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STUDIES OF ACRYLAMIDE LEVEL IN COFFEE AND COFFEE SUBSTITUTES: INFLUENCE OF RAW MATERIAL AND MANUFACTURING CONDITIONS

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ABSTRACT

Background. Many animal studies have shown that acrylamide is both neurotoxic and carcinogenic. The first reports of acrylamide actually having been found in foodstuffs were published in 2002 by the Swedish National Food Agency in conjunction with scientists from the University of Stockholm. It has since been demonstrated that acrylamide arises in foodstuffs by the *Maillard* reaction, ie. between free asparagine and reducing sugars at temperatures >120ºC. Coffee in fact, forms one of the principal dietary sources of acrylamide, where it is normally drunk in large quantities throughout many countries worldwide that includes Poland. Thus, it constitutes a major dietary component in a wide range of population groups, mainly ranging from late adolescents to the elderly.

Objectives. To determine the acrylamide level in commercial samples of roasted and instant coffee and in coffee substitutes by LC-MS/MS method. The influence of coffee species and colour intensity of coffee on acrylamide level was also detailed.

Materials and methods. A total of 42 samples of coffee were analysed which included 28 that were ground roasted coffee, 11 instant coffees and 3 coffee substitutes (grain coffee). Analytical separation of acrylamide from coffee was performed by liquid chromatography followed by tandem mass spectrometry (LC-MS/MS). To evaluate the colour intensity of ground roasted coffee and instant coffee we used method of arranging (sequence).

Results. The highest mean acrylamide concentrations were found in coffee substitutes (818 µg/kg) followed by instant coffee (358 µg/kg) and then roasted coffee (179 µg/kg). One single cup of coffee (160 ml) delivered on average from 0.45 µg acrylamide in roasted coffee to 3.21 µg in coffee substitutes. There were no significant differences in acrylamide level between the coffee species ie. *Arabica* vs *Robusta* or a mixture thereof. The various methods of coffee manufacture also showed no differences in acrylamide (ie. freeze-dried coffee vs agglomerated coffee). A significant negative correlation was observed between acrylamide levels and the intensity of colour in roasted coffee; this was not the case however for instant coffee.

Conclusions. It was demonstrated that roasting process had the most significant effect on acrylamide levels in natural coffee, however there were no relationships found with coffee species. Due to the high acrylamide levels demonstrated in coffee substitutes, recommended amounts should be defined and manufacturers should be obliged to reduce such levels in these products.

Key words*: acrylamide, different types of coffee, LC-MS/MS method, coffee species, color intensity*

STRESZCZENIE

Wprowadzenie. W licznych badaniach na zwierzętach wykazano, że akryloamid ma działanie neurotoksyczne i kancerogenne. W kwietniu 2002 r. Szwedzka Narodowa Agencja ds. Żywności oraz naukowcy z Uniwersytetu w Sztokholmie po raz pierwszy opublikowali dane o zawartości akryloamidu w produktach spożywczych. Akryloamid powstaje w żywności w reakcji *Maillarda* pomiędzy wolną asparaginą i cukrami redukującymi pod wpływem temperatury > 120°C. Wysokie spożycie tego napoju w wielu krajach, tym także w Polsce, sprawia że produkt ten może być znaczącym źródłem akryloamidu w diecie, zwłaszcza u osób dorosłych, ale także starszej młodzieży.

Cel badań. Oznaczenie zawartości akryloamidu w różnych rodzajach kawy metodą LC-MS/MS oraz ocena wpływu gatunku kawy, sposobu produkcji oraz barwy produktu finalnego na poziom badanego związku.

Materiał i metody. Materiał do badań stanowiły 42 próbki kawy, w tym 28 próbek mielonej kawy palonej, 11 próbek kawy rozpuszczalnej i 3 próbki substytutów kawy (kawa zbożowa). Akryloamid oznaczono metodą chromatografii cieczowej sprzężonej z tandemową spektrometrią mas (LC-MS/MS). Do oceny barwy próbek kawy wykorzystano metodę kolejności (szeregowania).

