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

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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.

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Dr. Rakesh K. Singh
Editor-in-Chief
LWT - Food Science and Technology

October 29, 2019

Dear Dr. Rakesh K. Singh:

I am pleased to submit an original research article entitled "Breadmaking with ancient wholewheat flour: optimization of ingredients to improve bread quality" for consideration for publication in *LWT - Food Science and Technology*.

This manuscript investigated the effects of seven bread improvers on the performance of ancient wholewheat (cv. Verna) doughs and breads in order to optimize the final product quality. Two different experimental trials were performed: (i) a screen design trial (T1) allowed us to select the improvers with the best effect on bread quality (i.e. sucrose, extra virgin olive oil and ice); (ii) a full factorial design trial (T2) studied the effect of these three improvers on dough rheology and bread quality. The optimization of bread quality was obtained with the addition of sucrose (2%) and extra virgin olive oil (3%). The present study presents a two-step optimization approach that could be applied to other ancient wheat varieties, to improve the quality of their final products and promote the consumption of healthy foods.

We believe that this manuscript is appropriate for publication by *LWT - Food Science and Technology* because it is innovative in the fields of food technology. To the author knowledge, the effect of common bread improvers on the performance of an ancient wholewheat flour has never been tested before. The relevance of this study is the optimization method for the improvement of ancient wholewheat bread quality.

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

Thank you for your consideration!

Sincerely,

Dr. Lorenzo Guerrini
Department of Agricultural, Food and Forestry Systems Management (DAGRI)
University of Florence, Italy

***Detailed Response to Reviewers**

The authors would like to thank Reviewers, for the time spent in improving the paper and for the important suggestions and corrections proposed. We hope to have addressed all the issues that reviewers outlined. Here following, a point by point reply to the comments received.

Reviewer #1: Dear Authors,

the paper gives important knowledge to the field of old wheat varieties, is well written, statistical analysis is appropriate and results and discussion adequately presented and discussed. The paer may be accepted, rpvided some minor revisions are made:

- Please change ancient with old, you used and old variety, the term acncient is related to ancient wheat species.

-We changed the word "ancient" with "old", accordingly with reviewer observation.

- 2.4.2 - Change rheological properties with lare deformatin tests.

-2.4.2 According to reviewer suggestion, we changed the "rheological properties" with "large deformation tests"

- Some important recent references are missing, please add in the intridution section and discuss the two following:
Mefleh M., Conte P., Fadda C., Giunta F., Piga A., Hassoun G., Motzo R. 2019. From ancient to old and modern durum wheat varieties: interaction among cultivar traits, management, and technological quality. Journal of the Science of Food and Agriculture, 99:2059-2067.
Farbo M.G, Fadda C., Marceddu S., Conte P., Del Caro A., Piga A. 2020. Improving the quality of dough obtained with old durum wheat using hydrocolloids. Food Hydrocolloids, 101:1-8.

-We added the references that reviewer proposed in the Introduction section (L-52-53 and L-60).

Reviewer #2: Review LWT

The paper presented on the use of ancient wheat is an interesting study and well written in general.
Still I would like to rise a few issues and give some comments/suggestions for improvement:

General: ancient wheats are interesting and their use for human nutrition is justified for increasing the diversity in nutrition. Their chemical composition differs from other cereals, so they can offer "other" nutrients and thus increase the variability in nutrients (e.g. other phytochemicals, different ratio of dietary fibre fractions, etc.), BUT to say that they are "healthier" or "better" than modern cereals is NOT correct and NOT scientifically justified. This is what consumer think are what they are made to think, but scientific papers should underline all these statements with facts and data. I would thus indeed request the authors to deliver proven facts and to deliver sincere evaluation.
In particular: Abstract: line 13: ancient wheat show positive effects on human health, line 26 "healthier breads" introduction line 45 "high nutritional value" line 50 "rich nutritional profile" - None of these statements is proven here in the text with scientific data!

The paper need to define and explain ancient cereals more detailed. In particular intro line 45-51 should be extended, clarified, improved, explaining exactly WHAT are ancient cereals, WHAT is the difference to modern cereals, BASED ON SCIENTIFIC DATA.

General:

As reviewer rightly pointed out, the paper focused on the use of an old wholewheat flour in the breadmaking process. The authors completely agree with the reviewer that we can not generally define old wheat as better than modern, but this was not the authors' intention.

Probably, the authors did not clearly explain the characteristics of the raw material used for the study, which may justify the statements that reviewer pointed out. The flour selected was characterised by two distinctive features: (i) it was "wholewheat" as regard to the flour refinement degree; the consumption of wholewheat products has been associated to health benefits in several scientific studies; and (ii) it was "old" as regard to the time of this wheat cultivar selection; in recent years some scientific studies have compared the characteristics of some old wheat cultivars to modern wheat cultivars.

In detail, these studies investigated the nutritional composition, especially focusing on phenolic compounds, of some old wheat varieties as well as they tested the effect of the regular consumption of some old wheat cultivars in intervention studies on humans (Leoncini et al., 2012; Dinelli et al., 2011; Sofi et al., 2010; Gotti et al., 2018; Sereni et al., 2016). These papers have shown that the tested old wheat cultivars (including *cv* Verna – the same of our tests) could present an interesting nutritional composition, particularly rich in phenolic compounds (Leoncini et al., 2012; Dinelli et al., 2011; Gotti et al., 2018). Furthermore, the regular consumption of this old variety has been associated to nutritional benefits, specifically for the prevention of chronic diseases (i.e. cardiovascular diseases, type 2 diabetes, various type of cancers) (Sofi et al., 2010; Sereni et al., 2016).

