

Article

Evaluation of the Fermentation Dynamics of Commercial Baker's Yeast in Presence of Pistachio Powder to Produce Lysine-Enriched Breads

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Abstract: The present work was carried out to evaluate the microbiological, physicochemical, and sensory characteristics of fortified pistachio breads. Pistachio powder (5% *w/w*) was added to flour or semolina and fermented by a commercial baker's yeast (*Saccharomyces cerevisiae*). Pistachio powder did not influence the biological leavening of the doughs. The kinetics of pH and total titratable acidity (TTA) during dough fermentation showed that the leavening process occurred similarly for all trials. The concentration of yeasts increased during fermentation and reached levels of 10^8 CFU/g after 2 h. Pistachio powder decreased the height and softness of the final breads and increased cell density of the central slices. The amount of lysine after baking increased in pistachio breads and this effect was stronger for semolina rather than flour trials. Sensory evaluation indicated that fortified breads processed from semolina were those more appreciated by the judges. This work clearly indicated that the addition of pistachio powder in bread production represents a promising strategy to increase the availability of lysine in cereal-based fermented products.

Keywords: biological leavening; bread fortification; commercial baker's yeast; lysine; pistachio powder

1. Introduction

In recent years, consumers are more and more integrating their diet with functional foods [1]. As a consequence, while high-calorie foods are considered unhealthy [2], the request for novel foods with functional and nutraceutical properties is increasing rapidly [3].

Bread is one of the world's widely consumed food; it represents a good source of carbohydrate, minerals, and proteins [4], but it is poor in some amino acids, especially lysine [5,6]. To this purpose, the recent dietary programs of several countries are recommending the nutritional enrichment of cereal-based foods with essential amino acids [7].

The fortification of cereal-based foods with non-conventional ingredients such as legumes, plant-derived foods, and fungi [8–11] has been recognized as a good strategy to enrich the nutritional quality of bread. The addition of several protein ingredients such as legumes and edible nuts exerts several beneficial effects on the human health, including the prevention of diabetes and coronary heart diseases [12].

Pistachios are dry edible nuts consumed by humans since prehistoric times [13]. Pistachios are a good source of macronutrients and micronutrients and contain high levels of lysine [14]. Recently, the consumption of pistachio nuts increased due to their potential health benefits and common

association with “healthy” food diets [15]. Pistachio nuts are well appreciated for their organoleptic characteristics; they are consumed entire or in powder form in a large variety of non-fermented foods such as chocolate, ice cream, and pastry desserts [16,17].

The addition of pistachio powder in sourdough production was recently investigated by Gaglio and co-workers [18] in order to evaluate the effect of a mixed lactic acid bacteria (LAB) starter culture during fermentation on the availability of lysine in breads. So far, the fermentation of commercial baker’s yeast in the presence of pistachio powder has not been investigated. To this purpose, in this work pistachio powder was used to fortify wheat flour and semolina breads. Dough and breads were analyzed for several microbiological, physical and chemical parameters.

2. Materials and Methods

2.1. Raw Materials and Dough Production

Wheat flour (Grandi Molini Italiani, Siracusa, Italy), durum wheat semolina (Salvia Gaspare, Partinico, Italy), and pistachio powder (L’agricola di Cartillone, Bronte, Italy) were all produced and purchased in Sicily (Italy). Baker’s yeast (La Parisienne, Casteggio, Italy) composed of *Saccharomyces cerevisiae* cells was used as leavening agent.

The experimental plan included four different dough productions: CF, control dough prepared from wheat flour; CS, control dough prepared from semolina; EFP, experimental dough prepared from wheat flour and 5% (*w/w*) pistachio powder; ESP, experimental dough prepared from semolina and 5% (*w/w*) pistachio powder.

Each dough of 406 g was produced following the protocol reported by Gaglio et al. [11] with a dough yield (weight of the dough/weight of the flour \times 100) of 160. Briefly, control doughs (CF and CS) were obtained from 246 g of wheat flour or semolina, 150 mL of tap water, 4 g of baker’s yeast and 6 g of NaCl while experimental doughs (EFP and ESP) were obtained with the same amount of tap water, baker’s yeast and NaCl added to 233.7 g of wheat flour or semolina and 12.3 g (5% of wheat flour/semolina weight) of pistachio powder. Fermentation was carried out for 2 h at 28 °C. Dough productions were carried out in duplicate and repeated twice after two weeks.