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Wyniki. Wśród przebadanych próbek różnych rodzajów kawy, najwyższą średnią zawartość akryloamidu stwierdzono w kawie zbożowej (818 μg/kg), a następnie w kawie rozpuszczalnej (358 μg/kg) i kawie palonej (179 μg/kg). Jedna filiżanka (160 ml) naparu kawy dostarczała, w zależności od rodzaju kawy, przeciętnie od 0,45 μg (kawa palona) do 3,21 μg (kawa zbożowa) akryloamidu. Nie stwierdzono istotnych różnic w zawartości akryloamidu w zależności od gatunku ziarna (*Arabica* vs *Robusta* vs mieszanka *Arabica* i *Robusta*). Sposób produkcji kawy rozpuszczalnej (liofilizowana vs. aglomeryzowana) nie wpływał na zawartość akryloamidu w produkcie finalnym. Stwierdzono istotną ($p < 0,05$) ujemną korelację pomiędzy zawartością akryloamidu a intensywnością barwy kawy palonej. Związku takiego nie stwierdzono dla kawy rozpuszczalnej.
Wnioski. Uzyskane wyniki potwierdzają, że proces wypalania kawy ma najistotniejszy wpływ na zawartość akryloamidu w kawie naturalnej. Nie potwierdzono natomiast związku pomiędzy poziomem akryloamidu a gatunkiem kawy. Z uwagi na wysoką zawartość akryloamidu w substytutach kawy (kawa zbożowa) zasadne wydaje się opracowanie wartości wskaźnikowych dla tej grupy produktów spożywczych oraz podjęcie przez producentów działań na rzecz obniżenia akryloamidu, szczególnie w tym rodzaju kawy.

Słowa kluczowe*: akryloamid, różne rodzaje kawy, metoda LC-MS/MS, gatunek kawy, intensywność barwy*

INTRODUCTION

For over the last 50 years, acrylamide has been manufactured on an industrial scale as a substrate for synthesising polyacrylamide polymers; this being used in industrial and drinking water filtration (as fillings), as well as in the paper, textile and cosmetics industries. Numerous studies have demonstrated that acrylamide is neurotoxic and may damage the central and peripheral nervous systems (respectively CNS and PNS) in both animal models and humans exposed to acrylamide at the workplace [5, 18].

Cell culture studies show that acrylamide is genotoxic and amongst other things it results in chromosomal aberrations, sister chromatid exchange, gene polyploidy and other mitotic disruption in mammalian cells [19]. Animal studies demonstrate an increase in multi-organ tumours that include the forestomach, testes, clictorial gland, mammary gland, oral cavity, skin and thyroid gland when acrylamide was supplied in the drinking water [4, 14, 22].

Already in 1994, the International Agency for Research on Cancer had assigned acrylamide to those substances that are 'probably carcinogenic to humans' (group 2A) [20]. The Joint FAO/WHO Expert Committee on Food Additives in 2011 stated that the estimated exposure limits to dietary acrylamide are low for such a substance that exhibits genotoxicity and cancerogenicity and thus constitutes a human health hazard [21].

In foodstuffs, acrylamide forms in the *Maillard* reaction between free asparagine and reducing sugars. The main dietary sources of acrylamide are potato products (French fries and potato crisps), cereals (eg. bread, breakfast cereal flakes and cakes), together with coffee [13].

When foodstuff acrylamide levels were monitored in Europe during 2007-2009, it was found that an average of 221 μg/kg were present in roasted coffee with maximum of 2200 μg/kg of product. Much higher amounts were measured in instant coffee (mean of 481 μg/kg) and in coffee substitutes (mean of 1500 μg/kg). In the latter case the acrylamide content reached a maximum of above 4500 μg/kg in individual samples [13]. Up till now in Poland, there is however a lack of any data on acrylamide levels in instant coffee and in coffee substitutes.

Many studies demonstrate that coffee to be a significant dietary source of acrylamide, especially in those countries with a high coffee consumption. The presence of coffee in an adult diet may deliver up 39% of the entire daily intake of acrylamide [6, 7, 12, 17, 23, 27, 30].

When coffee is roasted, the largest amounts of acrylamide form at the initial stage and swiftly attain maximum levels. Nevertheless, the longer the roasting continues, then acrylamide levels decrease [3, 24, 28, 31]. It also appears that the amounts of acrylamide may depend on the coffee species. When the acrylamide content of *Arabica* and *Robusta* coffees are compared, then the latter show raised levels; in most cases however these are not statistically significant [3, 24]. Another point that needs stressing, is that at present there are as yet no effective tools available for decreasing acrylamide levels in coffee, unlike those in the other affected foodstuffs such as cereals and potato products [32].

The aim of this study was to determine the acrylamide level in commercial samples of roasted and instant coffee and in coffee substitutes by LC-MS/MS method. The influence of coffee species and colour intensity of coffee powder on acrylamide level was also detailed.