Accordingly, we modified the Manuscript.

Abstract:

L 13-16 According to reviewer suggestion the authors changed Line 13 "Ancient wholewheat flours (AWWF) show positive effects on human health" into "Processing strategies are necessary to improve the quality of baked old wholewheat flour products, since they are increasingly required by consumers but have poor technological properties." We change the focus of the sentence from the nutritional benefits to the market's requests.

L 26 Line The sentence has been rephrased.

Introduction:

According to the reviewer comment, the authors clearly define old wheats at L 48-50. At L 50-53 it is now explained that "the old wheat term includes a large number of cultivars, with a broad genetic base, and therefore showing a broad range of characteristics (Dinu, Whittaker, Pagliai, Benedettelli, & Sofi, 2018; Mefleh, Conte, Fadda, & Giunta, 2018)", and finally at L 53-55 we explained that "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; Sereni et al., 2016; Gotti et al., 2018)." Furthermore, the authors added several scientific papers to support the statements in the Introduction.

Line 51: different quality and quantity of gluten: that is correct, but this is not really and advantage. Gluten in ancient wheat is less suitable for baking (THAT is one issue that has been improved by breeding to modern cereals) and they are NOT necessarily better digestible or tolerable. (still not gluten-free).

L 51 The authors totally agree with reviewer observation; in fact, the word “different” has not a meaning of an advantage, but it just outlines a characteristic of ancient cultivars compared to the modern ones. However, the sentence has been removed.

Describe in detail the used cereals. Where and when grown, year? Where? Experimental farms? Etc.

According with reviewer suggestion the authors added the specifications about the wheat used in the study (L 74-76).

Why have you used to different batches? To mix these batches and then use a mixture of it for T1 and T2 would have been better.

As the reviewer pointed out, the authors used two different batches of the same wheat (i.e. cv Verna, grown the same year in the same location) for the two experimental trials they performed (T1 and T2). This choice was made in order to increase the robustness of the Validation trail (T2) by increasing the variability of the raw matter. In fact, T2 showed that results were consistent with T1 independently from the inherent variability of the two flour batches.

Line 67-72: define or write complete the used abbreviations (you do so later in results, but it should already be here)

L 67-72: the authors specified the used abbreviations (now at L 75-77).

Line 79: a short description here would be better, so one is not urged to retrieve another paper.

L 79: according to reviewer suggestion, the authors added the description of the gelatinization process in the material and method section (now at L 81-84).

Experimental Design:
Principally well chosen. Only T1: to evaluate 7 parameters at once is a bit risky, in particularly in baking and limits the evaluation or results. But authors have stated it also themselves. Single addition would have made it easier (and not really much more work)

Experimental Design:
When we decided the experimental design, we also evaluated the possibility of testing the single addition of improvers instead of using the Screening Design approach.
First of all, we observed that in breadmaking the use of many improvers into the bread formulation is very common.
Testing the improvers one at time allowed to isolate the effect of each of them, but in real recipes they are often used in combination, and their effects cannot be considered additive *a priori* (later, in the full factorial validation we demonstrated that the effect were not additive). In fact, the

addition of one improver may masks the effect of the addition of another one, and *vice versa*. To avoid this problem, we should test the improvers in a full factorial design. To test the effects of the 7 improvers in recipes where they are simultaneously present in a full factorial design, we needed 2^7 (128 x number of replicates) tests.
Hence, we decided to split the investigation in 2 trials. In the first one we applied the Screening Design since it allows a direct comparison between improvers in recipes when they are simultaneously present in 2^3 (8 x number of replicates) tests. In the second one we focused on the most promising improvers with a full factorial (allowing us to test the selected improvers on more than 2 levels). Hence, the choice of these way of work allowed us, an evaluation of the improver main effect in the presence of other additives, and better mimic the real working conditions in breadmaking.

Line 97: mention T2 info here (move the section line 247-253 here), mention the name of the design.

L 97: according with reviewer suggestion we moved the section L 247-253 into Material and Methods (now at L 110-115).

Line 114: bread making machine is not really scientific baking equipment. At least describe here the exact conditions applied: kneading parameters, fermentation time, T, baking time, T, etc.

L 114: The authors added specifications about the exact conditions applied during the breadmaking with bread making machine. We chose to use bread machine because they allow to standardize the breadmaking procedure reducing the errors and differences connected with a straight dough method carried out manually. This allows to assess the effects of the improvers reducing possible causes of interference. Furthermore, bread machines are usually accepted in scientific literature. Several papers used them, for example see Martins et al., “Original article Effect of spent yeast fortification on physical parameters, volatiles and sensorial characteristics of home-made bread”. *International Journal of Food Science and Technology* (2015), 50, 1855–1863; Lodi et al., “Characterization of water distribution in bread during storage using magnetic resonance imaging”. *Magnetic resonance Imaging* (2007), 25, 1449–1458; Wang et al., (2004). “Water dynamics in white bread and starch gels as affected by water and gluten content” *LWT - Food Science and Technology*, 37, 377–384; Kadan et al., “Effects of Yeast and Bran on Phytate Degradation and Minerals in Rice Bread Materials and Methods” *Journal of Food Science* (2007), 72, 208–211; Noonan et al., “The Determination of Semicarbazide (N-Aminourea) in Commercial Bread Products by Liquid Chromatography-Mass Spectrometry” *Journal of Agricultural and Food Chemistry,* (2005), 53, 2064–2067; Noonan et al., Semicarbazide Formation in Flour and Bread, *Journal of Agricultural and Food Chemistry* (2008), 56, 2064–2067.

