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### **Sprouting of oats: a review on the relationship between processing conditions and chemico-physical properties of flour**

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4 **Sprouting of oats: a review on the relationship between processing**  
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6 **conditions and chemico-physical properties of flour**  
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4 1 **Abstract:** Despite its nutritional value, oat is underutilized for human consumption due to  
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6 2 sensory and technological issues. To increase oat use in food products, sprouting has been  
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8 3 proposed to enhance its compositional, sensory and functional properties. This review  
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10 4 examined 31 papers from the last two decades on the impact of sprouting and processing  
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12 5 conditions on oat chemical, physical, and technological attributes. The review outlines the  
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14 6 biochemical changes during sprouting and the mechanisms behind them, critically evaluating  
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16 7 the impact of sprouting duration and temperature. Results showed high variability in processing  
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18 8 conditions, often lacking details about raw material origin and conditions like relative humidity.  
19  
20 9 Most studies focused on small-scale production, with no evidence if changes are maintained at  
21  
22 10 a large scale. Common sprouting conditions identified were soaking for 12 h at 20°C, sprouting  
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24 11 for 48 h at 25°C, and freeze-drying or drying for 24 h at 50°C. This review provides valuable  
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26 12 insights into the interplay between processing conditions and chemico-physical properties of  
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28 13 sprouted oat, noting that few studies investigated the functional properties of flour and related  
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30 14 products. Understanding these relationships is crucial for harnessing sprouting's potential as a  
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32 15 sustainable approach to oat flour production, enhancing the quality and functionality of various  
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34 16 food products.

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38 17 **Keyword:** germination; oat; chemical composition; enzymatic activity; functional properties.  
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## 18 1. INTRODUCTION

19 Oat, like all cereals, is an annual plant belonging to the *Poaceae* family. The most known,  
20 cultivated and consumed species in the world is *Avena sativa* L. Currently 26 million tons of  
21 oats are produced globally, and the European Union is the world's largest producer with 7.5  
22 million tons (FAOSTAT, 2022). Compared to other cereals, by world production, oat ranks  
23 sixth followed by rye (FAOSTAT, 2022). Oat is commonly cultivated in temperate regions  
24 characterized by relatively high levels of rainfall and generally within areas exhibiting moderate  
25 temperatures (Sánchez-Martín et al. 2014). The primary production regions encompass mid to  
26 northern Europe, specifically Poland and Finland, the Russian Federation, as well as the USA  
27 and Canada (Stewart and McDougall 2014).

28 Oat over the years has been widely used for animal feed; although to date the percentage of oat  
29 destined for animal feed has declined significantly, it remains the main destination of use. The  
30 increase in oats destined for the food sector and thus for food production is mainly attributable  
31 to the composition of this cereal, which makes it very interesting from a nutritional point of  
32 view. From a quantitative point of view, the main component of oat is starch, which accounts  
33 for about 60% of the dry weight and possesses structural and physicochemical characteristics  
34 that differentiate it from the starches of other cereals in terms of granule size and gelatinization  
35 temperature (Punia et al. 2020). The protein content of oat is generally high, between 9 and  
36 17% depending on the variety considered (Sterna, Zute, and Brunava 2016). Compared to other  
37 cereals, oat is particularly rich in lipids, with an average content of 7% (80% of which  
38 represented by unsaturated fatty acids), which, when combined with a high intrinsic lipolytic  
39 activity, can easily lead to negative phenomena such as rancidity (Zhou et al. 1999). Among  
40 the fibers, that ranges between 9 and 15% (Németh et al. 2021), the most interesting component  
41 belonging to the fraction of soluble fiber is  $\beta$ -glucan, which is found to be notably high, with  
42 an average content ranging from 1.8 to 5.5%, when compared to the fiber content of other

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4 43 cereals (Miller et al. 1993). The consumption of  $\beta$ -glucan leads to a reduction of postprandial  
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6 44 glycemic and insulinemic responses, lowers total cholesterol and low-density lipoprotein  
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8 45 cholesterol levels, and enhances high-density lipoprotein cholesterol and blood lipid profiles  
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10 46 (Daou and Zhang 2012). It also helps in maintaining body weight, making it beneficial for  
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12 47 preventing, treating, and managing diabetes and cardiovascular diseases (Mathews, Shete, and  
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14 48 Chu 2023; Shen et al. 2016; Nwachukwu et al. 2015). Regarding micronutrients, oat is notably  
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16 49 rich in vitamin E, potassium, and phosphorus (White, Fisk, and Gray 2006; Gangopadhyay et  
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18 50 al. 2015); it also contains other minor components such as phenols, among which  
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20 51 avenanthramides, characteristic of this cereal, have sparked considerable interest due to their  
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22 52 antioxidant capacity (Bratt et al. 2003).

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25 53 Despite these compositional traits that make oat unique among grains, its use remains limited  
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27 54 to plant-based beverages, porridge or breakfast cereals. Among the main reasons for the limited  
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29 55 use of oat in baked goods are: the lack of gluten-forming proteins, the fiber-wheat gluten  
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31 56 interference, the bitter and rancid taste, and the lipid oxidation and hydrolysis (Heiniö et al.  
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33 57 2002; Heiniö et al. 2001; Cao et al. 2023). Thermal (such as kilning, roasting, extrusion,  
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35 58 superheated steam treatment, and microwave) and non-thermal (such as ultrasound, high  
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37 59 hydrostatic pressure, and cold plasma) treatments have been recently shown to be effective in  
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39 60 solving the issues related to sensory acceptability and kernel storage (Yang et al. 2023). Besides  
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41 61 such treatments, biotechnological processes (such as enzymatic treatments, fermentation and  
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43 62 germination) have been introduced as strategies to enhance oat stability while improving some  
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45 63 sensory and nutritional traits (Yang et al. 2023).

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48 64 Germination (or sprouting) is the natural process that allows seeds to grow. The germination  
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50 65 process itself can be broken down into several stages, each characterized by a well-defined  
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52 66 mechanism that enables the seed to develop (Han, and Yang 2015). It begins with the soaking  
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54 67 phase, during which the seeds absorb all the necessary water to activate the endogenous  
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4 68 metabolism required to mobilize the reserve material and thereby promote seed development.  
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6 69 Once the endogenous metabolism is activated, the radicle starts to grow from the seed.  
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8 70 Subsequently, increased mobilization of reserves allows the seedling to grow (Tuan et al. 2019).  
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10 71 From a nutritional standpoint, the main outcomes of the sprouting process are the decrease in  
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12 72 anti-nutritional factors, the increase in mineral bioavailability, and the increase in bioactive  
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14 73 compounds (Hübner and Arendt 2013). Such changes are the result of a complex of biochemical  
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16 74 changes triggered by the endogenous enzymatic activity developed during sprouting (Guzmán-  
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18 75 Ortiz et al. 2019). Thus, it is expected that such changes are dependent on the enzymatic  
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20 76 activity, thus on sprouting conditions. However, up to now there are no systematic reviews on  
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22 77 the relationship between processing conditions and the physico-chemical features of oat.  
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24 78 In this context, the aim of the present study was to provide a systematic overview of the relation  
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26 79 between sprouting conditions and the main changes occurring in oat, and to highlight potential  
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28 80 unexplored aspects that indeed would deserve attention. Improving the understanding of these  
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30 81 aspects can enhance the standardization of sprouting and the implementation of a tailored-made  
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32 82 biotechnological process, based on the desired modifications, and thus encourage the use of oat  
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34 83 in food production.  
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## 39 84 **2. MATERIALS AND METHODS**

### 40 85 **2.1 Search strategy and study selection**

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43 86 This study was conducted using the Preferred Reporting Items for Systematic Reviews and  
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45 87 Meta-Analyses (PRISMA) (Moher et al. 2009). The literature search was conducted by  
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47 88 consulting both Web of Science and PubMed databases using the combination of the following  
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49 89 words in the title, keywords and abstracts: (sprout\* OR germinat\*) AND (oat) NOT (pre-  
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51 90 harvest OR preharvest). The search was carried out in January 2024, with the last update in  
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53 91 March 2024.  
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4 92 Specific inclusion/exclusion criteria were chosen to improve the quality of the research. The  
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6 93 inclusion criteria adopted were (i) only research articles published in English; (ii) studies  
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8 94 conducted since 2003 (i.e., in the last 20 years); (iii) studies containing relevant information on  
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10 95 the effect of sprouting on chemical composition, nutritional traits, and technological properties.  
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12 96 Instead, the exclusion criteria were as follows: (i) studies other than original journal articles;  
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14 97 (ii) studies that did not report data on the unsprouted oats; (iii) studies that addressed other  
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16 98 thematic areas (e.g., botany, genetics).

## 19 20 99 **2.2 Data extraction and presentation**

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22 100 Two reviewers (A.S. and A.B.) independently extracted data from the included papers. In cases  
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24 101 of disagreement, consensus was reached through consultation with a third, independent author  
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26 102 (A.M.).

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28 103 All potentially eligible items were imported into Endnote Web data management software to  
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30 104 facilitate the first selection step. Then, the eligible studies selected after phase two were  
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32 105 categorized in Microsoft Excel according to publication details (authors, title, year of  
33  
34 106 publication) and type of variables/factors investigated. After an initial classification of studies,  
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36 107 creating a comprehensive database, the subsequent step involved extracting information  
37  
38 108 regarding the sprouting methodology. Specifically, details were gathered on: i) type of oat used;  
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40 109 ii) quantity of oat utilized; iii) information pertaining to the soaking, sprouting, and stabilization  
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42 110 phases; iv) indices monitored.

## 43 44 45 46 111 **3. RESULTS AND DISCUSSION**

### 47 48 112 **3.1 Study selection**

49  
50 113 The initial literature review yielded 789 potentially eligible scientific articles according to the  
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52 114 search string. All studies obtained underwent a selection process characterized by two stages to  
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54 115 ensure their relevance: the first step involved reading the titles and abstracts, while the second  
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56 116 step critically read the full texts of the articles selected in the first step. Based on the exclusion  
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4 117 criteria described above, all irrelevant records were discarded. In addition, the following were  
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6 118 not considered in this systematic search: review and meta-analysis articles, book chapters,  
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8 119 opinion/commentary, and conference papers. **Figure 1** details the PRISMA flowchart of the  
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10 120 systematic review study design.

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12 121 After removing duplicates through Endnote Web, 661 research articles were identified. Next,  
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14 122 based on screening of titles, 59 articles were found to be potentially eligible, while 602 were  
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16 123 excluded as irrelevant to the research question. Then, based on screening of abstracts, 31 articles  
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18 124 were found to be eligible, while 28 were excluded. The full texts of the 31 articles were  
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20 125 examined and they are the results of relevant studies at the end of the selection process.

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22  
23 126 *[Figure 1]*

24  
25 127 Almost 50% of relevant articles were published from 2019 to 2023, with the highest  
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27 128 number (n=6) being published in 2020. This growth in recent years indicates a gradual increase  
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29 129 in interest from the scientific community and industry in the application of bioprocesses such  
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31 130 as sprouting.

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34 131 Considering the geographic spread of publications, there are three continents involved  
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36 132 (Europe, Asia and America), however, most of the studies were carried out in Europe, followed  
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38 133 by Asia and America. Specifically, the countries that have mostly studied the effects of oat  
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40 134 sprouting are China (seven studies) followed by Korea (four studies), Ireland and Spain (three  
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42 135 studies), Sweden and Czech Republic (two studies), Mexico, Netherlands, Finland, India,  
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44 136 Japan, Serbia, Germany, Romania, USA and Turkey (one study). In many Asian countries,  
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46 137 sprouting has always been considered an economic as well as excellent process to improve the  
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48 138 nutritional value, flavor, taste and decrease the amount of anti-nutrients of grains (Waliat et al.  
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50 139 2023).

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53 140 The included studies focused on monitoring the technological properties and  
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55 141 compositional changes occurring in oat during sprouting with regard to macronutrients,  
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4 142 micronutrients, bioactive compounds, enzyme activities and  $\gamma$ -aminobutyric acid (GABA). As  
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6 143 shown in **Figure 2**, most of the studies focused on evaluating the effect of sprouting on  
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8 144 compounds with antioxidant activities (including phenolic compounds) and plant secondary  
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10 145 metabolism compounds. Very few studies investigated the effect of sprouting on the functional  
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12 146 and rheological properties of oat flour and its use in the reformulation of baked products. The  
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14 147 main changes occurring upon sprouting will be discussed in **section 3.3**.

16  
17 148 [Figure 2]

## 19 149 **3.2 Raw materials and main processing conditions used for oat sprouting**

### 21 150 **3.2.1 Raw material**

22  
23 151 Genotype plays a crucial role in determining the ultimate outcome of sprouting in relation to  
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25 152 chemical and functional modifications (Benincasa et al. 2019). Differences in kernel structure  
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27 153 (e.g., tissue) and composition (e.g., protein, sugar content) might account for differences in  
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29 154 germinability among different species and/or different varieties.

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31 155 With regard to the raw material, 16% of the studies did not indicate the type of oat used (neither  
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33 156 the species or the cultivar). By comparing sprouted *Avena sativa* L. with sprouted *Avena nuda*  
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35 157 L., the main changes in protein, fiber, and vitamins content after 4 days of sprouting were  
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37 158 measured in naked oat (Gabrovska et al. 2004). However, the authors did not state any  
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39 159 hypotheses about the reasons for the increase (expressed in percentage of original content) in  
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41 160 the above-mentioned nutrients between naked and common oat.

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44 161 Aparicio-García et al. (2020) evaluated differences in  $\beta$ -glucans, phenolic compounds,  
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46 162 antioxidant activity and enzymatic activity due to oat species. Specifically a hulled (variety  
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48 163 Barra) and a dehulled (variety Meeri) were studied. Results highlighted how the presence or  
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50 164 absence of the hulls impact on the biochemical changes occurring during sprouting, so that  
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52 165 optimal conditions (time and temperature) for each variety should be selected: 216 h at 16 °C  
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4 166 for Barra variety and for 156 h at 18 °C for Meeri (Aparicio-García et al. 2020). This study  
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6 167 suggests that sprouting conditions should be optimized based on the oat variety/species.

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8 168 Hulled varieties are more common than naked ones. In the former, the hulls are strongly adhered  
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10 169 to the kernel so that a mechanical process, known as dehulling or debranning, is necessary to  
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12 170 remove the hulls before using oats for consumption or for further processing, including  
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14 171 sprouting.

