




Research Article

Influence of the fermentation methods on the resistant starch formation by X-ray diffraction

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Abstract

This work consists of a study about the influence of the fermentation methods on the resistant starch (RS) formation. Crystalline phases at the white bread after production and during the staling were evaluated. The bakery formulation was proportionally the same for the two fermentation methods used, except for the yeast. A commercial dry yeast and sourdough were used for the direct method (DM) and indirect method (IM), respectively. The temperature and cooking time, as well as the packaging and storage, were kept constant for all samples. Characterizations were performed on both samples by XRD and FTIR-ATR. It is possible to affirm that the fermentation method greatly influences the crystalline and microstructural properties of bread, mainly on the RS. The bread produced by IM presented more significant changes in its microstructure and degree of crystallinity than bread made by DM. These microstructural differences may be directly related to the microorganisms present in the sourdough, which can influence the bread resistant starch during the cooking and storage time.

Keywords Crystallinity · Direct method · Indirect method · Sourdough · Staling

1 Introduction

Bread is considered the first food processed by man. However, we do not know the exact outset of the processing of bread as a fermentation product. According to Arranz-Otaegui et al. [1], the citizens of Jordan, the first producers of wheat in the world, were the pioneers in consuming bread-like products; hence considering bread as an essential food. Since then, both wheat production and bread-making process have been expanded all over the world. Currently, the bread-making process is well known and widely industrialized.

The fermentative process is vital for bread production; it is responsible for the bulk, smell, and flavor of the bread. There are two general kinds of fermentative processes: direct method (DM), which applies biological pressed or

chemical yeasts, and indirect (IM) method, which involve a dough obtained from a portion of a dough that separated from the last batch of the previous day. This portion of dough is mixed again with water and flour and is conveniently kneaded; after, it ferments naturally. This portion of dough is called sourdough [2].

The broad diversification of the bread flavors and aroma, mainly the starch digestibility, has led countless authors to focus their studies on analyzing the physicochemical and sensory characteristics associated with bread, related to the starch structure and its origin. For example, the role of amylases, amylopectin, and the influence of enzymes, which participate in starch degradation, influenced by the aging time of the bread was studied by Hug-Iten et al. [3]. Aguirre et al. [4, 5] studied bread crumbs to verify the influence of the storage-related with crystallinity and amylopectin on the

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bread consistency. Another work, also widely referenced, is one published by Sullivan et al. [6]. They reported the effects of the temperature on crystallinity properties and resistant starch during white bread storage. These studies are focused on the nutritional point of view, the microstructural changes during the aging time, and the influence of the bread extrinsic factors, for example, the temperature of storage.

Regarding the resistant starch (RS), this kind of starch is divided into five types, considered slowly digestible starches. According to Villarroel et al. [7], this kind of starch is formed during the cooling of the starch processed. Among the five types of starches, the most important is the 3-type resistant starch. It is considered a retrograde starch and is formed during the heating/cooling cycles, but it is possible to obtain it from recrystallization during the storage. The regular consumption of foods containing resistant starch is a good alternative for massifying the benefits that come with their consumption, mainly in reducing chronic non-transmissible diseases.

The fibers present in food have a role in human health that has gained importance in recent years due to their intestinal transit benefits. Particular interest is observed in food fiber present in the RS, which is generated during starch retrogradation [8]. It resists primary fermentation in the intestine, passing directly to the intestinal colon and being fermented by the colonic microbiota [9]. During its stay in the colon, it causes short-chain fatty acids, associated with numerous health benefits, to be generated [10]. The studies of Sotelo et al. [11] demonstrated an increase in resistant starch content during the wheat transformation into bread.

The aim of this work is to study the influence of the fermentation process on the formation of resistant starch by XRD, considering the action of microorganisms present in



yeast, which alter the physicochemical and sensory characteristics of bread. In this work, it was possible to observe fundamental differences between the bread produced by direct and indirect fermentation methods and which changes remain during the storage. Additionally, it was possible to associate the crystallographic phases and degradation changes. Bread samples were characterized by XRD and FTIR-ATR. The results clearly show that there are differences between the bread obtained by direct and indirect methods. Furthermore, it was possible to verify that the indirect method favors the resistant starch formation. It can be supposed that the microorganisms present in the sourdough can influence the starch retrogradation.

2 Materials and methods

The dry yeast used as ferment for the direct method presented a uniform color visually without the presence of microorganisms related to decomposition; the wheat flour of type 000 had a yellowish-white color with its characteristic aroma and appearance. Moreover, it presented the following composition: ferrous sulfate, niacin, thiamine, riboflavin, and folic acid; and centesimal composition: carbohydrates, 74%; humidity, 13.6%; proteins, 9.8%; Total fats, 1%; Food fiber, 1% and ashes, 0.55%. Additional information includes Bakery quality: falling number: 370 s, Wet Gluten: 29.87%, Dry Gluten: 9.89%, Gluten Index: 95.68%, Alveogram: W: 341, P: 135, L: 60, P/L: 2.25. Table 1 presents the baking formulation for the manufacture of the bread.

