INVESTIGATIONS REGARDING THE INFLUENCE OF CERTAIN TYPES OF FAT CONTENT ON ACRYLAMIDE LEVEL IN BISCUITS

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Abstract

Acrylamide (AA) in 1999 has been classified by the International Agency for Research on Cancer (IARC) as „potentially carcinogenic to humans” (Group 2A) and in 2002 was recognized as a genotoxic carcinogen by the European Union (EU), the Scientific Committee on Food. The aim of this study was to evaluate the correlation between the AA level formed in biscuits and colour difference (ΔE), the lipid content of fats used, respectively the water content of biscuits.

In the formulation of biscuits were used vegetable oils (sunflower oil, margarine, palm oil) and animal fat (butter, lard). For each type of fat used in biscuits were used three types of wheat flour with different ash content: 0.88% d.m. (F1), 1.27% d.m. (F2), respectively 2.2% d.m. (F3). The acrylamide level was determined by gas chromatography tandem mass spectrometry (GC-MS/MS) using internal standard (isotopically labelled AA 1, 2, 3-13C).

The AA level from the assortment of biscuits obtained with those 5 types of fat varied similarly, increasing for all types of wheat flour studied, as follows: palm oil > sunflower oil > lard > butter > margarine. When biscuits were obtained from the same type of fat and various types of wheat flour, the AA level increased for the wheat flour as follows: F3 > F2 > F1. Using in the recipe for the manufacture of biscuits of different types of fat (the same amount of fat with different lipid content) resulted in visible changes in the colour of products and changes in the AA level (F1 - 14.63 - 95.84 mg/kg, F2 - 153.26 - 608.87 mg/kg, F3 - 166.03 - 667.67 mg/kg). When the biscuits samples were obtained by using the wheat flour F2 and different amounts of fat with the same lipid content, the AA level ranged from 151.38 to 407.51 mg/kg.

The acrylamide level from biscuits is influenced by the type of fat and wheat flour used in their formulation, in this study the best combination to obtain a lower level of acrylamide was the use of margarine and flour with the lowest ash content.

Key words: Acrylamide, Biscuits, Fat, Wheat Flour, GC-MS/MS.

1. Introduction

When developing or improving the formulation recipe of food products, the manufacturer should choose carefully the ingredients used in order to obtain healthier products, sensory quality being as well an important factor to take in consideration.

Biscuits are one of the most popular bakery products, daily consumed mainly by infants, with a long shelf-life, many formulation of this food matrix being obtained. Several researchers tried to improve the functional and nutritional properties of biscuits [1, 2, 3, 4, 5]. Tarancón et al., [6] investigated the consumer perception of the biscuits made with olive oil and sunflower oil and concluded that the perception is based on the label information.

Biscuits characteristics are influenced mostly by the flour used in the formulation recipe, the fat type used and their fatty acids profile. Biscuits contain a considerably level of fats, lipids playing an important role in the quality of the final product.

Avoiding the use of trans fatty acids in the formulation recipe and the formation of chemical contaminants in the food matrix is an important factor in obtaining healthier products. Acrylamide (2-propenamide) is a process contaminant that forms in starchy food products at high temperature cooking (more than 120 °C) and low moisture [7]. This compound is formed mainly during Maillard reaction from asparagine and
carbonyl sources, as reducing sugars. The level of AA formed in the bakery products is dependent of the free asparagine present in the cereals, respectively the flours used and the reducing sugars used in the recipe. Maillard reaction is also responsible for the product flavour and surface browning. Another alternative for AA formation is through the oxidation process of fats. Triglycerides are formed from fatty acids and glycerol. During the baking process it takes place the oxidation of fats resulting the dehydration of glycerol, like this being obtained acrolein and acrylic acid [8]. Acrolein is then transformed to acrylamide by a series of reactions [9]. This reaction is accelerated by high temperature and exposure to light and oxygen [10]. Establishing the influence of the fat type on the AA level in biscuits is important for manufacturers of bakery products for highlighting the factors leading to the formation of a high AA level in food systems such as biscuits and similar products, and to establish the best mitigation strategy.