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16 172 In naked oats the hull is genetically removed, allowing food industries to reduce processing  
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18 173 time and costs by avoiding the dehulling step (Kirkkari, 2008). Additionally, this type of oat is  
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20 174 more suitable for sprouting and thus to malting processes, but it shows lower yields and is  
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22 175 more prone to kernel damage during harvesting, as well as mold (Kirkkari, Peltonen-Sainio,  
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24 176 and Rita 2001). Dehulled oat varieties, on the other hand, at the time of harvesting, still have  
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26 177 the hull, which, being inedible, must be removed through hulling. During the de-hulling  
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28 178 process, germ can be damaged, thus reducing the germinability of the seeds. From a nutritional  
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30 179 point of view, the two species of oat are different and naked oat has garnered significant interest  
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32 180 in recent years due to the nutritional profile, as their fiber, protein and lipid content and  
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34 181 antioxidant properties is generally higher compared to dehulled oats (Biel, Jacyno, and  
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36 182 Kawecka 2014; Boeck et al. 2018).

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38 183 Another important variable is the amount of sample used during sprouting. The amounts of oat  
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40 184 grains used during experiments ranged from as little as 5 g (one studies) to 1 kg (one study)  
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42 185 (Table 1). The most used amount was 200 g of sample per single trial. The quantity of seeds  
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44 186 used for sprouting tests, in addition to being imported to better simulate the process that would  
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46 187 take place on an industrial scale, is an aspect that could influence the efficiency of sprouting in  
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48 188 terms of oxygen available to the seeds and humidity of the environment for example.

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53 189 **3.2.2 Sprouting Conditions**  
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4 190 In addition to the type of oats used, another important factor influencing sprouting of seeds is  
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6 191 the processing conditions to which they are subjected. Various biochemical changes have been  
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8 192 observed to depend on time and temperature and that these conditions can be adjusted to achieve  
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10 193 the desirable target (e.g., reduction of antinutrients and/or the increase in specific  
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12 194 macro/micronutrients) (Krapf et al. 2019). **Table 1** summarizes the main sprouting conditions  
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14 195 applied to oats that will be discussed in the following section.

16  
17 196 *[Table 1]*

18  
19 197 Sprouting process involves three stages: soaking, sprouting and stabilization (**Figure 3**).

20  
21 198 *[Figure 3]*

22  
23 199 Seeds are soaked in water to reach a moisture content suitable for initiating the seedling growth  
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25 200 (generally 30-50%) (Marti, Cardone, and Pagani 2020). After that, the soaking water is drained  
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27 201 and the grains are left to germinate under controlled conditions. Controlling the parameters (e.g.  
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29 202 time, temperature, and relative humidity) during the sprouting phase determine the development  
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31 203 of enzyme activity that will affect the hydrolysis of macronutrients and the release of secondary  
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33 204 compounds that might affect color and/or flavor of the final product. Sprouted grains are used  
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35 205 as it is, i.e., in the form of sprouts, or are stabilized to decrease the moisture content to guarantee  
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37 206 the shelf life of the seeds.

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40 207 As regards **soaking**, the main processing conditions in terms of time, temperature, seed-water  
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42 208 ratio and light-darkness conditions, are summarized in **Table 1**. In general, the most common  
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44 209 conditions used in the selected studies were: (i) seed:water ratio from 1:2 to a 1:6; (ii) 20 °C;  
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46 210 (iii) soaking time between 2 h and 72 h with the most used condition being around 12 h. Few  
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48 211 studies specified whether soaking was carried out applying dark conditions; in most of the cases  
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50 212 this information was not reported by the authors. The extent of sprouting modifications depends  
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52 213 on darkness or the intensity and source of light used (Mastropasqua, Dipierro, and Paciolla  
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54 214 2020). Specifically, sprouting carried out in light conditions promoted the accumulation of  
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4 215 polyphenols (He et al. 2023); thus the use of light is widely used in sprouts production  
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6 216 (Benincasa et al. 2019).

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8 217 As stated above, the minimum moisture content to activate the sprouting process is 30%;  
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10 218 however, few studies reported the seed moisture after soaking: the value was about 40-50% (Li  
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12 219 et al. 2017; Hübner et al. 2010; Skoglund et al. 2008).

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14 220 The germination process starts precisely with the activation of the gibberellin hormone. For  
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16 221 simplicity's sake, it is common to refer to the germination period as that time span from the end  
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18 222 of the soaking phase, then when excess water is removed from the seeds to the beginning of the  
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20 223 seed stabilization phase. During sprouting, the most common conditions used in the selected  
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22 224 studies were: (i) sprouting temperature between 11 and 30 °C, with 25 °C being the most  
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24 225 commonly used; (ii) sprouting time between 7 and 216 h with the most commonly used  
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26 226 conditions 48 h; (iii) relative humidity between 60 and 99 %, with 95% as the most commonly  
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28 227 used condition. Specifically, as regard the sprouting time, 14 studies sprouted for 48 h, 13  
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30 228 papers focused on 24 h-sprouting time, and 11 studies evaluated the effect of sprouting after 72  
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32 229 h and 96 h. Some studies have evaluated multiple germination conditions in terms of time and  
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34 230 temperature, to better elucidate the impact of time and/or temperature on macro- and/or  
35  
36 231 micronutrients; but, none of the studies evaluated the impact of relative humidity on the  
37  
38 232 sprouting process. When sprouting was carried out at 10, 14, 20, 25, and 30°C for 1, 2, and 3  
39  
40 233 days, it is found that for a process duration of 3 days, temperatures between 20 and 25°C yield  
41  
42 234 the most significant changes in the properties of sprouted grains (Krapf et al. 2019).

43  
44 235 Despite what was found for soaking, for the sprouting phase, more studies specified whether  
45  
46 236 the sprouting was carried out in darkness or light conditions and most of them carried this step  
47  
48 237 in darkness.

49  
50 238 One of the objective parameters used to determine the degree of germination is the length of  
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52 239 the radicle. Parameters reported by various authors are: 2 mm after 48 h at 16 °C (Xu et al.,  
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4 240 2009); up to 6 mm after 60 h at 25 °C (Li et al. 2020); up to 65 mm after 120 h at 25 °C  
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6 241 (Damazo-Lima et al. 2020) and 144 h at 16 °C (Tian et al. 2010). In this regard, in early 2008,  
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8 242 Cereals & Grains' Board of Directors decreed that "malted or sprouted grains containing all of  
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10 243 the original bran, germ, and endosperm shall be considered whole grains as long as sprout  
11  
12 244 growth does not exceed kernel length and nutrient values have not diminished. These grains  
13  
14 245 should be labeled as malted or sprouted whole grain". Krapf et al. (2019) defined the degree of  
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16 246 germination through a numerical scale from 0 to 5 that takes into account radicle length and  
17  
18 247 coleoptile. Other parameters used to determine the degree of germination were the weight of  
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20 248 1,000 seeds and the ratio of radicle weight to coleoptile weight. Results showed that 1,000 grain  
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22 249 weight is insufficiently linked to quality parameters, whereas the mass fraction of radicle and  
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24 250 coleoptile in the grain and the degree of germination correlated very well with the  $\beta$ -glucan  
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26 251 level (Krapf et al. 2019).

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29 252 After sprouting, a stabilization step is necessary to lower the moisture content of sprouted seeds.  
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31 253 In the selected studies, drying and freeze-drying were the most common approaches. Regarding  
32  
33 254 drying, the temperature used will affect the enzymatic activity developed during the sprouting  
34  
35 255 phase, as well as the development of specific color and flavors (Heiniö et al. 2001). In general,  
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37 256 the most commonly used drying conditions range from 50 to 60°C for between 12 and 24 h.  
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39 257 One critical point of sprouting is bacterial growth (Miyahira and Antunes 2021), especially if  
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41 258 the initial total bacteria count of the seeds is high; indeed, sprouting conditions can promote  
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43 259 their proliferation (US FDA, 1999). There are several techniques for the containment and  
44  
45 260 abatement of microbial load of sprouted seeds. These may relate to containment as early in seed  
46  
47 261 collection, transport and storage as to specific treatments applied before and after sprouting.  
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49 262 Washing the seeds before their sprouting is generally the most common action taken to  
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51 263 decontaminate the seeds (Caetano Anollés, Favelukes, and Bauer 1990), but it can not be  
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53 264 sufficient. Thus, other treatments should be used, although it seems very complex to select an  
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4 265 effective treatment in inactivating pathogens and microbial load present in seeds. This will  
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6 266 certainly depend on the characteristics of the seed surface as well as the germinability of the  
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8 267 seed, that should not be negatively affected by the treatment. One of the most commonly used  
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10 268 chemical treatments to decontaminate seeds before the germination stage is the treatments with  
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12 269 chlorine. Several studies have applied seed pretreatments with chlorine and one of the most  
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14 270 commonly used conditions at this stage is 1% sodium hypochlorite solution at a 1:2  
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16 271 (seed:solution) ratio for 8 h at 20 °C. There are other strategies for microbial load reduction that  
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18 272 exploit physical (e.g., heat), biological, and chemical principles applicable to seeds before or  
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20 273 after sprouting. For example, drying of sprouted seeds can be modulated to achieve microbial  
21  
22 274 load reduction (Douglas and Flannigan 1988). Lipiec et al. (2005) evaluated the positive effects  
23  
24 275 of oscillating magnetic field pulses on decreasing the microbial load in sprouted naked oats.  
25  
26 276 There are no studies that compared different decontamination methods applied during oat  
27  
28 277 sprouting. Moreover, none of the studies that used sodium hypochlorite during soaking did not  
29  
30 278 report data about the microbial growth before and after treatment with sodium hypochlorite.  
31  
32 279 However, the positive effect of sodium hypochlorite on microbial growth is widely recognized  
33  
34 280 (Mir et al., 2021). While selecting the method for limiting the microbial growth during  
35  
36 281 sprouting, several aspects should be considered, including its effectiveness, country regulations,  
37  
38 282 and costs.

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42 283 Among the studies included in the systematic search, none of them evaluated the evolution of  
43  
44 284 microbial load after the sprouting process. In general, after sprouting, seeds have higher counts  
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46 285 than the starting raw material by 2-3 logs (Thompson and Powell 2000). As an example, we  
47  
48 286 reported the microbial load of oat seeds sprouted at lab scale (**Table 2**). The overall microbial  
49  
50 287 scenario of sprouted oats was good. Sprouting for 72 h at 22 °C increased the amount of yeasts  
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52 288 and lactic bacteria but no pathogens were found. In detail, after sprouting there is a one-log  
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4 289 increase in total bacterial and yeast counts, and 2-log increase in lactic acid bacteria. On the  
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6 290 other hand, molds decreased probably due to the drying phase conducted at 60 °C.  
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9 291 [Table 2]  
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### 15 293 3.3 Effect of sprouting on chemical composition

16  
17 294 The main changes occurring during sprouting on macronutrients, micronutrients,  $\gamma$ -  
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19 295 aminobutyric acid, and phenolic compounds and the related mechanism are summarized in  
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21 296 **Table 3** and discussed in the following paragraphs.  
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23

24 297 [Table 3]  
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#### 26 298 3.3.1 Proteins

27  
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29 299 The protein content of oat ranges from 10 to 17%, depending on several factors including  
30  
31 300 agronomic and environmental conditions, as well as the type of species (*Avena sativa* L. versus  
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33 301 *Avena nuda* L.) and varieties (Alemayehu et al. 2023). It was reported that dehulled oats are  
34  
35 302 characterized by a higher protein content than naked oats (Biel, Jacyno, and Kawecka 2014).  
36

37 303 A couple of papers (Aparicio-García et al. 2020; Tian et al. 2010) showed a 10% increase in  
38  
39 304 protein in the first 24 h of sprouting at 16 °C. As germination time increased, keeping the  
40  
41 305 temperature constant at 16 °C, a further increase in protein content was observed, up to 50%  
42  
43 306 after 216 h (i.e., 9 days, which represent an extreme sprouting time, even higher than malting).  
44  
45 307 Increases between 10 and 20% were measured by increasing the temperature from 16 °C to 20  
46  
47 308 °C after 96 h of sprouting, and from 16 °C to 25 °C for 120 h. However, at 25 °C, a decrease in  
48  
49 309 proteins (by 26%) occurred within the first 72 h of sprouting; after that, protein content  
50  
51 310 increased, reaching the maximum increase of about 40% after 125 h.  
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53  
54 311 Differences in changes in protein content upon sprouting can be due to differences in the raw  
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56 312 materials used. However, a third processing parameter - often neglected by the selected studies  
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4 313 - is the relative humidity which has an effect on sprouting kinetics (Limwiwattana, Tongkhao,  
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6 314 and Na Jom 2016). Specifically, only 6 studies (Aparicio-García et al. 2021; Damazo-Lima et  
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8 315 al. 2020; Khang, Vasiljevic, and Xuan 2016; Tang et al. 2020; Jiménez-Pulido et al. 2022; He  
9  
10 316 et al. 2023) out of 12 have reported the relative humidity used during sprouting (60% or 90%).  
11  
12 317 However, none of the studies made a comparison between different relative humidity values.  
13  
14 318 The decrease in protein content measured in the early stages of sprouting could be justified by  
15  
16 319 use of proteins by the plant to grow (Jiménez-Pulido et al. 2022). After that, the increase in  
17  
18 320 protein content could be explained by two reasons: i) decrease in carbohydrates due to starch  
19  
20 321 hydrolysis by amylases (Tian et al. 2010; Wu, 1983); ii) synthesis of new proteins (Aparicio-  
21  
22 322 García et al. 2020).  
23  
24 323 Besides a change in protein content (evaluated as nitrogen content), sprouting promotes changes  
25  
26 324 in protein structure. Indeed, protein hydrolysis by proteases increases the amount of amino acids  
27  
28 325 and soluble peptides (Tian et al. 2010), that in the early stages of sprouting are used by the  
29  
30 326 embryo to form structural proteins (Mäkinen, Zannini, and Arendt 2013; Aparicio-García et al.  
31  
32 327 2021).  
33  
34 328 Among the few studies that evaluated the effect of sprouting on amino acids (5 studies), that of  
35  
36 329 Tian et al. (2010) was the most complete. Overall, total essential amino acids increased up to 3  
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38 330 times after sprouting at 20 °C for 48 h (Tang et al. 2020).  
39  
40 331 Although it is well known that changes in amino acids and protein content could be due to the  
41  
42 332 proteolytic activity developed during sprouting, only few studies have monitored the enzymatic  
43  
44 333 activity during germination. Proteolytic activity increased from 3 to 40 times during sprouting  
45  
46 334 (Aparicio-García et al. 2020; Mäkinen, Zannini, and Arendt 2013). It was also found to have a  
47  
48 335 higher proteolytic activity in hulled varieties than dehulled ones: the presence of the hulls  
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50 336 resulted in different moisture retention during sprouting of oats, a phenomenon that could  
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4 337 contribute to the observed differences in protease activity between hulled and dehulled varieties  
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6 338 (Aparicio-García et al., 2020).  
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9 **339 3.3.2 Starch**

