

THE EFFECTS OF PARTIALLY DEFATTED MILK THISTLE (*SILYBUM MARIANUM*) SEED FLOUR ON WHEAT FLOUR

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Abstract

Milk thistle (Silybum marianum) is a rich source of ingredients, such as aminoacids, fatty acids, minerals, and phytochemicals exhibiting nutraceutical effects on human health. In this work, partially defatted milk thistle seed flour, a by-product obtained during the manufacture of milk thistle seed oil, was studied as an additive for common wheat flour.

The influence of the addition of partially defatted milk thistle seed flour was studied by analyzing the colour, mineral content and rheological properties of dough in order to further exploit the functionality of partially defatted milk thistle seed in bakery products.

Mixtures containing 5%, 10% and 15% partially defatted milk thistle added to wheat flour maintained rheological parameters within recommended limits for a good technological behaviour and, consequently, a good quality of bakery products. Mineral contents, especially calcium, magnesium, iron and potassium, were also significantly increased.

Key words: *Silybum marianum*, milk thistle seed, color, mineral content, dough, rheological properties

1. INTRODUCTION

Milk thistle (*Silybum marianum*, family: *Asteraceae*) is an annual or biennial plant, native to the Mediterranean area, which nowadays has spread out to the warm and dry regions of Southern Europe and Asia. It is cultivated as an ornamental species, a medicinal herb, and as a minor vegetable.

Milk thistle has been used in folk medicine for over 2,000 years as a remedy for a variety of medical conditions, especially liver, kidney and gallbladder ailments (Wang et al. 2014).

The roots can be consumed raw, boiled or fried. In springtime, young sprouts can be cut down to their roots and boiled. Thorny bracts at the base of the inflorescence were consumed in the past as an alternative to artichokes, and stems (after cleaning) were soaked overnight in water to eliminate their bitterness and then fried. Leaves can be boiled to obtain a suitable replacement of spinach and can also be added into raw salads.

The cholagogue and diuretic effects of milk thistle have been described as far back as 1554 by Mattioli.

There are numerous pharmacology and toxicology studies with regard to milk thistle. Both *in vitro* and animal-based research studies have suggested that milk thistle can exhibit hepatoprotective (antihepatotoxic) properties, i.e. protection of liver cells against toxins. (Al-Anati et al. 2009; Jayaraj et al. 2009).

Milk thistle seeds are the main source of silymarin (4-6%), with applications in the pharmaceutical industry. Silymarin is a flavonoid complex containing several flavonolignans, including silybinin, isosilybinin, silydianin, and silychristin. (S.Eskandari Nasrabadi et al. 2014).

The main reason for the widespread cultivation of this plant species is its importance for the treatment of liver and biliary diseases, as well as for prevention of liver cancer (S. Eskandari Nasrabadi et al. 2014). It is believed that the silymarin extracted from the ripe seeds of milk thistle is the biologically active component. The whole plant is used for medicinal purposes, but the highest content of silymarin is in the seeds. Silymarin exerts its antitoxic effect by stabilizing intermembrane exchanges and via its

antioxidant effect on free radicals, while the regenerative effect of silymarin on liver cells is achieved by stimulating protein biosynthesis.

The efficacy of silymarin in chronic liver diseases was demonstrated back in the 80s, through ample studies over the course of up to 4 years. Toxicology tests have demonstrated that, upon administration of silymarin, the liver tissue is protected better against toxic substances such as carbon tetrachloride, galactosamine, and thioacetamide. (Hahn et al. 1968).

The regenerative action of silymarin comes from the curative properties of silybin, stimulating liver cell restoration. In biochemical terminology, tissue regeneration is based on stimulation of cell metabolism in the direction of macromolecular synthesis.

The most frequent cause of chronic liver ailments was, until recently, alcohol abuse. However, nowadays we are confronted with preservatives, poor quality foods, and viral hepatitis. Irrespective of cause, based on statistical and clinical studies, it can be concluded that silymarin is not only a hepatoprotectant, but also a regenerator for liver cells.

Thus, as a result of its qualities demonstrated through numerous studies, milk thistle has been introduced around the world as a widespread crop.

