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Impact of organic and conventional farming practices on the multidimensional characteristics of flour and indirectly on bread

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ABSTRACT

The demand for organic and locally sourced products is on the rise. This study investigates the impact of farming practices (organic versus conventional) on the microbiological, microscopic, and physical-chemical properties of 'Caaveiro' flour, alongside the physical-chemical, instrumental texture parameters, and sensory attributes of breads, using two fermentation methods (sourdough versus mixed leavening). 'Caaveiro' flour and bread, recognized under the Protected Geographical Indication 'Galician Bread', were employed as a case study. Organic flours exhibited higher counts of aerobic plate microorganisms, yeasts, and molds, yet these levels posed no health risks. Overall, farming practices demonstrated negligible influence on the evaluated flour and bread characteristics. Instead, fermentation method emerged as the predominant factor shaping bread attributes. Consequently, promoting organic production could help maintain the distinctive qualities of traditional breads under Protected Geographical Indications.

1. Introduction

Bread is probably the oldest 'processed' food product (Katsi et al., 2021) and it plays an essential role in the diet of different cultures and regions throughout the world (Zamaratskaia et al., 2021). In recent centuries, modern agriculture has prioritized allocating resources towards attaining higher yields in wheat production (Molfetta et al., 2021). This has involved selecting genetically homogeneous varieties that are well-suited for cropping systems demanding substantial inputs such as fertilizers, herbicides, insecticides, and fungicides. These varieties are commonly referred to as modern varieties (González-García et al., 2021; Zamaratskaia et al., 2021).

Nevertheless, local or traditional varieties, which have remained unchanged for centuries (Dinu et al., 2018), possess inherent adaptability to diverse environments (Migliorini et al., 2016). Typically, these older varieties are well-suited for organic farming practices as they exhibit reduced dependency on external inputs (Zamaratskaia et al., 2021).

In general, traditional varieties are better adapted to organic farming conditions because they do not require as many inputs. Promoting this type of cultivation represents a way to protect and improve biodiversity, thus achieving environmental and agronomic benefits (Câmara-Salim et al., 2020, 2021).

Undoubtedly, there is a clear and growing consumer demand for products that are produced in a sustainable way. The world market for organic food and beverages reached 124.8 billion euros in 2021. The largest single market was the United States (39%), followed by the European Union (37%) and China (9.1%). The importance of organic production at a global level is reflected in the increase in the area devoted to organic cultivation, being 11 million ha in 1999, while in 2021 it reached 76.4 million ha (FiBL & IFOAM- Organics International, 2023).

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In addition to the agronomic and economic benefits, the traditional wheat varieties show a better nutritional profile than modern ones and consumers appreciate the organoleptic characteristics of the products obtained from them (Vindras-Fouillet et al., 2021; Zamaratskaia et al., 2021).

Breads made from local wheat flours may carry quality seals guaranteeing their exclusivity, such as the Protected Geographical Indication (PGI) 'Galician Bread'. This bread, containing at least 25% Galician wheat like 'Caaveiro', is known for its crunchy crust, spongy crumb, irregular cells, and color ranging from golden to dark brown. Its quality stems from local wheat varieties and traditional methods, including sourdough fermentation and stone oven baking (García-Gómez et al., 2022). In terms of color, the crust can range from golden to dark brown and the crumb from dark white to pale cream (Regulation (EU) 2019/2182). The quality of this bread is associated with the use of autochthonous varieties of wheat that give it differentiated organoleptic characteristics as well as the production process, where sourdough is used, with long fermentation times and cooking in stone ovens (Câmara-Salim et al., 2020).

Therefore, the quality of the bread depends both on the quality of the raw materials and on the production process applied (Popov-Raljić et al., 2009). In flour and bread production, numerous control factors are involved, with water activity (a_w) emerging as a key determinant. The level of a_w plays a significant role in effectively managing microbial growth in various food products (Liu et al., 2018). Wheat is a product that can be contaminated throughout the crop production chain including the pre-harvest, harvest, transport, storage and processing stages (Magallanes & Simsek, 2021). Low a_w flour is not compatible with the growth of pathogenic microorganisms, but there are spores and certain latent microorganisms that can survive (Eglezos, 2010; Magallanes & Simsek, 2021). In recent years, foodborne illness outbreaks linked to wheat flour have been reported in many countries such as Germany or the United States where outbreaks of *Salmonella,* and Shiga toxin-producing *Escherichia coli* (STEC) were detected (Zhang et al., 2021). STEC is normally associated with foods of animal origin but there is increasing interest in the food safety of plant-based foods associated with these outbreaks (Mäde et al., 2017).

Another important factor is the color of both the flour and the bread. The white color of wheat flour is attributed to the separation of the starch-rich endosperm from the pigmented bran while the yellow color is related to the content of pigments like carotenoids in the endosperm of the grain (Onyango et al., 2020; Zhai et al., 2018). The color of bread is intricately linked to the baking process along with the flour origin. The baking stage is very important, since various physicochemical and organoleptic changes take place in the bread: volume, texture, alveolate formation and also the coloration of the crust (Castro et al., 2017). These parameters play an important role in the acceptance of the product by consumers (Purlis, 2012) and can be evaluated both instrumentally and by sensory evaluation.

Despite the nutritional importance of wheat bread and its role in shaping consumer perception and preference in organic food production, there is a noticeable scarcity of studies addressing quality control at both the physicochemical and sensory levels for organic flour and bread. Existing research on the impact of cultivation methods focuses primarily on texture and other organoleptic characteristics, leaving broader quality aspects underexplored. Pontonio et al. (2021) compared the effect of the cultivation method on bread texture, although their research was more oriented towards studying the effect of sourdough on breads made with organic *vs*. conventional commercial flour.

The influence of the cultivation method on organoleptic characteristics such as appearance (like color intensity), aroma (for instance as toasted or intensity of wheat bread aroma), texture (for example denseness or cohesiveness) and flavor (as wheaty, salty or yeasty) were studied by Annett et al. (2007), Haglund et al. (1998) or Kihlberg et al. (2004, 2006). In general, not many significant differences were found at the sensory level depending on the farming system.

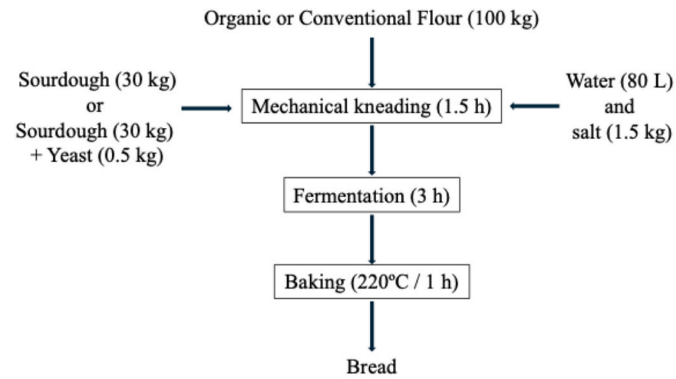

Fig. 1. Block diagram of the method of producing the resulting breads.

