Effect of Storage on Acrylamide and 5-hydroxymethylfurfural Contents in Selected Processed Plant Products with Long Shelf-life

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Abstract This study investigated the effects of storage and temperature duration on the stability of acrylamide (AA) and 5-hydroxymethylfurfural (HMF) in selected foods with long shelf-life. Products were analysed fresh and stored at temperatures of 4 and 25 °C after 6 and 12 months (with the exception of soft bread samples, which were analysed after 15 and 30 days). The AA and HMF contents were determined with RP-HPLC coupled to a diode array detector (DAD). AA and HMF were not stable in many processed plant products with a long shelf-life. The highest AA reduction and the largest increase in HMF content were observed in the samples stored at a higher temperature (25 °C) for 12 months. It was found that an initial water activity of 0.4 is favourable to HMF formation and that AA reduction may be considerably greater in stored products with a low initial water activity. The kind of product and its composition may also have a significant impact on acrylamide content in stored food. In the final period of storage at 25 °C, acrylamide content in 100 % cocoa powder, instant baby foods, 20 % cocoa powder and instant coffee was 51, 39, 35 and 33 % lower than in products before storage, respectively. It was observed that a large quantity of ε-NH₂ and SH groups of amino acids in some products can be assumed as the reason for the significant AA degradation.

Keywords Acrylamide · 5-hydroxymethylfurfural · Storage conditions · Processed plant products

Abbreviations

AA Acrylamide
ANOVA Analysis of variance
aw Water activity
DAD Diode array detector
HMF 5-hydroxymethylfurfural
LSD Least significant difference test
RP-HPLC Reversed phase-high performance liquid chromatography
SDs Standard deviations

Introduction

The Maillard reaction is considered to be one of the most important chemical reactions taking place during food processing. Some Maillard reaction products may have high toxicological potential [1, 2]. Acrylamide (AA) has been added to the list of food-borne toxicants since 2002. It is found in concentrations up to a few mg/kg in a wide range of foods, mainly carbohydrate-rich foods subjected to high thermal processing [1, 3, 4]. The acrylamide content in foods is defined as the net amount of acrylamide, i.e., what remains after formation and degradation. In general, it has been found that prolonged heating and storage can also cause AA degradation. Opinions, however, differ and there is little data available on this subject. The type of reaction responsible for the degradation is also still unclear and it is believed that these reactions may be heterogeneous for different products [5–7]. Another compound formed during the Maillard reactions is 5-hydroxymethylfurfural (HMF). HMF concentrations in foods vary widely, sometimes exceeding 1 g/kg in certain dried fruits and caramel products. However, the most important contributors to dietary HMF intake are bread and coffee [8, 9]. Many studies have shown that it can be toxic, mutagenic...
and carcinogenic to the human body. However, other researchers have found that HMF and its derivatives may be an anti-carcinogenic factor [8, 10, 11]. To date, there are no available mitigation strategies specifically addressed to reduce HMF content in foods [12].

Plant foods are important in human and animal nutrition [13]. However, AA and HMF are present in high quantities in many plant-based processed foods [8]. Plant-based processed foods include snacks, pasta and breakfast cereals, as well as breads, coffee, cocoa and baby food. Since some processed plant foods are stored for long periods of time, it is important to investigate AA and HMF changes during storage. In several previous studies, the effect of some storage variables on AA and HMF formation was investigated and the effects were individually tested but not in combination with each other. Therefore, from these studies, it has not been possible to understand the most optimum conditions leading to minimizing HMF formation and maximizing AA degradation in products during storage. Thus, in this study, the effect of storage on acrylamide and 5-hydroxymethylfurfural contents in different food matrices with long shelf-life was investigated. The influence of initial water activity of products upon acrylamide and HMF kinetics was also examined.

**Materials and Methods**

**Samples** The test material included 90 samples of commercially-produced plant-based foods: a) cereal-based foods for infants such as: (10) jarred baby foods (ready-to-eat), (10) instant baby foods (in powder products) and (10) biscuits for infants. The major ingredients of these foods were cereals, milk powder and various sugars, in some cases follow-on formula and dried fruit and/or natural fruit powder; b) (10) cacao (100 % cocoa powder), (10) cacao (cocoa-containing beverages powder: sugars and 20 % cocoa powder); c) (10) instant (soluble) coffee, (10) coffee substitutes containing different proportions of coffee and sugars, chicory and other substitutes such as rye, barley, malt; d) (10) crisp bread (wheat, rye, multigrain bread) and (10) soft bread (also wheat, rye, multi-grain bread). The soft bread with a prolonged shelf-life was modified in packed atmosphere.