MATERIAL AND METHODS

Sampling

Analyses were performed on 42 coffee samples consisting of 28 samples of ground roasted coffee, 11 instant coffee and 3 coffee substitutes made by various manufacturers. The roasted coffee samples were randomly selected as part of a monitoring programme in accordance with European Commission (EC) recommendations

No. 2007/331/EC [10]. This had been performed by the State Sanitary Inspectorate during 2008-2009 by sampling from grocery shops and supermarkets throughout the whole of Poland. The sampled coffee were from 14 brands that originated from 8 manufacturers of which 3 were Polish coffee roasting companies. One sample comprised at least two commercially available packages of the same name, manufacturer and batch number in the minimum quantity of 1 kg. The methods of sampling and amounts taken were in accordance with EC Regulation Part B No. 333/2007 [11].

The 28 roasted coffee samples consisted of 6 *Arabica* species (*Coffea Arabica*), 10 *Robusta* (*Coffea canephora*) and 12 were a mixture of both. There were 11 samples of instant coffee of which 9 were a mixture of *Arabica* and *Robusta*. The coffee substitutes samples were principally composed of roasted cereals (ie. rye and/or barley) and chicory root. One sample contained also roasted beetroot. In accordance with specifications provided by manufacturers, the degree of coffee roasting was moderate in twenty four samples, light to moderate in two cases and light to dark also in two. Of the eleven instant coffee samples, seven were freeze-drying whilst four were agglomerated.

All samples were transported and stored under conditions stated on the product labels. Prior to analysis, sample replicates were mixed together from which a representative portion was taken which then constituted the laboratory sample.

Sample preparation

The laboratory samples consisted of 1.8 to 3 g coffee placed in centrifuge tubes, to which 360 to 600 µL of internal standard were added (AAd_3 at a concentration of 1000 µg/L). Next, deionised water and hexane were added, followed by centrifugation (10 mins at 10,000 rpm). After the phases had separated, the organic layer was removed. The aqueous layer was incubated in an ultrasonic water bath. After cooling the samples to room temperature, Carrez I and II solutions were added. The sample was then further purified on a carbon column (Bakerbond Carbon, 1000 mg, 6 ml) as previously described [15]. The methanol fraction so obtained was evaporated to dryness under nitrogen and then reconstituted in 1 ml of mixture of water and methanol (9:1; v/v), of which 20 µL was used as the injection volume for chromatographic analysis.

Analysis of acrylamide in coffee by LC-MS/MS

Acrylamide in coffee was determined a previously optimised and validated LC-MS/MS method [15]. Chromatographic separation was achieved by the Dionex UltiMate 3000 system on a Hypercarb column (150 mm x 2.1mm; 5µm; Thermo Scientific) after a guard column with the same absorbent. Analytical conditions: flow rate of 350 µL/min, column temperature of 20ºC, sample injection volume - 20 µL and an isocratic mobile phase of water methanol (9:1; v/v) with 0.1% formic acid. Run time was 5 minutes. Detection and quantification were performed on an ABSciex 3200 QTrap mass spectrometer using multiple reaction monitoring, (MRM) in the positive ion mode with an electrospray capillary voltage (IS) of 5000 V. The carrier gas was nitrogen (CUR = 40) and the ion source temperature was 600ºC. As previously described [15, 26], acrylamide concentrations were calculated by the peak areas under the curve originating from the following ion transitions; m/z 72.1 → 55.2 (AA), m/z 75.1 →58.1 (AAd_3), whereas the ions 72.1 → 44.1 (AA) and m/z 75.1 → 47.1 (AAd_3) were used for verification.

Assessing roasted and instant coffee colouring

This was performed according to an established method using an method of arranging (sequence) [8]. Here, a ten member team rated the grades of colourings in order of intensity (shown in photos 1 & 2). Samples

