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MONITORING GAS EXPANSION AND BUBBLE EVOLUTION OF WHEAT BREAD DOUGH DURING LEAVENING USING LIQUID NITROGEN FOLLOWED BY FREEZE-DRYING AND IMAGE ANALYSIS

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Abstract

Description of the subject: The use of a new technique to evaluate wheat bread dough fermentation
Objective: monitor the fermentation process of wheat bread dough using a new technique based on immersing the dough in liquid nitrogen; then, freeze-drying and image analysing.
Methods: Eight dough lumps are prepared corresponding to different times of leavening (0, 10, 20, 30, 60, 90, 120, 150 min). The dough lumps are immersed into liquid nitrogen, then, dried by a lyophilizer. The expansion ratio of different dried dough lumps is calculated: 2D images are captured.
Results: Results reveal that gas expansion of the dough lumps increased from 0 to 60 min with an expansion ratio of 181.13%. A slow growth continued until 120min with 200.15%. After that, a decline in the volume expansion is observed until 150 min. Image analysis showed that the void size and fraction increased (p<0.05) within the increasing time of leavening. A decrease in these parameters was noted at 150 min corresponding to a decrease of the volume of expansion.
Conclusion: a new technique using liquid nitrogen followed by freeze-drying and image analysis is successfully developed to monitor and evaluate the fermentation stage of bread dough.
Keywords: leavening; wheat bread dough; freeze-drying; dough expansion; image analysis.

SUIVI DE L'EXPANSION GAZEUSE ET DE L'ÉVOLUTION DES ALVÉOLES AU SEIN DE LA PATE Á BASE DE BLÉ AU COURS DE LA FERMENTATION PAR UTILISATION DE L'AZOTE LIQUIDE SUIVI PAR LA LYOPHILISATION ET L'ANALYSE D'IMAGE

Résumé

Description du sujet : utilisation d'une nouvelle technique pour l'évaluation de la fermentation des pâtes boulangères
Objectifs : suivi la cinétique de fermentation et le développement des alvéoles gazeuses des pâtes boulangères par utilisation d'une nouvelle technique basée sur l'immersion dans l'azote liquide suivie par la lyophilisation et l'analyse d'image
Méthodes : Huit pâtons ont été préparés correspondant à différents temps de fermentation (0, 10, 20, 30, 60, 90, 120, 150 min). Les pâtons ont été immergés dans de l'azote liquide puis séchés par un lyophilisateur. Le taux d'expansion de différents pâtons séchés a été calculé : des images 2D ont été prises.
Résultats : Les résultats ont révélé que l'expansion gazeuse des pâtons est passée de 0 à 60 min avec un taux d'expansion de 181,13%. Une croissance lente a été poursuivie jusqu'à 120 min avec 200,15% et après cela une diminution de l'expansion de volume a été notée jusqu'à 150 min. L'analyse des images a montré que la taille et la fraction de vide augmentaient (p<0,05) avec l'augmentation du temps de fermentation. Une diminution de ces paramètres a été notée à 150 min correspondant à une diminution de l'expansion volumique.
Conclusion : une nouvelle technique utilisant l'azote liquide suivi de la lyophilisation et d'analyse d'images a été développée avec succès pour suivre et évaluer l'étape de fermentation de la pâte à pain.
Mots clés: fermentation; pâtes à pain de blé ; lyophilisation; expansion de la pâte; l'analyse d'image.

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INTRODUCTION

Dough fermentation is one of the key unit operations in the process of bread-making. Yeast metabolizes flour sugars into carbon dioxide gas, which diffuses toward the air nuclei embedded in the dough during mixing, causing the growth and the expansion of the gas bubbles [1-6]. In this way, fermentation is responsible for the growing volume of the dough. The stability of the gas bubbles during leavening strongly affects the final quality of baked bread such as volume and texture [3, 5, 7, 8].The creation of the cellular structure is affected by bubbles' growth including phenomena such as disproportionation and coalescence [1, 8, 9].