Numerous studies have shown that seeds can be regarded as safe, since no health hazard or side effects are known to date and they are rich in proteins, lipids and total carbohydrates, with concentrations from 19.1 to 30.0%, 20.0 to 30.0%, and 24.2 to 26.3%, respectively (Wichtl and Bisset 1994; Abu Jadayil et al. 1999; Khalil 2008; Montvale 2000).

In their study, El-haak et al. (2015) showed that milk thistle seeds are a good source of lipids (29.68%), proteins (25.25%), total carbohydrates (38.16%) and crude fibre (29.95%). Furthermore, the seed proteins contained have markedly high amounts of essential amino acids such as lysine, isolucine, leucine, valine and threonine.

In Europe, it is cultivated widely for production of raw materials used in the pharmaceutical industry, especially in Austria, Germany, Hungary and Poland.



Figure 1. Milk thistle flower and seed

In Romania, milk thistle is a niche crop with huge potential, especially considering that is not a very demanding plant, which can bring smallholders profits upwards of 2,500 euros per hectare.

Milk thistle seeds contain a relatively significant amount of oil. Although the defatted milk thistle seed flour is a by-product and is typically eliminated as waste, it may contain beneficial components such as proteins, carbohydrates (especially crude fibres), minerals, and some phytochemicals that have antioxidant or antimicrobial properties (Parry et al . 2008).

The objective of this work was to characterize mixtures of wheat flour and partially defatted milk thistle seed flour through evaluation of their colour and rheological properties, which are two main quality attributes of these mixtures for use in bakery.

2. MATERIALS AND METHODS

2.1. Materials

Defatted milk thistle seed flour, a by-product obtained during manufacture of milk thistle seed oil, was kindly supplied by SC Oleomet-sa SRL (Bucharest, Romania). This product was obtained from milk thistle (*Silybum marianum*) seeds on a large scale through hulling, grinding and degreasing at low temperatures (below 45° C). The level of degradation of the components of this material may be considered low because all steps were performed at low temperature.

Wheat flour used in the study was 550 type (ash, d.m. – 0.55%) and was provided by Titan S.A. (Bucharest, Romania).

2.2. Preparation of wheat flour mixtures enriched in bioactive compounds

Three types of mixtures of 550 type wheat flour (ash, d.m. - 0.55%) and different proportions of partially defatted milk thistle seed flour were obtained, in the following ratios: 95:5, 90:10, and 85:15 (w/w). The types of flour mixtures used in this study are presented in Table 1.

Table 1. The types of mixtures of flours used in this study

P1	100% wheat flour type 550
P2	95% wheat flour type 550+5% partially defatted milk thistle seed flour
P3	90% wheat flour type 550+10% partially defatted milk thistle seed flour
P4	85% wheat flour type 550+15% partially defatted milk thistle seed flour
P5	100% partially defatted milk thistle seed flour

2.3. Colour evaluation

Colour was evaluated instrumentally using the CIELAB coordinate system defined by the Commission Internationale de L'éclairage - 1976. CIE L*, a* and b* were measured using a colorimeter CN5 from Konica Minolta, protocol Software SpectraMagic NX. Black and white calibration tiles were used to calibrate the instrument before analysis.

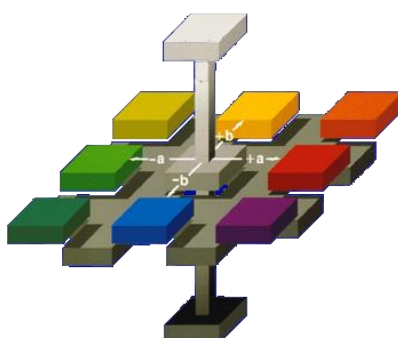


Figure 2. Colour space of CIE L*, a*, b*.

The parameters determined were: L* (lightness factor, 0 = black, 100 = white), a* (-a = green, +a = red) and b* (-b = blue, +b = yellow) (fig.2.). The colour coordinates a* and b* were used to calculate chroma

$C^* = \sqrt{a^{*2} + b^{*2}}$. The total colour difference (ΔE) between the control (wheat flour) and the mixture formulations using wheat flour and partially defatted milk thistle seed flour was calculated as: $\Delta E = \sqrt{(L_C^* - L_P^*)^2 + (a_C^* - a_P^*)^2 + (b_C^* - b_P^*)^2}$, where subscripts C and P refer to the control and to the various mixture formulations, respectively (Raquel P. F. Guiné; Duta et al., 2015). Each value was the average of 10 different measurements.

