

## VALORIZATION OF PRICKLY PEAR PEELS & SEED PRESS-CAKE IN TRADITIONAL SOURDOUGHS AND EVALUATION OF THEIR BREAD-MAKING CAPACITIES

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### ABSTRACT

The prickly pear fruit's peel (PPP) and seed press-cake (SPC) are discarded during the pulp and the seed-oil processing. This study incorporated these two by-products in the preparation of sourdoughs and evaluated their bread-making capacities. The seed press-cake was recovered during the mechanical extraction of the seed oil, ground and used to produce S1 sourdough. The peels were milled and their puree was utilized to produce firm and liquid sourdoughs S2 and S3, respectively. The prepared sourdoughs had an average pH of 3.24 and a total titratable acidity (TTA) between 13.4 and 27.4 mL NaOH 0.1M/10g. The doughs fermented with these sourdoughs had different rising capacities (167 to 259%) and specific volumes (1.53 to 2.26 cm<sup>3</sup>/g). The sourdough-fermented doughs produced equivalent amounts of CO<sub>2</sub>. The doughs fermented with the peels sourdoughs produced more ethanol than that with press-cake sourdough. Fermented doughs had a final TTA between 7.8 and 11.5 mL NaOH 0.1M/10g and a final mean pH of 3.47. The control dough fermented with baker's yeast alone had a low final TTA of 4.5 mL NaOH 0.1M/10g and a comparable pH to that of the sourdough-fermented doughs. Our results suggest the incorporation of prickly pear by-products in sourdoughs formulation and use to inoculate bread dough to decrease food waste and improve the quality of baked products.

**Keywords:** Prickly pear, peels, seed press-cake, fermentation, sourdough, bread-making

### INTRODUCTION

*Opuntia spp.* is a plant that grows in wild, dry, and semi-dry environments. While its fruit and cladodes are widely acknowledged as precious resources, they are still significantly underutilized (Daniloski *et al.*, 2022). The prickly pear fruits are consumed fresh or processed for their pulp, which is the edible part. The peel and seeds are considered as waste or as derivative products, even though they are actually rich in minerals, dietary fiber, phenolic compounds, and flavonoids. As a result, they have the potential to serve as valuable sources of functional components. (M Jimenez-Aguilar *et al.*, 2014).

According to previous research, the peel and seeds together constitute over 50% of the fruit (Daniloski *et al.*, 2022; Sawaya *et al.*, 1983). Their valorization can increase the food production chain's economic and environmental sustainability (Ribeiro *et al.*, 2022). Several experiments relating to their use, in particular for the extraction of natural dyes, pectin, edible and cosmetic oil and their incorporation in animal feed, have been reported in the literature (Bourhia *et al.*, 2020; Habibi *et al.*, 2009; Melgar *et al.*, 2017; Todaro *et al.*, 2020).

Currently, the need to find a sustainable use of food by-products and to meet the real needs of consumers in terms of diversification of healthy and tasty foods, with extended shelf life and without adding chemical preservatives, is a real challenge for food industries, especially for baked products (Israr *et al.*, 2016). In the Mediterranean food tradition, bread plays an important role and has long been a component of the diet (Parafati *et al.*, 2020). The importance of sourdough is due to the popularity of bread in many world cultures, where it is one of the most popular meals. The utilization of sourdough is one of the earliest biotechnology methods used in the manufacturing of traditional cereal foods and baked products (Sanmartin *et al.*, 2020). In fact, sourdough fermentation improves bread taste, nutritional content, volume gain and shelf life by inhibiting spoilage bacteria and mold development. It enhances loaf volume, baking evenness, color, aroma, flavor, and texture (Chavan & Chavan, 2011). In this regard, the elaboration of traditional sourdoughs based on food by-products remained an innovative solution to address the issue of food waste while also creating highly nutritious products that capitalize on the nutritious content of these by-products.

Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB) (De Vuyst & Neysens, 2005), it has been used in bread production to enhance the flavor and texture of baked cereals (Hansen & Schieberle, 2005). Aneur *et al.* (2022) noted that some researchers reevaluated the potential of bread to deal with future food supply challenges. Although several difficulties, such as

the sustainability of the supply chain, the coverage of nutrients, and the digestion of bread, still need to be addressed (Thielecke *et al.*, 2021; Weegels, 2019).

Despite the increasing consumer interest in sourdough bread in recent years, baker's yeast has mostly replaced sourdough as the primary leavening agent for bread over the past century. However, the practice of making sourdough using wheat or rye flour has a rich history in bread technology. However, the interest in making sourdough with non-conventional flours has recently gained great importance. Sorghum, buckwheat, oat, barley, spelt, rye, quinoa, and amaranth flour can be used for sourdough preparation and then combined with wheat flour for bread making to produce products with functional properties and improved nutritional value, and can be used for making baked products for special diets, like gluten-free foods (Catzeddu, 2019). When sourdough is employed in place of baker's yeast *Saccharomyces (S.) cerevisiae* during the fermentation of bread dough, it has been shown that both bread quality and technological attributes are improved (Kezer, 2022).

Another advantage of using sourdoughs consists in the degradation of phytate by the phytase enzyme. According to Rodriguez-Ramiro *et al.* (2017), recent studies have shown that the activity of wheat phytase is predominant compared to that of sourdough microorganisms. However, the endogenous cereal phytase benefits from the sourdough's pH being lowered as a result of the bacteria's metabolism during fermentation. Using an intestine cellular model, it was shown that sourdough bread had a higher iron bioavailability than yeasted bread (Rodriguez-Ramiro *et al.*, 2017). The main protein found in wheat, rye, barley, and other cereals is, gluten, which causes a chronic inflammatory process in genetically predisposed people that results in lesions in the small intestine and difficulty in nutrient absorption, a condition known as celiac disease, and immune-mediated enteropathy (Catassi *et al.*, 2014; Lamacchia *et al.*, 2014). In order to create gluten-free wheat flour-based bread, the ability of sourdough microflora to hydrolyze gluten proteins has been exploited. It has been confirmed that this bread is safe for people suffering from celiac disease (Greco *et al.*, 2011; Rizzello *et al.*, 2014).

