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Sprouted grains in product development. Case studies of sprouted wheat for baking flours and fermented beverages.

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Sprouted grains in product development. Case studies of sprouted wheat for baking flours and fermented beverages.

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1. Introduction

Nowadays, the food sector is oriented to develop healthier products closer to new consumer needs. In recent years, sprouted grains have appeared in the market as a new ingredient for the food industry. The increased nutrient value, the lower quantity of antinutrients, the better source of bioactive molecules and the sweeter taste than in no sprouted grains make them a potential novel ingredient for the actual food market (Ding & Feng, 2019).

In the last years, certain indicators have been observed for sprouted grains as a possible future trend in the food industry. One of them is the number of scientific papers published in the food field (Benincasa et al., 2019; Wu & Xu, 2019; Finnie et al., 2019; Pagand et al., 2017). Recently, a book called Sprouted Grains: Nutritional Value, Production and Applications was launched by Elsevier (Feng et al., 2018) pointing out the properties and possibilities of these ingredients.

On the other hand, according to an analysis carried out by the trend agency Mintel, the launch of products with sprouted grains has increased exponentially since 2006, for all food categories (Pagand et al., 2017). The largest change occurred between 2012 and 2016, observing an increase of 26%. This trend has been mainly reflected in the American, European, and Australian markets with brands like Food for Life Baking Co, Silver Hills Sprouted Bakery, Angelic Bakehouse and Spring Whole foods in the bakery category.

According to Pagand et al., 2017 most of the products launched with sprouted ingredients in the last years have been snacks (22%), followed by flours (19%) and bakery products (15%). For the beverage category, no big brands have been found in Europe while countries in Asia like India seems to have products positioned for the drinking category with Heinz Power Sprouts®, Sprouvita® and Prana Sprouted Malt Drink®.

Currently, there is no a clear definition of sprouted grains yet. In 2008, the American Association of Cereal Chemists (AACC) proposed a definition for sprouted grains***: "Malted or sprouted grains containing all of the original bran, germ and endosperm shall be considered whole grains as long as sprout growth does not exceed kernel length, and nutrient values are not diminished*****"** (Oldways Whole Grains Council, n.d.).

Actually, germination is a complex process, run by different phytohormones and enzymes, during the seed must pass from a dormant phase (I), where it starts to absorb water to a wake-up phase (II) where all the metabolic processes start, to be able to develop the radicle (III) (Nonogaki et al., 2010). This enzymatic activity is key to understand the nutritional, technological and sensorial properties of sprouted grains (Nelson et al., 2013) and the reason to study its potential interest in the food industry to create new products.

This process is crucial for the final quality of sprouted grains, and consequently the quality of the final food products containing this ingredient. One of the most important factors involved is the microbiological contamination that can be attributable to many potential pre-harvest and post-harvest sources of contaminants. All the employed strategies to reduce health risks associated include physical, biological, and chemical applications (Benincasa et al., 2019; Ding et al., 2018).

During germination, the enzymes involved, such as amylases or invertases, have a very important role since they break down the starch and sucrose into mono and disaccharides, thus, this process allows creating products with a lower content of added sugars without reducing the perception of sweetness (Desai et al., 2010). On the other hand, it has been observed that this higher concentration of simple sugars, free amino acids and peptides can act as flavour precursors through the aromatic compounds generated in the Maillard reactions, improving sensory perception compared to ungerminated products (Pagand et al., 2017). It is important to note that the drying and cooking process of the grains also directly influences the organoleptic characteristics and the quality of the final products. Hence, different times and temperatures of processing would influence the flavour and aroma of the final products. (Finnie et al., 2019). Fermentation is also a common technique for cereal and pseudo-cereals processing, to better preserve or promote nutritionally interesting compounds (Benincasa et al., 2019; Marti et al., 2018).

The greater capacity of water absorption by sprouted grain flours is a very important variable in the production of doughs and especially bread. Besides, this absorption is determined by other factors, which differ greatly depending on the manufacturer, such as particle size, starch breakdown, gluten content, enzyme activity, proteins, and the germination process itself (Finnie et al., 2019).

The present study is focused on bakery and fermented beverages. Two applications of sprouted wheat have been studied: the effect of wheat germination time in the preparation of new flours with applications in bakery that was not deeply studied in the scientific literature; and the evaluation of safety production of rejuvelac, a sprouted wheat-based popular fermented drink that have not been studied yet by science.

The use of sprouted grains as a new ingredient in baking flours is an important research topic in the bakery industry. The study aimed to identify how wheat germination time (12 and 24 h.) affects the quality of sprouted wheat flour, as well as its mixed use with other commercial strength flour, in relation to hardness changes in the breads produced. The research also aimed to know how strength flour with sprouted flour behaves during all the phases in the bakery process.

Also, nutritional functional beverages obtained by lactic acid fermentation of mixture based on sprouted grains and flours represent a possible future perspective. In line with this, one of the most commonly used sprouted-grain-based fermented beverages is rejuvelac. This drink is usually made of germinated quinoa or wheat grains, introducing them in water and anaerobically fermented at room temperature (Wigmore, 1986). It is used as a starter in the production of vegan cheeses as it is supposed to provide the lactic acid bacteria needed for the fermentation in the food matrix (Chen et al., n.d.). However, it has not yet been studied the rejuvelac food safety in the scientific literature. After observing the procedure of its development, under no microbiological control (temperature, pH, salt concentration, etc.) and the published report by EFSA on November 15, 2015, expressing the health risk of sprouted seeds, it is clearly thought that products made by rejuvelac (juices, cheeses, rejuvelac itself, etc.) may pose a risk to public health. On the other hand, the properties and applications of rejuvelac for food preparation have not been deeply studied.