In the same way, no differences associated with the crop were found in other vegetable products such as potatoes (Lombardo et al., 2012), fresh kiwi (Nunes-Damaceno et al., 2013) or transformed kiwi (García-Quiroga et al., 2015). On the other hand, other authors found certain differences in organic blackberries that were related to the attributes of sweet taste and taste intensity (Pinto et al., 2018). Raffo et al. (2014) found significant differences in 10 out of 14 sensory attributes measured in apples (green, citrus and floral odors, sweet and sour tastes, overall, fruity and green flavors, hardness and mouthfeel).

Given the significance of autochthonous cereals in local agriculture, organic production, and consumer demand for such products, it becomes imperative to enhance our understanding of the impact of cultivation method on instrumental and sensory characteristics of flour and indirectly of bread. Therefore, the objective of this study was to investigate the influence of the cultivation method on the microbiological, physical-chemical, microscopic, and sensory attributes of both organic and conventional flours, as well as indirectly the resulting breads conforming to the PGI Pan Galego as a case study. The type of fermentation (sourdough *vs.* mixed leavening) were also evaluated.

2. Materials and methods

2.1. Samples

'Caaveiro' autochthonous wheat flour was supplied by the company Dacunha, which came from two cultivation method: organic and conventional. A completely randomized block design was done. The type of cultivation method (organic and conventional) was evaluated with 4 repetitions per crop in each type of crop. In total, 8 subplots were cultivated (2 types of cultivation x 4 repetitions). These plots were located in the northwestern of Spain (43°15′20″ N, 8°21′18″ W). Each subplot was 10 × 6 m.

The differences between organic and conventional wheat cultivation concerned the type of herbicide. The herbicidal pre-emergence treatment for conventional subplots was pendimethalin 40% + glyphosate 36%. Post-emergence herbicidal treatment was with prosulfocarb 80% + diflufenican 50% + clorsulfuron 75%. Whereas in the organic plots no herbicide treatment was applied. In order to evaluate the color and physicochemical characteristics of the flour and elaborate the breads, the grain samples were collected and ground in a stone mill.

Four types of bread were prepared in accordance with the conditions of the Protected Geographical Indication 'Pan Galego' (Regulation (EU) 2019/2182) (organic and conventional). Two types of bread were made with both conventional and organic flours, one with sourdough and the other with a mixed method (sourdough and biological yeast). Thus, it is possible to compare the cultivation method and the two fermentation methods allowed by the IGP Pan Galego. Breads were prepared in a traditional bakery, under our supervision following the process describe in Fig. 1 using 100 % 'Caaveiro' flour. The formula per 100 kg of flour was 80 L of water, 30 kg of sourdough or 30 kg of sourdough and 0.5 kg of biological yeast (mixed method) and salt (1.5 kg). It was kneaded for 1.5 h in a kneading machine and left to ferment for 3 h (1.5 h in hopper fermentation and 1.5 h in ball-shaped pieces). Baking was carried out in a stone oven for 1 h at 220°±5 °C. The bread was shaped like a bun and weighed 1.2 kg. The process was done under the same conditions for flours from both farming systems (organic and conventional) and both fermentation systems (sourdough and mixed method).

2.2. Microbiological analyses

Flour samples (10 g) were suspended in 90 mL of sterile peptone solution (Scharlau, Spain) and homogenized in a Stomacher lab blender for 1 min at room temperature. Afterwards, serial dilutions were made with sterile peptone solution.

Aerobic plate count (APC) was measured as following: 1 mL of each dilution was transferred to Petri dishes. Then, added 20 mL of Plate Count Agar (PCA; Scharlau, Barcelona, Spain) and mixed. The plates were further incubated in reversed position at 30 °C for 72 h and counted according to ISO 4833–1:2013 (ISO 4833-1, 2013).

Coliforms was measured as following: 1 mL of each dilution was transferred to Petri dishes in triplicate. Next, 20 mL of Violet Red Bile Agar Mug (VRBL; Liofilchem, Roseto degli Abruzzi, Italy) was poured, swirled to mix and let to solidify. The plates were incubated in reversed position at 30 °C for 48 h (ISO 4832, 2006).

Escherichia coli was measured as following: 1 mL of each suspension was mixed with Tryptone Bile Glucuronic A. (TBX; Scharlau, Barcelona, Spain). The plates were further incubated at 30 °C for 48 h and counted according to ISO 4832:2006 (ISO 4832, 2006).

Yeasts and molds were measured as following: 0.2 mL of each suspension was spread in petri dishes containing 20 mL of Dichloran Glycerol Agar Base (DG18; Scharlau, Barcelona, Spain). The plates were further incubated in upright position at 25 °C for 180 h for yeast counting and 300 h for mold counting, according to ISO 21527-1,2:2008 (ISO 21527-1,2, 2008).

Sulphite-reducing clostridia (SRC) was measured as following: 5 mL of each suspension were transferred to a 50 mL falcon tube and further heat-treated in a water bath at 80 °C for 10 min. The suspension was immediately cooled in ice and 25 mL of Iron Sulfite Agar (ISA; Scharlau, Barcelona, Spain) were added. The mixture was homogenized and allowed to solidify. Afterwards, 5 mL of ISA medium was added to create anaerobiosis. The falcon tubes were incubated at 30 °C for 24–48 h and black spots were counted, according to ISO 15213:2003 (ISO 15213, 2003).

Salmonella spp was measured as following: 25 g of flour was homogenized in 225 mL Bouillon Salmonella Enrichissent (Biokar, Allonne, France). The solution was incubated for 24 h at 40 °C. Afterwards, the solution spread with an inoculation loop. It was incubated for 24 h at 37 °C. Results were reported as detected or not detected (ISO 6579-1, 2017).

2.3. pH determination and water activity

The freeze-dried flour and bread samples (10 g) were macerated in 100 mL of distilled water for 30 min. The pH was measured using a digital pH meter (GLP21 Crison) according to AOAC method 981.12. It was determined in triplicate. Water activity was determined in flour and bread with an AquaLab meter (Pullman, Washington, USA) according to AOAC method 978.18. A_w was determined in sextuplicate.