Five units of each product type were purchased from local supermarkets. The production date of these products was not longer than seven days. One was analysed immediately after buying (zero time). The first two units were stored at 4 °C (fridge) and the second two units were stored at 25 °C (thermostat). The samples were stored in commercial package in the dark and were analysed after 6 and 12 months of storage. The soft bread samples, due to their relatively short shelf-life, were analysed immediately after the production (zero time) and after 15 and 30 days. The instant samples (instant baby foods, cacao, coffee) were analysed as bought after being thoroughly stirred. Other products were crushed or/and homogenized in a Masterchef 8000 FP664 Moulinex multi-function food processor (Grupe SEB Poland Sp. z o.o., Warsaw, Poland). All samples were analysed before the expiry date. Sample preparation and analytical determinations were carried out in triplicate.

**Reagents and Chemicals** The acrylamide standard (99.8 %, catalogue No. 23701), 5-hydroxymethylfurfural (≥98.0 %, catalogue No. 53407) and all chemicals of HPLC analytical grade were obtained from Sigma–Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

**Determination of Water Activity (a<sub>w</sub>)** Water activity (a<sub>w</sub>) was determined using a resistive electrolytic humidity measuring system (Novasina LabMaster AW, Novatron Scientific Ltd., Horsham, England).

**Determination of Acrylamide** The AA content in all food products was determined with the method developed by Michalak et al. [14] using the ion-pair RP–HPLC (Shimadzu LC–10A, Kyoto, Japan). The quality parameters were as follows: detection limit 10 μg/kg, the linear range: 10.0–40, 000 μg/kg with satisfactory linearity (R<sup>2</sup> = 0.9997) and 106 % recovery.

**Determination of 5-hydroxymethylfurfural** 5-hydroxymethylfurfural was determined by the procedure of Ferrer et al. [15] and Chavez-Servin et al. [16] using the RP–HPLC (Agilent 1200, Santa Clara, CA, USA). The quality parameters of the method were: detection limit 0.01 mg/kg, the linear range of 0.03–250 mg/kg with satisfactory linearity (R<sup>2</sup> = 0.9989), recovery - 101 %.

**Statistical Analysis**

The data were analysed using the Statistica 9.0 software package (StatSoft, Poland). Significant differences were calculated according to Duncan’s multiple range test with a 5 % level considered statistically significant. The results were submitted to a bi-factorial (time and temperature) analysis of variance (ANOVA). The mean values were compared using the least significant difference test (LSD) at the 5 % level. Correlations among HMF, AA and water activity were determined by Pearson’s correlation analysis at the p < 0.05 confidence level.

**Results and Discussion**

During storage, no change was detected in the HMF content of the jarred baby foods analysed (Table 1). According to Ramírez-Jiménez et al. [17], storing products with high water
activity at relatively low temperature (25 °C) does not favour HMF formation. As can be seen in Table 1, acrylamide contents also did not significantly differ after 6 and 12 months of storage at 4 °C. At a higher temperature (25 °C), acrylamide loss during storage was observed. The initial acrylamide content of jarred baby foods decreased by 9 % and 15 % after 6 and 12 months of storage, respectively. It was found that storing jarred baby foods with high water activity (0.987) at a relatively low temperature (4 °C and 25 °C) did not favour HMF formation or a high reduction of AA during the 12-month study period.

At the same time, another baby product with very low water activity (0.166) was examined (Table 1). In this case, the storage of instant baby foods in powder also did not significantly affect HMF formation. It is believed that in these products, with very high and low water activity and stored at 4 °C and 25 °C, HMF formation is limited. The AA content in the examined instant baby foods was 34.7 μg/kg and during storage for 12 months at a temperature of 4 °C and 25 °C, it significantly decreased to 25.3 μg/kg and 21.1 μg/kg, respectively (Table 1). AA losses were greater at the higher storage temperature, reaching 39 % after 12 months. It was observed that a higher temperature and longer storage time had a greater impact on the degree of acrylamide reduction in the products.