10  
11 340 Starch is the main component of oat and can reach up to 65% of its weight (Doehlert et al.  
12  
13 341 2013). Generally, starch content is between 44 and 62% for naked oats and 55 and 70% for  
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15 342 dehulled oats (Antonini et al. 2016). Among the selected studies, only six of them reported and  
16  
17 343 discussed the effect of sprouting on starch content. A decrease by around 10% was observed in  
18  
19 344 the first 24 h, regardless of sprouting temperature (16 °C or 25 °C) (Kaur and Gill 2020; Tian  
20  
21 345 et al. 2010). As sprouting time increased, the extent of the decrease in starch content increased,  
22  
23 346 reaching the maximum values of -20% after 72 h at 25 °C and -65% at 144 h at 16 °C (Kaur  
24  
25 347 and Gill 2020; Tian et al. 2010). Only one study (Khang, Vasiljevic, and Xuan 2016) observed  
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27 348 an increase around 13% when sprouting was carried out at 25 °C for 125 h; although starch  
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29 349 content after sprouting was not statistically different from the value measured in the unsprouted  
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31 350 sample.  
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35 351 As with other cereals (Marti et al. 2017; Ayernor and Ocloo 2007), the decrease in starch  
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37 352 content was matched by an increase in simple sugar; in particular an increase in glucose and  
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39 353 maltose was observed after 96 h of sprouting, at the expense of fructose content (Yıltırak et al.  
40  
41 354 2021). In some cases, after 24 h of sprouting, the content of free sugars (Tian et al., 2010),  
42  
43 355 including glucose and maltose (Yıltırak et al. 2021), turned out to be equal to or slightly lower  
44  
45 356 than the sugar content in the unsprouted sample. This could be due to the fact that at this stage  
46  
47 357 the consumption of free sugars by the seed is faster than their production (Aldasoro and Nicolas  
48  
49 358 1980). As sprouting time increases, it is the increased amylase activity accounting for starch  
50  
51 359 hydrolysis into simple sugars (Tian et al. 2010; Krapf et al. 2019; Jiménez-Pulido et al. 2022).  
52  
53 360 By investigating the amylase activity during sprouting at 16 °C, Watt et al. (2021) found that  
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55 361 the highest  $\alpha$ -amylase activity was reached after 120 h, whereas in the case of  $\beta$ -amylases the  
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4 362 maximum value was achieved at 72 h, that resulted to be lower than that of  $\alpha$ -amylases, as also  
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6 363 found by Mäkinen et al. (2013). On the other hand, Tian et al. (2010) highlighted that  $\alpha$ -amylase  
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8 364 activity increased up to 20-fold after 144 h of sprouting reaching a climax of 80 mg/g min,  
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10 365 while  $\beta$ -amylase activity reached the maximum value of 228.9 mg/g min (i.e., four times higher  
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12 366 than in unsprouted oat) after 96 h of sprouting.

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14 367 Usually, during sprouting the  $\alpha$ -amylase activity increased whereas the  $\beta$ -amylases, that  
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16 368 initially are only partially present in a free or soluble form, during sprouting they are  
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18 369 progressively released in a soluble form, presumably due to proteases secreted by the aleurone  
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20 370 and/or scutellum (Ziegler, 1995).

21  
22 371 Differences among the studies can not be explained solely by differences in oat varieties,  
23  
24 372 because no relation was found between enzymatic activity and presence or absence of hulls was  
25  
26 373 found (Watt et al. 2021).

### 27 28 29 30 374 **3.3.3 Fiber**

31  
32 375 The total fiber content in hulled and naked oat cultivars are comparable (Givens, Davies, and  
33  
34 376 Laverick 2000). As regards the effect of sprouting on fiber content, there are conflicting results;  
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36 377 indeed, some studies highlighted an increase in fiber, others reported a decrease or no change  
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38 378 in the amount of this component. For example, after 72 h at 25 °C, fiber decreased by 14%  
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40 379 (Kaur and Gill 2020) and up to 55% after 120 h (Damazo-Lima et al. 2020), suggesting that  
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42 380 fiber content decreases as sprouting time increases. Temperature also affects the decrease in  
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44 381 fiber: when sprouting was carried out at 21 °C (instead of 25 °C), the decrease was of about  
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46 382 30% after 120 h (instead of 55%) (Jiménez-Pulido et al. 2022). The relation between  
47  
48 383 temperature and changes in fiber content might explain why Aparicio-García et al. (2021) did  
49  
50 384 not observe any changes in fiber content at 18 °C and 96 h.

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52 385 The decrease in fiber was associated with degradation of cell wall material during plant growth  
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54 386 (Jiménez-Pulido et al. 2022). Whereas a significant increase in total fiber content (up to 23%)  
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4 387 was measured after 144 hours of germination at 20 °C (Hübner et al. 2010). Increase in fiber  
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6 388 content was explained as a consequence of the decrease in starch content (Hübner et al. 2010),  
7  
8 389 which is one of explanations proposed for accounting for the increase in protein content. In the  
9  
10 390 study of Hübner et al. (2010) it was the insoluble fraction that increased up to 40% after 144 h,  
11  
12 391 whereas the soluble fraction decreased by 65% already after 96 h (Hübner et al. 2010) and by  
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14 392 88% after 120 h (Damazo-Lima et al. 2020). Decrease in soluble fiber could be explained as a  
15  
16 393 consequence of the degradation of  $\beta$ -glucans because of the increased  $\beta$ -glucanase activity  
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18 394 (Damazo-Lima et al. 2020).

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20  
21 395 On the contrary, the study of Aparicio-García et al. (2021) stated that, although a substantial  
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23 396 decrease in  $\beta$ -glucans is observed with germination, this does not affect the total soluble fiber  
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25 397 content, likely because of the formation of smaller fragments of oligosaccharides derived from  
26  
27 398 the degradation of  $\beta$ -glucans that fall within the soluble fiber.

28  
29 399 With the exception of the work of Aparicio-García et al. (2020) who found an increase in  $\beta$ -  
30  
31 400 glucans by 14% and 37% in naked and hulled oats, respectively, sprouted at 14 °C for 60 h, in  
32  
33 401 all other studies the content of  $\beta$ -glucans significantly decreased, reaching even losses of 95%,  
34  
35 402 generally for times longer than 96 h (Hübner et al. 2010). Besides the relation between sprouting  
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37 403 time and  $\beta$ -glucans decrease, a relation between the latter and sprouting temperature was  
38  
39 404 observed: specifically at low sprouting temperature (10 and 14 °C), the decrease in  $\beta$ -glucan  
40  
41 405 content during sprouting is of smaller entity (Krapf et al. 2019).

42  
43 406  $\beta$ -glucans are one of the most interesting bioactive compounds in oats. Generally there are no  
44  
45 407 differences between naked and dehulled varieties (Antonini et al. 2016). However, in some  
46  
47 408 cases differences have been found based on presence or absence of hulls, which being  
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49 409 composed mainly of insoluble fiber can affect the overall beta-glucan content (Aparicio-García  
50  
51 410 et al. 2020). On a dehulled basis, traditional hulled cultivars contain more  $\beta$ -glucans than the  
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53 411 naked types (Givens, Davies, and Laverick 2000).  
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### 412 3.3.4 Lipids

413 Compared with other grains, oats have a higher lipid content that ranges from 3 to 9% (Pokhrel  
414 et al. 2022), which negatively affects the grain shelf-life and sensory profile due to rancidity  
415 phenomena.

416 During sprouting at 25 °C, lipid content decreased by 18% and 40% after 24 h and 72 h,  
417 respectively (Kaur and Gill 2020) while it increased up to 26% and 35% after 120 h and 125 h,  
418 respectively (Damazo-Lima et al. 2020; Khang, Vasiljevic and Xuan 2016). Also at 21 °C, a  
419 decrease in lipids occurred in the first three days of germination, whereas an increase in this  
420 component was observed at longer germination times (greater than 72 h) and reaching an  
421 increase by 40% after 120 h (Jiménez-Pulido et al. 2022). The decrease in lipid content is the  
422 result of increased lipase activity, which leads to breakdown of lipids into glycerol and fatty  
423 acids (Kaur and Gill 2020).

424 As regards the impact of sprouting temperature, at lower germination temperatures (16-18 °C),  
425 no major changes in lipid content were found after 50 h (Dlouhá et al. 2020).

426 Regarding the fatty acid composition of oats, linoleic and linolenic acids are the essential fatty  
427 acids present in the greatest amount; whereas palmitic acid is the most abundant saturated fatty  
428 acid (Saastamoinen, Kumpulainen, and Nummela 1989). Mono- to polyunsaturated fatty acids  
429 ratio did not change when sprouting was carried out at 18 °C for 96 h (Aparicio-García et al.  
430 2021). However, other findings reported a significant increase in saturated and  
431 monounsaturated fatty acids content (Jiménez-Pulido et al. 2022), as well as an increase in  
432 palmitoleic and  $\alpha$ -linolenic acids by 12% and 17%, respectively (Aparicio-García et al. 2021).

433 Such changes might be attributed to the activation of desaturase enzymes during germination  
434 that lead to the conversion from oleic to  $\alpha$ -linoleic (Aparicio-García et al. 2021).

435 However, other authors reported no lipid degradation during sprouting, with no formation of  
436 free fatty acids (Jiménez-Pulido et al. 2022), which was related to a stable or even lower

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4 437 lipolytic activity after germination, depending on the type of oats. Hulled oats seem to have  
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6 438 greater lipolytic activity than dehulled grains, reaching the maximum activity at long  
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8 439 germination times (96-216 h) (Aparicio-García et al. 2020). Using a dehulled oat, the study  
9  
10 440 even finds a decrease in lipase enzyme activity upon sprouting. This finding could explain how  
11  
12 441 using dehulled oats could provide advantages attributable to greater stability, thus with less  
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14 442 rancidity and a longer shelf life.

### 17 443 **3.3.5 Micronutrients**

18  
19 444 Oats contain a variety of micronutrients, including vitamins (vitamin B1, B6, folate,  
20  
21 445 pantothenic acid) and minerals (Mn, Mg, Se, Fe, Zn, and Cu) (Chen et al. 2021). Unlike  
22  
23 446 macronutrients, the effect of sprouting on micronutrients has been little studied. Thiamine  
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25 447 content was not affected by 96 h sprouting at 18°C, while riboflavin increased up to 50%  
26  
27 448 (Aparicio-García et al. 2021). To provide more insights on the effect of sprouting on  
28  
29 449 micronutrients, we monitored changes in some vitamins and minerals in commercial oats after  
30  
31 450 67-72 h of sprouting. Specifically, vitamin B2, B5 and B9 greatly increased (**Table 4**). The  
32  
33 451 increase in vitamin B9 with sprouting would appear to be due to de novo synthesis due to the  
34  
35 452 demand for methyl groups as a result of increased metabolic activities of the developing seed  
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37 453 (Jabrin et al. 2003).

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41  
42 454 *[Table 4]*

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44  
45 455 As regards minerals, a couple of studies reported an increase in these micronutrients, especially  
46  
47 456 in calcium, magnesium, sodium, zinc and copper (Aparicio-García et al. 2021; Hübner et al.  
48  
49 457 2010). After 48-72 h-sprouting, in a commercial sample we found an increase in copper and  
50  
51 458 calcium by 6 and 25%, respectively, but a decrease in magnesium and zinc, by 24 and 48%,  
52  
53 459 respectively (**Table 4**). Differences in mineral composition might be affected by mineral  
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55 460 content of soaked water, as well as by leaching phenomena during the soaking phase. Loss in  
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4 461 mineral content might be also due to loss in the rootlets during seed handling and processing;  
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6 462 indeed, during sprouting, minerals are transported from the seed into the rootlets (Hübner et al.  
7  
8 463 2010). On the other hand, the increase in mineral content might reflect the loss of organic  
9  
10 464 material by respiration. Since higher losses are expected for longer germination periods as well  
11  
12 465 as higher temperatures, this might result in increased mineral contents (Hübner et al. 2010).  
13  
14 466 Furthermore, the decrease in phytates, and their role in limiting mineral absorption, should be  
15  
16 467 also considered. Indeed, sprouting resulted in a decrease in phytates from 12% (Jiménez-Pulido  
17  
18 468 et al. 2022) up to 91% (Damazo-Lima et al. 2020) after 120 h at 25°C.

### 469 **3.3.6 $\gamma$ -aminobutyric acid (GABA)**

470 Interest in GABA has increased in recent years due to the health benefits associated with this  
471 component (Liska et al. 2022). GABA increases upon sprouting (Aparicio-García et al. 2021;  
472 Tang et al. 2020; Xu et al. 2010). Specifically, GABA content increased from 10 to 30 times  
473 after 48 and 62 hours of sprouting at 20°C, respectively (Tang et al. 2020; Xu et al. 2010). Less  
474 modest increases (around 37%) were found by Khang et al. (2016) after sprouting oat at 25°C  
475 for 120 h. Such increase is due to the partial hydrolysis of proteins and the increased availability  
476 of free amino acids and glutamate decarboxylase activity occurring during sprouting (Aparicio-  
477 García et al. 2021). Indeed, a relation between glutamate decarboxylase activity and GABA  
478 content has been reported upon sprouting, reaching a plateau after 60 h (Xu et al. 2010).

### 479 **3.3.7 Phenolic compounds**

480 Oat is a good source of phenolic compounds, such as avenanthramides, saponins,  
481 hydroxybenzoic acids, flavonols, and flavones. In addition, other families, which include  
482 mainly isoflavones, lignans, and phytosterols, were found in sprouted oat (Damazo-Lima et al.  
483 2020).