According to Gobetti *et al.* (2020), the challenge for ecological friendly and sustainable baking industries is the development of approaches to supplement wheat flour with others ingredients from non-wheat cereals, pseudo-cereals, legumes, oilseeds, non-cereal plants, or agri-food by-products. Parafati *et al.* (2020) evaluated the addition of 10% prickly pear peel flour to bread dough as a source of nutrient and bioactive compounds; they reported that the final bread had

the best sensory evaluation and the highest values for the specific volume ( $3.09 \pm 0.24 \text{ cm}^3/\text{g}$ ) and leaving capacity of the dough between 100% and 120%.

In the present work, the prickly pear peel and the press-cake obtained after seeds oil extraction were incorporated into the traditional sourdough preparation and their bread-making performance was evaluated. Our objective was to characterize the prepared sourdoughs and their fermented doughs.

## MATERIALS & METHODS

### Plant material

*Opuntia dillenii* (Ker Gawl.) Haw., 1819 fruits obtained from the woodlot established for mass production of *Opuntia spp.* cactus resistant to *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) in the experimental station of ORMVAD-Zemamra, Doukkala, Morocco ( $32^{\circ}37'48'' \text{ N}$ ,  $8^{\circ}42'0'' \text{ W}$ ) were well-cleaned of their glochids, peeled and the seeds were separated from the pulp using a plastic sieve, washed and sun-dried. The seeds have undergone a mechanical extraction by press to obtain the press-cake and the oil. The press-cake and peels were stored tightly in plastic containers and placed in a refrigerator ( $6^{\circ}\text{C}$ ) and a freezer ( $-18^{\circ}\text{C}$ ), respectively before further use.

### Sourdough preparation and refreshment

The seed press-cake was incorporated in the preparation mix of S1 sourdough while the peel puree was used in the preparation of the S2 and S3 sourdoughs. The sourdoughs formulation is shown in Table 1. The S1 and S2 sourdoughs were refreshed every 24 hours for 10 days. The S3 liquid sourdough was fermented under agitation, then stored at  $6^{\circ}\text{C}$  until use. The fermentation of all sourdoughs was carried out in an oven at  $30^{\circ}\text{C}$ .

**Table 1** Composition of prepared sourdough (%).

Sourdough	Water	Soft wheat flour T55	Peel puree	Press-cake	Raisin
S1	54,86	23,49	-	20,04	1,32
S2	35,00	47,00	16,00	-	1,00
S3	72,99	14,00	12,00	-	1,00

### Dough preparation

Three different doughs were prepared, and 220 g of each dough was used for monitoring the bread-making. The control dough was prepared with the same compositions excluding the sourdough, as shown in the Table 2. Baking yeast (*Saccharomyces cerevisiae*) was added to the dough at a percentage lower than 0.2%, in order to respect the denomination of "sourdough bread" according to the French legislation (article 4 of the Decree n°93-1074 of September 13, 1993). The doughs fermentation was carried out at  $30^{\circ}\text{C}$ .

**Table 2** Composition of prepared doughs (%).

Dough	Water	Soft wheat flour T55	Salt (NaCl)	Baker's yeast	Sourdough
Dough S1		55.5	1.0	0.1	
Dough S2	35.5	55.5	1.0	0.1	9.0
Dough S3		55.5	1.0	0.1	
Control	38.9	60.0	1.0	0.1	0.0

### Fermentation Monitoring System

A smart fermentation oven (Patent application no. MA57946), equipped with multiple sensors, was used for an online monitoring and tracking of fermentation parameters, such as dough rise,  $\text{CO}_2$  and ethanol release, pH and mass loss kinetics. All data collected were stored in a database using a computer-based data acquisition program.

### Production of $\text{CO}_2$ and ethanol

Based on the values of the volume concentrations measured by the  $\text{CO}_2$  and ethanol sensors, their respective productions were calculated using the following formula by (Zhang et al., 2007):

$$\text{Volume of the gas (ml)} = \text{Concentration of the gas (ppmv)} \times V_{\text{oven}} (\text{m}^3)$$

### Total titratable acidity (TTA)

The tested doughs (10 g) were well mixed with 90 ml of distilled water and 2 to 3 drops of phenolphthalein solution were added to the mixture. The total titratable acidity (TTA) was estimated as the amount (mL) of 0.1 M NaOH required to neutralize the mixture (Rizzello et al., 2019).

### Dough Raising Capacity (DRC)

The Dough Raising Capacity was determined according to the formula cited by Bhatt & Gupta (2015), with a small modification that consists in replacing the volume by the height of the dough, since all the fermentation trials were conducted in the same cylindrical container:

$$\text{DRC} = 100 * (\text{H}_f - \text{H}_i) / \text{H}_i$$

With:

- H<sub>f</sub>: Final height of the dough
- H<sub>i</sub>: Initial height of the dough

### Dough yield (DY)

Depending on how much flour and water are used in the preparation, the sourdough might have a firm or liquid consistency. This proportion is called the dough yield and is defined as follows (Chavan & Chavan, 2011):

$$\text{Dough Yield (\%)} = 100 * (\text{Amount of flour} + \text{Amount of water}) / (\text{Amount of flour}).$$

### Moisture content

The moisture content of sourdoughs and fermented doughs samples was measured using an OHAUS MB45 moisture meter.

### Statistical analysis

At least three replications were performed for each experiment and results are expressed as mean  $\pm$  standard deviation. Data were analyzed using one-factor ANOVA followed by Tukey's test at a significance level of  $p \leq 0.05$  using EXCELSTAT v.2016.02 add-on. Data measured in percentages were subjected to an arcsine transformation in order to approximate the normal distribution before analysis.

## RESULTS & DISCUSSION

### Sourdoughs characterization

The surrounding bacteria and yeasts colonize the flour-water combination in the initial sourdough mix and digest the available carbohydrates and other nutrients. The microbial flora is fed by continuously adding flour and water over a period of 5–15 days. This process is also called "back slopping" or "propagation," and during that time, part of the mixture is discarded (Amr & Alkhamaiseh, 2022).

The major factors used to describe a mature sourdough starter, which is very acidic, are generally the number of bubbles, volume/height, smell, and taste (Amr & Alkhamaiseh, 2022). Table 3 shows the main characterization parameters of the prepared sourdoughs.