In the case of stored biscuits for infants with an initial water activity of 0.366, HMF formation was observed (Table 1). The storage of instant baby foods in powder also did not significantly affect HMF formation. It is believed that in these products, with very high and low water activity and stored at 4 °C and 25 °C, HMF formation is limited. The AA content in the examined instant baby foods was 34.7 μg/kg and during storage for 12 months at a temperature of 4 °C and 25 °C, it significantly decreased to 25.3 μg/kg and 21.1 μg/kg, respectively (Table 1). AA losses were greater at the higher storage temperature, reaching 39 % after 12 months. It was observed that a higher temperature and longer storage time had a greater impact on the degree of acrylamide reduction in the products.

Table 1 Changes of HMF (mg/kg), AA (μg/kg) and water activity (aw) in cereal-based foods for infants during storage

| Type of product | Storage time (months) | Parameter | Temperature |
|-----------------|-----------------------|-----------|-------------|
| Jarred baby foods (ready-to-eat) (n = 10) | 0 | HMF | 4 °C | 25 °C |
| | | 25.1 ± 1.1 Aa | 25.1 ± 1.1 Aa |
| | | AA | 15.1 ± 1.2 Aa | 15.1 ± 1.2 Aa |
| | | aw | 0.987 ± 0.005 Aa | 0.987 ± 0.005 Aa |
| | 6 | HMF | 25.3 ± 1.1 Aa | 25.6 ± 1.2 Aa |
| | | AA | 14.5 ± 1.0 Aa | 13.7 ± 0.9 Ab |
| | | aw | 0.985 ± 0.005 Aa | 0.983 ± 0.005 Aa |
| | 12 | HMF | 25.7 ± 1.4 Aa | 26.3 ± 1.5 Aa |
| | | AA | 14.3 ± 0.9 Aa | 12.9 ± 0.4 Aa |
| | | aw | 0.983 ± 0.005 Aa | 0.981 ± 0.005 Aa |
| Instant baby foods (in powder products) (n = 10) | 0 | HMF | 52.1 ± 13.9 Aa | 52.1 ± 13.9 Aa |
| | | AA | 34.7 ± 5.8 Aa | 34.7 ± 5.8 Aa |
| | | aw | 0.166 ± 0.028 Aa | 0.166 ± 0.028 Aa |
| | 6 | HMF | 55.3 ± 10.0 Aa | 56.6 ± 9.1 Aa |
| | | AA | 28.7 ± 2.1 Aab | 26.3 ± 2.2 Aab |
| | | aw | 0.161 ± 0.025 Aa | 0.160 ± 0.023 Aa |
| | 12 | HMF | 56.7 ± 11.4 Aa | 60.0 ± 10.5 Aa |
| | | AA | 25.3 ± 1.4 Ab | 21.1 ± 0.9 Bc |
| | | aw | 0.159 ± 0.035 Aa | 0.159 ± 0.033 Aa |
| Biscuits for infants (n = 10) | 0 | HMF | 87.2 ± 3.2 Ab | 87.2 ± 3.2 Ac |
| | | AA | 73.2 ± 5.8 Aa | 73.2 ± 5.8 Aa |
| | | aw | 0.366 ± 0.040 Ab | 0.366 ± 0.040 Aa |
| | 6 | HMF | 90.9 ± 4.1 Bb | 101.0 ± 5.9 Ab |
| | | AA | 68.0 ± 2.1 Aa | 64.1 ± 1.9 Ab |
| | | aw | 0.344 ± 0.035 Aa | 0.341 ± 0.033 Aa |
| | 12 | HMF | 96.7 ± 4.5 Bb | 113.0 ± 5.0 Aa |
| | | AA | 61.6 ± 3.8 Ab | 55.7 ± 3.1 Bc |
| | | aw | 0.339 ± 0.068 Aa | 0.331 ± 0.063 Aa |

1 Mean values ± standard deviation (n = 10, n = the number of the same kind products from different producers) A–B in a row, different letters indicate significant differences (p < 0.05)

A–C in a column within the same parameter, different letters indicate significant differences (p < 0.05) for the same type of products; LSD interaction time–temperature factor of HMF, AA and aw are 1.14, 0.95, 1.50, respectively for jarred baby foods; LSD interaction time–temperature factor of HMF, AA and aw are 1.37, 0.69, 1.45, respectively for instant baby foods; LSD interaction time–temperature factor of HMF, AA and aw are 1.17, 0.65, 1.55, respectively for biscuits.
results showed that intermediate water activity, i.e., about 0.4, can be more favourable to HMF formation than lower and higher \( a_w \) values. Acrylamide in biscuits was not a stable compound. AA content in biscuits stored at 25 °C for 12 months was about 24 % lower than before storage. The effect of storage on AA content in baby foods has not been intensely studied, but some studies have found no common pattern in the degree of AA reduction. The reduction of AA in different biscuits has been found to range from no decrease to a decrease of 20 % [7].

