The Effect of Milk Thistle (Silybum marianum L.) Fortification, Rich in Dietary Fibre and Antioxidants, on Structure and Physicochemical Properties of Biscuits

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Abstract: In the present study, an attempt was made to enrich cookies with ground seeds of milk thistle (Silybum marianum L.) and to determine the effect of this addition on the quality of cookies. The content of nutrients, fibre, and calorific value, as well as, texture, spreadability factor, volume, colour and sensory parameters were tested. The biscuits were characterized by lower caloric value, hardness and volume, but higher spreadability. The cookies with 5% of milk thistle gained high consumer acceptance, but greater than 20% addition caused a worsening quality of the product. The biscuit fortification with ground milk thistle seeds seems to be justified, through enrichment of the products with fibre and the antioxidant compounds having health-promoting properties.

Keywords: polysaccharides; dietary fibre; antioxidant activity; food fortification; biscuits; milk thistle

1. Introduction

Confectionery production is an important branch of the food industry as the consumption of biscuits, sponge cakes and other confectionery products is constantly growing. It is forecast that the global confectionery market will reach $270.5 billion by 2027 [1]. Foods containing sugar and those rich in fat are blamed for obesity in society. Therefore, food producers have been trying to change this negative perception of confectionery goods by introducing ecological, pro-health products, enriched with nutrients, with reduced calories, among others, by eliminating sugar or replacing it with other sweeteners, introducing vitamins, fibre and antioxidant compounds. Efforts are also being made to replace the traditional flour used in sweet flour-based bakery products with others of higher nutritional value. Thanks to such treatments, it is possible to enrich the final product with bioactive compounds, vitamins, minerals, protein and dietary fibre, while reducing the calorific value. It is extremely important as it enables the introduction of new products to the market, as well as allowing expansion of the range of products for people suffering from diet-related diseases and those who care about a healthy lifestyle. An important form of food enrichment is the introduction of ingredients that have not been attractive or known to producers so far. This mainly concerns plants that are not common in the world and occur only in a particular narrow area or continent, are not very popular or their culinary potential is not known. Examples of such plants are açai (Euterpe oleracea L.), psyllium (Plantago psyllium L.), chia (Salvia hispanica L.) or Lycium barbarum, popular in Asia, Africa or America, while in Europe their qualities have been appreciated only in recent years [2–6]. One such raw material, little recognized so far, is the seed of milk thistle (Silybum marianum L.), an annual plant, found throughout Europe, Africa, the Americas and Asia [7]. The seeds of the plant are rich in flavonolignans, referred to as silymarin [8], which mainly contains compounds such as: silibinin, silychristin, silidianin and isosilybin [9]. Studies carried out in the last decade confirm that thistle seeds have antioxidant...
activity, anti-inflammatory, anticarcinogenic, antiatherosclerotic, antihypertensive, anti-diabetic and anti-obesity properties [8,10–12]. Silymarin compounds show high antioxidant activity, therefore, they can inhibit the formation of free radicals and lipid peroxidation in the presence of liver toxicity. It was found that silymarin can protect the membranes of lipid hepatocytes by limiting cell lysis [13], as well as chelate iron and copper ions and affect the biosynthesis of molecules that protect our bodies from stressful stimuli [12]. Dietary fibre consists of soluble (SDF) and insoluble (IDF) fractions. Soluble fibre fractions include oligosaccharides and some indigestible polysaccharides (e.g., arabic gum, pectins, galactomannan, inulin and β-glucans). SDF increases viscosity in the intestines, slows down the speed of passage of food content through our digestive tract and slows intestinal absorption of glucose and sterols. Valuable sources of the soluble fibre fraction are oat bran, barley, beans, lentils, peas and some fruits and vegetables [14–21]. In turn, the insoluble fibre (IDF) primarily consists of cellulose, insoluble hemicelluloses and lignin. Rich sources of this fraction are wheat bran, whole grains, nuts and seeds. It has been proven that consuming IDF can benefit our bodies by reducing weight gain, improving insulin sensitivity, lowering blood pressure and improving immune function. In order to achieve the desired results, it is recommended that the daily intake of dietary fibre by an adult should be approximately 25–35 g, of which about 50–75% should be IDF [20–25].