Likewise, the use of sprouted grains as a new ingredient for preparing functional beverages could gain relevance in the near future. Some studies have been done on the probiotic drink made of sprouted grains (Chavan et al., 2018; Mridula & Sharma, 2015). For this reason and because there is still a lot to investigate in relation to rejuvelac -one of the most widely used sprouted-grain-based fermented drinks- the aim of this study is to verify the possible growth of pathogens during its producing as well as to propose a safety methodology for its preparation at home or in the gastronomy field.

2. Material and Methods

2.1 Materials and wheat germination requirements.

The raw materials for this study were procured from Basque Culinary Center, San Sebastian, Spain. Organic wheat grains (*Triticum aestivum)* were supplied from Biogran, S.L. Company in January 2019. Compositions of the wheat were: 1.8g fats, 59.9g carbohydrates, 13.3 g dietary fiber, 10.9 proteins. Sprouting process was performed according to Baranzelli et al., 2018 with some modifications and parameters described by Hung et al., 2011. Wheat grains were immersed for 15 minutes in a Hypochlorite solution 1% (v/v) to eliminate possible microbiological contamination and after washed a minimum of five times with water until a pH 7.0. Later, grains were soaked in water at 26 ± 2 °C for 6 h. The samples were then placed in germination trays that were placed in a controlled growth room, germination was performed at 20 ± 2 °C, and relative humidity of 80 ± 3%. The sprouted wheat was then removed from the germination room at selected germinating times from 12 and 24 h.

2.2 Drying and milling of grains – Making flours.

Wheat grains were dried and ground according to the methodologies of Baranzelli et al., 2018 and Hung et al., 2011 with some modifications. The wheat was dried in a Siemens controlled air circulation oven for 7 hours at a temperature of 50 ºC until reaching 10–12% moisture. Measurements were made using a Thermo Scale (COBOS, 330MX series, Switzerland). Then grains were milled in a flour mill (KOMO, Fidibus 21, Germany). With both flours obtained from germination after 12 and 24 hours and after a milling process, mixtures of germinated flour (GF) and strength flour (SF) were made in the proportions (GF/SF) of 10/90, 20/80, 30/70, 40/60 and 50/50, respectively.

2.3 Mixolab™ mixing properties of the flours.

Mixolab™ (Chopin) test with 75 g of the flour samples was performed in duplicate for each flour to assess the dough mixing properties and thermomechanical characteristics. The flours selected for analysis were 100%, and 50/50 % GF/SF for 12 and 24 h germination time using the Mixolab™ analyzer (Chopin Technologies, Villeneuve-La-Garenne, France). Wheat flour without the addition of germinated flour was used as a standard sample **(S0)**. The premixes selected for the Mixolab™ were performed according to the germination time and mixes flours of GF/SF for a total of 5 samples to analyse at Mixolab™ (**S0** = SF / **S12.50** = GF 12h seeds germination and 50/50 GF/SF / **S12.100** = 12h seed germination and 100% GF / **S24.50** = GF 24h seed germination and 50/50 GF/SF / **S24.100**= 24h seed germination and 100% GF). The

AACC International Method 54-60.01 with the Chopin wheat protocol was used (Ding et al., 2018). The moisture of the sample was determined first. The parameters obtained from the Mixolab™ included water absorption, dough development time, protein weakening, starch gelatinization, the stability of the hot-formed gel, and starch retrogradation.

2.4 Bread-making

Breads were made according to the following mixtures of flour: 10/90, 20/80, 30/70, 40/60 and 50/50 of GF/SF, for a total of 10 different blends. Five samples from germination after 12h, five samples from germination after 24 h as well as a sample with 100% SF and samples with 100% GF (12 and 24 h germination time) were included to establish comparisons. Similar breads were made in the previous studies of Marti et al., 2017 and similar blends of sprouted wheat flour were also used to study characteristics of tortillas (Liu et al., 2017). The formula of the breads consisted of wheat flour (500g), refined salt (10g), yeast (10g) and water (350g) at 4 °C. The dough was fermented in a resting cool chamber for 24 h at 5 °C and 80% of RH. The baking process was carried out in an electric conventional oven (Siemens, Germany) at 230 °C for 25 min. Figure 1 shows the breads with 12 h germinated wheat flour. The bread was evaluated following a hardness texture test.

"Insert Figure 1 here"

2.5 Texture analysis of breads

The hardness texture test was performed using the TA XTPlus Texture Analyzer (Stable Micro Systems, Godalming, UK) equipped with a P/36 R diameter acrylic cylindrical probe. Steamed bread was sliced horizontally and a flat piece of 25mm thickness was compressed to 50% of its original height. The conditions for the test were: pre-test speed, 2 mm/s; test speed, 1mm/s; post-test speed, 1 mm/s; trigger force 5 g. The measure was made by triplicate for every sample and results are presented as mean ± SEM. A linearity analysis was made to determine the blend in which linearity is lost.

2.6 Preparation of rejuvelac and second fermentation

Homemade rejuvelac (R) was obtained according to the popular method (Wigmore, 1986). In this case, sprouted wheat grains obtained according to the germination method described below, were immersed in hypochlorite solution (1% v/v), then washed and covered with sterile water (20 % w/v) and fermented in sterilized bottles for 24 hours at 20ºC. Sterilized rejuvelac (SR) was obtained from sterilizing R sample in a pressure cooker at 115 ºC for 15 min. This sample was then inoculated with 1% (m/v) of Lactobacillus acidophilus (Perez & Tan, 2005) and fermented again for 24 h at room temperature (25ºC.). This sample will be referred as Inoculated rejuvelac (IR) during the study. A flow chart of the process is shown in Figure 2. The purpose of performing these two treatments, Sterilization and Fermentation, was to improve the safety of Rejuvelac production and to increase its functionality when adding Lactobacillus acidophilus. Given that sprouted-seeds have very particular organoleptic characteristics and that these treatments can impact the sensorial properties, a sensory evaluation was carried out as well.