2.2. Monitoring of Acidification and Biological Leavening

Dough fermentation was followed by pH, total titratable acidity (TTA), and microbiological analyses conducted soon after production (0 h) and at the end of fermentation (2 h). Drop in pH was determined electrometrically using the pH meter pH 70 + DHS (XS Instruments, Carpi, Italy). TTA was determined by titration with 0.1 N NaOH (expressed in terms of mL of NaOH) on 10 g of dough.

The microbiological counts were performed on 15 g of each dough. Dough samples were suspended in 135 mL Ringer’s solution (Sigma-Aldrich, Milan, Italy), homogenized by means of a stomacher (BagMixer[®] 400, Interscience, Saint Nom, France) at the highest speed (blending power 4) for 2 min, and then serially diluted. The inoculation, cultivation, and incubation of the different microbial groups occurred as follows: total mesophilic microorganisms (TMM) were spread plated on plate count agar (PCA) and incubated aerobically at 30 °C for 72 h; total LAB were pour plated on modified de Man, Rogosa, and Sharpe (mMRS) agar prepared as described by Corsetti et al. [19], incubated anaerobically at 30 °C for 48 h; sourdough LAB on sour dough bacteria (SDB) agar prepared as described by Kline and Sugihara [20], incubated aerobically at 30 °C for 48 h; and total yeasts on yeast extract peptone dextrose (YPD) agar, incubated at 28 °C for 48 h. To avoid fungal growth, cycloheximide (10 mg/mL) was added to mMRS and SDB while YPD was supplemented with chloramphenicol (0.05 mg/mL) to inhibit bacterial growth. All media were purchased from Oxoid (Milan, Italy). Plate counts were performed in triplicate.

2.3. Dough Moulding, Baking and Analyses of Bread Attributes

After preparation, 100 g of each dough was transferred into rectangular stainless steel baking pans of dimensions (143 × 79 mm, top inside; 129 × 64 mm, bottom outside; and depth inside 57 mm) indicated by the Method 10-10B of the American Association of Cereal Chemists [21]. At the end of fermentation, all doughs were baked at 200 °C in the semi-industrial oven Compact Combi (Electrolux, Pordenone, Italy) applying the following two-step baking program: 5 min with the “combi cooking” function and 15 min with the “convection heat” function. Bread samples after cooling at room temperature were evaluated for their quality attributes such as weight loss, height, colorimetric parameters, hardness of crumb, and image analysis (void fraction, cell density, and mean cell area) as described by Liguori et al. [22].

2.4. Sample Preparation and Lysine Content Determination

Lysine was extracted and detected according to the method described by Gaglio et al. [18]. Briefly, 2 g of wheat flour and semolina bread samples were weighed and transferred into a 50 mL polypropylene tube to which 10 mL of 0.2 mM acetic acid was added. The suspension was homogenized for 2 min in an Ultra-Turrax system (T 25 basic IKA Labortechnik, Staufen, Germany). The homogenate was centrifuged at 2800× *g* for 10 min at −5 °C and the supernatant was recovered and filtered through a 0.45 µm filter (Sartorius, Muggiò, Italy). The identification and quantification of lysine were obtained using a G6120B single quadrupole LC/MS system equipped with an electrospray ionization source (ESI). The analytical separation of lysine was carried out on a Zorbax Eclipse XDB C18 (75 mm × 4.6 mm, 5µm) supplied by Agilent Technologies (Wilmington, DE, USA) at 30 °C. Twenty microliters of the extracts were eluted at 0.3 mL/min with water/formic acid (A), pH 3.1, and acetonitrile (B), using the following gradients: 0–15 min, 10–60% B and 15–20 min, 60–10% B. In order to optimize the acquisition parameters of the lysine, individual standard solutions prepared in starting mobile phase were infused into the instrument. The potential chosen was 200 V. ESI work conditions were as follows: capillary voltage 5000 V, gas flow rate 13 L/min, gas temperature 300 °C, and nebulizer pressure 60 Psi. In order to enhance the sensitivity, the quadrupole was used in SIM mode. The characteristic fragments (SIM mode) of lysine amino acids and ions for quantification were as follows: fragment ions *m/z* 147, 130, and 84; selected ion *m/z* 147; retention time 6.2. min. A peak corresponding to M+1 was found which was attributed to the formation of [M+H]⁺. The identification was performed by comparing the analyte mass spectra determined from standard solutions. Lysine was quantified using an external calibration obtained by analyzing nine standard solutions at different concentrations. To ensure stability and reproducibility of the method the analyses were performed in triplicate. For lysine extraction and determination, lysine standards (99%) (Sigma-Aldrich), formic acid (98%), and acetic acid (glacial) at analytical grade (Merck, Darmstadt, Germany) were used. Ultra-pure water (Milli-Q system, Millipore, Bedford, MA, USA) was used throughout the experiments.

