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Breadmaking with an old wholewheat flour: Optimization of ingredients to improve bread quality

Ottavia Parenti, Lorenzo Guerrini*, Benedetta Cavallini, Fabio Baldi, Bruno Zanoni

Department of Agricultural, Food and Forestry Systems Management (DAGRI), University of Florence, Piazzale Delle Cascine 16, 50144, Florence, Italy

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ABSTRACT

Processing strategies are necessary to improve the quality of baked old wholewheat flour products, since they are required by consumers but have poor technological properties. The present study tested the addition of common improvers on an old wholewheat flour performance to optimize bread quality. At first, the effect of seven improvers on dough rheology and bread specific volume was evaluated using a screening design method. All of the improvers affected the farinographic parameters; the most promising effects were shown by sucrose, salt and guar gum. Bread specific volume was significantly improved by sucrose, extra virgin olive oil and ice; hence, the effects of these variables on dough rheology and bread quality were evaluated in-depth in a full factorial trial. Dough stability and dough weakening were significantly improved by sucrose and extra virgin olive oil. Sucrose and extra virgin olive oil interaction optimized bread specific volume, crumb specific volume and hardness. The addition of 2% sucrose and 3% extra virgin olive oil resulted in optimized bread, on which a qualitative sensory evaluation was performed. This optimization approach could be applied to other wholewheat flours to improve product quality, hence promoting the consumption of high nutritional value breads.

1. Introduction

Wheat bread represents the staple food in many diets, with a far-reaching impact on human health. Depending on the degree of refinement of the flour used in the bread recipe, the composition of the final product changes immensely.

Refined flours are mainly composed of the starchy endosperm, while they are deprived of the germ fraction and the outer kernel layers. Conversely, unrefined flours are extremely rich in compounds such as dietary fibres, fats, minerals, vitamins, lignans and phenolic compounds, which are positive for human health (Zhou, Therdthai, & Hui, 2014).

In recent years, several scientific studies have shown that a regular consumption of wholewheat products protects from chronic diseases such as cardiovascular disease, type 2 diabetes and some types of cancers (Ye, Chacko, Chou, Kugizaki, & Liu, 2012). Unfortunately, unrefined flours show a poor technological performance, since the presence of the bran fraction has a negative effect on the breadmaking process, and changes the taste and flavour of the resulting bread (Gómez, Ronda, Blanco, Caballero, & Apesteguía, 2003). Therefore, refined wheat flour still represents the preferred choice for bread production.

Due to increasing consumer attention towards healthy food, in the recent years there has been renewed interest in old wheats (Guerrini, Parenti, Angeloni, & Zanoni, 2019). Old wheats are generally defined as those wheat varieties cultivated before the intense genetic selection that took place during the Green Revolution of the 1960s (Dinu, Whittaker, Pagliai, Benedettelli, & Sofi, 2018). Hence, the old wheat term includes a large number of cultivars, with a broad genetic base, and therefore showing a broad range of characteristics (Dinu et al., 2018; Mefleh et al., 2019). Within them, some varieties were reported to have high nutritional value and potential health benefits (Leoncini et al., 2012; Dinelli et al., 2011; Sofi et al., 2010; Gotti et al., 2018; Sereni et al., 2017).

Considering the poor technological properties of old wholewheat flours compared to conventional flour blends, it is still a challenge to use them in breadmaking (Cappelli et al., 2018; Farbo, Fadda, Marceddu, Conte, & Del, 2020). Thus, different operating procedures should be specifically designed to maximize the technological performance of old wheat flour, for example by using some ingredients in the recipe with the aim of ameliorating the final product quality (i.e. improvers).

In this study an optimization approach was carried out to find the best combination of improvers employed in breadmaking on sp. *Triticum aestivum* L., cv. Verna old wholewheat flour, evaluating their effects on the quality of dough and bread. At first, seven common

* Corresponding author.
E-mail address: lorenzo.guerrini@unifi.it (L. Guerrini).

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improvers were evaluated following an optimized experimental design, in order to reveal which of them had the greatest effect on bread quality. This evaluation enabled the selection of three bread improvers, which were evaluated in-depth in a full factorial design trial. Finally, an optimized bread recipe was identified.

2. Materials and methods

2.1. Materials

Experimental trials were carried out with two batches (*V1* and *V2* batches) of a sp. *Triticum aestivum* L., cv. Verna *old wholewheat flour*; wheat seeds were grown in Montespertoli (Florence, Italy), during the growing season 2018–2019. The chemical and physical characterization of the old wholewheat flour Verna batch 1 *"V1"* and Verna batch 2 *"V2"* was as follows: moisture (*V1* = 12.83g/100g, *V2* = 13.46g/100g), ash (*V1* = 1.01g/100 g d.m., *V2* = 1.28g/100 g d.m.) and protein (*V1* = 12.3g/100 g d.m., *V2* = 10.5g/100 g d.m.) contents; WA (*V1* = 57.75%, *V2* = 55.00%), DDT (*V1* = 3.00min, *V2* = 2.50min), DS (*V1* = 2.00min, *V2* = 1.17min) and DW (*V1* = 165BU, *V2* = 203BU); P (*V1* = 39.0mmH_2O, *V2* = 46.0mmH_2O), L (*V1* = 30.0 mm, *V2* = 25.0 mm) and W (*V1* = 42.3 10^{-4}J, *V2* = 44.8 10^{-4}J) and P/L (*V1* = 1.3, *V2* = 1.9).

The old wholewheat flours were processed using a stone grinding mill and a sieve (two consecutive passages through a 1,100–1,200 μm sieve) at the Molino Paciscopi (Montespertoli, Florence, Italy). Mineral water (Levissima, Bormio, Italy), fresh brewer's yeast (Lievital, Trecasali, Italy), extra virgin olive oil (*EVOO*), guar gum (*GG*), sucrose (*Suc*) and sodium chloride (*NaCl*) were purchased at a local market (Florence, Italy). Ascorbic acid (AH_2) was purchased in a drugstore. *Ice* (prepared with the above mineral water) and gelatinized flour (*GF*) were prepared in the lab the day before each trial. The *GF* was prepared with a 1:4 ratio of old wholewheat flour to mineral water (Levissima, Bormio, Italy). The mixture was continuously stirred as it was heated to 85 °C for 3 min. Temperature was measured with a Type J penetration probe (Testo, Lenzkirch, Germany). GF was cooled to room temperature, stored at 4 °C and used the following day as bread improver (Parenti et al., 2019).

2.2. The experimental design

2.2.1. The screening design trial (T1)

A Plackett-Burman screening design (Antony, 2014) was adopted to simultaneously test the main effects of the seven bread improvers on dough performance and bread quality. The screening design allowed the seven factors to be tested at two levels using only eight samples. The chosen variables, their level settings and the combinations used in the eight trials are shown in Table 1.

The *T1* trial was carried out on the *V1* Verna old wholewheat flour batch. Rheological analyses of doughs were carried out using a Farinograph (Brabender, Duisburg, Germany). The baking process was standardized as reported below. Bread quality was evaluated by measuring the bread specific volume immediately after baking.

2.2.2. The full factorial design trial (T2)

The screening design made it possible to evaluate a large number of factors with a small number of tests. However, there are several limitations. Specifically, the design is a resolution III design (Antony, 2014), meaning that the main effects could be confused with two-factor and higher order interactions. Hence, the three variables with the highest impact on bread quality in *T1* were tested in detail in a validation trial (*T2*), following a full factorial design. The experimental design is shown in Table 3. The chosen maximum level of *EVOO* (2%) and *Ice* (20%) was the same as in the *T1* trial, while the chosen maximum level of *Suc* was lowered from 6% to 4%. This choice was made since the addition of 6% *Suc* resulted in the excessive browning of the bread crust and the perception of too much sweetness during the bread tasting, while 4% *Suc* did not show these drawbacks (data not shown). Moreover, a medium level of *Suc* (i.e., 2%) and *Ice* (i.e., 10%) was also included.