The instant cacao (100 % cocoa) contained, on average, 2.0 mg HMF/kg (Table 2). In other cacao products (cocoa-containing beverages powder: sugars and 20 % of cocoa), the mean HMF concentration was 153 mg/kg. In instant cacao samples, HMF content did not significantly differ after 6 and 12 months of storage at 4 °C and 25 °C (Table 2). The highest HMF content was found for the sweetened cacao products after 12 months of storage, both at 4 °C and 25 °C, (a 28 % and 46 % increase, respectively). HMF presence in foods is usually the result of the Maillard reaction or caramelization processes. Most likely, in the case of instant cacao (100 % cocoa), HMF was formed during the roasting process of the cocoa beans, whereas in sweetened cacao, HMF could be formed by both reactions. According to Abraham et al. [18], HMF concentration in cocoa-containing beverages powder and sugars can reach 503.8 mg/kg.

During the roasting process of cacao beans, acrylamide is also formed. For instant cacao (100 % cocoa), the average acrylamide concentration was 347 µg/kg, while in sweetened cacao it was 248 µg/kg. After storage at 4 °C and 25 °C for 12 months, AA concentrations in instant cacao significantly dropped to 202 µg/kg (42 % decrease) and 169 µg/kg (51 % decrease), respectively; in sweetened cacao to 191 µg/kg (23 % decrease) and 162 µg/kg (35 % decrease), respectively (Table 2). This finding was similar to the observation made by Hoenicke and Gatermann [6], who showed that the acrylamide concentrations in cacao powder stored at 10–12 °C for 6 months dropped significantly from 265 µg/kg to 180 µg/kg (a 32 % decrease).

Table 3 shows that the amount of HMF was significantly higher in the coffee substitutes (397 mg/kg) than in the instant coffee (120 mg/kg). Husøy et al. [19] and Del Campo et al. [20] reported a great variation of HMF levels within instant coffee food products: from 97 to 6180 mg/kg. As shown in Table 3, significant formation of HMF during storage at 25 °C for 12 months was only noted for coffee substitutes (32 %

| Table 2 Changes of HMF (mg/kg), AA (µg/kg) and water activity (\( a_w \)) in cocoa products during storage |
|------------------------------------------|---------------------|---------------------|
| **Type of product**                     | **Time (months)**   | **Parameter**       | **Temperature** |
|                                        |                     |                     | 4 °C             | 25 °C            |
|                                        |                     |                     |                  |                  |
| Cacao (100 % cocoa powder)              | 0                   | HMF                 | 2.0 ± 0.5<sup>Aa</sup> | 2.0 ± 0.5<sup>Aa</sup> |
| (\( n = 10 \))                          |                     | AA                  | 347.0 ± 42.0<sup>Aa</sup> | 347.0 ± 42.0<sup>Aa</sup> |
|                                        |                     | \( a_w \)           | 0.155 ± 0.015<sup>Aa</sup> | 0.155 ± 0.015<sup>Aa</sup> |
|                                        | 6                   | HMF                 | 2.1 ± 0.3<sup>Aa</sup>  | 2.1 ± 0.2<sup>Aa</sup>  |
|                                        |                     | AA                  | 268.0 ± 34.0<sup>Ab</sup> | 225.0 ± 34.0<sup>Ab</sup> |
|                                        |                     | \( a_w \)           | 0.154 ± 0.013<sup>Aa</sup> | 0.154 ± 0.014<sup>Aa</sup> |
|                                        | 12                  | HMF                 | 2.1 ± 0.2<sup>Aa</sup>  | 2.3 ± 0.2<sup>Aa</sup>  |
|                                        |                     | AA                  | 202.0 ± 32.0<sup>Ac</sup> | 169.0 ± 31.0<sup>Bc</sup> |
|                                        |                     | \( a_w \)           | 0.153 ± 0.018<sup>Aa</sup> | 0.152 ± 0.013<sup>Aa</sup> |
| Cacao (cocoa-containing beverages powder: sugars and 20 % cocoa powder) (\( n = 10 \)) | 0  | HMF                 | 153.0 ± 8.0<sup>Bc</sup> | 153.0 ± 8.0<sup>Bc</sup> |
|                                        |                     | AA                  | 248.0 ± 29.0<sup>Aa</sup> | 248.0 ± 29.0<sup>Aa</sup> |
|                                        |                     | \( a_w \)           | 0.324 ± 0.083<sup>Aa</sup> | 0.324 ± 0.083<sup>Aa</sup> |
|                                        | 6                   | HMF                 | 175.0 ± 7.0<sup>Bb</sup> | 192.0 ± 9.0<sup>Bb</sup> |
|                                        |                     | AA                  | 228.0 ± 12.0<sup>Bb</sup> | 215.0 ± 10.0<sup>Bb</sup> |
|                                        |                     | \( a_w \)           | 0.321 ± 0.075<sup>Aa</sup> | 0.320 ± 0.083<sup>Aa</sup> |
|                                        | 12                  | HMF                 | 196.0 ± 9.0<sup>Ba</sup> | 224.0 ± 8.0<sup>Ba</sup> |
|                                        |                     | AA                  | 191.0 ± 19.0<sup>Bb</sup> | 162.0 ± 15.0<sup>Bc</sup> |
|                                        |                     | \( a_w \)           | 0.319 ± 0.078<sup>Aa</sup> | 0.319 ± 0.072<sup>Aa</sup> |

