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Potravinarstvo Slovak Journal of Food Sciences
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Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 14, 2020, p. 788-797
https://doi.org/10.5219/1365
Received: 26 February 2020. Accepted: 1 July 2020.
Available online: 28 September 2020 at www.potravinarstvo.com

ISSN 1337-0960 (online)

MILK THISTLE FLOUR EFFECT ON DOUGH RHEOLOGICAL PROPERTIES

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ABSTRACT
The influence of the addition of partially defatted milk thistle seed flour was studied by analyzing the rheological properties of dough in order to further exploit the functionality of partially defatted milk thistle flour in bakery products. The rheological properties of dough were monitored using Mixolab 2 (Chopin Technologies, France). A rheofermentometer F4 (Chopin Technologies, France) was used to check the dough fermentation, and for the baking trials wheat flour, rye flour, and milk thistle flour were kept in the portion: 50:50:0 (control flour); 50:45:5; 50:40:10 and 50:35:15. The addition of different milk thistle flour in the mixtures resulted in a difference in the viscoelastic properties of the dough. The results showed a weakening of the gluten network in all trial mixtures evaluated. The dough development time values of the control flour were 1.20 min, while an addition of milk thistle flour in portions of 5, 10, and 15% increased these values to 1.30 min, 1.90 min, and 2,80 min, respectively. In addition to higher dough development time values, all trial mixtures exhibited also higher stability (5.07 min; 6.25 min and 8.03 min), when compared to the control flour (4.63 min). The trial mixture with 15% milk thistle flour had different characteristics of gelatinization and retrogradation. The rheofermentometer measured the dough characteristics during proofing, and the trial mixtures with the addition of MTF had a retention volume at approximately the same level as the control flour (WRF). The Volscan profiler was used to determine the bread volume and other parameters. All breads had high volume and specific volume values and can be rated as good, with good porosity and ratio. Mixtures containing 5%, 10% and 15% milk thistle flour added to wheat flour + rye flour maintained rheological parameters within the recommended limits for good technological behavior and, consequently, good quality of bakery products. 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.

Keywords: wheat-rye-milk thistle composite; Chopin+ protocol; rheofermentometer; bread quality

INTRODUCTION
Food not only supplies human beings with nutritive components that are indispensable for maintaining a healthy daily life, it also contains many types of biofunctional components that help to maintain good physical and mental health. In the last three decades, a growing interest has occurred in the use of active compounds from natural sources that can contribute to promoting consumer's health and preventing diseases. One opportunity is milk thistle (*Silybum marianum* L. Gaertn); an annual or biennial plant belonging to the family *Asteraceae* and usually growing in dry, sunny areas. The plant native to the Mediterranean area has now spread to other warm and dry regions. It is a plant that has been used in folk medicine for over 2,000 years as a remedy for a variety of medical conditions, especially the liver, kidney, and gallbladder ailments **(Anthony et al., 2013; Eskandari Nasrabadi et al., 2014; Wang et al., 2014; El-haak, Atta and Abd Rabo, 2015; Saad-Allah, Fetouh and Elhaak, 2017; Abenavoli et al., 2018)**.

In Europe, it is cultivated widely for the production of raw materials used in the pharmaceutical industry, especially in Austria, Germany, Hungary, and Poland. Over-the-counter nutritional or dietary supplements are becoming extremely popular in the United States, Europe, and many other countries for liver enhancement and recovery **(Anthony et al., 2013; Jedlinszki et al., 2016; Salomone, Godos and Zelber-Sagi, 2016; Choe et al., 2019; Çeribasi et al., 2020)**.

Milk thistle is a rich source of ingredients, such as amino acids, fatty acids, minerals, and phytochemicals exhibiting nutraceutical effects on human health. The whole plant is used for medicinal purposes. Its seeds (there is confusion about whether milk thistle has fruits or seeds – botanically correct, this plant has a cypselae, which looks like a seed but is technically a fruit.) 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. Furthermore, the seed proteins have markedly high amounts of essential amino acids such as lysine, isoleucine, leucine, valine, and threonine, however, they are a poor source of prolin and histidin **(El-haak, Atta and Abd Rabo, 2015; Apostol et al., 2017; Saad-Allah, Fetouh and Elhaak, 2017)**.

In milk thistle seeds linoleic and oleic acids are the predominant fatty acids in its oil **(Tuğba- Çelik and Gürü, 2015)** (Table 1). Milk thistle oil has significant scavenging effects on the DPPH radical. This antioxidant activity reflects the bioactive molecules composition in oil extracted from milk thistle (1.1 ±0.6% of polyphenols on dried material, 13.4 mg.100g^{-1} beta-carotene of carotenoids and 70 – 85% of unsaturated fatty acids) **(Li et al., 2012; Anthony et al., 2013; Rahal et al., 2015; Abenavoli et al., 2018)**.