The *T2* trial was carried out on the *V2* Verna old wholewheat flour batch. Rheological analyses of the dough were carried out using a Farinograph (Brabender, Duisburg, Germany). The baking process was standardized as reported below. The bread quality parameters were evaluated immediately after baking. Bread specific volume, crumb specific volume, crumb and crust moisture, instrumental bread texture (Texture Profile Analysis - TPA), crumb image analysis and bread colour were evaluated. A sensory evaluation was also carried out on the optimized sample.

2.3. Preparation methods

2.3.1. Breadmaking

The bread dough was prepared in 500g batches. The basic formulation was: flour (310g), fresh brewer's yeast (13g) and the amount of water required to reach the farinograph consistency value of 500BU (51–59.5%, w/flour w). The straight dough method was applied.

The improvers were added together with the main ingredients. The *GF* was warmed to room temperature, the *Ice* was finely broken up in a mixer and the AH_2 was carefully solubilized in mineral water before adding the improvers to the bread dough. The breadmaking phases were all carried out with a bread machine (Pain doré, Moulinex, Ecully, France) using the WWF programme (mixing step: 25 min at room T, resting and leavening: 1h and 20 min at 40 °C, baking: 55 min at 180 °C). The bread samples were cooled to room temperature prior to the bread quality evaluation. Two replicates were performed in the *T1* trial, and four in the *T2* trial.

2.4. Measurement method

2.4.1. Chemical characterization of old wholewheat flour

Moisture (AACC 44–15.02), protein (ISTISAN 1996/34, N x 6.25) and ash (ISTISAN 1996/34) contents were measured according to AACC International Approved Methods.

Table 1
T1 trial settings; gelatinized flour = *GF*; extra virgin olive oil = *EVOO*, sucrose = *Suc*, ascorbic acid = AH_2, guar gum = *GG*. The symbol "-" represents the lowest level of each factor (i.e. 0%), the symbol "+" represents the highest level of each factor, which is shown in the table.

Samples	*GF* (6%, w/flour w)	*EVOO* (3%, w/flour w)	*Suc* (6%, w/flour w)	AH_2 (100 ppm)	*GG* (1%, w/flour w)	*NaCl* (2%, w/flour w)	*Ice* (20%, w/water w)
1	+	+	+	–	–	–	+
2	–	+	+	+	+	–	–
3	+	–	+	+	–	+	–
4	–	–	+	–	+	+	+
5	+	+	–	–	+	+	–
6	–	+	–	+	–	+	+
7	+	–	–	+	+	–	+
8	–	–	–	–	–	–	–

Table 2
Farinographic parameters of *T1* trial dough samples with addition "+" or not "-" of the seven improvers.

FACTOR	WA (%)	P WA	DDT (MIN)	P DDT	DS (MIN)	P DS	DW (BU)	P DW
GF +	56.81 ± 1.03[a]	*	2.50 ± 0.01[a]	***	4.37 ± 0.41[a]	n.s.	118 ± 12[a]	n.s.
GF -	55.19 ± 1.03[b]		3.37 ± 0.01[b]		4.44 ± 0.41[a]		101 ± 12[a]	
EVOO +	55.25 ± 1.03[a]	*	2.87 ± 0.01[a]	***	4.31 ± 0.41[a]	n.s.	102 ± 12[a]	n.s.
EVOO -	56.75 ± 1.03[b]		3.00 ± 0.01[b]		4.50 ± 0.41[a]		117 ± 12[a]	
Suc +	54.94 ± 1.03[a]	**	2.25 ± 0.01[a]	***	4.87 ± 0.41[a]	**	94 ± 12[a]	**
Suc -	57.06 ± 1.03[b]		3.62 ± 0.01[b]		3.94 ± 0.41[b]		126 ± 12[b]	
AH_2 +	55.56 ± 1.03[a]	n.s.	2.87 ± 0.01[a]	***	3.94 ± 0.41[a]	**	116 ± 12[a]	n.s.
AH_2 -	56.44 ± 1.03[a]		3.00 ± 0.01[b]		4.87 ± 0.41[b]		103 ± 12[a]	
GG +	56.86 ± 1.03[a]	*	3.50 ± 0.01[a]	***	4.87 ± 0.41[a]	**	107 ± 12[a]	n.s.
GG -	55.37 ± 1.03[b]		2.37 ± 0.01[b]		3.94 ± 0.41[b]		112 ± 12[a]	
NaCl +	55.19 ± 1.03[a]	*	3.37 ± 0.01[a]	***	6.69 ± 0.41[a]	***	63 ± 12[a]	***
NaCl -	56.81 ± 1.03[b]		2.50 ± 0.01[b]		2.12 ± 0.41[b]		156 ± 12[b]	

Selected factors: gelatinized flour = *GF*; extra virgin olive oil = *EVOO*, sucrose = *Suc*, ascorbic acid = AH_2, guar gum = *GG* and salt = *NaCl*. Experimental data are expressed as mean ± standard error. *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. "n.s." indicates no significant difference at $p < 0.05$. Means in column with different superscripts are significantly different at $p < 0.05$. Specifically, "a" and "b" refer to main effect of each factor.

Table 3
T2 trial settings showing all 18 variable combinations. The variables tested in *T2* were: sucrose = *Suc* (3 levels: 0%, 2% and 4%, w/flour w); extra virgin olive oil = *EVOO* (2 levels: 0% and 3%, w/flour w) and *Ice* (3 levels: 0%, 10% and 20%, w/water w).

Sample	*Suc* (w/flour w)	*EVOO* (w/flour w)	*Ice* (w/water w)
1	0%	0%	0%
2	0%	3%	0%
3	0%	0%	10%
4	0%	3%	10%
5	0%	0%	20%
6	0%	3%	20%
7	2%	0%	0%
8	2%	3%	0%
9	2%	0%	10%
10	2%	3%	10%
11	2%	0%	20%
12	2%	3%	20%
13	4%	0%	0%
14	4%	3%	0%
15	4%	0%	10%
16	4%	3%	10%
17	4%	0%	20%
18	4%	3%	20%

2.4.2. Large deformation tests

Old wholewheat flour rheological characterization was performed according to the official method using a Farinograph (AACC 54-21.02) and Alveograph (AACC 54-30.02). Dough farinographic analyses were carried out in two replicates in the *T1* trial and three replicates in the *T2* trial.

2.4.3. Bread quality measurements

Bread volume (L) was measured using the standard millet displacement method (AACC, 2000). Specific volume (L/kg) was determined as the ratio between total volume and mass. Crumb specific volume (L/kg) was determined by cutting a small piece of crumb (5–10 g) and determining the ratio between its volume (L) (calculated using the standard millet displacement method (AACC, 2000)) and its mass (kg).

Crumb and crust moisture (g/100 g) were measured by gravimetry at 105 °C until constant weights were reached. Since the dough was prepared with different amounts of water (i.e. the quantity to reach 500BU), comparison between moisture parameters was made using the ratio between the crumb or crust bread moisture (g/100g) and the original dough moisture (g/100g).

The Texture Profile Analysis (TPA) of the bread samples was carried out by two-bite compression using a Texture Analyzer (Stable Micro Systems, UK), equipped with a circular flat-plate probe (diameter: 30 mm). Hardness (N), cohesiveness, gumminess (N), chewiness (N*mm) and springiness (mm) were measured on three slices (1.5 cm thickness) of each bread sample in five replicates.