<sup>1</sup> Mean values ± standard deviation (\( n = 10 \), \( n \) = the number of the same kind products from different producers)

\(<A>B</A>\) in a row, different letters indicate significant differences (\( p < 0.05 \))

\(<A>C</A>\) in a column within the same parameter, different letters indicate significant differences (\( p < 0.05 \)) for the same type of products; LSD interaction time–temperature factor of HMF, AA and \( a_w \) are 0.88, 0.35, 1.50, respectively for cocoa powder; LSD interaction time–temperature factor of HMF, AA and \( a_w \) are 0.73, 0.42, 1.45, respectively for cocoa 20 % powder
increase). Other studies have shown a slight HMF formation during storage at low temperatures, but a significant increase at 35 °C [21]. For the instant coffee and for coffee substitutes, the average acrylamide concentration was 145 μg/kg and 68.4 μg/kg before storage, respectively (Table 3). During storage at 25 °C for 12 months, AA concentrations in instant coffee and coffee substitutes significantly dropped to 97.0 (33 % reduction) and 49.2 μg/kg (28 % reduction), respectively, indicating the instability of acrylamide in coffee during storage. The same observation was noted in other studies. Delatour et al. [5] reported that a sample of roasted coffee with 203 μg AA/kg, after storage for seven months contained only 147 μg AA/kg. Hoenicke and Gatermann [7] reported AA reduction from 305 μg/kg to 210 μg/kg in roasted coffee for three months of storage at 10–12 °C. A reduction of AA in dried chicory and instant coffee was also found. During storage for several months, acrylamide concentrations dropped significantly, from 214 to 174 μg/kg and 771 to 256 μg/kg, respectively [5].

Crisp bread contained 330 mg HMF/kg, on average, while soft bread had 130 mg/kg (Table 4). According to Abraham et al. [18], the HMF content in soft bread can ranged from 14 to 53 mg/kg. The highest HMF content was determined in crisp bread, where the toasting temperature and other production technological parameters differed from those used in traditional bread production and were generally more drastic [18]. The initial content of HMF in crisp bread increased throughout the 12 months of storage with statistically significant changes at high temperature (25 °C) (Table 4). Storage at low temperature (4 °C) did not favour HMF formation throughout the entire studied period. In the case of soft bread with high water activity (0.940), storage at both temperatures (4 and 25 °C) did not favour HMF formation during the 30-day period (Table 4).

