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REVIEW ARTICLES

A systematic review of drying methods and their impact on technological characteristics of sourdough type III

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Highlights

- Choosing the best drying method enables good cell viability.
- Application of protective agents results in a viable type III sourdough.
- Process parameter optimization results in increased cell viability.
- Spray-drying is a promising technique that can replace freeze-drying.

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KEYWORDS
Bread;
Spray-drying;
Freeze-drying;
Bakery;
Cell viability;
Baked goods.

Abstract: Type I sourdough refers to a natural fermentation of flour and water used to manufacture baked goods, with specific sensory, technological, and nutritional attributes. However, it is a slow process, quite laborious, and that occurs without standardization. Thus, on an industrial scale, sourdough fermentation becomes viable from the use of type III sourdough, in which microbial cultures are selected and stabilized by drying, and the results in terms of quality of the final product are standardized. However, dry sourdough is challenging; it is preferable to preserve viable cells for further fermentation and the aromas generated in the first fermentation. The objective of this review was to address the influence of drying methods on the technological characteristics of type III sourdough. This study was based on the PRISMA methodology, searching for scientific articles in four databases: Scielo, Science Direct, Scopus, and PubMed, with the descriptors: "sourdough" OR "sourdough drying" OR "sourdough dried". The search resulted in 6,429, of which only 23 articles addressed the researched topic. The main sourdough drying methods found were oven drying, freeze-drying, and spray-drying. It is noteworthy that low temperatures and vacuum during the freeze-drying, and the sample's short-residency time during the spray-drying process, better preserve nutrients and microorganisms viability. Methods that include protective agents can increase cell viability and extend the storage time of dehydrated sourdough. The physicochemical characteristics of type III sourdough and baked goods, in addition to sensory analysis, indicate a promising industrial application. Large-scale production of type III sourdough from spray-drying is a viable alternative for operational costs and continuous production; however, studies should focus on obtaining better microbial survival.

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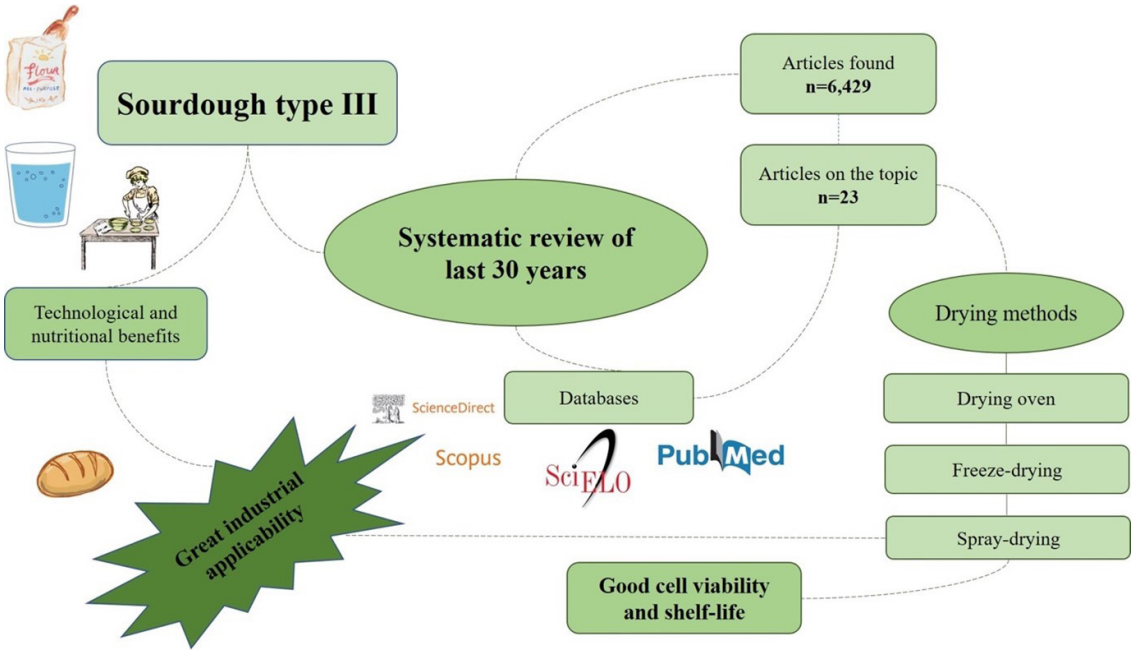
GRAPHICAL ABSTRACT



Introduction

Sourdough is a complex biological ecosystem made from a mixture of water and wheat flour and/or other cereal, which ferments naturally by the action of lactic acid bacteria (LAB) and yeasts present in the raw material and production environment (Arendt et al., 2007). This traditional process has been perpetuated for centuries; however, more recently, due to an increased interest in the topic, numerous scientific research has shown that sourdough fermentation provides unique technological and nutritional characteristics to bakery products (Arora et al., 2021).

Currently, sourdough is classified into four types (I, II, III, IV) (Figure 1). The traditional sourdough (type I) is the result of spontaneous fermentation – microorganisms from water, flour, and production environment - that requires a continuous maintenance process (known as backslopping, in which a portion of the previous ferment is used to inoculate a new portion of flour and water, daily). The propagation of this type of sourdough occurs slowly, taking five to ten days for the microbiota stabilization. So, the technological parameters such as aroma, acidity, and gluten structure take the same time to be minimally stable (Brandt, 2019).

Sourdough type II, recognized as industrial sourdough, is a liquid mass with a reduced fermentation time than type I sourdough, resulting from the addition of starter cultures to the flour and water mixture. Its elaboration avoids the backslopping steps, in addition to controlling microbial fluctuations over time, allowing the development of aroma, flavor, and other specific characteristics of the product. When type II is dried, *e.g.*, by hot air drying oven, freeze-drying, spray-drying, or fluidized bed, it results in type III sourdough (Brandt, 2019; De Vuyst et al., 2017). Finally, the combination of sourdough types I and II gives sourdough type IV, propagated employing backslop, conventionally used in artisanal bakeries and scientific studies (Catzeddu, 2019; Siepmann et al., 2018).

Microbiota associated with type I sourdough is broad, with a predominance of homofermentative and/or heterofermentative LAB symbiosis with yeast (Gänzle & Ripari, 2016). The vast biodiversity is due to factors such as the substrate present in the dough and the process parameters used, such as temperature and fermentation time (Menezes et al., 2020). The main LAB strains found in type I sourdough and strongly applied to the other types of sourdough are *Fructilactobacillus sanfranciscensis*, *Lactiplantibacillus plantarum*, *Companilactobacillus paralimentarius*, *Levilactobacillus brevis*, *Pediococcus pentosaceus*, *Limosilactobacillus fermentum*, and *Lacticaseibacillus casei* (Gänzle & Ripari, 2016; Menezes et al., 2021). The composition of the sourdough's microbiota influences the characteristics of the baked product, bringing many benefits arising mainly from the metabolic processes of these microorganisms. For example, amino acid catabolism can generate aromatic and flavor compounds, improving the sensory profile; produce exopolysaccharides and enzymatic reactions improve texture, with higher volumes and soft crumb breads; released antimicrobial compounds slow spoiling by fungi, extending shelf-life.