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4 484 Phenolic content increased with sprouting time till doubling up to 72 h of germination at 25 °C  
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6 485 (Kaur and Gill 2020; Lee et al. 2019) or increasing 3-4 times for after 120 h (Damazo-Lima et  
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8 486 al. 2020; Tian et al. 2010). The lower the sprouting temperature, the lower the change rate: for  
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10 487 example, when sporting was carried out at 20 °C, a 50% increase of total phenolic compounds  
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12 488 was measured after 96 h (Kim et al. 2013).

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14 489 As regards the effect of sprouting on bound/free phenolic compounds ratio, previous studies  
15  
16 490 reported contrasting results. For example, Khang et al. (2016) found that the bound phenolic  
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18 491 compounds increased 1.5 times after 120 h of germination at 25 °C, while free phenolic  
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20 492 compounds did not change. On the contrary, other studies reported an increase in free phenolic  
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22 493 compounds (i.e., 3 times after 156-216 h at 16-18 °C (Aparicio-García et al. 2020) or after 48  
23  
24 494 h at 16 °C (Xu et al. 2009). Most phenolic compounds in oats, as in other cereals, particularly  
25  
26 495 low molecular weight phenolic compounds, are bound or attached through covalent interactions  
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28 496 with cell wall polysaccharides (Xu et al. 2009; Adom and Liu 2002). Enzymatic activity  
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30 497 developed during sprouting can be responsible for breaking down the cell walls surrounding  
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32 498 the phenolic compounds, so that more free and bound phenolics can be released, resulting in  
33  
34 499 increased total phenolics (Xu et al. 2009; Tian et al. 2010). In addition, part of the increases in  
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36 500 phenolic compounds could be attributed to *de novo* synthesis through activation of the  
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38 501 shikimate and phenylpropanoid pathways (Aparicio-García et al. 2020).

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40 502 The most abundant phenolic acids in oats are ferulic and gallic acids, followed by p-coumaric  
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42 503 and caffeic acids (Xu et al. 2009). The levels of these compounds varied between germination  
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44 504 stages: gallic and caffeic acid content increased by 13% and 6% after 24 h at 16 °C, respectively  
45  
46 505 after 24 h, and then decreased after 48 h, while p-coumaric and ferulic acid content significantly  
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48 506 increased by 77% and 67%, respectively after 48 h of sprouting at 16°C, respectively (Xu et al.  
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50 507 2009). Caffeic and p-coumaric acids decreased in 120 h-sprouted oat (Skoglund et al. 2008),  
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4 508 regardless of the type of varieties. On the other hand, changes in ferulic acid seem to be variety-  
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6 509 dependent (Skoglund et al. 2008).

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8 510 Increase in phenolic acids could be due to the release of bound forms that through the activity  
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10 511 of enzymes lead to the breakdown of various cell walls during germination. On the other hand,  
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12 512 the decreases may be attributable to the activities of peroxidase and phenoloxidase enzymes  
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14 513 that during germination may catalyze the oxidation of phenolic substrates (Xu et al. 2009).  
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16 514 Some of these acids for example could be used in the biosynthesis of avenanthramides  
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18 515 (Skoglund et al. 2008).

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20 516 Within the phenolic compounds, oats possess unique molecules not found in other grains, called  
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22 517 avenanthramides, known for their antioxidant, antiproliferative and anti-inflammatory  
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24 518 properties (Perrelli et al. 2018). They are soluble, low molecular weight molecules that are  
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26 519 produced by the plant itself when subjected to particular stress conditions (Meydani, 2009).

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29 520 Germination time positively affects the content of avenanthramides, particularly the A, B, and  
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31 521 C isoforms, which are the predominant ones in oats (Lee et al. 2019). In particular, the C-type  
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33 522 isoform, which is the most abundant in oats, increases up to 40% after 24 h, and it doubles after  
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35 523 48 h. Also Xu et al. (2009) found an increase in total avenanthramides up to 48% after 48 h of  
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37 524 germination. Specifically, among the various types of avenanthramides monitored (2c, 2p and  
38  
39 525 2f) the one that contributes most to the increase in total avenanthramides is 2f, which increases  
40  
41 526 up to 89% after 48 h of germination. As reported above, the significant increase in  
42  
43 527 avenanthramide content during germination could be explained by the activity of the  
44  
45 528 avenanthramide synthesizing enzyme, which utilizes pre-existing free precursors such as  
46  
47 529 caffeic acid and p-coumaric acid (Xu et al. 2009).

### 50 51 52 **3.3.8 Antioxidant capacity**

53  
54 531 The antioxidant capacity of oats increases during sprouting. For example, it increased up to 4.5  
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56 532 times (after 156 h at 18 °C), however, the magnitude of the increase was found to depend on  
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4 533 the variety (hulled or dehulled) of oats and the germination conditions (time and temperature).  
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6 534 Likely, the dehulled variety, having a higher free phenolic and  $\beta$ -glucans content, exhibits a  
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8 535 greater antioxidant capacity compared to the hulled variety. (Aparicio-García et al. 2020). Also  
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10 536 Dlouhá et al. (2020) found an increase in antioxidant activity in sprouted oats after 40-50 h at  
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12 537 16-18 °C, much greater than that found in other grains such as buckwheat and quinoa.  
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14 538 Increase in antioxidant capacity in germinated oats could be attributed to the release of bound  
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16 539 phenolic compounds due to modifications occurring in the endosperm particularly from the  
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18 540 activation of endogenous esterases (Aparicio-García et al. 2020; Kaur and Gill 2020). Indeed,  
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20 541 a positive correlation between radical scavenging activity (DPPH) and polyphenol content was  
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22 542 found, especially by presence and position of phenolic hydroxyl groups as the DPPH activity  
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24 543 depends on the availability of phenolic hydrogen and the ability to stabilize the resulting  
25  
26 544 phenoxyl radicals formed by hydrogen donation (Kim et al. 2013). In addition, the *de novo*  
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28 545 synthesis of avenanthramides but also the hydrolysis of  $\beta$ -glucans, as the oligosaccharides of  
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30 546 the latter would appear to have high radical scavenging activity and possess reducing power, as  
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32 547 well as vitamin E content (Aparicio-García et al. 2020; Dlouhá et al. 2020).  
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#### 36 548 **3.4 Effect of sprouting on functional properties of oat flour**

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38 549 The enzymatic activities developed during sprouting not only promote the chemical changes  
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40 550 described in the previous sections, but they also might affect the functional and sensory  
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42 551 properties of the raw material, and thus the overall quality of sprouted oat-enriched products.  
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44 552 In the following section, the main changes in techno-functional properties of sprouted oats will  
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46 553 be discussed, as well as how these changes affect the quality of flour and baked goods.  
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48 554 As described in **section 3.3.1**, protein degradation by proteases would increase the amount of  
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50 555 soluble proteins which play a key role in enhancing the functionality of oat flour (Wu, 1983).  
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52 556 However, none of the selected studies investigated the impact of sprouting on oat functional  
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4 557 properties. Thus, this aspect deserves further investigation, also considering the recent interest  
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6 558 in using oat proteins in food formulation (Boukid, 2021).  
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8 559 Changes in starch properties upon sprouting have been studied more than changes in protein  
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10 560 properties. Starch hydrolysis promoted by  $\alpha$ -amylases is responsible for changes in  
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12 561 gelatinization and retrogradation properties, which were mainly studied measuring changes in  
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14 562 viscosity during heating and cooling. Overall, a decrease in flour peak viscosity, breakdown,  
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16 563 final viscosity and setback values was observed as a function of sprouting time (Li et al. 2020;  
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18 564 Kaur and Gill 2020). The decrease in gelatinization and retrogradation properties had been  
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20 565 associated with the decrease in amylose content (from 17.2% to 15% and 14.3% after 24h and  
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22 566 60 h, respectively) (Li et al. 2020) and to other changes, including the decrease in granule size  
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24 567 and relative crystallinity (from 27.44% to 26.78% and 25.05% after 24 h and 60 h, respectively)  
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26 568 (Li et al. 2017).  
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28  
29 569 In the case of starch isolated from sprouted oats, the increase in pasting temperature (from 91.5  
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31 570 °C to 93.2 °C and 94.7 °C after 24 h and 60 h at 25 °C, respectively) was attributed to the  
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33 571 formation of a more rigid crystalline structure (Li et al. 2017). Conflicting results were obtained  
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35 572 for peak viscosity, which increased after 24 h and decreased after 60 h. Comparing the data on  
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37 573 starch (Li et al. 2020) and flour (both from sprouted oats), higher peak viscosity was measured  
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39 574 in starch compared to flour (Li et al. 2017). Lower viscosity in sprouted flour has been explained  
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41 575 by the hydrolysis of proteins that were not anymore able to surround the starch granules, that  
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43 576 becomes less stable during heating and mixing resulting in lower viscosity (Li et al. 2020).  
44

45  
46 577 In addition, the decrease in pasting properties are due to starch hydrolysis that occurs during  
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48 578 sprouting, but also to that one occurring during the test. Indeed, all experiments aimed at  
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50 579 evaluating the pasting properties of germinated oat were conducted by dispersing the sample in  
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52 580 water. This ensured that the residual intrinsic enzyme activity developed during germination  
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54 581 also had a chance to act during the test. None of the authors of the papers analyzed in this review  
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4 582 utilized an agent (e.g., silver nitrate) that would allow the enzymes to remain inactive during  
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6 583 the test, and thus directly assess the effects only of the germination process on starch properties  
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8 584 (Grassi et al. 2018). Changes in viscosity during heating and cooling of commercial oats before  
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10 585 and after sprouting is reported in **Figure 4**. The test was performed both in distilled water  
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12 586 (Figure 4a) and in AgNO<sub>3</sub> (Figure 4b); the latter was used to inactivate amylases and thus to  
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14 587 get information only on starch changes occurring during sprouting. 48-72 h-sprouting  
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16 588 significantly impacts on the pasting properties, decreasing in peak viscosity, breakdown, final  
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18 589 viscosity and setback. The decrease in the ability of starch granules to swell (i.e., peak viscosity  
19  
20 590 decreased) and retrograde (setback and final viscosity decreased) in sprouted oats - as also  
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22 591 found by Li et al. (2020) and Kaur and Giull (2020) - is probably related to the breaking of the  
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24 592 bonds of starch molecules by enzymes activated during the sprouting process.  
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27  
28 *[Figure 4]*

29 594 Sprouting also affected the sensory properties of oats. Specifically, 72 h-sprouted oats resulted  
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31 595 to be sweeter and more crispy and crunchy (Salmenkallio-Marttila et al. 2004). The sweet note  
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33 596 can be due to the production of sugars upon starch hydrolysis, whereas crispness and  
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35 597 crunchiness might be attributed to the drying conditions (indeed, after sprouting, seeds were  
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37 598 dried for over 19 h between 65 and 93 °C). In addition, a decrease in the bitter and rancid taste  
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39 599 typical of oats was observed and attributed to the inactivation of lipolytic enzymes (Heiniö et  
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41 600 al. 2002). Rancidity in sprouted oats developed more slowly over time than in unsprouted  
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43 601 grains, resulting in an increase in flour stability during 12 months of shelf-life. The stabilization  
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45 602 treatment has a significant impact on the sensory profile of oats after sprouting. As shown by  
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47 603 Heiniö et al. (2001), different combinations of time and temperature during drying generated  
48  
49 604 variable sensory profiles. The most valued sensory attributes in processed oats include a roasted  
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51 605 odor and flavor, a sweet taste, an intense aftertaste, and hard, crisp, brittle texture. Production  
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53 606 of these characteristics requires high temperatures (>85°C) and rapid drying following  
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4 607 germination. Changes in the sensory profile of oat after sprouting for 48 and 72 h at 22°C and  
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6 608 drying at 50°C for 16 h are shown in **Figure 5**. Sprouting caused a decrease in bitter, “uncooked  
7  
8 609 dough” and “oatmeal” attributes. In contrast, salty/umami, acid, grass/green, pulse/chickpea  
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10 610 and fermented attributes increased. Sugar production and increase in acidity after sprouting  
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12 611 could be responsible for the decrease in perceived bitterness, as was seen in quinoa (Suárez-  
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14 612 Estrella et al. 2021).

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17 613 *[Figure 5]*

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19 614 Changes in color were also observed. Specifically, luminosity (L\*) decreased whereas redness  
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21 615 (a\*) and yellowness (b\*) increased when oat was sprouted longer than 24 h at 16 °C; moreover,  
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23 616 the longer the sprouting time, the greater the color changes (Tian et al. 2010). Similar results  
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25 617 were confirmed more recently by Kaur and Gill (2020) and Li et al. (2020) who carried out the  
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27 618 sprouting at 25°C up to 72 h and up to 60 °C, respectively. Such changes were attributed to the  
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29 619 Maillard reaction occurring during drying between simple sugars and lysine derived from starch  
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31 620 and protein hydrolysis (Tian et al. 2010).