2.4. Analytical methods
Line 137-138. The right measurement for this is bake loss (weight of bread before and after baking)

L 137-138 did not refer to the bake loss, but in these lines, we wanted to point out on the different losses of water from bread crumb and crust due to the improvers. On the other hand, bake loss is comprehensive of the total water losses of bread.

Line 139: describe method here, instead of referring to another paper.

L 139: the authors added the explanation of the method (now at L 158-161).

Good: number of replications for baking and methods.
Addition of ice: have you measured T of water before and after its addition? Dough T before and after kneading?

Addition of Ice: in a preliminary trial we tested different levels of Ice addition and we measured the water T as well as the dough T before and after kneading. In detail, the water was at room T (20°C); the sample containing 20% of Ice with the rest part of water at 20°C was at 14°C before the kneading step and at the end of the kneading step its T was 20°C. The T of the control sample was 20°C before the kneading step and 25°C after the kneading step. We did it in a separate preliminary test (with the same flour, yeasts, and procedures) to avoid interferences during the presented experimental trials. The authors added this specification at L 211-212.

Line 160- 162: delete this sentence

L 160-162: the authors delated the sentence.

3.1. I think there was interaction of GF and GG and these two have strong interaction with water ….

Reviewer observation is true, and for this reason the authors decided to perform the farinographic test on the dough samples in the presence of the different combination of improvers, according to the Screening Design adopted for T1. This rheological analysis allowed us to estimate WA in the presence of the different improvers. One of the limitations of using the Screening Design method is that it does not allow the estimation of the interactions between the tested variables. The authors already explained these limitations in the Material and Method section.
Moreover, according to the confounding pattern of T1 experimental design, the interaction between GG and GF may result in an overstate of the Suc effect. However, we confirmed the robustness of our results with T2 trail, where Suc was tested without interferences neither from GG nor from GF having the same results.

EVOO: this is characterized by long chain fatty acids, and has a very different effect to bread then short chain fats like shortening or butter. This should be considered in the results. (e.g. line 214)

L 214: The authors reworded the sentence according to reviewer comment (now at L 238-240).

Line 234… growth of microorganisms - there was not sourdough addition, so SUC could only have supported yeast growth.

L 234 the authors modified the sentence according to the reviewer comment (now at L 260).

Line 246. Why did you measure rheology in T1 if you then did not consider these results at all for T2?

L 246: In T1 the authors measured dough rheology in order to evaluate the effects of the improvers on the dough behaviour, which reflects the dough workability and predicts the breadmaking performance. In the authors' opinion, since the scientific literature about breadmaking with old wholewheat flour is poorly investigated, it is important to report the effects of the tested improvers on dough rheology, independently of their effects on bread specific volume. However, since the aim of the study was the optimization of the final product quality, for T2, the authors decided to focus on the improvers that showed positive effect on one of the most important parameter of bread quality, i.e. bread volume.

Chapter 3.2.2:
Authors do not describe the interaction effects correctly. E.g. Line 283: how was the interaction effect? (does the statistical evaluation tool shows graphs where this can be seen? Then add them here). Also true for Line 296, line 305, 316, 321: - interaction effect of addition effect? (is not the same)

Chapter 3.2.2: Experimental data described in L 283, 296, 305, 316 and 321 are reported in Table 4 and the effects on bread specific volume, crumb specific volume, crumb hardness and chewiness are represented as graphs shown in Fig. 3; the paper reported it in L 281-282.
In the Discussion section, the significant effects of the interactions are reported, but the paper did not aim to explain the interaction effect. Conversely, it followed the optimization approach, focusing on the recipe that allowed the obtainment of the best bread quality. With this approach we just measured the effect of the interactions to consider them in the final recipe. However, in the first version of the paper the explanations of the interactions were included, but we had to remove them due to the limited number of words allowed by the journal.

Line 285: increase of 11% - where can this be seen? (table)

L 285: the increase of 11% can be seen in Table 4 as stated in L 281-282.

Line 286: ß'crystals in EVOO??? (normally in short chain fatty acids)

L 286: we reworded the sentence about the use lipids in breadmaking which probably was misleading; ß'crystals were related to the use of shortening not to the composition of EVOO (now at L 312).

Line 289: unique compostion of EVOO - all fats have unique composition…. Peculiar effects - which?

L 289: we reworded the sentence according to reviewer observation (now at L 315).

Tables: T1: lowest level was 0?

Table 1: Yes, in T1 each variable was tested in 2 levels; the lowest level is 0 and the highest was selected according to the scientific literature as reported in L 111-116.

Table 2: T1 trials in T1 were only 8 trials, here in Table 2, which results are here presented? Is this the right way to evaluate this statistical test?