2.4. Flour and bread color measurements

Flour and bread crust and crumb color were measured with Color-Striker (Mathai GmbH, Sydney, Germany) using the CIE L*a*b* system. The color parameters, lightness (L*) and chromaticity (coordinates a* and b*) were obtained. The coordinates of CIE L*a*b* C* and H° were computed from a* and b* values based on the following equations: $C^* = (a^{*2}+b^{*2})^{1/2}$ and $H^\circ = \tan^{-1}(b^*/a^*)$. L* is a measure of brightness, ranging from 0 (black) to 100 (white); a* quantifies redness (positive) or greenness (negative); and b* assesses yellowness (positive) or blueness (negative) (Zhai et al., 2018). Chroma (C*) evaluates intensity or saturation and H° indicates hue angle which is expressed on a 360° grid, with 0° and 180° corresponding to + a* axis (red) and − a* (green), respectively, 90° and 270° for the + b* axis (yellow) and − b* (blue), respectively (Sui et al., 2015). Crust color was evaluated on top of the whole loaves, while the crumb color was determined after cutting each loaf into in different slices. Both were analyzed on twenty samples.

2.5. Microscopic characteristics

Samples of 10 mg of flour were weighed, introduced into an eppendorf and 1 mL of gelatine solution was added and mixed homogeneously on a vortex mixer at 15 Hz for 10 min. Then, 200 μL of the suspension was distributed onto a microscope slide and allowed to dry at room temperature according to Fernández-Canto et al. (2023). Light microscopy was used to observe the appearance and shape of starch granules and to find morphological differences between the samples using a Nikon Type 104 microscope. (40x) In addition, these samples were also characterized under polarized light by adding polarizing filters.

2.6. Bread instrumental texture

All analyses were performed on the day of baking. Instrumental texture evaluation of crust and crumb bread was performed using a TA-Xtplus texturometer (Stable Micro System, Viena Court, UK) equipped with a 30 kg load cell (Stable Micro Systems, Goldalming, UK). The crumb samples were cut into small cubes (20 × 20 × 20 mm). Texture profile analysis (TPA) was used a cylindrical aluminum probe (P/50) with 50 mm diameter. The compression speed was 1 mm/min. Samples were compressed to 75% of the original height. It was measured hardness (g), springiness, cohesiveness and chewiness (g). Hardness was measured as the force at the maximum height of the first compression. Springiness was calculated as the height of the product on the second compression divided by the height of the first peak. Cohesiveness was calculated as the ratio of the areas of the second and the first peak. Chewiness was calculated as hardness*cohesiveness*springiness (García-Gómez et al., 2019). Fifteen samples were analyzed for each bread type.

A puncture test was used to measure crust hardness (g) (the maximum force required to rupture the crust bread (Grotte et al., 2001) and the work of penetration (g·s) (as the work required to penetrate the sample to a given depth (Lis et al., 2021)) using a stainless-steel probe and a test speed of 1 mm s^{-1}. The top crust samples measured 30 × 30 mm. Fifteen samples were analyzed for each bread type.

2.7. Sensory analyses

Sensory tests were carried out in a standard tasting room equipped with individual taste booths separated by screens, high and wide enough to isolate the different judges (ISO 8589:2007/Amd 1:2014). Samples were served at room temperature (about 20 °C) on black dishes and they were presented in a sequential monadic way. Twelve judges, aged 50 to 65, were selected and trained for sensory bread evaluation, having successfully passed a validation process before conducting the assessments (Estévez-López et al., 2021).

A quantitative descriptive analysis (QDA) was carried out using the tasting sheet previously developed (Estévez-López et al., 2021). Each sample was labelled with random 3-digit codes and the evaluations were performed in accordance with a randomized complete block experimental design to assess the efficacy of the panel as well as establishing the sensory profile. Water was provided to clear the assessor palates

Table 1
Results of the microbiological control of 'Caaveiro' wheat flours from organic and conventional cultivation method. Significant differences were determined based on a p-value threshold of less than 0.05.

Parameter	Organic wheat (mean ± standard deviation)	Conventional wheat (mean ± standard deviation)	p-value
APC (Log_{10} CFU/g)	4.70 ± 0.31	3.80 ± 0.14	0.030
Coliforms (Log_{10} CFU/g)	1.55 ± 0.37	2.00 ± 0.29	0.110
Yeasts (Log_{10} CFU/g)	2.60 ± 0.14	1.65 ± 0.26	0.010
Molds (Log_{10} CFU/g)	2.75 ± 0.58	2.10 ± 0.16	0.000
Escherichia coli (Log_{10} CFU/g)	Absent	Absent	
Clostridium (Log_{10} CFU/g)	Absent	Absent	
Salmonella spp. (Log_{10} CFUg)	Absent	Absent	

between sample evaluations. For taste, aroma, odor and texture attributes, the judges received a piece of bread of each type with dimensions measuring 10 × 3 cm (including crumb and crust). Reference standards were provided at each session, and assessors were required to refer to these prior to each evaluation. In addition to the quantitative sensory characterization of the breads, trained assessors performed a qualitative evaluation of the loaves. For this, the judges evaluated the presence/absence of the descriptors of the adapted bread wheel (Kleinert et al., 2009) in nose (odor), in mouth (aroma) and aftertaste.

2.8. Statistical analyses

The mean value and standard deviation of the physical-chemical, sensory and microbiological parameters were calculated. In the case of flours, a Student's t-test for independent samples was applied to identify any difference between the two types of flour (organic and conventional). To analyze the results obtained in the breads, an analysis of variance (two-way ANOVA) was accomplished. The cultivation method (organic *vs*. conventional), and type of fermentation (sourdough *vs*. mixed leavening), and their interaction as fixed sources of variation were studied. When the interaction cultivation method * type of fermentation was significant for some descriptors, those descriptors were studied more in depth, segmenting the matrix data in different levels of crop systems and types of fermentation. Student's t-test for independent samples in the case of physical-chemical parameters and for paired samples in the case of sensory parameters was performed. In this study, significant differences were considered at a p-value threshold of less than 0.05. Statistical analyses were carried out using SPSS 25 (IBM, Armonk, NY, USA).

3. Results and discussion

3.1. Flour characterization

The nutritional characterization of both flours and bread has been described previously (Fernández-Canto et al., 2024). No significant differences were found in the protein content of the flours depending on the cultivation system, which indicates that it is possible to obtain the same amount of protein in the flours using organic practices. However, organic breads contained more protein than conventional.

The results regarding the microorganisms analyzed in the wheat flours are shown in Table 1. Significant differences were observed between organic flour and conventional flour in terms of the concentration of APC microorganisms, yeast, and molds, with organic flour exhibiting the highest levels. No differences were found in the case of coliforms.

Table 2
Instrumental parameters of organic and conventional 'Caaveiro' wheat flours. Significant differences were determined based on a p-value threshold of less than 0.05.