The assessment of the fermentation process is an important point to understand the growth of bubbles in the dough during leavening. Several techniques have been used to monitor the fermentation process and to study the aeration of the dough [10, 11]. Different studies are based on the measurement of gas production by different instruments, such as, the fermentometer Burrows and Harrison, the fermentographes SJA, the Brabender, the zymatochygraphe and the Rheofermentometer Chopin [12, 13, 2]. Other methods are based on monitoring the fermentation through 2D and 3D images using different devices: digital camera, Magnetic Resonance Imaging (IRM), X-ray tomography, scanning electronmicroscopy and confocal laser scanning microscopy [14- 20, 11, 9]. A morphological analysis of the obtained images is applied to determine the cellular structure in the wheat flour dough [8, 19]. In these cases, image analysis (IA)proved to be a good alternative for quantifying the structure of gas bubbles during leavening by determining parameters such as the cell size, cell size distribution, void fraction and morphology of bubbles [8, 21- 23]. Despite of the progress achieved in research to monitor the fermentation process, only few research methods focused on bubbles evolution during leavening and these methods remain very expensive; therefore, it was inevitable to use a simpler and a useful method.

The purpose of this study is to use a new technique based on image analysis to monitor the fermentation process and to describe gas bubbles evolution during leavening. The technique involves monitoring the expansion of the wheat dough at various times of fermentation using liquid nitrogen that is followed by drying to freeze the structure of the fermented dough.

MATERIALS AND METHODS

1. Raw Materials

All materials included, wheat flour, instant dry yeast (saf-instant, France), and salt (Enasel. Algeria) were purchased from an Algerian local market.

2. Proximate analysis of wheat flour

The moisture content was determined by the AFNOR NF VO3-707 [24], the ash content was identified by the AFNOR NFV03-720 [24], the lipid content was found using the Soxhlet method [25] and the protein content was discovered using the Kjeldahl method (AFNOR NF V03-050) [24]. The wheat flour used was characterized also by wet, dry and gluten index (Glutomatic, ICC nº 107/1) [26] and , alpha-amylase activity (Falling Number, ICC nº 107/1) [26].

3. Dough and Lumps Preparation

The dough was prepared according to Benatallah *et al.* [27] by mixing 200 g of flour, 4g of dry yeast, 4g of salt, and 116 g of water (fixed according to preliminary experiments). All ingredients were kneaded using a mechanical dough kneader (Kenwood KM300, 80 turns/min) for two times, each of which lasted15 min and separated by a rest of 5 min. The final temperature of the dough was 27+/- 1°C.

After mixing, the dough was taken and shaped into a square mold (18×18×1.2) cm^3 using a roller. Then, eight cylindrical molds (4.0cm of diameter and 7.5cm of height), corresponding to fixed proofing times according to preliminary experiments (0, 10, 20, 30, 60, 90, 120 and 150 min), were used to take volumes of dough lumps of 15 cm^3 for each one. The resulting dough lumps (4.0 cm diameter and 1 cm height) were proofed (38°C, 80%-90% RH) in cylindrical molds in a fermentation cabinet. After each fermentation time, the dough lumps were rapidly immersed into liquid nitrogen (about 1 min) for the aim of determining their volume. After that, the samples of dough used for the layout of the kinetics of fermentation were preserved for 24 hours in liquid nitrogen at -196° C, and then, the fixed samples were freeze-dried by a lyophilizer (Alpha1-4 CHRIST, with - 57°C, under a pressure of 0.05 mbar) for 10 hours.

4. Measurements of the Dough's Volume Expansion

The volume of dough lumps was determined by the rapeseed displacement method according to the AACC Approved Method 10.05. [28] as follows: for each fermentation time, a cylindrical mold containing dough was delicately taken and immersed into liquid nitrogen for at least 1 min to fix and solidify the structure of the dough. The mold was entirely filled with the rapeseed, then, it was emptied of rapeseed in a measuring cylinder (100 cm^3). The volume of the dough lump is the difference between the average volume of the molds (83 cm^3) and the volume of the rapeseed in the measuring cylinder following this formula: $\boldsymbol{V = V_{av} - V_{rp}}$. Where, $\boldsymbol{V}$: volume of dough lump (cm^3); $\boldsymbol{V_{av}}$: average molds volume (83 cm^3); $\boldsymbol{V_{rp}}$: rapeseed volume (cm^3), corresponding to each proofing time.

The gas expansion of dough lump for each proofing time was calculated as follows: $\boldsymbol{EXP}\ (\%) = [(\boldsymbol{V_t - V_0}) \times \boldsymbol{100}]/\boldsymbol{V_0}$. Where, $\boldsymbol{V_0}$: dough lump volume before proofing t_0=0min; $\boldsymbol{V_t}$: dough lump volume at t time of proofing.