"Insert Figure 2 here"

2.7 Microbiological analysis of rejuvelac

For microbiological analysis, the rejuvelac samples (R, SR and IR) were diluted in sterile peptone water and analyzed in several mediums according to the respective ISO standard (ISO 11290-1). Samples were cultured in triplicates in Chromogenic Media Salmonella (CMS, ITW Reagents), Eosin Methylene Blue Agar (EMB, ITW Reagents) deMan Rogosa Sharpe (MRS, VWR Chemicals), Baird Parker Agar (BP, VWR Chemicals) and Listeria Chromogenic Agar (LC, ITW Reagents) (ISO 11290-1), and incubated at 37ºC for 24 h. Lactobacillus colony count was done in MRS agar and incubated at 37ºC for 48h, according to Süle et al., 2014 method. All the data was treated in IBM SPSS Statistics®. Results were expressed as mean ± SD. Means of two independent samples were compared by a Student's T-test analysis. P-value < 0.05 was considered significant.

2.8 Sensory evaluation of rejuvelac

The acceptance level of sensory characteristics was tested in 75 consumers according to the UNE-ISO 8587:2010. 100 ml of each beverage sample (IR and SR) was presented in plastic glasses with three digits random number (Chavan et al., 2018). Each consumer evaluated the samples by rating them using a nine-point scale, where 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely. The measured attributes were appearance, odour, colour, mouthfeel and preference. Three descriptors were asked for each beverage and a voluntary comment section was included in the survey in order to obtain some qualitative information (Adelekan et al., 2013). A word cloud was used to synthesize and show the information regarding the descriptors. Word clouds are a way to show qualitative data in sensory analysis, using the size of each word to indicate the frequency count of words written by the consumer panel. The larger a word is in the cloud, the more frequent was in the sensory evaluation. This word cloud was constructed using an online tool called WordArt in which the word cloud feature takes as input a vector of terms and a term frequency matrix. In addition, and to support the value of qualitative data provided by our consumer panel, comments quoted literally are shown in Table 3 to reflect some general impressions and ideas of the products tested.

3. Results and discussion

3.1 Dough mixing properties and thermomechanical characteristics.

Figure 3 displays the Mixolab™ curves for two samples showing the different phases during the analyses. The green curve indicates the torque recorded by the sensor in Nm, the red curve denotes the mixer temperature in °C, the pink curve denotes the dough temperature in °C, and the purple horizontal line shows the target consistency (1.1 Nm) that requires to be achieved during the hydration process. The typical Mixolab™ chart, Phase 1 (p1) is for the initial mixing, where the parameters include the percentage of water required for the dough to produce a torque within the target range 1.1 ± 0.07 Nm (water absorption, %), maximum torque during p1 (C1, Nm), the time to reach the C1 at 30 °C (T1, dough development time, min), and the mixing time that the torque stayed within the target range (stability, min). Phase 2 (p2) represents protein weakening, with a minimum torque at 30–60 °C (C2, Nm). Phase 3 (p3) features starch gelatinization, with minimum consistency at 60–90 °C (C3, Nm). Phase 4 (p4) highlights the stability of the hot formed gel, with minimum consistency at 90 °C (C4, Nm). Lastly, phase 5 (p5) shows starch retrogradation during the cooling phase from 90 °C to 50 °C (C5, Nm).

"Insert Figure 3 here"

In Figure 3, the differences between **S0** and **S24.50** samples are shown. The principal difference is found in *p1* related to dough development time and stability. For the mixture of S24.50, the dough needs more time to get stability (the elapsed time that the torque was kept at 1.1 Nm), probably due to the difficulties produced during the germination on proteins. This also influences the hydration and development of the gluten network. Similar results were obtained for S12.50, S12.100, S24.100, blends in which wheat flour was partially or totally substituted by sprouted wheat flour. Previous studies showed similar outcomes using sprouted wheat flour mixes as a substitute for wheat flour (Liu et al., 2017). The C2 value is higher in S0 during p2, because of heating breaks the peptide links of the protein; a higher C2 means gluten is less weakened, so more stable gluten structure during dough heating stage is formed.

As it is shown in Figure 3, the C3 (pasting viscosity peak), C4 (pasting viscosity minimum torque), and C5 (retrogradation final torque) decreased for S24.50, this also occurs for S12.50, S12.100, S24.100, and the same findings were found by (Liu et al., 2017) in similar sprouted wheat flours blends. The increase of the dough temperature during p3 contributed to the breakdown of starch particles, resulting in lower pasting temperature and higher paste consistency. C3 indicates the maximum viscosity during p3, the decline of C3 in S24.50 was mainly due to the higher amount of degraded starch in sprouted wheat. The high temperature of the dough and water released by the denatured proteins caused the starch to swell and rupture, thus inducing a slightly increased dough consistency. The C3-C4 torque (hot gel stability, Nm) reflects the amylolytic activity, where a bigger difference in C3 and C4 indicates higher amylolytic activity (Erukainure et al., 2016; Liu et al., 2017). A reduction in viscosity caused by the physical breakdown of starch granules is observed in p4, leading to a minimum value for the torque. During cooling in p5, gelatinized amylose molecules in the dough begin to recrystallize, leading to the retrogradation of the end products. A higher C5 means more starch retrogradation, thus the decreased value of C5 represents longer shelf stability that may be beneficial for slowing the staling of bakery products during storage, leading to a better texture of the end products. Similar findings and behaviours in flours with geminated wheat were found (Ding et al., 2018; Liu et al., 2017; Schmiele et al., 2017).