**Table 3** Main characterization parameters of the prepared sourdoughs.

	S1	S2	S3
Sourdough Type	Type I	Type I	Type II
Dough Yield (DY) (%)	228.26 <sup>b</sup>	157.13 <sup>a</sup>	384.54 <sup>c</sup>
Sourdough consistency	Liquid	Firm	Liquid
Moisture (%)	54.26 $\pm$ 0.29 <sup>a</sup>	52.85 $\pm$ 1.83 <sup>a</sup>	86.10 $\pm$ 0.23 <sup>b</sup>
pH	3.27 $\pm$ 0.01 <sup>a</sup>	3.24 $\pm$ 0.05 <sup>a</sup>	3.20 $\pm$ 0.01 <sup>a</sup>
TTA (mL NaOH 0.1M/10g)	15.5 $\pm$ 0.7 <sup>b</sup>	27.4 $\pm$ 0.6 <sup>c</sup>	13.4 $\pm$ 0.1 <sup>a</sup>

S1: Sourdough prepared from seed press-cake; S2 and S3: Sourdoughs prepared from peels. Results are expressed as mean  $\pm$  standard deviation. Values in the same row with at least one letter in common are not significantly different at the 5% probability level.

### Sourdough Type

The S1 and S2 sourdoughs are of type I, this type is usually used in artisanal bakeries and is typically maintained at room temperature ( $20\text{--}30^{\circ}\text{C}$ ). However, it can be stored in the refrigerator when it is not in use (Amr & Alkhamaiseh, 2022). The S3 sourdough is of type II, it was processed in the same technique as S1 and S2, with a prolonged fermentation time and a high water content (Amr & Alkhamaiseh, 2022). In industrial contexts, this type is produced in bioreactors or tanks. Its usage is intended to accelerate the fermentation process (De Vuyst & Neysens, 2005; Decock & Cappelle, 2005).

In industrial point of view, the management of sourdough type I is time-consuming, requires experienced workers, and interferes with microbiological stability and optimal performance during bread-making. Thus, liquid-sourdough fermentation was just recently provided as a technological solution for bakeries

using sourdough type I standard (Brandt, 2007; Carnevali et al., 2007; Lacaze et al., 2007).

Yildiz et al. (2019) reported that sourdoughs type I are acidic and traditionally produced, sourdoughs type II are acidic, semi-fluid and industrially produced.

### Dough yield & sourdough consistency

Dough Yield (DY), defined as a ratio between water and flour in the dough and calculated during preparation, determines sourdough's consistency (Decock & Cappelle, 2005) and influences its quality and handling (Barber et al., 1992). Firm and liquid dough yield values are around 150 - 160 and more than 200%, respectively (Galli et al., 2019; Kulp & Lorenz, 2003).

The S1, S2 and S3 sourdoughs had a dough yields of 228.26, 157.13 and 384.54%, respectively. As a result, the sourdoughs S1 and S3 had a liquid consistency, however the sourdough S2 was firm.

Decock & Cappelle (2005) reported that Spicher & Stephan (1987) stated that the DY value of a sourdough has a considerable impact on its taste profile. The sourdough produces more acetic acid and less lactic acid as its DY value decreases. For high DY values, the rate of acidification is quick, which may be explained by the better diffusion of organic acids produced in the medium.

### Sourdoughs moisture content

Sourdoughs S1 and S2 had statistically similar moisture content of 54.26 and 52.85, respectively. The sourdough S3 had the highest value of 86.10%. The water absorption capacity of the flours influences the DY of the dough. As a result, various dough consistencies may be obtained with the same DY (Amr & Alkhamaiseh, 2022).

Water content and DY value are positively correlated; high water content impacts sourdough acidity and slightly, water activity values (De Vuyst et al., 2014; Gianotti et al., 1997).

### pH and TTA of sourdoughs

The basic biochemical parameters such as pH and TTA describe the performance of a sourdough (Arora et al., 2021). The results of Table 3 show that there was no significant difference between the final pH values of the studied sourdoughs. The average pH of the three sourdoughs was 3.24, compared to a value of 4.0 of a sourdough made from date seed flour as reported by Ameer et al. (2022). According to Amr & Alkhamaiseh (2022), starters generally begin with a near neutral pH that subsequently decreases due to organic acids produced by lactic acid and acetic acid bacteria until final maturation.

The total titratable acidity of the three sourdoughs were statistically different. The S3 liquid sourdough had the lowest value of 13.4 mL NaOH 0.1M/10g followed by the S1 sourdough with 15.5 mL NaOH 0.1M/10g. Ameer et al. (2022) reported a value of 13.3 mL NaOH 0.1M/10g for date seed powder sourdough, without giving any information about the consistency of the latter. The TTA of the firm S2 sourdough was 27.4 mL NaOH 0.1M/10g, which was twice as that of S3 sourdough. Di Cagno et al. (2014) reported that this could be explained by higher concentration of lactic and, in particular, acetic acids. Thus, the S2 sourdough could have the highest concentrations of lactic and, in particular, acetic acids

### Physical parameters of dough fermentation

According to Amr & Alkhamaiseh (2022), fermented products have different characteristics (volume, structure, flavor, color and shelf life) depending on the yeast used. Table 4 shows the physical characterization of the fermented dough using S1, S2 and S3 sourdoughs.

**Table 4** Physical characterization of the fermented dough.

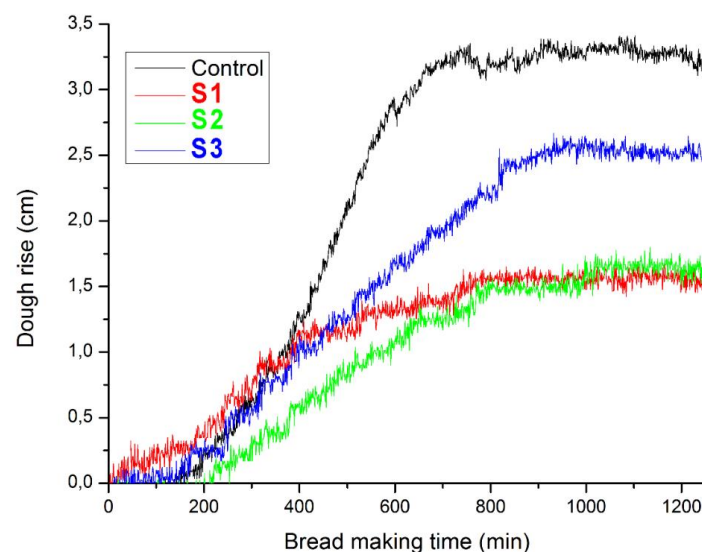
	Control	Dough-S1	Dough-S2	Dough-S3
End of bread making (min)	656 ± 56 <sup>a</sup>	716 ± 49 <sup>a</sup>	930 ± 129 <sup>b</sup>	859 ± 49 <sup>ab</sup>
Mass loss (%)	2.18 ± 0.13 <sup>a</sup>	1.73 ± 0.17 <sup>a</sup>	2.18 ± 0.23 <sup>a</sup>	2.24 ± 0.09 <sup>a</sup>
Dough rise (cm)	3.44 ± 0.66 <sup>b</sup>	1.67 ± 0.35 <sup>a</sup>	1.79 ± 0.26 <sup>a</sup>	2.59 ± 0.15 <sup>ab</sup>
DRC (%)	344 ± 66 <sup>b</sup>	167 ± 34 <sup>a</sup>	179 ± 26 <sup>a</sup>	259 ± 15 <sup>ab</sup>
Specific volume (cm <sup>3</sup> /g)	2.78 ± 0.51 <sup>b</sup>	1.53 ± 0.28 <sup>a</sup>	1.61 ± 0.21 <sup>a</sup>	2.26 ± 0.13 <sup>ab</sup>