5. Image Analysis

Image analysis aimed to extract the quantitative information in relation to the cells' size and shape from the visual texture of fermented freeze-dried dough lumps. This aimed to show the evolution of the cellular structure during leavening. Lyophilized dough lumps were cut using a thin knife at 25 °C. The images in the middle sections of the loaf were captured by a digital Camera (20.1 MP) in the presence of a scale. The images were saved in true color at 300 dots per inch resolution. All images were saved in uncompressed TIFF format. Cell analysis was performed using Image J software (version 1.43, National Institutes of Health, USA) as described by Gonzales-Barron and Butler [21]. The first steps of analysis involved the selection of the region of interest (crumb area), and then the original images (RGB) were converted into monochromatic with grayscale (8 bits forma). Each pixel assigned a color coded by a computer, generally ranged from 0 to 255 for the images in a grayscale. The images were, then, adjusted and transformed into binary images in which the cells were presented as black holes. The analysis of the binary images allowed determining the cellular structure of freeze-dried crumb. The number of cells, the average size, the fraction area, the morphology and the shape of cells (circularity) were calculated. The cell analysis was performed on two slices for each dough lump. Four replicates were used for each slice analysis.

6. Statistical Analysis

The Mean of the three replicates was calculated along with the standard deviation (SD) unless otherwise stated. The data were averaged and the means were compared using one-way analysis of variance (ANOVA), followed by the Fisher Least Significant Differences (LSD) *post hoc* test. A statistical difference at $p<0.05$ was considered significant. Pearson's Correlation Coefficients were also determined. The data were statistically analyzed using Statistica version 7.0 software (StatSoft, Inc., Tulsa, OK, USA).

RESULTS

1. Proximate Composition of Wheat Flour

Table 1 shows the proximate composition of the wheat flour used. Wheat flour had 14.85±0.05%, moisture, 0.02±0.01% ash, and 0.8±0.02% lipid and 9.29±0.32% protein. The wheat flour used was characterized by 22.15±1.62% of wet gluten, 7.45±0.21% of dry gluten and 96±2.82% of gluten index and alpha-amylase activity of 327±1.2 seconds.

Table 1: proximate composition of wheat flour (per 100g)

	Wheat flour
Moisture (%)	14.85±0.05
Ash (%)	0.02±0.01
Lipid (%)	0.8±0.02
Protein (%)	9.29±0.32
Wet gluten (%)	22.15±1.62
Dry gluten (%)	7.45±0.21
Gluten index (%)	96±2.82
Alpha-amylase activity (sec)	327±1.2

2. Dough's Expansion during Leavening

Fig.1 shows the volume expansion in time of dough lumps during the leavening stage, determined in terms of volume expansion ratio (percent). Regarding Fig.1, the curve of gas expansion of the dough lumps in time is similar to the curve of microbial growth. It is characterized by three distinct phases: (a) an exponential phase (b) a slow growth phase (stationary); and (c) a decline phase. During the first minutes of leavening, the volume increased exponentially and rapidly until 60 min indicating a strong production of CO_2 and a good retention of gas; at 60 min, the expansion reached 181.13±12.47%. During this phase, we notice a continuous and an intense production of CO_2 that is related to the beginning of the fermentation process. Between 60 and 120 min, the dough continued to rise, but got slightly constant. In this period, the gas expansion slightly increased from 181.13±12.47% to 200.28±15.56% (from 60 to 120min), which presented an optimum time of fermentation in this interval with maximum values of gas expansion. After 120min and until the final stage of leavening (150min), a slight decrease in gas expansion was noted from 200.28 ±15.56% (for 120 min) to 185.12±10.56% (for 150 min).