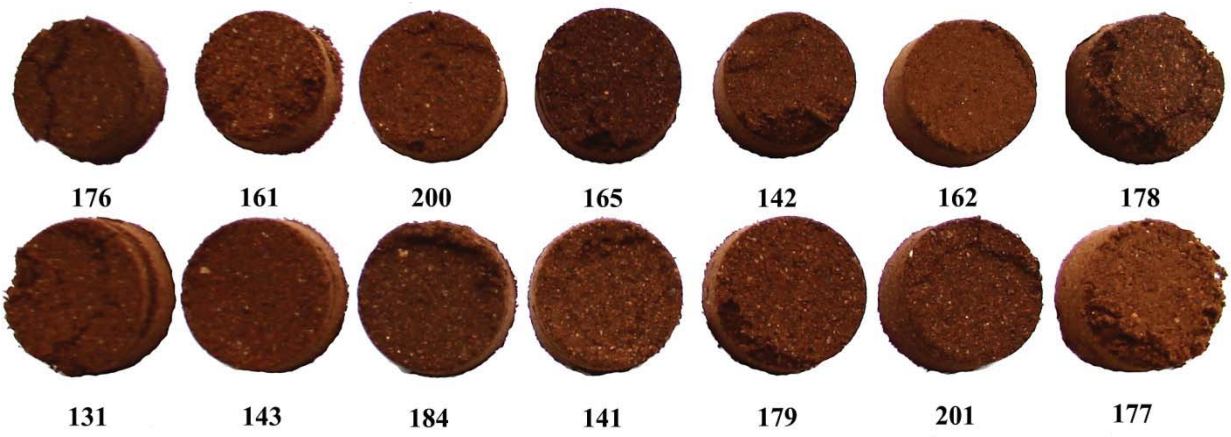

Photo 1. Samples of ground roasted coffee
Samples significantly lightest: 161, 162, 141, 177
Samples significantly moderately dark: 200, 178, 131, 143, 179
Samples significantly darkest: 176, 165, 142, 184, 201

Photo 2. Samples of instant coffee
Samples significantly lightest: 11, 16, 18, 20
Samples significantly moderately dark: 10,12, 19
Samples significantly darkest: 13, 14, 15, 17

were arranged by members in order of increasing intensity according to a 1 to 14 scale for roasted coffee and a 1 to 11 one for instant coffee. Results were recorded in custom-made reporting cards and then analysed by *Kramer's* tables where the upper division served to determine if there were any significantly lighter or darker colourings in samples amongst all those tested. The lower division was used to establish which of the samples differed.

Statistical analysis

Results were calculated on a Microsoft Excel spreadsheet and statistically analysed by the Statistica 6.0 version (Statsoft, Inc). Acrylamide concentrations are given as the mean ± SD. ANOVA analysis was performed to first determine if any significant differences existed between samples. When this proved to be the case, a post-hoc NIR test was used to find where they lay. Linear relationships were assessed by the *Pearson* correlation coefficient.

RESULTS

Concentrations of acrylamide in all the sample types of commercial coffee studied are shown in Table 1.

The highest mean level values was in coffee substitutes (818 µg/kg), followed by instant coffee (358 µg/kg), whilst the lowest were for roasted coffee at 179 µg/kg (range: 61 – 397 µg/kg). The ANOVA analyses indicated that there were significant differences present between samples ($p < 0.005$).

In most of the roasted coffee samples (n=20) acrylamide levels were below 200 µg/kg of which 3 were lower than 100 µg/kg. Acrylamide concentrations in a further six samples ranged between 200 to 300 µg/kg whilst only two were higher at 386 and 397 µg/kg (Figure 1). For the instant coffee, there were 2 samples with acrylamide levels below 200 µg/kg and 7 ones that ranged between 300 – 400 µg/kg. In a further two samples, acrylamide levels were as high as 513 and 830 µg/kg (Figure 2). As aforementioned, coffee substitutes had the highest acrylamide levels, where two samples from the same manufacturer, but from different batches showed 781 and 1145 µg/kg when compared to a sample from another manufacturer at 528 µg/kg.

Because acrylamide is highly water soluble, it can be assumed that it the entire acrylamide content of commercial products will be present in any coffee infusion. Taking into account the variations of acrylamide levels observed, an single cup of drinking coffee is expected

Table 1. Comparison of acrylamide level in different types of coffee and coffee substitutes

Type of coffee	Acrylamide content			
	[µg/kg of coffee]		[µg/cup of coffee]	
	x ± SD	min ÷ max	x ± SD	min ÷ max
ground roasted coffee[1]	179 ± 85[a*]	61 ÷ 397	0.45 ± 0.21[a*]	0.15 ÷ 0.99
instant coffee[2]	358 ± 188[b]	152 ÷ 830	0.93 ± 0.49[b]	0.40 ÷ 2.16
coffee substitutes[3]	818 ± 310[c]	528 ÷ 1145	3.21 ± 1.25[c]	2.11 ÷ 4.58