Silymarin represents 1.5 – 6% of the fruit's dry weight and is an isomeric mixture of unique flavonoid complexes – flavonolignans **(Eskandari Nasrabadi et al., 2014; Bijak, 2017)**. The main representatives of this group presented in silymarin are silybin, isosilybin, silychristin, isosilychristin, silydianin, and silimonin. The chemical composition of milk thistle fruit besides flavonolignans also includes other flavonoids, such as taxifolin, quercetin, dihydrokaempferol, kaempferol, apigenin, naringin, eriodyctiol, and chrysoeriol **(Kvasnička et al., 2003; Bijak, 2017)**. The whole plant is used for medicinal purposes, but the highest content of silymarin is in the seeds. Silybin, a secondary metabolite isolated from the seeds of milk thistle was discovered as the first member of a new family of natural compounds called flavonolignans in 1959 **(Biedermann et al., 2014)**. Since the 1970s, silybin has been regarded in official medicine as a substance with hepatoprotective properties. There is a large body of research that demonstrates silybin's many other healthy properties, but there is still a lack of papers focusing on its molecular structure, chemistry, metabolism, and novel form of administration **(Bijak, 2017)**.

Currently, the most important medicinal application of milk thistle is its use as a hepatoprotective, antioxidant, antiradical, and free radical scavenging food supplement. Silymarin is a natural antioxidant and this action is believed to contribute to the hepatoprotective effects of milk thistle preparations. Cold-pressed milk thistle seed flour was evaluated for its phytochemical composition, and gut microbiota modulating, free radical scavenging, anti-inflammatory, and anti-proliferative capacities **(Surai, 2015)**. The results suggest milk thistle seed flour's potential health benefits in functional foods **(Choe et al., 2019; Menasra and Fahloul, 2019)**, despite its properties observed in experimental studies, the current efficacy of milk thistle preparations in patients with liver diseases is not fully compelling **(Salomone, Godos and Zelber-Sagi, 2016; Abenavoli et al., 2018)**.

Cytotoxic activities of silychristin, silydianin, and silybinin were evaluated against Caco-2 cells (colon cancer cell line) **(Rahal et al., 2015)**, but the anticancer activity of silymarin, as well as silibinin, was demonstrated against various cancer cells such as breast, skin, cervix, ovary, prostate, lung and hepatocellular cancers **(Bosch-Barrera and Menendez, 2015)**.

Most of the existing researches on the milk thistle plant dealt only with pharmacological and medicinal studies due to the production of silymarin and its use for healing some hepatic diseases. However, the defatted milk thistle seed flour which is a by-product, and is typically eliminated as waste, may contain beneficial components such as proteins, carbohydrates (especially crude fibres), minerals, and some phytochemicals and be utilized as a suitable food ingredient in low fibre food **(Li et al. 2012; El-haak, Atta and Abd Rabo, 2015)**. Milk thistle leaves and flowers have been used as a vegetable for salads and a substitute for spinach. On the other hand, milk thistle seeds are roasted for use as a coffee substitute.

The fundamental food in many parts of the world is bread. Wheat flour dough is a viscoelastic system that exhibits an intermediate rheological behavior between a viscous liquid and elastic solid. The viscoelastic protein network plays a predominant role in dough processing as well as in textural characteristics of the finished bread. The gluten network in wheat is constructed from gliadins and glutenins, which are responsible for the dough resistance to extension, thus providing the dough with its unique viscoelastic properties and its ability to retain gases produced during the yeast fermentation process. Therefore, gluten is a fundamental component, which is responsible for the overall quality and structure of bread **(Fendri et al., 2016)**.

The information necessary for the evaluation of the flour quality is obtained on the basis of some indices determined through the chemical, rheological and technological analyses. Rheological characteristics, such as elasticity, viscosity, and extensibility, are important for the milling and bakery industry, used for a prediction of the dough processing parameters and the end product quality **(Jirsa, Hrušková and Švec, 2007; Ziobro et al., 2013; Švec and Hrušková, 2015)**.

Composite flours on the basis of wheat and other cereals and non-grain seeds became popular in the baking technology. The purpose of this research was to identify the percentage of bread flour as well as to find a mixture of flours, the composition of which combines the nutritional value with adequate processing properties in an optimal way. The objective of this work was to characterize mixtures of wheat flour + rye flour, with a replacement of 5, 10, and 15% of milk thistle seed flour, with regard to the technological characteristics of dough (rheological properties) and objective properties of the final product.

Scientific hypothesis

We expect some effect of milk thistle seed flour in addition to baker's flour (wheat flour and rye flour) on the rheological properties of the dough, its ability to retain fermentation gases, and the objective properties of the final product.