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34 621 Despite the positive effect of sprouting on sensory attributes and starch retrogradation (that  
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36 622 decreased upon sprouting), so far, very few studies have developed bakery products from  
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38 623 sprouted oats (Cao et al. 2022; He et al. 2023; Cao et al. 2023; Mäkinen, Zannini, and Arendt  
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40 624 2013). Cao et al. (2022) and Cao et al. (2023) evaluated how wheat dough was affected by  
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42 625 adding 20% of oats that was sprouted at 25°C for different times (24, 48, 72, 96 and 120 h).  
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44 626 Sprouting time affects the technological properties of the wheat-based dough and thus the  
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46 627 qualitative indices of the bread. The right trade-off between improving nutritional properties  
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48 628 and weakening technological properties was found with oats sprouted for 72 h and a substitution  
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50 629 level of 20% (Cao et al. 2022). Compared to unsprouted oat-enriched dough, a reduction in the  
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52 630 amount of water required to reach optimal consistency and also in the development time was  
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54 631 observed when 72 h-sprouted oat was used at 20% level, and it was attributed to the degradation  
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4 632 of soluble fiber upon sprouting (Cao et al. 2023). The ability of dough to withstand mechanical  
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6 633 stress (i.e., dough stability) increased when up to 72-sprouted oats was used; longer sprouting  
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8 634 time (96-120 h) decreased dough stability. Adding oats, and thus fiber, causes a reduction in  
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10 635 stability as an effect of gluten dilution. This is all the more evident, the less the oats are  
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12 636 germinated; while by increasing the degree of germination the effect of  $\beta$ -glucans is less  
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14 637 evident. In addition,  $\beta$ -glucans can form hydrophobic interactions with nonpolar amino acids  
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16 638 of gluten supporting the formation of a protein network. In summary, high  $\beta$ -glucans result in  
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18 639 competition with gluten for water, as well as a dilution effect, while a few, in contrast, may help  
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20 640 bond formation through hydrophobic interactions with some amino acids (Cao et al. 2023).  
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22 641 To better understand the impact of sprouting on dough rheological properties, the gluten  
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24 642 aggregation properties (by GlutoPeak), mixing properties (by Farinograph) and extensibility  
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26 643 (by Alveograph and Extensograph) of wheat dough enriched with unsprouted and sprouted (at  
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28 644 lab scale for 48 h and 72 h) oats at 10, 20, and 30% were evaluated. The related indices, together  
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30 645 with those of dough enriched in commercial sprouted oats, were analyzed by Principal  
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32 646 Component Analysis (PCA) to provide additional discriminatory power. The PCA in **Figure**  
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34 647 **6a** shows the distribution of oat-doughs according to the indices described above. The first two  
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36 648 principal components provided a good summary of the data, accounting for 85% of the total  
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38 649 variance (PC 1 = 56%; PC 2 = 29%). Moreover, the loading plot (**Figure 6b**) distinguishes the  
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40 650 variables affecting sample distributions, which are those more distant from the origin of the  
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42 651 plot. Specifically, the first principal component (PC1) differentiates the samples according to  
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44 652 the sprouting time, whereas the second principal component (PC2) differentiates the samples  
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46 653 based on the enrichment level. The indices that better explain the effect of sprouting time on  
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48 654 dough rheology are related to gluten aggregation properties (peak maximum time, maximum  
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50 655 torque, and aggregation energy), mixing (water absorption, development time, stability and  
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52 656 degree of softening), and extensional (maximum resistance, resistance at 5 cm, energy and ratio)  
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4 657 properties. Along PC2, the level of integration is well described especially by some indices  
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6 658 such as extensibility (measured by both extensograph and alveograph) and ratios (maximum  
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8 659 ratio of extensibility to maximum strength for extensograph and tenacity to extensibility ratio  
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10 660 for alveograph). Mixtures containing commercial sprouted oat rank very close to those  
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12 661 containing oat sprouted for 72 h on a laboratory scale.

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15 662 *[Figure 6]*

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17 663 As regards bakery products, so far bread was the only system studied. Bread produced with  
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19 664 sprouted oats exhibited an overall lower specific volume compared to bread prepared solely  
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21 665 with wheat. However, the inclusion of oats sprouted for 72 h showed a specific volume more  
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23 666 closely approaching that of wheat bread (4.02 vs. 3.78 mg/L for 100% wheat bread and bread  
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25 667 with oats sprouted for 72 h, respectively). Sprouting increased cell density and decreased their  
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27 668 average area, indicating that sprouting promoted the loss of the inner structure of the bread (Cao  
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29 669 et al. 2022). Compared to wheat-only bread, the addition of unsprouted oats decreased the  
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31 670 quality of the bread in terms of hardness, springiness, cohesiveness, and cohesion. However,  
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33 671 sprouting led to an improvement by reducing hardness, chewiness, and gumminess, albeit with  
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35 672 lower values compared to wheat-only bread, with more promising results observed for the 72 h  
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37 673 sprouting period (Cao et al. 2023).

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40 674 He et al. (2023) investigated the characteristics of bread produced with the addition of sprouted  
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42 675 oats under light and dark conditions. A deterioration in the physical characteristics of bread  
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44 676 produced with 10% sprouted oats for 72 h compared to bread made solely with wheat was  
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46 677 observed. The results of specific volume and texture are consistent with those found by Cao et  
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48 678 al. (2022, 2023), showing a substantial worsening. The different light and dark conditions used  
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50 679 for oat germination did not lead to substantial differences in the bread. Furthermore, the bread  
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52 680 after baking has a lighter crumb compared to a non-sprouted control sample. This characteristic  
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4 681 is probably due to the leaching of some pigments during soaking, while no differences in  
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6 682 coloring were observed in the crust.

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8 683 As regards gluten-free formulation, Mäkinen et al. (2013) explored the use of sprouted oats in  
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10 684 gluten-free bread. The study revealed that incorporating sprouted oats could enhance the  
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12 685 volume and texture of gluten-free bread when used at levels below 1%. However, using  
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14 686 amounts above 1% (2%) the crumb structure had large holes. The pivotal factor influencing  
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16 687 altered technological properties was the activity of  $\alpha$ -amylase. Interestingly, proteolytic activity  
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18 688 did not significantly impact the properties of the gluten-free formulation.

#### 19 20 21 22 23 689 **4. Conclusions and Perspectives**

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25 690 This study reviewed 31 papers published in the last two decades that have evaluated the effect  
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27 691 of sprouting on chemical/physical and technological attributes of oat flour. Analysis of the  
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29 692 selected papers revealed a high variability of process conditions in terms of time and  
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31 693 temperature that influence the degree of germination and inevitably the extent of observed  
32  
33 694 changes. Variations in sprouting conditions increase the need for research to standardize the  
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35 695 characteristics of the sprouted grains. This is because the composition and technological  
36  
37 696 properties of sprouted seeds depend on the sprouting conditions: soaking phase, time and  
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39 697 temperature primarily during the germination phase, and the conditions used during the seed  
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41 698 stabilization phase. From the high variability in processing conditions comes a great limitation  
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43 699 in terms of comparing results given the complexity and difficulty in obtaining well-repeatable  
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45 700 and reproducible data across germination studies. Indeed, most of the studies involved  
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47 701 sprouting trials on a laboratory scale, where the process was carried out in thermostatic cells or  
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49 702 under uncontrolled environmental conditions; for example, relative humidity is often not  
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51 703 controlled, and in some cases the temperature is referred to as “room temperature”, without  
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53 704 reporting the value.  
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4 705 Although some more common conditions can be identified (soaking phase: 12 h at 20°C;  
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6 706 sprouting phase: 48 h at 25°C; stabilization phase: freeze-drying or drying for 24 h at 50°C),  
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8 707 some questions remain to be answered including:

- 10 708 ● What is the effect of process conditions on microbiological aspects?  
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12 709 ● Does variety/species of oats or process conditions matter more?  
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14 710 ● What is the role of different hydration kinetics during soaking on seed characteristics  
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16 711 after germination?  
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18 712 ● What is the relationship between relative humidity during the sprouting stage and  
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20 713 chemico-physical changes?  
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22 714 ● What is the relationship between process conditions (including the drying stage) and  
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24 715 sensory characteristics?  
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27 716 Beside that, the combination of sprouting to other processes, for example ultrasound technique,  
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29 717 should be further investigated also in the case of oats. Indeed, the study of Ding et al. (2019)  
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31 718 highlight the positive effects of power ultrasound (25 kHz), with notable enhancements in both  
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33 719 nutritional properties (e.g., increased levels of GABA, avenanthramides, and phenolic  
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35 720 compounds) and microstructural characteristics. Considering that, the effectiveness of  
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37 721 ultrasound treatment during sprouting is influenced by the timing of application and the  
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39 722 duration of exposure, further optimization of these parameters is required to maximize its  
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41 723 benefits in enhancing metabolic and structural changes during sprouting (Ding et al., 2019).  
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44 724 Finally, considering that all the work considered conducted germination on a laboratory scale  
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46 725 and on small batches (between 5 g and 1 kg), it remains to evaluate the scale-up of the process  
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48 726 and its repeatability to enable the production of sprouted oats at large scale and its use for  
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50 727 reformulation of baked goods. Moreover, besides bread, the reformulation of other products  
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52 728 (such as cookies, cakes, snacks) deserve attention. This would allow for the enhancement of a  
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54 729 crop that is nutritionally interesting but still underutilized in the food industry.  
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12 734 acknowledged.

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15 735 **CRedit authorship contribution statement**

16  
17  
18 736 A.S.: methodology, investigation, formal analysis, visualization, writing—original draft  
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20 737 preparation; A.B.: investigation, formal analysis, writing—review and editing; A.M.:  
21  
22 738 conceptualization, supervision, writing—review and editing, founding acquisition. All authors  
23  
24 739 have read and agreed to the published version of the manuscript.

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28 740 **Declaration of Competing Interest**

29  
30  
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32  
33 742 relationships that could have appeared to influence the work reported in this paper.

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16 1022 Yıltrak, S., Kocadağlı, T., Çelik, E. E., Özkaynak Kanmaz, E., and Gökmen, V. 2021. Effects  
17 1023 of Sprouting and Fermentation on Free Asparagine and Reducing Sugars in Wheat, Einkorn,  
18 1024 Oat, Rye, Barley, and Buckwheat and on Acrylamide and 5-Hydroxymethylfurfural Formation  
19 1025 during Heating. *Journal of Agricultural and Food Chemistry*, 69(32), 9419–9433.  
20 1026 <https://doi.org/10.1021/acs.jafc.1c03316>  
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23 1027 Zhou, M. (Charles S. U., Robards, K., Glennie-Holmes, M., and Helliwell, S. 1999. Oat lipids.  
24 1028 *Journal of the American Oil Chemists' Society*, 76(2), 159–169.  
25 1029 <https://doi.org/10.1007/s11746-999-0213-1>  
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31 1030 Ziegler, P. 1995. Carbohydrate degradation during seed development. *Seed Development and*  
32 1031 *Germination*  
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**Table 1.** Raw material and processing conditions used to sprout oat.

Raw material		Pre-treatment	Soaking				Sprouting				Post sprouting	References
Type	Amount		Seed:water (w/w)	Time	Temperature	Light (L)/Darkness (D)	Time	Temperature	Relative humidity	Light (L)/Darkness (D)		
Avena sativa L. (cv Sang) A. sativa L. nuda (cv Bullion)	5 g	Surface-sterilization	1:3	2 h 6 h 10 h 24 h 30 h	Room	-	24 h	-	-	D	-	Bryngelsson et al. (2003)
Hulled oat (cv Veli) Hull-less oat (cv Lisbeth)	-	-	-	-	-	-	72 h	15 °C	-	-	Drying (19 h, 65-93 °C, 5% moisture)	Salmenkallio-Marttila et al. (2004)
Common oat (cv Auron) Naked oat (cv Abel)	-	-	-	3 h	Room	-	96 h 144 h 192 h	20 °C	-	L	-	Gabrovskaa et al. (2004)
Avena sativa L. (cvs Vista, Gem, Dane)	-	1% NaClO 30 s	-	10 h 12 h 14 h	16 °C 20 °C	-	12 h 24 h 48 h 72 h	16 °C 20 °C	-	-	Freeze-drying	Skoglund et al. (2008)
Oat	-	-	-	6 h	-	-	48 h 62 h	10 °C 12 °C	-	-	Drying	Hübner et al. (2009)

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							96 h 130 h 144 h	15 °C 18 °C 20 °C			(8h, 45 °C + 15 h, 55 °C)	
Avena nuda L. cv Baiyan II	500 g	1% NaClO 30 s	1:2	8 h 16 h 24 h	20 °C	-	12 h 24 h 36 h 48 h	16 °C	95%	-	Freeze-drying	Xu et al. (2009)
-	-	-	-	3h (wet and dry phases; 7 cycles)	-	-	48 h 62 h 96 h 130 h 144 h	10 °C 12 °C, 15 °C 18 °C 20 °C	-	-	Drying (8h, 45 °C + 15 h, 55 °C)	Hübner et al. (2010)
Avena nuda L.	-	2% NaClO 15 min	-	24 h	16 °C	D	24 h 48 h 72 h 96 h 124 h 144 h	16 °C	-	D	Drying (24 h, 50 °C)	Tian et al. (2010)
Avena nuda L. (cv Bayou I and Baiyan II)	200 g	1% NaClO 30 s	1:5	2 h 4 h 7 h	20 °C	-	7 h 13 h 25 h 37 h 48 h 60 h 72 h	20 °C	98%	-	Freeze-drying	Xu et al. (2010)
Avena sativa L.	-	-	-	72 h	Room	-	96 h	-	-	-	Drying (24 h, 60 °C)	Kim et al. (2013)

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Oat (cv Lutz)	-	-	-	-	-	-	120 h	15 °C	-	-	Drying (35 °C + 50 °C + 60 °C)	Mäkinen et al. (2013)
Oat (cv Lovrin 1, Jeremy)	-	-	-	-	-	-	-	-	-	-	Drying	Panfil et al. (2014)
Avena sativa L.	50 g	0.1% NaClO 30 min	-	24 h	28 - 30 °C	-	120 h	25 °C	90%	-	Freeze- drying (10% moisture )	Khang et al. (2016)
Oat (cv Choyang)	200 g	1% NaClO 30 min	1:5	8 h	25°C	D	24h 60 h	25°C	95%	-	Drying (50 °C, < 10% moisture )	Li et al. (2017)
Avena sativa L. nuda (cv Golozrni)	40 g	5% H <sub>2</sub> O <sub>2</sub>	-	6h	-	D	168 h	21 +/- 1°C during 18 h, and dark modes at 17 +/- 1°C for 6 h.	-	D L	Freeze- drying	Aborus et al. (2018)
Avena sativa L.	150 g	Washing (70% ethanol) + 1.4% NaClO 20 min	-	20 h	20 °C	-	168 h	25 °C the first 72h of sprouting; 30°C the last 96 h of spouting	>70%	D	Freezing	de Bruijn et al. (2019)
Huskless oat (cv Gehl)	500 g	Washing (tap water) 30 min	-	4.5 h + 19 h rest +	20 °C	-	24 h 48 h 72 h	10 °C 14 °C 20 °C	-	D	Freeze- drying	Krapf et al. (2019)