3.2 Hardness properties of bread formulations

The hardness values obtained in the different bread formulations are shown in Table 1. A one-way ANOVA showed that the variable proportion of germinated wheat flour significantly explains the hardness of the bread, both after germination time 12 h and 24 h (p-value <0.05). According to the texture profile analysis and hardness values, it was observed that 12 hour of seed germination represents greater stability in each of the mixtures since data showed higher linearity with an R^2 value of 0.9532 (Figure 4). As the proportion of germinated flour increases, the hardness of the bread also increases. The results in the present study indicate that there are no significant differences (p-value >0.05) between germination time after 12 and 24 hours, especially in values of 30% and 40% GF, with similar values of hardness between 6.15 (N) to 6,75 (N). Figure 1 shows the physical aspect of the bread made with 12 h germinated wheat flour and it is possible to appreciate the decrease in bread size as the GF flour content in the mixture increases. Colour appearance is also shown, for more GF content, bread is darker. Similar findings were showed in the study made by Liu et al., 2017 using blends of GF.

"Insert Table 1 here"

"Insert Figure 4 here"

Regarding the global quality of the GF and its use for bread recipes, it was observed that the bread with a higher content of germinated flour was relatively harder and more aromatic. In general, the hardness of 50% of GF is among the accepted values for a bread, however, for a greater increase in the percentage of GF the hardness rises too much, which makes the texture of the bread unpleasant. These breads tend to be smaller without losing too much fluffiness (Figure 1).

3.3 Potentially harmful microorganisms in rejuvelac

The results of the microbiology tests showed significant differences (p-value<0.05) between R, SR and IR samples. Presence of Salmonella sp. (4.4 ± 0.19 Log cfu / ml), Enterobacter sp. (4.8 ± 0.13 Log cfu / ml) and Listeria sp. (5.0 ± 0.25 Log cfu / ml) was observed on R sample. However, no presence of pathogens was found for SR and IR. The recount of Lactobacillus spp. was positive for 2 samples without significant differences: 6.64 ± 0.12 Log cfu/ml for R sample and 6.56 ± 0.02 Log cfu/ml for IR sample. Nevertheless, SR did not present Lactobacillus spp. growth due to the sterilization.

The germination process presents the perfect conditions for the growth of bacterial pathogens, as they need high humidity, high nutrient biodisponibility and a temperature between 20-25ºC. The most known outbreak caused by sprouted seeds occurred in Germany and France on May 2011 (Soon et al., 2013). In line with this, this research has shown that Homemade rejuvelac is not safe and should be prepared under better controlled conditions. The new method proposed for its preparation is using sterile material, cleaning the seeds before sprouting, applying a thermal treatment or inoculating it with a pure culture of lactic acid bacteria to ensure its safe consumption and avoid food security related issues.

3.4 Sensory attributes of wheat fermented beverages formulations

No significant differences (P-value > 0.05) were found in consumer acceptance for both samples. The mean scores of measured attributes are shown in Table 2. In total, 55,7 % of consumers chose sterilized rejuvelac (SR) as the preferred sample.

"Insert Table 2 here"

Qualitative results (Figure 5) shows that IR was mainly "acid", "soft" and "intense" while SR was "soft", "light" and "sweet". Despite the fact these data are difficult to compare, it serves to give a general overview of the product perception and may explain the slight preference for SR as consumers tend to prefer sweeter tastes over acids. The acidity found in IR could be explained to its inoculation by L.acidophilus while the perceived sweetness in SR could be due to the cooking of grains during its sterilization.

"Insert Figure 5 here"

Some other minor descriptors found in SR were "pleasant", "watery" and "tasteless" while IR presented minor descriptors like "aromatic", "fresh" and "corn", as well as "tasteless" which could reflect the possible use of these beverages as a neutral base for the preparation of more complex drinks as referred by some consumers in the sensory evaluation comments section (Table 3). The general perception extracted from this comments section is that both beverages need to be improved in terms of flavour, colour and texture. Two main suggestions to enhance its overall perception were related to drink both beverages at a hot temperature and use them as a starter neutral ingredient to work with.

"Insert Table 3 here"

In fact, the development of probiotic drinks is promising as shown by some articles that have studied probiotic drinks properties made by germinated seeds (Chavan et al., 2018; Mridula & Sharma, 2015). For example, Chavan et al., 2018 fermented germinated and no-germinated barely, ragi and moth bean with sugar and cardamom, using Lactobacillus acidophilus with distilled water, soymilk, almond milk and coconut milk. Another non-dairy probiotic drink was developed utilizing sprouted wheat, barley, pearl millet and green gram separately with oat flour, stabilizer and sugar using L. acidophilus to ferment soy milk. Previous studies describe that the variation of the food matrix or culture used modifies the texture, the taste and consequently the final perception of the product (Coda et al., 2012). Moreover, there are some traditional fermented beverages around the world as Boza, a fermented wheat/corn beverage made with part of germinated wheat from Eastern Europe, or an African non-alcoholic fermented beverage called Mahewu, made by sorghum, milled malt, or wheat flour (Blandino et al., 2003).

The appearance on the market of probiotic drinks based on sprouted seeds seems to be promising in the near future, so these new product developments will have to be accompanied by good sensory evaluations in order to know what preferences the consumers have.