Considering that cookies are one of the highly popular snacks among the world’s sweet products, but on the other hand, rich in sugar and poor in nutrients, such technological solutions should be searched for in order to increase their quality and nutritional value. Taking into account the above-mentioned health benefits of consuming milk thistle seeds, an attempt was made to enrich wheat biscuits with bioactive ingredients and dietary fibre contained in this plant. It was also necessary to identify the effect of flour from thistle seeds on the physical and sensory parameters of the product.

2. Materials and Methods

2.1. Materials

The ground seeds of milk thistle (Silybum marianum L.) (MT) were bought from Intenson Europe company (Józefów, Poland). The material was characterized by the following parameters (per d.w.): moisture 8.80%, proteins 21.05%, total carbohydrates 29.81%, fats 34.59%, total dietary fibre (TDF) 27.95%, insoluble dietary fibre (IDF) 24.19%, ash 5.75%. Wheat flour (type 550) was bought in the Polish Cereal Plant PZZ (Krakow, Poland), and was characterized by the parameters (per d.w.): moisture 11.70%, proteins 9.15%, total carbohydrates 76.87%, fats 1.77%, total dietary fibre 0.94%, soluble dietary fibre 0.40%, insoluble dietary fibre 0.54%, ash 0.34%. The other ingredients: sugar, potato starch, margarine, eggs, milk (1.5% fat), salt and baking powder have been purchased at the local market.

2.2. The Biscuits Preparation

The preparation of the dough and the baking of biscuits were carried out according to the previously developed methodology [3]. The amount of ingredients used in the basic recipe (control biscuits) was as follows: 100 g of wheat flour, 17.8 g of sugar, 11.5 g of potato starch, 24.1 g of margarine, 4.6 g of eggs, 29.9 g of milk (cont. 1.5%) and 0.3 g of salt and 3.3 g of baking powder. In the recipe of fortified biscuits, wheat flour was replaced with ground milk thistle seeds (MT) in variable amounts, i.e., 5, 10, 15 and 20% (samples MT5, MT10, MT15 and MT20, respectively). Each repetition consisted of cookies that were made from separate batches of dough. Analyses of the cookies were carried out on fresh product, i.e., on the day they were baked.

2.3. The Chemical Composition of Biscuits

The analyses were carried out according to the Association of Official Analytical Chemists International methods [26]. The Kjeldahl procedure (using the Büchi B324 extraction system) with nitrogen was used to determine the total proteins content (protein
conversion factor was 6.25, method number 950.36). Analysis of the total dietary fibre (DF) and its fractions: soluble (SDF) and insoluble (IDF) was carried out by the enzymatic-gravimetric method (method number 991.43). The Soxhlet method (using the Büchi B811 extraction system) (method no. 935.38) was used to determine the fats content. The ash content was examined by carbonization (method number 923.03). The content of total carbohydrates is the difference after taking into account the moisture, protein, fat and ash content. The caloric value = \( (4 \times \text{protein}) + (9 \times \text{fat}) + (\text{total carbohydrates} – \text{total dietary fibre}) \times 4 \) [27]. The final result is the average of three repetitions.

2.4. Total Polyphenol Content and Antioxidant Activity of Biscuits

The sample of biscuits was ground (0.6 g) and extracted for 2 h, at temperature 23 °C, using 30 mL of 80% ethanol. Then sample was centrifuged (MPW-350 centrifuge) at 2500 \( \times \) g for 15 min. The extract was stored in a freezer at temp. of −20 °C. The content of total polyphenols was carried out according to Singleton et al. [28] by spectrophotometric method, flavonoids content according to El Hariri et al. [29] by spectrophotometric method. The antiradical activity was determined with the use of ABTS method [30].

2.5. Volume of Biscuits

The volume of fresh products was carried out according to the procedure of Krystyjan et al. [3] with a laser volume meter (Volscan profiler 600, Stable Micro Systems, London, UK). The final result is the average of three repetitions.

2.6. Colour of Biscuits

The upper surface colour of fresh biscuits was carried out according to the procedure of Krystyjan et al. [31] using Konica MINOLTA CM-3500d equipment (Konica Minolta Inc., Tokyo, Japan), with reference to illuminant D65 and a visual angle of 10°. Using the CIELab system, the color parameters have been determined: \( L^* \) (\( L^* = 0 \) black, \( L^* = 100 \) white), \( a^* \)—share of the green colour (\( a^* < 0 \)) or red (\( a^* > 0 \)), \( b^* \)—share of blue (\( b^* < 0 \)) or yellow (\( b^* > 0 \)). The final result is the average of four repetitions.