As the acrylamide content of the final product is dependent on the formulation recipe, the aim of this study was to determine the influence of five types of fat (sunflower oil, palm oil, margarine, lard and butter) on the acrylamide content of biscuits and to evaluate the correlation between the AA level and total colour difference ($\Delta E^*$), lipid content of the fat used in the formulation recipe, and water content of the biscuit.

2. Materials and Methods

2.1 Raw and auxiliary materials

For the experimental samples were used raw and auxiliary materials as: wheat flour, sugar, edible fats, eggs, leavening agents, salt and vinegar. Wheat flour was the main raw material, which came in the highest proportion in the composition of biscuit assortments, made in experimental variants. Based on the extraction degree [11] were used three types of wheat flours: semi-white flour, 75 - 85% (F1), black flour, 85 - 95% (F2), dietetic flour, 95 - 100% (F3). Sugar was introduced in the form of tiny white crystals (granulated sugar), odorless and tasteless.

Fat foods contribute to improve the taste and tenderness of the final products. In this study were used fats with different lipid content: margarine, 60% lipid; butter, 65% lipid; lard, 100% lipid; sunflower oil, 100% lipid; palm oil, 100% lipid. Eggs were used as additive in dough to improve food value and biscuits colour. In the formulation recipe of biscuit assortments was used as chemical leavening agent, baking soda (sodium bicarbonate).

2.2 Biscuits preparation

The technological process to obtain the biscuit assortments in experimental variants included the following main phases: preparation and dosing of raw and auxiliary materials, dough kneading, resting/dough maturation, dough shaping, baking, cooling, packaging (Figure 1).

There were achieved 20 variants of the formulation recipe:

• 3 types of wheat flour x 5 types of fat (same amount of fat/ different lipid content) (Table 1).
• 1 type of wheat flour x 5 types of fat (different amounts of fat/ same lipid content) (Table 2).

The fat was mixed with sugar until a creamy mass was obtained, then eggs, the baking powder mixed with
vinegar, salt and finally flour were added. Kneading lasted for about 10 - 15 min., to obtain a loose dough of suitable consistency which can be easily modeled. After kneading, the dough was packed in polyethylene bags and was allowed to stand in a refrigerator at 4 °C for 60 - 120 min. After depletion of resting time, the dough was shaped in the form of biscuits (3 x 8 cm), by a modeling machine and biscuits were then placed in trays lined with parchment paper. Biscuit trays were placed in an oven at 220 °C for 30 min.

The experimental variants were performed in three separate batches, and for the chemical analysis was used the average media of three batches. To obtain the biscuit assortments in the experimental variants were used specific technological equipment for the manufacture of bakery products, from the Pilot Experiments Plant for Cereals and Flours Processing from IBA Bucharest: dough mixer (Diosna, DM 08 - 4/6), dough roller, electric rotary oven with proofer, shaping machine, rotary kiln oven with controlled temperature and baking time (model ROTOR) etc.

2.3 Reagents and Consumables

For the AA content determination of biscuits were used a native AA standard, min. 99% purity, of concentration 1000 μg mL⁻¹ in methanol (1000 Ultra Scientific Analytical Solution), internal standard (IS) of labeled acrylamide (1,2,3-¹³C), min. 99% purity (+100 ppm hydroquinone), of concentration 1000 μg mL⁻¹ in methanol (Cambridge Isotope Laboratories, Inc.). All chemicals, solvents and reagents used to analyze the AA were of chromatographic purity and were purchased from Merck (Darmstadt, Germany), LGC Promochem GmbH (Wesel, Germany) and Scharflai (Spain). The water used was obtained from a purification system, PURELAB Option-S7/15 and PURELAB Ultra Ionic.

2.4 Acrylamide analyses

The acrylamide level of biscuits was quantified by GC-MS/MS [12]. The calibration solutions and the derivatized extracts sample were analyzed using a gas chromatograph, type TRACE GC Ultra, coupled with triple quadrupole mass spectrometer (TSQ Quantum XLS) type Thermo Fisher Scientific (USA). The analyses were performed as described by Negoiță and Culețu [13].

AA concentration values of the experimental variants of biscuit samples were expressed both on dry matter of biscuits (% d.m.) and as μg kg⁻¹ AA of biscuits.