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				4 h				25 °C 30 °C			(60 h, 3-8% moisture )	
Avena sativa L. (cv. Daeyang)	-	-	-	-	-	-	24 h 48 h	-	-	-	Freeze-drying	Lee et al. (2019)
Hulled oat (cv Barra) Dehulled (cv Meeri)	20 g	-	1:6	4 h	20 °C	-	24 h 60 h 96 h 156 h 216 h	12 °C 14 °C 16 °C 18 °C 20 °C	-	D	Freeze-drying	Aparicio-García et al. (2020)
Avena sativa L. (cv Turquesa)	100 g	1.5% NaClO 30 min, 30 °C	1:6	12 h	-	-	120 h	25 °C	60%	D	Drying (12 h, 50 °C)	Damazo-Lima et al. (2020)
Naked oat	-	-	-	8 h	-	-	40-50 h	16-18 °C			Drying	Dlouha (2020)
Avena sativa (cv OL-9)	-	-	-	12 h	25 °C	-	24 h 48 h 72 h	25 °C	-	-	Drying (45 °C, 8% moisture )	Kaur & Gill (2020)
Oat (cv Choyang)	200 g	1% NaClO 30 min	1:5	8 h	25 °C	D	24 h 60 h	25 °C	95%	-	Drying (50 °C, < 10% moisture )	Li et al. (2020)
Oat	200 g	1% NaClO 15 min	1:2	12 h	11 °C 14 °C 17 °C	-	48 h	11 °C 15 °C 19 °C	95%	-	Freezing	Tang et al. (2020)

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					19 °C 21 °C			23 °C 27 °C				
Dehulled oat (cv Meeri)	20 g	0.1% NaClO 30 min	1:6	4 h	Room	-	96 h	18 °C	≥90%	D	Freeze- drying	Aparicio- García, et al. (2021)
Hulless oat (cv Paul Streaker, AC Gehl, Sun Organic) In- hull oat (cv Leggett, Beach, Rockford, NDSU 901, Betagene, Souris, GMI 423, Horsepowe, Morgan, Deon)	100 g	2% NaClO	1:2	6-8 h (hulled oats) 3-5 h (hulless oats)	22 °C	-	72 h 96 h 120 h	16 °C	98%	-	Freeze- drying	Watt et al. (2021)
Oat	1000 g	2% NaClO 10 min	1:2	7 h	20 °C	-	24 h 48 h 60 h 72 h 84 h 96 h	20 °C	95%	D	Freeze- drying	Yıltrık. et al. (2021)
Avena sativa L.	-	0.5% NaClO 30 min	1:6	4 h	Room	-	120 h	21 °C	>90%	D	Freeze- drying	Jiménez- Pulido et al. (2022)

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(cv Chimene) -dehulled grain													
Oat	-	2% NaClO 10 min + 0.1% H <sub>2</sub> O <sub>2</sub> 10 min	-	12 h	-	-	24 h 48 h 72 h 96 h 120 h	25 °C	-	-	Freeze- drying	Cao et al. (2022)	
Oat	200 g	2% NaClO 30 s		24 h	20 °C	-	72 h	20 °C	95%	D L	Freeze- drying	He et al. (2023)	
Oat	20 g	2% NaClO 10 min + 0.1% H <sub>2</sub> O <sub>2</sub> 10 min	-	12 h	-	-	24 h 48 h 72 h 96 h 120 h	25 °C	-	-	Freeze- drying	Cao et al. (2023)	

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**Table 2.** Effect of sprouting (72 h at 22 °C) on the microbial contamination in oats.

	Unsprouted oat	Sprouted oat
Total bacteria count (cfu/g)	3.8 * 10 <sup>5</sup>	> 3 * 10 <sup>6</sup>
Coliforms (cfu/g)	-	-
E. Coli (cfu/g)	-	-
Molds (cfu/g)	1700	< 100
Yeasts (cfu/g)	4200	15000
Salmonella (cfu/ 25g)	-	-
S. Aureus (cfu/g)	-	-
Lactic bacteria (cfu/g)	2300	360000

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**Table 3.** Effects of sprouting on the main components of oats

Component	Modification	Mechanism
Starch	↓	Hydrolysis by the increased amylase activity with sugar production
Protein	↑	(i) Decrease in starch content (ii) Synthesis of new proteins
Fiber	↓	Use of proteins by the plant to grow
	↑	Decrease in starch content
	↓	Degradation of cell wall material by increased cellulase and other enzymatic activities
Soluble fraction	↓	(i) Degradation of $\beta$ -glucans by the increased $\beta$ -glucanase activity
	↔	Compensation between decrease in $\beta$ -glucans and formation of smaller fragments of oligosaccharides
Lipids	↑	Remobilization
	↓	Hydrolysis by the increased lipase activity with production of saturated and monounsaturated fatty acids
Minerals	↑	(i) Migration from soaked water (ii) Hydrolysis of phytic acid by the increased phytase activity
	↓	Leaching into soaking water Remove of radicle
Vitamins	↑	De novo synthesis
	↓	Leaching into soaking water Remove of radicle
GABA	↑	Partial hydrolysis of proteins and increased availability of free amino acids and glutamate decarboxylase activity
Phenolic compounds	↑	(i) Degradation of cell wall material by increased cellulase and other enzymatic activities (ii) Cleavage of intermolecular bonds and the breakdown of protein structures (iii) Synthesis of new molecules

1037 ↑ = increase; ↓ = decrease

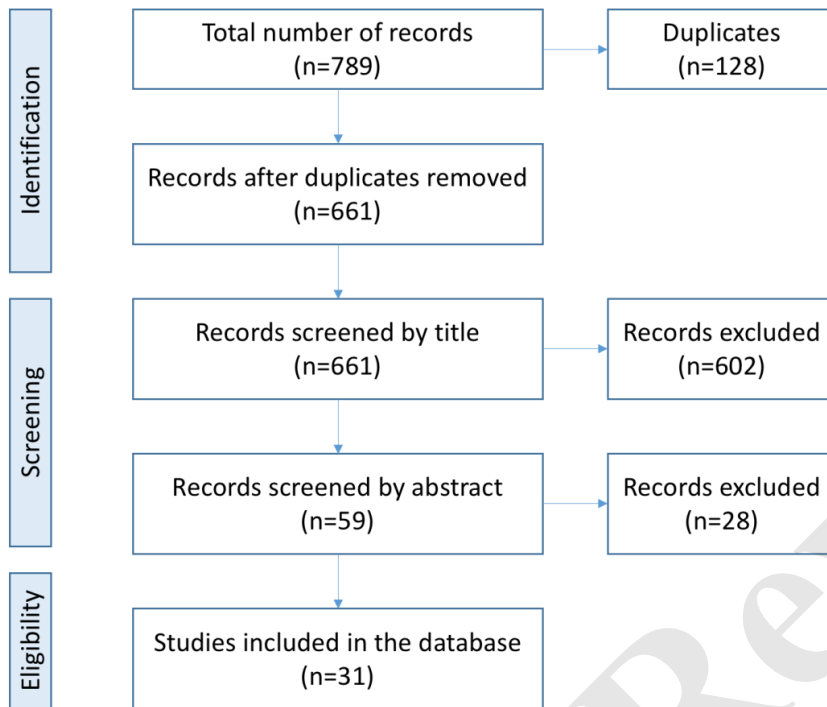
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1039 **Table 4.** Effect of sprouting (67-72 h at 20-25 °C) on specific vitamins and minerals content  
1040 in oats (*Avena nuda* L.)

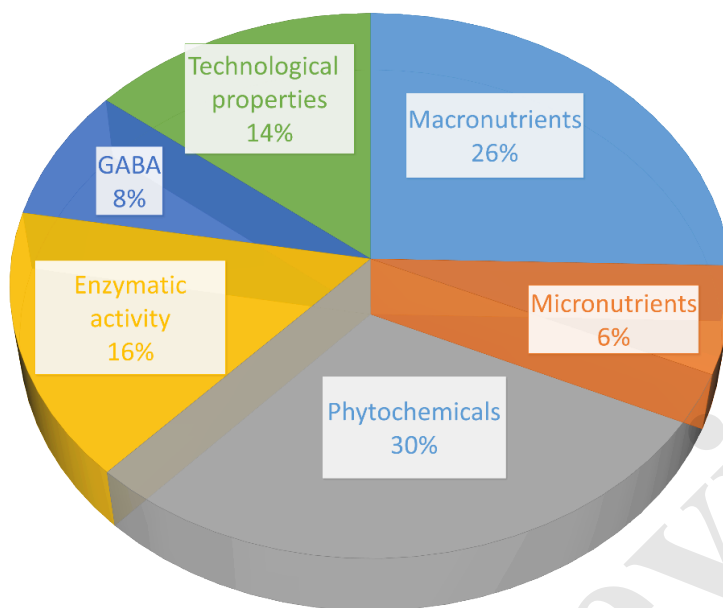
	Unsprouted oat	Sprouted oat
Vitamin B2	1.2 ± 0.1 mg/100g	4.1 ± 0.1 mg/100
Vitamin B5	0.86 ± 0.33 mg/100g	2.1 ± 0.80 mg/100g
Vitamin B9	20.1 ± 4.5 µg/100g	37.2 ± 8.5 µg/100g
Calcium	312 ± 42 mg/kg	390 ± 51 mg/kg
Zinc	30.9 ± 5.9 mg/kg	16.2 ± 3.4 mg/kg
Magnesium	579 ± 71 mg/kg	721 ± 8 mg/kg
Copper	3.1 ± 0.8 mg/kg	3.3 ± 0.9 mg/kg

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**Figure 1.** Flowchart of the study design of systematic review.

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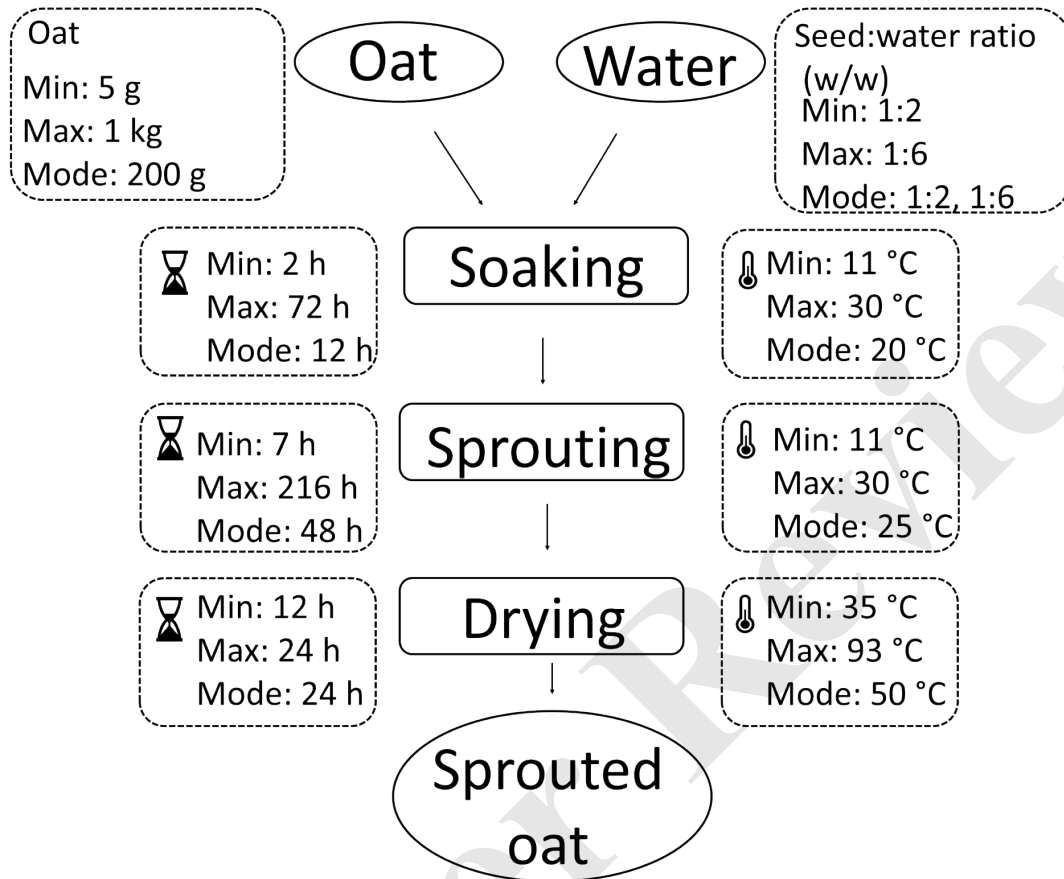


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1081 **Figure 2.** Main attributes investigated in oat before and after sprouting.