4. Conclusions

Substitution of germinate flour affected the rheological behaviours of flour blends and bread properties like hardness, volume and colour. Breads made with higher levels of sprouted wheat flour were smaller and harder. Furthermore, the addition of germinated flour would bring benefits in nutritional values of final products. The findings of this study indicate that the use of a mixture of germinated wheat flour and wheat flour improve dough functionality i.e., less starch retrogradation during gelatinizing, improved gluten quality with less weakening, and longer mixing stability time during dough mixing. There were no statistically significant differences between bread hardness produced with flours after 12 and 24 hours of seeds germination, thus a germination time of 12 h is enough to get such benefits. Germinated flours are promising ingredients for baked products to improve its end-use functionality or enhance health benefices.

According to the results shown for the evaluation of homemade rejuvelac, some measures have to be taken into account for its safe production, for example, the use of sterile material, as well as a disinfectant for cleaning the seeds before sprouting. However, the development of a safe procedure for rejuvelac was possible thanks to the sterilization of the beverage after fermentation (SR). Additionally to this step, it was decided to develop an inoculated alternative (IR) with a commercial strain of L. acidophilus due to the growing interest for fermented beverages. No significant differences were found in the sensory evaluation but the suggestions received from consumers showed that this procedure could open a safe and novel way to develop fermented beverages based on sprouted grains.

This article highlights sprouted grains as a very beneficial ingredient to create new products and to ferment with, thanks to its high nutrient content and healthy properties. In fact, if the germination and fermentation process is controlled, applying the correct food safety practices, sprouted grains could be used to prepare new beverages and bakery-based products for the European market in the upcoming years, as the demand for healthier, more nutritious, and tastier products is increasing.

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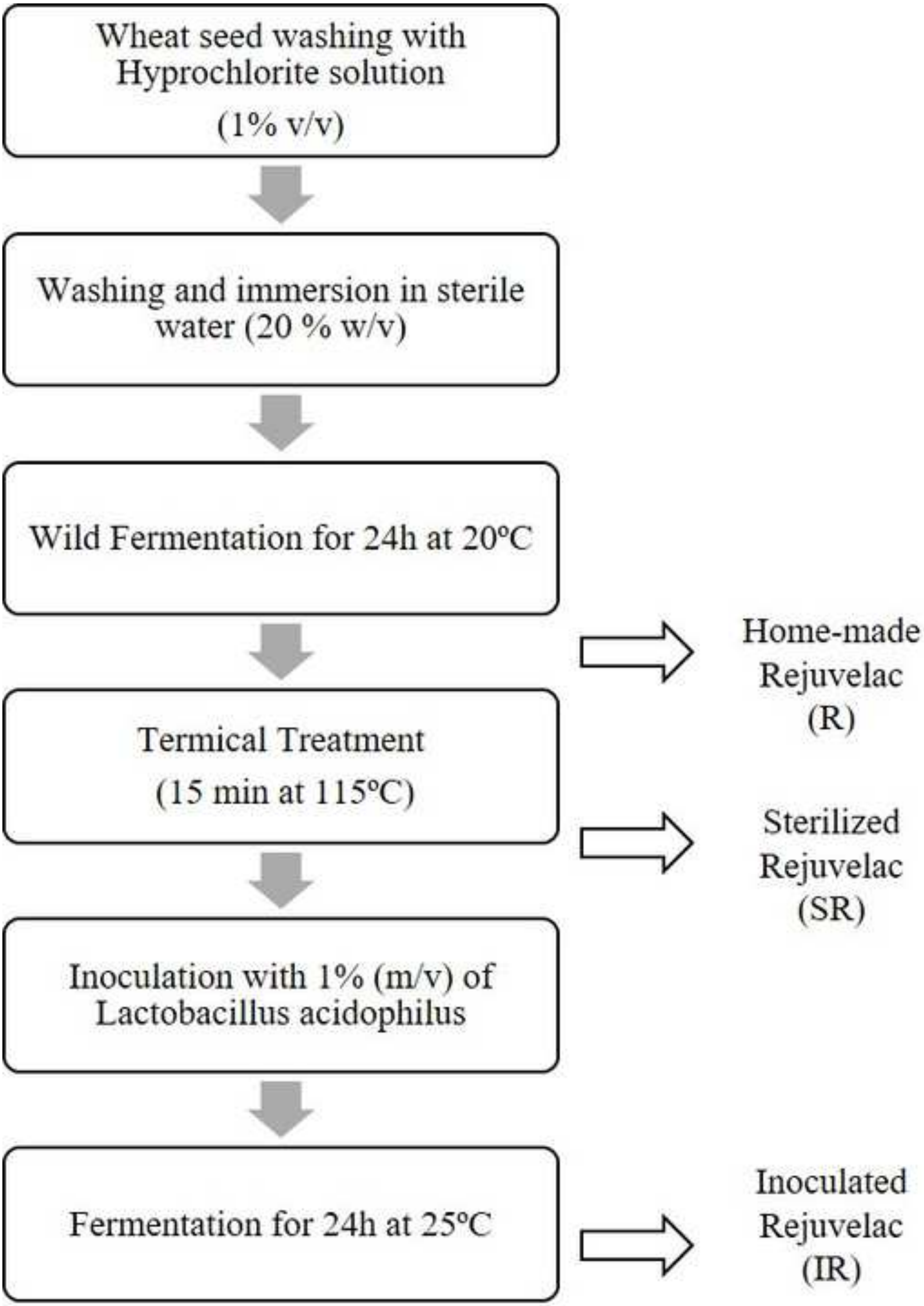
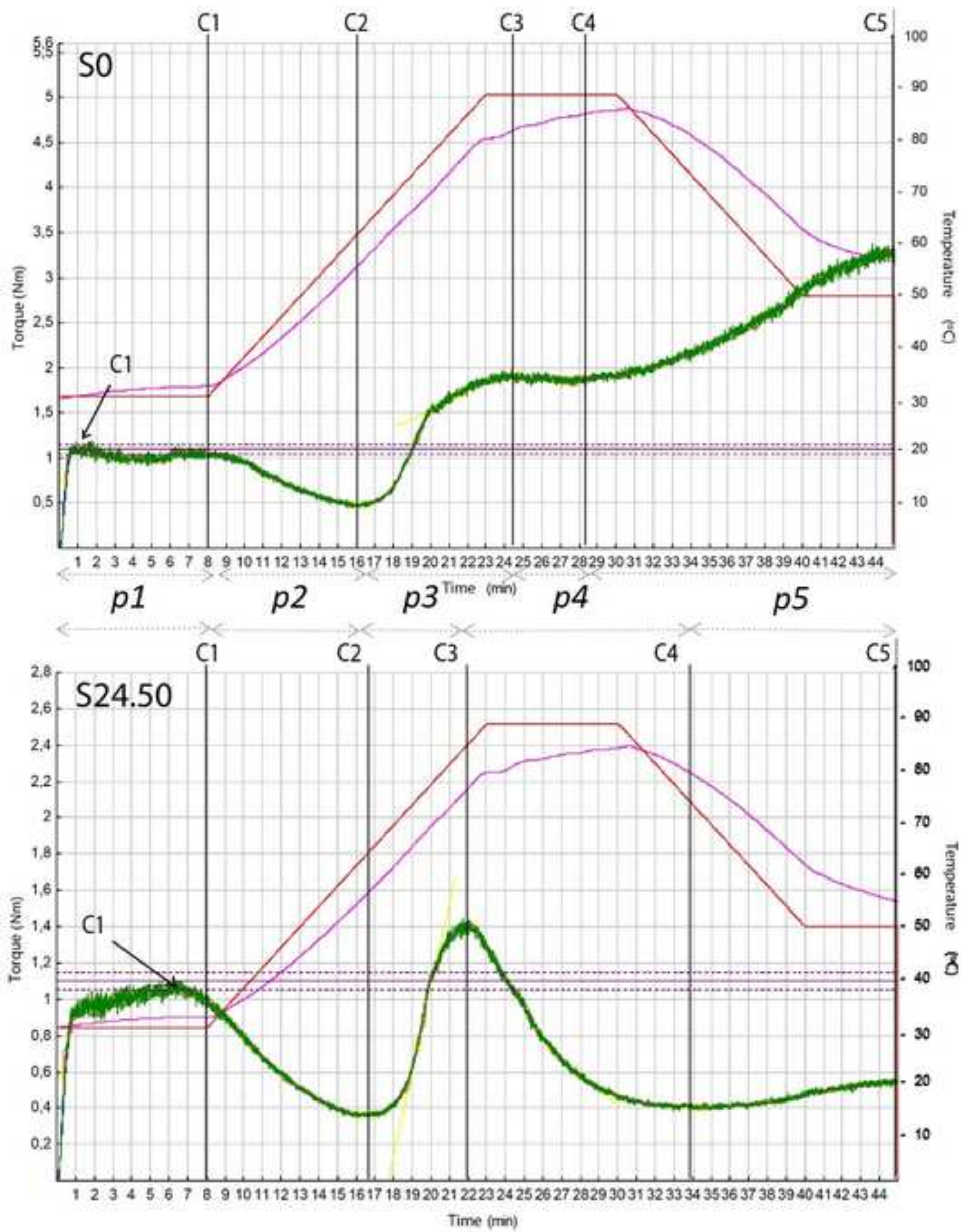
Figure