Table 2: Rheological results are shown in Table 2. The statistical analysis was made according to Antony (2014) as reported in L 108.

Fig 1. Table values would be clearer to see results.

Fig 1. We modified Fig. 1 in order to make table values clearer, according to reviewer comment.

Editorial comments:

L.29: remove

L 29 We removed the line and the abbreviations throughout the Manuscript.

L.37, elsewhere: All citations in the text should refer to:
1. Single author: the author's name (without initials, unless there is ambiguity) and the year of publication (Smith, 2003);
2. Two authors: both authors' names and the year of publication (Smith & Jones, 2004);
3. Three, four or five authors: all authors names and year of publication (Smith, Jones, & Brown, 2005). For all subsequent citations of this work use et al. (Smith et al., 2005).
4. Six or more authors: first author's name followed by et al. and the year of publication (Black et al., 2007).

-All citations in the text were changed in journal style.

- L.68, elsewhere: do not use "%" for concentration (here for moisture), replace by e.g. g/100 g as for other data. Check that there is always a space between number and unit (e.g. L.69: V2=10.5g/100g -> V2 = 10.5 g/100 g). L.110: g water per 100 g flour

-The authors modified the moisture unit of measurement according to Editorial comment. We also delated the space between the number and the unit.

- L.121: include AACC methods manual in references

-We included AACC methods in the references.

- References: give journal issue numbers (in brackets) for all references, or remove from all

-We modified the references according to Editorial comment.

- Prepare tables in journal style (check current publications in LWT please - e.g. no vertical lines)

-We modified the tables in journal style

Highlights

- Two trials (T1, T2) tested common bread improvers on an ancient wholewheat flour
- Salt, sucrose and guar gum improved dough rheology in the fractional trial (T1)
- Sugar, extra virgin olive oil and ice increased bread specific volume in T1
- A full factorial trial (T2) was performed to optimize bread quality
- 2% sucrose and 3% extra virgin olive oil gave the best bread quality

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

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Abstract

~~The consumption of old wholewheat flours have shown positive effects on human health, but they have poor technological properties.~~ 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 ~~in order~~ 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 ~~the above three~~ 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.

Key words: unrefined flour, bread improvers, Brabender Farinograph, healthy foods

~~**Abbreviations:** OWF, old wheat flour; old wholewheat flour, old wholewheat flour~~

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 et al., 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 et al., 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, Whittaker, Pagliai, Benedettelli, & Sofi, 2018; Mefleh, Conte, Fadda, & Giunta, 2018). 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., 2016). ~~These varieties show lower yields and poor technological performance compared to modern wheats, but they are characterized by a broader genetic base, making them an important safeguard of *Triticum* genus biodiversity and giving them a rich nutritional profile as well as a different quality/quantity of gluten~~

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; Fabro et al., 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 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 ~~flour~~ 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/100g d.m., *V2*=1.28g/100g d.m.) and protein (*V1*=12.3g/100g d.m., *V2*=10.5g/100g 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*=30.0mm, *V2*=25.0mm) 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~~, as explained in the Results and Discussion section~~. 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: 25min at room T, resting and leavening: 1h and 20min at 40°C, baking: 55min 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.

2.4.2 Large deformation tests ~~Rheological analysis of OLD WHOLEWHEAT FLOUR and dough samples~~

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). ~~their moisture~~

~~was calculated as the ratio between the final crumb or crust moisture (g/100g) and the original dough moisture (g/100g), in order to allow a proper comparison between the tested samples.~~ 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. ~~according to Parenti et al. (2019).~~ 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 1.2MP. 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 & 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. ~~The participants were informed about the procedures and were asked to sign an informed consent form when they agreed on participation.~~ 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 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), 100ppm AH_2 (Tebben, Shen, & Li, 2018); 1%w/flour w *GG* (Tebben, Shen, & Li, 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, Axel, Zannini, & Arendt, 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, Shen, & Li, 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 & 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~~;~~ ~~hence~~, while ~~there has been little investigation into the effect of *EVOO*~~, 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 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, Shen, & Li, 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 ~~microorganisms~~ 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, Finnie, Putseys, & Delcour, 2011). Conversely, Matsakidou, Blekas, & 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.

~~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.~~

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, Li, Ding, & Yang (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, Finnie, Putseys, & Delcour, 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 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; ~~indeed~~ 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, Finnie, Putseys, & Delcour, 2011). However, the literature also reports that different lipid typologies show very different effects (Autio & Laurikainen, 1997). Considering the unique chemical composition of *EVOO*, ~~peculiar~~ different effects may be associated with this improver, as shown by Matsakidou, Blekas, & Paraskevopoulou (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, Li, Ding, & Yang, 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, Finnie, Putseys, & Delcour, 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%).

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% and 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 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.

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FIGURE CAPTIONS

FIGURE 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).

FIGURE 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.

FIGURE 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).

FIGURE 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.

Table 1

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

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%

Table 4

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*.

Table 5

Table 5. *T2* trials bread quality evaluation.

Sample	*Suc*	*EVOO*	*Ice*	*Pore area*	*Pore area/area tot*	***Crumb***			*Crust*		
	(w/flour w)	*(w/flour w)*	*(w/water w)*	*0.5 (mm^2)*	*(%)*	***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*.