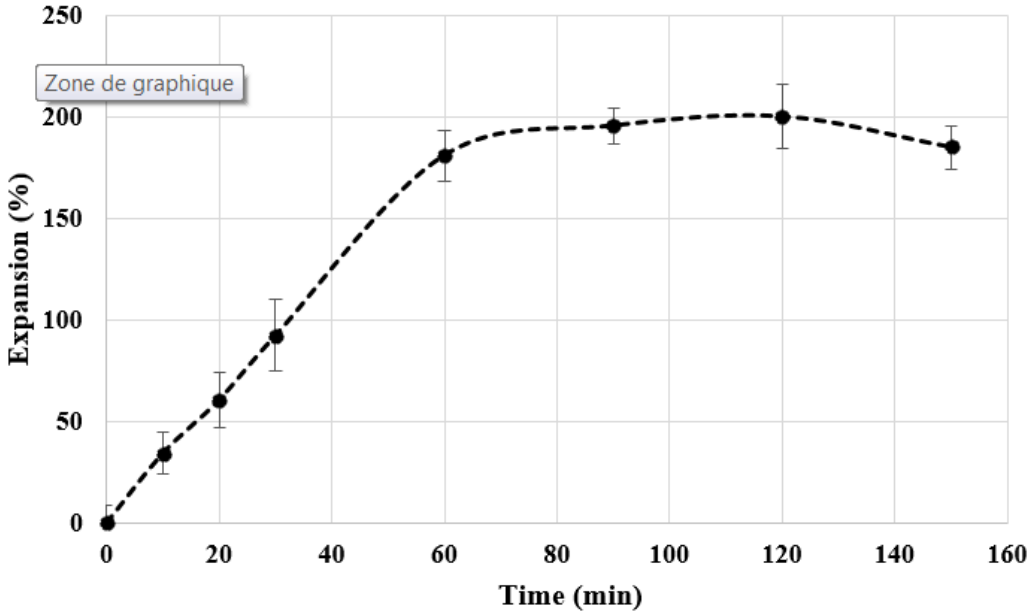

Figure 1: Gas expansion change in time of the dough during leavening (0 min to 150min).

3. Structural Image Analysis of the Bubbles' Growth

3.1. Images

The 2D images of the dough at different times of leavening are shown in Fig. 2. The Figure above shows that at different times of leavening, the cellular structure of the dough is presented as a complex phenomenon. By the increasing time of leavening, the bubbles increase in structure. At 0 min of fermentation and just after mixing, the cellular structure (Fig. 2.a) is characterized by the presence of millipores, confirming their formation during mixing. After 10 min of leavening, it shows the presence of micronuclei and some cells in the middle of the structure. From 20 min to the final stage of leavening, the cellular network structure begins to be formed by the presence of round cells of heterogeneous dimensions. At 150 min, the phenomenon of coalescence becomes more important which allows for the decrease in the volume of the dough. Digital images in Fig. 2 allow describing the cellular structure of the dough during leavening, but a statistical quantitative analysis is important to provide an analytical description of the cellular structure by detailing information about the size, the number, and the morphology of the cells during leavening.

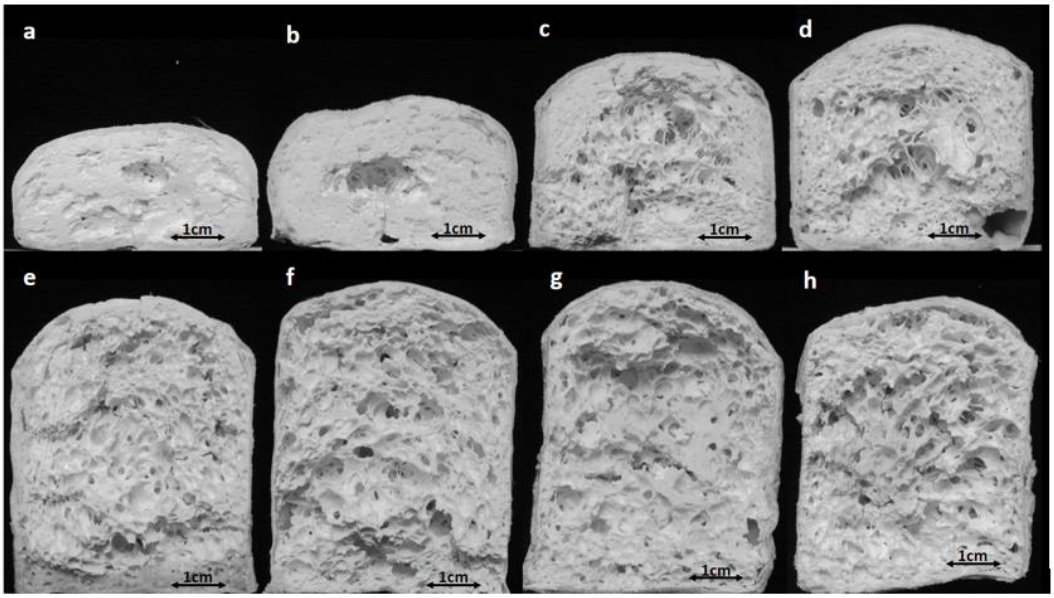

Figure 2: Digital images of the dough's sections during leavening:
a: 0 min; **b**: 10 min; **c**: 20min; **d**: 30min; **e**: 60min; **f**: 90min; **g**: 120min; **h**: 150min.