	Organic wheat (mean ± standard deviation)	Conventional wheat (mean ± standard deviation)	p-value
a_w	0.54 ± 0.01	0.52 ± 0.02	0.00
pH	6.23 ± 0.04	6.25 ± 0.03	0.229
L*	84.95 ± 1.31	84.48 ± 2.67	0.485
a*	0.90 ± 0.25	1.03 ± 0.29	0.124
b*	7.65 ± 0.26	8.12 ± 0.77	0.170
C*	7.71 ± 0.27	8.19 ± 0.73	0.010
H°	83.36 ± 1.81	82.62 ± 2.65	0.305

None of the samples tested positive for *Clostridium*, *E. coli*, or *Salmonella*, indicating that they are safe for human consumption. In organic soft and durum wheat flours, a higher concentration of molds was discovered, while in organic rye flours, the mold concentration was lower. However, no significant difference was observed between organic and conventional flours in terms of mesophilic acid bacteria and yeast content (Pontonio et al., 2021).

The APC obtained in the present study were consistent with findings reported by other authors. For example, 3.33–4.44 log_{10} (CFU/g) was reported for refined and whole wheat flours (Cardoso et al., 2019; Eglezos, 2010). The greater use of slurry and manure as fertilizers or avoiding the use of chemical products (such as chemical fungicides) could be a possible source of contamination in organic farming (Pontonio et al., 2016). This work showed that the risk of contamination by pathogens in organic flours is not greater than in conventional ones, even though significant differences were detected in some microbial parameters between the cultivation method, the values were low and in accordance with other vegetable products (Oliveira et al., 2010).

Overall, the physical-chemical and color parameters of the flours were not influenced by the cultivation method, excepting for a_w and C* (Table 2). The pH values of these flours (Table 2) were similar to those reported by Cardoso et al. (2019), in which the range was 6.05–6.41. These authors suggested the existence of few publications on the study of pH in wheat flour, although it is an important parameter to detect possible alterations in its quality due to the development of microorganisms. To the authors' knowledge, no study was published comparing the a_w and the pH of the flours depending on the cultivation method. Although the values were very similar, the flour grown under organic conditions had significantly higher a_w (0.54 ± 0.01) than in conventional system (0.52 ± 0.02). A_w of flour was low (a_w <0.60), with values similar to those obtained by other authors (Magallanes & Simsek, 2021; Syamaladevi et al., 2016). The absence of pathogenic microorganisms observed earlier can be related with the low a_w detected.

Regarding the instrumental analysis of the color, only differences were found in C* value. Conventional flours showed higher values than organic flours (Table 2), although this difference was lower than 2 units (C* = 7.71 ± 0.27 *vs*. 8.19 ± 0.73), which are not perceptible by the human eye (Reche et al., 2019). The results of the present study were in agreement with those reported by Crecente-Campo et al. (2012), because organic strawberry was less vivid than the conventional one (lower C* value).

In the rest of the color parameters, evidence of the cultivation method (organic *vs*. conventional) effects in wheat flour were not found. Jaskulska et al. (2018) evaluated the effect of the environment and agronomic management practices on the baking value of the grain of the wheat. They studied the effect of three tillage methods (conventional moldboard plow, reduced ploughless, and strip-till) and two nitrogen fertilization rates (100 kg N·ha^{-1} and 200 kg N·ha^{-1}) for two years, and they did not observe differences in terms of the color parameters of the flours.

Regarding the morphology of the starch grains, no differences were found between organic and conventional flours. In both cases the

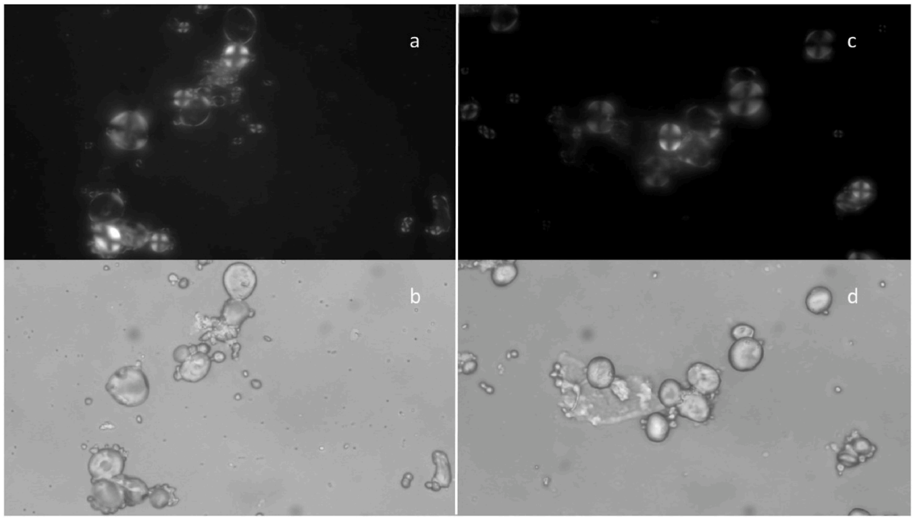

Fig. 2. Organic (a and b) and conventional flour (c and d). Photographs correspond to the same field of each sample observed under polarized light (a, c) and bright field (b, d) in an optical microscope (40x).

Table 3
Instrumental parameters of breads made with flours from organic and conventional 'Caaveiro' wheat. The effect of cultivation method, type of fermentation and interaction between those design variables were considered.