The complex microbiota also favors nutritional aspects. This may increase mineral bioavailability and protein digestibility and reduce the fermentable content of oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs), and phytates (Ertop & Coşkun, 2018; Gobbetti et al., 2005; Gocmen et al., 2007; Menezes et al., 2021). Another relevant feature of sourdough fermentation is the variation of the volatile organic compounds (VOCs) profile present in the products since the synthesis of VOCs depends on both the

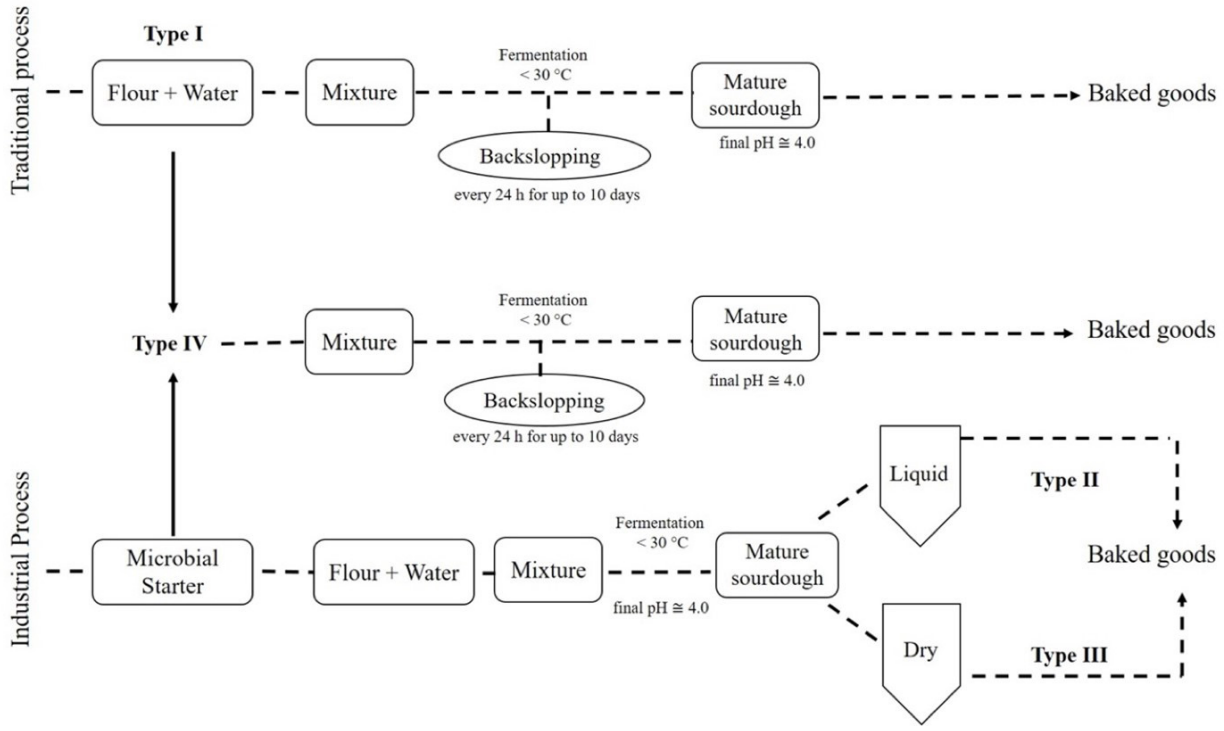


Figure 1. Flowchart of sourdough types I, II, III, and IV production processes. Adapted from Chavan & Chavan (2011).

existing microbiota and the chemical reactions that occur during fermentation. In the literature, major compounds such as aldehydes, ketones, alcohols, and acids, among others (Arora et al., 2021; Liu et al., 2020), are related to a profile highly appreciated by consumers found in sourdough products.

The drying process is widely used in food preservation. Different techniques can be applied to dry sourdough, such as freeze-, spray-, drum-, oven-, and fluidized bed drying (Brandt, 2019). However, to obtain a quality type III sourdough, the method chosen needs to be efficient to the point of achieving a reduction in moisture (<7%) and, mainly, in water activity (a_w) (below 0.3) sufficient to prevent reactions from occurring biochemical and microbiological processes in the dried powder, thus ensuring stability and extended shelf-life (Reale et al., 2019). Even though type III sourdough is easier to store, transport, and market than type II, high-temperature drying may negatively affect the viability of yeast and LAB due to heat stress during dehydration (Tan et al., 2018). In the literature, there are few studies on the drying of this specific matrix, with freeze-drying being the preferred method due to greater cell preservation. However, this process is expensive and time-consuming. In contrast, spray-drying is a cheaper method, with continuous production, and operates on a large scale (Caglar et al., 2021). A good cell survival rate depends on optimizing drying parameters and using cell-protective agents (Mantzourani et al., 2019; Mohd Roby et al., 2020; Peighambardoust et al., 2011; Stefanello et al., 2019).

The objective of this systematic review was to compile the results of sourdough type III, found in the last thirty years, identifying the parameters of the different drying techniques, the physical-chemical properties, and its use as a starter for the production of bakery products. In addition, the main characteristics of food products made from type III sourdough were addressed.

Method

The survey of articles for this systematic review was conducted through four bibliographic databases: Scielo, Science Direct, Scopus, and PubMed. The stipulated search period was set for the last 30 years (December 1991 to January 2021). Although there are several studies in the literature related to the drying of LAB, for the most varied applications, we aimed to search only for articles associated with the drying of the sourdough to describe the characteristics of type III sourdough. The descriptors used in this research were: “sourdough” OR “sourdough drying” OR “dried sourdough”, which are intentionally comprehensive. There was no language restriction. The process of selection and inclusion of articles was recommended by the PRISMA method guidelines (Page et al., 2021), following the steps described in Figure 2. Two authors performed all steps, including inclusion and exclusion criteria and data extraction, to avoid bias.

Results

Databases search resulted in 6,429 articles. Nine hundred seventeen articles were excluded from the study by duplication, resulting in 5,512. The title and abstracts of the remaining articles were carefully studied and selected

according to the adequacy of the objectives of this review. One hundred twenty-five articles were separated for full reading, excluding 5,387. At the last stage, 23 articles were chosen for this systematic review. A flowchart showing the study selection process is shown in Figure 2.

Of the 23 studies selected from 1991 to 2021, only 43% (n=10) were published in the last five years, and 17% (n=5) were published in the 1990s. A timeline showing the years and the number of publications is shown in Figure 3.

The language was not a requirement for selection, so we found two articles in Persian (Khorasanchi et al., 2011; Peighambardoust et al., 2011) and the rest written in English. All studies have been published in scientific journals, except that of Ćurić et al. (2006), which is published in the annals of a congress. All articles are original research. A summary of the study characteristics and the main objectives of each study is included in Table 1. The included studies were conducted in different countries, namely: five in Turkey (Caglar et al., 2021; Ertop et al., 2018; Ertop & Coşkun, 2018; Gul et al., 2020a, b), four in Iran (Khorasanchi et al., 2011; Peighambardoust et al., 2011; Tafti et al., 2013a, b), three in Brazil (Aplevicz et al., 2014; Stefanello et al., 2018, 2019) and Italy (Cossignani et al., 1996; Lattanzi et al., 2014; Reale et al., 2019), two in Germany (De Valdez & Diekmann, 1993; Meuser et al., 1995) and Poland (Różyło et al., 2015a, b), one in Croatia (Ćurić et al., 2006), Greece (Mantzourani et al.,