Dough-S1: Dough fermented with S1-Sourdough; Dough-S2: Dough fermented with S2-Sourdough; Dough-S3: Dough fermented with S3-Sourdough. Control: Dough fermented without sourdough. Results are expressed as mean ± standard deviation. Values in the same row with at least one letter in common are not significantly different at the 5% probability level.

### Dough rising capacity (DRC)

The dough fermented with S1 and S2 sourdoughs had similar dough rises, with an average of 173%, which is lower than the rise of the dough fermented with S3

liquid sourdough (259%). The dough fermented with baker's yeast alone showed a higher dough rise (344%). The stabilization of the dough rise parameter at a final value, represented by the plateau zone of the curve (Figure 1), corresponds to the end of fermentation.



**Figure 1** Rising monitoring of dough with sourdoughs (S1, S2 and S3) and baking yeast (control) during fermentation process.

Clarke et al. (2004) reported that fermentation reduces elasticity and viscosity of dough, but adding sourdough to the bread dough results in less elastic and softer dough. The fermentation process influences the rheology of both the sourdough and the dough inoculated with the sourdough. According to Venturi et al. (2013), the leavening process is due to carbon dioxide which is produced at an equimolar amount of ethanol by yeasts.

### Bread making time

The monitoring of the kinetics of dough rising (Figure 1), allowed determining the end of fermentation. No significant difference ( $p > 0.05$ ) was observed between fermentation time of the control, S1 and S3. The S3 sourdough fermented the dough in 859 ± 49 min.

These fermentation times are considered to be quite long. According to Decock & Cappelle, (2005), the traditional bread production method consists of fermenting at very low temperatures for a long period.

### Specific volume

Results of Table 4 show no significant difference between the specific volumes of the fermented doughs with the addition of S1, S2 and S3 sourdoughs, their average specific volume was 1.8 cm<sup>3</sup>/g, which is slightly higher than 1.4 cm<sup>3</sup>/g, reported by Ameer et al. (2022) for wheat sourdough bread. However, doughs with S1 and S2 sourdoughs had a significant lower specific volume than the control (2.78 cm<sup>3</sup>/g). The natural microflora (yeast and bacteria) present in the dough produce a significant amount of gas that makes the dough rise (Amr & Alkhamaiseh, 2022). The reduction in specific volume in wheat flour dough has been previously reported, it could be explained by the addition of fibers (Gómez et al., 2003; Pomeranz et al., 1976), the dilution of gluten and the interaction between gluten and fiber (Borchani et al., 2011; Chen et al., 1988). According to Gómez et al. (2003), fibers generally have a significant impacts on the characteristics of the dough, resulting in great water absorption, a dough that is more resistant to kneading, and a bread that is less extensible than the control bread to which no fiber is added.

### Dough mass loss

No significant difference ( $p > 0.05$ ) in weight loss was observed between the control dough and those with S1, S2 and S3 sourdoughs. The lower and higher values were 1.73 % and 2.24 % for doughs corresponding to S1 and S3, respectively, with an average value of 2.08 %. This result is in accordance with the finding of Borchani et al. (2011), who reported that the addition of date seed flour did not have a significant effect on dough mass loss. The same authors reported that the amount of water needed to hydrate the dough depends on its fiber content. Therefore, the weight loss of breads is strongly influenced by the fiber rate.

### Biochemical parameters of dough fermentation

Yeast plays a significant role in fermentation, producing more than just carbon dioxide and ethanol; it also creates numerous secondary metabolites that might

affect the end product's quality. Alcoholic fermentation is carried out by naturally existing yeasts, which results in the production of one mole of ethanol and one mole of carbon dioxide per each mole of glucose (Amr & Alkhamaiseh, 2022). The unit used to quantify the volume concentration of the produced gases is ppmv (Brovkin et al., 2004; Van Kerrebroeck et al., 2018).

**CO<sub>2</sub> production**

Carbon dioxide (CO<sub>2</sub>) plays an important role in the expansion of bubbles during the fermentation of the dough (Lucas et al., 2007). The creation of an internal pressure gradient and the rupture of cell membranes, which creates an open porous structure connected to the environment, both provide an explanation for the mechanism of CO<sub>2</sub> release (Zhang et al., 2007). Table 5 shows the concentrations and productions of CO<sub>2</sub> at different stage of the fermentation process.