Parameter	Organic mixed leavening	Conventional mixed leavening	Organic sourdough	Conventional sourdough	Cultivation method		Type of fermentation		Interaction (Cultivation method*Type of fermentation)	
	mean ± standard deviation	mean ± standard deviation	mean ± standard deviation	mean ± standard deviation	F	p-value	F	p-value	F	p-value
a_w	0.89 ± 0.14	0.95 ± 0.00	0.97 ± 0.00	0.96 ± 0.00	0.840	0.370	2.469	0.132	1.183	0.290
pH	5.64 ± 0.00	5.70 ± 0.00	5.64 ± 0.00	5.63 ± 0.00	241.935	0.000	474.194	0.000	474.194	0.000
C* top crust	23.07 ± 5.06	21.10 ± 3.52	22.55 ± 4.56	22.15 ± 4.94	1.698	0.196	0.085	0.772	0.740	0.392
H° top crust	63.78 ± 5.57	64.05 ± 5.70	65.53 ± 4.56	67.74 ± 8.51	0.780	0.380	3.783	0.055	0.482	0.490
L* top crust	39.39 ± 4.80	39.04 ± 6.95	46.19 ± 5.04	52.54 ± 11.54	3.127	0.081	35.814	0.000	3.907	0.052
a* top crust	10.05 ± 2.65	9.12 ± 2.20	9.42 ± 2.25	8.90 ± 4.64	1.088	0.300	0.388	0.535	0.088	0.767
b* top crust	20.66 ± 4.83	18.91 ± 3.48	20.42 ± 1.48	20.05 ± 3.54	1.780	0.186	0.329	0.568	0.759	0.386
C* crumb	19.01 ± 0.72	18.88 ± 0.94	14.63 ± 4.56	15.66 ± 0.93	5.818	0.018	421.321	0.000	9.868	0.002
H° crumb	82.05 ± 0.86	82.17 ± 1.07	84.82 ± 0.89	84.41 ± 0.79	0.523	0.472	151.507	0.000	1.733	0.192
L* crumb	59.25 ± 1.00	60.02 ± 7.62	68.37 ± 1.28	67.51 ± 1.54	0.003	0.960	87.481	0.000	0.846	0.360
a* crumb	2.64 ± 0.30	2.59 ± 0.43	1.34 ± 0.27	1.55 ± 0.28	1.157	0.285	259.074	0.000	3.150	0.080
b* crumb	18.83 ± 0.71	18.70 ± 0.91	14.57 ± 0.68	15.58 ± 0.91	5.955	0.017	415.348	0.000	9.993	0.002
Hardness (g)	8929.10 ± 1802.78	6348.77 ± 1318.80	11360.55 ± 1782.60	12714.96 ± 1702.60	1306	0.261	67251	0.000	13452	0.001
Adhesiveness (g.s)	−3.84 ± 1.08	−4.43 ± 4.67	−2.03 ± 0.66	−2.09 ± 1.17	0.151	0.702	6338	0.020	0.105	0.749
Springiness	0.52 ± 0.04	0.70 ± 0.09	0.63 ± 0.09	0.60 ± 0.05	9964	0.003	0.000	0.984	20964	0.000
Cohesiveness	0.49 ± 0.03	0.52 ± 0.03	0.40 ± 0.03	0.39 ± 0.02	2884	0.097	144955	0.000	4977	0.031
Chewiness (g)	2112.40 ± 463.59	2184.55 ± 412.40	2619.47 ± 707.51	3018.10 ± 443.31	2115	0.154	17143	0.000	1016	0.320
Crust hardness (g)	4962.37 ± 1547.30	3548.23 ± 1029.81	5644.65 ± 1319.74	5694.89 ± 919.45	3410	0.072	14669	0.000	3930	0.054
Crust work of penetration (g.s)	39455.60 ± 8244.96	29619.70 ± 7330.57	46767.27 ± 21594.60	45854.45 ± 11205.29	1177	0.289	5646	0.026	0.811	0.377

granules had round and elliptical shape, although there were a high number of granules with irregular shape. No differences were found under polarized illumination (Fig. 2). Both organic and conventional flours presented granules with a perfectly defined Maltese cross, while other granules did not present the Maltese cross. Instead, they had a well delineated peripheral halo, as previously reported by Fernández-Canto et al. (2023). Thus, these results confirm the usefulness of the microscopy technique as a traceability tool for autochthonous 'Caaveiro' wheat in flours.

Other authors have also used microscopy to compare the cultivation method in other food products. Ordóñez-Santos et al. (2009) did not find any structural differences after the microscopic examination of peel, flesh and seed coat of the tomato depending on the cultivation method. On the contrary, the size of the mesocarp cells of cherry tomato presented differences in size depending on the cultivation method (Stertz et al., 2005). Nakamura et al. (2007) reported that after a long storage time (9 years at room temperature), conventional soybean seeds presented collapsed tissue and gaps in the internal and external parts of seeds, which could be associated with the lower Ca content of the cell wall structure.

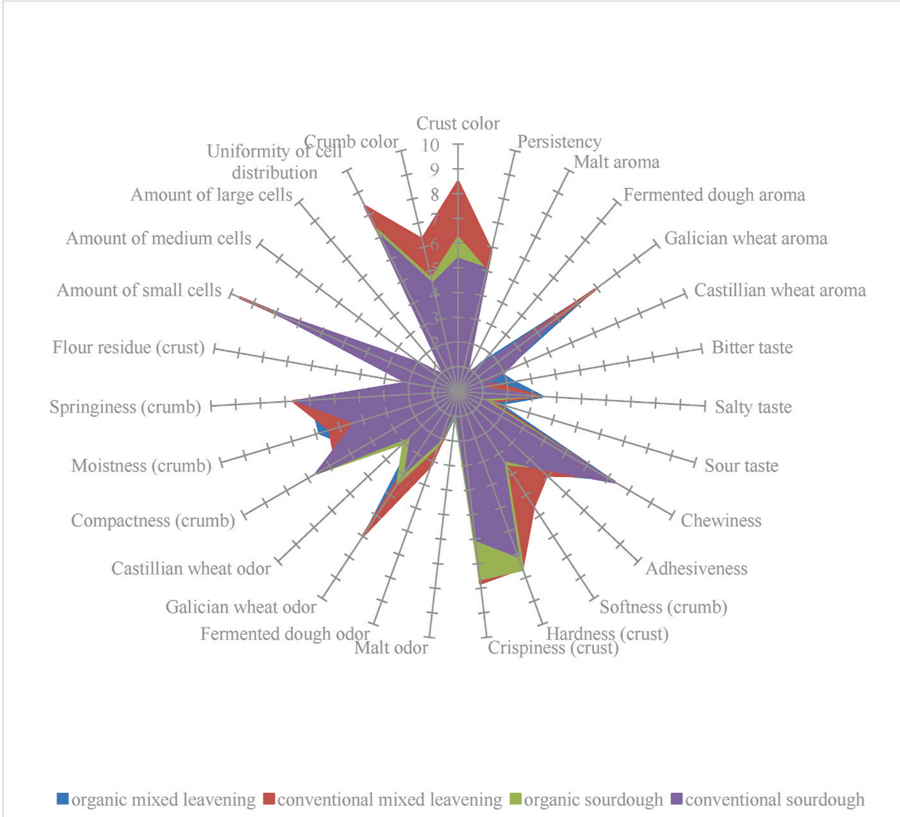

Fig. 3. Sensory profile of breads made with flours from organic and conventional 'Caaveiro' wheat and different ferment used.

3.2. Bread characterization

3.2.1. pH and water activity of bread

The values obtained for the instrumental parameters of breads made with flours from organic and conventional wheat are shown in Table 3. The cultivation method*type of fermentation interaction was not significant for the a_w, however it was significant for pH. According to the data obtained, the values of a_w have not been affected by the cultivation method or the type of ferment. Bread is a food known for having high moisture levels and a_w greater than 0.85, which can generally reach values of 0.95–0.99 (Smith et al., 2004).