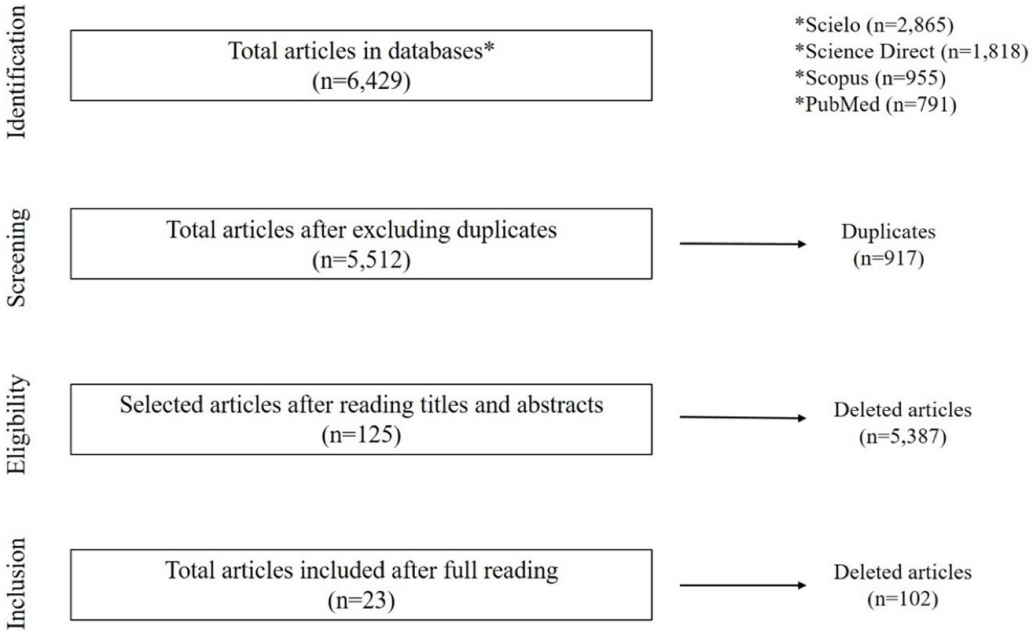


Figure 2. Flowchart of the article inclusion process by the PRISMA method. Adapted from Page et al. (2021).

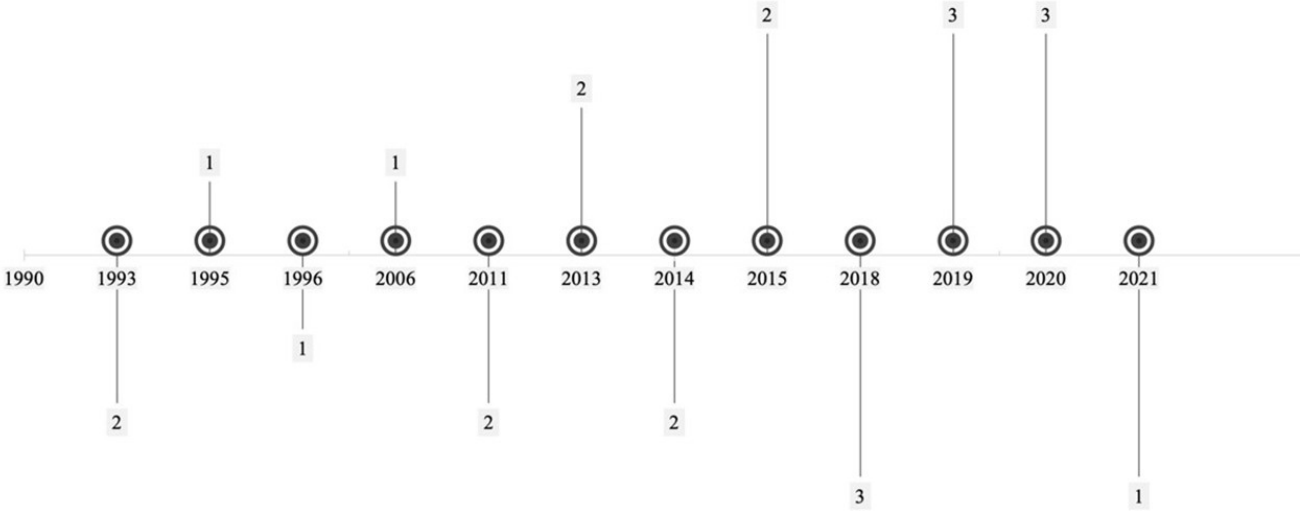


Figure 3. Timeline of the sourdough drying publications between the years 1990 and 2021.

Table 1. Revised studies on sourdough drying by different methods.

Method	Reference	Microbial starter	Raw material	Protective agents	Process parameters	Initial viability (log CFU/g)	Viability after drying (log CFU/g)	Shelf-life (log CFU/g)	The objective of the study
Drying oven	Reale et al. (2019)	Sourdough type I	Wheat	N.A	Temperature of 40 °C/48 h under vacuum (-87 kPa)	9.17 - LAB 7.53 - yeast	5.77 - LAB 4.53 - yeast	Unrealized	Evaluate the performance of dry sourdough by different drying methods regarding its fermentation capacity and the sensory characteristics of the breads
	Lattanzi et al. (2014)	*Lac. plantarum; Fru. sanfranciscensis; Fur. rossiae; S. cerevisiae* combined to form the starter pool	Wheat	N.A	Temperature of 40 °C/48 h	≅ 8.50 - LAB ≅ 7.30 - yeast	Unrealized	≅ 8.50 - LAB ≅ 5.00 - yeast (after 3 months of storage)	Test the influence of different storage conditions (4 °C, -20 °C, and dry) on microbial survival, and the technological performance in sourdough fermentation of three associations of LAB and yeast
	Ćurić et al. (2006)	*Lev. brevis* + *Lac. plantarum* + *Fru. sanfranciscensis*	Rye + Wheat	N.A	Oven at a temperature of 40 °C/24 h	9.07 - LAB	8.36 - LAB	Unrealized	Three drying methods were applied to determine the best cell viability and the concentration of organic acids in sourdough type III, in addition to evaluating the sensory properties of the breads produced
					Vacuum oven at a temperature of 40 °C/3.5 h	9.07 - LAB	8.77 - LAB	Unrealized	
	Ertop & Coşkun (2018)	Sourdough type I	Chickpea	N.A	Temperature of 35 °C until moisture content is reached de 4-5%.	9.47 - LAB 5.30 - yeast	8.25 - LAB 1.30 - yeast	Unrealized	A chickpeas-based sourdough, spontaneously fermented and oven-dried, was applied in the formulation of breads and the physical-chemical properties and shelf-life were evaluated
	Ertop et al. (2018)	Sourdough type I	Wheat	N.A	Temperature of 37 °C until moisture content is reached de 4-5%	9.30 - LAB	7.70 - LAB	Unrealized	Obtain two sourdough type III (one spontaneous fermentation and one with starter culture) dried by different drying methods. Physical-chemical and sensory characteristics of the bread were evaluated with different proportions of dry sourdough (3%, 6% and 12%)
		L. delbrueckii + *Lev. brevis* + *Lac. plantarum*	Wheat	N.A	Temperature of 37 °C until moisture content is reached de 4-5%	8.05 - LAB	7.48 - LAB	Unrealized	

N.A: not applied.

Table 1. Continued...