The average acrylamide concentration in the analysed crisp bread was 443 μg/kg before storage while for soft bread it was 55.5 μg/kg (Table 4). Low moisture and large specific surface area of crisp bread contribute to the generally higher acrylamide level in these products. In another study, the content of AA in soft bread and crisp bread was 30–160 and 30–1900 μg/kg, respectively [22]. The highest levels of AA reduction in crisp bread were found in samples stored at 25 °C for 6 months (14 %) and 12 months (27 %), while in the samples stored at 4 °C it was only 3 % and 6 %, respectively (Table 4). In the case of soft bread with high water activity (0.940), a short storage time (15 and 30 days) at both temperatures did not

### Table 3: Changes of HMF (mg/kg), AA (μg/kg) and water activity (aw) in coffee products during storage

| Type of product          | Storage time (months) | Parameter | Temperature |
|-------------------------|-----------------------|-----------|-------------|
|                         |                       |           | 4°C         | 25°C        |
| Instant (soluble) coffee| 0                     | HMF       | 120.0 ± 46.0 Aa | 120.0 ± 46.0 Aa |
|                         |                       | AA        | 145.0 ± 31.0 Aaa | 145.0 ± 31.0 Aaa |
|                         |                       | aw        | 0.320 ± 0.070 Aaa | 0.320 ± 0.070 Aaa |
|                         | 6                     | HMF       | 128.0 ± 34.0 Aaa | 136.0 ± 32.0 Aaa |
|                         |                       | AA        | 128.0 ± 26.0 Aaa | 116.0 ± 17.0 Aaa |
|                         |                       | aw        | 0.319 ± 0.035 Aaa | 0.319 ± 0.033 Aaa |
|                         | 12                    | HMF       | 135.0 ± 24.0 Aaa | 154.0 ± 27.0 Aaa |
|                         |                       | AA        | 119.0 ± 16.0 Aaa | 97.0 ± 12.0 Ab |
|                         |                       | aw        | 0.319 ± 0.027 Aaa | 0.318 ± 0.026 Aaa |
| Coffee substitutes      | 0                     | HMF       | 397.0 ± 71.0 Aaa | 397.0 ± 71.0 Ab |
|                         |                       | AA        | 68.4 ± 10.9 Aaa  | 68.4 ± 10.9 Aaa |
|                         |                       | aw        | 0.350 ± 0.100 Aaa | 0.350 ± 0.100 Aaa |
|                         | 6                     | HMF       | 432.0 ± 67.0 Aaa | 456.02 ± 62.0 Ab |
|                         |                       | AA        | 63.5 ± 8.5 Aaa   | 58.1 ± 7.7 Aaa |
|                         |                       | aw        | 0.349 ± 0.075 Aaa | 0.347 ± 0.083 Aaa |
|                         | 12                    | HMF       | 469.0 ± 54.0 Aaa | 524.0 ± 51.0 Aaa |
|                         |                       | AA        | 59.9 ± 8.3 Aaa  | 49.2 ± 7.1 Ab |
|                         |                       | aw        | 0.348 ± 0.066 Aaa | 0.346 ± 0.069 Aaa |

1 Mean values ± standard deviation (n = 10, n = the number of the same kind products from different producers) A–B in a row, different letters indicate significant differences (p < 0.05)

a–c in a column within the same parameter, different letters indicate significant differences (p < 0.05) for the same type of products; LSD interaction time–temperature factor of HMF, AA and aw are 1.21, 0.75 1.40 respectively for instant coffee; LSD interaction time–temperature factor of HMF, AA and aw are 0.90, 0.55 1.45 respectively for coffee substitutes.
favour AA degradation. The changes in AA content during storage of cereal-based products has been studied by other researchers. They found no common pattern in the degree of AA reduction. The level of AA in some of the cereal-based products was quite stable [5, 6], while others showed a wide range of decrease [3].

Our study showed that acrylamide is not a stable compound in many processed plant products with a long shelf-life. In food products during storage, probably a partial AA degradation takes place and its content decreases. The mechanism of these reactions is not clear. Acrylamide evaporation should be excluded since the products were stored in retailed closed packaging. AA reduction during storage as a result of its polymerization under UV light also has to be excluded because the package of the stored products protected them from light. According to Hoenicke and Gatermann [7], acrylamide reactions with compounds containing SH groups, which are particularly present in food products such as coffee and cocoa or milk powder, can have a significant impact on AA reduction during storage in these products or in foods with their addition. Storage affects AA degradation in coffee (45–60 %), cacao (32 %) and foods fortified with milk powder (29 %). Generally, it is believed that the high reactivity of acrylamide toward nucleophilic food components, such as sulphydryl, amino and hydroxyl groups of peptides, proteins, and melanoids in acrylamide-containing foods, might be responsible for the reduction of AA in stored food products [7]. Consequently, acrylamide could be quite stable in food such as cereal-based products (bread, breakfast cereals and biscuits), that usually do not consist of sulphur-containing substances [5, 6]. On the other hand, some authors have reported that storage temperature affects AA degradation in the crisp bread (37 %) and baby and dietary biscuits (7 and 11 %, respectively) [3, 7]. They suggested that water content could be the major factor responsible for AA reduction during storage. It is believed that the varying results of AA found in stored food products of some researchers depend not only on differences in food composition, but also on the water content in these products. It seems that processes for acrylamide elimination are more complicated.