3.2. Bubbles' Evolution by Image Analysis

The number of cells, the size of cells, the fraction area, and the circularity during leavening are shown in Table2. ANOVA results indicate significant differences ($p \leq 0.05$) in the mean number, the mean size of bubbles, the mean fraction area, and the mean circularity. These differences are supported by the visual description of images in Fig. 2. Image analysis revealed changes in the cellular structure of the dough within the increasing time of leavening. All the dough lumps leavened at 10 min until the end of fermentation resulted in significant larger slice areas compared to dough lumps at 0min of leavening and (just after mixing). The number of the cells increases within the increasing time of leavening. The highest value is noted for 120 min with 362.30±5.459 of cells against 121.33±5.556 of cells for 0 min of leavening. Concerning the size of the cells (alveoli), it is noticed to highly increase with the increasing time of leavening compared to 0 min (0.195±0.008 mm^2). The same observations are drawn from the fraction area that presents the proportion of the cells in the measured area (void fraction).The fraction area is noted to be the maximum value with 38.6±4.475% corresponding to the highest size of cells (1.212±0.110 mm^2) at 150 min of leavening. With reference to the morphology of bubbles presented by the circularity parameters, bubbles of 30 and 60 min of leavening seem to be more rounded than bubbles in other times, but in general, all bubbles during leavening present the same morphology and are presented by a round shape. From the beginning of leavening, a continuous increase in the number and the size of bubbles is observed until 120min of leavening; consequently, the spherical surface area of the cells increases. After120 min and until the final stage of leavening (150min), a decrease in the number and the size of cells appears. Correlation analysis between dough volume expansion and image analysis characteristics for different times of leavening was performed to statistically discover conclusive relationships. Dough expansion was found to be positively correlated with the number and the size of cells as with the fraction area (R= 0.86, 0.95 and 0.91 respectively).

Table 2: Image analysis of the dough's parameters at different times of fermentation (from 0 to 150min).

Time (min)	Number of cells	size (mm^2)	Area fraction (%)	Circularity
0	121.33±5.556^a	0.195±0.008^a	12.05±0.212^a	0.757±0.013^a
10	212.05±9.916^b	0.457±0.036^b	14.6±5.233^b	0.736±0.005^a
20	313.10±8.735^c	0.770±0.033^c	29.5±4.879^c	0.750±0.016^a
30	318.12±9.459^c	0.804±0.095^d	31.4±4.101^d	0.776±0.006^b
60	360.32±3.936^e	1.111±0.211^e	35.9±2.546^e	0.775±0.011^b
90	360.00±4.41^e	1.134±0.214^f	36.4±3.818^f	0.740±0.002^a
120	362.30±5.459^e	1.146±0.090^g	36.5±1.061^f	0.760±0.011^a
150	333.00±6.054^d	1.212±0.110^h	38.6±4.475^g	0.765±0.002^a

Values followed by the same letter in the same column are not significantly different ($p<0.05$).

DISCUSSION

Proximate composition of wheat flour used in this study indicates a good quality of this flour. Gluten is the functional component of wheat protein; it is the determining factor of the strength of flour which improves water absorption capacity, extensibility and elasticity of the dough. The quantity and quality of gluten are responsible for the viscoelastic properties of the dough [29].