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1085 **Figure 3.** Flow-sheet of sprouting and elaboration of the main processing conditions reported  
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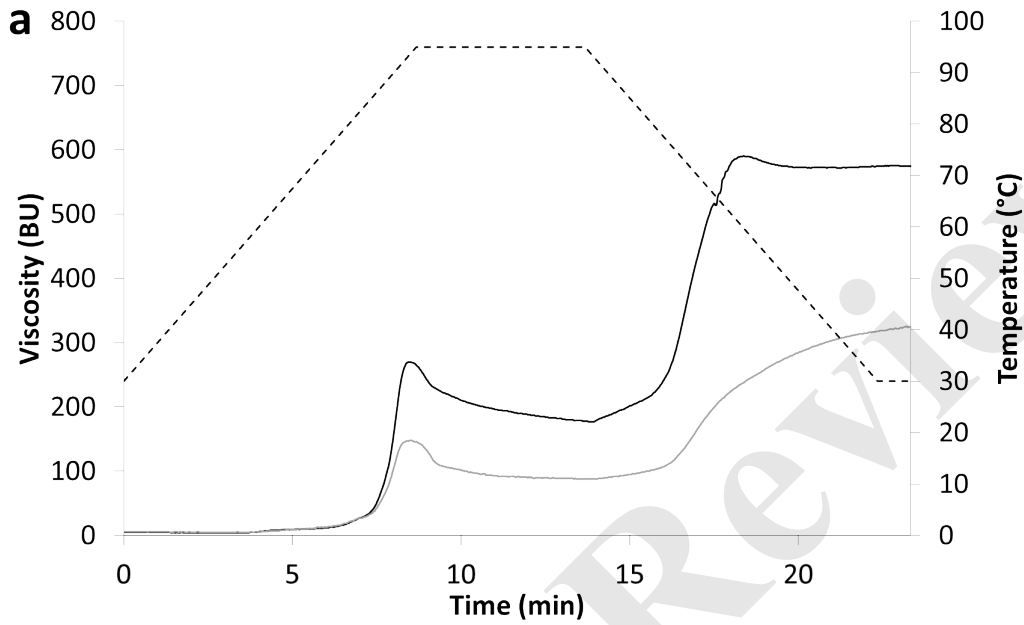
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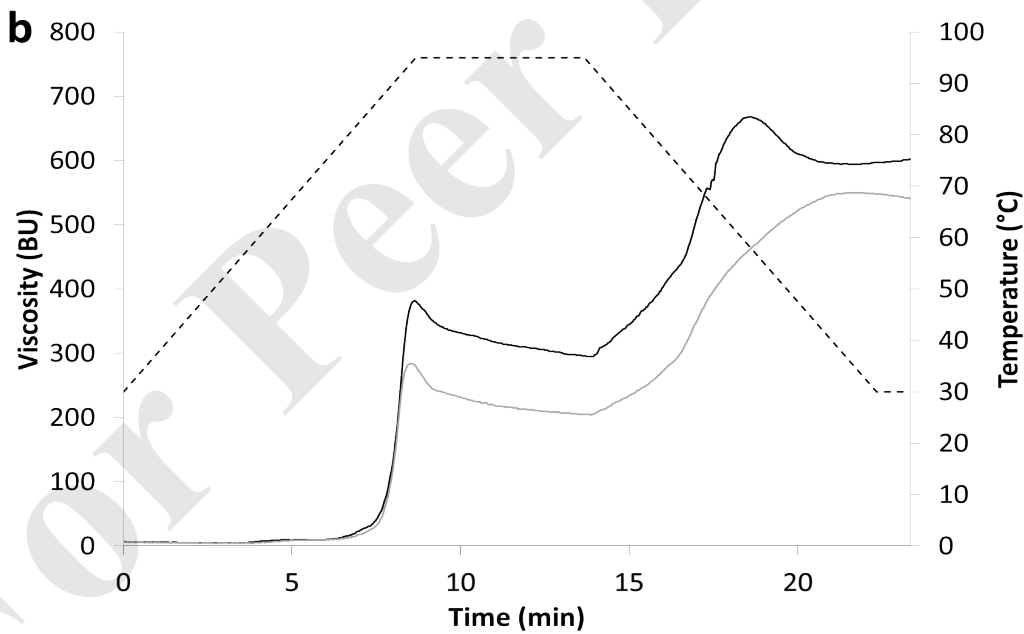
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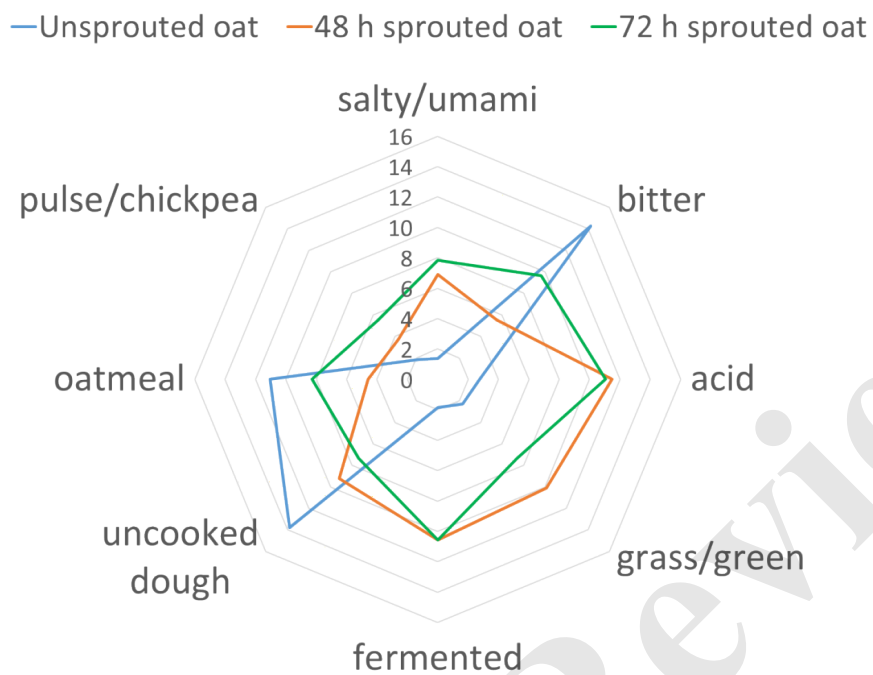
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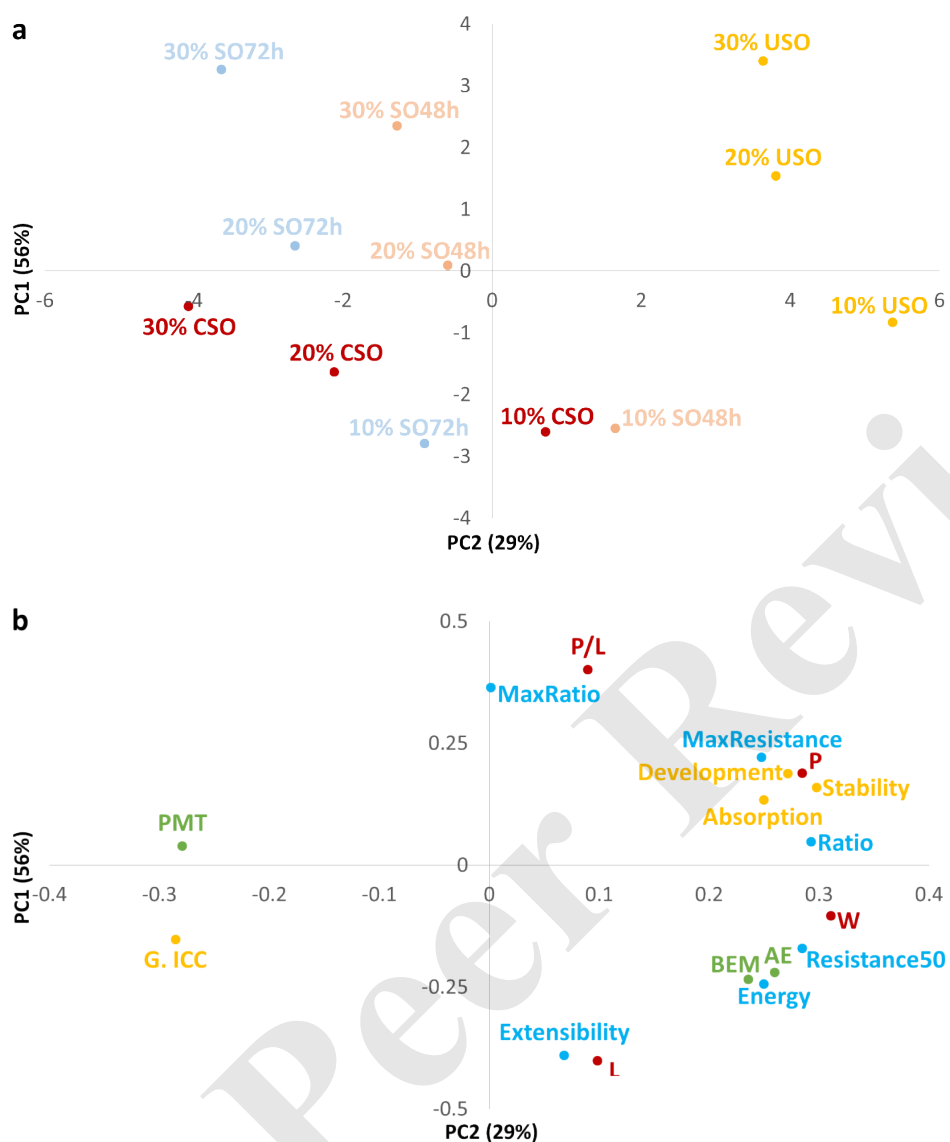
1102 **Figure 4.** Pasting profile of flours from commercial unsprouted and sprouted oat for 48-72 h  
1103 by using H<sub>2</sub>O (a) and AgNO<sub>3</sub> (b) as solvent. Unsprouted oat: black line; sprouted oat for 48-  
1104 72 h: gray line

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**Figure 5.** Sensory profile of oat before and after sprouting (48 and 72 h, followed by drying at 50°C for 16 h).



**Figure 6.** Distribution of the mixtures of wheat flour with 10, 20 and 30% of unsprouted oat (USO), sprouted (SO) on laboratory scale for 48 and 72 h and commercial sprouted oat (CSO) (loadings plot, Figure 6a) for PC1-PC2, generated from a correlation-matrix PCA. Score plot of the samples on PC1-PC2, generated from a correlation-matrix PCA (Figure 6b). Gluten aggregation properties were evaluated by GlutoPeak, indices in green (PMT: peak maximum time; BEM: maximum torque and AE: aggregation energy), mixing properties by Farinograph, indices in yellow (WA: water absorption; DT: development time; STB: stability and G. ICC: degree of softening), extensibility properties by Alveograph, indices in red (P: tenacity; L: extensibility; W: strength and P/L: tenacity/extensibility ratio) and by Extensograph, indices in light blue (ENR: energy; MRST: max resistance; RST50: resistance at 50 mm; EXT: extensibility; RT: resistance/extensibility ratio and MRT: max resistance/extensibility ratio).