MATERIAL AND METHODOLOGY

In this study, commercial blend flours (Miroslav Grznár MLYN ZRNO, Slovak Republic), were used: defatted milk thistle seed flour (8.4% fat) in mixtures with wheat flour (ash 0.65%) and rye flour (ash 0.96%). Three types of mixtures of wheat flour + rye flour and different proportions of partially defatted milk thistle seed flour were obtained, and a control flour without milk thistle flour in the following ratios: 50:45:5, 50:40:10, 50:35:15, and 50:50:0, respectively. Further materials for dough and bread formulation were: salt (K.S. Czech Republic, a. s.), sucrose (Slovenské cukrovary s. r. o.), and dry yeast of the species *Saccharomyces cerevisiae* (Ruf, sušené droždie).

Rheological properties of dough were monitored using Mixolab 2 (Chopin Technologies, France) applying the "Chopin+" protocol. 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. In „Chopin+" protocol Mixolab recorded changes of torque in five defined points as follows: C1 – water absorption; C2 – weakening of the protein-based on mechanical stress at increasing temperature; C3 – the rate of starch gelatinization; C4 – stability of the formed gel; C5 – starch retrogradation during the cooling period.

A Rheofermentometer F4 (Chopin Technologies, France) was used to check the dough fermentation, to measure the dough parameters: the maximum dough height (Hm, mm), the maximum height of the gas release curve (Hm′, mm), the time required to obtain H´m (Tx, hours), total volume (total volume of gas produced in mL), the volume of CO_2 lost (carbon dioxide volume in mL that the dough has lost during proofing), retention volume (carbon dioxide volume in mL still retained in the dough at the end of the test). The dough (315 g) was placed in a movable basket of the gas meter with a 2000 g cylindrical weight, and the cover of the vat was fitted with an optical sensor. The test was conducted at 28.5 °C for 3 h (according to the conditions of the Chopin protocol reported in the rheofermentometer instruction manual). The method conforms to the AACC 89-01 **(AACC, 2000)** standard for the measurement of yeast activity and gas production.

Bread making procedure: for baking trials wheat flour, rye flour, and milk thistle flour (MTF) were kept in the portion: 50:50:0 (control flour WRF); 50:45:5; 50:40:10 and 50:35:15. Other recipe compounds were: water (the level of water used in baking experiments, to obtain the dough suitable for bread baking – the water addition to individual dough mixes ranged between 61 to 64%, 2.0% NaCl, and 1.4% dry yeast. The percentages are based on 100% of the flour mixture. All ingredients were kneaded 3 minutes at lower speed and 4 minutes at higher speed in a spiral kneader type SP 12 D (Diosna Dierks & Söhne GmbH, Osnabrück, Germany). After 40 min fermentation (35 °C) samples were baked for 40 min in mode: 180 °C 7 minutes, 200 °C 20 minutes, and 160 °C 13 minutes, with stream (250 mL) (MIWE Condo). The bread loaves were cooled at room temperature and analyzed by a Volscan Profiler volume analyzer (Stable Mycrosystems, Surrey, UK) one hour after baking (weight of the bread (g), bread volume (mL), specific volume ($mL.g^{-1}$), volume-yield ($mL.100g^{-1}$ flour), the aspect ratio of a middle slice ect.).

Statistical analysis

The results of the technological measurement were statistically analyzed using XLSTAT for Excel (version 2015). The data were subjected to the Z. test at significance level 0.05 to determine differences between samples.

RESULTS AND DISCUSSION

The effect of adding milk thistle flour on the dough properties and bread parameters was investigated. Bread is widely consumed as a staple food across many cultures and countries worldwide. There are three major stages of bread making: mixing, fermentation (i.e. proofing), and thermal setting (i.e. baking/steaming). The mechanical energy imparted during mixing induces the formation of a viscoelastic dough matrix. Besides the proper development of the gluten network, the initial gas inclusion during mixing and the yeast activity during proofing also greatly affect the bread quality **(Gao et al., 2017)**. Gluten is a major protein component of the same cereals which is responsible for flour processing characteristics in the bakery industry. The reduction of gluten often results in baked bread with a crumbling texture, poor color, and other post-baking quality defects **(Schmiele et al., 2017)**.

In the trial breads prepared with milk thistle, the proportion of gluten gradually decreased with an increasing amount of the addition, which affected the rheological behavior of the dough. The preliminary rheological analysis indicates the influence of applied MTF on the dough properties.

Monitoring the rheological properties of dough is very important for the overall technology to estimate the mechanical properties of dough and to imitate its behavior during its processing or even to anticipate the quality of the final product **(Dapčević-Hadnađev et al., 2014; Torbica, Belović, and Tomić, 2019)**.