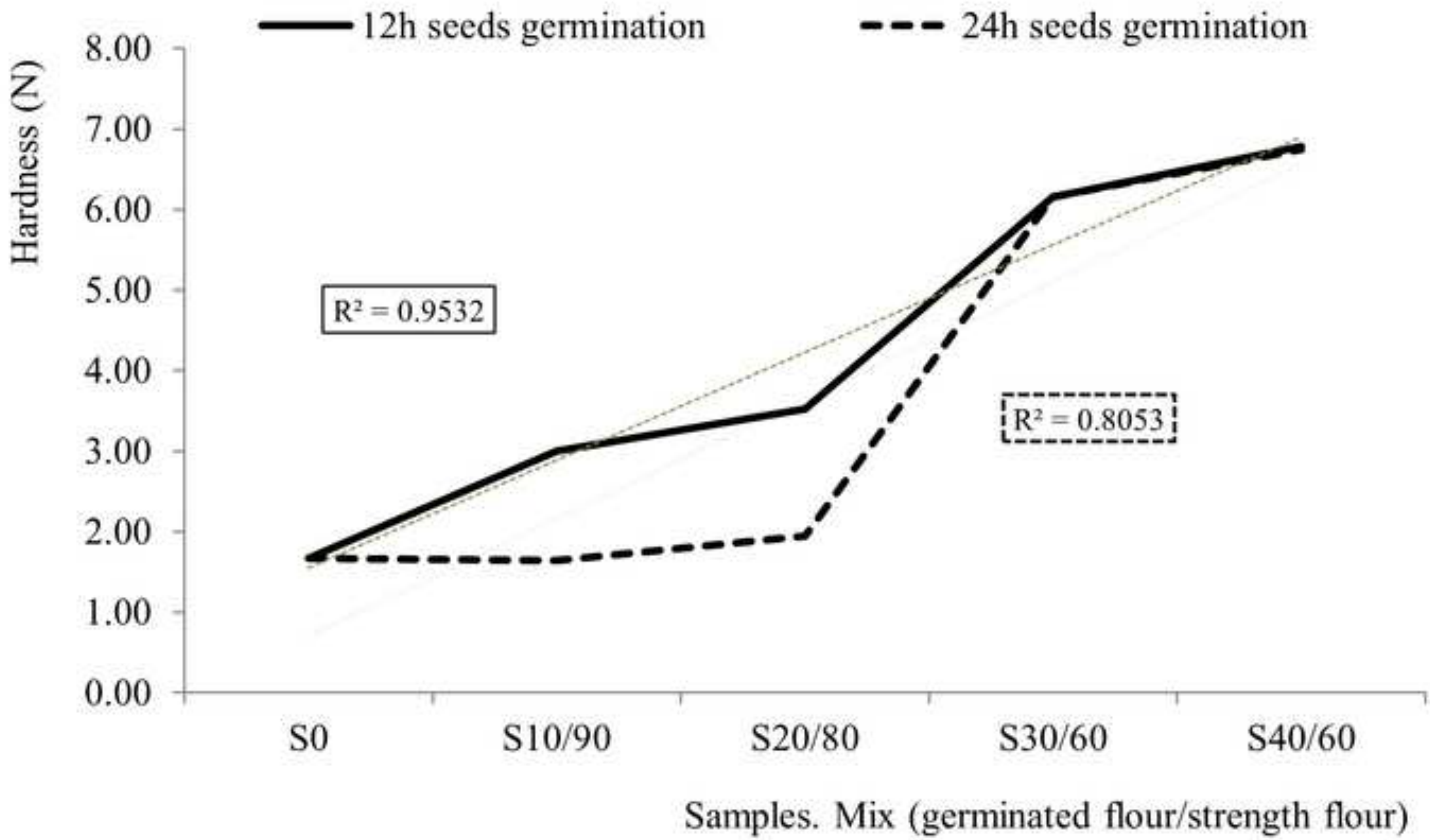
Click here to access/download;Figure;Figure 1.jpg