**Table 5** Effect of sourdough on CO<sub>2</sub> production during dough fermentation.

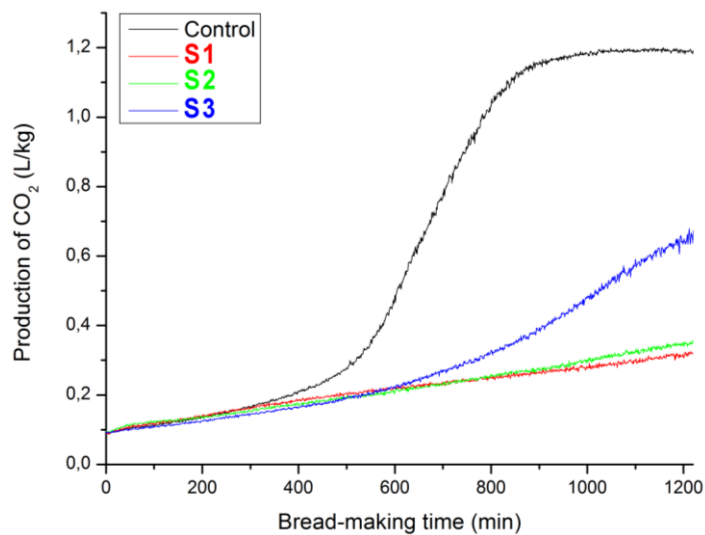
Fermented doughs	[CO <sub>2</sub> ] <sub>EBM</sub> (ppm)	[CO <sub>2</sub> ] <sub>MEE</sub> (ppm)	CO <sub>2</sub> production L/kg of dough
Control	2864.00 ± 453.10 <sup>b</sup>	6009.66 ± 607.52 <sup>c</sup>	0,622 ± 0,067 <sup>a</sup>
Dough+ S1	965.00 ± 188.80 <sup>a</sup>	1435.00 ± 249.27 <sup>a</sup>	0,237 ± 0,031 <sup>b</sup>
Dough+ S2	1208.66 ± 375.07 <sup>a</sup>	1614.66 ± 375.07 <sup>a</sup>	0,291 ± 0,053 <sup>b</sup>
Dough+ S3	1537.00 ± 136.04 <sup>a</sup>	3162.00 ± 136.04 <sup>b</sup>	0,359 ± 0,030 <sup>b</sup>

[CO<sub>2</sub>]<sub>EBM</sub>: Concentration at the end of bread making; [CO<sub>2</sub>]<sub>MEE</sub>: Maximum concentration at end of experiment;

CO<sub>2</sub> production: Production at the end of bread making; Control: Dough fermented without sourdough; Dough + S<sub>i</sub>: Dough fermented with S<sub>i</sub> sourdough (i: 1-3). Results are expressed as mean ± standard deviation. Values in the same column with at least one letter in common are not significantly different at the 5% probability level.

There was no significant difference between the doughs with S1, S2 and S3 sourdoughs in terms of CO<sub>2</sub> production at the end of fermentation. But at the end of the experiment, the dough with S3 liquid sourdough produced more CO<sub>2</sub> than the doughs with S1 and S2 sourdoughs. The release of CO<sub>2</sub> during the fermentation process of the dough is determined by two physical processes: the production via the activities of the yeast's glycolytic enzymes and the retention of gas by the gluten network, which depends on the flour used in the dough (Amr & Alkhamaiseh, 2022).

Figure 2 shows the experimental CO<sub>2</sub> production kinetics. The general trend consists of three distinct phases, namely an induction period followed by a sudden linear increase in CO<sub>2</sub> production and ending with a plateau zone. The obtained measurements of CO<sub>2</sub> were correlated with the specific volume and dough rise of the fermented dough (Istudor et al., 2020).



**Figure 2** Production of CO<sub>2</sub> during the dough's fermentation process

According to Istudor et al. (2020), the physico-chemical properties of the flour used to prepare the dough, the recipe, particularly the dough's consistency, and the quantity of yeast all have a significant impact on the production of CO<sub>2</sub>. Another crucial factor is the dough's temperature at the beginning of fermentation. Therefore, using a softer dough and more yeast will produce a higher level of CO<sub>2</sub> throughout the fermentation process.

The slope of the control dough curve is higher in the linear phase, compared to the other doughs fermented by sourdoughs. This is due to the high production of CO<sub>2</sub> by the yeasts in the control, which are the major producer of CO<sub>2</sub> and are considered responsible for dough leavening (Catzeddu, 2019). According to Paramithiotis et al. (2006), the symbiosis of certain hetero- and homo-fermentative lactic acid bacteria with specific yeasts, most notably *Saccharomyces cerevisiae*, is a common phenomenon in sourdough fermentations. Gobetti et al.

(1994) found that the growth of lactic acid bacteria was promoted by co-culture with yeast, due to the latter's (yeast) excretion of certain amino acids and small peptides, regardless of antagonism for the main carbon source. This may explain the low amounts of CO<sub>2</sub> released by sourdough fermented doughs, which contain both bacteria and yeast.

**Ethanol production**

Sourdoughs can be used as a leavening agent or as a fermentation improver to give bread a specific flavor (Catzeddu, 2019). According to Amr & Alkhamaiseh (2022), the natural microflora (yeast and bacteria) of traditional sourdoughs give the dough a pronounced alcoholic and acidic smell. Van Kerrebroeck et al. (2018) reported that ethanol was the main volatile compound detected during most of the fermentations.

As shown in Table 6, neither the S2 and S3 sourdoughs, which are made from the same flour (fruit peels), nor the S1 and S2 sourdoughs show significant differences regarding the amount of ethanol produced during fermentation. The average final production of ethanol ranged between 2.1 and 3.5 L/kg. Venturi et al. (2013) reported a final production of 1.5 to 2.0 L/kg of ethanol per kg of soft wheat flour based sourdough.

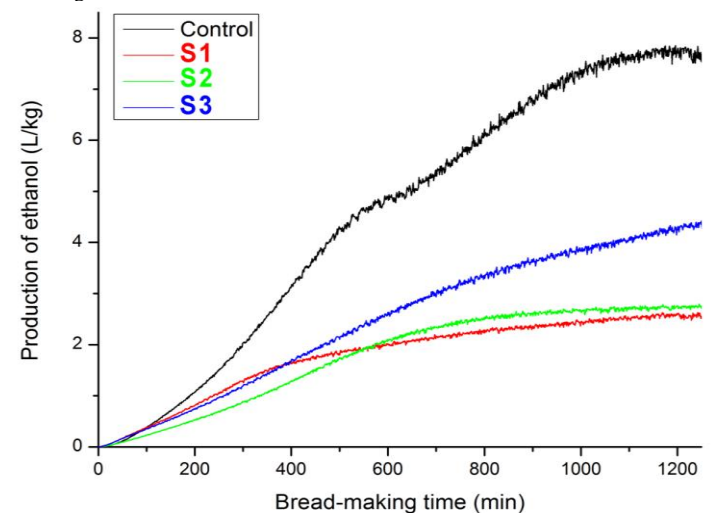
The control dough showed high production level of both CO<sub>2</sub> (Table 5) and ethanol (Table 6), this may be explained from a metabolic point of view by the yeast's ability to ferment a wide range of sugars, such as glucose, fructose, sucrose, maltose, and maltotriose, which are present in both ripe fruits and cereal flours. Additionally, the yeasts may survive in conditions at pH as low as 3.5 (Maicas, 2020).