Crumb porosity was evaluated by digital image analysis (Image J software, Color Inspector 3D.jar). Images of the central bread slice (thickness 1.0 cm) were acquired at a resolution of 1.2 MP. Rectangular sections of the bread crumb were selected, converted into an 8bit grey scale and subjected to spatial calibration before the analysis. The threshold was chosen according to Gonzales-Barron and Butler (2006), using the Otsu method. The following measurements were determined: pore area at the 50th percentile (mm^2), and total pore area (%), determined as the ratio between the total pore area (mm^2) in the analysed bread crumb section and the total area of the analysed bread crumb section (mm^2). Three replicates were performed on each bread sample. Crumb and crust colour were determined by digital image analysis. Photos of the bread samples were taken in standard light conditions. The crumb colour was evaluated on the central slice of the bread, while crust colour was assessed on the upper surface of the bread. L* or lightness (black 0/white 100), a* (green-/red+) and b* (blue-/yellow+) values were calculated according to (CIE Commission, 1978). All measurements were carried out in triplicate.

2.4.4. Bread sensory evaluation - a descriptive analysis

The sensory profile of the optimized sample was compared to the control sample (i.e. bread without improvers - CTR) and a qualitative analysis was performed (Dinnella, Borgogno, Picchi, & Monteleone, 2010). Fresh bread samples were prepared on the same day as the test, allowed to cool at room temperature and then used for the sensory evaluation. The descriptive panel consisted of seven panellists (3 males and 4 females, age 20–40) familiar with cereal products. A training before the test was performed to define the sensory attributes (Table S1 in the supplementary material). A nine-point scale (1–9, from extremely weak to extremely strong, respectively) was used to rate intensity. The freshly baked bread samples were given three-digit codes and 2.5 cm slices were presented to the assessors in random order. Water was provided to cleanse the palate between the samples. The panel was instructed to smell each sample before tasting it, and then they were requested to swallow the samples. A qualitative evaluation was performed using the medians of the raw data obtained.

2.4.5. Data processing

Two replicates were carried out to in the *T1* trial. A multi-factor ANOVA was performed to assess significant differences ($p < 0.05$) resulting from the seven tested factors.

In the *T2* trial three replicates were carried out for dough rheology

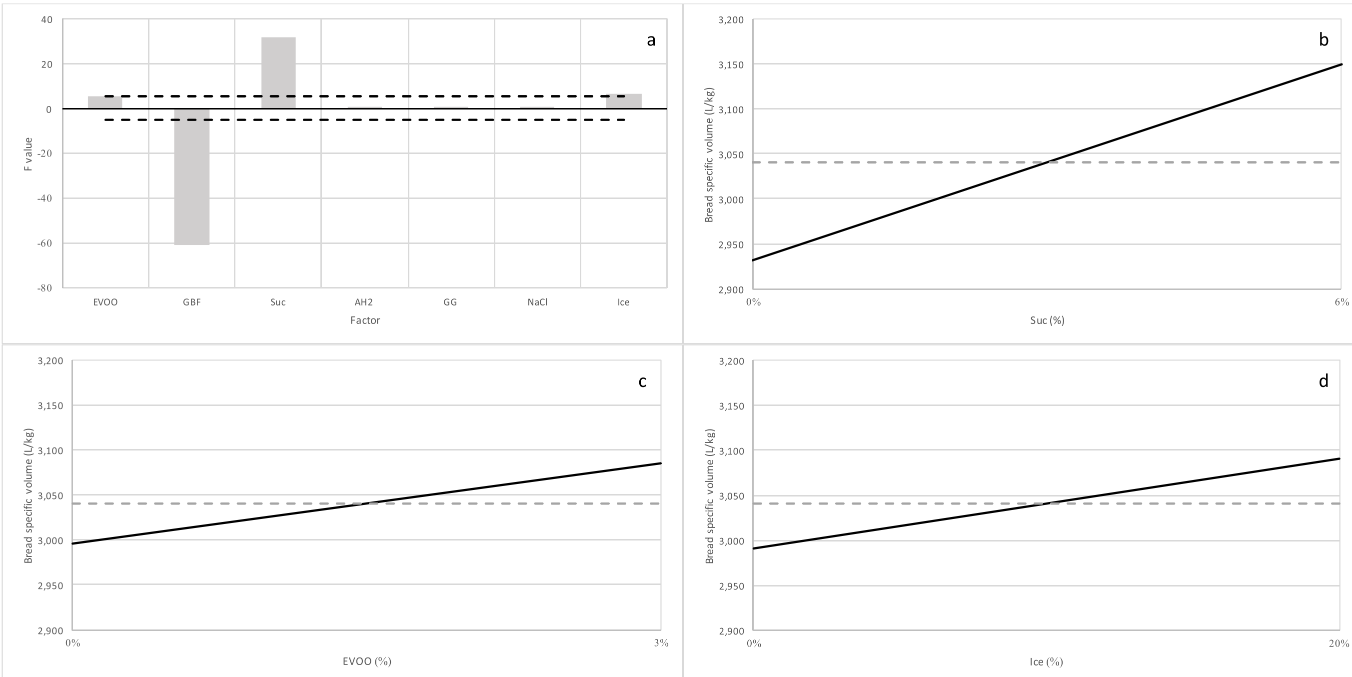

Fig. 1. Bar charts of T1 factors affecting bread specific volume (L/kg) (a). Line charts show the effect of addition of *Suc* (b), *EVOO* (c), and *Ice* (d) on bread specific volume (L/kg). Dashed line represents mean value of bread specific volume. The x-axis reports tested levels of each factor (*Suc* −1 = 0%, +1 = 6% w/flour w; *EVOO* -1 = 0%, +1 = 3% w/flour w; *Ice* −1 = 0%, +1 = 20% w/water w).

and four replicates for bread quality evaluation. A three-way ANOVA was performed to assess significant differences ($p < 0.05$) resulting from these factors and their two-factor and three-factor interactions. The Tukey HSD test was used as the post-hoc test.

3. Results and discussion

3.1. The T1 trial

Seven bread improvers were simultaneously tested on old wholewheat flour performance. Five of the seven improvers can be considered well-known bread improvers (i.e., *EVOO*, *Suc*, AH_2, *GG* and *NaCl*); *GF* and *Ice*, were also included.

GF from different sources has been tested in breadmaking (Carrillo-Navas et al., 2016; Fu, Che, Li, Wang, & Adhikari, 2016; Kim, Kwak, & Jeong, 2017). In particular, the addition of *GF* showed a significant improvement in the quality of the bread from brown wheat (Parenti et al., 2019).

The inclusion of *Ice* can be seen as a way to control a crucial factor of the kneading step: the temperature (Zhou et al., 2014). In preliminary trials different amounts of *Ice* (data not shown) in the breadmaking process were tested. The best result was obtained with a ratio of 20% (w/water w) of *Ice*: it reduced the dough temperature during the kneading step (20% of *Ice* addition reduced dough T before dough kneading from 20 °C to 14 °C and after dough kneading from 25 °C to 20 °C), without affecting this parameter during the leavening step and it gave the highest bread specific volume and softness.

The highest level of each factor was selected according to the literature as follows: 3%w/flour w *EVOO* (Pareyt, Finnie, Putseys, & Delcour, 2011), 2%w/flour w *NaCl* (Silow, Axel, Zannini, & Arendt, 2016); 6%w/flour w *Suc* (Zhou et al., 2014), 100 ppm AH_2 (Tebben, Shen, & Li, 2018); 1%w/flour w *GG* (Tebben et al., 2018); 6% of the total flour added to the bread dough was used to prepare the *GF* (Parenti et al., 2019).

3.1.1. Rheological characteristics of old wholewheat flour and the dough samples

The farinographic values showed that the *V1* batch of Verna old wholewheat flour was consistent with the "weak flour" definition: the reference consistency is reached quickly, to then decline considerably, with little or no stability (Zhou et al., 2014). Then, in "weak flours" an improvement in dough performance is usually related to an increase in dough stability (DS) and a reduction in dough weakening (DW). The alveographic values also showed a low value of dough strength (W) and an unbalanced ratio between dough tenacity and extensibility (P/L).

Addition of the improvers affected dough behaviour during the kneading step (Table 2).