Wheat seed washing with Hyprochlorite solution (1% v/v)
Washing and immersion in sterile water (20 % w/v)
Wild Fermentation for 24h at 20ºC
Termical Treatment (15 min at 115ºC)
Inoculation with 1% (m/v) of Lactobacillus acidophilus
Fermentation for 24h at 25ºC
Home-made Rejuvelac (R)
Sterilized Rejuvelac (SR)
Inoculated Rejuvelac (IR)


S0
C1
C2
C3
C4
C5
C1
Torque (Nm)
Temperature (°C)
Time (min)
p1
p2
p3
p4
p5
S24.50
C1
C2
C3
C4
C5
C1
Torque (Nm)
Temperature (°C)
Time (min)

Figure

Click here to access/download;Figure;Figure 4.jpg




Concentrated
Transparent
Different
Genuine
Strong
Satiating
Organic
Soft
Coco water
Murky
Horchata
Soda
Persistent
Whitish
Fresh
Aromatic
Germinated
Unappealing
Brine
Cloying
Grass
Thick
Bitter
Field
Corn
Acid
Astringent
Neutral
Salty
Impure
Watery
Alive
Mucilage
Odorous
Pale
Cereal
Sweet
Fruity
Silky
Nice
Tasty
Vegetal
Strange
Dry
Bad
Opaque
Oat
Pleasant
Fermented
Interesting
Plant
Intense
Unpleasant
Sparkly
Lactic
Humidity
Appealing
Colorless
Tasteless
Viscous
Appealing
Odorless
Sugar
Wood
Light
Field
Odorous
Intense
Horchata
Innovative
Refreshing
Simple
Pleasant
Nutritious
Rural
Floral
Crystalline
Complete
Aftertaste
Watery
Body
Pale
Horse pasture
Translucent
Sour
Unappealing
Cider
Ground
Corn
Different
Soft
Soda
Fresh
Grass
Transparent
Silky
Cereal
Aromatic
Beer
Murky
Dry
Fruity
Bodiless
Synthetic
Vegetal
Clean
Colorless
Tasty
Acid
Sweet
Neutral
Wheat
Healthy
Tasteless
Fermented

Figure Captions

Figure 1. Breads with 12 h germinated wheat flour. Mixtures of germinated flour/strength flour (GF/SF), from left to right: 100 SF, 10/90, 20/80, 30/70, 40/60, 50/50, 100 GF.

Figure 2. Flow chart of the production process of R (Home-made Rejuvelac) SR (Sterilized Rejuvelac) and IR (Inoculated Rejuvelac).

Figure 3. Mixolab™ curves and parameters analysed from S0 (standard sample) and S24.50 (24h germination time; mixed flours 50/50 germinated flour/strength flour, respectively).

Figure 4. Linear behaviour of hardness values for the bread samples until mixture 40/60 (germinated flour/strength flour). The 50/50 (GF/SF) mixture shows lower bread volume and loss of linearity.

Figure 5. Qualitative descriptors for inoculated rejuvelac (IR; left image) and sterilized rejuvelac (SR; right image).

Table 1. Texture profile analysis (hardness values¸ mean ± SD) of bread made from wheat flours after 12 and 24 h germination time.

Sample	**Hardness (N) – 12 h**	**Sample**	**Hardness (N) – 24 h**
S0	1.67 ± 0.05	S0	1.67 ± 0.05
S12-10	3.00 ± 0.53	S24-10	1.64 ± 0.19
S12.20	3.53 ± 0.83	S24.20	1.95 ± 0.45
S12.30	6.15 ± 1.05	S22.30	6.16 ± 1.49
S12.40	6.79 ± 3.38	S24.40	6.75 ± 2.96
S12.50	12.29 ± 1.60	S24.50	19.06 ± 9.16
S12.100	77.58 ± 14.56	S24.100	137.81 ± 33.24

Table 2. Sensory evaluation (N=75; 9-point hedonic scale) of global acceptance and sensory attributes for inoculated rejuvelac and sterilized rejuvelac. Results are presented as mean ± SD.

