightly positive or near-zero PC2 loadings.

3.5. Sensorial Parameters

In this context, as highlighted by two-way ANOVA analysis (with the panelists and bread recipe as main effects), most of the parameters evaluated during tasting sessions showed significant differences for both quantitative (Table 8) and hedonic (Figure 5a,b) parameters.

Table 8. Sensorial expression of cooked breads evaluated by panelists during tasting sessions.

Parameter Evaluated by Panelist	*p*-Value [1]	SB0	SB5	SB7.5	SB10	YB0	YB5	YB7.5	YB10
				Crumb					
Crumb color intensity (White scale)	***	5.85 ^{a}	0.00 ^{b}	0.00 ^{b}	0.00^{b}	6.12 ^{a}	0.00 ^{b}	0.00 ^{b}	0.00 ^{b}
Crumb color intensity (Brown scale)	***	0.00 ^{d}	5.23 ^{c}	6.55 ^{a,b,c}	6.83 ^{a,b}	0.00 ^{d}	5.92 ^{b,c}	7.38 ^{a}	7.88 ^{a}
Alveoles dimension	***	6.63 ^{a}	1.28 ^{c}	1.10 ^{c}	1.05 ^{c}	2.95 ^{b}	0.62 ^{c}	0.74 ^{c}	1.40 ^{c}
Homogeneity of alveolation	***	2.1 ^{b}	7.30 ^{a}	6.08 ^{a}	8.53 ^{a}	6.00 ^{a}	8.32 ^{a}	8.05 ^{a}	7.08 ^{a}
Smell intensity	**	5.48 ^{b}	8.03 ^{a}	7.28 ^{a}	7.13 ^{a,b}	5.43 ^{b}	6.45 ^{a,b}	7.47 ^{a}	6.70 ^{a,b}
Wheat smell	***	5.87 ^{a}	0.62 ^{b}	2.57 ^{b}	1.33 ^{b}	5.98 ^{a}	3.22 ^{a,b}	2.48 ^{b}	3.40 ^{a,b}
Yeast smell	***	2.03 ^{b}	0.47 ^{b}	2.10 ^{b}	1.13 ^{b}	4.65 ^{a}	2.75 ^{a,b}	2.42 ^{a,b}	2.58 ^{a,b}
Acetic smell	***	3.40 ^{b}	2.27 ^{b,c}	2.87 ^{b,c}	6.30 ^{a}	0.17 ^{c}	1.75 ^{b,c}	2.12 ^{b,c}	1.25 ^{b,c}
Frankness	**	8.72 ^{a}	7.97 ^{a,b}	7.28 ^{a,b}	5.85 ^{a,b}	8.05 ^{a,b}	7.53 ^{a,b}	6.55 ^{a,b}	4.78 ^{b}
Salted taste	***	3.17 ^{a,b}	4.52 ^{a}	2.98 ^{a,b}	3.23 ^{a,b}	1.98 ^{b,c}	0.72 ^{c}	0.78 ^{c}	2.48 ^{b,c}