Method	Reference	Microbial starter	Raw material	Protective agents	Process parameters	Initial viability (log CFU/g)	Viability after drying (log CFU/g)	Shelf-life (log CFU/g)	The objective of the study
Freeze-drying	Reale et al. (2019)	Sourdough type I	Wheat	N.A	Temperature of 20 °C /48 h under a vacuum of 15 Pa	9.17 - LAB 7.53 - yeast	6.07 - LAB 5.03 - yeast	Unrealized	Evaluate the performance of dry sourdough by different drying methods regarding its fermentation capacity and the sensory characteristics of the breads
	Ertop et al. (2018)	Sourdough type I	Wheat	N.A	Temperature of -68 °C under vacuum, up to 4-5% moisture content.	9.30 - LAB	8.60 - LAB	Unrealized	Obtain two sourdough type III (one spontaneous fermentation and one with starter culture) dried by different drying methods. Physical-chemical and sensory characteristics of the bread were evaluated with different proportions of dry sourdough (3%, 6% and 12%)
		L. delbrueckii + *Lev. brevis* + *Lac. plantarum*	Wheat	N.A	Temperature of -68 °C under vacuum, up to 4-5% moisture content	8.05 - LAB	8.30 - LAB	Unrealized	
	Różyło et al. (2015a)	Commercial bakery starter	Buckwheat	N.A	Temperature in the ice condensator: -55 °C; Different heating temperatures (20, 40 e 60 °C); pressure in the drying chambre: -63 Pa. Moisture 3%	Unrealized	Unrealized	Unrealized	Examine the rheological and sensory properties of gluten-free breads prepared with buckwheat sourdough and commercial starter culture. Sourdough dehydration was carried out at different freeze-drying temperatures
	Różyło et al. (2015b)	Commercial bakery starter	Amaranth	N.A	Temperatures of 20 and 40 °C and with a constant pressure of 52 Pa	Unrealized	Unrealized	Unrealized	Sourdough from amaranth, fermented by starter cultures, was freeze-drying at different temperatures, and its performance was evaluated for rheological and sensory properties in breads
	Caglar et al. (2021)	Sourdough type I	Wheat	N.A	56 h drying under vacuum at 15 Pa	9.70 - LAB 9.70 - yeast	8.00 - LAB 8.00 - yeast	Unrealized	Produce dry sourdough using different drying methods, assessing fermentation capacity and cell viability, as well as rheological properties of the dough and bread production

N.A: not applied.

Table 1. Continued...

Method	Reference	Microbial starter	Raw material	Protective agents	Process parameters	Initial viability (log CFU/g)	Viability after drying (log CFU/g)	Shelf-life (log CFU/g)	The objective of the study
Freeze-drying	Mantzourani et al. (2019)	*Lact. paracasei* + *L. bulgaricus*	Wheat	Wheat bran	Uninformed	8.90 – LAB 7.50 - yeast	9.00 – LAB 7.70 – yeast	Unrealized	Strains of *L. paracasei* and *L. bulgaricus* immobilized in wheat bran were freeze dried and applied as biocatalysts for the manufacture of breads. Physical-chemical characteristics, shelf-life of the breads and aromatic compounds were analyzed
	Gul et al. (2020a)	*Lev. brevis* ED25	N.A	skim milk; lactose;sucrose	Temperature of -80°C/ 18 h chamber pressure of 8 Pa	9.50 - LAB	9.30 - LAB	9.1 – LAB (after 6 months of storage at 4 °C)	Evaluate the effect of cryoprotectants on the viability of *L. brevis* ED25 during freezing and freeze-drying, performing the characterization of freeze dried powders, stability during storage (4 and 25 °C) for 6 months, acidification and fermentation capacity of the sourdough freeze dried
	Gul et al. (2020b)	*Lat. curvatus* N19	Wheat	Skim milk + lactose + sucrose	Temperature -55 °C/18 h, with chamber pressure of 6.1 Pa	9.50 – LAB	9.30 – LAB	8.80– LAB (stored at 4 °C for 6 months)	Use cryoprotectants to increase the cell viability of *L. curvatus* during freeze-drying and the stability during storage. In addition, determined the kinetics of acidification of the cryopreserved strain during sourdough fermentation
	Martínez-Anaya et al. (1993)	*S. cerevisiae; S. fructuum; C. boidinii; Lev. brevis e Lac. plantarum* combined to form the starter pool	Wheat	Skim milk	Undescribed	8.0 – LAB 5.0 – yeast	Unrealized	Unrealized	Evaluate the dehydrated starter pool regarding the production of organic acids, sugars and volatile composition in the bread dough, in addition to the fermentative and rheological performance in the bread

N.A: not applied.

Table 1. Continued...

Method	Reference	Microbial starter	Raw material	Protective agents	Process parameters	Initial viability (log CFU/g)	Viability after drying (log CFU/g)	Shelf-life (log CFU/g)	The objective of the study
Freeze-drying	Cossignani et al. (1996)	*Lac. plantarum; Fru. sanfranciscensis; S. cerevisiae; S. exiguus*	Wheat	Glycine 1%; glutamic acid 1%; maltose 3%; bovine serum albumin 3%	Heater temperature -40 and 20 °C and condenser temperature -45 °C. Pressure from 133,32 to 13,33 Pa/18 h	≅ 9.00 - LAB ≅ 8.00 - yeast	≅ 8.80 -LAB ≅ 7.00 - yeast	Unrealized	From freeze dried and fresh strains of LAB and yeasts have evaluated the microbiological characteristics, physical-chemical, aromatic composition and organic acid production in sourdough
	De Valdez & Diekmann (1993)	*Lim. reuteri*	wheat	Glutamate 5%; glucose 7%; maltose 7%; skim milk 10% (control)	Uninformed	10.00 - LAB	9.80 - LAB	9.00 - LAB(after 6 months of storage at 4°C with 5% glutamate cryoprotectant solution)	Evaluate the efficiency of different protective agents in cell viability of *L. reuteri* after freeze-drying
	Stefanello et al. (2018)	Sourdough type I	Wheat + whole wheat + malt extract	Trehalose three concentrations (0, 10 and 15%)	Temperature -37 °C / 60 h with camera pressure of 40 Pa	9.02 - LAB 7.44 - yeast	9.00 - LAB 7.00 - yeast	7.52 - LAB 4.00 - yeast(after 45 days of storage at room temperature)	Evaluate the cryoprotectant effect of trehalose on the survival of microorganisms present in type I sourdough and identified the bacteria and yeasts present in type I sourdough
	Stefanello et al. (2019)	*Lim. fermentum* + *W. anomalus*	N.A	Four cryoprotectable solutions: peptone 0.1%; sucrose 10%; trehalose 5%; skim milk 10%;sodium L-glutamate 5% + skim milk powder	Temperature -50 °C/24 h with camera pressure of 15 Pa	10.0 - LAB 9.0 - yeast	10.0 - LAB 8.7 - yeast	8.7 - LAB 7.0 - yeast (after 12 months of storage at room temperature with the sodium cryoprotectant solution L-glutamate 5% + skim milk powder)	Evaluate the survival assessment of *L. fermentum* and *W. anomalus* freeze dried using four cryoprotectant solutions, and their influence on shelf-life for 12 months
	Khorasanchi et al. (2011)	*Lac. plantarum, Lim. reuteri*	Wheat	N.A	Undescribed	8.0 - LAB	7.5 - LAB	Unrealized	The strains *L. plantarum*, *L. reuteri* and a mixture of both bacteria after freeze-drying were evaluated for fermentation capacity and cell viability
	Peighambardoust et al. (2011)	*Lac. plantarum* +*Lim. reuteri*	Wheat	N.A	Undescribed	Unrealized	Unrealized	Unrealized	Evaluate the effect of adding freeze dried sourdough containing *L. plantarum* and *L. reuteri* on the physical-chemical and sensory characteristics of bread

N.A: not applied.