Except for the reduction of WA (approx. 2.9%), *NaCl* effect was consistent with the literature (Silow et al., 2016): it strengthened the dough, increased DDT (approx. 1 min), triplicated DS and greatly reduced DW (approx. 90 DU). Similarly, *GG* significantly extended the DDT (more than 1 min), and increased DS (approx. 1 min). All these effects were consistent with previous studies (Tebben et al., 2018).

Considering the *Suc* effect, consistent with the literature (Peng, Li, Ding, & Yang, 2017) a decrease in WA, an increase in DS (approx. 1 min) and a decrease in DW (approx. 30 BU) were observed. Conversely, the decrease in DDT (approx. 1.5 min) was not in accordance with Mariotti and Alamprese (2012).

The addition of *EVOO*, *GF* and AH_2 did not result in an improvement in the farinographic performance. Specifically, *EVOO* decreased WA (2.6%) and slightly reduced DDT, without affecting DS or DW. The most common lipids used in breadmaking are shortening and surfactants, while, very little investigation has been performed on the long chain fatty acids EVOO. The decrease in DDT could be the direct consequence of the lower amount of water required by dough with added *EVOO*.

GF significantly increased the WA parameter (2.9%), consistently with Parenti et al. (2019), and reduced the DDT (approx. 1 min), whereas no significant effects were observed on DS or DW.

Finally, AH_2 significantly decreased DDT and DS (approx. 1 min) without affecting the other parameters, worsening the old wholewheat flour's technological properties. These results were in contrast to the

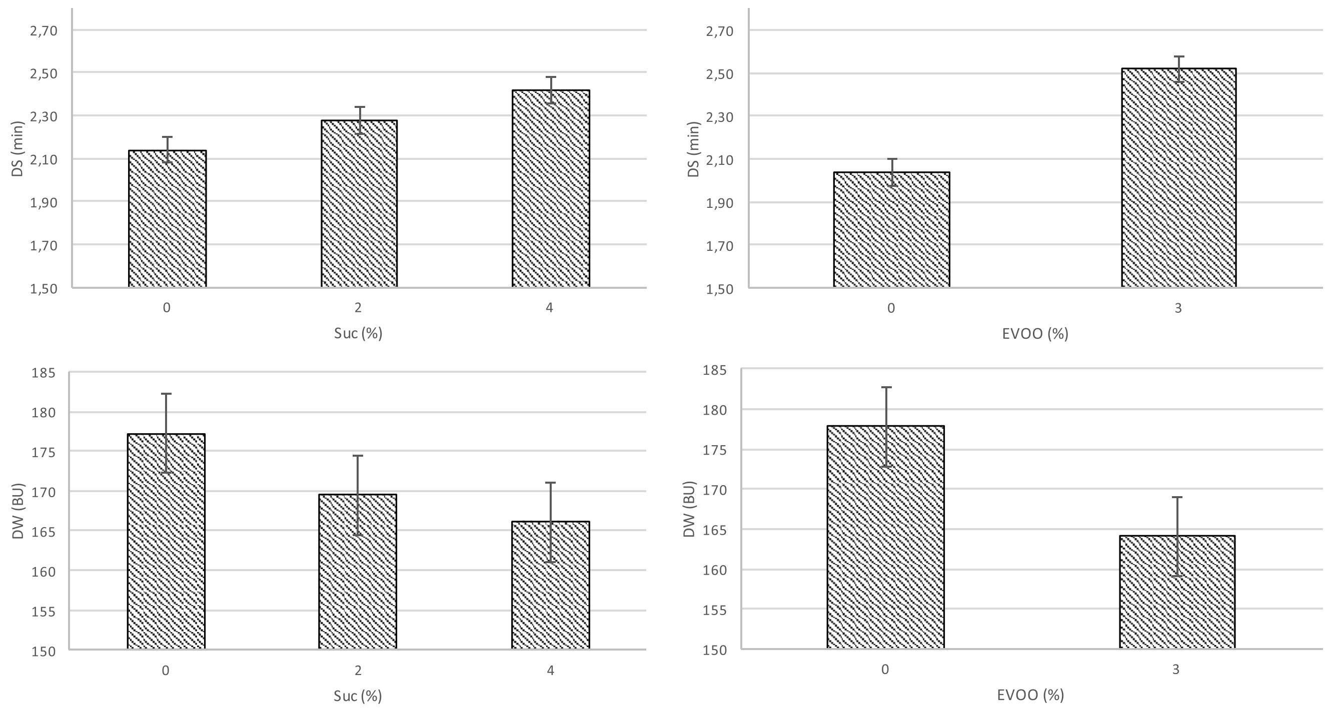

Fig. 2. Dough Stability (DS) and Dough Weakening (DW) farinographic parameters as affected by the addition of *Suc* (0%, 2%, 4% w/flour w) in a and c, and *EVOO* (0%, 3% w/flour w) in b and d.

positive effect of an oxidant agents on white flours. It is likely that the fibre fraction of old wholewheat flour containing a high quantity of reducing compounds, lowered the effects of oxidant agents (Tebben et al., 2018).

Boosting dough rheological parameters thanks to *NaCl*, *GG* and *Suc* improvers could be seen as a good strategy to facilitate dough workability for the old wholewheat flour breadmaking process.

3.1.2. Bread quality

Fig. 1 compares the effects of the improvers on bread specific volume. An effect was observed for *GF*, *EVOO*, *Suc* and *Ice*, while the other improvers did not significantly affect the bread volume. Specifically, a significant increase was obtained with *Suc* (from 2.93 ± 0.08 L/kg to 3.15 ± 0.08 L/kg), *EVOO* (from 3.00 ± 0.08 L/kg to 3.09 ± 0.08 L/kg), and *Ice* (from 3.00 ± 0.08 L/kg to 3.09 ± 0.08 L/kg), while *GF* decreased the parameter from 3.19 ± 0.08 L/kg to 2.89 ± 0.08 L/kg.

The greatest rise in bread specific volume was obtained with *Suc* (7.4%), whereas *EVOO* and *Ice* produced a similar increase (3%). The effect of *Suc* probably promoted the growth of yeasts, which led to a better performance during the leavening step (Zhou et al., 2014). The literature has reported no effect or a worsening effect on bread volume when vegetable oils are added to bread dough (Pareyt et al., 2011). Conversely, Matsakidou, Blekas, and Paraskevopoulou (2010), observed a significant volume increase when *EVOO* was added to cake dough production. The inclusion of *Ice*, which lowered the mixing temperature, could have improved the gluten matrix development (Quayson, Marti, Bonomi, Atwell, & Seetharaman, 2016). The negative effect of the *GF*, inconsistent with the literature (Parenti et al., 2019), could be the result of the different amylose/amylopectin ratio, which is a genetic characteristic of each wheat variety and deeply influences the starch gelatinization process (Goesaert et al., 2005).

3.2. The T2 trial

This study aimed to optimize the bread quality, hence, only the improvers that positively affected the bread specific volume (i.e. *Suc*, *EVOO* and *Ice*) were selected for the *T2* trial.

3.2.1. Rheological characteristics of old wholewheat flour and dough samples

According to the *T1* trial, the farinographic test only considered *Suc* and *EVOO* as factors, while the addition of *Ice* was not tested. The *V2* batch of Verna old wholewheat flour showed rheological properties consistent with the *V1* batch.

All of the farinographic parameters were affected by *Suc*; *EVOO* significantly changed the WA, DS and DW. WA was significantly reduced by both factors (data not shown), in accordance with the *T1* trial.

These results were consistent with the scientific literature; Peng et al. (2017) reported a decrease in the WA parameter when a sugar (i.e. trehalose) was added to the bread dough; lipid improvers (i.e. shortening) decrease the flour components' adsorption capacity by settling around the starch granules and the gluten protein during the hydration phase (Pareyt et al., 2011).