2.5. Sensory Evaluation

All breads were also evaluated for their sensory characteristics. The descriptive panel consisted of 13 assessors including seven women and six men (20–62 years old) familiar with the sensory analysis of bread. The analysis was performed following the guidelines of the International Organization for Standardization (ISO) [23]. Twenty descriptive attributes were judged among those reported by Comendador et al. [24] and evaluated by other authors [25,26]. In particular, the evaluation considered the following aspects of the breads: color and thickness of crust, color, porosity, alveolation and alveolation uniformity of crumb, intensity, bread and strange odor, intensity, bread and strange aroma, salty, acid, astringent, bitter, taste persistency, adhesiveness in mouth, crispness, and the overall assessment.

Each aspect was scored using a line scale anchored on the left (visual analogue scale) with dislike/low quality and on the right with like/high quality. The hedonic scale results were converted as distance (cm) of mark from the left end of the line.

2.6. Statistical Analyses

Data on dough acidification (pH, TTA, and microbial load) and bread attributes (height, color, firmness, void fraction, cell density, and mean cell area) of the resulting breads were statistically analyzed using the one-way analysis of variance (ANOVA) procedure with the software STATISTICA, version 10 StatSoft Inc., Tulsa, OK, USA. The post-hoc Tukey's multiple range was applied to determine the significance and the difference between control and pistachio-enriched breads. Significance level was $p < 0.05$.

3. Results

3.1. Leavening Process

The kinetics of acidification for the four dough trials are reported in Table 1. No significant differences ($p > 0.05$) in pH and TTA values were found among the four trials. The doughs were characterized by pH values almost superimposable in all trials just after production (around 6.0) and after 2 h of fermentation (almost 5.6). TTA data confirmed the acidification trend displayed by pH. In particular, the decrease of pH values corresponded to the increase of TTA and the highest values were registered for the trials carried out with semolina (0.8 mL 0.1 N NaOH of increase from 0 to 2 h).

Table 1. Kinetics of acidification during fermentation.

Samples	pH		TTA	
	0 h	2 h	0 h	2 h
CF	6.00 ± 0.05 ^a	5.66 ± 0.02 ^a	1.00 ± 0.10 ^a	1.40 ± 0.10 ^a
EFP	5.97 ± 0.03 ^a	5.67 ± 0.03 ^a	0.90 ± 0.20 ^a	1.40 ± 0.10 ^a
CS	5.98 ± 0.04 ^a	5.57 ± 0.02 ^a	1.00 ± 0.00 ^a	1.70 ± 0.10 ^a
ESP	5.95 ± 0.02 ^a	5.58 ± 0.04 ^a	1.00 ± 0.10 ^a	1.70 ± 0.20 ^a
<i>p</i> value	0.456	0.244	0.693	0.156

Results indicate mean values ± standard deviation of four determinations (carried out in duplicate for two independent productions). ^a Data within a column followed by the same letter were not significantly different according to Tukey's test. Abbreviations: CF, control dough prepared from wheat flour; EFP, experimental dough prepared from wheat flour + 5% (*w/w*) pistachio powder; CS, control dough prepared from semolina; ESP, experimental dough prepared from semolina + 5% (*w/w*) pistachio powder; and TTA, total titratable acidity.