Considering that the temperature plays an essential role in the crystalline properties of the bread [6], during the storage experiments, the temperature was fixed and

Table 1 Formulation for the manufacture of the breads (%)

Ingredients	Direct M.*	Indirect M
		
Wheat flour 000	59.40	49.50
Water	39.60	34.65
Salt	0.9	0.99
Sourdough	–	14.85**
Total	100	100

*Amount of yeast used was 0.4%, approximately

**Sourdough was composed of 9.90% of wheat flour and 4.95% of water

monitored ($27.25 \pm 1.19^\circ\text{C}$). Manufacture conditions of the bread, on direct and indirect methods, were maintained the same: temperature (230°C) and baking time (20 min). After obtaining the bread, they were kept at rest until cold. The first samples were collected immediately after cooled of the bread, (D or IM)_01 samples at time 0 s, for both manufacturing methods. Each sample was stored separately in polyethylene bags for each storage time considered (0, 24, 72, and 168 h). It was not possible to observe any changes in the physical appearance of the bread until three days. Within seven days of storage, it was possible to verify the formation of dark spots, characteristic of the decomposition state. All samples were lyophilized (Libras L101, Brazil) before X-ray diffraction analysis (X'Pert3 Powder, Panalytical, Cu source, $\lambda = 0.15405\text{ nm}$, K α radiation, 40 kV and 40 mA with 0.01° step and time of 1.5 s/step, range of $5^\circ \leq 2\theta \leq 40^\circ$).

The crystalline phases (CP%, Eq. 1) were determined from the crystalline and amorphous phases relation.

$$CP\% = \frac{P_a}{TP_a} \times 100 \quad (1)$$

where P_a is the sum of all crystalline peaks areas, and TP_a is the total area above the diffraction curve, which corresponds to the crystalline and amorphous phases.

The Scherrer equation (Eq. 2) was used to calculate the crystallite size (D) of the wheat flour and the samples prepared by DM and IM methods.

$$D = \frac{K \times \lambda}{\beta \times \cos\theta} \text{ (nm)} \quad (2)$$

where $K = 0.9$ is the Scherrer constant; $\lambda = 0.15406\text{ nm}$ is the wavelength of the X-ray source; β is the full width

at half maximum (FWHM) in radians and θ is the Bragg angle, it can be in radians or degrees since the $\cos\theta$ corresponds to the same number. The fitting was performed, firstly subtracting the diffusion halo, using Gaussian law applied by Origin Pro 2020 software. FTIR-ATR (Thermo Scientific, Nicolet iD5-ATR model) analysis was performed between 650 and 4000 cm^{-1} , with 4 cm^{-1} of resolution and 100 scans.

3 Results and discussion

Figure 1 presents the X-ray diffractogram, wheat flour and bread, with the baseline subtracted, which identifies the crystalline and amorphous regions and the deconvolution of the highest peaks.

The X-ray diffraction spectrum, Fig. 1(a), reveals the semi-crystalline nature of wheat flour. The results indicate that the peaks around 15 , 17 , 18 , and 23° are characteristic of the crystalline structure of A-type starch [4, 12]. These results correspond to a flour typically obtained from cereals [12]. An additional peak is observed around 20° attributed to the type V_h structure, which corresponds to the amylose–lipid complex, resulting from the reaction between starch and native lipids [13]. Saiah et al. [14] attribute the peak around 17° to the content of water present in wheat flour, which disappears as the water is eliminated, or contrariwise. This peak may also be related to the aging process of bread [4]; that is, during the time, this peak tends to increase at an intensity as it absorbs water from the environment.

Another important information that can be obtained from the diffraction spectrum is the crystallite size. Peaks weaker and broader indicate that crystal size is small. In