Figure 1

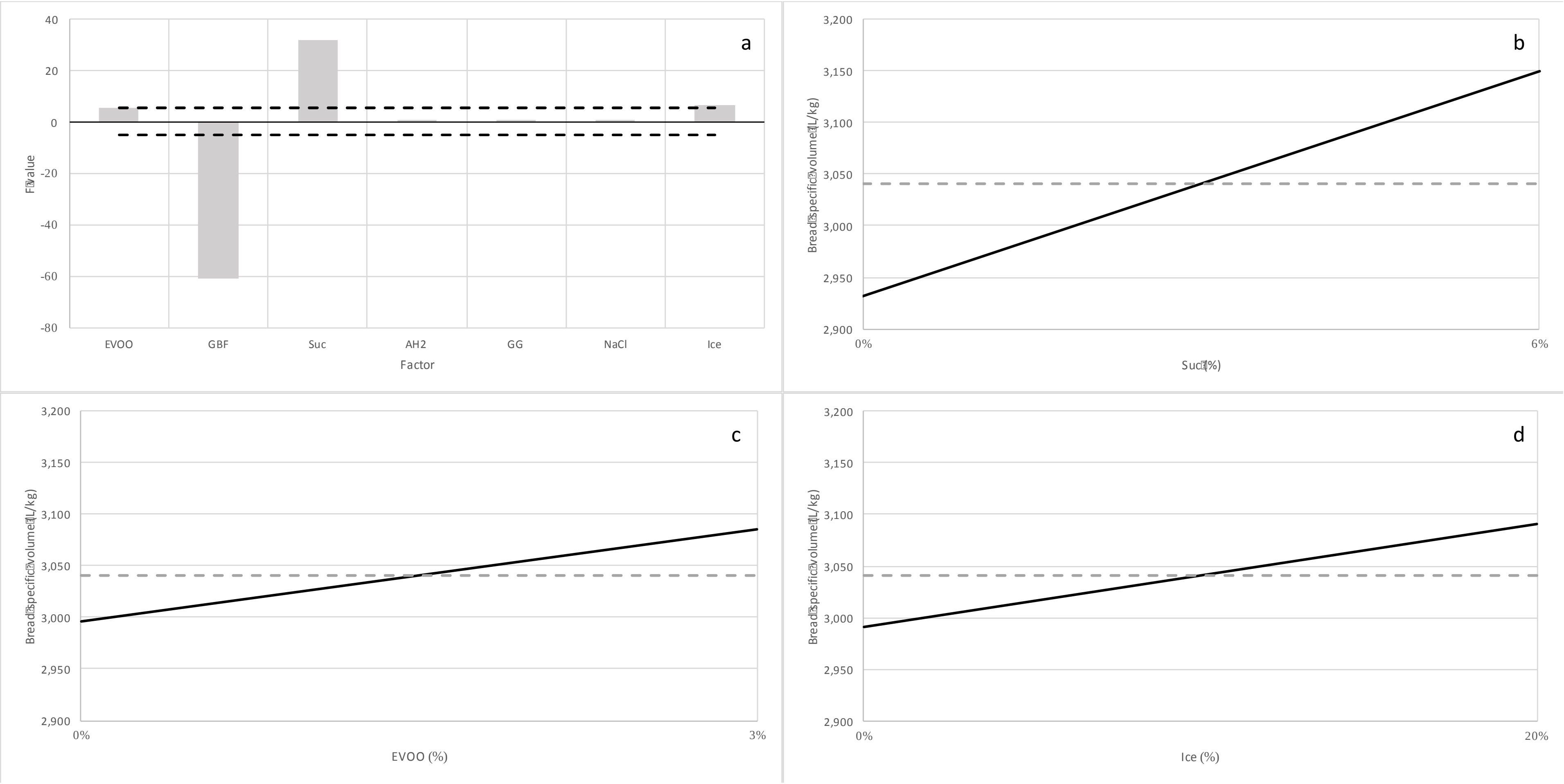


Figure 1.