2.7. Hardness of Biscuits

The analyses were carried out using the texture analyzer (TA-XT plus, Stable Micro Systems, Haslemere, UK) according to Krystyjan et al. [3]. In brief, a P/6-cylinder probe was penetrated the product with 1 mm per second speed, at a distance of 20 mm. The value of maximum force (hardness) was measured. The final result is the average of ten repetitions.

2.8. Sensory Analysis of Biscuits

The sensory analyses were carried out according to our previous paper [32] by a panel of 13 persons (6 men and 7 women between the ages 25–55) with adequate sensory sensitivity [33]. A five-point evaluation scale with quality descriptors and weighting coefficients: shape (0.1), colour (0.1), surface (0.15), consistency (0.15), fracturability (0.1), smell (0.1) and taste (0.3) were used [34]. The overall acceptance of fresh biscuits was assessed on the basis of the total score: <2.9 unacceptable, 3.0–3.5 acceptable, 3.51–4.5 good, and 4.51–5.0 very good, according to Gambuś et al. [34].

2.9. Statistical Analysis

The experimental data was subjected to one-way analysis of variance (ANOVA), using Statistica v. 8.0 software (Statsoft, Inc., Tulsa, OK, USA) and Fisher’s test was applied to calculate the least significant difference (\( p = 0.05 \)).

3. Results

3.1. Chemical Composition of Biscuits

Table 1 shows the results of the analysis of content of nutrient and dietary fibre in the product. The enrichment of flour with milk thistle led to a statistically significant
increase in the fat content of the biscuit, by about 5.3% with the lowest additive used, and as much as 26.8% with 20% fortification. Despite the increased content of fat, the caloric value of the milk thistle cookies decreased compared to the control. The decrease was statistically significant, and there was a 2.7% reduction in calories at 5% substitution, and a 5.3% reduction at 20% substitution. The content of the other essential nutrients, protein and ash, have also increased, but not as significantly as for fat (Table 1). Thus, in the case of protein, there was an increase from 3.0% to 8.7%, although the 5% substitution did not change the protein content of the product. In the conducted research, a statistically significant and uniform increase in fibre was observed, both its soluble and insoluble fractions, along with the increasing addition of ground milk thistle seeds (Table 1). At the lowest MT addition to the cookies, there was an increase in TDF by as much as 45%. The maximum MT substitution increased overall fibre content by 170%. It should also be noted that the applied substitution contributed to simultaneous decrease in caloric value (from 2.6 to 5.3%).

Table 1. The chemical composition of fortified biscuits.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fat [g/100 g d.m.]</th>
<th>Total Carbohydrates [g/100 g d.m.]</th>
<th>Protein [g/100 g d.m.]</th>
<th>Ash [g/100 g d.m.]</th>
<th>Dietary Fibre [g/100 g d.m.]</th>
<th>Calorific Value [kcal/100 g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.99 ± 0.1 a</td>
<td>70.53 ± 0.16 b</td>
<td>7.89 ± 0.00 c</td>
<td>1.65 ± 0.01 b</td>
<td>1.22 ± 0.01 a</td>
<td>414 ± 0.8 d</td>
</tr>
<tr>
<td>MT 5</td>
<td>13.68 ± 0.32 d</td>
<td>68.36 ± 0.46 b</td>
<td>7.88 ± 0.11 c</td>
<td>1.48 ± 0.02 d</td>
<td>1.47 ± 0.07 d</td>
<td>403 ± 2.1 b</td>
</tr>
<tr>
<td>MT 10</td>
<td>14.85 ± 0.12 c</td>
<td>66.37 ± 0.12 b</td>
<td>8.13 ± 0.12 b</td>
<td>1.58 ± 0.01 c</td>
<td>1.86 ± 0.02 c</td>
<td>398 ± 1.1 c</td>
</tr>
<tr>
<td>MT 15</td>
<td>15.47 ± 0.01 b</td>
<td>66.52 ± 0.08 c</td>
<td>8.12 ± 0.01 b</td>
<td>1.67 ± 0.03 b</td>
<td>2.58 ± 0.05 b</td>
<td>368 ± 0.6 c</td>
</tr>
<tr>
<td>MT 20</td>
<td>16.47 ± 0.05 a</td>
<td>64.71 ± 0.08 d</td>
<td>8.58 ± 0.05 e</td>
<td>1.81 ± 0.01 a</td>
<td>3.18 ± 0.04 a</td>
<td>392 ± 0.6 d</td>
</tr>
</tbody>
</table>

The parameters in columns with the same letters (a, b, c, d or e) do not differ statistically.