2.4. Mineral content analysis

Mineral content was determined using an atomic absorption spectrophotometer (ContrAA 700; Analytikjena). Total ash was determined by incineration at 550 °C, in an oven. Analysis was performed using an external standard (Merck, multi element standard solution) and calibration curves for all minerals were obtained using 6 different concentrations. Dried samples were digested in a mixture of concentrated HCl.

2.5. Rheological properties evaluation

The rheological behavior of doughs was analyzed using the predefined “Chopin +” protocol on Mixolab, a new equipment of CHOPIN Technologies. The international standard ICC-Standard Method No. 173, a protocol for complete characterization of flours, was used, and a simplified graphic interpretation of the results was performed. The Mixolab is an apparatus used to characterize the rheological behaviour of dough subjected to a dual mixing and temperature constraint. It measures in real time the torque (expressed in Nm) produced by passage of the dough between the two kneading arms, thus allowing study of rheological and enzymatic parameters: dough rheological characteristics (development time, hydration capacity, etc.), protein reduction, enzymatic activity, gelatinisation and gelling of starch. The Mixolab can work with a constant dough weight to eliminate the influence of the mixer filling ratio (www.chopin.fr).

The procedure parameters used for analysis of the rheological behavior in the Mixolab were as follows: tank temperature 30°C, mixing speed 80 min⁻¹, heating rate 2°C/min, total analysis time 45 minutes.

Table 2. Mixolab curves interpretation

Point	Significance	Associated parameters	
C1	Used to calculate water absorption	T°C 1 and T1	Dough temperature and the time taken for different types of torque to appear
C2	Measures protein weakening as a function of mechanical work and temperature	T°C 2 and T2	
C3	Measures starch gelatinization	T°C 3 and T3	
C4	Measures the stability of the hot-formed gel	T°C 4 and T4	
C5	Measures starch retrogradation during the cooling period	T°C 5 and T5	

Mixolab curves recorded (Table 2) are essentially characterized by torque values in five defined points (C1- C5, N·m), temperatures and processing times corresponding to those points. The correlation between parameters (Table 3.) is tested during mixing and heating of dough by Mixolab.

Table 3. Mixolab parameters correlation and significance

Parameter	Calculation method	Significance
Water Absorption (%)	Quantity of water required to obtain C1 = 1.1 Nm +/- 0.05	Quantity of water that the flour can absorb to achieve a given consistency during the constant temperature phase.
Time for C1 (min)	Time required to obtain C1	Dough formation time: The stronger the flour, the longer it takes.
Stability (min)	Time during which torque is > C1 – 11% (constant T° phase)	Dough resistance to kneading: The longer it takes the "stronger" the dough.
Amplitude (Nm)	Curve width at C1	Dough elasticity: The higher the value, the greater the flour elasticity.

The parameters obtained from the recorded curves are: water absorption (%) or percentage of water required for the dough to produce a torque (C1) of 1.1 N·m, mixing stability (min) or elapsed time at which the torque produced is kept at 1.1 N·m, protein weakening (C2, N·m and the difference between points C1-2, N·m), starch gelatinisation (C3, N·m and the difference between points C3-2, N·m), amylolytic activity (C4, N·m and the difference between points C3-4, N·m), starch gelling (C5, N·m and the difference between points C5-4, N·m).

Mixolab “Chopin +” transforms the standard curve into six quality indicators, expressed on a scale of 0-9 (Mixolab index) regarding:

- Water Absorption Index (a function of the composition of the flour (protein, starch, fiber). It affects dough yield. The higher the value, the more water is absorbed by flour.
- Mixing Index represents the behaviour of the dough during mixing at 30°C (stability, development time and weakening). A high value corresponds to high dough stability in mixing.
- Gluten+ Index represents the behaviour of gluten when heating the dough. A high value corresponds to high gluten resistance to heating.
- Viscosity Index represents the increase in viscosity during heating. It depends on both amylase activity and starch quality. A high value corresponds to high dough viscosity during heating.
- Amylolysis Index, the starch's ability to withstand amylolysis. A high value corresponds to low amylase activity.
- Retrogradation Index represents the characteristics of starch and its hydrolysis during the test. A high value corresponds to a low shelf life of the end product.