**Table 6** Effect of sourdough on ethanol production during dough fermentation.

Fermented doughs	[Ethanol] <sub>EBM</sub> (ppm)	[Ethanol] <sub>MEE</sub> (ppm)	Ethanol production L/kg of dough
Control	25698 ± 4039 <sup>c</sup>	42101 ± 5607 <sup>c</sup>	5.58 ± 0,65 <sup>c</sup>
Dough + S1	8781 ± 712 <sup>a</sup>	10759 ± 324 <sup>a</sup>	2.16 ± 0,07 <sup>a</sup>
Dough + S2	10896 ± 1025 <sup>ab</sup>	11617 ± 535 <sup>a</sup>	2.63 ± 0,15 <sup>ab</sup>
Dough+ S3	15183 ± 1679 <sup>b</sup>	19331 ± 957 <sup>b</sup>	3.55 ± 0,32 <sup>b</sup>

[Ethanol]<sub>EBM</sub>: Concentration at the end of bread making; [Ethanol]<sub>MEE</sub>: Maximum concentration at end of experiment; Ethanol production: Production at the end of bread making; Control: Dough fermented without sourdough; Dough+S<sub>i</sub>: Dough fermented with S<sub>i</sub> sourdough (i: 1-3). Results are expressed as mean ± standard deviation. Values in the same column with at least one letter in common are not significantly different at the 5% probability level.

Figure 3 shows the production kinetics of ethanol released into the oven atmosphere during the fermentation process in the doughs. The ethanol production rate of the control dough is higher than that of the sourdough fermented doughs. According to Van Kerrebroeck et al. (2018), the ethanol determined in the headspace of the fermenter, measured by SIFT-MS, and the ethanol in the flour-water mixture, as determined by other techniques, are well correlated. Thus, the monitoring of the production of this gas during fermentation was facilitated by the sensor of this gas in the atmosphere of the fermenter used in the present work. This dosing technique can be an alternative to other techniques of analysis of this gas in the dough.



**Figure 3** Production of ethanol during dough fermentation process

**Total titratable acidity**

The TTA value represents the total amount of organic acids produced during fermentation (Decock & Cappelle, 2005). The results presented in Table 7 show that there is a significant effect of sourdough on the TTA values of the dough. In fact, the doughs fermented by sourdoughs (S1, S2 and S3) had TTA values that are

significantly higher than those of the control dough fermented by baking yeast. S1 and S3 sourdoughs exhibited similar TTA values, both at the beginning (3.2 mL NaOH 0.1M/10g) and at the end of the fermentation (8.2 mL NaOH 0.1M/10g). Our results are in accordance with those reported by Mueen-ud-Din et al. (2009), who reported a final TTA value that ranged between 3.5 and 9.7 mL NaOH 0.1M/10g, depending on the cultures used to prepare sourdoughs. Also, De Luca et al. (2021) reported that final TTA ranged between 6.32 and 7.32 mL NaOH 0.1M/10g in wheat flour based sourdoughs.

The direct influence of organic acids on the rheological properties of the dough has been intensively examined (Arendt et al., 2007). Yeasts work synergistically and antagonistically with LAB, producing organic acids that impact bacterial activity and sourdough flavor (Carbonetto et al., 2018). The fungistatic effect of sourdough has been attributed, in particular, to acetic acid produced by lactic acid bacteria (Corsetti et al., 1998).

**Table 7** Effect of sourdough on TTA (mL NaOH 0.1M/10g) of the dough.

Fermented doughs	TTA <sub>i</sub>	TTA <sub>f</sub>	Δ TTA
Control	2.4 ± 0.2 <sup>a</sup>	4.5 ± 0.1 <sup>a</sup>	2.1 ± 0.3 <sup>a</sup>
Dough+S1	3.1 ± 0.1 <sup>b</sup>	8.6 ± 0.8 <sup>b</sup>	5.4 ± 0.7 <sup>b</sup>
Dough+S2	4.1 ± 0.1 <sup>c</sup>	11.5 ± 1. <sup>c</sup>	7.4 ± 1.1 <sup>c</sup>
Dough+S3	3.2 ± 0.3 <sup>b</sup>	7.9 ± 0.3 <sup>b</sup>	4.6 ± 0.5 <sup>b</sup>

TTA<sub>i</sub>: Initial total titratable acidity; TTA<sub>f</sub>: final total titratable acidity; Δ TTA: Difference between TTA<sub>f</sub> and TTA<sub>i</sub>; Control: Dough fermented without sourdough; Dough+S<sub>i</sub>: Dough fermented with S<sub>i</sub>-sourdough (i: 1-3). Results are expressed as mean ± standard deviation. Values in the same column with at least one letter in common are not significantly different at the 5% probability level.

**pH kinetics**

The acidification of bread dough with added sourdough is mainly due to lactobacilli present in the sourdough (Lopez et al., 2001). A lowering of the pH is observed in the dough due to the production of biogenic acids including lactic and acetic acids by the microorganisms (Blandino et al., 2013), as well as by the carbon dioxide produced by the yeast. Indeed, acidifying power is the main metabolic property sought from lactic acid bacteria.

Although the initial pH values of the different doughs were different, there was no significant difference between their final pH values, whether they were fermented with sourdough or with baker's yeast (Table 8). Final pH values were 3.42 ± 0.02 and 3.53 ± 0.03 for the S2 and S3 doughs, respectively. The average final pH of doughs prepared with the sourdoughs was 3.47 and it is slightly lower than the pH of the control (pH = 3.74) and the value of 4.69 reported by Ameer et al. (2022) for durum wheat flour fermented with date seeds flour based sourdough. pH values between 4.22 and 4.30 have been reported by De Luca et al. (2021) for a fermented dough composed of wheat flour and Manitoba flour. Fermented doughs with sourdoughs S1, S2 and S3 had an average pH variation of 1.04 ± 0.05, while the control had the higher pH variation of 1.86 ± 0.17 due to its high initial value (5.58).