Acid taste	***	3.95 ^{a,b}	5.10 ^{a}	2.85 ^{b}	4.83 ^{a}	0.37 ^{c}	0.82 ^{c}	0.80 ^{c}	0.95 ^{c}
Bitter taste	***	0.23 ^{d}	1.12 ^{c,d}	2.67 ^{b,c}	5.30 ^{a}	0.10 ^{d}	0.53 ^{c,d}	1.68 ^{b,c,d}	3.65 ^{a,b}
Aftertaste	**	0.00 ^{b}	0.23 ^{a,b}	1.37 ^{a,b}	1.63 ^{a,b}	0.22 ^{b}	0.20 ^{b}	0.12 ^{b}	2.08 ^{a}
Springiness	n.s.	7.53	6.25	6.63	6.28	7.00	6.87	7.03	7.05
Humidity of surface	n.s.	3.67	4.27	5.05	4.28	3.63	4.37	4.33	4.53
Crumb residual	n.s.	1.43	1.30	2.58	1.70	2.58	2.85	2.32	2.55
Resistance to chewing	***	6.80 ^{a}	5.58 ^{a,b}	3.68 ^{b,c}	1.88 ^{c}	4.30 ^{b}	3.92 ^{b,c}	3.98 ^{b,c}	4.73 ^{a,b}
Juiciness	n.s.	2.17	3.53	2.70	2.95	3.25	3.77	2.82	3.25
Adhesiveness	**	5.83 ^{a}	4.90 ^{a,b}	3.80 ^{a,b,c}	2.45 ^{c}	3.68 ^{a,b,c}	3.50 ^{a,b,c}	3.40 ^{b,c}	3.70 ^{a,b,c}
				Crust					
Crispiness	***	4.90 ^{d}	6.37 ^{a,b,c,d}	7.02 ^{a,b,c}	5.95 ^{a,b,c,d}	5.55 ^{c,d}	7.32 ^{a,b}	7.63 ^{a}	5.70 ^{b,c,d}
Hardness	***	3.10 ^{b,c}	4.00 ^{a,b,c}	4.95 ^{a,b,c}	2.88 ^{b,c}	2.77 ^{c}	4.88 ^{a,b,c}	5.33 ^{a,b}	6.10 ^{a}
Smell intensity	***	4.34 ^{a,b}	6.05 ^{a}	5.53 ^{a,b}	3.03 ^{b}	4.07 ^{a,b}	5.80 ^{a}	6.57 ^{a}	3.10 ^{b}
Salted taste	**	2.00 ^{b}	3.13 ^{a,b}	4.20 ^{a}	3.60 ^{a,b}	1.80 ^{b}	2.43 ^{a,b}	2.47 ^{a,b}	2.45 ^{a,b}
Toasted taste	*	1.98 ^{b}	4.48 ^{a,b}	5.30 ^{a}	4.73 ^{a,b}	4.82 ^{a,b}	4.38 ^{a,b}	4.92 ^{a,b}	5.10 ^{a}
Bitter taste	***	0.12 ^{c}	0.57 ^{b,c}	2.80 ^{a,b}	5.05 ^{a}	0.55 ^{b,c}	0.40 ^{b,c}	0.92 ^{b,c}	2.78 ^{a,b}
Aftertaste	***	0.00 ^{b}	0.00 ^{b}	1.02 ^{a,b}	2.00 ^{a}	0.00 ^{b}	0.07 ^{b}	0.03 ^{b}	1.23 ^{a,b}