[*] statistically significant difference ($p < 0.005$)
[1] one teaspoon (3.2 g) of ground roasted coffee was used to prepare a cup of coffee (160 ml)
[2] one teaspoon (2.6 g) of instant coffee was used to prepare a cup of coffee (160 ml)
[3] one teaspoon (4.0 g) of coffee substitutes was used to prepare a cup of coffee (160 ml)

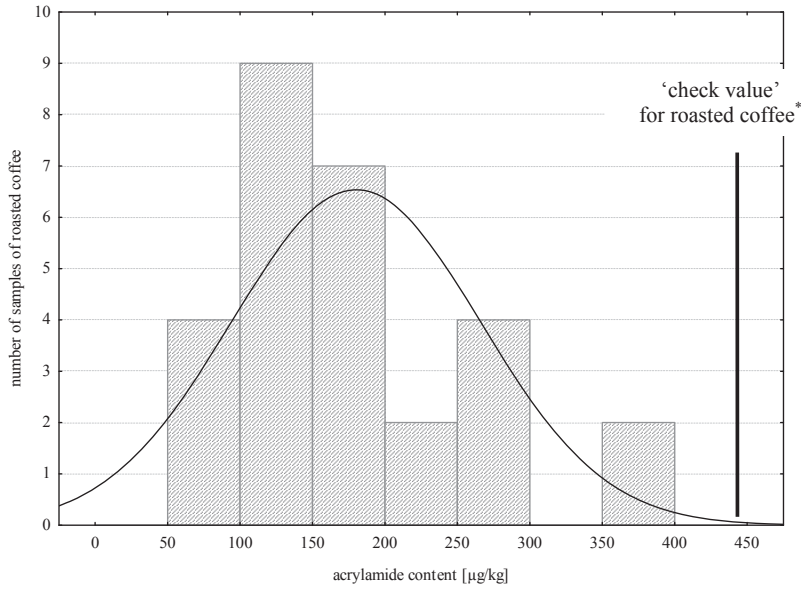

Figure 1. Distribution of samples of roasted coffee according to the content of acrylamide (n = 28)
* source: [9]

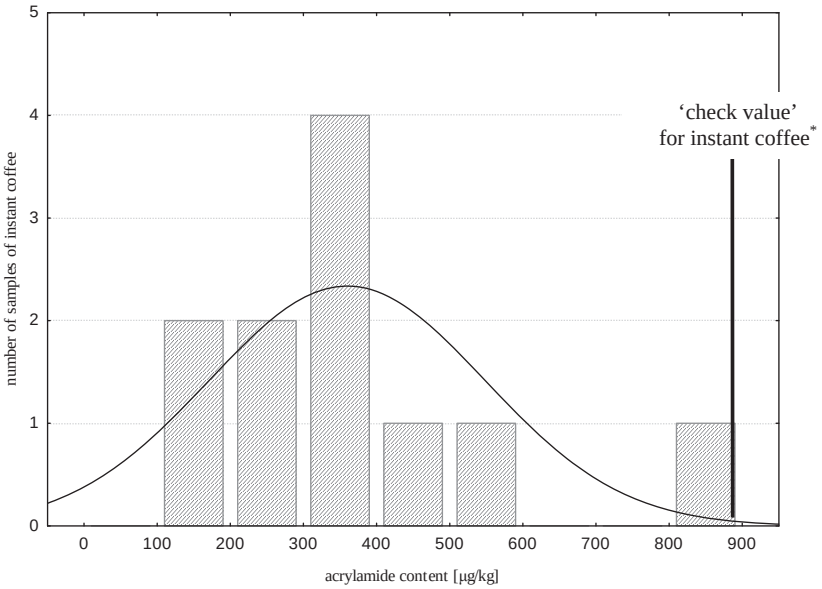

Figure 2. Distribution of samples of instant coffee according to the content of acrylamide (n = 11)
* source: [9]