The DDT was significantly enhanced by the addition of 4% *Suc* (from 2.8 ± 0.3 min to 3.1 ± 0.3 min): the greater the addition of the improver, the lower the water availability for the development of the gluten network, which requires a longer time (Mariotti & Alamprese, 2012).

DS was significantly improved by the highest level of *Suc* (from 2.1 ± 0.3 min to 2.4 ± 0.3 min) as well as by *EVOO* (from 2.0 ± 0.3 min to 2.5 ± 0.3 min). These results confirmed the effect of *Suc* already observed in the *T1* trial. Furthermore, they revealed that *EVOO* exercised a comparable role. Finally, both improvers were effective in reducing DW: the highest level of *Suc* decreased the value from 177 ± 10BU to 166 ± 10BU, in accordance with the literature (Mariotti & Alamprese, 2012); a similar decrease was also observed with the inclusion of *EVOO* (from 177 ± 10BU to 164 ± 10BU). Hence, a general improvement of the rheological properties can be obtained by supplementing *Suc* and *EVOO* (Fig. 2). The positive effects exercised by *Suc* to the tested old wholewheat flour were consistent

Table 4
T2 trials bread quality evaluation.

Sample	*Suc (w/flour w)*	*EVOO (w/flour w)*	*Ice (w/water w)*	*Bread specific volume (L/kg)*	*Crumb specific volume (L/kg)*	*Crumb/dough moisture*	*Crust/dough moisture*	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (N mm)
1	0%	0%	0%	3.184 ± 0.097^{ax}	3.322 ± 0.252	96.9 ± 0.8^{ai}	67.4 ± 4.4^{x}	3.69 ± 0.73^{ax}	0.235 ± 0.058^{xi}	0.764 ± 0.066^{xi}	0.638 ± 0.256^{abxi}
2	0%	3%	0%	3.188 ± 0.097^{ay}	2.968 ± 0.252	96.9 ± 0.8^{ai}	55.8 ± 4.4^{y}	5.24 ± 0.73^{ay}	0.185 ± 0.058^{yi}	0.679 ± 0.066^{yi}	0.661 ± 0.256^{abyi}
3	0%	0%	10%	3.172 ± 0.097^{ax}	3.562 ± 0.252	97.6 ± 0.8^{aj}	66.3 ± 4.4^{x}	3.73 ± 0.73^{ax}	0.221 ± 0.058^{xi}	0.725 ± 0.066^{xij}	0.594 ± 0.256^{abxij}
4	0%	3%	10%	3.133 ± 0.097^{ay}	3.023 ± 0.252	97.4 ± 0.8^{aj}	62.0 ± 4.4^{y}	4.49 ± 0.73^{ay}	0.207 ± 0.058^{yi}	0.743 ± 0.066^{yij}	0.776 ± 0.256^{abyij}
5	0%	0%	20%	3.238 ± 0.097^{ax}	3.325 ± 0.252	97.5 ± 0.8^{ak}	70.5 ± 4.4^{x}	4.36 ± 0.73^{ax}	0.271 ± 0.058^{xj}	0.826 ± 0.066^{xj}	0.981 ± 0.256^{abxj}
6	0%	3%	20%	3.248 ± 0.097^{ay}	3.001 ± 0.252	97.4 ± 0.8^{ak}	59.5 ± 4.4^{y}	4.15 ± 0.73^{ay}	0.249 ± 0.058^{yj}	0.782 ± 0.066^{yj}	0.812 ± 0.256^{abyj}
7	2%	0%	0%	2.850 ± 0.097^{bx}	2.856 ± 0.252	97.3 ± 0.8^{abi}	66.6 ± 4.4^{x}	5.51 ± 0.73^{ax}	0.239 ± 0.058^{xi}	0.728 ± 0.066^{xi}	0.950 ± 0.256^{axi}
8	2%	3%	0%	3.215 ± 0.097^{by}	3.196 ± 0.252	98.2 ± 0.8^{abi}	61.6 ± 4.4^{y}	2.92 ± 0.73^{ay}	0.204 ± 0.058^{yi}	0.739 ± 0.066^{yi}	0.439 ± 0.256^{ayi}
9	2%	0%	10%	2.888 ± 0.097^{bx}	3.093 ± 0.252	98.6 ± 0.8^{abj}	68.5 ± 4.4^{x}	5.26 ± 0.73^{ax}	0.300 ± 0.058^{xi}	0.822 ± 0.066^{xij}	1.346 ± 0.256^{axij}
10	2%	3%	10%	3.149 ± 0.097^{by}	3.176 ± 0.252	98.5 ± 0.8^{abj}	66.2 ± 4.4^{y}	3.16 ± 0.73^{ay}	0.212 ± 0.058^{yi}	0.739 ± 0.066^{yij}	0.491 ± 0.256^{ayij}
11	2%	0%	20%	2.987 ± 0.097^{bx}	2.926 ± 0.252	98.8 ± 0.8^{abk}	71.4 ± 4.4^{x}	3.96 ± 0.73^{ax}	0.381 ± 0.058^{xj}	0.896 ± 0.066^{xj}	1.361 ± 0.256^{axj}
12	2%	3%	20%	3.048 ± 0.097^{by}	3.225 ± 0.252	98.2 ± 0.8^{abk}	62.2 ± 4.4^{y}	3.68 ± 0.73^{ay}	0.182 ± 0.058^{yj}	0.648 ± 0.066^{yj}	0.440 ± 0.256^{ayj}
13	4%	0%	0%	3.050 ± 0.097^{cx}	3.148 ± 0.252	98.1 ± 0.8^{bi}	67.8 ± 4.4^{x}	3.65 ± 0.73^{bx}	0.252 ± 0.058^{xi}	0.769 ± 0.066^{xi}	0.653 ± 0.256^{bxi}
14	4%	3%	0%	3.185 ± 0.097^{cy}	3.055 ± 0.252	98.0 ± 0.8^{bi}	64.5 ± 4.4^{y}	3.16 ± 0.73^{by}	0.144 ± 0.058^{yi}	0.655 ± 0.066^{yi}	0.279 ± 0.256^{byi}
15	4%	0%	10%	3.045 ± 0.097^{cx}	3.021 ± 0.252	98.9 ± 0.8^{bj}	72.2 ± 4.4^{x}	4.07 ± 0.73^{bx}	0.232 ± 0.058^{xi}	0.793 ± 0.066^{xij}	0.759 ± 0.256^{bxij}
16	4%	3%	10%	3.207 ± 0.097^{cy}	3.280 ± 0.252	98.5 ± 0.8^{bj}	61.1 ± 4.4^{y}	2.42 ± 0.73^{by}	0.198 ± 0.058^{yi}	0.754 ± 0.066^{yij}	0.355 ± 0.256^{byij}
17	4%	0%	20%	3.093 ± 0.097^{cx}	2.968 ± 0.252	98.9 ± 0.8^{bk}	69.6 ± 4.4^{x}	3.87 ± 0.73^{bx}	0.336 ± 0.058^{xj}	0.835 ± 0.066^{xj}	1.086 ± 0.256^{bxj}
18	4%	3%	20%	3.183 ± 0.097^{cy}	3.231 ± 0.252	98.2 ± 0.8^{bk}	61.5 ± 4.4^{y}	3.41 ± 0.73^{by}	0.195 ± 0.058^{yj}	0.689 ± 0.066^{yj}	0.446 ± 0.256^{byj}
p	*Suc (a,b,c)*			***	n.s.	***	n.s.	***	n.s.	n.s.	**
p	*EVOO (x,y)*			***	n.s.	n.s.	***	***	***	***	***
p	*Ice (i,j,k)*			n.s.	n.s.	*	n.s.	n.s.	**	*	**
p	*Suc*EVOO*			***	***	n.s.	n.s.	***	n.s.	n.s.	***
p	*Suc*Ice*			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p	*EVOO*Ice*			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
p	*EVOO*Suc*Ice*			n.s.	n.s.	n.s.	n.s.	** ?	n.s.	*?	n.s.