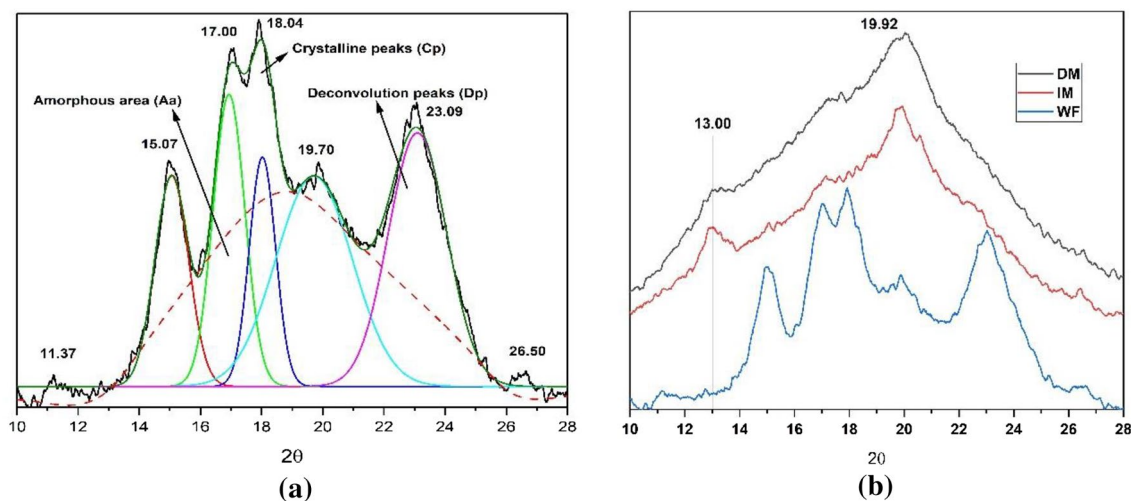


Fig. 1 X-ray diffractogram of the wheat flour **a** and bread crumb—DM, and IM 4 methods **b** at time 0 h

this study, the bread made by the direct method (2.10 nm) shows a smaller crystal size than that of wheat flour (5.40 nm) and the bread obtained by the indirect method (4.68 nm).

In Fig. 1.b, it is possible to observe the X-ray diffractograms of the bread after cooling, considering the time 0 h of the storage period. Comparing the spectra of wheat flour and bread, practically, all peaks disappeared, which causes a reduction in crystallinity of approximately 48% to 22.42 (DM) and 35.18% (IM), respectively. The only remaining peak was around 20° for both fermentation methods, which remains practically constant during the storage period, Fig. 2. A new peak appears around 13°, with high intensity for the indirect method sample, which may be related to the growth of lamellar crystals of the starch gel [15], possibly originated in the fermentation process influenced by the microorganisms present in the sourdough. This peak coincides with the V-type structure associated with a type of resistant starch, as reported by Shen et al. [16] and Simsek and El [17]. During the baking process, the bread undergoes several changes in the starch retrogradation, crystalline structure, amylose (AP), and amylopectin (AMP) phase. These changes not only take place in the baking process but during the cooling [4] and storage, too. Both molecules (AP and AMP) are associated with generating a new crystalline order, with a B-type structure. It is possible to verify that the peak around 17° is more pronounced in bread manufactured by the indirect method.

It is not only during cooking and cooling that bread undergoes structural changes. During the storage period, bread also undergoes various modifications in organoleptic properties such as texture, color, softness, and flavors, as well as microstructural characteristics, which

directly influence the organoleptic properties of bread. According to the results of Fig. 2, it is possible to observe for IM bread made with sourdough, the Bragg reflection angles at $2\theta = 13, 15, 17, 20$ and a weak at 23° . According to Bail et al. [18], in their study about the transformation of the V_h -structure obtained from different polymerization degrees. They report that the reflections $2\theta = 7.45, 12.93,$ and 19.84° are characteristics of the amylose fractions. Morphology and crystallinity depend on the presence of amylose. Sullivan et al. [6] demonstrated that there is a relationship between resistant starch (RS) content and total crystallinity, measured by XRD. They report that at least a portion of the degree of total crystallinity is due to RS formation. Figure 3 shows the evolution of the total crystallinity for both fermentation methods. We can confirm that the bread obtained from the sourdough (IM) presents a higher total crystallinity after cooking until the