**Table 8** Effect of sourdough on pH of the dough during the fermentation process

Fermented doughs	pH <sub>i</sub>	pH <sub>f</sub>	Δ pH
Control	5.58 ± 0.03 <sup>c</sup>	3.74 ± 0.16 <sup>a</sup>	1.86 ± 0.17 <sup>c</sup>
Dough+S1	4.62 ± 0.02 <sup>b</sup>	3.47 ± 0.05 <sup>a</sup>	1.15 ± 0.05 <sup>b</sup>
Dough+S2	4.26 ± 0.06 <sup>a</sup>	3.42 ± 0.02 <sup>a</sup>	0.85 ± 0.07 <sup>a</sup>
Dough+S3	4.65 ± 0.01 <sup>b</sup>	3.53 ± 0.03 <sup>a</sup>	1.12 ± 0.03 <sup>ab</sup>

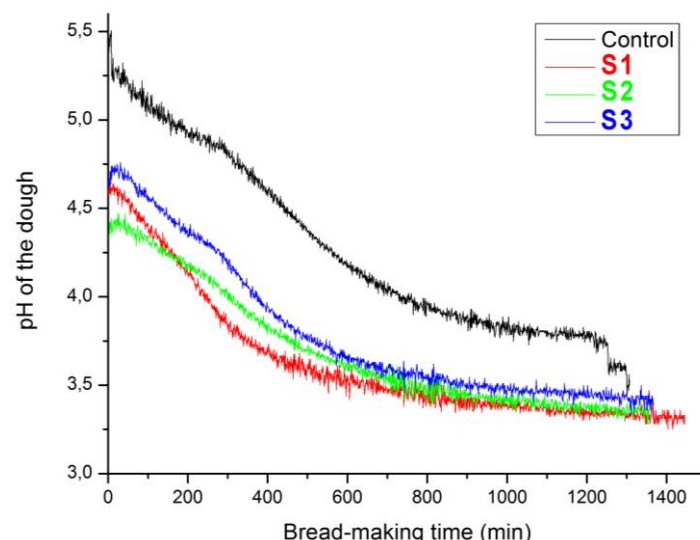
pH<sub>i</sub>: Initial pH of the fermented dough; pH<sub>f</sub>: final pH of the fermented dough; Δ TTA: Difference between TTA<sub>f</sub> and TTA<sub>i</sub>; Dough+S<sub>i</sub>: Dough fermented with S<sub>i</sub> sourdough (i: 1 - 3). Results are expressed as mean ± standard deviation. Values in the same column with at least one letter in common are not significantly different at the 5% probability level.

The pH trend of the dough during fermentation is shown in Figure 4. At the beginning of fermentation, the pH values of the sourdough-fermented doughs ranged from 4.26 to 4.65, while at the end of fermentation, all doughs had an average pH of 3.47. Also, the pH values of the sourdough-fermented doughs were lower than those of the control throughout the fermentation period. Torrieri et al. (2014) reported a pH between 4.73 and 5.03 depending on the concentration of the sourdough used to prepare the bread.

The addition of prickly pear peels (PPP) and seeds press-cake (SPC) in a fermentation process can reduce the initial pH value. According to Martău et al. (2021), a similar decline in pH was reported for different types of sourdoughs in various fermentation conditions (Hashemi et al., 2019; Kocková et al., 2013; Teleky et al., 2020). Reducing pH is crucial for the activity of endogenous wheat proteases, which act on the gluten together with bacterial hydrolytic enzymes and decreasing dough firmness (Amr & Alkhamaiseh, 2022).

The lower pH and higher TTA of dough with PPP and SPC sourdoughs can be explained by more activation of microflora due to compounds brought by the added peel puree in the sourdough formulation. (Catzeddu, 2019; Martín-García et al., 2022) reported that the gradual decrease in pH during fermentation promotes the

activity of several enzymes, such as amylases and proteinases, which are active at low pH values (Catzeddu, 2019; Martín-García et al., 2022). Acidity and pH are stable in the initial phase, then followed by a rise during the intermediate phase. According to (Chavan & Chavan, 2011), the titratable acidity and pH of the dough are key parameters during fermentation. These authors reported that during the long-term dough fermentation phase, the presence of yeast becomes negative, and the dough's titratable acidity and pH mostly rely on the amount of LAB in the system. However, acetic acid has a considerably greater impact on the yeasts in sourdough than does lactic acid Sourdoughs performance.

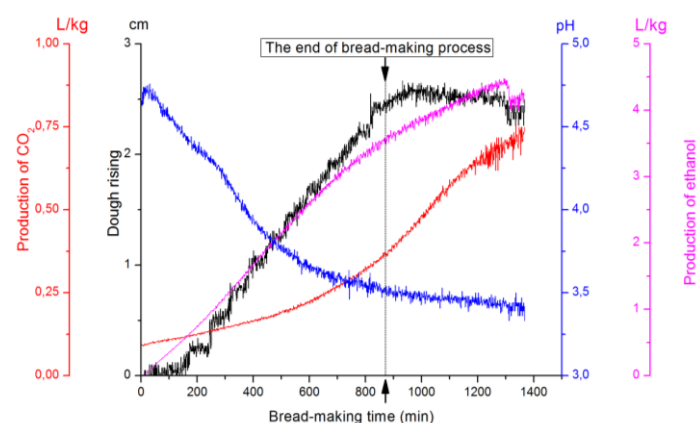


**Figure 4** Evolution of pH during the dough fermentation process

**Sourdough's thumbprint**

According to Carnevali et al. (2007), there is still much to discover about liquid sourdough. It is a promising, natural, and scalable technology that, because of the difficulty of its reproduction, may provide a high degree of authenticity and act as an effective tool for customizing the quality of baked products. Figure 5 shows the trend of the main performance parameters of the S3 liquid sourdough-fermented dough. This visual representation makes it easy to evaluate the performance of the sourdoughs and constitutes a fingerprint, which could be considered as a unique identifier of each sourdough.

Compared to other sourdoughs, the S3 liquid sourdough showed better performance in terms of bread making time, specific volume, dough rising capacity and production of ethanol and CO<sub>2</sub>. The industrial use of liquid sourdough can offer several advantages to the baking industry and really contribute to the creation of a wide variety of products that are characterized by their specific flavor, texture and health benefits (Carnevali et al., 2007).



**Figure 5** Performance thumbprint for S3 sourdough-fermented dough

**Comparison of fermented dough's profiles**

According to Amr & Alkhamaiseh (2022), the microbial flora in sourdough is generally different from that of the flour used as a raw material, which explains the variation in the values of the fermentation parameters when utilizing various sourdoughs. Figure 6 shows the trends for the main parameters of the S1, S2, S3 and control doughs. This representation makes it easy to compare the performance of the sourdoughs for each parameter, according to the profile of each fermented dough.