The analytical procedure to determine the AA level from biscuits was characterized by a high degree of sensitivity (LOD = 4.63 μg kg⁻¹, LOQ = 13.89 μg kg⁻¹) and good precision (RSD (r) ≤ 5%, RSD (R) ≤ 6%) [13].

2.5 Colour analysis

The most common way of measuring food colour was introduced by Commission Internationale de L’éclairage (CIE) in 1976, in which colour is described in a three-dimensional scale, the amounts of L*, a*, b* [14 and 15].

Table 1. Formula used for the biscuits preparation achieved with the same amount of fat/ different lipid content

<table>
<thead>
<tr>
<th>No.</th>
<th>Ingredients</th>
<th>Margarine</th>
<th>Butter</th>
<th>Sunflower oil</th>
<th>Palm oil</th>
<th>Lard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Flour F1, kg, max.</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Flour F2, kg, max.</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Flour F3, kg, max.</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Margarine, kg (% lipids)</td>
<td>1 (60)</td>
<td>1 (60)</td>
<td>1 (60)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Butter, kg (% lipids)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (65)</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Sunflower oil, kg (% lipids)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Palm oil, kg (% lipides)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Lard, kg (% lipids)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Sugar, kg</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10.</td>
<td>Eggs, pieces</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>11.</td>
<td>Baking soda, g</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>12.</td>
<td>Salt, g</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>13.</td>
<td>Vinegar, mL</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

*Fats will be marked in the graphical representations with the following colours: margarine – purple, butter – yellow, sunflower oil – green, palm oil – red, lard – blue
The colour analysis was performed using a HunterLab colorimeter (Universal Software V4.01 Miniscan XE Plus). There were calculated CIELAB’76 colour parameters: L*, a*, b*. According to CIE, L* measured the object luminance intensity on a scale from 0 to 100, where 0 represents black and 100 white, a* represents the colour position of the object on a scale from pure red and pure green, where pure green is -127, and pure red is +127, and b* represents the position of the object on a scale colour of pure blue and pure yellow, where pure blue is -127 and pure yellow is +127. For each sample, measurements were made on 10 different points, and the mean value was determined. Total colour difference, ΔE* was calculated using the following equation:

\[ \Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \]

Where \( \Delta L^* = L^*_1 - L^*_0, \Delta a^* = a^*_1 - a^*_0, \Delta b^* = b^*_1 - b^*_0 \)

\( L^*_0, a^*_0, b^*_0 \) it refers to the standard colour (black, where \( L^*_0 = 0, a^*_0 = 0, b^*_0 = 0 \)), \( L^*_1, a^*_1, b^*_1 \) refers to the sample colour.

Instrument calibration was done with black and white pads supplied by the manufacturer. The colour of the samples was measured using illuminate D65 with an angle of view of 10°.

2.6 Statistical analysis

AA content was expressed as mean ± standard deviation. The differences among sample groups were analyzed by one-way ANOVA (analysis of variance) followed by Tukey’s test, \( p < 0.05 \) was considered statistically significant.

3. Results and Discussion

3.1 Influence of fat type on the formation of AA level from biscuits

The experimental samples of biscuit (Figure 2, 3, 4 and 5) have been analyzed in terms of water content [16], AA level and colour parameters (\( L^*, a^*, b^*, \Delta E^* \)).

The physico-chemical properties of the biscuit samples are shown in Tables 3, 4, and 5. The AA level was shown as average concentration ± standard deviation (\( n = 2, 3, 4 \)).