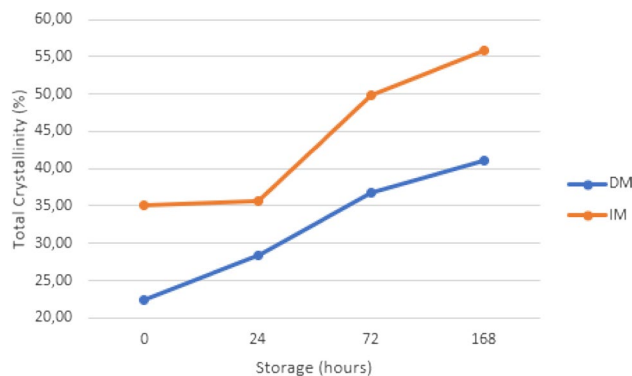


Fig. 3 Evolution of the total crystallinity of the breads

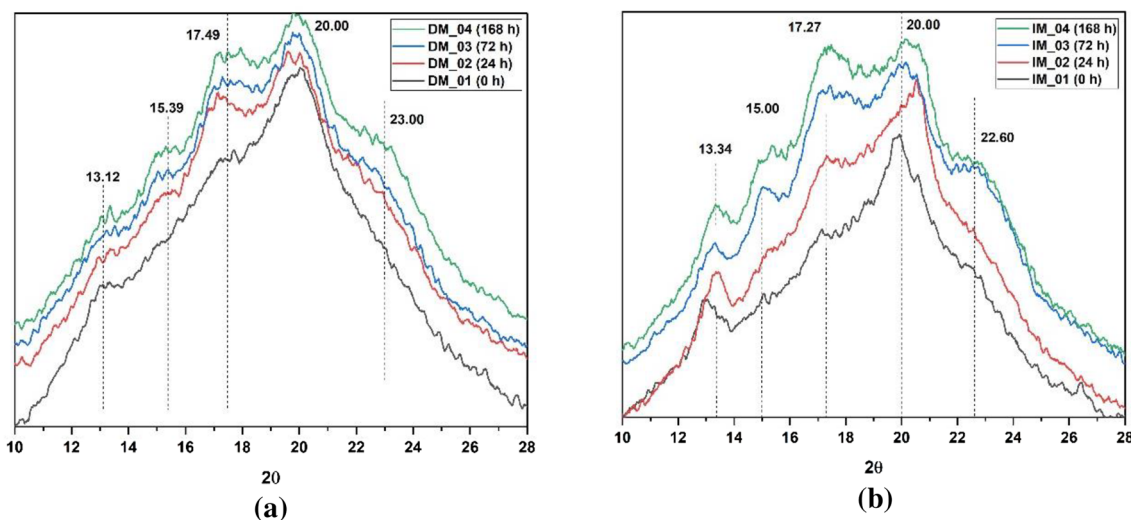


Fig. 2 X-ray diffractogram of the bread prepared by a direct (DM) and b indirect (IM) methods at different times

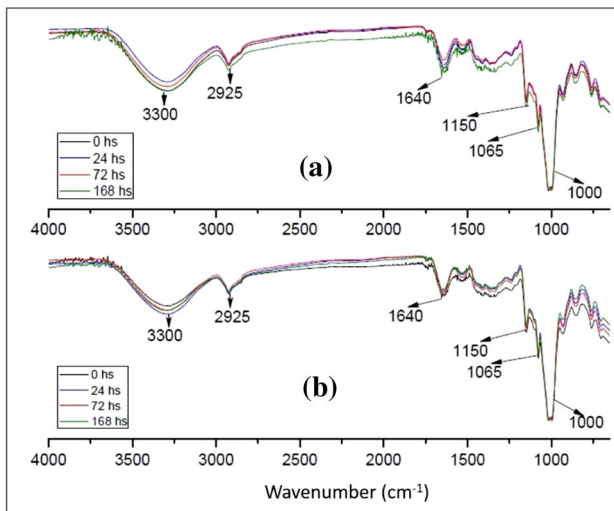


Fig. 4 FTIR spectra of the breads during storage time, **a** indirect and **b** direct methods

seventh day of storage, which leads us to assume that IM has a higher RS content.

From the FTIR spectra, Fig. 4, it is possible to appreciate the results during storage time. For both production methods, the breads have shown the same bands. It is observed the -OH bond $3000\text{--}3600\text{ cm}^{-1}$ corresponds to the stretching vibration band and the $900\text{--}1150\text{ cm}^{-1}$ bands, which correspond to the saccharides group. Al-Mahsaneh et al. [19] reported that these groups increase during the storage time based on their absorbance study. The -OH group is associated with the effect of water mobility on water-starch-protein interaction. On the other hand, the change on the saccharides bands indicating progressive starch crystallization during the storage time. It can explain the bread staling due to starch retrogradation.

From Fig. 4, it was not possible to find a correlation between the intensity variation of the FTIR bands and the aging bread. Since that, the increase observed in the intensity of the bands does not present a chronological sequence. More detailed studies are needed.

4 Conclusions

From the results obtained from XRD, it was observed that the fermentation method has a strong influence on the bread staling process. For both methods, the crystallinity degree increased with the storage time, which can be related to the staling process. The indirect method preparation (sourdough) presented a higher crystallinity degree than the direct method. Consequently, it is possible to affirm that the bread obtained from the indirect method has a higher content of the resistant starch and

retrogradation degree, which can be associated with the fermentation method and the microorganisms present in sourdough. Besides, this can be related to acidification of the sourdough from the lactic acid, which inhibits the yeast growth due to competition between alcoholic and acetic fermentation. That can explain, too, the different sensory characteristics of the bread produced by both methods. From the FTIR results, it was impossible to determine the relationship between FTIR bands behavior and the bread changes during the storage time. More detailed studies are needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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