3.2. Total Polyphenol Content and Antioxidant Activity of Biscuits

The content of total phenolic compounds (TPC) in milk thistle was on the level 4.57 mg catechin/g d.m. and the number of flavonoids was quite substantial and equal to 4.35 mg rutin/g d.m. It was observed that the amount of polyphenols increased, in a range from 31 to 258% in relation to the control, with the introduction of 5 to 20% milk thistle seeds to biscuits (Table 2). A similar trend was observed in the case of flavonoids in biscuits with milk thistle supplementation. The largest amount of flavonoids was recorded in biscuits with a 20% share of MS, two times greater than in the control, and the smallest in biscuits with 5% milk thistle addition (about 30% greater than the control). Taking into account the antioxidant activity, its increase was also noted in biscuits with ground milk thistle seeds fortification, adequate to the level of supplement share. The correlation between ABTS and TPC and flavonoids was, respectively, 0.980 and 0.961.

Table 2. The total polyphenol content and antioxidant activity of biscuits.

<table>
<thead>
<tr>
<th>Samples</th>
<th>TPC (mg Catechin/g d.m.)</th>
<th>Flavonoids (mg Rutin/g d.m.)</th>
<th>ABTS (µmol/kg d.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.58 ± 0.01 a</td>
<td>0.53 ± 0.03 d</td>
<td>14.98 ± 0.47 a</td>
</tr>
<tr>
<td>MT 5</td>
<td>0.76 ± 0.09 b</td>
<td>0.74 ± 0.07 b</td>
<td>20.97 ± 1.12 b</td>
</tr>
<tr>
<td>MT 10</td>
<td>1.16 ± 0.10 c</td>
<td>0.84 ± 0.01 c</td>
<td>39.75 ± 2.20 c</td>
</tr>
<tr>
<td>MT 15</td>
<td>1.59 ± 0.05 d</td>
<td>0.95 ± 0.00 d</td>
<td>50.14 ± 1.60 d</td>
</tr>
<tr>
<td>MT 20</td>
<td>2.08 ± 0.12 e</td>
<td>1.15 ± 0.06 e</td>
<td>58.92 ± 1.13 e</td>
</tr>
</tbody>
</table>

The parameters in columns with the same letters (a, b, c, d or e) do not differ statistically.

3.3. Physical Parameters of Biscuits

Table 3 shows the spreadability of the biscuits with increasing amount of ground milk thistle seed. The control sample was characterized by the lowest spreadability. The gradual increase in the amount of added milk thistle seeds contributed to the increase in this parameter by 23.4–30.3% compared to the control.
Table 3. Physical parameters of biscuits: colour, texture and volume.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spread Ratio (-)</th>
<th>Volume (mL)</th>
<th>Colour</th>
<th>Hardness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>L⁺</td>
<td>a⁺</td>
</tr>
<tr>
<td>Control</td>
<td>5.22 ± 0.34 b</td>
<td>75.1 ± 3.7 a</td>
<td>72.33 ± 0.56 a</td>
<td>6.66 ± 0.37 a</td>
</tr>
<tr>
<td>MT 5</td>
<td>6.44 ± 0.42 a</td>
<td>70.3 ± 0.8 b</td>
<td>71.85 ± 0.14 a</td>
<td>1.81 ± 0.36 b</td>
</tr>
<tr>
<td>MT 10</td>
<td>6.78 ± 0.35 a</td>
<td>69.7 ± 0.3 b</td>
<td>69.71 ± 0.44 a</td>
<td>1.92 ± 0.16 b</td>
</tr>
<tr>
<td>MT 15</td>
<td>6.79 ± 0.53 a</td>
<td>68.5 ± 2.6 bc</td>
<td>68.93 ± 1.07 b</td>
<td>1.82 ± 0.56 b</td>
</tr>
<tr>
<td>MT 20</td>
<td>6.80 ± 0.37 a</td>
<td>65.2 ± 1.0 c</td>
<td>65.23 ± 1.81 c</td>
<td>2.06 ± 0.35 b</td>
</tr>
</tbody>
</table>

The parameters in columns with the same letters (a, b, c or d) do not differ statistically.