Notes: [1] Significance level *** $p < 0.001$, ** $p < 0.01$; * $p < 0.05$; ns: not significant ($p > 0.05$). In the same row, different letters indicate significant differences among samples; no letters were reported when differences were not significant.

The sensory profile of the cooked breads was deeply influenced by the fortification, starting from the evident change in color in accordance with instrumental data. Furthermore, the effects on the crumb and crust sensorial expression by the fortification level seem to be higher than those linked to the leavening agent used, mainly on the rheological properties, such as the alveoles dimension and resistance to chewing for crumb and crispness and hardness for crust, which were closely followed by the smell intensity and complexity. In particular, the fortification seemed to determine higher smell intensity as well as a tendentially lowering effect on wheat and yeast smell perception, irrespective of the percentage of flaxseed cake added.

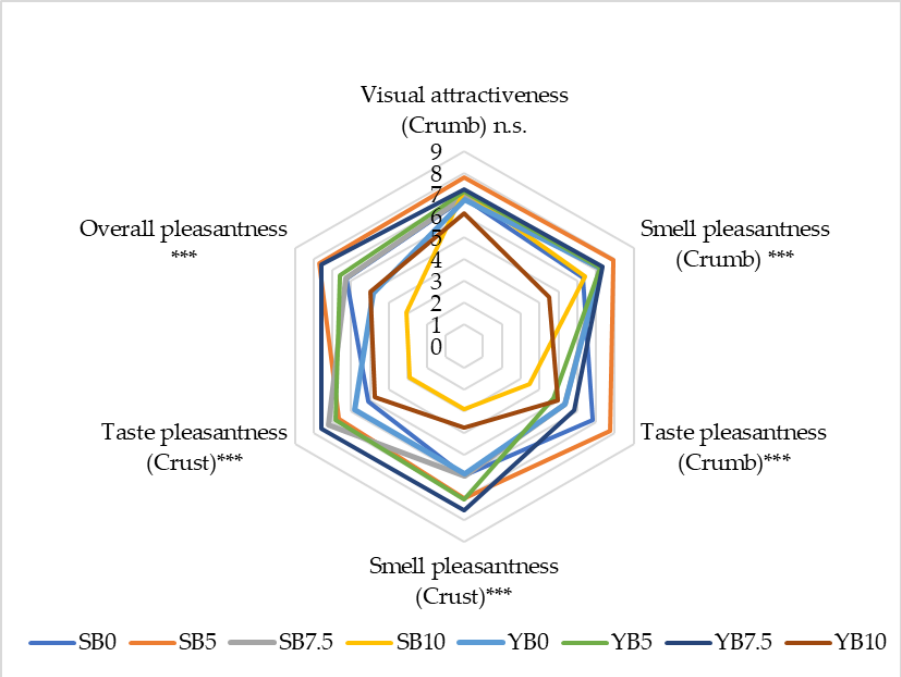
As regards crumb taste, the higher the flaxseed cake percentage, the higher the value attributed by panelists to the bitter taste (both in crumb and crust), regardless of the leavening agent. On the contrary, when the same percentage of flaxseed cake was used in the recipe, an improved perception of bitter was detected when sourdough was used as the leavening agent, which was probably because of a synergistic effect between the acid taste of the dough and the bitterness associated to the phenolic compounds added with flaxseed cake.

On the basis of the above-mentioned synergistic effect between acid and bitter taste, also the bad aftertaste detected in all the fortified sourdough breads (both in crumb and crust fractions) could be

explained, while this negative final sensation was detected only at the highest percentage of flaxseed cake added when the baker's yeasts were used as a leavening agent. Furthermore, although no salt was added at all, a salted taste was especially perceived in the crumb fraction of sourdough breads, because of the recognized combined effect of acidification and proteolysis promoted by LAB activity, which could even mask the absence of salt [61], thus further increasing the complexity of the perceived taste.

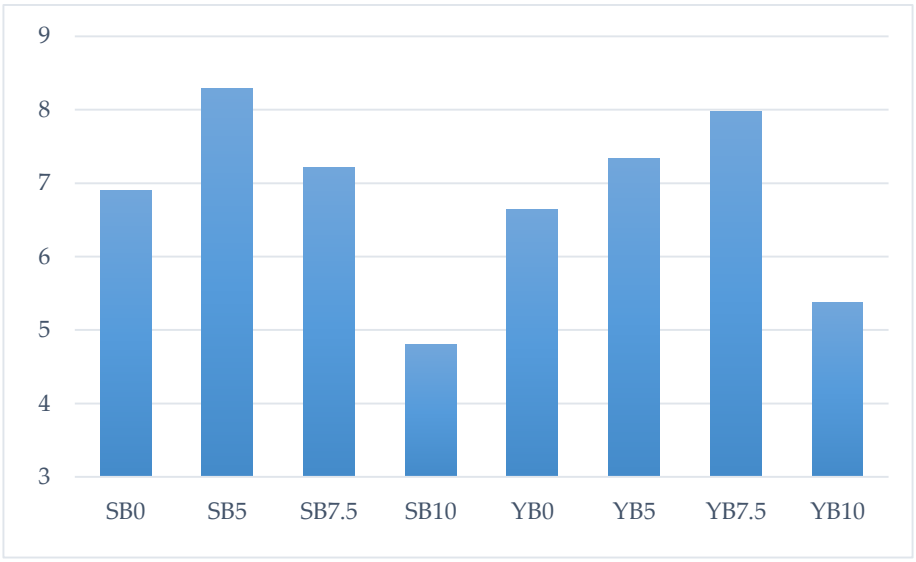
When a new formulation is explored, the level of hedonic quality expressed by the new product is fundamental to determining its consumer's acceptability [39]; therefore, despite the panel test being performed by trained judges, some hedonic parameters related to the view, smell, taste, and overall pleasantness were also evaluated (Figure 5a) to collect some preliminary indications about the organoleptic appeal of the different recipes. Moreover, as shown in Figure 5b, through the means of the values attributed during panel tests to each hedonic parameter converted on a scale from 0 to 10 (Equation (2)), it was possible to calculate an overall hedonic index for each recipe, which was useful to measure the whole organoleptic quality of all the fortified breads evaluated.