Table 1. Continued...

Method	Reference	Microbial starter	Raw material	Protective agents	Process parameters	Initial viability (log CFU/g)	Viability after drying (log CFU/g)	Shelf-life (log CFU/g)	The objective of the study
Freeze-drying	Aplevicz et al. (2014)	*Lact. paracasei LP1Lact. paracasei LP2 S. cerevisiae SC1S. cerevisiae SC2*	Grape	N.A	Undescribed	9.40 - LP1 11.00 - LP2 8.18 - SC1 7.16 - SC2	Unrealized	Unrealized	*L. paracasei and S. cerevisiae* isolated from grape sourdough were freezer dried and applied in the production of breads. The physical-chemical, rheological and sensory properties of the loaves were analyzed
Spray-drying	Ertop et al. (2018)	Sourdough type I	Wheat	N.A	Inlet air Temperature 130 °C; Outlet air Temperature 65 °C, moisture content 4-5%	9.30 - LAB	9.70 - LAB	Unrealized	Obtain two sourdough type III (one spontaneous fermentation and one with starter culture) dried by different drying methods. Physical-chemical and sensory characteristics of the bread were evaluated with different proportions of dry sourdough (3%, 6% and 12%)
		L. delbrueckii + Lev. brevis + Lac. plantarum	Wheat	N.A	Inlet air Temperature 130 °C; Outlet air Temperature 65 °C, moisture content 4-5%	8.05 - LAB	9.60 - LAB	Unrealized	
	Tafti et al. (2013a)	*Com. paralimentarius*	Whole wheat	N.A	Inlet air Temperature 180 °C	8.0 - LAB	Unrealized	Unrealized	Evaluate the effect of sourdough type III dried by spray-drying prepared from *C. paralimentarius*, and its effects on the physical-chemical and rheological characteristics of the flour added different concentrations of dry sourdough (3, 6, 9 and 15%)
	Tafti et al. (2013b)	*Com. paralimentarius*	Wheat	N.A	Inlet air Temperature 180 °C;Outlet air Temperature 90 °C;	9.30 - LAB	5.30 - LAB	Unrealized	Produce spray-dried sourdough type III containing *C. paralimentarius* and investigated the physical-chemical and microbiological properties. In addition, the functionality of dry sourdough in improving the quality of Sangak flat bread was studied
	Reale et al. (2019)	Sourdough type I	Wheat	N.A	Inlet air Temperature 130 °C;Outlet air Temperature 54 °C; Aspiration rate 95%;Feed flow rate 8 mL/min.	9.17 - LAB 7.53 - yeast	8.07 -LAB 5.93 - yeast	7.0 - LAB 5.1 - yeast (after 6 months)	Evaluate the performance of dry sourdough by different drying methods regarding its fermentation capacity and the sensory characteristics of the breads

N.A: not applied.

Table 1. Continued...

Method	Reference	Microbial starter	Raw material	Protective agents	Process parameters	Initial viability (log CFU/g)	Viability after drying (log CFU/g)	Shelf-life (log CFU/g)	The objective of the study
	Caglar et al. (2021)	Sourdough type I	wheat	N.A	Inlet air Temperature 160 °C;Outlet air Temperature 90 °C; Feed flow rate 34 mL/ min. moisture content of the final product below 5%	9.7 - LAB 9.7 - yeast	5.0 - LAB 4,9 - yeast	Unrealized	Produced dry sourdough using different drying methods, assessing fermentation capacity and cell viability, as well as rheological properties of the dough and bread production
Spray-drying	Mohd Roby et al. (2020)	Sourdough type I prepared with kombucha	Wheat	Arabic gum (7%)	Inlet air Temperature 140 °C;Outlet air Temperature 100 °C;Feed flow rate: 30 rpm	11.00 - LAB 10.50 - yeast	9.93 - LAB 9.40 - yeast	unrealized	Identify the potential for fermentation and bread production with sourdough prepared from Kombucha and dried by spray-drying technique. Characteristics such as specific volume, firmness of the kernel, water activity, shelf-life and consumer acceptance were some of them was applied in the analyses

N.A: not applied.

2019), Spain (Martínez-Anaya et al., 1993), and Malaysia (Mohd Roby et al., 2020).

Discussion

Drying methods for sourdough: advantages and disadvantages

Drying oven

A drying oven is a batch process that can operate by convection or vacuum. In the first case, it is based on the removal of moisture, creating a heat transfer gradient from the surface to the geometric point (α) of the food at atmospheric pressure. The use of forced air at high-speed circulating favors the exchange of heat. In the vacuum, drying chamber pressure is reduced, and consequently, the vaporization temperature is also reduced, causing water removal at low temperatures, protecting thermosensitive compounds, and avoiding drastic reductions of microorganisms and oxidation reactions. The method has few parameters to be controlled, such as air temperature and speed and/or vacuum, but the process requires a longer drying time and requires specific care regarding non-enzymatic browning resulting from the Maillard reaction (Santivarangkna et al., 2008).

Organic acids produced by LAB during fermentation are proton carriers essential for triggering one of the stages of the Maillard reaction, the Amadori rearrangement (El-Dash, 1971). Ertop et al. (2018) reported that the colorimetric parameters, a* (red to green axis) and b* (blue to yellow axis), were increased in breads produced with sourdough dried in a drying oven at 36 - 38 °C for 6 h. These values were visualized in breads prepared with type I and type II sourdough (starters: *Lactobacillus delbrueckii*, *Lev. Brevis,* and *Lac. plantarum*). That is related to melanoidin pigment formation, which is usually polymers with nitrogen in their structure and is the result of compounds degradation that occurs during the Maillard reaction. Thus, both long-term oven drying and acidified products from sourdough are factors that tend to accentuate the brown color of baked goods. Staining is a criterion to be verified for obtaining a quality product (Ertop et al., 2018) since the intensity with which it occurs may influence the consumer's acceptance of the product.

Freeze-drying

Freeze-drying is composed of two steps, sample freezing, and gradual temperature rise, which occurs in primary and secondary dryings. Water removal occurs due to sublimation of the substance, which is at a temperature and partial pressure of water vapor below the triple point of water. In the first stage, freezing can be performed inside the freeze-drying itself or in external equipment, considering the desired freezing rate (°C/min), that is, slow or fast freezing, which will influence the position of the crystals and thus the formation of pores (Bhatta et al., 2020). The slow freezing promotes the formation of large ice crystals outside the cell, with crystalline regions. However, it may damage the lipid structure of the cell membranes of microorganisms, decreasing their viability. In rapid freezing, intracellular, smaller, amorphous ice crystals form are created, and cell is preserved (Stefanello et al., 2019; Stephan et al., 2016).

The drying mechanism occurs due to the presence of a vacuum and heat source, whether conduction, convection, or radiation. After primary drying, controlled by vacuum pressure, secondary drying or desorption occurs, with bonded water being removed from the solid matrix for the necessary time until the moisture is stabilized (Bhatta et al., 2020; Morgan et al., 2006). Although this technique presents a high cost, it promotes the final product easier rehydration, avoids oxidation reactions, preserves nutrients, VOCs, and thermosensitive substances, thanks to low temperatures, and preserves cell viability (Santivarangkna et al., 2007).