2.6. Statistical Analysis

All analyses were performed in triplicate and the mean values with the standard deviations were reported. Microsoft Excel 2003 Program was employed for statistical analysis of the data with the level of significance set at 95%. Analysis of variance (ANOVA) followed by Tukey’s test was used to assess statistical differences between samples. Differences were considered significant for a value of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of incorporation of partially defatted milk thistle seed flour on the color of wheat flour

Colour measurements for flour mixtures are presented in figure 3. The lightness parameter L^* decreased as more partially defatted milk thistle seed flour was added to the wheat flour, sample P4 having the darkest colour ($L = 82,47$). The flour mixtures with the highest percentage of added ingredient showed significantly higher redness values (a^*) compared to the control. The yellowness values (b^*) slightly decreased by increasing the amount of ingredient added, low b^* values corresponding to low L^* values (darker samples).

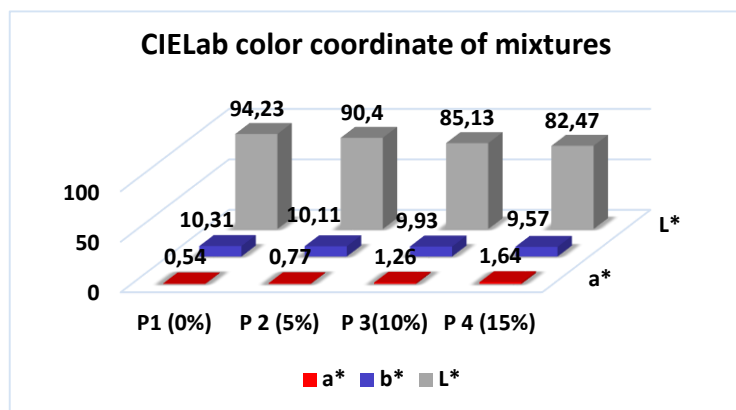


Figure 3. Influence percentage of partially defatted milk thistle seed flour on CIELab color coordinate

The total colour difference (ΔE) (figure 2) was calculated to establish whether colour differences between the control and the mixtures of flours are noticeable with the human eye. Thus, it can be seen that all ΔE values are greater than 3 and the differences are detectable with the human eye (Francis and Clydesdale 1975).

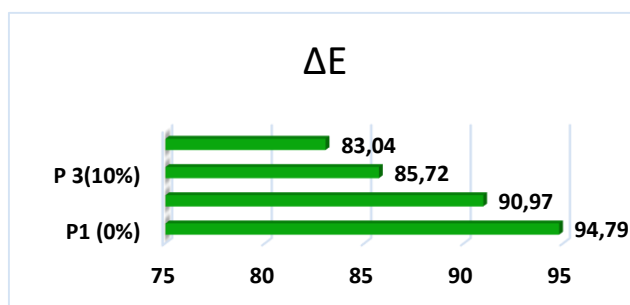


Figure 4. Influence of percentage of partially defatted milk thistle seed flour on „ ΔE ”.

Mixtures of wheat flours with different ingredients (oat, bran and barley) with darker colour were also reported (Duta et al. 2015, Sudha et al. 2007).

3.2. Effect of incorporation of partially defatted milk thistle seed flour on the minerals content of wheat flour

The mineral contents of the samples are given in Table 4. Their profiles in the samples of wheat flour and partially defatted milk thistle seed flour were typical for these plant species.

Table 4. Mineral content of wheat flour, partially defatted milk thistle seed flour and mixtures thereof

Sample	Constitutes (mg/100g):							
	Ca	Mg	Na	K	Cu	Zn	Mn	Fe
P1	43.80±0.69	47.70±0.71	30.60±2.95	187±0.91	0.78±0.09	5.45±1.02	0.56±0.30	1.10±0.35
P 2	87.21±0.93	66.97±0.89	29.63±2.69	217±1.11	0.88±0.11	5.55±1.02	0.93±0.36	5.07±0.41
P 3	130.62±1.23	86.23±0.92	28.66±2.15	247±1.12	0.97±0.28	5.64±1.03	1.30±0.39	9.04±0.49
P 4	174.03±1.78	105.50±1.45	27.69±1.78	277±1.15	1.07±0.45	5.74±1.03	1.67±0.54	13.01±0.68
P5	912±2.19	433±2,20	11.2±2.60	790±1.65	2.69± 0.54	7.38± 1.70	7.97±1.91	80.5±1.85

It is easily noticeable that, compared to the low mineral content of the wheat flour sample (P1), mixtures of wheat flour and partially defatted milk thistle seed flour have higher contents of minerals, in direct proportionality with the percentage increase of partially defatted milk thistle seed flour added in the flour mixtures.