Data are expressed as mean ± standard error. *Suc* = sucrose, *EVOO* = extra virgin olive oil and *Ice* = ice. *p Suc, p EVOO, p Ice, p Suc*EVOO, p Suc*Ice, p EVOO*Ice* and *p EVOO*Suc*Ice* refer to main effects of *Suc (p Suc), EVOO (p EVOO)* and *Ice (p Ice)* factors and their two-factor *(p Suc*EVOO, p Suc*Ice, p EVOO*Ice)* and three-factor *(p EVOO*Suc*Ice)* interactions. *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; "n.s." indicates no significant difference at $p < 0.05$. Means in a column with different superscripts are significantly different ($p < 0.05$). Specifically, "a", "b" and "c" refer to main effect of *Suc*, "x" and "y" refer to main effect of *EVOO* and "i", "j" and "k" refer to main effect of *Ice*.

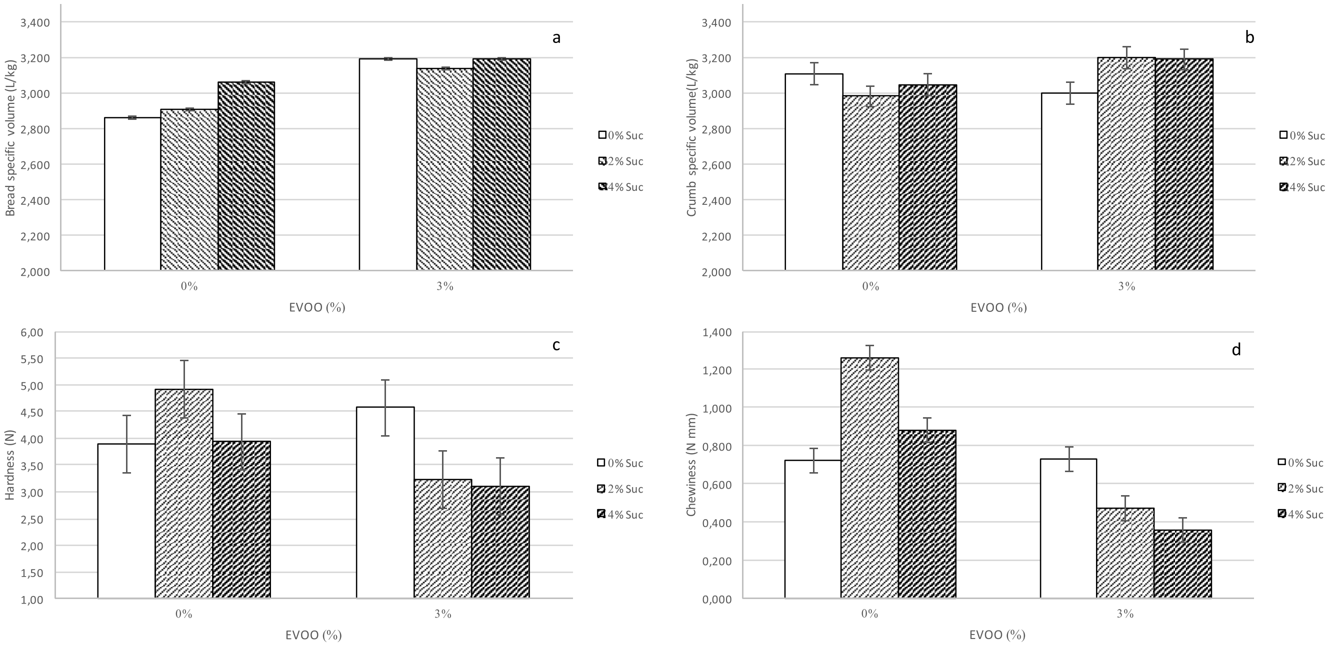

Fig. 3. Effects of *Suc*EVOO* interaction on: a) bread specific volume (L/kg), b) crumb specific volume (L/kg), c) hardness (N) and d) chewiness (Nmm).

with those reported in the literature for conventional flour blends. Considering that there are few descriptions of the effects of *EVOO* in the literature, the results revealed it to be an improver of particular interest for old wholewheat flour rheological performance.

3.2.2. Bread quality

The experimental data of the bread quality characteristics are shown in Table 4.

Considering bread specific volume, the *Suc*EVOO* interaction had a significant effect (Fig. 3). Specifically, the above parameter was optimized by *EVOO*, since regardless of *Suc* levels, the value increased by approx. 11%. This effect was not consistent with the literature on vegetable oils; furthermore, the presence of solid ß' crystals in the shortening seemed crucial for the stabilization of gas bubbles and the increase in bread volume (Pareyt et al., 2011). However, the literature also reports that different lipid typologies show very different effects (Autio & Laurikainen, 1997). Considering the unique chemical composition of *EVOO*, different effects may be associated with this improver, as shown by Matsakidou et al. (2010).

The highest level of *Suc* significantly increased bread specific volume (7%). This result was probably linked to the well-known effects of *Suc* on the breadmaking process: (i) an increase in starch gelatinization temperature, resulting in a higher crumb porosity (Psimouli & Oreopoulou, 2012), (ii) higher fermentative activity with a rise in CO_2 production and (iii) a greater increase in the volume of the final product (Zhou et al., 2014).

The *Suc*EVOO* interaction had a significant effect on the crumb specific volume. In contrast with the bread specific volume, the inclusion of *Suc* as a single improver reduced the parameter. The addition of *EVOO* together with *Suc*, regardless of the level of *Suc*, gave the best result, increasing the crumb specific volume (Fig. 3). Probably, a synergic effect between the two improvers occurred.

Looking at the moisture parameters, *Suc* and *Ice* slightly but significantly increased the crumb moisture (1%), whereas *EVOO* significantly reduced the crust moisture, lowering the value by around 10%.

All the improvers had a significant effect in the TPA analysis. The hardness was significantly affected by the *Suc*EVOO* interaction (Fig. 3). The parameter was optimized with both *Suc* and *EVOO*, which reduced the value by about 17–20%. Considering cohesiveness, *EVOO* and *Ice* had a significant effect. Specifically, *EVOO* significantly reduced the parameter, while *Ice* determined a significant increase. Since cohesiveness is inversely related to water content, these results are consistent with the amount of water in the sample; indeed, the addition of *EVOO* significantly lowered the dough water requirement (WA), while *Ice* significantly increased crumb moisture. With regard to springiness, the *EVOO*Ice* interaction had a significant effect: without *EVOO* addition, the highest level of *Ice* boosted springiness by about 24%. Chewiness was significantly affected by *EVOO*Suc* and *Suc*Ice* interactions. The best value, the lowest one according to the literature (Peng et al., 2017), was achieved by adding *EVOO* and the highest level of *Suc* (50.6%). Interestingly, the best improvement in chewiness was achieved with the combination of *Suc* and *EVOO*, as already observed on the specific volume parameters (Fig. 3). The *Suc*Ice* interaction showed that the highest level of *Ice* only combined with the highest level of *Suc* increased chewiness (58%), hence reducing the product quality.

Table 5 reports the experimental data on bread image and bread colour analysis. Considering the median pore area, the *EVOO*Ice* and *Suc*Ice* interactions exercised a significant effect. In detail, the highest level of *Ice* significantly reduced the parameter when combined with *EVOO* as compared to the value observed without the addition of *EVOO*. The second interaction showed that the highest level of *Ice* increased the pore area when *Ice* was the sole improver added. The addition of *EVOO* reduced the ratio between pore area/total pore area, revealing a similar effect to that of shortening in decreasing the pore size and probably improving crumb evenness (Pareyt et al., 2011).