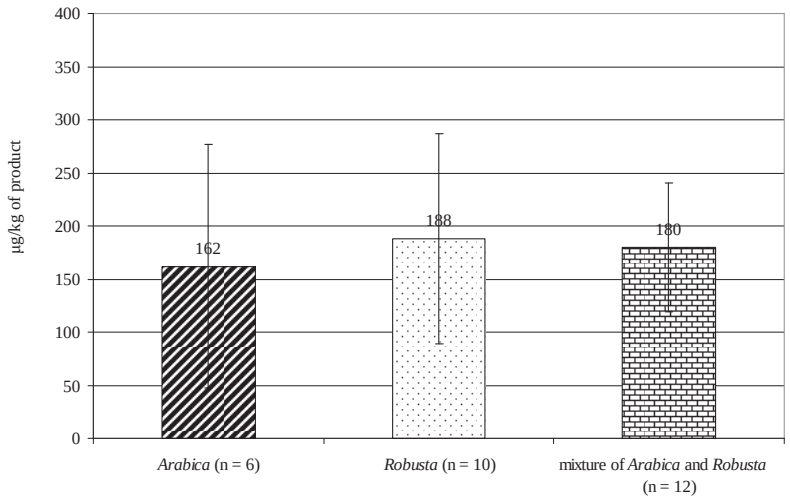

Figure 3. Comparison of acrylamide level in ground roasted coffee depending on the coffee species

to contain between 0.15 µg in roasted coffee, to 4.58 µg in coffee substitutes (Table 1).

The mean amount of acrylamide present in the *Arabica* roasted coffee was 162 µg/kg which was lower, but not significantly, than mean levels measured in the *Robusta* species at 180 µg/kg (Figure 3).

Coffee originating from roasting facilities in Poland, had slightly higher mean acrylamide levels at 194 µg/kg compared to those of other manufacturers at 173 µg/kg, but as before this difference was not significant.

Likewise, the higher levels of acrylamide found in freeze-dried coffee compared to agglomerated coffee (379 µg/kg vs 320 µg/kg) were also not significant (Table 2).

Table 2. Comparison of acrylamide level in instant coffee depending on the way of production

Type of coffee	Number of samples	Acrylamide content [µg/kg of coffee]	
		x ± SD	min ÷ max
freeze-dried	7	379 ± 214	153 ÷ 830
agglomerated	4	320 ± 150	152 ÷ 513

The intensity of coffee colouring in the studied samples are shown in photos 1 and 2. The results demonstrate significant differences of intensity in roasted coffees irrespective of the coffee species. In fact a negative correlation was observed (r = - 0.5569, p <0.05), between acrylamide levels and the intensity of coffee colourings (Figure 4). The highest statistically significant (p <0.05) acrylamide levels were seen when the colour was lightest at 323 µg/kg, compared to those

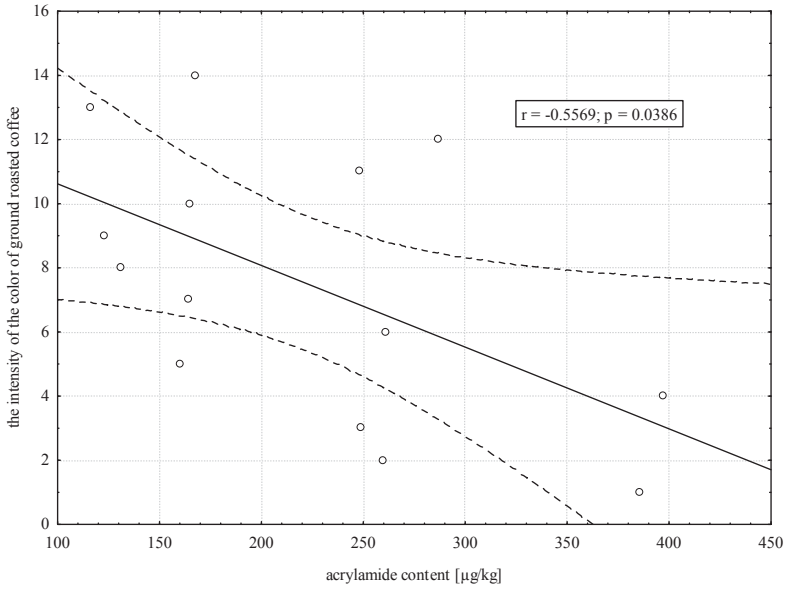

Figure 4. The correlation between acrylamide content in coffee and color intensity of ground roasted coffee