Figure 2

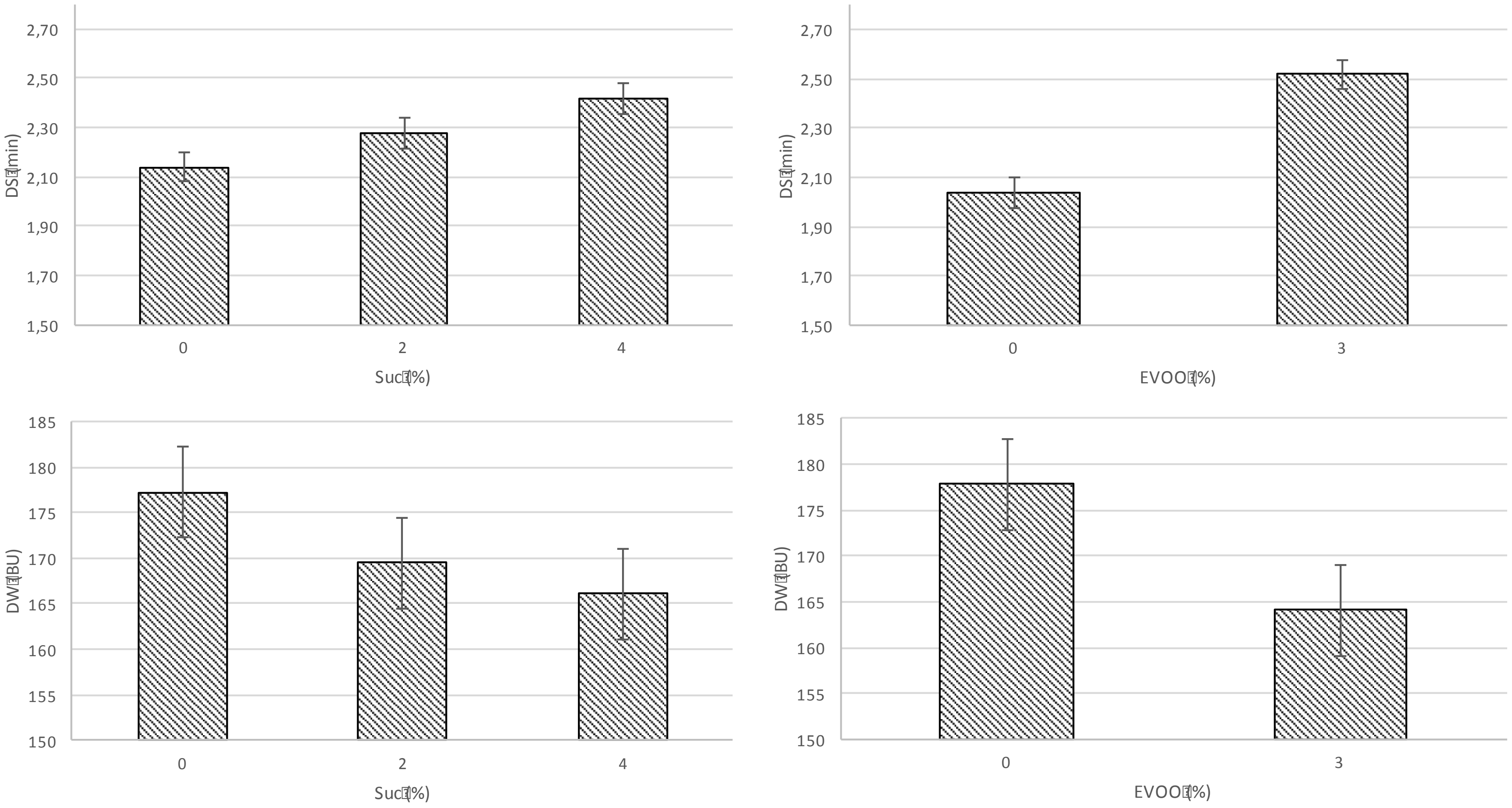


Figure 2.