Since the interaction was significant in the case of pH, data matrix was segmented in different levels for cultivation method and type of fermentation. Segmenting by cultivation method, in breads made with organic flour, it was observed that no differences were found depending on the type of ferment used, whereas breads made with conventional flour using the mixed method showed higher values than if sourdough was used. Segmenting by type of leaven, no clear trend was observed; when a mixed leaven was used, the conventional breads had a higher pH than the organic ones, while in the bread made with sourdough the organic breads had higher values.

However, the differences between the pH of the different breads, although significant, were not important from a technological point of view and they were found within the ranges reported by other authors (Katsi et al., 2021; Smith et al., 2004). No studies were found that compared the pH and a_w of bread depending on the cultivation method. Only Pontonio et al. (2021) compared the pH of sourdoughs made with organic and conventional flours. In sourdoughs with 1 day of propagation, no differences in pH were found. In contrast, if analyzed after 10-day activation, conventional wheat sourdoughs (soft wheat and durum wheat) had a higher pH than those made with organic flour. In the case of rye sourdough, the observed behavior was the opposite.

3.2.2. Color measurements of bread

Color is indeed a crucial property that significantly impacts the market acceptance of bakery products. The visual appearance of baked goods, including their color, plays a vital role in consumer perception and purchase decisions. The color of bakery products can indicate freshness, quality, and even flavor. Therefore, achieving the desired color in bakery products is an important aspect of product development and can contribute to consumer satisfaction and market success (Yalcin et al., 2021). According to the information provided in Tables 3 and it can be concluded that, similar to the flours, the color parameters of the bread were not significantly affected by the cultivation method. The results indicate that regardless of the cultivation method, the color of the bread remained consistent across different cultivation method. The only color parameter influenced by the culture system in the flours (C*) was overridden by the baking process, where non-enzymatic chemical reactions take place and produce colored compounds such as caramelization and Maillard reactions (Purlis, 2010), reducing the influence of the flour coloration (Gómez et al., 2011).

In those variables in which the interaction (cultivation method and type of fermentation) has been significant (C* and b* crumb) when segmenting by crop management type, there were differences in these variables. The breads made with conventional and organic flours followed the same trend, showing higher values of C* and b*crumb in the mixed method (more color intensity and more yellowish color). On the other hand, if segmented by type of ferment, breads made by the mixed method did not show significant differences in these variables, whereas breads made with sourdough got higher C* and b*crumb in conventional than organic breads.

In those variables in which the interaction (cultivation method and type of fermentation) was not significant, the effect of the factors was analyzed independently, and the ferment type factor was significant for the variables L* top crust, H° crumb, L* crumb and a* crumb (Table 3). Higher values were observed in breads made with sourdough than in

Table 4
Sensory descriptors of breads made with flours from organic and conventional 'Caaveiro' wheat.

Descriptors	Cultivation method		Type of fermentation		Interaction (Cultivation method*Type of fermentation)	
	F	p-value	F	p-value	F	p-value
Crust color	0.083	0.775	30.243	0	2.843	0.099
Crumb color	0.071	0.791	20.571	0	0.805	0.374
Uniformity of cell distribution	0.078	0.782	0.847	0.362	0.371	0.546
Amount of large cells	0.205	0.653	12.285	0.001	0.251	0.619
Amount of medium cells	0.032	0.858	13.615	0.001	1.271	0.266
Amount of small cells	0.419	0.521	17.249	0	0.891	0.35
Flour residue (crust)	0.302	0.585	4.323	0.043	1.985	0.166
Springiness (crumb)	0	0.996	1.344	0.253	0.127	0.723
Moistness (crumb)	0.197	0.659	0.02	0.889	0.001	0.974
Compactness (crumb)	1.696	0.2	3.964	0.053	0.424	0.518
Castillian wheat odor	0.023	0.88	10.929	0.002	0.365	0.549
Galician wheat odor	1.23	0.274	0.126	0.724	0.028	0.869
Fermented dough odor	0.02	0.889	6.301	0.016	0.378	0.542
Malt odor	2.093	0.155	13.983	0.001	1.904	0.175
Crispiness (crust)	0.715	0.402	1.323	0.256	4.451	0.041
Hardness (crust)	0.137	0.713	0.028	0.869	2.012	0.163
Softness (crumb)	0.015	0.902	12.085	0.001	0.251	0.619
Adhesiveness	0.023	0.881	0.571	0.454	0.001	0.976
Chewiness	0.159	0.692	0.055	0.816	1.018	0.318
Sour taste	3.103	0.085	10.613	0.002	0.005	0.943
Salty taste	0.027	0.871	0.284	0.597	0.002	0.961
Bitter taste	0.637	0.429	7.246	0.01	0.539	0.467
Castillian wheat aroma	0.042	0.838	0.103	0.75	0.838	0.365
Galician wheat aroma	0.021	0.887	0.276	0.602	1.351	0.251
Fermented dough aroma	0.991	0.325	2.001	0.164	1.761	0.191
Malt aroma	0.196	0.66	6.622	0.014	0.004	0.95
Persistency	0.089	0.767	1.392	0.244	0.022	0.882

those made by the mixed method, except for a* crumb. Therefore, the differences found were due to the type of ferment since the culture system had no influence. This study was in agreement with the results of Pontonio et al. (2021), since they could not relate the color differences found in breads made with different flours (soft wheat, durum wheat or rye) to the type of cultivation method.

3.2.3. Instrumental texture of bread

Textural characteristics of breads were summarized in Table 3. Cohesiveness, springiness, chewiness and hardness were measured in crumb bread. The bread crust was determined by work of penetration and hardness. In general terms, the differences found were not due to the cultivation method but rather to the type of ferment. In those variables in which the interaction (cultivation method*type of fermentation) was significant (hardness, springiness and cohesiveness), when segmenting by type of crop management type, there were differences in all the variables depending on the ferment. The behavior in breads made with conventional and organic flours showed the same trend in cohesiveness, with higher values for breads made with the mixed leavening method. The same trend was also observed in hardness, although the values in this case were higher in breads made with sourdough. In the case of springiness, no clear trend was observed. On the other hand, when segmenting by type of ferment, there were no differences depending on the cultivation method in these three variables in breads made with sourdough. In the mixed leavening method, springiness and cohesiveness presented higher values in conventional breads while hardness presented lower values.

The variables in which the interaction (cultivation method*type of fermentation) was not significant (Table 3) the effect of the factors was analyzed independently. When the effect of each of the factors is analyzed independently, only a significant effect of the fermentation type factor was observed for the adhesiveness, chewiness, hardness and work of penetration variables (Table 3). Higher values were observed in breads made with sourdough than those made by the mixed method, except for crumb's adhesiveness.