The Mixolab measures in real time the torque (expressed in Nm) produced by the passage of the dough between the two kneading arms, thus allowing the study of rheological and enzymatic parameters: dough rheological characteristics (development time, hydration capacity, etc.), protein reduction, enzymatic activity, gelatinization and gelling of starch. Figure 1 exhibits the Mixolab curve (Chopin+ protocol) and parameters of the analyzed sample (WRF 50:50 = control flour). The results confirm that the control flour has a short dough development time (1.2 min) and only average stability (4.63 min), which is due to its composition, especially the high proportion of rye flour which cannot form gluten. During the heating and cooling stages, the control flour showed a high viscosity of starch gelatinization and retrogradation.

The addition of different MTF (5%, 10%, 15%) in the mixtures resulted in a difference in the viscoelastic properties of dough (Figure 2). The results showed a weakening of the gluten network in all trial mixtures evaluated.

Water absorption, amplitude, dough development time, stability, and C1 Mixolab parameters are used to evaluate the gluten network formation, dough development during mixing, and stability at constant mechanical shear. The first stage of the Mixolab curve is related to the farinograph analysis, which allows evaluating the viscoelastic properties of dough including hydration and mixing tolerance index. In Mixolab, the first stage temperature is kept at 30 °C. The mixing process after the first stage may give extra energy to the dough system; therefore, these results should be used with caution because they do not represent the breadmaking process, since the dough can no longer pass through the mixing process after the formation of the gluten network, without weakening the structure during the mixing of the dough **(Schmiele et al., 2017)**.

Table 1 Fatty acid composition milk thistle oil, %.

	Fatty acid	%		Fatty acid	%
C 18:2	**Linoleic acid**	**54.97**	C 20:1	Gadoleic acid	0.95
C 18:1	**Oleic acid**	**24.10**	C 24:0	Lignoceric acid	0.59
C 16:0	Palmitic acid	8.15	C 18:3	Linolenic acid	0.17
C 18:0	Stearic acid	5.51	C 16:0	Palmitoleic acid	0.10
C 20:0	Arachidic acid	3.03	C 14:0	Myristic acid	0.09
C 22:0	Behenic acid	2.27	C 17:0	Margaric acid	0.07
			C 17:1	Heptadesenoic acid	0.03

Note: **(Tugba-Celik and Gürü, 2015)**.

Table 2 Rheofermentometer parameters of dough from the trial mixtures and the control flour.

Sample	H´m (mm)	Tx (time)	Total volume (mL)	Retention volume (mL)	Volume of CO^2 lost (mL)	Retention coefficient
Control flour (50:50:0)	60.50$_a$	0:54:00$_a$	1 569$_a$	1 247$_a$	322$_a$	79.50$_a$
50:45:5	74.20$_b$	0:34:30$_b$	1 779$_b$	1 230$_a$	549$_b$	69.10$_a$
50:40:10	79.30$_b$	0:39:00$_a$	1 803$_b$	1 228$_a$	575$_b$	68.10$_a$
50:35:15	83.50$_b$	0:46:30$_a$	1 927$_b$	1 307$_a$	621$_b$	67.80$_a$

Note: The following indices were measured: H´m – maximum height of the gas release curve; Tx – time required to obtain H´m; Total volume – volume of gas produced, Retention volume – CO_2 volume in mL still retained in the dough at the end of the test; Volume of CO_2 lost – CO_2volume in mL that the dough has lost during proofing; Gas retention coefficient (Retention volume/Total volume). Means followed by different letters are significantly different (*p* <0.05).

Table 3 Bread quality parameters.

Trial	Weight, g	Volume, mL	Specific volume, mL.g^{-1}	Volume-yield, mL.100g^{-1} flour	Aspect ratio of middle slice
Control bread (WRF)	805$_a$	2 126$_a$	2.64$_a$	425.17$_a$	0.68$_a$
Bread with 5% MTF	964$_b$	2 040$_a$	2.12$_a$	408.02$_a$	0.75$_a$
Bread with 10% MTF	883$_b$	1 749$_b$	1.98$_a$	349.88$_b$	0.64$_a$
Bread with 15% MTF	881$_b$	1 734$_b$	1.97$_a$	346.80$_b$	0.87$_a$

Note: Means followed by different letters are significantly different (*p* <0.05).