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4 1128 **Figure 1.** Flowchart of the study design of systematic review.  
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6 1129 **Figure 2.** Main attributes investigated in oat before and after sprouting.  
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8 1130 **Figure 3.** Flow-sheet of sprouting and elaboration of the main processing conditions reported  
9 1131 in the selected studies.  
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11 1132 **Figure 4.** Pasting profile of commercial flours from unsprouted and sprouted oat for 48-72 h  
12 1133 by using H<sub>2</sub>O (a) and AgNO<sub>3</sub> (b) as solvent. Unsprouted oat: black line; sprouted oat for 48-  
13 1134 72 h: gray line  
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15 1135 **Figure 5.** Sensory profile of oat before and after sprouting (48 and 72 h, followed by drying  
16 1136 at 50°C for 16 h).  
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18 1137 **Figure 6.** Distribution of the mixtures of wheat flour with 10, 20 and 30% of unsprouted oat  
19 1138 (USO), sprouted (SO) on laboratory scale for 48 and 72 h and commercial sprouted oat (CSO)  
20 1139 (loadings plot, Figure 6a) for PC1-PC2, generated from a correlation-matrix PCA. Score plot  
21 1140 of the samples on PC1-PC2, generated from a correlation-matrix PCA (Figure 6b). Gluten  
22 1141 aggregation properties were evaluated by GlutoPeak, indices in green (PMT: peak maximum  
23 1142 time; BEM: maximum torque and AE: aggregation energy), mixing properties by Farinograph,  
24 1143 indices in yellow (WA: water absorption; DT: development time; STB: stability and G. ICC:  
25 1144 degree of softening), extensibility properties by Alveograph, indices in red (P: tenacity; L:  
26 1145 extensibility; W: strength and P/L: tenacity/extensibility ratio) and by Extensograph, indices in  
27 1146 light blue (ENR: energy; MRST: max resistance; RST50: resistance at 50 mm; EXT:  
28 1147 extensibility; RT: resistance/extensibility ratio and MRT: max resistance/extensibility ratio).  
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4 Dear Reviewer,  
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6 As regard your comment (*"The author refused to do the necessary revision based on the suggestions. For example, the author said that this review focus on the sprouting conditions, but in the abstract the author said "This review examined 31 papers from the last two decades on the impact of sprouting and processing conditions on oat chemical, physical, and technological attributes." So the processing conditions are included in this review and the pre-treatment method, like ultrasound, soaking in salt belong to the processing methods. Therefore, this review is not comprehensive. In L259-275, the author talked about the decontaminate methods, but in the response letter, the author said there are no studies that compared different decontamination methods applied during oat sprouting. Although the research did not do the comparison, as a review, the author should make your own judgment based on previous research and discuss the possible pros and cons of these research*), the sentence has been changed as follows:  
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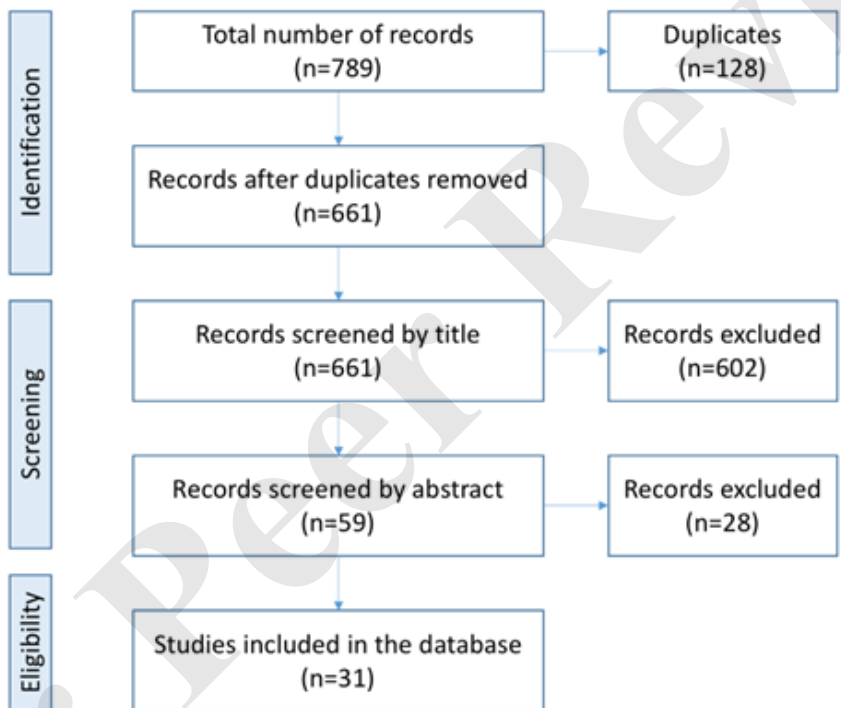
21 *"There are no studies that compared different decontamination methods applied during oat sprouting. Moreover, none of the studies that used sodium hypochlorite during soaking did not report data about the microbial growth before and after treatment with sodium hypochlorite. However, the positive effect of sodium hypochlorite on microbial growth is widely recognized (Mir et al., 2021). While selecting the method for limiting the microbial growth during sprouting, several aspects should be considered, including its effectiveness, country regulations, and costs."*  
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31 We hope we clarified the point that remained unclear from the previous revision process.  
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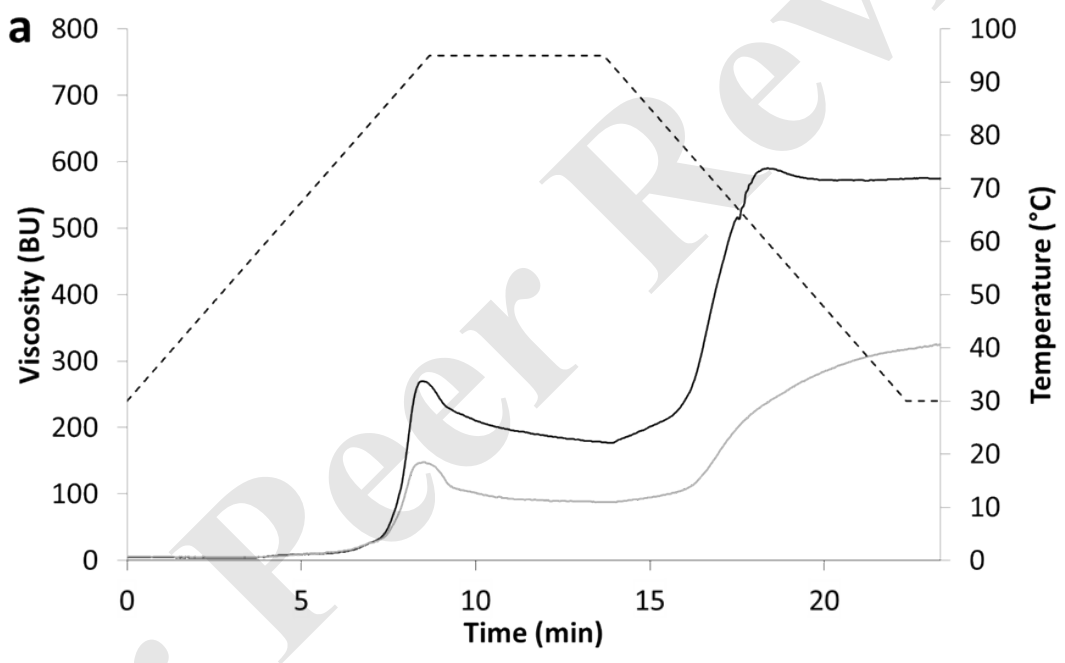
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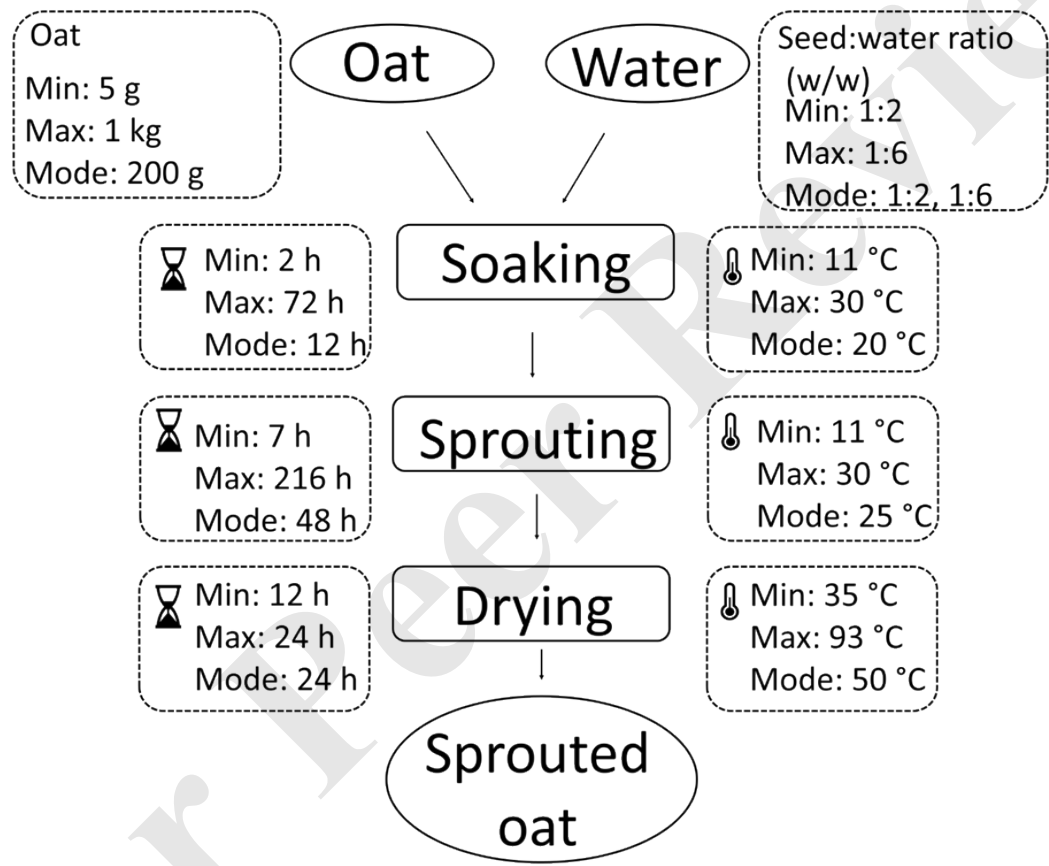
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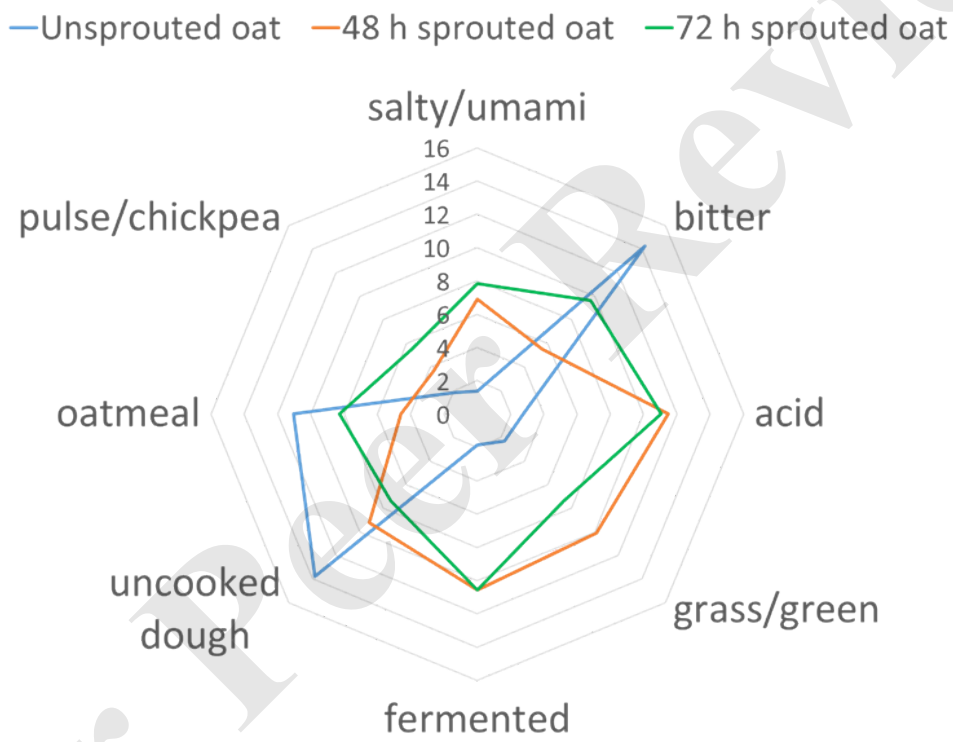
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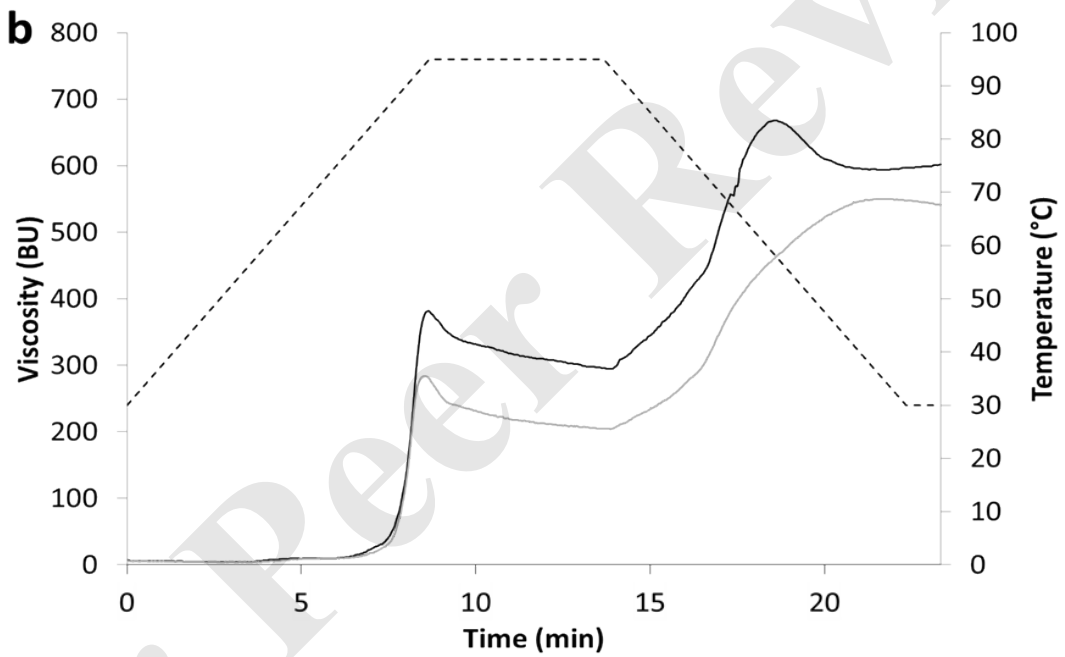
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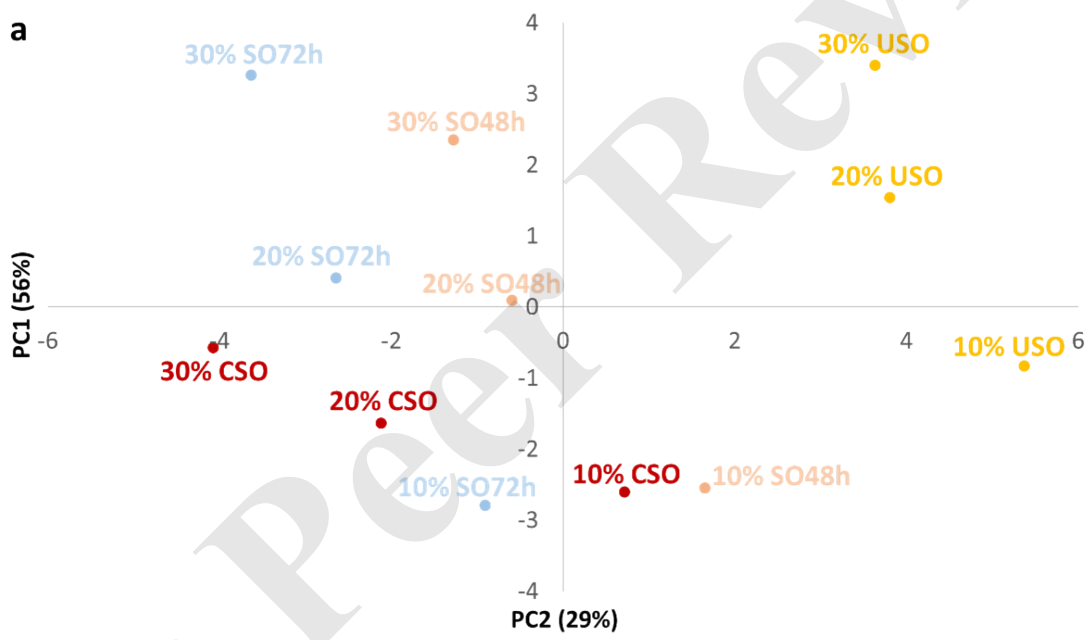
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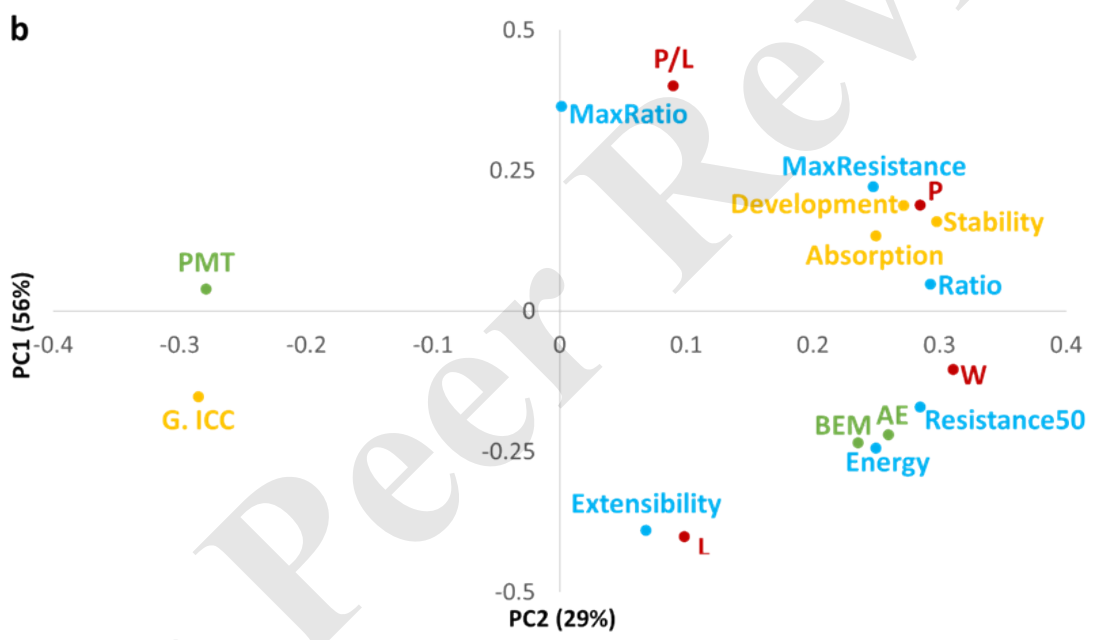
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