The growing importance of sourdoughs is a consequence of consumer demand for breads that are flavorful, additive-free, and have a long shelf life. Furthermore, the usage of sourdough and the advancement of modern sourdough manufacturing tools and procedures have simplified the management of sourdough production in both artisan and commercial bakeries (Amr & Alkhamaiseh, 2022). The selection of the best sourdoughs would be made much easier by comparisons based on scientific parameters using sourdough fingerprints (Figure 5) and profiles (Figure 6). The composition and preparation methods may have an impact on

sourdough performance. The main goal is to find a sourdough with microbial flora that are well-adapted to produce enough CO<sub>2</sub> to allow the dough to rise, as well as organic acids and other metabolites to provide bread good texture, sensory characteristics, and a longer shelf life (Häggman & Salovaara, 2008). The performance of a sourdough is mainly influenced by its flora, which varies according to the flour, the ingredients used to prepare the dough and the environment used during the bread-making process (Kezer, 2022).

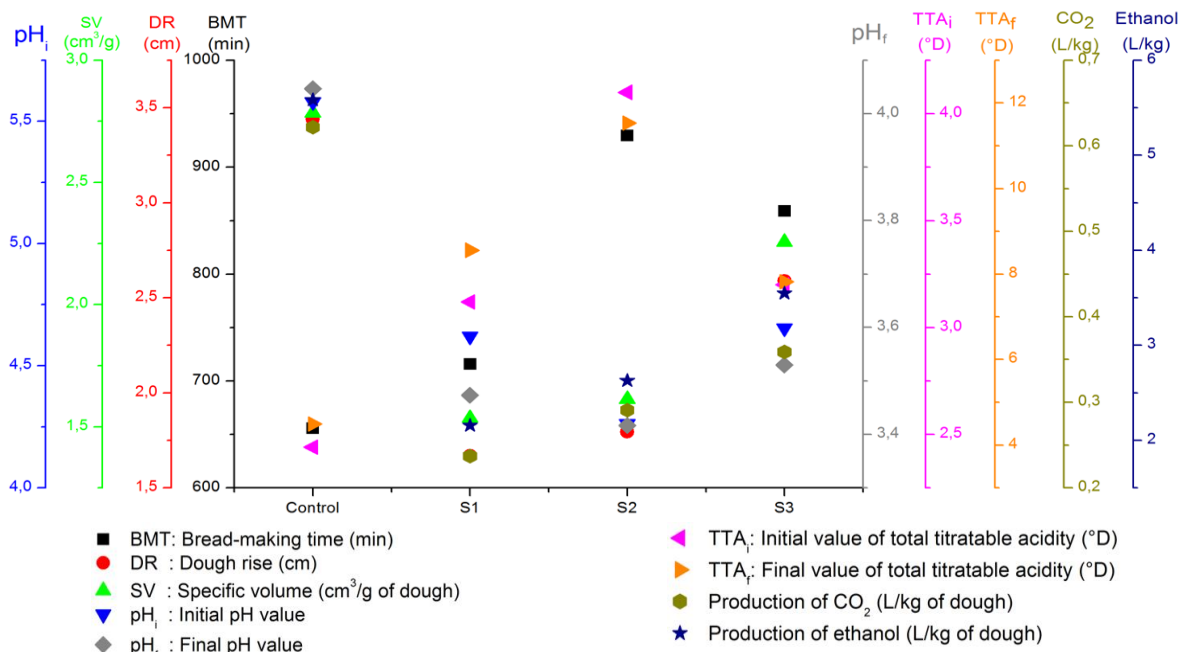


Figure 6 Physico-chemical parameters of fermented doughs.

CONCLUSION

The prickly pear fruit's peel (PPP) and seed press-cake (SPC) are discarded during the pulp and the seed-oil processing. This research investigated the bread-making capacity of sourdoughs containing these two by-products. The utilization of sourdough is one of the first biotechnology methods used in the preparation of traditional cereal based foods and baked products.

The pH values of the prepared sourdoughs were statistically similar with an average of 3.24. The TTA values for the S1, S2 and S3 sourdoughs were 15.5, 27.4 and 13.4 mL of 0.1M NaOH/10g, respectively. The Type II liquid sourdough (S3) had the highest dough yield value of 384% when compared to S1 (228%) and S2 (157%). The Sourdough-fermented doughs had different rising capacities (167-259%) and specific volumes (1.53-2.26 cm<sup>3</sup>/g). The dough fermented with liquid sourdough (S3) recorded the highest specific volume, which is comparable to that of the control dough (2.78 cm<sup>3</sup>/g) fermented with baker's yeast alone. The S1 and S3 liquid sourdoughs required comparable dough fermentation time to the control dough with an average of (787 min). However, the S2 sourdough showed higher fermentation time (930 min)

The sourdough-fermented doughs showed similar CO<sub>2</sub> quantities released at the end of fermentation process, but less than the control dough. More ethanol was produced by the doughs fermented with the peel-based sourdoughs (S2 and S3) than those containing seed press-cake sourdough (S1) and control dough. The final mean pH of fermented doughs was 3.47 and their final TTA ranged from 7.8 to 11.5 mL NaOH 0.1M/10g. The control dough, which was fermented using just baker's yeast, had a similar pH to the sourdough-fermented doughs and a low final TTA of 4.5 mL NaOH 0.1M/10g.

The studied sourdoughs showed a good capacity to ferment the dough, compared to baker's yeast. Thus, this research proposed a sustainable bio-recycling of PPP and SPC as innovative sourdough bread ingredients. Our results suggest using prickly pear by-products in sourdoughs to decrease food waste and increase the quality of baked products. Volatile substances and sourdough bread sensory analysis are needed to get consumer acceptance. Also, the microorganisms in sourdough and bread should be investigated to understand how PPP and SPC impact bread aroma and flavor.

**Authors contribution:** SBAGHI Mohamed and El-AALAOUI Mohamed provided the plant material and reviewed the manuscript. CHAFAI Youssef and RAFFAK Anas conducted the experiments. CHAFAI Youssef wrote the main manuscript text. DJERRARI Abdellatif and ZAHAR Mohamed supervised the study and reviewed the manuscript.

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**Data Availability:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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