$$\text{Overall hedonic index} = MEAN[\textit{Hedonic indices}] * 1,11 \quad (2)$$



(**a**)

Figure 5. *Cont.*



(**b**)

Figure 5. (**a**) Hedonic profile of cooked breads (for both crumb and crust). Significance level *** $p < 0.001$; ns: not significant ($p > 0.05$). (**b**) Overall hedonic indices of cooked breads (for both crumb and crust).

The use of the highest percentage of flaxseed cake determined the worst values in terms of visual attractiveness, smell, taste and, consequently, overall pleasantness attributed by panelists to SB10 and YB10, with the overall hedonic indices below the acceptability level, regardless of the leavening agent utilized. On the contrary, higher ratings were attributed to YB7.5 and SB5, which showed the best overall hedonic index. Moreover, when the same percentage of flaxseed was used in the recipe sourdough breads tendentially exhibited better evaluations compared to the baker's yeast ones.

4. Discussion

Bread is eaten worldwide; thus, it is a useful vehicle to improve the nutritional status of the population, as its enrichment may be achieved through the incorporation of value-added compounds in the recipe as extra ingredients [62–64].

In this context, the fortification with oilseeds [6] and by-products obtained from the oilseed and olive oil industry [39,65–67] is the focus of growing interest for the production of healthier bread, due to their content in proteins, fibers, vitamins, minerals, essential fatty acids, and bioactive compounds.

In particular, the phytochemical composition of flaxseed and its byproducts has recently gained attention due to the interesting results obtained in bakery product fortification, which showed improved nutraceutical features [5,6,23,30,68–71]. According to this evidence, our results showed a significant improvement of nutraceutical profile of the bread fortified with flaxseed cake in a dose-dependent manner, regardless of the leavening agent utilized. Particularly encouraging was also the significant decrease of the n-6:n-3 ratio observed together with the raise of the fortification level until the values fell in the recommended range from 3:1 to 1:1, as expected considering the scientific evidences available in the literature [5,6,30,69–71]. In this regard, recent studies have shown that a dietary imbalance of n-6:n-3 PUFA ratio, which is often found in the n-3-deficient Western diet of today, with values between 10 and 25 [72,73], can promote the pathogenesis of many diseases, as it can lead to an increased production of cytokines and eicosanoids, thus determining a prothrombotic, proconstrictive, and proinflammatory state [74,75].

However, as any modification of the bread-making process or recipe leads to changes in the quality of the final product and in its shelf life [3], their inclusion determines changes in the rheology of bread dough, which also depend on their relative percentage used combined with the dough characteristics (i.e., the nature of the leavening agent).