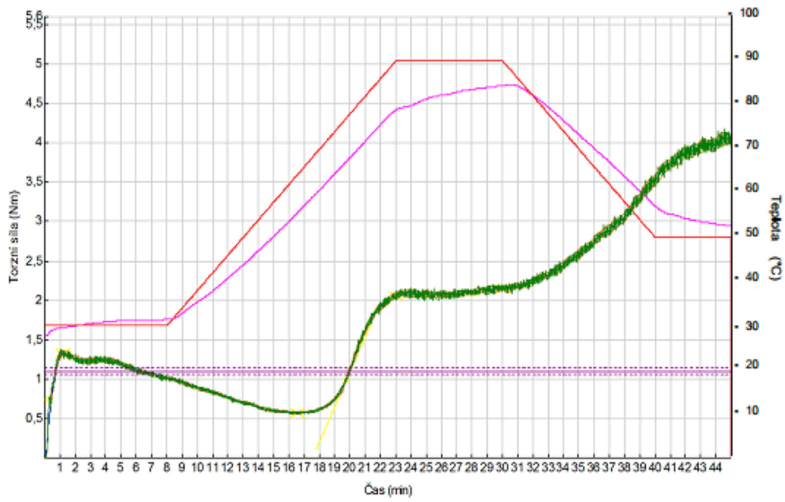


Figure 1 Mixolab curve Chopin+ protocol, control flour (WRF 50:50).

The rheological properties are provided in Figure 3. The dough development time values of the control flour were 1.20 min, while addition milk thistle flour increased these values to 1.30 min, 1.90 min, and 2.80 min, respectively. In addition to higher dough development time values, all trial mixtures exhibited also higher stability (5.07min; 6.25 min and 8.03 min) when compared to the control flour (4.63 min). In general, the increase of the dough development time indicates that higher fibre content slows down the rate of hydration and the development of gluten **(Kohajdova et al., 2011)**. At large, the stability of the dough is attributed to protein poor in sulfhydryl groups, which normally causes dough softening or even degradation. The gluten network is stronger and has higher resistance to shear stress at higher protein content **(Kaur et al., 2016)**. Although milk thistle flour contains a high level of protein, a higher level of fibre may weaken the gluten network **(Shahat Mohamed, Hassein Ahmed and Hady Essam, 2016)**.

The dough development time parameter and stability are very important for bread making because the development of the gluten network should be optimized to ensure loaves with a high volume and soft texture **(Rosell, Collar, and Haros, 2007)**. The enhancing ability of MTF is surprising as this raw material does not bring into the system gluten-forming proteins. Nevertheless, for flours with high rye content, the MTF had, besides its nutritional benefits, also a technological benefit related to a higher mixing tolerance (Figure 2). Similar results were found by other authors **(Apostol et al., 2017; Bortlíková, Kolarič, and Šimko, 2019)**.

The parameters slope-α, C2, and C2-C1 were recorded when the heating reached 52 – 57 °C at the beginning of the second stage. In this phase, starch started to gelatinize, and the proteins changed their quaternary, tertiary, and secondary structures due to protein denaturation. Also, swelling of the starch granules was observed, mainly for the damaged granules, and the changes along the mixing process modified the dough consistency **(Schmiele et al., 2017)**. C2 values, representing the weakening of the protein network, decreased from 1.332 Nm to 0.571 Nm (control flour), or from 1.572 Nm to 0.774 Nm (50:35:15), and then remained at an almost constant value indicating a certain compatibility level of gluten and milk thistle flour proteins during the dough formation.

In the third stage, where the evaluated parameters were slope-β, C3, and C3-C2, the heating step began until reaching 70 °C while the dough remained under constant mixing, stopping protein denaturation and starch gelatinization. Higher C3 values were observed for all trial mixtures when compared to the control flour, but the difference for consecutive samples is rather small.

The fourth stage assessed the parameters slope-γ, C4, and C4-C3, and evaluated the enzymatic activity and heat stability of the starch gel, at temperatures above 80 °C. In this sense, a dependence of the determined values on the dough formulation was sought. A decrease in C4 was observed in the trial mixture 50:35:15 (15% milk thistle flour), and the torque value was significantly lower compared to all other samples during the last stage too.

In the last stage the dough was cooled to 58 – 60 °C, and the parameters C5 and C5-C4 were assessed. The retrogradation stage of starch (C5) for the tested WRF and MTF mixtures demonstrated similar differences as for the starch gel stability. Significant differences in C5 can be seen between the trial mixture 50:35:15 and all other samples.

The trial mixture 50:40:10 (10% MTF) presented the highest C5 values, exhibited the highest retrogradation in the cooling phase, due to the higher degree of starch gelatinization in the heating phase. The differences between this trial mixture, the mixture 50:40:5, and the control flour 50:50:0 were small. This is also documented in Figure 4, because no changes in slope-α and slope γ was observed for the trial mixture 5%, 10%, and 10% MTF, and the control flour. The results from slope-β confirmed that the trial mixture 50:35:15 (15% MTF) has different gelatinization characteristics at a temperature above 52 – 57 °C.

The rheofermentometer analysis of flour and dough enables accurate simulation of the processing conditions during the production of baked goods containing yeast **(Dapčevič-Hadnađev et al., 2011)**. The rheofermentometer measures the characteristics of dough during proofing (the dough development, the production of gas due to yeast action, the porosity of the dough, the tolerance of the dough during proofing), and the analysis determines the total gas production of yeast and dough volume at standard barometric pressure over time. The instrument records two curves during the dough fermentation and rising, one describing the development of the dough and another depicting the production and retention of gas.