Even though the processing conditions were the same, it is noted from the results shown in Tables 3, 4, 5, that for every type of flour, the use of the same amount of fat (margarine/ butter/ sunflower oil/ palm oil/ lard) in the formulation recipe (1 kg), but with different lipid content (60 - 100%) resulted in an increase of AA level as follows:

\[ \text{Margarine} < \text{butter} < \text{lard} < \text{sunflower oil} < \text{palm oil} \]

It is noted that the lowest values of the AA concentration (14.63 to 95.84 μg/kg) were obtained for biscuit assortments made with semi-white flour with a lower extraction degree (F1). On the contrary, the highest concentrations of AA were obtained for the biscuit assortments made with flour with an extraction degree higher, black flour (F2) (153.26 to 608.87 μg/kg) and dietetic flour (F3) (166.03 to 667.67 μg/kg) probably due to the fact that flours with a higher extraction degree contain more precursors of AA. A greater asparagine level is present in the outer layers of grains so a higher content of asparagine is found in flours with a higher extraction degree. Also, the ash content, respectively the extraction degree of the flour is in direct ratio with the reducing sugars, these compounds being responsible for the Maillard reaction [8].

Similar results were obtained by Przygodzka et al., [17] who showed that as the flour extraction rate is high-
Figure 2. Experimental variants of biscuits obtained with five types of fat (same amount of fat/different lipid content) and flour F1

Figure 3. Experimental variants of biscuits obtained with five types of fat (same amount of fat/different lipid content) and flour F2

Figure 4. Experimental variants of biscuits obtained with five types of fat (same amount of fat/different lipid content) and flour F3

Figure 5. Experimental variants of biscuits obtained with five types of fat (different amounts of fat/same lipid content) and flour F2

Table 3. Physico-chemical characteristics of experimental variants obtained with five types of fat (same amount of fat/different lipid content) and flour F1

<table>
<thead>
<tr>
<th>Experimental variant/ fat type</th>
<th>Biscuits moisture, %</th>
<th>AA (C ± S_p), µg/kg</th>
<th>Colour parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>V16/margarine</td>
<td>4.03</td>
<td>14.63 ± 3.07</td>
<td>72.56 3.39 17.12 80.77</td>
</tr>
<tr>
<td>V26/butter</td>
<td>3.95</td>
<td>24.33 ± 5.11</td>
<td>71.82 3.61 17.44 80.34</td>
</tr>
<tr>
<td>V36/sunflower oil</td>
<td>3.02</td>
<td>63.29 ± 13.29</td>
<td>54.69 8.46 19.74 69.44</td>
</tr>
<tr>
<td>V46/palm oil</td>
<td>2.37</td>
<td>95.84 ± 20.13</td>
<td>49.35 8.59 18.77 65.03</td>
</tr>
<tr>
<td>V56/lard</td>
<td>3.10</td>
<td>55.35 ± 11.62</td>
<td>59.12 6.42 19.26 71.88</td>
</tr>
</tbody>
</table>

Table 4. Physico-chemical characteristics of experimental variants obtained with five types of fat (same amount of fat/different lipid content) and flour F2

<table>
<thead>
<tr>
<th>Experimental variant/ fat type</th>
<th>Biscuits moisture, %</th>
<th>AA (C ± S_p), µg/kg</th>
<th>Colour parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>V18/margarine</td>
<td>3.99</td>
<td>153.26 ± 4.27</td>
<td>60.23 8.04 18.87 72.62</td>
</tr>
<tr>
<td>V28/butter</td>
<td>2.40</td>
<td>200.03 ± 7.68</td>
<td>54.08 9.6 19.21 68.75</td>
</tr>
<tr>
<td>V38/sunflower oil</td>
<td>0.91</td>
<td>449.19 ± 10.0</td>
<td>36.37 11.42 15.42 54.04</td>
</tr>
<tr>
<td>V48/palm oil</td>
<td>0.81</td>
<td>608.87 ± 1.49</td>
<td>32.32 11.24 14.12 50.06</td>
</tr>
<tr>
<td>V58/lard</td>
<td>1.24</td>
<td>389.70 ± 4.88</td>
<td>40.71 11.45 17.14 58.7</td>
</tr>
</tbody>
</table>
er the asparagine level and total sugar is higher, thus resulting the increase of AA level of bread.

By applying the same baking conditions (time, temperature) but using in the biscuits formulation recipe of smaller quantities of fat/same content of lipids (60%) for the variants presented in Table 2, it lead to the decrease of AA content comparing to variants where a large amount of fat with different lipid content (60 - 100%) was used in their formulation, as shown in Figure 6. It is known that the AA content is higher as the fat contain a greater level of tryglicerides, respectively more unsaturated fatty acids. Tryglicerides are responsible for the AA formation, an increase in tryglicerides meaning a higher level of acrolein formed through the oxidation process [9].