Dough volume expansion, in which CO_2 production is associated with yeast activity, is related to fermentation time. The increasing volume of the dough during the first minutes of leavening indicates that yeast quickly adapts to their environment and begins the synthesis of enzymes which is necessary in producing carbon dioxide (CO_2) from pre-existing sugars in the dough provided by flour [16, 30, 31]. Richness of flour in enzymes, such as α-amylase (alpha-amylase activity of 327 seconds), results in an increase in fermentable sugars and, thus, shows a positive effect on the yeast fermentative activity. The ability of the dough to retain the gas in bubbles is strongly related to the fermentative gas production by yeast [5, 32]. This period is related to the start of the fermentation process which characterized by the diffusion of carbon dioxide to the alveoli [8]. The second phase (between 60 and 120 min) is characterized by a lower slope that can be explained by the depletion of the substrate (sugar) and, hence, low CO_2 production. The CO_2 production decreases but still grows. In this case, yeasts take longer to produce the CO_2 gas [9]. Different factors take place to determine this phase, such as the biochemical composition of the used flour. According to Alais and linden [33], the flour rich in sugar and damaged starch favor the fermentation activity of yeast. Wheat variety used can also affect the action of yeast. After 120min, the decline in gas expansion maybe explained by the coalescence phenomenon: the merging of two or more gas bubbles in the dough, due to the rupture of cellular walls, causes the diffusion of CO_2 through other thinner walls, which results in an increasing size and a decreasing number of cells. For this, the entire dough must be well structured: it must be sufficiently extensible and elastic while presenting a certain rigidity, capable of ensuring the stability of the cells embedded in the protein network which carbon dioxide (CO_2) accumulates [9, 34].

Monitoring the fermentation process using the technique of freezing in liquid nitrogen and drying in a lyophilizer allows distinguishing three characteristic periods. These results are consistent with the findings of Babin *et al*. [16], who found that the fermentation process is characterized by three periods: gradual, stationary, and decline periods. Romano *et al*. [8] indicated that the curve of volume expansion ratio (point) shows a sigmoid shape and it is characterized by three distinct regions: lag phase; growth phase and stationary phase. These differences may be related to the characteristics of wheat flours used in each study especially the strength of gluten and the biochemical compound such as protein, lipid, etc which enter into the formation of the interfacial film which prevents the CO_2 gas from escaping. Although the dough expansion measurement gives information about monitoring the fermentation process in the wheat dough, it cannot provide information about bubbles' evolution in the dough during leavening, such as void fraction, and the number and the size of the gas bubbles. Therefore, it is necessary to assess this point by an analytical analysis based on digital images of the freeze-dried dough to extract parameters in relation to bubbles' evolution during leavening. The cellular structure of the dough changes with the increasing time of leavening; bubbles increase in structure to reflect an important production of CO_2 during leavening.

A continuous increase in the number and the size of detected bubbles can be observed during leavening which would reflect the strength of the gluten network. At 150 min, a decrease in the volume of the dough is observed. These observations are supported by the results shown in Fig. 1 for expansion ration of the dough during leavening. Image analysis of the cellular structure of the dough is an important parameter to predict and provide a more detailed view of the texture of the final product [35, 36]. From the beginning of leavening, a continuous increase in the number and the size of bubbles is observed until 120min of leavening; consequently, the spherical surface area of the cells increases. This result reflects the network strength of gluten and the dough's viscoelasticity, which causes the expansion of bubbles and the retention of gas in the dough by the increasing time of leavening.

The gas bubbles which already exist in the dough can be subdivided and thus, their number and size as well the surface area of bubbles are improved during leavening [5, 8, 37]. The bubble evolution depends on the amount of gas produced by the yeast during leavening which allow to a growth of bubbles and a developed alveolar structure of the dough [8]. After120 min and until the final stage of leavening (150min), a decrease in the number and the size of cells is shown. This may be explained by the coalescence phenomena of bubbles that reduces their spherical surface. This phenomenon can cause a decrease in the volume of the bread results and an increase of the heterogeneity of the cellular structure [9, 16].Correlation analysis between the expansion of the dough volume and the characteristics of the image analysis indicate that the expanded dough is characterized by an aerated cellular structure.

CONCLUSION

The objective of this study was to use a new technique to monitor the fermentation kinetics of the dough based on the freezing of the dough in liquid nitrogen and then freeze-drying. It also aimedat investigating image analysis methods to characterize the cellular structure of the fermented dough. Technical monitoring of the fermentation kinetics by this method allows to trace the gas expansion change during leavening and to capture images of the frozen dough in each time of leavening. Image analysis confirms the results of gas expansion traced and gives information about the number, the size and the shape of bubbles during leavening. This technique is a simple tool which, not only, allowed to monitor the fermentation process, but also enabled to observe and describe the bubble's evolution and the cellular structure change of the dough during leavening. Understanding the fermentation step with such methods is important to improve the quality of the resulted baked bread.

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