As reported in the literature [76,77], the addition to bread of insoluble fibers, lipids, and proteins coming from different sources affects the physicochemical properties of the fortified product to various extents due to the interaction of lipids with proteins and starch, through amylose inclusion complexes and the interference in the gluten network formation. Therefore, a negative effect, in particular at the highest levels of oilseed addition, was highlighted on bread volume, with a resulting increase of crumb hardness and a possible faster staling of the loaves [5,23]. However, this effect is not clearly defined as, in contrast to these results, a softer texture and a delayed staling of bread supplemented with uncoated flaxseed or coated ground flaxseed at 10% and 15%, respectively, was observed [33]. This result can probably be ascribed to an increase in bread moisture content or the presence of some flaxseed compounds such as flaxseed gum, proteins, and fats, which contributed to the improvement of the dough structure.

On the other hand, a general problem is the low stability to lipid oxidation of n-3 polyunsaturated fatty acids of which oilseeds are an important source [6,78,79]. Indeed, breads enriched with 10% roasted ground flaxseed showed a significant increase in the peroxide value starting from only two days of storage [79], confirming the evidence described in previous research studies for similar products [78]. As previously mentioned, the incorporation of antioxidant substances such as phenols, tocopherols, and ascorbic acid that can interrupt lipid autoxidation by interfering either with the chain propagation or the decomposition process could represent a partial solution to this problem [80,81]. Feizollahi et al. evaluated the use of encapsulated n-3 fatty acids for bread fortification as their degradation during storage time has proven to decrease, although negative changes in the texture and color of bread have been also observed during storage [82]. Besides, we must consider that the extent of lipid degradation in these products is usually determined by peroxide value measurements, but this could not give a reliable answer, as peroxides may be subjected to further degradation. In this context, more complete information regarding the oxidative stability during storage should be therefore obtained, and the study of the best storage conditions to preserve the quality of the product, according to the percentage of flaxseed cake and the different leavening agent used, is currently in progress.

Sourdough has a long tradition and is largely used as a leavening agent since it provides many technological advantages and higher overall quality compared to baker's yeast [83,84]. The complex microflora characterizing sourdough includes several strains of both yeasts and LAB and is deeply influenced by both endogenous factors such as raw material composition (carbohydrates, nitrogen sources, minerals, lipids, free fatty acids, and enzyme activities) and exogenous factors such as process parameters (temperature, dough yield, fermentation time, and refreshment procedure) [13,84]. Many properties of sourdough are related to the metabolites produced by resident LAB (organic acids, exopolysaccharides (EPS), enzymes, bactericidal and antimicrobial compounds).

To the best of our knowledge, the effect of flaxseed cake fortification as a function of the leavening agents adopted has not yet been investigated. In this context, our findings showed that the leavening agent was able to affect the levels of health-promoting nutraceutical compounds, with an improvement of phenols, flavonoids, and TEAC in sourdough breads, as already reported for various legumes and pseudo-cereals used for the fortification of bakery products [61,85]. These interesting and beneficial properties, here highlighted, are added to others that are well known and related to the use of sourdough in breadmaking [4,40,41], such as the ability to improve the final product shelf life thanks to its acidity [86], as well as the antifungal [87] and antimicrobial activity [88]. Moreover, in our experience, the leavening agent significantly affected both the organoleptic expression and the aroma profile of the fortified bread at different levels of flaxseed concentration.

As in many other areas of food processing, the challenge in fortified cereal food lies in the ability to combine nutritional and health benefits with good sensory quality. In the experimental conditions here adopted, it was possible to select the best recipe in terms of percentage of flaxseed cake added to the flour mix as a function of leavening agent utilized (5% and 7.5% when sourdough and baker's yeast were used respectively).

Based on this evidence, once the preferred quality levels of both the nutritional and organoleptic profile of the fortified bread are guaranteed to the consumers, the bakers will be able to choose the leavening system (sourdough versus baker's yeast) best suited to their production needs simply by varying the percentage of flaxseed cake added to the recipe.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/15/5235/s1, Table S1: Complete headspace compositions of cooked breads (w = whole or s = sliced) as a function of flaxseed percentage and leavening agent used. Data reported in italics were already published in Sanmartin et al., 2020 [39].

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