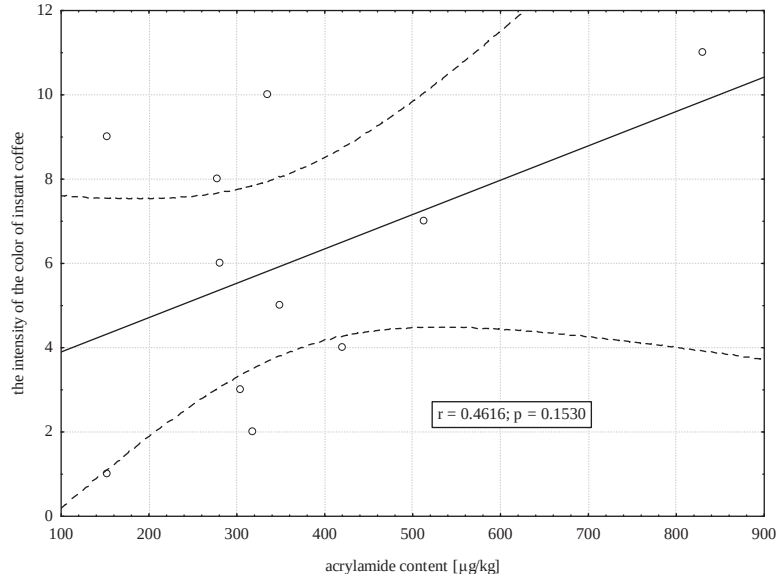

Figure 5. The correlation between acrylamide content in coffee and color intensity of instant coffee

Table 3. Comparison of acrylamide level in roasted and instant coffee depending on the intensity of colour

Intensity of coffee color[1]	Roasted coffee (n = 14)		Instant coffee (n = 11)	
	Number of samples	x ± SD	Number of samples	x ± SD
significantly lightest	4	323 ± 79*	4	299 ± 110
significantly moderately dark	5	168 ± 55	3	381 ± 119
significantly darkest	5	197 ± 69	4	399 ± 297

[1] evaluated on the basis of *Kramer's* tables
* statistically significant difference ($p < 0.05$)

where the colour were moderately dark (197 µg/kg) or dark (168 µg/kg) (Table 3).

In contrast to roasted coffee, there was no relationship observed between acrylamide content and the intensity of colour for instant coffee (Figure 5). Less acrylamide was in instant coffee with the lightest colourings (299 µg/kg), than coffee with moderately dark or dark colourings at 381 µg/kg and 399 µg/kg respectively, although these differences were not significant (Table 3).

DISCUSSION

The mean acrylamide content in the roasted coffee samples was 179 µg/kg ranging from 61 to 397 µg/kg depending on the product. Similar findings were observed in a study by *Andrzejewski* et al [2] which tested 31 different coffee types available on the USA market, where acrylamide levels ranged from 45 to 374 µg/kg. Higher amounts of acrylamide were found in various coffee types in Denmark by *Granby* and *Fagh* [17]. According to this study [17], one litre of expresso coffee contains on average 9.5 µg of acrylamide (range 7 to 15 µg), which in terms of a 1 kg of coffee gives an average equivalent of 237.5 µg (it was assumed that 40 g of roasted coffee were required to make up 1 litre of drinking coffee). A smaller amount was demonstrated by another study conducted in Portugal, *Soares* et al. [29], where expresso coffee contained acrylamide from 57 to 181 µg/kg coffee (the authors claiming that an expresso coffee is made up of 6 g extract with 30 ml water). However, much lower acrylamide concentrations were demonstrated by *Senyuva* and *Gokmen* [28] in Turkey, with levels in ground roasted coffee ranging from 12 to 29 µg/kg.

The mean levels of acrylamide in instant coffee were found to be 358 µg/kg which was twice that in roasted coffee (179 µg/kg). Similar results were obtained by other studies; *Andrzejewski* et al. [2] and *Soares* et al [29]. In the USA, the acrylamide content of instant coffee ranged from 169 to 539 µg/kg, whereas the mean acrylamide levels in commercial products in Portugal were 362 µg/kg [29]. Lower acrylamide concentrations than those presented were seen in instant coffee studies from Turkey [28] ie. of 42 to 338 µg/kg. Somewhat higher acrylamide levels (8 µg per litre of drinking coffee) were seen in the aforementioned Danish study [17] of *Grandy* and *Fagh*. Taking into account that 13.4 g of coffee are used per litre in drinking coffee, then instant coffee in the Danish study contained 597 µg acrylamide/kg of commercial product. A monitoring study conducted throughout Europe [13] during 2007-2009 demonstrated slightly higher acrylamide levels, where on average, instant coffee contained 481 µg/kg, ranging between 293 to 1470 µg/kg.