The results of the plate counts of the doughs are reported graphically in Figure 1. According to Tukey's test, no statistically significant differences were found for any of the microbial groups that were the object of investigation among the four trials. The levels of TMM were almost superimposable to those of yeasts in all dough samples analysed. After 2 h of fermentation, TMM increased of about 0.5 Log cycles (Figure 1a). Total food LAB (enumerated on mMRS) and sourdough LAB (enumerated on SDB) were found at similar levels (about 10⁵ CFU/g) in dough samples just after production and after 2 h of fermentation (Figure 1b,c). Regarding yeasts, their levels increased during fermentation. In particular, after 2 h of fermentation this microbial group was counted at 10⁸ CFU/g in all dough trials (Figure 1d). These results confirmed that the addition of 5% (*w/w*) pistachio powder to flour or semolina did not influence the fermentation process carried out by the commercial baker's yeast.

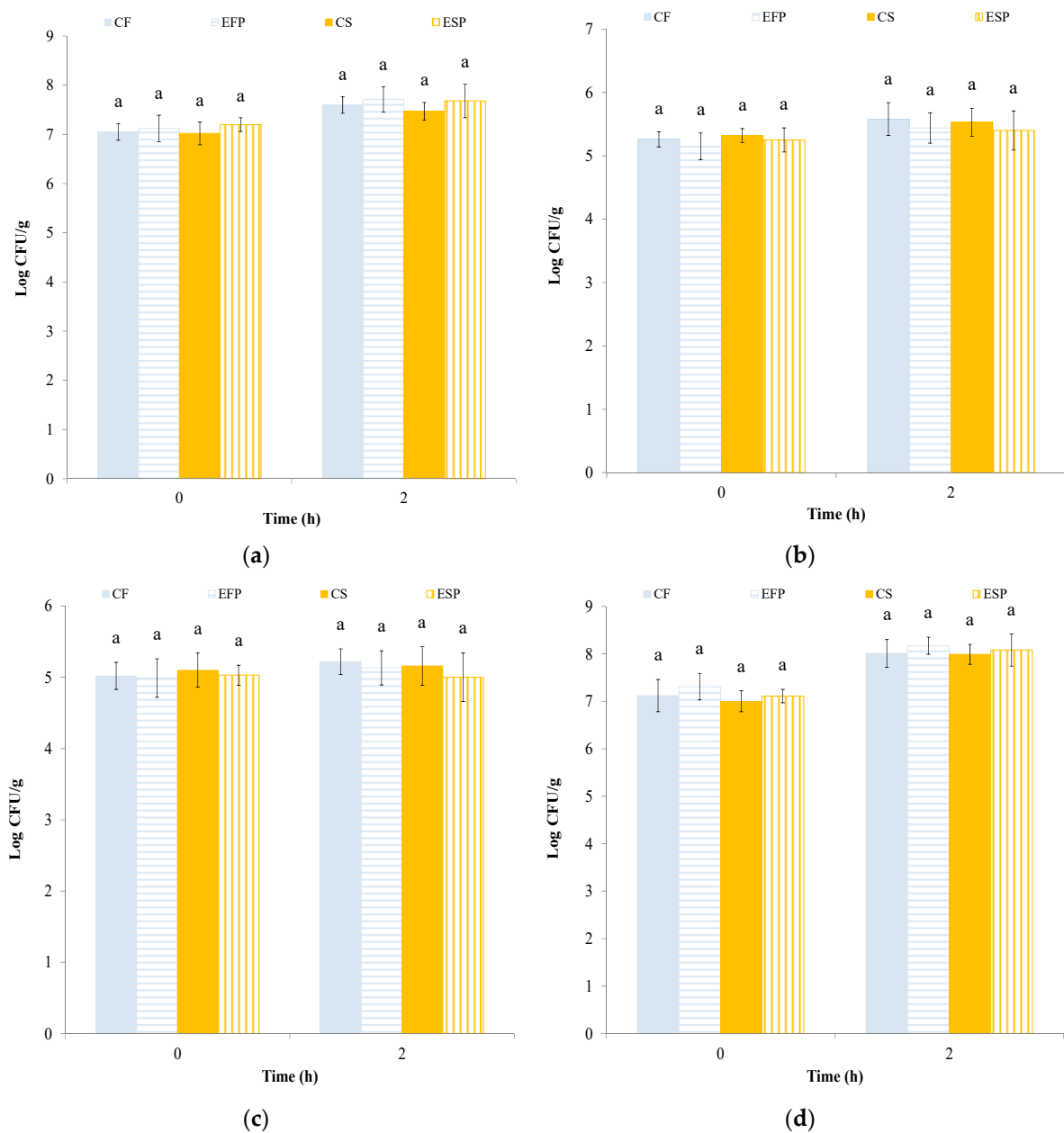


Figure 1. Microbiological counts during fermentation. (a) PCA, plate count agar for total mesophilic microorganisms (TMM); (b) mMRS, modified de Man, Rogosa, and Sharpe agar for generic food lactic acid bacteria (LAB); (c) SDB, sour dough bacteria agar for sourdough LAB; and (d) YPD, yeast extract peptone dextrose agar. for yeasts. Results indicate mean values and standard deviation of four determinations (carried out in duplicate for two independent productions). ^a Histograms followed by the same letter are not significantly different according to Tukey’s test. Abbreviations: CF, control dough prepared from wheat flour; EFP, experimental dough prepared from wheat flour + 5% (w/w) pistachio powder; CS, control dough prepared from semolina; and ESP, experimental dough prepared from semolina + 5% (w/w) pistachio powder.

3.2. Bread Attributes