Spray-drying

Another method of interest to the industry, with regard to its efficiency, is spray-drying, an atomization process that takes place in several stages. The product to be atomized is sprinkled and droplets form in a chamber in which there is the circulation of heated air, thus, the transfer of heat and evaporation of the solvent in the outer layer occurs until the water is removed from the particle, making it solid. Therefore, the formed powder falls vertically and sediment in the equipment chamber (Azhar et al., 2021).

The spray-drying has a reasonable rate of cell survival and is a continuous process but requires a comprehensive study on drying conditions, equipment configurations, considering the raw material, staining, yield, and powder quality (Peighambardoust et al., 2011). Among the adjustable parameters of the equipment, for drying sourdough, the temperature of the outlet air is crucial because it affects the microbial survival, that is, the lower the outlet temperature, the greater cell viability (Huang et al., 2017). Relatively small adjustments in outlet air temperatures may show significant results in LAB and yeast viability (Reale et al., 2019). Also, it may influence the aroma produced.

According to Ertop et al. (2018), the exposure of sourdough in spray-drying for about 2 to 3 seconds can preserve the white color of the final product. In Table 1, it is possible to find a summary of studies related to obtaining type III sourdough by different drying techniques, main parameters employed, starters used, presence or absence of protective agents, cell viability before and after drying, as well as the main objectives of each study.

Influence of sourdough drying methods on microbial viability and shelf-life

Cell viability during drying involves several related conditions such as the applied method, process parameters, and the use or absence of protectant compounds (Huang et al., 2017). Intrinsic factors that can influence cell death are thermal, osmotic, and oxidative stresses, increased pH, and salt concentration, promoted by heating and dehydration of cells (Fu & Chen, 2011). Extrinsic factors are different for each stage of the drying process, ranging from environmental (e.g., temperature and relative moisture) to material conditions, exposure time, and drying rate (Tan et al., 2018). Tan et al. (2018) also report that extrinsic factors that influence cell survival are not only observed during drying but rather in the stages that precede or follow such a process, and it is essential to mitigate these factors.

In the pre-drying, it is possible to perform some procedures with the objective of prior adaptation of the microorganism to the drying stage and the possibility of developing greater thermal tolerance. The post-drying processes also aim to preserve cell viability by controlling the presence of oxygen, moisture, and temperature (Fu & Chen, 2011). During the drying, several studies (Table 1) presented an experimental project, in search of optimizing the parameters involved, according to the applied method, to increase the cell viability and yield of type III sourdough, with the premise of reducing costs.

Survival in the drying process can also be influenced by the stress tolerance of each type of strain and/or microbiota present in sourdough, resulting in cell viability differences (Table 1). Ertop et al. (2018) evaluated the LAB viability of type I and II sourdoughs, which after drying in an oven at 37 °C, found reductions of 1.60 and 0.57 log CFU/g, respectively. In this same study, the freeze-drying and spray-drying methods were also applied, which did not influence the survival of the LAB in the sourdough. Such results point to the importance of understanding which factors affect the survival of microorganisms and only then comparing the different drying methods regarding the capacity of cell preservation. In other words, the proper drying technique should maintain survival and, mainly, cell activity as close as possible to those originally performed by microorganisms (Tan et al., 2018).

Among the 23 studies found (Table 1), 65% (n=15) evaluated cell viability before and after the drying process, of which 33% (n=5) were type I sourdough. When comparing cell viability between drying techniques, freeze-drying was the most efficient, with small reductions in LAB and yeast counts (Table 1). But there are exceptions where the freeze-drying process did not guarantee the expected viability, Reale et al. (2019), for example, prepared a type I sourdough with initial viability of 9.17 and 7.53 log CFU/g for LAB and yeast, respectively, and after the freeze-drying process, a drastic reduction of 3 log CFU/g have been observed for both groups of microorganisms researched.

Reductions of 1.7 log CFU/g for both LAB and yeast were also observed by Caglar et al. (2021) after freeze-drying of type I sourdough. These losses in the microbial viability may be conditioned time of permanence of the sample in the equipment (48 and 60 hours, respectively) without application of protective agents, which may cause microbial inactivation by cryogenic lesions (Santivarangkna et al., 2007). An example of the effectiveness in the use of protectors is shown by Stefanello et al. (2018), in which the freeze-drying type I sourdough showed reductions of 2.0 log CFU/g for LAB and yeast in the samples of sourdough without cryoprotection, while samples with 15% trehalose application remained at the same initial microbial concentration. Similar results using protective agents are also observed in studies with type II sourdough (with starter strains) (Table 1) (Cossignani et al., 1996; De Valdez & Diekmann, 1993; Gul et al., 2020a, b; Khorasanchi et al., 2011; Mantzourani et al., 2019; Stefanello et al., 2019).

Although freeze-drying is chosen as the best in preserving microorganisms, it still has a high cost and drying time (Huang et al., 2017). Because of this, spray-drying has been an advantageous method. However, as discussed above, process parameters must be studied to achieve desired cell viability. In the studies found for this review (Table 1), different viability can be related to the parameters used in the equipment, it is worth remembering that the air outlet temperature is paramount for a reasonable survival rate. The studies that used lower air outlet temperatures were the ones that obtained better results; Ertop et al. (2018) did not observe reductions in the counts when using 65 °C as air outlet temperature. However, slight decreases were reported in the study by Reale et al. (2019) when the air outlet temperature of 54 °C was sufficient to reduce 1.1 and 1.6 log CFU/g of LAB and yeast, respectively. In studies where the output temperature was 90 °C, loss of viability was observed in the 4.0 log CFU/g (Caglar et al., 2021; Tafti et al., 2013b). Mohd Roby et al. (2020) used an air outlet temperature of 100 °C, but with the addition of 7% Arabic gum as a protective agent for drying sourdough fermented with Kombucha, this prevented drastic microbial reductions caused by thermal injuries from occurring, having at the end of the drying a type III sourdough with 9.0 log CFU/g viability.

Storage is an important post-drying step and requires the study of factors to minimize significant cell reduction. Optimal storage temperature, controlled moisture, and microorganism tolerance are factors to be studied. The suitability of high barrier packaging, whether plastic, blister, or glass, allows for minimizing the effects of reactive agents such as temperature, moisture, light, and oxygen (Morgan et al., 2006). It is recommended to improve the stability conditions during storage that the moisture content is up to 7% and the a_w below 0.2 (Tafti et al., 2013b; Reale et al., 2019).

The storage temperature was studied by Gul et al. (2020b), obtaining greater cell viability when type III sourdough, prepared with *Latilactobacillus curvatus* and dried by spray-drying, was stored at 4 °C. At a temperature of 25 °C, as expected, there was an increase in reactions that impaired cell survival. After accelerated storage experiments at temperatures of 50, 60, and 70 °C, researchers noted a decrease in cell count, promoted by increased molecular water movement and changes in the glassy matrix structure, established with increased viscosity during drying and because the storage temperature is higher or closer to the glass transition temperature (Tg). In short, the ideal conservation structure of the particles is modified and affects cell viability.

The use of protective agents of high molecular weight before drying, to increase the Tg, associated with the storage carried out below the Tg of the powder substance, are strategies that can favor cell stability due to the reduction of molecular mobility and delay of the kinetics of reactions (Huang et al., 2017).