The presented study showed that coffee substitutes contain on average, 4.5 times the amount of acrylamide found in roasted coffee. The results are similar to that demonstrated in the aforementioned European study [13], where the mean acrylamide levels found in coffee substitutes were 1030 µg/kg, with a maximum of 4300 µg/kg. Acrylamide concentrations in coffee substitutes depend on the composition of products. These products contain chicory root and roasted cereals (eg, rye, barley) and some also roasted beetroot. All these ingredients contain acrylamide precursors ie. reducing sugars such as glucose and fructose as well as asparagine. The differences in acrylamide levels found may be accounted for by the variety of coffee substitutes sampled and of having different manufacturers and differing raw materials that thereby influence the final composition of product. For example, chicory roots contain twice as much fructose as glucose [25], where the former is more reactive than the latter during acrylamide formation. Furthermore, it has been noted by *Worobiej* and *Relidzyńska* [33], that there are large differences in reducing sugars present in coffee substitutes, which thus effect the levels of acrylamide found in a given final product.

It should be stated that none of the roasted coffee or instant coffee samples showed acrylamide levels that exceeded the check value of 450 and 900 µg/kg respectively, as stated in EC recommendations from 2011 [9]. At present, there are however no such check values existing for coffee substitutes.

Although the study showed that acrylamide levels were lower in *Arabica* coffee than in *Robusta* or mixtures thereof, these differences were not significant. However, like findings have been shown in another study by *Lantz* et al. [24], with acrylamide levels of 378 and 251 µg/kg in respectively *Robusta* and *Arabica* coffee;

the differences also being not significant. In addition, a study by *Alves* et al. [1] demonstrated that *Robusta* coffee had significantly higher acrylamide than *Arabica* coffee; 564 vs 183-279 μg/kg; the latter depending on the country from which the beans originated. It appears that during roasting, more acrylamide is formed in *Robusta* coffee than *Arabica* coffee, but up till now, the determining factors remain unexplained. Nevertheless it has been suggested that fresh *Robusta* coffee beans contain more asparagine [3, 24] than *Arabica* ones.

The instant coffee products that appear on the Polish market vary in the methods of manufacture, especially in how the coffee is dehydrated. When coffee is freeze--drying from frozen extracts, the ice is removed though water evaporation, however in agglomerated coffee, the extract is dried under streams of hot air up to the moment that the dissolvable granules are formed. Despite such differences, the current study did not show any significant association between these methods of production with acrylamide levels; the apparent 18% increase of acrylamide in freeze-dried coffee was not significant.

A negative and significant correlation, ($p<0.05$) was demonstrated between acrylamide concentrations and the intensity of color which was consistent with other work; *Taeymans* et al [31] and *Gokmen & Senyuva* [16]. Lower acrylamide levels in dark coloured coffee compared to the light coloured ones reflect the sensitivity of acrylamide to decompose during roasting; acrylamide is principally formed at the start of the roasting process but its content decreases the longer the roasting continues [3, 24, 31]. Even though there were differences in colouring within the instant coffee samples, this was found not to be linked to acrylamide concentrations.

The presented study confirms that the roasting process is a key factor in determining the acrylamide levels in the finished product. The species bean (ie. raw material), has a smaller effect, although it would be worth checking the acrylamide content in *Arabica* and *Robusta* coffee according to country of origin.

It can be summarised that coffee forms a significant source of dietary acrylamide within the population of persons consuming high amounts of coffee. Of special concern is the high acrylamide levels in coffee substitutes which often takes the place of natural coffee, especially in the more vulnerable population groups such as children and pregnant or breast feeding women. It is therefore vital that check values are established for coffee substitutes, that nutritional recommendations are modified accordingly in affected groups and that targeted education is undertaken. Furthermore, because of the wide variations of acrylamide concentrations within the same group of coffee products, manufacturers should take whatever steps are necessary to reduce such levels.

CONCLUSIONS

1. The study findings confirm that coffee can be an important dietary source of acrylamide. Amongst the various coffee types, coffee substitutes had the highest mean acrylamide concentrations (818 μg/kg) followed by instant coffee (358 μg/kg) and then by roasted coffee (179 μg/kg).
2. We did not find significant difference in acrylamide content between coffee types or mixtures (ie. *Arabica* vs *Robusta* vs mixtures thereof) nor in the methods of manufacturing instant coffee (ie. freeze--drying vs agglomeration).
3. We found a negative correlation between acrylamide levels in roasted coffee and the intensity of color of coffee. This was not observed with instant coffee.
4. The presented study confirms that the process of roasting coffee beans is a significant factor in determining the acrylamide content in the final product. In coffee substitutes, this critical factor is the composition of the raw material.

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Conflict of interest

The authors declare no conflict of interest.

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