The results of the quality characteristics of the final breads obtained with and without the addition of pistachio powder are reported in Table 2. Statistically significant differences were found among the final breads for all the quality characteristics analyzed.

Table 2. Bread attributes.

Trials	Height (mm)	Crust Color			Crumb Color			Firmness Value (N)	Void Fraction (%)	Cell Density (n.cm ⁻²)	Mean Cell Area (mm ²)
		L*	a*	b*	L*	a*	b*				
CF	41.00 ± 1.44 ^a	68.98 ± 1.26 ^{ab}	1.56 ± 0.91 ^b	23.96 ± 2.80 ^b	72.01 ± 3.44 ^a	-1.63 ± 0.08 ^a	14.21 ± 0.47 ^c	15.33 ± 0.04 ^c	2.31 ± 0.25 ^c	0.16 ± 0.02 ^b	0.30 ± 0.07 ^{ab}
EFP	36.00 ± 1.02 ^{ac}	64.33 ± 1.74 ^c	3.79 ± 1.60 ^a	27.04 ± 2.11 ^b	64.89 ± 1.50 ^b	-1.81 ± 0.30 ^a	15.30 ± 0.41 ^c	19.01 ± 0.07 ^a	0.93 ± 0.02 ^d	0.24 ± 0.10 ^b	0.04 ± 0.00 ^c
CS	38.00 ± 1.41 ^{ab}	70.76 ± 0.73 ^a	1.42 ± 0.37 ^b	35.06 ± 1.99 ^a	71.82 ± 3.35 ^a	-3.84 ± 0.14 ^b	22.01 ± 0.62 ^a	18.21 ± 0.08 ^b	4.30 ± 0.10 ^b	0.36 ± 0.05 ^b	0.40 ± 0.02 ^a
ESP	33.00 ± 1.51 ^c	65.62 ± 2.14 ^{bc}	0.23 ± 0.93 ^b	34.49 ± 1.89 ^a	67.08 ± 0.44 ^{ab}	-3.20 ± 0.33 ^c	20.35 ± 0.26 ^b	19.17 ± 0.11 ^a	5.37 ± 0.01 ^a	1.10 ± 0.34 ^a	0.20 ± 0.03 ^b
<i>p</i> value	0.001	0.003	0.004	0.001	0.02	0.0001	0.0001	0.0001	0.0001	0.001	0.0001

Results indicate mean values ± SD of four determinations (carried out in duplicate for two independent productions). Data within a column followed by the same letter (superscript letters) were not significantly different according to Tukey's test. Abbreviations: CF, control bread prepared from wheat flour; EFP, experimental bread prepared from wheat flour + 5% (*w/w*) pistachio powder; CS, control bread prepared from semolina; ESP, experimental bread prepared from semolina + 5% (*w/w*) pistachio powder; L*, lightness; a*, redness; and b*, yellowness.

The height of the central slices ranged between 33.00 and 41.00 mm. The highest values were reached by the breads produced from wheat flour (with and without pistachio powder). However, pistachio powder limited the height of the breads.