In contrast with our results where the highest AA level was obtained when using palm oil, Muchtaridi et al., [9] showed that when coconut oil, palm oil and corn oil were used in fried tempe as frying media, the lowest AA level was obtained for palm oil.

With the same baking parameters (time, temperature) and using in the formulation recipe of a lower amount of fat but with the same content of lipids, resulted the decrease of the AA level compared to variants where in the formulation recipe was used a higher amount of fat with different lipid content. When using in the biscuits formula of the same amount of fat but with different lipid content, the difference between samples was significantly different (p < 0.05), but the difference between samples with the same amount of fat and the ones with different amounts of fat was not statistically different.

Except V18 and V18a variants, in which the amount of fat, respectively the lipid content was almost similar, the AA level of variants, V28a, V58a, V38a, V48a, in which have been used different amounts of fat (0.6 - 0.923 kg) with the same lipid content (60%) was reduced with about 11 - 15% compared to the variants, V28, V58, V38, V48, in which was used the same quantity of fat (1 kg) with different lipid content (60 - 100%).

### 3.2 Correlations between AA levels and total colour difference

In addition to AA formation, the Maillard reaction is considered to be one of the most important chemical reaction to establish the quality of heat-treated foods, which is also responsible for colour development of processed food products. Therefore control of this reaction is an important aspect in food processing [18]. This lead to the question whether there is a correlation between the AA content and colour intensity of final product. Studies on the relationship between the colour and the content of AA in the bread crust, have shown that there is a significant correlation between these parameters [19]. Thus, in the present study, the use of different types of fat in the formulation recipe had as a result, a distinct change in colour of the final products.

From the results presented in Tables 3, 4, 5 it can be noted that, as the AA content increases, colour difference decreases. From the five types of fat used in the experimental variants, biscuit samples with margarine (V16, V18, V18a, V17) show the lowest level of AA (14.63 - 166.03 μg/kg) and the lightest colour (high values of total colour difference, ΔE* = 80.77 - 72.26), and biscuit samples with palm oil (V46, V48, V48a, V47), present the highest level of AA (95.84 - 667.67 μg/kg) and the darkest colour (low values of total colour difference, ΔE* = 50.06 - 65.03).

These changes in the AA level and total colour difference are shown in a similar manner for all the three types of wheat flour. The highest values of AA content and the lowest values of the total colour difference were obtained for the biscuits made with dietetic flour F3, with a high extraction degree.

![Figure 6. The AA variation for the experimental variants achieved with flour F2](image_url)
The correlation between the AA level formed in the experimental variants and the total colour difference, $\Delta E^*$, was plotted for each type of flour used and presented in Figures 7, 8, 9, and 10.

From Figures 7, 8, 9, and 10 it is noted that the increase in total colour difference, $\Delta E^*$, produced the decrease of AA level.

Between the AA level and the total colour difference, $\Delta E^*$, of the biscuits samples obtained in the experimental variants with the three types of wheat flour used with different extraction degrees (F1, F2, F3), there were obtained negative linear correlation, represented by the regression equations and correlation coefficients:

- Semi-white flour F1: $y = -4.6386x + 391.58$, $R = 0.9862$ (Figure 7).
- Black flour F2: $y = -19.148x + 1525.1$, $R = 0.9854$ (Figure 8).
- Dietetic flour F3: $y = -23.597x + 1885.6$, $R = 0.9941$ (Figure 9).
- Black flour F2: $y = -21.486x + 1694.1$, $R = 0.9920$ (Figure 10).

The correlation increases as the extraction degree of wheat flour is higher.

Except for the first two variants, margarine and butter, in the case of experiments carried out with black flour, F2, when in biscuits formulation recipe were used smaller amounts of fat, 0.6 kg (Figure 10), with the same lipid content (60%), the AA level of samples with lard decreased from 389.7 μg/kg to 260.44 μg/kg, from 449.19 μg/kg to 349.75 μg/kg for sunflower oil samples, respectively from 608.87 μg/kg to 407.51 μg/kg for palm oil samples.