Biscuits baked with the addition of milk thistle seeds had a smaller volume in comparison with the control; 20% addition lowered this parameter by 12.8%.

The results of the color analysis of biscuits enriched with milk thistle seeds are shown in Table 3. Values of the L⁺ parameter, describing the brightness of the color of the subject biscuits, was the largest for the control sample. The values of this parameter for fortified cookies turned out to be lower compared to the control and decreased with increasing addition of milk thistle by 0.7–9.8%. The a⁺ component, responsible for the red or green color, also obtained the highest value for the control sample, which proves a greater share of red color in the tested sample. The fortified samples were characterized by approximately 28.6% smaller proportion of red color. The b⁺ parameter, describing the share of blue or yellow color, similarly to the parameters described above, reached the highest value in the control sample. The b⁺ values for fortified biscuits gradually decreased in relation to the control by 20.9–38.5%.

Another important physical parameter of biscuits is their hardness. The share of 5–20% milk thistle in biscuits reduced their hardness by 50.2–65.3% successively. However, no statistically significant differences were found between the biscuits with 10, 15 and 20% addition.

3.4. Sensory Parameters of Biscuits

Sensory analysis showed that the control sample obtained the highest rating, at the level of 4.5 (Table 4). The acceptance of biscuits was rated as [35]: <2.9 unacceptable, 3.0–3.5 acceptable, 3.51–4.5 good, and 4.51–5.0 very good (Figure 1). The remaining samples received comparable notes. Biscuits with 5% milk thistle addition were rated 4.3, with 10% addition achieved a rating of 4.1, and containing 15 and 20%—4.0 and 3.8, respectively.

Table 4. The score of sensory analysis of biscuits.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Shape x(0.1)</th>
<th>Colour x(0.15)</th>
<th>Surface x(0.15)</th>
<th>Consistency x(0.15)</th>
<th>Fracturability x(0.1)</th>
<th>Smell x(0.1)</th>
<th>Taste x(0.3)</th>
<th>Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5 ± 0.0 a</td>
<td>0.5 ± 0.0 a</td>
<td>0.8 ± 0.0 a</td>
<td>0.6 ± 0.1 a</td>
<td>0.4 ± 0.1 a</td>
<td>1.3 ± 0.2 a</td>
<td>4.5 ± 0.3 a</td>
<td></td>
</tr>
<tr>
<td>MT 5</td>
<td>0.5 ± 0.0 a</td>
<td>0.4 ± 0.1 b</td>
<td>0.7 ± 0.1 ab</td>
<td>0.5 ± 0.1 a</td>
<td>0.4 ± 0.1 a</td>
<td>1.4 ± 0.2 a</td>
<td>4.3 ± 0.5 ab</td>
<td></td>
</tr>
<tr>
<td>MT 10</td>
<td>0.5 ± 0.0 a</td>
<td>0.4 ± 0.1 b</td>
<td>0.7 ± 0.1 ab</td>
<td>0.5 ± 0.2 a</td>
<td>0.4 ± 0.1 a</td>
<td>1.3 ± 0.2 a</td>
<td>4.1 ± 0.6 abc</td>
<td></td>
</tr>
<tr>
<td>MT 15</td>
<td>0.5 ± 0.0 a</td>
<td>0.4 ± 0.1 b</td>
<td>0.6 ± 0.2 b</td>
<td>0.5 ± 0.1 a</td>
<td>0.4 ± 0.1 a</td>
<td>1.2 ± 0.2 ab</td>
<td>4.0 ± 0.7 bc</td>
<td></td>
</tr>
<tr>
<td>MT 20</td>
<td>0.4 ± 0.1 b</td>
<td>0.4 ± 0.1 b</td>
<td>0.6 ± 0.1 b</td>
<td>0.5 ± 0.1 a</td>
<td>0.3 ± 0.1 a</td>
<td>1.1 ± 0.2 b</td>
<td>3.8 ± 0.3 c</td>
<td></td>
</tr>
</tbody>
</table>

The parameters in columns with the same letters (a, b or c) do not differ statistically.