The firmness of the breads increased with the addition of pistachio powder to both flour and semolina. In particular, the softest breads were those obtained from wheat flour without the addition of pistachio powder.

The evaluation of the three color parameters showed that crumb color was more influenced than crust color by pistachio powder. Regarding crust, yellowness was the only parameter not affected by pistachio powder; as expected the highest yellowness values were registered for the breads produced from semolina. Regarding yellowness, the same trend was observed for crumb, with semolina trials being characterized by the highest values. The other color parameters showed statistically significant differences among the trials both for crust and crumb highlighting that they are particularly influenced by the type of flour as well as pistachio addition. The redness values of crumb were negative for all breads.

The image analysis indicated that all breads were different for the three parameters evaluated i.e. void fraction, cell density, and mean cell area. However, the highest levels of all parameters were found in breads produced with semolina without the addition of pistachio powder.

3.3. Available Lysine in Bread

The results of lysine content determined for the four bread productions are reported in Figure 2. Statistically significant differences ($p < 0.0001$) were found among breads. As expected, the amount of lysine detected in flour and semolina control breads was lower than that registered in the corresponding pistachio breads. In particular, the available lysine in control breads was 0.93 and 0.74 mg/100 g for flour and semolina trials, respectively. The addition of 5% (w/w) of pistachio powder determined a higher increase of lysine in semolina (1.45 mg/100 g) rather than flour (1.18 mg/100 g) breads.

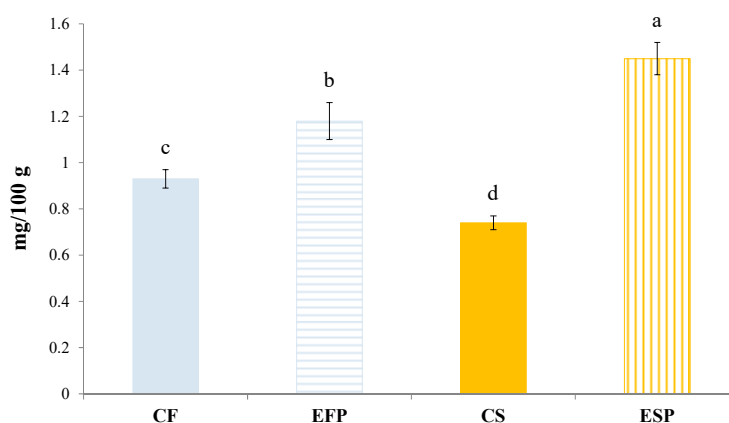


Figure 2. Lysine content of final breads. Abbreviations: CF, control bread prepared from wheat flour; EFP, experimental bread prepared from wheat flour + 5% (w/w) of pistachio powder; CS, control bread prepared from semolina; and ESP, experimental bread prepared from semolina + 5% (w/w) of pistachio powder. Histograms followed by the same letter are not significantly different according to Tukey's test.

3.4. Sensory Evaluation

After baking, all breads were cooled for about 2 h at room temperature and subjected to sensory evaluation by the judges. The results of the sensory tests are reported in Figure 3. Except for strange odor, strange aroma and bitter that scored at zero in all four trials, all other sensory characteristics that were objects of evaluation were perceived as different by the judges. The highest scores for crust color, crumb color, odor intensity, aroma intensity and taste persistency were registered for the breads produced from semolina and supplemented with pistachio powder (ESP), while the breads

produced with wheat flour and pistachio powder (EFP) showed the highest evaluation for sweetness and adhesiveness. The thickness of the crust of both bread trials supplemented with pistachio was lower than that of the corresponding control breads (CF and CS). Porosity and crumb elasticity were registered at higher levels in CF and CS. Salty attribute decreased with the addition of pistachio powder in both wheat raw materials. Finally, the highest score for the overall assessment was displayed by the breads supplemented with pistachio powder and, in particular, for the trial ESP, followed by the breads of the control productions.