During the sourdough drying processes, the aim is to minimize the osmotic stress on the microorganisms present to preserve their cell viability. Some protective agents induce an increase in viscosity inside and outside the cell to decrease solute mobility, protect the membrane, and preserve microorganisms during the storage period, considering the agent with the highest Tg to be more efficient for matrix stability (Reyes et al., 2018). Cell survival is influenced by the type of agent used and by the microorganism's tolerance (Morgan et al., 2006). The application of low molecular weight carbohydrates and high Tg or low melting point fat

can promote greater cell stability, functioning as protective agents (Huang et al., 2017).

Gul et al. (2020a) carried out an experimental design to optimize the use of cryoprotectants and increase the cell viability of *Lev. brevis* ED25 during freeze-drying. The best response found was in the composition containing 17.28% skim milk, 2.12% lactose, and 10% sucrose, which resulted in cell survival above 94%, while the viability in the strain without cryoprotection was 72% after drying. The most significant protective effect was attributed to skimmed milk due to the presence of milk proteins overlapping the cell surface and inhibiting the formation of ice crystals, increasing matrix viscosity and reducing cell damage, while lactose acted as a dehydrator, promoting stability and prolonging shelf-life, resulting in an approximate reduction of 0.4 log CFU/g after storage at 4 °C for 180 days. Cossignani et al. (1996), using freeze-drying cultures with cryoprotection (e.g., glycine, glutamic acid, maltose, and bovine serum albumin), observed better rheological aspects of the dough, a greater presence of aromatic compounds and cell viability when compared to the elaboration of a traditional Italian fermented dough.

The freeze-drying of sourdough with trehalose, a cryoprotectant capable of binding to water and preventing the formation of ice crystals inside and outside the cell, was investigated by Stefanello et al. (2018). The microbial survival over 45 days of storage increased according to the concentration of added trehalose, reaching the end of the storage period with reductions of 1.5 and 3, 0 log CFU/g for LAB and yeast in sourdough added 15% trehalose. Control samples without cryoprotection had reductions of 3.0 and 5.0 log CFU/g, respectively. De Valdez & Diekmann (1993) compared the effect of different cryoprotectants (glucose, maltose, and glutamate) on cell viability after freeze-drying of type II sourdough fermented by *Limosilactobacillus reuteri*. The highest cell survival rate (80%) was attributed to glutamate cryoprotection, and even after six months of storage at different temperature conditions (-20, 4, and 25 °C), cell counts were higher than 8.0 log CFU/g for both temperatures.

Data published by Stefanello et al. (2019) showed that the co-culture of *Lim. fermentum* and *Wickerhamomyces anomalus*, freeze-drying with different cryoprotectants, obtained better survival rates, in 12 months of storage at room temperature, for two protective solutions, one of skimmed milk powder combined with sodium glutamate (87%) and the other with only skimmed milk powder (74%). Meanwhile, samples without cryoprotectants lost their complete viability after 90 days of storage. According to the authors, the adequate choice of cryoprotectant is essential to preserve viability, given that the use of 10% sucrose proved to be the least protective agent. Interestingly, although cell viability is better maintained with the use of cryoprotectants, the surfaces of cryoprotected sourdough samples analyzed by scanning electron microscopy in the study by Gul et al. (2020a) were not fully covered by the cryoprotectant, indicating that there is an irregular water loss during the drying process, greater gas permeability, and less cell protection. However, in most studies, the protective effect was proportional to the concentration of added protectors, that is, the higher the percentage of the added protective agent, the greater the cell viability.

Although type III sourdough has a low cell concentration, without an adequate fermentation capacity, it is still capable of promoting aroma and flavor and can be used as an additive to improve the sensory characteristics of baked goods (Khorasanchi et al., 2011; Siepmann et al., 2018), with the advantage of being a stable product, which does not require backslopping steps and can be incorporated directly into the dough quite easily, with the fermenting agent, in this case, being baker's yeast (*Saccharomyces cerevisiae*).

Technological characteristics in the use of type III sourdough

The surface properties and morphology of each dry sample interfere with the rehydration and reactivation conditions of the sourdough (Tafti et al., 2013a). Before the preparation of the baked product, it is necessary to carry out the reactivation of the type III sourdough. In the articles reviewed in this work, reactivations from 12 to 32 h were used, and this step influences the cost-benefit perception, as the shorter the time required, the greater the efficiency of the process. In the study by Meuser et al. (1995), there was little microbial multiplication activity in the first three hours, thus, they concluded that type III sourdough must be mixed with flour before being added to the bread dough so that the microbiota of the flour itself also helps in the yeast reactivation process.

The apparent density (mass/volume) seems to be directly related to the particle geometry. Caglar et al. (2021) described that the apparent density of sourdough powders ranged from 450 to 700 kg/m^3 and showed more irregular particles in freeze-drying. The results by spray-drying ranged from 525 to 775 kg/m^3 and more spherical particles were observed. In this way, the lower apparent density generated greater fluidity due to the irregular particles covering a greater portion of air, resulting from the freeze-drying method. Gul et al. (2020a), when drying sourdough with strain *Lev. brevis* ED25 by freeze-drying, observed amorphous regions in samples with cryoprotection, which facilitates rehydration, while samples without cryoprotection presented crystalline arrangements.

The wettability and dispersibility of the powder can be changed according to the particle size, favored when they are in larger, irregular, and agglomerated forms of particles, allowing greater internal space for rehydration. For example, in the spray-drying method, the increase in pressure can influence this parameter by generating finer particles with less wettability, increasing the possibility of regulating the equipment according to the interest in the product results (Tafti et al., 2013b).

The acidification rate is an important parameter to estimate cell activity after the thermal or cryogenic stress it undergoes during drying (Tan et al., 2018). Its role during sourdough fermentation reflects on the technological and sensory characteristics of the final product (Stefanello et al., 2019). The slower acidification rate compared to fresh sourdough was obtained in freeze-dried type III sourdough (added with *Lev. brevis* ED25), justifying this fact for the use of cryoprotection (Gul et al., 2020a). In another subsequent study, Gul et al. (2020b) observed this same slowness, now with the strain of *Lat. curvatus*, where the storage period (six months) and temperature (4 and 25 °C) had a negative

influence on sourdough fermentation. The type of flour used can also modify the acidification rate due to the ash content presented. In the study by Tafti et al. (2013a), the use of whole wheat flour that had a higher ash content resulted in less acidification when compared to a common flour, which has a lower ash content.

At the end of reactivation, all studies evaluated in this review, as expected, reported a reduction in pH promoted by LAB, and an increase in total titratable acidity, corresponding to the increase in VOCs developed during prolonged fermentation, compared to control samples with baker's yeast (Martínez-Anaya et al., 1993; Meuser et al., 1995). This situation indicates that the sourdough remained active after drying and reactivation. A good sensory indicator to observe that the activation of type III sourdough was efficient is when the mixture with flour and water doubles in volume and exudes a characteristic sweet odor. However, for control in commercial bakeries or industrial scale, other parameters can be evaluated, such as the pH reduction (Mohd Roby et al., 2020).

Desirable characteristics in breads made with sourdough include the presence of a range of aromatic compounds and a complex flavor, low pH, soft crumbs, and brown color (Brandt, 2019). The rheology of sourdough acidified dough is altered due to the activation of proteinases by reducing the pH and changing the gluten protein fractions present in cereal flours, which consequently promotes dough relaxation. In the study of Tafti et al. (2013b) with spray-drying of type II sourdough (inoculated with *Com. paralimentarius*) observed a reduction from 26 to 16% of wet gluten in white flour, with the gluten that came into contact with water, underwent mechanical action and formed the network protein. The sample containing the highest percentage of type III sourdough (15%) had the lowest gluten content. The higher the percentage of type III sourdough in the dough, the greater the water absorption, stability, and degree of relaxation of the dough.