In the only work in which these parameters were evaluated using a texturometer, the data indicate that there was no trend depending on the cultivation method (Pontonio et al., 2021). Gonzales-Barron and Butler (2008) evaluated the appearance of bread loaves prepared with different organic flours and a control flour (conventional system) using image texture analysis methods. These authors stated that in general the organic bread loaves presented a more heterogeneous appearance and the alveoli were larger. This is possibly due to the fact that these parameters were not only influenced by the cultivation method, but also by other factors such as the variety and the quality of the proteins.

3.2.4. Sensory analyses of bread: quantitative descriptive analyses

A quantitative descriptive analysis (QDA) of the breads was carried out to identify which specific sensory attributes were affected by the cultivation method to obtain the sensory profile of organic and conventional breads following the two fermentation methods accepted by the PGI Pan Galego (Fig. 3).

Crispiness crust was the only sensory descriptor in which the interaction (cultivation method*type of fermentation) has been significant (Table 4). However, when it was segmented by type of cultivation method, there was no significant differences. When segmenting by type of fermentation, significant differences were found, although a clear pattern was not observed. No significant differences were found in the breads obtained by the mixed method, whereas the breads made with sourdough presented lower values of crispiness crust in conventional and higher values in organic management system.

The effect of the factors, in the variables in which the interaction (cultivation method*type of fermentation) was not significant, was analyzed independently. Regarding the type of ferment used, the breads made by the mixed method presented higher values of crust and crumb color, in the amount of small cells, Galician wheat odor, moistness (crumb), softness (crumb), sour taste and bitter taste, but less amount of large and medium cells, flour residue (crust) and compactness (crumb) than breads made with sourdough.

As in the instrumental texture analysis and color, the cultivation method did not influence the sensory attributes of the bread (Table 4).

There is scarce research on the effect of the cultivation method on the sensory qualities of bread. Kihlberg et al. (2006) evaluated the management system in uncommon breads since it had added lard and sugar (Real Decreto 308/2019) and found differences in terms of the elasticity and juiciness, as well as rancid flavor. Breads baked with conventional flour had significantly lowest springiness and rancid flavor but significantly higher juiciness than breads baked with organic flour. This is related to the higher protein content (and perhaps protein quality) in conventional breads. As in the present study, they found no significant differences in wheat aroma.

Annett et al. (2007) found significant differences in surface and denseness texture of sliced bread, a different product from the present study since it was made with sugar, vegetable fat and powdered milk. Organic bread had a denser texture and smaller air cells in the crumb, so the volume of the loaf was lower, which was associated with the difference in the quality of the proteins and the dough properties. These results agreed with those of Haglund et al. (1998), where organic wholemeal breads have a lower volume. They have not found differences in taste or aroma, identical to the present study.

Kihlberg et al. (2004) investigated the effect of the cultivation method, milling technique, and variation in formulation on the sensory attributes of whole wheat uncommon bread. Conventional breads were associated with a crumb with greater intensity of aromas of wheat and toasted cereals and a crispier crust (unlike the present study). Instead,

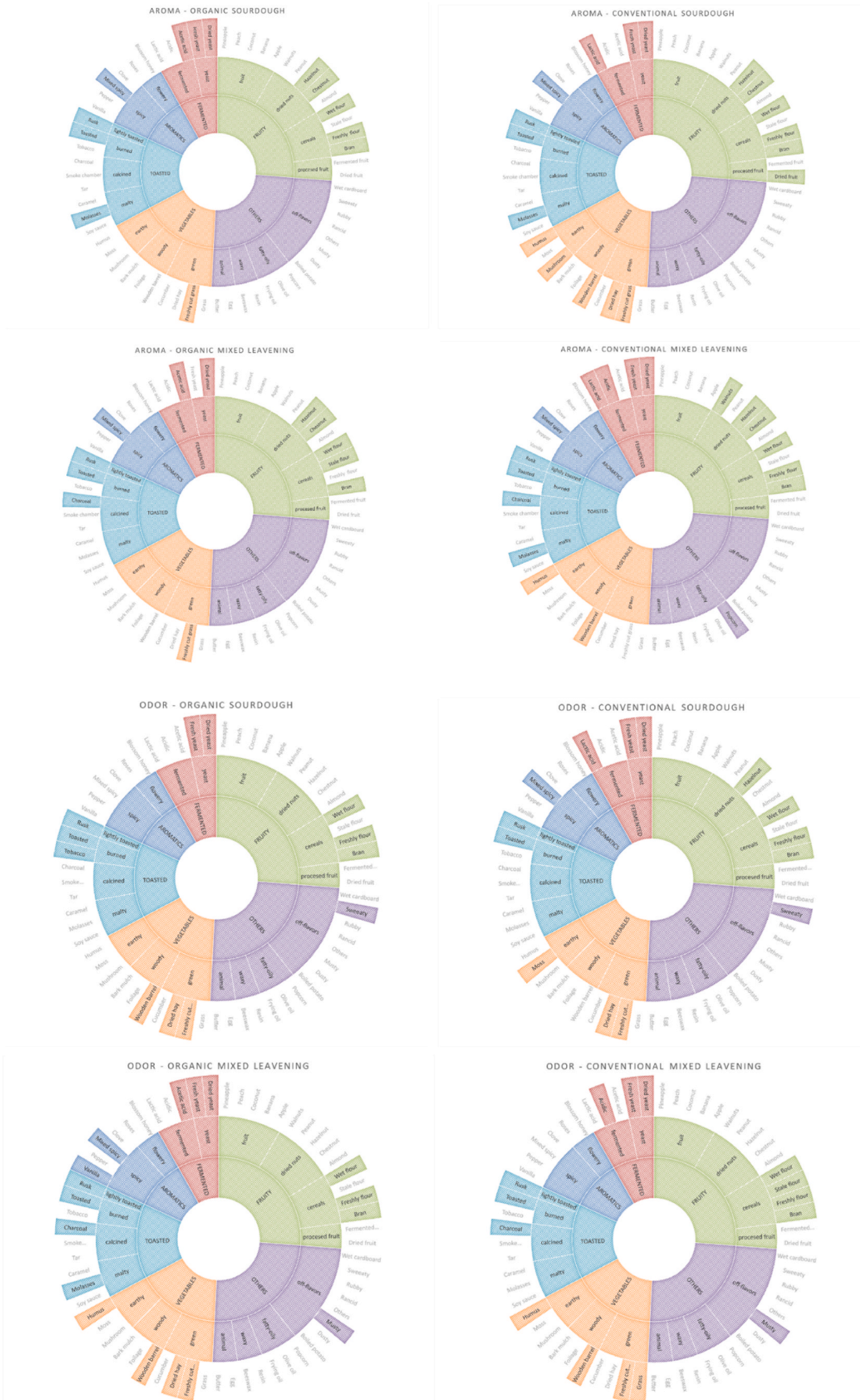

Fig. 4. Bread wheel descriptors selected at least once by one of the trained assessors, both for odor and aroma.