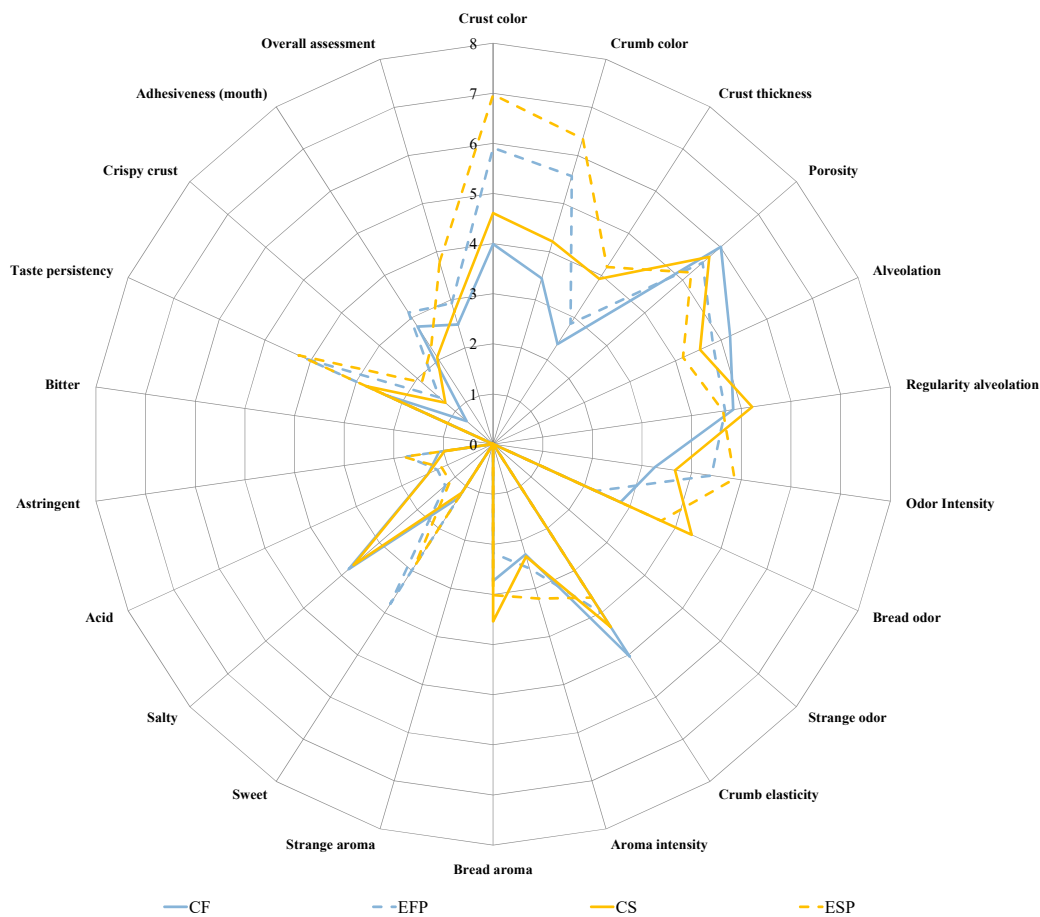


Figure 3. Spider diagrams of descriptive sensory evaluation of breads. Abbreviations: CF, control bread prepared from wheat flour; EFP, experimental bread prepared from wheat flour + 5% (*w/w*) pistachio powder; CS, control bread prepared from semolina; and ESP, experimental bread prepared from semolina + 5% (*w/w*) pistachio powder.

4. Discussion

Recently, besides enhancing the nutritional profile of breads, the practice of fortification with biologically active compounds [27] has also become common. Indeed, consumers are more and more aware of food composition and are demanding foods with no chemical preservatives [28] that are able to prevent health problems or diseases [29]. Several recent studies focused on fermentation by commercial baker’s yeast to produce fortified bread with dietary fibers, vitamins, and antioxidants [11,22,30]. Furthermore, these studies have investigated the microbiological and physicochemical parameters of fortified bread with legumes, fungi, and vegetative part of plants, but very little is known about bread fortification with edible nuts, such as pistachio, in order to produce lysine- enriched breads. With this in mind, the present study was carried out to evaluate the microbiological and physicochemical parameters of breads produced with and without addition of pistachio powder to wheat flour and semolina using baker’s yeast as leavening agent.