Dough development is a function of both yeast gassing power and gluten network integrity. The rheofermentometer analysis allows simultaneous observation of both yeast fermentation and dough growth, which provides direct evidence on the correlation between the two factors. The dough development curves of the control sample (WRF) and trial mixture sample with the addition of MTF 15 % are shown in Figure 5 and Figure 6, respectively. It is evident that the trial mixture dough sample with the addition of milk thistle 15% rose to a greater height (83.5 mm) than the control dough (60.5 mm).

The rising of the control dough can be characterized as insufficient and slow, which is associated with a high proportion of rye in the flour. The results recorded throughout the tests are shown in Table 2, and it can be concluded that the addition of MTF to bread flour (wheat flour and rye flour) influenced all evaluated parameters.

Rheofermentometer curve trial mixtures with MTF 15% showed that the dough after the fermentation completely retained 67.8% of the total CO_2 produced. In comparison, the rheofermentometer curve WRF retained 79.5% of the total CO_2 produced. The maximum dough volume MTF 15% of 1927 mL was reached after 1 hour, 10 minutes, and 30 seconds, in comparison with WRF (1569 mL after 1 hour and 15 minutes).

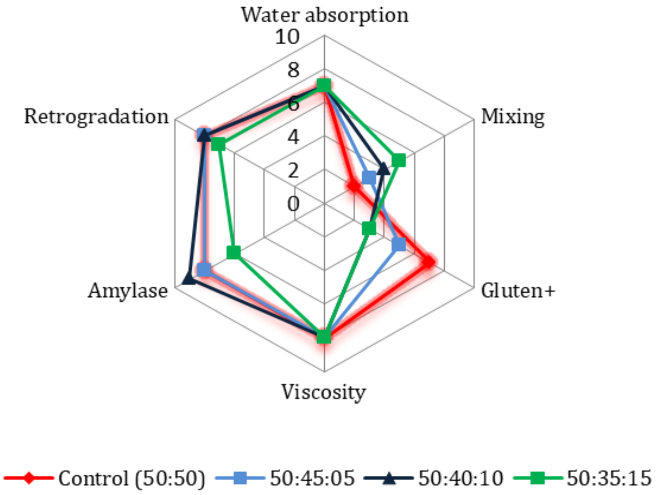
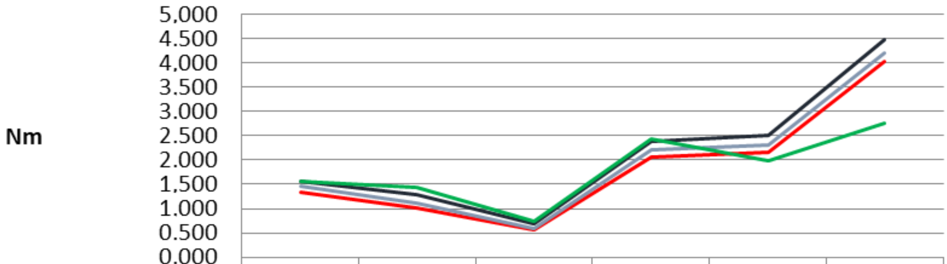


Figure 2 Mixolab profiler.



	C1	C1´8	C2	C3	C4	C5
Control (50:50)	1,332	1,015	0,571	2.061	2,149	4,034
50:45:05	1.460	1,109	0,593	2,214	2,303	4,201
50:40:10	1.564	1.293	0.690	2,393	2,501	4,477
50:35:15	1,572	1.445	0,744	2.423	1,981	2,749

Figure 3 Influence of milk thistle seed flour added to wheat flour + rye flour in different proportions in Mixolab characteristics (torque during mixing).

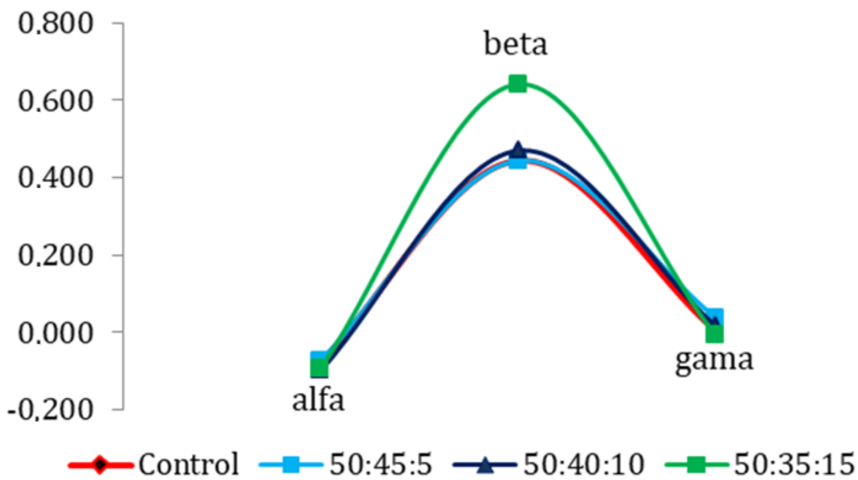


Figure 4 Slope alfa, beta and gamma during the mixing process of dough.