The results of this study showed that the AA content in the examined products was stable during storage at low temperature. A higher storage temperature, however, resulted in a reduction of AA content. Statistical analysis revealed that the interaction time temperature factor had a very strong effect

| Type of product | Storage time (months/days) | Parameter | Temperature |
|-----------------|---------------------------|-----------|-------------|
| Crisp bread (n = 10) | 0 | HMF | 4 °C | 25 °C |
| | | AA | | |
| | | aw | | |
| | 6 | HMF | 330.0 ± 80.0 Aa | 330.0 ± 80.0 Aa |
| | | AA | 443.0 ± 110.0 Aa | 443.0 ± 110.0 Aa |
| | | aw | 0.282 ± 0.098 Aa | 0.282 ± 0.098 Aa |
| | 12 | HMF | 354.0 ± 41.0 Aa | 396.0 ± 39.0 Ab |
| | | AA | 428.0 ± 94.0 Aa | 374.0 ± 91.0 Ab |
| | | aw | 0.290 ± 0.095 Aa | 0.292 ± 0.093 Aa |
| Soft bread (n = 10) | 0 | HMF | 130.0 ± 80.0 Aa | 130.0 ± 80.0 Aa |
| | | AA | 55.5 ± 15.5 Aa | 55.5 ± 15.5 Aa |
| | | aw | 0.940 ± 0.021 Aa | 0.940 ± 0.021 Aa |
| | 15 | HMF | 134.0 ± 70.0 Aa | 137.0 ± 58.0 Aa |
| | | AA | 56.8 ± 14.3 Aa | 55.3 ± 14.1 Aa |
| | | aw | 0.938 ± 0.015 Aa | 0.931 ± 0.023 Aa |
| | 30 | HMF | 140.0 ± 61.0 Aa | 144.0 ± 69.0 Aa |
| | | AA | 59.2 ± 13.8 Aa | 53.7 ± 13.1 Aa |
| | | aw | 0.937 ± 0.018 Aa | 0.921 ± 0.020 Aa |

1 Mean values ± standard deviation (n = 10, n = the number of the same kind products from different producers);
2 Storage time of soft bread
A–B in a row, different letters indicate significant differences (p < 0.05)
a–c in a column within the same parameter, different letters indicate significant differences (p < 0.05) for the same type of products; LSD interaction time–temperature factor of HMF, AA and aw are 1.47, 0.48, 1.35, respectively for crisp bread; LSD interaction time–temperature factor of HMF, AA and aw are 1.02, 0.85, 1.15, respectively for soft bread
Acrylamide content decreases in some foods during long storage, particularly at higher temperatures. Further studies are needed to investigate the mechanism of acrylamide reduction during warm storage and the possibility of applying this procedure to appropriate products. The challenge is to find a balanced approach between acrylamide reduction and HMF formations in products during storage.

Conclusions

Acrylamide content decreases in some foods during long storage, particularly at higher temperatures. Further studies are needed to investigate the mechanism of acrylamide reduction during warm storage and the possibility of applying this procedure to appropriate products. The challenge is to find a balanced approach between acrylamide reduction and HMF formations in products during storage.

Compliance with Ethical Standards

Conflict of Interest The author(s) declare that they have no competing interests.

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Table 5 Correlation matrix between HMF, AA and initial water activity (aw)

|        | HMF  | AA   | aw  |
|--------|------|------|-----|
| HMF    | 1.00 |      |     |
| AA     | -0.74*| 1.00 |     |
| aw     | -0.36*| -0.66*| 1.00 |

*Correlation coefficients statistically significant at p < 0.05
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