	Inoculated Rejuvelac	Sterilized Rejuvelac
Global Acceptance	5.79 ± 1.24	5.69 ± 1.37
Appearance	5.68 ± 1.54	5.76 ± 1.67
Color	5.56 ± 1,40	5.63 ± 1.63
Odor	5.72 ± 2,03	5.21 ± 1.77
Flavor	5.29 ± 1,66	5.31 ± 1.77
Texture	6.35 ± 1.49	6.41 ± 1.77

Table 3. Written comments by consumers panel during the sensory evaluation of rejuvelac. Sample 567 is referred to inoculated rejuvelac. Sample 694 is referred to sterilized rejuvelac.

"The flavours are very pleasant in both cases. They have very pleasant herbal nuances. The texture should improve as its flavour asks for a better one."
"Both samples need more intensity and complexity. They taste like flavoured water."
"I preferred sample 694 for its transparency and for being a more neutral drink."
"You can modify the texture of sample 567 making it more refreshing while drinking. The colour could be more transparent in both cases to attract more consumers' attention. The 694's smell is earthy, which is not very pleasant. It is reasonable to understand that they are sprouted but it is a matter of working better with the aroma. They are both interesting. Good job!"
"Sample 694 reminds me of fermentation and Sample 567 of vegetables (soil)."
"Very interesting tasting. I liked sample 694 a lot better. Good appearance. Very pleasant on the palate."
"I preferred sample 694 as it has a lighter colour which makes it more appealing for drinking and its taste is not as intense as sample 567"
"Sample 567 was my favourite although it would be an opportunity to enhance the white colour that it has."
"Lack of aroma and flavour."
"They are not very palatable. I would use them as an ingredient."
"Sample 694 is less bitter and has a rounded taste. Sample 567 has less flavour and is more bitter."
"Consider improving colour and enhancing smell and taste. The texture is correct."
"I like the textures and the flavours are peculiar."
"The flavour could be improved so it doesn't taste so fermented."
"Sample 694 has worse appearance but the taste is better. Sample 567 called my attention because the smell was much nicer but not the taste."
"If it is a drink to the mainstream consumer, try with something more palatable, sweet or acid touches. Sample 694 goes well for me."
"I would not buy them. They still lack flavour and personality but it is interesting to continue working on it. Cheer up!"
"Both samples have a fairly watery taste. Sample 694 has a cleaner appearance and a slightly stronger aroma."
"Maybe both samples are better if drunk hot."
"In my opinion, the taste of sample 694 is more intense and bold."
"Sample 694 seems stronger but I don't really like the smell. Both samples look like corn chowder. Sample 567 is better in my opinion."
"I don't really understand the product but I think it would be a good base for a soda drink."
"Both samples taste strange and slightly unpleasant to me. As such I don't like them very much but they may have potential in other applications."
"Sample 567 taste like green olives maceration liquid. Sample 694 taste like canned corn liquid, it is cooler."
"I think both samples would taste better if drunk hot."
"I think sample 694 does barely smell but the taste is much perceived. The smell of sample 567 is more powerful but I don't like the texture very much."
"Sample 567 has an unattractive appearance and an intense aroma of ferment. On the other hand, the taste is good, the cereal notes are noticeable. Sample 694 has barely any flavour or aroma."

Implications for gastronomy

This article research came up as a result from the great interest for authors to diversify uses of sprouted grains in food and gastronomy development. Seeds, some cereals and legumes are rich sources of nutrients and it is noteworthy that bioavailability of nutrients and bioactive compounds is enhanced during the sprouting process by the enzymatic activity developed during the germination. In addition to the nutritional values, we highlight the technological and sensory properties of sprouted grains as ingredients with a big interest for food industry and gastronomy innovation.

We firstly focus our attention on the use of germinated grains for the preparation of flours for bakery industry. We aimed to identify how the wheat germination time (12 and 24 h.) affects quality of wheat germination flour, as well as the use of this flour as ingredient to mix with other commercial strength flour in bread products. After investigation, the mixture of 12 h germinated flour and strength flour (50/50) produced less starch retrogradation and enhanced gluten quality, with less weakening and longtime stability during dough mixing. Thus, we promote the use of germinated wheat to create new flours in bread products, with technological and health benefits.

Exploring other sprouted grains opportunities, we realised that homemade wild fermented rejuvelac or other sprouted grain-based fermented drink may present a risk to consumers health. These drinks are used, mostly by vegan people, to make vegan cheeses and this product is also being used as a beverage for its supposed healthy benefits. We demostrate that if the fermentation process is done at environmental temperature with any microbiological control pathogenic microorganisms as Listeria sp., and Salmonella sp. will be present in the final product. Thus, we advise it is neccesary to ensure the production of a safety sprouted grain fermented beverage, in order to be able to carry it out in homes, industries or restaurants. We describe a controlled germination and fermentation process, applying the correct food safety practices, and showing that sprouted grains could be used to prepare new beverages and bakery-based products.

We also focus the mentioned applications in encouraging industry and gastronomic interaction in this field, so chefs, master bakers, pastry chefs, food scientists, nutritionists and/or food technologists can be inspired and work together.

Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript
- The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript:

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Patricio David Pérez: Resources, Analysis.

Laura Perezábad: Conceptualization, Supervision, Writing-Reviewing and Editing.