The degree of softening increases proportionally as the percentage of type III sourdough increases. In this way, the raw material applied for the production of breads must be carefully selected, as the sourdough can lower the pH and lead to excessive water absorption, an increase in the development time, and excessive relaxation of the dough. In addition, the final pH of the dough can be controlled by the percentage of sourdough that is added to the raw material at the time of preparation (Brandt, 2019; Tafti et al., 2013a).

The aromatic complexity and flavor of the final product are also consumer choice criteria. As is already known, type I and II sourdoughs have a range of different VOCs, their origins stem from several biochemical processes such as lipid oxidation, LAB and yeast metabolism, and enzymatic reactions that take place at the time of sourdough and bread dough fermentation (Siepmann et al., 2019; Yan et al., 2019). One hundred ninety-six VOCs originated from sourdough fermentation have already been identified, including 43 aldehydes, 35 alcohols, 33 esters, 19 ketones, 14 acids, 13 furans, 11 pyrazines, two lactones, two sulfur compounds, and others in lower concentrations (Pétel et al., 2017). A longer fermentation, combined with microbiota, fermentation temperature, and raw material, results in a complex chain of VOCs that provide special aromas to the final product, such as the presence of esters in the dough, releasing fruit and flower aromas in the product (Pétel et al., 2017).

For this review, we found three studies (13%) that determined the composition of VOCs in type III sourdough (Cossignani et al., 1996; Mantzourani et al., 2019; Martínez-Anaya et al., 1993). None exclusively compared the VOCs of sourdoughs before and after the drying process, considering that volatile compounds can be created, such as by the Maillard reaction, and/or lost during the drying process. In this way, the drying technique can strongly influence the complexity of VOCs, as is the case of acetic acid that is evaporated in methods that use higher temperatures. The concentration reduction of acetic acid in the dry sourdough can negatively affect the flavor, inhibition of the rope, and shelf-life of baked goods (Brandt, 2007; Brandt, 2019).

Cossignani et al. (1996) reported the composition of VOCs in doughs fermented with freeze-dried sourdough containing *Fru. sanfranciscensis*, *Lac. plantarum*, *S. cerevisiae*, and *Saccharomyces exiguus* strains showed lower values of volatile compounds when compared to fresh sourdough doughs (control). In the same study, the fermented doughs that contained higher concentrations of yeasts had higher percentages of 1-propanol, 2-methyl-l-propanol, and 3-methyl-1-butanol, corresponding to the typical fermentation process of yeasts. The ethyl acetate compound, which originated from the LAB metabolism, was predominant when its concentrations were higher in the fermented doughs. Similar results were reported by Martínez-Anaya et al. (1993) in fermented doughs combined by strains of *Lac. plantarum, Lev. brevis*, *S. cerevisiae*, *Saccharomyces fructuum*, and *Candida boidinii*.

In the study by Mantzourani et al. (2019), the composition of VOCs was evaluated in breads fermented with strains of *Lacticaseibacillus paracasei* and *L. delbrueckii* ssp. *bulgaricus* freeze-dried, having as main VOCs heptanol, 2-phenylethyl acetate, hexanal, 1-octen-3-ol, benzaldehyde, 2-nonenal, and furfural. The bread produced with 1% of the powder containing *Lact. paracasei* showed the highest number of VOCs (12 alcohols, 13 esters, and 11 carbonyl compounds), registering the highest concentrations for esters (1.15 µg/g) and carbonyl compounds (3.77 µg/g). These values being satisfactory for obtaining a good aroma in the bread.

The increase in the shelf-life of bakery products is another advantage attributed to sourdough, not requiring the application of additives. Breads stored at room temperature showed spoilage by fungi only after the 14th day, which was attributed to the presence of organic acids, especially acetic acid, which still favors the flavor of the bread (Mantzourani et al., 2019). Mohd Roby et al. (2020) observed an increase in the shelf-life of bread made with sourdough added to spray-drying kombucha, compared to the control sample of type I sourdough, in the range of 5 to 10 days, possibly due to the presence of antifungal metabolites.

Acidity can delay the microbial spoilage of bread, which is attributed to LAB, as demonstrated by the Caglar et al. (2021) research, where the shelf-life was positively influenced according to the increase in the proportion of sourdough powder (3, 6, 9, and 15%) added to the dough. In summary, the result of fermentation, final pH, and formation of organic acids with antifungal activity influence the conservation of the product obtained (Arora et al., 2021). Extending the shelf-

life of additive-free baked goods is a gap where sourdough technology can be timely, particularly up to the sixth day of manufacture (Ertop & Coşkun, 2018). The increase in bread shelf-life through natural ingredients such as sourdough is of great interest to the industry and the consumer market. For a type III sourdough to be well-accepted for industrial application, a positive effect on bread shelf-life is paramount.

Costs and applicability

The choice of a suitable method for drying sourdough, which gives it cellular viability and desirable technological properties, must be carefully studied. In addition, the choice of method needs to consider its industrial scalability and operational and installation costs.

Due to its relatively simple operation, oven drying generates low operating costs of about 25% compared to freeze-drying. In addition, the drying time is variable, considering that to obtain good cell viability, milder temperatures should be applied (Ertop et al., 2018; Tan et al., 2018). Although dry products have a shelf-life, their quality is still lower than the original product, with volume reduction, changes in color, and problems with powder rehydration (Barbosa et al., 2015).

Freeze-drying is still the most used drying method to preserve microorganisms (Huang et al., 2017). Although freeze-drying has numerous advantages, as already mentioned in this review, it is one of the most expensive techniques due to the lowest operating parameters of temperature and longer periods of vacuum application, which generates higher operating costs (Tan et al., 2018). It is estimated that freeze-drying consumes 3.3 MJ/h for each kilogram of wet product, disregarding the pre-drying step, which would further increase its energy cost (Rudy, 2009). In addition, the need for an operating unit with low temperatures for good equipment performance makes the installation cost twice as high compared to spray-drying (Foerst & Santivarangkna, 2015).

Spray-drying is a potential substitute for freeze-drying, its operation has a reduced energy cost, about 3.05 MJ/h for each kilogram of wet product (Rudy, 2009). Depending on the equipment, production scale, and market demand, it is possible to obtain an average production of 50,000 tons/year while freeze-drying only 10,000 tons/year (Fu et al., 2018). Due to the continuous process pattern, spray-drying is the technique that guarantees cost reduction and high productivity. However, strategies to optimize process parameters and the application of protective agents are required for good cell viability during and after the atomization process.

Final considerations

Sourdough biotechnology had its benefits evidenced by several studies, both in terms of technological and nutritional aspects. On the other hand, sourdough processing time and lack of standardization have been inconvenient, making the process not very scalable for the bakery industry. The use of type III sourdough can be an opportunity to leverage industrial production, not restricted only to the formulation of breads, but with vast possibilities of use, such as, for example, in panettone, pasta, and pizza.

The drying methods presented in this review showed different degrees of applicability. When evaluated about microbial survival, freeze-drying proved to be more efficient. In terms of applicability on an industrial scale, spray-drying is a promising method for obtaining a type III sourdough with good cell viability and technology. However, future studies are required to optimize parameters and cereal raw materials involved in improving this product.

Conflict of interests

The authors declare that there are no conflicts of interest.

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