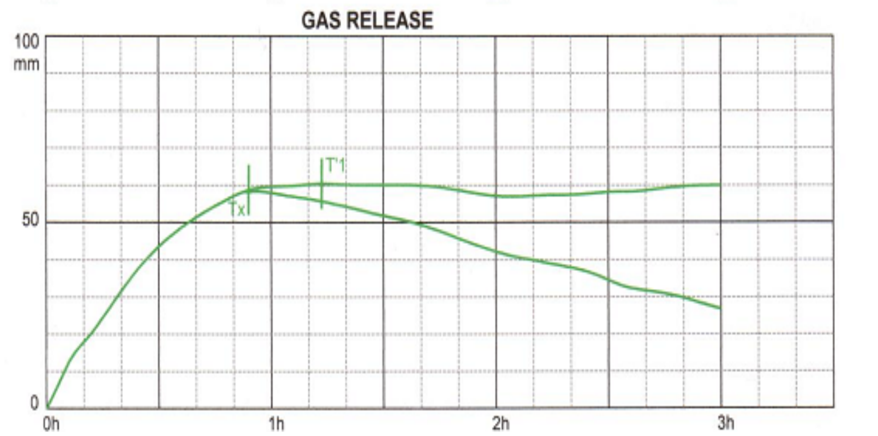


Figure 5 Rheofermentometer curve WRF.

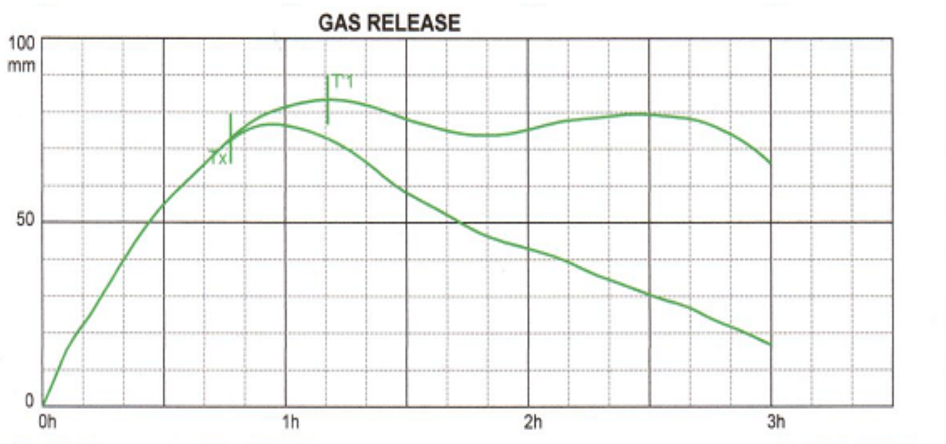


Figure 6 Rheofermentometer curve trial mixtures with MTF 15%.

a **b** **c** **d**

Figure 7 Bread volume and bread aspect ratio by Volscan profiler.
Note: a – control bread; b – bread with 5% MTF; 7c – bread with 10% MTF; d – bread with 15% MTF.

In general, the trials with the addition of MTF began to release CO_2 too early, probably related to the reduction of gluten, but the total gas produced in these samples was higher than in the control flour (Table 2). During rheofermentometer measurements, the trial mixtures with the addition of MTF have lost significantly higher amounts of CO_2 in comparison with the control flour, which is documented by the retention coefficient lower by 13.1%, 14.3%, and 14.7%, respectively. However, it is important to note that the retention volume at the end of the measurement was at approximately the same level as the control flour (WRF) in all evaluated mixtures, with an MTF addition of 15% even higher than the control flour.

Monitoring the rheological properties of dough is very important for the overall technology to estimate the mechanical properties of dough and to imitate its behavior during its processing or even to anticipate the quality of the final product **(Dapčevič-Hadnađev et al., 2011)**. 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.

Bread volume is one of the major quality-determining criteria for bread quality. Bread with a larger volume is always preferred. A Volscan profiler was used to determine the bread volume, and the result was divided by the sample's weight to obtain its specific volume. The form ratio was also calculated by taking the maximum height divided by the maximum width of the sample, of which the measurements were determined by the Volscan profiler.