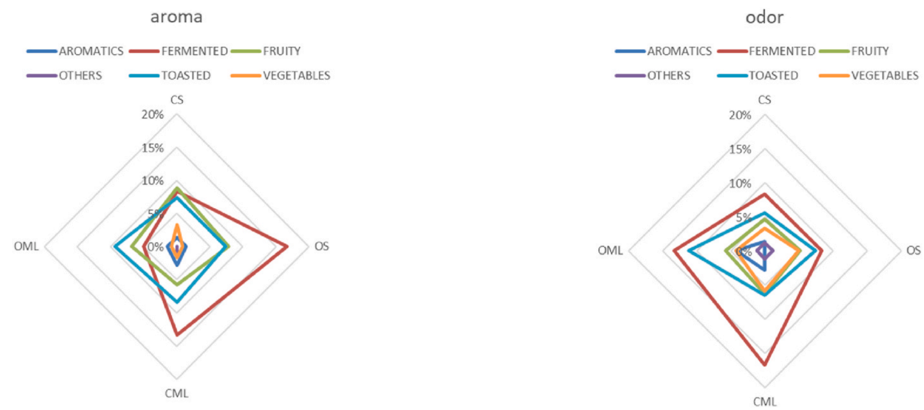

Fig. 5. Count of descriptors by families in odor (a) and aroma (b) in the different breads analyzed: organic mixed leavening (OML), organic sourdough (OS), conventional mixed leavening (CML) and conventional sourdough (CS).

trained assessors linked organic farming with a higher nut and cereal flavor, whiteness/blackness, yellow-red tone, color intensity, compactness, raw streak and aftertaste of the crumb. These authors stated that the grinding technique had a greater impact on the sensory qualities of the wholemeal bread than the effect of the crop management system.

3.2.5. *Sensory analyses of bread: Qualitative sensory analyses*

In order to deepen the sensory characterization at the level of the presence of volatile compounds, the results of the qualitative analysis of odor, aroma and aftertaste are presented (Fig. 4). In general, regarding odor, the breads with the greatest variety of descriptors selected by the trained panel were those made with the mixed leavening (organic and conventional management) with 18 descriptors out of the total 69. The organic sourdough bread presented the least variety of descriptors (12 descriptors out of 69). However, in terms of aroma, a greater variety of aromas was observed in breads made with conventional flour (both with sourdough and mixed leavening) (22 descriptors out of 69) than in organic ones (18 and 17 descriptors for sourdough and mixed leavening, respectively).

Due to the large number of descriptors present in the wheel, a count was made by families. And since all families have not the same number of descriptors, a weighted count was performed (Fig. 5).

The family with the highest presence of odor and aroma descriptors was fermented mainly in mixed leavening breads made with conventional flour. The odor is a very important descriptor because it is one of the first characteristics perceived when bread is being bought (Pico et al., 2015). These samples presented the odor of fermented family with percentages around 15%, while the aromas of the fermented family presented 13.3% (although they are mentioned at a slightly lower level than that corresponding to the sample made with organic flour and sourdough (16.6%).

A multitude of volatile compounds have been identified in wheat bread, significantly influencing its flavor and aroma. They originate from enzymatic reactions during kneading and also mainly due to fermentation of sugars in the dough by yeasts and/or lactic acid bacteria, or occur during the baking process such as non-enzymatic Maillard reactions (Pico et al., 2015; Zolfaghari et al., 2017). These volatile compounds include alcohols, aldehydes, esters, ethers, ketones, acids, hydrocarbons, pyrazines, pyrrolines, furans, lactones, or sulfur compounds (Pico et al., 2015). The non-volatile compounds that influence the flavor of wheat bread are lactic acid and salt (Heitmann et al., 2017). Pico et al. (2015) showed a total of 326 volatile compounds identified in wheat bread, and they reported that the 2-octen-4-one (ketones) was associated with yeasty odor type and 2-pentanol (volatile ketones alcohol) was associated with odor fermented type.

Regarding the aftertaste (data not shown), the tasters barely indicated the presence of descriptors except for some isolated mention. In all four loaves there was some mention of the descriptor freshly ground flour, but also isolated.

In the present study, the sensory characteristics were more influenced by the fermentation system than by the cultivation method. Like other authors (Kihlberg et al., 2004, 2006), the cultivation method had less impact than other factors such as milling techniques or the year of cultivation.

4. Conclusion

After a comprehensive analysis of the characteristics of flours and breads, it was determined that the differences observed in the instrumental parameters, with respect to the cultivation method employed, were generally minimal for both flours and breads. These findings were further supported by an evaluation of the sensory attributes of the breads. Moreover, it was evident that the fermentation process had a more significant impact compared to the cultivation method, as no discernible differences related to the type of management were found. Furthermore, although organic flours exhibited slightly higher concentrations of APC microorganisms, yeast, and molds, the contamination levels remained within the permissible limits established by regulations. Notably, none of the samples tested positive for *Clostridium*, *E. coli*, or *Salmonella*. This study has significantly contributed to the expanding knowledge surrounding the effects of cultivation method on microbiological, microscopic, sensory, and instrumental aspects in a field that increasingly values proximity and organic products. Given that the disparities associated with the management system were minimal, it is recommended to promote the organic production of Galician bread. This approach effectively addresses environmental concerns associated with intensive agricultural practices, such as soil degradation and the loss of biodiversity, thereby enhancing overall environmental sustainability.

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Ethical statement

During the execution of the research, appropriate protocols were used to protect the rights and privacy of all panellists, without coercion to participate, and including full disclosure of the requirements and absence of risk of the study, the written consent and non-disclosure of your data without your knowledge, and the ability to withdraw from the study at any time.

CRediT authorship contribution statement

Nerea Fernández-Canto: Writing – original draft, Investigation. **María Belén García-Gómez:** Writing – review & editing, Validation, Investigation. **María Lourdes Vázquez-Odériz:** Writing – review & editing, Conceptualization. **Matilde Lombardero-Fernández:** Writing – review & editing, Supervision, Methodology. **Santiago Pereira-Lorenzo:** Writing – review & editing, Supervision, Project administration. **Ángel Cobos:** Writing – review & editing, Validation, Conceptualization. **Olga Díaz:** Writing – review & editing, Methodology, Formal analysis. **Manuel Vázquez:** Writing – review & editing, Supervision, Resources, Conceptualization. **Ma Ángeles Romero-Rodríguez:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declared that they had no conflict of interests with respect to their authorship or the publication of this article.

Data availability

Data will be made available on request.

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