Concerning colour analysis, all of the bread samples displayed an acceptable both crust and crumb colour. The crumb colour results outlined a significant increase in the L^* parameter, as a consequence of the highest level of *Suc* (4.2%). All parameters related to crust colour were significantly affected by *Suc* and *EVOO*. Specifically, L^* was reduced by *Suc* (6.4%), and increased by *EVOO* (5.2%). Moreover, the a^* parameter was increased by *Suc* (62.3%), while *EVOO* lowered it (19.1%). Finally, the b^* parameter showed a similar trend to a^*: an increase with *Suc* (15.2%) and a reduction with *EVOO* (6.7%).

Table 5
T2 trials bread quality evaluation.

Sample	*Suc (w/ flour w)*	*EVOO (w/ flour w)*	*Ice (w/ water w)*	Pore area 0.5 (mm^2)	*Pore area/area tot (%)*	Crumb			Crust		
						L*	a*	b*	L*	a*	b*
1	0%	0%	0%	2.71 ± 0.35^{x}	29.55 ± 2.88^{x}	60.00 ± 2.97^{a}	4.75 ± 0.96	15.50 ± 3.79	52.00 ± 2.29^{ax}	9.75 ± 1.80^{ax}	30.25 ± 3.10^{ax}
2	0%	3%	0%	2.88 ± 0.35^{y}	27.33 ± 2.88^{y}	63.75 ± 2.97^{a}	4.00 ± 0.96	14.75 ± 3.79	55.00 ± 2.29^{ay}	6.50 ± 1.80^{ay}	27.50 ± 3.10^{ay}
3	0%	0%	10%	3.05 ± 0.35^{x}	29.55 ± 2.88^{x}	59.75 ± 2.97^{a}	3.50 ± 0.96	12.75 ± 3.79	50.25 ± 2.29^{ax}	9.00 ± 1.80^{ax}	29.50 ± 3.10^{ax}
4	0%	3%	10%	2.91 ± 0.35^{y}	29.74 ± 2.88^{y}	59.75 ± 2.97^{a}	4.00 ± 0.96	16.25 ± 3.79	55.50 ± 2.29^{ay}	7.00 ± 1.80^{ay}	27.75 ± 3.10^{ay}
5	0%	0%	20%	3.76 ± 0.35^{x}	28.39 ± 2.88^{x}	58.13 ± 2.97^{a}	4.13 ± 0.96	13.50 ± 3.79	51.75 ± 2.29^{ax}	8.63 ± 1.80^{ax}	29.13 ± 3.10^{ax}
6	0%	3%	20%	2.98 ± 0.35^{y}	26.23 ± 2.88^{y}	64.00 ± 2.97^{a}	3.25 ± 0.96	13.50 ± 3.79	56.25 ± 2.29^{ay}	5.50 ± 1.80^{ay}	27.25 ± 3.10^{ay}
7	2%	0%	0%	3.04 ± 0.35^{x}	28.28 ± 2.88^{x}	63.50 ± 2.97^{ab}	3.50 ± 0.96	14.25 ± 3.79	50.50 ± 2.29^{ax}	9.50 ± 1.80^{ax}	31.00 ± 3.10^{ax}
8	2%	3%	0%	2.98 ± 0.35^{y}	26.60 ± 2.88^{y}	62.25 ± 2.97^{ab}	3.50 ± 0.96	13.25 ± 3.79	54.40 ± 2.29^{ay}	7.00 ± 1.80^{ay}	27.00 ± 3.10^{ay}
9	2%	0%	10%	2.95 ± 0.35^{x}	28.36 ± 2.88^{x}	61.75 ± 2.97^{ab}	4.00 ± 0.96	14.50 ± 3.79	51.50 ± 2.29^{ax}	9.75 ± 1.80^{ax}	31.75 ± 3.10^{ax}
10	2%	3%	10%	2.80 ± 0.35^{y}	24.91 ± 2.88^{y}	61.75 ± 2.97^{ab}	3.75 ± 0.96	16.75 ± 3.79	52.25 ± 2.29^{ay}	7.75 ± 1.80^{ay}	27.25 ± 3.10^{ay}
11	2%	0%	20%	3.20 ± 0.35^{x}	30.18 ± 2.88^{x}	61.75 ± 2.97^{ab}	3.25 ± 0.96	14.25 ± 3.79	52.00 ± 2.29^{ax}	9.50 ± 1.80^{ax}	30.50 ± 3.10^{ax}
12	2%	3%	20%	2.60 ± 0.35^{y}	26.97 ± 2.88^{y}	62.50 ± 2.97^{ab}	3.50 ± 0.96	15.00 ± 3.79	53.75 ± 2.29^{ay}	8.75 ± 1.80^{ay}	32.00 ± 3.10^{ay}
13	4%	0%	0%	3.15 ± 0.35^{x}	29.03 ± 2.88^{x}	65.50 ± 2.97^{b}	4.00 ± 0.96	15.25 ± 3.79	50.75 ± 2.29^{bx}	13.00 ± 1.80^{bx}	34.00 ± 3.10^{bx}
14	4%	3%	0%	2.91 ± 0.35^{y}	28.77 ± 2.88^{y}	63.75 ± 2.97^{b}	3.50 ± 0.96	17.00 ± 3.79	52.00 ± 2.29^{by}	9.75 ± 1.80^{by}	29.00 ± 3.10^{by}
15	4%	0%	10%	3.09 ± 0.35^{x}	30.96 ± 2.88^{x}	61.25 ± 2.97^{b}	3.75 ± 0.96	16.25 ± 3.79	48.00 ± 2.29^{bx}	14.25 ± 1.80^{bx}	33.50 ± 3.10^{bx}
16	4%	3%	10%	3.19 ± 0.35^{y}	27.74 ± 2.88^{y}	62.75 ± 2.97^{b}	3.50 ± 0.96	14.25 ± 3.79	50.25 ± 2.29^{by}	12.75 ± 1.80^{by}	33.75 ± 3.10^{by}
17	4%	0%	20%	2.95 ± 0.35^{x}	30.93 ± 2.88^{x}	64.25 ± 2.97^{b}	3.75 ± 0.96	13.50 ± 3.79	49.25 ± 2.29^{bx}	12.75 ± 1.80^{bx}	34.00 ± 3.10^{bx}
18	4%	3%	20%	2.74 ± 0.35^{y}	28.68 ± 2.88^{y}	63.25 ± 2.97^{b}	3.75 ± 0.96	16.50 ± 3.79	50.00 ± 2.29^{by}	12.75 ± 1.80^{by}	33.25 ± 3.10^{by}
p	*Suc (a,b,c)*			n.s.	n.s.	*	n.s.	n.s.	***	***.	***
p	*EVOO (x,y)*			*	**	n.s.	n.s.	n.s.	***	***	**
p	*Ice (i,j,k)*			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p	*Suc*EVOO*			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p	*Suc*Ice*			*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p	*EVOO*Ice*			*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
p	*EVOO*Suc*Ice*			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Data are expressed as mean ± standard error. *Suc* = sucrose, *EVOO* = extra virgin olive oil and *Ice* = Ice. *p Suc, p EVOO* and *p Ice* refer to the main effects of these factors; *p Suc*EVOO, p Suc*Ice* and *p EVOO*Ice* refer to the effect of the two-factor interactions; *p EVOO*Suc*Ice Suc* refers to three-factor interaction. *, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; "n.s." indicates no significant difference at $p < 0.05$. Means in a column with different superscripts are significantly different ($p < 0.05$). Specifically, "a", "b" and "c" refer to main effect of *Suc*, "x" and "y" refer to main effect of *EVOO* and "i", "j" and "k" refer to main effect of *Ice*.

Hence, this analysis revealed that *Suc* had a significant effect: it enhanced crumb brightness, reduced crust brightness and increased its yellow and red components. However, only the highest level of *Suc* exercised a significant effect on bread colour, probably because the lower level was entirely depleted by yeasts during fermentation, without leaving any reducing sugars in the final dough for non-enzymatic browning reactions. The addition of *EVOO* significantly affected crust colour, too; it increased crust brightness as well as reduced the red and yellow components.