Figure 3

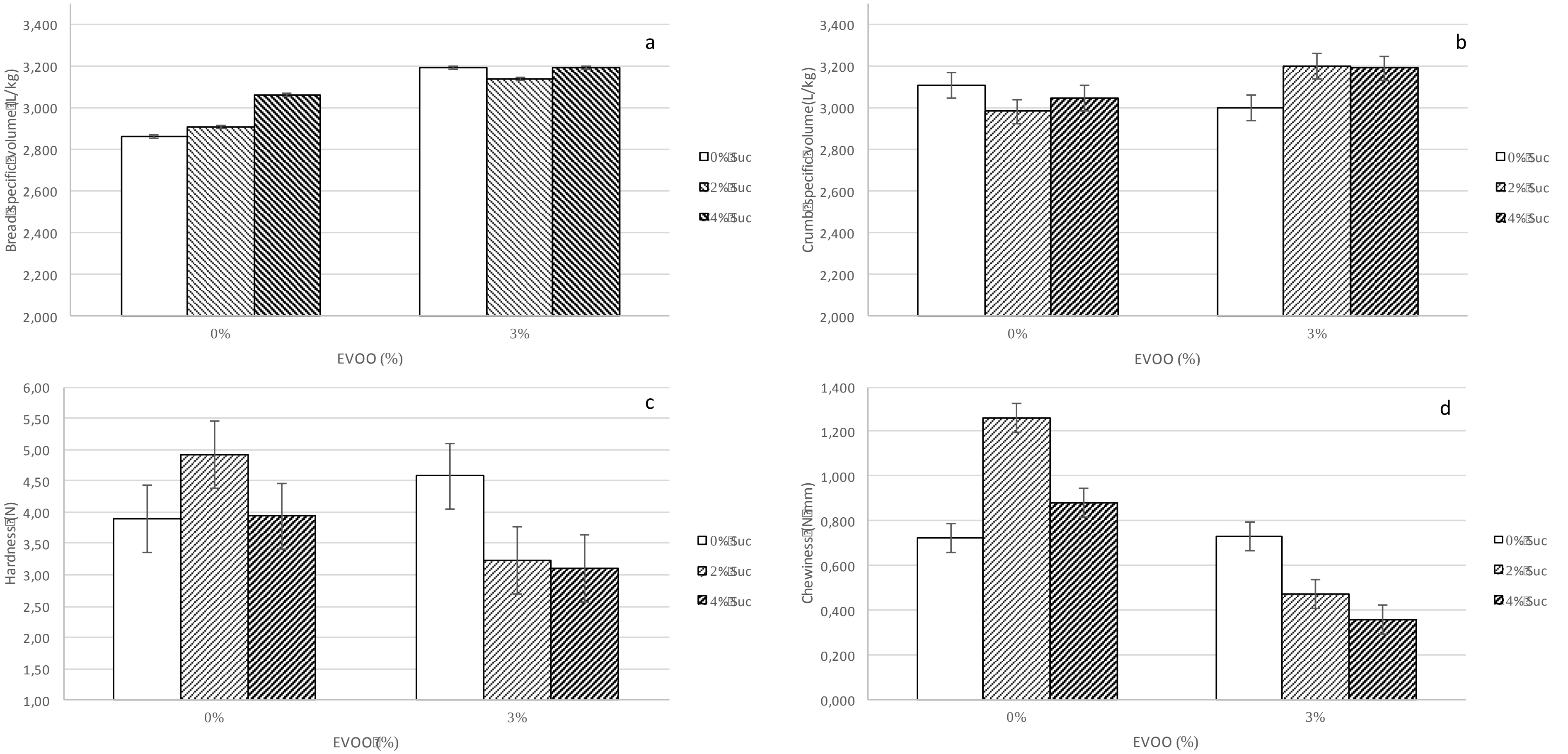
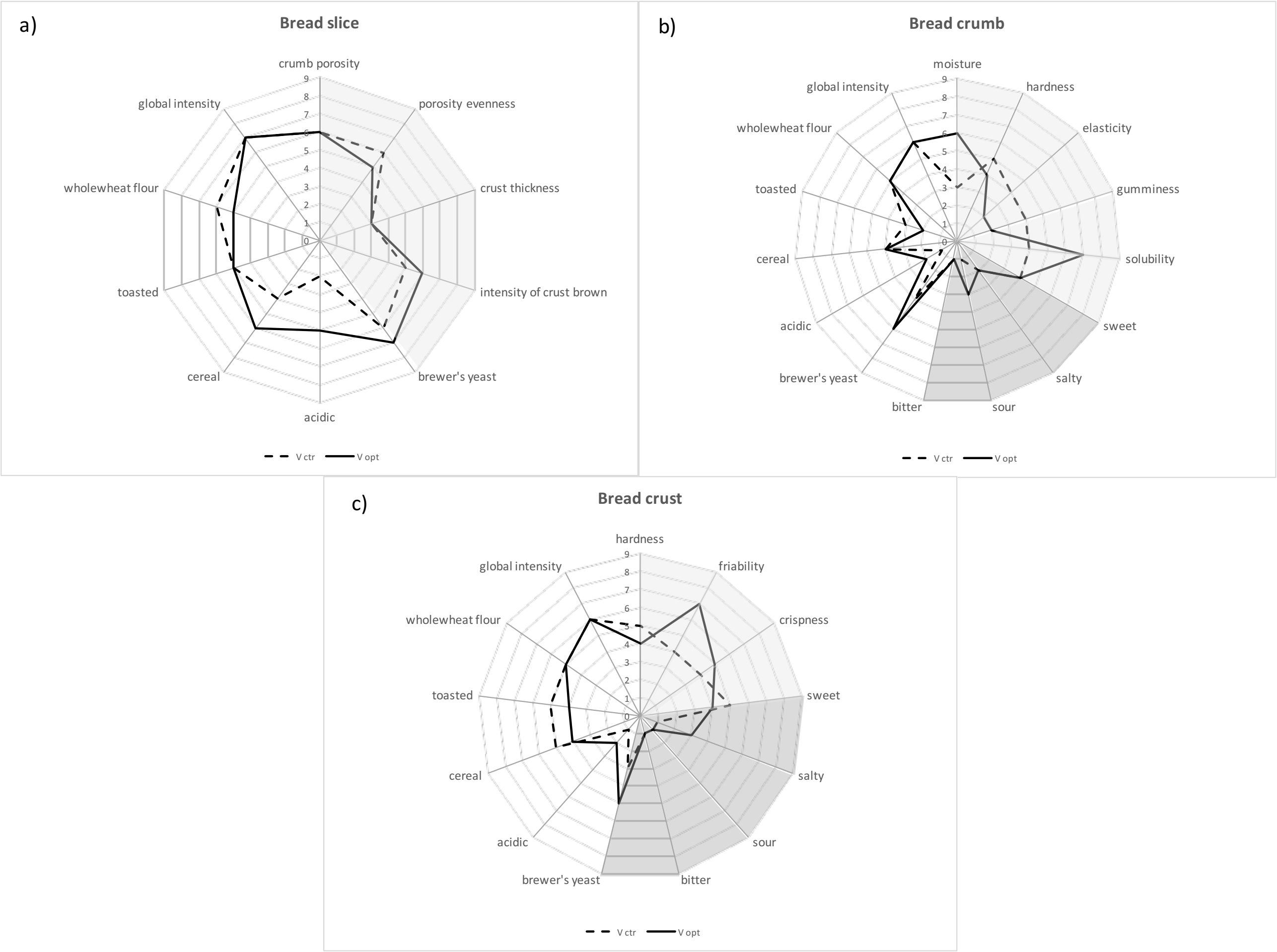


Figure 3.