Besides being influenced by Maillard reaction and caramelization reactions that occur during baking, biscuits color was influenced by the type of fat used and their lipid content.

The correlation between the AA level and total colour difference ($\Delta E^*$) of biscuits obtained in experimental variants (Figure 8 and Figure 10) is higher as the amount of fat was lower, respectively lipid content was lower.

### 3.3 Correlations between the AA levels and lipid content of fats used in the formulation recipe of biscuits

From the graphical representation from Figures 11, 13, and 15, it can be observed that:

- The correlation between the AA level and total colour difference ($\Delta E^*$) of biscuits obtained in experimental variants (Figure 8 and Figure 10) is higher as the amount of fat was lower, respectively lipid content was lower.

**Figure 7. Correlation between the AA level and total colour difference ($\Delta E^*$) when using flour F1 (same amount of fat/different lipid content)**

**Figure 8. Correlation between the AA level and total colour difference ($\Delta E^*$) when using flour F2 (same amount of fat/different lipid content)**

**Figure 9. Correlation between the AA level and total colour difference ($\Delta E^*$) when using flour F3 (same amount of fat/different lipid content)**

**Figure 10. Correlation between the AA level and total colour difference ($\Delta E^*$) when using flour F2 (different amounts of fat/same lipid content)**
and 15 in which is presented the variation of the AA level and lipid content of fats used in the manufacture of biscuits (the same amount of fat/different lipid content), for each type of flour used, it is highlighted that as the lipid content of fats used increases in the manufacture of biscuits, the level of AA increases.

In variants V16, V17, V18, in which was used margarine containing 60% lipid, respectively V26, V27, V28, in which was used butter, containing 65% lipid, there were obtained the lowest levels of AA. Biscuit samples made with lard (V56, V57, V58), with sunflower oil (V36, V37, V38), respectively with palm oil (V46, V47, V48), in which the lipid content of fats was 100% showed the highest level of AA.

The variation of the AA level was similar for all the wheat flour tested. For the same type of fat, for example palm oil, the highest value of the AA levels (667.67 μg/kg) was obtained when dietetic wheat flour (F3) was used (Figure 15), with the highest degree of extraction. The lowest AA level value (95.84 μg/kg) was obtained when using semi-white flour, F1, with the lowest degree of extraction (Figure 11).

In the case of experiments carried out with black flour, F2, the use in the formulation recipe of different amounts of fat (0.6 to 0.923 kg) with the same lipid content (60%) (Figure 17), the AA level was reduced compared to the variants in which was used the same amount of fat (1 kg) with different lipid content (60 - 100%) (Figure 13).

From the graphical representation of the AA level in experimental variants when using flour F2 and different amounts of fat with the same content of lipids (60%) (Figure 17) it is highlighted that by keeping constant the lipid content of fat used in the formulation recipe, the AA level increases, as follows:

Margarine < butter < lard < sunflower oil < palm oil

To determine whether there is a correlation between the AA level formed in biscuit samples and the lipid content of fats used in the biscuits recipe, regression lines were traced and straight line equations and correlation coefficients between these parameters of the three types of wheat flour were determine (F1 - Figure 12; F2 - Figure 14; F3 - Figure 16). This gives a positive linear correlation between the AA level and lipid content of the fats used in the manufacture of biscuits, represented by a correlation coefficient, R, between 0.8835 to 0.9667.
The increase of AA level is higher, as the extraction degree of flour is higher.

In the samples achieved with the black flour F2 (Figure 14 and 18), the use in the formulation recipe of different amounts of fat, 0.6 - 0.923 kg (Figure 18) with the same lipid content (60%), AA level was reduced (151.38 - 407.51 μg/kg) comparing with the variants in which was used the same amount of fat, 1 kg (Figure 14), and the lipid content varied between 60 - 100% (153.26 - 608.87 μg/kg).

Analyzing the results, it can be said that the level of AA formed in experimental variants it correlated with the lipid content of fats used in the formulation recipe of biscuits for all wheat flours studied. By using in the biscuits recipe of a higher amount of fat (1 kg) as, lard, sunflower oil, palm oil (containing 100% fat) resulted a greater level of AA compared with the use of a smaller quantity of the same type of fat (0.6 kg).