3.2.3. Optimization of bread ingredients and bread sensory evaluation

The results of the *T2* trial were analysed with the aim of optimizing bread quality. Bread specific volume, crumb specific volume and bread hardness were considered the most representative parameters of product quality. The bread specific volume was maximized with *EVOO*, while for the optimization of the crumb specific volume and hardness, the combination of *Suc* and *EVOO* was required. Indeed, the highest crumb specific volume and the lowest hardness was obtained with *Suc* 2% and *EVOO*. No significant difference was obtained when the *Suc* was increased from 2% to 4%.

Since the aim of the study was to combine the optimization of technological properties with the preservation of the nutritional value of old wholewheat flour, the choice was to minimize the addition of improvers. Hence, *Suc* at 2% and *EVOO* at 3% were chosen for the optimized recipe.

The optimized sample was subjected to a qualitative sensory evaluation in comparison to the control sample (i.e. without improvers). Fig. 4 outlines the bread slice, bread crumb and bread crust results. The panel perceived differences for all the bread portions analysed. For the bread slices, the attributes that most discriminated the two samples were acidulous and cereal aromas, both perceived as more intense in the optimized bread. The bread crumb revealed the greatest differences in the following attributes: elasticity, moisture, solubility, brewer's yeast flavour and sourness. All these attributes except elasticity resulted more intense in the optimized sample than in the control. Considering the crust evaluation, the greatest differences were perceived in the friability, saltiness and brewer's yeast flavour, which received a higher score for the optimized bread.

The highest intensity of acidulous aroma, sourness and brewer's yeast flavour could be linked to the inclusion of Suc, which probably increased the yeast growth and metabolic activity (Zhou et al., 2014).

The solubility descriptor of bread crumb was perceived as higher, in accordance with the TPA results, which showed the lowest hardness value. The highest value for the crumb moisture attribute is consistent with the physical parameter, which revealed an increase of 1%. The bread crust of the optimized sample, perceived as more friable, could be the result of its lower moisture content (10%). This moisture difference may also have emphasized the taste of the crust, making it seem saltier: the lower the water content, the higher the solute concentration. Finally, the elasticity value proved to be lower than the control sample, consistently with the TPA analysis.

4. Conclusions

Old wholewheat flours are characterized by an interesting nutritional profile, but they showed a very poor technological performance. Hence, the use of old wholewheat flour for the breadmaking process requires appropriate techniques, specifically designed for the different characteristics of the raw material compared to conventional flours.

By applying a two-step experiment (a screening step and a validation step), we selected the optimal combination of flour improvers to increase the bread quality. *Suc* (2%) and *EVOO* (3%) were identified as the optimized mixture of ingredients to improve bread quality.

The possibility of adopting this optimization method with other old

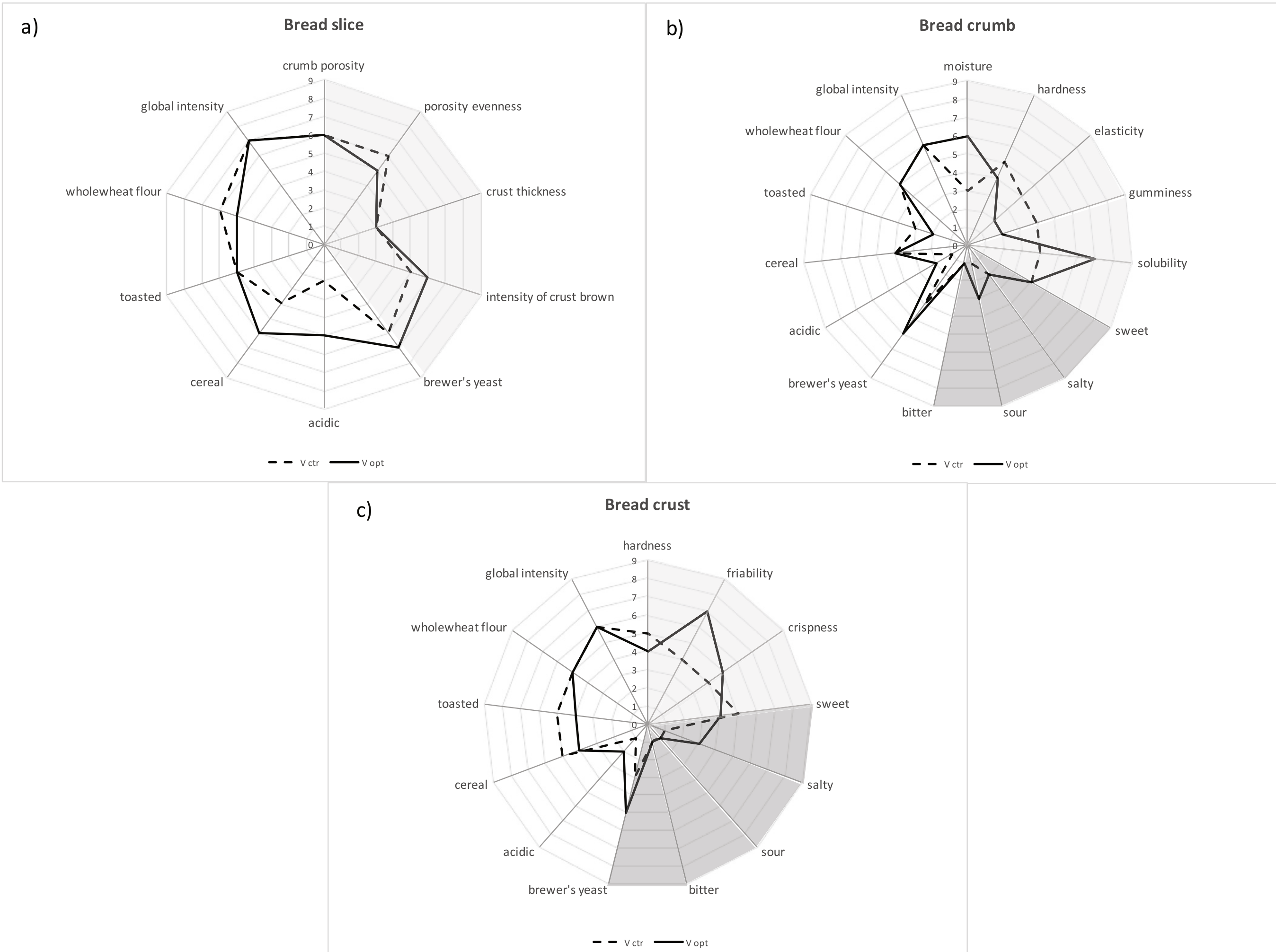

Fig. 4. Sensory evaluation of bread slices (a), bread crumb (b) and bread crust (c). Sectors with different colours correspond to different classes of descriptors: aroma (white) and appearance (light grey) descriptors for bread slices; touch (light grey), taste (grey) and flavour (white) descriptors for bread crumb and crust. Reported values are medians of the raw data.

wholewheat flours may be an interesting tool to design old wholewheat flour breadmaking. Indeed, if the breadmaking process is designed to optimize the specific characteristics of bread, an improvement could be obtained in product quality. Thereby, the use of old wholewheat flour in the bakery industry could be increased, promoting the consumption of healthier breads as well as safeguarding *Triticum* genus biodiversity.

Author contributions

Ottavia Parenti: Conceived and designed the analysism, Collected the data, Performed the analysis, Wrote the paper. Lorenzo Guerrini: Conceived and designed the analysis, Contributed data or analysis tools, Wrote the paper. Benedetta Cavallini: Collected the data, Performed the analysis. Fabio Baldi: Performed the analysis. Bruno Zanoni: Conceived and designed the analysis, Wrote the paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2019.108980.

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