Figure 4


a)
Bread slice
crumb porosity
porosity evenness
crust thickness
intensity of crust brown
brewer's yeast
acidic
cereal
toasted
wholewheat flour
global intensity
0
1
2
3
4
5
6
7
8
9
V ctr
V opt
b)
Bread crumb
moisture
hardness
elasticity
gumminess
solubility
sweet
salty
sour
bitter
brewer's yeast
acidic
cereal
toasted
wholewheat flour
global intensity
V ctr
V opt
c)
Bread crust
hardness
friability
crispness
sweet
salty
sour
bitter
brewer's yeast
acidic
cereal
toasted
wholewheat flour
global intensity
V ctr
V opt

Conflict of Interest and Authorship Conformation Form

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X All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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X The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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Author contributions

Use this form to specify the contribution of each author of your manuscript. A distinction is made between five types of contributions: Conceived and designed the analysis; Collected the data; Contributed data or analysis tools; Performed the analysis; Wrote the paper.

For each author of your manuscript, please indicate the types of contributions the author has made. An author may have made more than one type of contribution. Optionally, for each contribution type, you may specify the contribution of an author in more detail by providing a one-sentence statement in which the contribution is summarized. In the case of an author who contributed to performing the analysis, the author's contribution for instance could be specified in more detail as 'Performed the computer simulations', 'Performed the statistical analysis', or 'Performed the text mining analysis'.

If an author has made a contribution that is not covered by the five pre-defined contribution types, then please choose 'Other contribution' and provide a one-sentence statement summarizing the author's contribution.

Manuscript title: Breadmaking with an old wholewheat flour:optimization of ingredients to improve bread quality

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☒ Conceived and designed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☒ Collected the data
Specify contribution in more detail (optional; no more than one sentence)

☐ Contributed data or analysis tools
Specify contribution in more detail (optional; no more than one sentence)

☒ Performed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☒ Wrote the paper
Specify contribution in more detail (optional; no more than one sentence)

☐ Other contribution
Specify contribution in more detail (required; no more than one sentence)

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Specify contribution in more detail (optional; no more than one sentence)

☒ Contributed data or analysis tools
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☐ Performed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☒ Wrote the paper
Specify contribution in more detail (optional; no more than one sentence)

☐ Other contribution
Specify contribution in more detail (required; no more than one sentence)

Author 3: Benedetta Cavallini

☐ Conceived and designed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☒ Collected the data
Specify contribution in more detail (optional; no more than one sentence)

☐ Contributed data or analysis tools
Specify contribution in more detail (optional; no more than one sentence)

☒ Performed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Wrote the paper
Specify contribution in more detail (optional; no more than one sentence)

☐ Other contribution
Specify contribution in more detail (required; no more than one sentence)

Author 4: Fabio Baldi

☐ Conceived and designed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Collected the data
Specify contribution in more detail (optional; no more than one sentence)

☐ Contributed data or analysis tools
Specify contribution in more detail (optional; no more than one sentence)

☒ Performed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Wrote the paper
Specify contribution in more detail (optional; no more than one sentence)

☐ Other contribution
Specify contribution in more detail (required; no more than one sentence)

Author 5: Bruno Zanoni

☒ Conceived and designed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Collected the data
Specify contribution in more detail (optional; no more than one sentence)

☐ Contributed data or analysis tools
Specify contribution in more detail (optional; no more than one sentence)

☐ Performed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☒ Wrote the paper
Specify contribution in more detail (optional; no more than one sentence)

☐ Other contribution
Specify contribution in more detail (required; no more than one sentence)

Author 6: Enter author name

☐ Conceived and designed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Collected the data
Specify contribution in more detail (optional; no more than one sentence)

☐ Contributed data or analysis tools
Specify contribution in more detail (optional; no more than one sentence)

☐ Performed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Wrote the paper
Specify contribution in more detail (optional; no more than one sentence)

☐ Other contribution
Specify contribution in more detail (required; no more than one sentence)

Author 7: Enter author name

☐ Conceived and designed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Collected the data
Specify contribution in more detail (optional; no more than one sentence)

☐ Contributed data or analysis tools
Specify contribution in more detail (optional; no more than one sentence)

☐ Performed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Wrote the paper
Specify contribution in more detail (optional; no more than one sentence)

☐ Other contribution
Specify contribution in more detail (required; no more than one sentence)

Author 8: Enter author name

☐ Conceived and designed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Collected the data
Specify contribution in more detail (optional; no more than one sentence)

☐ Contributed data or analysis tools
Specify contribution in more detail (optional; no more than one sentence)

☐ Performed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Wrote the paper
Specify contribution in more detail (optional; no more than one sentence)

☐ Other contribution
Specify contribution in more detail (required; no more than one sentence)

Author 9: Enter author name

☐ Conceived and designed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Collected the data
Specify contribution in more detail (optional; no more than one sentence)

☐ Contributed data or analysis tools
Specify contribution in more detail (optional; no more than one sentence)

☐ Performed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Wrote the paper
Specify contribution in more detail (optional; no more than one sentence)

☐ Other contribution
Specify contribution in more detail (required; no more than one sentence)

Author 10: Enter author name

☐ Conceived and designed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Collected the data
Specify contribution in more detail (optional; no more than one sentence)

☐ Contributed data or analysis tools
Specify contribution in more detail (optional; no more than one sentence)

☐ Performed the analysis
Specify contribution in more detail (optional; no more than one sentence)

☐ Wrote the paper
Specify contribution in more detail (optional; no more than one sentence)

☐ Other contribution
Specify contribution in more detail (required; no more than one sentence)