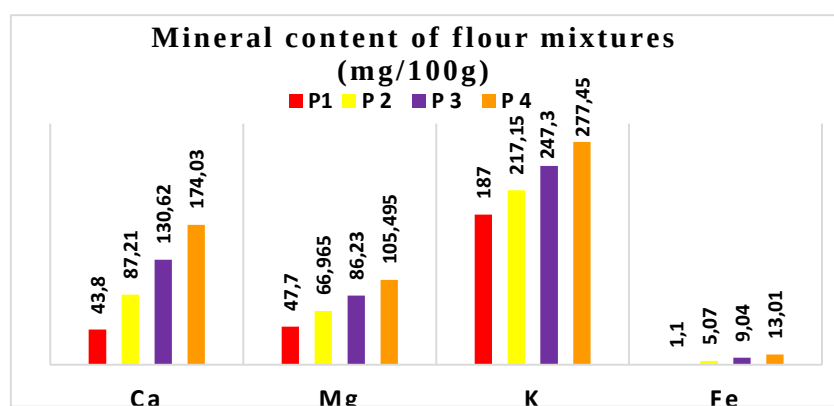


Figure 5. The influence of the addition of partially defatted milk thistle seed on the mineral content of flour mixtures

Thus, as can be seen in figure 5, calcium content increased almost four times, from 43.8 mg/100 g (P1) to 175.03 mg/100 g (P4), magnesium content increased 2.2 times from 47.7 mg/100 g (P1) to 105.5 mg/100 g (P4), potassium content increased significantly, from 187 mg/100 g (P1) to 277.45 mg/100 g (P4), and iron content also increased significantly, from 1.1 mg/100 g (P1) to 13.01 mg/100 g (P4).

3.3. Rheological properties of flour mixtures

The rheological behaviour of wheat flour dough (P1) and of all flour mixtures during the Mixolab test is illustrated in Table 4.

Table 5. Influence of partially defatted milk thistle seed flour added to wheat flour in different proportions on Mixolab characteristics (rheological behavior)

Parameter	Abrev name	P1	P2	P3	P4
Water absorption (%)	CH	60	59.5	58.9	58.1
Stability (min)	ST	9.43	8.73	8.07	7.85
Amplitude (Nm)	A	0.073	0.075	0.087	0.106
Maximum consistency during:					
– phase 1 (N·m)	C1	1.11	1,14	1,12	1,14
	TC1	5.55	5.42	5.38	4.67
– phase 2 (N·m)	C2	0.49	0.47	0.43	0.38
	TC2	16.73	16.76	16.78	17.02
– phase 3 (N·m)	C3	1.87	1.77	1.74	1.71
	TC3	23.43	23.12	23.18	22.97
– phase 4 (N·m)	C4	1.54	1.52	1.50	1.49
	TC4	30.83	30.82	30.78	29.88
– phase 5 (N·m)	C5	2.43	2.39	2.34	2.32
	TC5	45	45	45	45

Mixolab C1-C5 values of pure wheat dough (P1) were 1.11 N·m, 0.49 N·m, 1,87 N·m, 1,54 N·m, and 2,43 N·m, respectively (Table 4). Similar Mixolab behavior was mentioned by Papouskova et al. (2011) for three wheat varieties, where a small difference was shown (C1-C5 averages 1.12 N·m, 0.46 N·m, 2.04 N·m, 1.76 N·m, 2.44 N·m, respectively).

From the data obtained here, it can be seen that, as the amount of added partially defatted milk thistle seed flour increases, the water absorption capacity (CH) decreases from 60% (P1) to 59.5% (P2), 58.9% (P3) and 58.1% (P4), respectively. As such, with regard to water absorption capacity, the baking characteristics of flours did not decrease considerably as the percentage of partially defatted milk thistle seed flour increased. For bread flour, optimal CH values are between 55%-62% (Despina Bordei 2004).