Analyzing the sensory parameters assessed, it was found that the fortification used did not affect such quality descriptors of fortified biscuits as consistency and fracturability compared to the control. It slightly lowered the value of surface, but only at higher share of milk thistle—15 and 20%, as well as the shape of 20% fortified biscuits. On the other hand, biscuits with a 5–15% share of ground milk thistle seeds could boast similar notes for taste as the control sample.
4. Discussion

4.1. Chemical Composition of Biscuits

The fortification of ground milk thistle seeds led to an increase in the fat content of the biscuit. This impact is due to the difference in chemical composition between ground thistle seeds and wheat flour; the milk thistle flour contains 19.5 times more fat. Kazazis et al. [10] proved that milk thistle seed oil has unsaturated fatty acids, with the greatest share of linoleic and oleic acid, which demonstrate beneficial impacts on human health and protects against diabetes, arteriosclerosis and cancer. It should also be emphasized that, despite the increased content of fat, the caloric value of the milk thistle cookies decreased compared to the control. This was due to the lower content of carbohydrates in thistle seeds compared to wheat flour. The nutrient content of the raw materials used in baking has a significant impact on the physicochemical and health-promoting properties of the final product. Carbohydrates and fats control the flavour, aroma, color and texture of biscuits [32]. Mineral compounds have a regulating and hematopoietic role. In addition, they are cofactors of enzymes that are involved in the formation of the human antioxidant barrier [36].

An important food ingredient, due to its pro-health properties, is dietary fibre. Despite the fact that it is not a bioactive compound, it plays an important role in human nutrition [31]. The soluble dietary fibre shows hypoglycemic, hypocholesterolemic and antitumour effects [32]. According to the Institute of Medicine in the United States, the recommended daily intake of TDF by an adult is not less than 25 and not higher than 38 g/day (14 g/1000 kcal/day). Unfortunately, the reality shows that our diet is poor in this component, and average intake of dietary fibre range between 13 and 14 g/day [37].

4.2. Total Polyphenol Content and Antioxidant Activity of Biscuits

High content of flavonoids in milk thistle should be explained by a significant amount of a subclass, the flavonolignans, such as: silibinin, silychristin, silidianin and isosilybin [9,38,39]. The high content of polyphenols, including flavonoids, guarantees high anti-radical activity expressed as TEAC (70.11 micromoles/kg d.m.). The increased TPC in biscuits can be explained by the fact that, during baking, products of the Maillard reaction are formed. These products may react with Folin–Ciocalteu’s reagent used in the determination of polyphenols by a method proposed by Singleton et al. [28], thereby increasing the TPC in the samples, though actually polyphenols are introduced by milk thistle. Korus et al. [40] came to similar conclusions by testing gluten-free breads after the addition of defatted strawberry and blackcurrant seeds, among them a control sample that also contained TPC. In the study by Antoniewska et al. [41], after adding quince (Chaenomeles japonica) fruits (FJQ) to cookies, an increase in antioxidant properties was noted compared to the control. Even the addition of 0.5% FJQ caused a double increase of antioxidant activity of final
products. It should be emphasized that high antioxidant activity is related to the Maillard reaction products, that affect the antioxidant activity of the product.

An important bioactive component of thistle seeds is the aforementioned Silymarin, which belongs to the group of flavonolignans [9,38,39] and includes silybin and its isomer isosilybin, silyristin, as well as silydianin. Due to antioxidant activities, anti-inflammatory and antifibrotic effects, silymarin and its derivatives demonstrate protective effects on the liver in various forms of toxic hepatitis, fatty liver, cirrhosis, ischemic injury and virus-induced liver disease [42]. The polyphenols exhibit, among other things, anti-carcinogenic, antiradical, hypoglycemic, hypotensive, antiviral, antibacterial, anti-inflammatory effects, as well as reduce the risk of diseases such as diabetes, cardiovascular disease, atherosclerosis, genetic damage, degenerative bone changes, neurodegenerative diseases and act against obesity [43–45], hence they are very important compounds in the human diet.