3.4 Correlations between the AA levels and water content of biscuits

Acrylamide formation is influence by the product moisture. In a low-moisture system, the AA level is higher.

In Tables 3, 4, and 5 it is shown that as the moisture of biscuits sample drops, the AA content increases. Biscuit samples with margarine (V16, V18, V17) which presents the highest water content, so the lowest fat content had the lowest level of AA (14.63 μg/kg, 153.26 μg/kg, 166.03 μg/kg).

Biscuit samples with palm oil (V46, V48, V47) which presented the lowest water content, and the highest fat content, had the highest level of AA (95.84 μg/kg, 608.87 μg/kg, respectively 667.67 μg/kg).

The results are in agreement with the findings of Mulla et al., [20] who shown that acrylamide formation in extruded snacks increases from 704 ± 3 to 1560 ± 5 μg/kg as the water content decreased.

To determine whether there is a correlation between the AA level formed in biscuit samples and their water content, the regression lines were drawn, and the regression equations and correlation coefficient values between these parameters were determine for each type of flour used (F1 - Figure 19; F2 - Figures 20, 22; F3 - Figure 21).

A negative linear correlation between the AA level and water content of biscuit samples was obtained, when varying the type of fat (the same amount of fat/ different content of lipids). The correlation is represented
by the regression equation and the correlation coefficient, R, which for the 3 types of wheat flour used were:

- Semi-white flour F1: \( y = -46.379x + 203.46, \) \( R = 0.9947 \) (Figure 19).
- Black flour F2: 
  - \( y = -123.48x + 591.12, \) \( R = 0.8894 \) (same amount of fat/different lipid content) (Figure 20).
  - \( y = -79.51x + 437.84, \) \( R = 0.9004 \) (different amounts of fat/same lipid content) (Figure 22).
- Dietetic flour F3: \( y = -139.59x + 761.47, \) \( R = 0.9466 \) (Figure 21).

The correlation increases as the flour extraction degree used is higher.

Analyzing the results for all the flours used it can be said that the AA level formed in experimental variants is correlated with their water content:

- low biscuits moisture produced high levels of AA
- high biscuits moisture produced a low level of AA.

A low water content of biscuits was obtained for the experimental variants which were achieved with fats as, lard, sunflower oil, palm oil, which had the highest content of fat, 100%. A high water content of biscuits were obtained when in the experimental variants were used margarine and butter, which had a lower lipid content, 60 - 65%.

4. Conclusions

Based on the results, it can be said that:

- The type of fat used in the formulation recipe of biscuits, caused an increase in the AA level, as follows: Palm oil > Sunflower oil > lard > butter > margarine.
- A fat with a high lipid content, provide a higher level of AA compared to a type of fat with less lipid content.
- A greater amount of fat in the formulation recipe of biscuits caused an increase in the AA level.
- By using the same type of fat (margarine/butter/lard/sunflower oil/palm oil) in the formulation recipe and different types of wheat flour, with different extraction degrees caused an increase in the AA level, as follows: F3 (dietetic flour, 95-100%) > F2 (black flour, 85-95%) > F1 (semi-white flour, 75-85%).
- A negative linear correlation (R = 0.9854 to 0.9941) between the AA level and the total colour difference (ΔE*) of biscuit samples obtained with 5 types of fat was obtained. A dark colour of biscuits (ΔE*) caused a high levels of AA. The correlation increases as the flour extraction degree is higher.

- A positive linear correlation (R = 0.8835 to 0.9667) between the AA level and lipid content of the fats used in the formulation recipe of biscuits was obtained. A high lipid content of fats used in the formulation of biscuits, causes high levels of AA compared with the use of fat containing less lipid. The correlation increases as the extraction degree of the flour used is greater.

- A negative linear correlation (R = 0.8894 to 0.9947) between the AA and water content of biscuit samples obtained by varying the type of fat was obtained. A small biscuits water content caused high levels of AA and high biscuits water content caused a low level of AA. The correlation increases as the extraction degree of the flour used is higher.

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5. References