Dough stability had the following values: 9.43 min (P1), 8.73 min (P2), 8.07 min (P3) and 7.85 min (P4). It can be noticed that the addition of partially defatted milk thistle seed flour did not have a significant influence on the stability of the wheat flour sample, even for the sample with 15% added partially defatted milk thistle seed flour.

As the percentage of partially defatted milk thistle seed flour increased, the amplitude, i.e. the width of the curve during dough formation, increased, which suggests a higher elasticity of the dough, due to higher content of fat. This increase in fat content has a positive influence on doughs.

As the content of partially defatted milk thistle seed flour increases, the water absorption capacity and dough formation time (TC1) also increases (Table 4). A bigger resistance of dough to mixing is noticed.

A small decrease of consistency (C2 – the degree of dough weakening as a consequence of temperature) shows a higher softening of dough as an effect of temperature. This means some small negative qualitative changes in flour protein composition, i.e. dilution of gluten content and changes of gluten structures, can possibly occur.

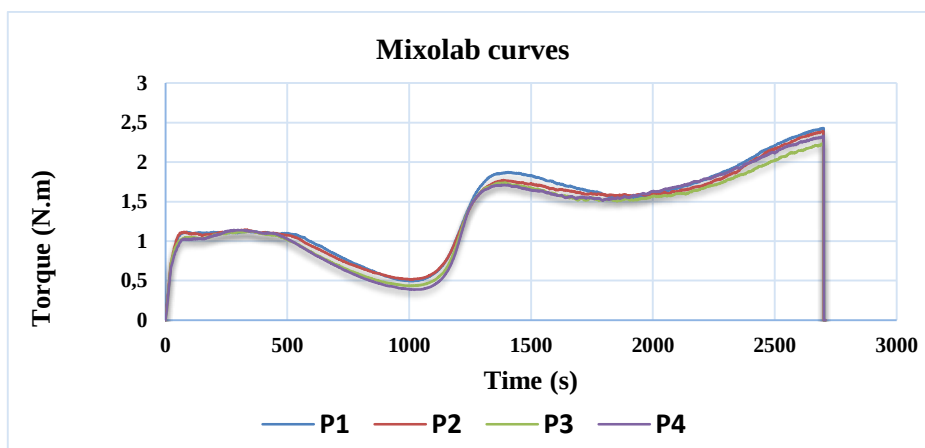


Figure 6. The influence of partially defatted milk thistle seed flour on the Mixolab curve of wheat flour

In phase 3, the starch gel formation, when the temperature reached 50-55 °C, the lowest C3 was observed for P4. The difference in C3 results between P1 and P4 samples was 0.16 N·m, so the influence of dough preparation recipe was low, similar to C2. The difference in C3 for consecutive samples is rather small, such that the influence of percentage of partially defatted milk thistle seed flour for the first two mixtures (P2 and P3) on the rheological quality of flour is minor. This will be tested by production of bakery products.

As mentioned above, the C4 parameter corresponds to the stability of the starch gel formed. In this sense, a dependence of determined values on dough formulation was sought. The lowest C4 was found for P4 (Table 4). The difference of C4 results between P1 and P4 samples was only 0.05 N·m, thus insignificant. The stability time of the gel (TC4) decreases as the percentage of partially defatted milk thistle seed flour increases.

The retrogradation stage of starch (C5) for the tested wheat flour and wheat-partially defatted milk thistle seed flour mixtures demonstrated similar differences as for starch gel stability. It can be seen that differences in C5 between consecutive samples are generally not significant, but some difference between P1 and P4 is registered (2.43 and 2.32 N·m, respectively).

From all of the above data, it can be stated that, with regard to their baking characteristics, these flour mixtures fall into the category of flours suitable for bakery products.

4. CONCLUSIONS

The present work led to the conclusion that, as the percent of partially defatted milk thistle seed flour added increased, the color of flour mixtures darkened compared to pure wheat flour.

The characterization performed in this study proved that the partially defatted milk thistle seed flour is a valuable source of minerals, especially calcium, magnesium, iron and potassium.

The main conclusion in our study concerning the rheological properties of dough (pure wheat flour and mixtures of wheat flour with partially defatted milk thistle seed flour) is that rheological parameters were maintained within limits that can assure a good technological behaviour towards obtaining high quality bakery products.

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