The positive effect of polyphenols on our health is related to their inhibitory effect on digestive enzymes. The molecular weight, number and position of substitutions, as well as flavonoid glycosylation seems to be the most important features that play a predominant role in antidiabetic activity. Numerous works have also confirmed that the mode of action and the final effect of polyphenols seems to be different depending on the type of enzymes, as it will be other for carbohydrate hydrolyzing enzymes and other for protease enzymes [46–48]. Interactions of polyphenol–digestive enzymes at the molecular level are regulated by hydrophobic bonds, electrostatic forces and hydrogen bonds. These interactions are mainly associated with non-competitive inhibition of enzyme activity [46–49]. Regarding the antidiabetic activity of polyphenols, it is mainly due to their potency in lowering postprandial glycemic levels, thanks to their effective reduction of glucose absorption by inhibiting digestive enzymes. Various possible mechanisms have been proposed that may be responsible for the anti-obesity effects of polyphenols. They control the expression of genes which regulate the thermogenesis, energy expenditure and metabolism of lipids. However, the binding of polyphenols to enzymes is regarded as the main mechanism controlling obesity. [46–52].

4.3. Physical Parameters of Biscuits

The spreadability of biscuits increased with the level of fortifications. The parameter is correlated with dough viscosity and depends on the amount of gluten in the recipe [3], which decreased due to the substitution of biscuits with milk thistle flour; thus, it resulted in greater spreadability of the final product obtained. Another factor that increased the spreadability of enriched biscuits was the increased fat content, additionally introduced to the product along with milk thistle seeds. It should be mentioned that the seeds of this plant contain as much as 34.59 g/100 g d.m. of fat. Fat plays an important role in the production of biscuits as it is the predominant ingredient. It reduces the formation of a gluten network and thus contributes to obtain less-elastic dough. In addition, the increased fat content in the dough limits the pasting and gelatinization of starch, giving crisp texture [53].

The fortification decreased the volume of the biscuits, because the addition of milk thistle reduced the amount of wheat flour in the recipe, and thus also the amount of gluten. As a result, supplementation with the biscuits’ gluten network structure was much weaker.

The color of the food has an essential function in assessing the quality and attractiveness of a product by the consumer. It often determines its purchase or rejection. It can also provide information on the freshness of food. In biscuit production, the color is produced in the final baking stage, as an effect of the Maillard reaction. As a result of numerous chemical changes related to the Maillard reaction, sensory changes occur in bakery foods that include brown color and flavours, but also nutritional changes [54].

The addition of MT darkened the biscuit and changed the a* and b* component in relation to the control sample. Comparing the literature data, it was noticed that similar results were obtained for biscuits enriched with plantago psyllium flour [3]. However, the addition of bee pollen to the confectionery products made them darker [32]. According to the authors, bee pollen increases the reducing sugars content in the dough; therefore,
in biscuits enriched with its addition more melanoidins, responsible for their darker color, are formed [32]. According to Mundt and Wedzicha [55], the main factor affecting the darker color of biscuits after baking is the non-enzymatic darkening reaction, the aforementioned Maillard reaction, which takes place between reducing sugars and amino acids, proteins or peptides with a free amino group. This complex reaction produces dark-colored melanoids and flavours. The content of free amino acids, reducing sugars and heat treatment parameters (temperature and time) determine the intensity of the color of cereal products [56,57].

Significant influence of milk thistle seeds on the texture of biscuits limits the possibility of adding more of them. As shown by the sensory evaluation data, the increased proportion of its addition significantly worsens the taste (Table 4). One of the factors affecting the hardness was the increase in fat content in the recipe, which softened the biscuit dough [58]. Comparing the results of other researchers, it can be concluded that not only the addition of milk thistle reduces the hardness of biscuits, but also plantago psyllium flour [3], as well as bee pollen [32].

4.4. Sensory Parameters of Biscuits

Many factors determine the marketing success of a product, but the most important is consumer acceptance. Sensory analysis showed that biscuits with a 5–15% addition of milk thistle were of good quality. Nevertheless, all of the supplemented biscuits received a rating above 4.0, meaning the products were of good quality.

5. Conclusions

The biscuit fortification with milk thistle flour seems to be justified, mainly due to the enrichment of the products with dietary fibre and the antioxidant compounds having health-promoting properties. This treatment increases the nutritional value of the product, which becomes richer in essential nutrients: protein, mineral compounds, and polyphenols. Biscuits improved with milk thistle seeds were characterized by lower hardness and volume, but higher spreadability, that increased with the increasing fortification. The color of the biscuits was lighter, but this fact had little effect on the sensory evaluation. As it was confirmed by sensory analyses, biscuits with a 5–15% addition of milk thistle were of good quality; above this level, the quality parameters of the product slightly deteriorated.

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