The fermentation process of all dough trials was followed by acidification kinetics and microbiological evolution. The change of pH in all trials followed the same trends commonly reported for dough samples supplemented with other ingredients [11,22]. TTA showed highest values in the trials carried out with semolina, due to its higher protein content compared to flour [31]. The microbiological evolution during dough fermentation followed the conventional approach applied to sourdoughs [20,32]. To this purpose, the samples were analyzed for total microbial count on PCA, for cultivable LAB on mMRS which is the medium generally used for total LAB in bread environment [19], for SDB which is specific for sourdough LAB [33], and for total yeasts on YPD. The highest levels of cell densities were found for TMM and yeasts, followed by LAB. In particular, yeasts increased their numbers during fermentation in all dough trials and reached values of about 10^8 CFU/g, indicating that the supplementation with pistachio powder at 5% (*w/w*) did not alter the development of commercial baker's yeast. LAB, commonly present in wheat flour and semolina [34], remained almost unchanged during fermentation. This trend was previously reported by other authors for dough prepared from wheat flour and dough prepared from wheat flour supplemented with *Pleurotus eryngii* powder or *Opuntia ficus-indica* mucilage [11,22], confirming that 2 h is not enough for the development of LAB.

The final characteristics of the breads confirmed the ability of the commercial baker's yeast to act as leavening agent in presence of pistachio, although the height of the breads was lower than control breads. This is not surprising, since Almorai [35] reported the decrease of the specific volume of bread due to the addition of walnut powder. The softness of the breads was directly correlated with their height as reported by other authors [36,37]. The addition of pistachio powder determined a little increase in the firmness of breads. However, with the exception of the breads fortified with the legume hulls or insoluble cotyledon fibers from peas, lentils, and chickpeas that do not determine an increase of firmness [30], our results are mostly in agreement with those of other authors showing that the fortification with different powders such as mushroom [38], coriander leaf [39], and fennel seed [40] determined an increase of this parameter in final breads. Some differences were registered among the breads for the crumb and crust color of the breads. However, it is well known that the use of different cereal material and the fortification have different effects on colorimetric parameters of bread. Alfonzo et al. [41] reported that the use of semolina from different genotypes of durum wheat cultivated in Sicily influenced color parameters in the final breads, even though the strongest effects on bread color are exerted by vegetative parts of plants [22,39] or fruit powders [42,43]. Statistically significant differences were also registered among void fraction, cell density, and mean cell area. These parameters are generally evaluated in breads obtained with different raw materials [41].

The determination of lysine content in the breads confirmed that a fortification with 5% (*w/w*) pistachio powder to wheat flour and semolina determined an increase of this amino acid in both pistachio breads. However, the higher increase of lysine content was estimated for semolina pistachio breads. This trend was reported by Gaglio et al. [18] for pistachio breads prepared from the same raw materials used in this study (wheat flour, semolina, and pistachio powder) but with the aim of evaluating the persistence of a mixed LAB starter culture populations during lysine fortification of sourdough breads by addition of pistachio powder. Moreover, the increase of lysine content in pistachio breads could be due to the ability of baker's yeast to ferment the pistachio proteins. The ability of *S. cerevisiae* to improve the lysine content in fermented foods was previously reported by different authors [44,45].

The judges evaluated all breads for their sensory profiles and evidenced several differences among the four trials. A higher complexity of odors (odor and aroma intensity) was registered for both pistachio breads. With regard to the overall assessment, both pistachio-enriched breads were preferred to the corresponding control breads. In particular, the breads produced with semolina and supplemented with pistachio powder were those most appreciated by the judges.

5. Conclusions

This study provided, for the first time, an extended analysis of the microbiological, physicochemical, and sensory characteristics of pistachio-fortified breads leavened by a commercial baker's yeast (*S. cerevisiae*). The fortification with pistachio powder increased the availability of lysine in the final breads. Indeed, it is well known that pistachios are rich in essential amino acids, especially lysine [14]. The supplementation did not alter the microbiological and acidification parameters during the fermentation carried out with baker's yeast. The quality characteristics of the final breads clearly showed that the addition of pistachio powder to wheat flour and semolina decreased the height and the softness of the breads and increased cell density of the crumb. Furthermore, the sensory evaluation determined the appreciation by the judges, with high values for overall acceptance of pistachio-enriched breads, preferably when prepared with semolina.

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