As can be seen in Figure 7 (by Volscan) and Table 3, bread volume and specific volume decreased with increased addition of MTF. This could be explained by a better gas retention ability of the gluten matrix in the dough, leading to bread with a larger volume in the control sample. The specific volume of bread decreased when the MTF was added. With 2.12 $mL.g^{-1}$, the specific volume of bread with 5% MTF approaches the value of standard wheat-rye bread (2.64 $mL.g^{-1}$) the most. In comparison, commercial wheat and bread with lower gluten content can only achieve a higher porosity by adding various supplements, which has a negative impact on nutrition and consumer acceptance. According to **Gao et al. (2017)**, the structural and textural attributes of baked bread were significantly correlated with the gluten development and the gas production of the dough. These findings suggest a great dependence on baked bread quality on its gluten network. **Kondakci, Wenjuan-Zhang, and Zhou (2015)** state that the low-, medium- and high-protein bread samples showed lower specific volumes compared to their corresponding control sample.

In general, all breads had high specific volume values and can be evaluated as of good quality, with good porosity and ratio. Even the nutritionally interesting addition of 15% MTF did not cause an important decrease in the quality of the trial bread, giving a good presumption for the application of MTF in baking flour.

The addition of MTF affects the color of the flour as indicated by **Apostol et al. (2017)**, color differences between the control flour and the flour mixtures are noticeable with the human eye. The flour mixtures with the highest percentage of added ingredient showed significantly higher redness values compared to the control flour. As the percentage of added partially defatted milk thistle seed flour increased, the color of the flour mixtures darkened compared to pure wheat flour. The overall acceptability of all experimental breads was evaluated as good.

From a nutritional point of view, in addition to silimarin MTF is a valuable source of minerals, especially calcium, magnesium, iron, and potassium. The mineral contents of the defatted milk thistle seed compared to wheat flour were referred to by **Apostol et al. (2017)**. The authors confirmed that compared to the low mineral content of wheat flour, samples of milk thistle seed flour were having higher levels of minerals. MTF is a good source of proteins, mineral compounds, and fats and therefore it can be used for human nutrition. Moreover, due to the high content of flavonolignans, summarily called silymarin, and their positive effects on the liver, it is also suitable for the production of functional foods **(Bortlíková, Kolarič, and Šimko, 2019; Menasra and Fahloul, 2019)**.

The addition of MTF to bread is in line with the trend of designing food according to consumers' needs, also those with special needs. Food thus becomes an active part of a progressive lifestyle and can help prevent certain diseases.

CONCLUSION

Modern foods are expected to contain nutritive components that are indispensable for maintaining a healthy daily life, but also contain many types of biofunctional components that help to maintain good physical and mental health. One opportunity is milk thistle (*Silybum marianum* L. Gaertn). It is a plant that has been used as a remedy for a variety of medical conditions, especially liver, kidney, and gallbladder ailments. The defatted milk thistle seed flour could be utilized as a suitable ingredient in food production, for example in the composite flours based on wheat and other cereals and non-grain seeds that have become popular in the baking technology.

The analysis of the viscoelastic properties gives information about the influence of the applied ingredients on the rheological behavior of the dough. Preliminary rheological analyses indicated a strong influence of the applied non-wheat flour on the dough properties.

Summarising the results and findings of this study, the following conclusions can be postulated:

-The suitability of WRF replacement with MTF in the range 5, 10, and 15% were studied for the preparation of bread as healthy bakery products,

-During the experiments, the effects of the WRF replacement on the technological properties of the dough were determined using Mixolab characteristics. The results showed that the trial mixture with 5 and 10% MTF had very similar results to the control flour (WRF). The trial mixture with 15% MTF has different characteristics of gelatinization and retrogradation,

-A rheofermentometer measures the dough characteristics during proofing, and the trial mixtures with the addition of MTF have created a large gas volume. During the rheofermentometer measurements, the MTFs lost significantly higher amounts of CO_2 compared to the control flour, but the retention volume at the end of the measurement was approximately the same as with the control flour (WRF) at all evaluated mixtures,

-The rheological analysis to predict the quality of the final product showed that it was possible to make bread with a WRF replacement by these functional ingredients in concentrations that allow maintaining the specific volume similar to the control bread,

-A Volscan profiler was used to determine the bread volume and other parameters and confirmed the predicted properties because all breads had high volume and specific volume values and can be evaluated as of good quality, with good porosity and ratio.

The main conclusion in our study concerning the rheological properties of dough WRF and mixtures of WRF with MTF is that the rheological parameters were maintained within limits that can assure a good technological behavior towards obtaining high-quality bakery products.

From all of the above data, it can be stated that, concerning their baking characteristics, these flour mixtures fall into the category of flours suitable for bakery products. The results suggest that milk thistle seed flours have health benefits potential to be used in functional foods.

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Acknowledgments:

This work was supported by project VEGA 1/0139/17 and ITEM 26220220180 Building Research Centre AgroBioTech.

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