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Acrylamide content of commercially available capsule coffees

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1. SUMMARY

The consumption of capsule coffees is becoming more and more common in everyday life. Today, a number of studies support the fact that there are benefits of consuming the right amount of coffee. Despite its beneficial effects, there are also disadvantages to drinking coffee. For example, the acrylamide found in roasted coffee, which is formed during the process of roasting, poses a health risk. Acrylamide has been classified by the International Agency for Research on Cancer (IARC) as a Group 2A substance, i.e., as an agent which is probably carcinogenic to humans [1]. The technological parameters of the roasting process affect the amount of acrylamide formed in the product. Light roasted coffees contain higher levels of this compound than dark roasted coffees.

Numerous studies have been conducted to investigate the acrylamide content of ground coffee products, however, capsule coffees have not yet received similar attention in this respect. In my study, the acrylamide content of various types of commercially available capsule coffees was investigated by HPLC-MS/MS measurements. Decaffeinated coffees are produced using a different technology, so some of these types were also tested.

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2. Introduction

2.1. Acrylamide, its formation and effects

Acrylamide is an organic compound with the molecular formula C_3H_5NO. Its IUPAC name is prop-2-enamide. It is a low molecular weight, odorless, white solid which is highly soluble in water but also soluble in organic solvents. It is used in industry in the production of polyacrylamides, which are used as water-soluble thickeners and flocculants. It is a highly toxic compound therefore it is mainly handled in the form of an aqueous solution **[2]**.

Acrylamide is a human neurotoxin, classified by the International Agency for Research on Cancer (IARC) as a Group 2A substance, i.e., as an agent which is probably carcinogenic to humans **[1]**. Acrylamide has been used in many industrial processes since the 1950s. An announcement was issued by the Swedish National Food Administration on April 24, 2002, about the discovery that it is formed as a byproduct in heat-treated foods with a high carbohydrate content **[3]** and can therefore be detected mainly in snack foods, potato chips, breads, cereal products and coffee. Following the discovery, more and more studies were launched to detect acrylamide content. An increasing number of researchers are looking for the answer to the question how it is formed in different foods.

Mottram et al. have conducted extensive studies on the formation of acrylamide from amino acids and reducing sugars during heat treatment as a result of the Maillard reaction. Asparagine, the amino acid most abundant in potatoes and cereals, has been found to contribute greatly to acrylamide formation. During baking and roasting, products of the Maillard reaction are responsible for the formation of flavor and aroma substances and the development of color. Strecker degradation of the amino acids also occurs at this time, during which amino acids are decarboxylated and then deaminated to form aldehydes. An outline of the process is shown in ***Figure 1*** **[4]**.

According to several studies, acrylamide is toxic because it forms adducts with compounds found in hemoglobin and also reacts with important functional proteins and DNA. Glycidamide, a metabolite of acrylamide, reacts similarly with hemoglobin as well **[5]**.

The area most studied is related to the neurotoxic properties of acrylamide, since these can be observed in both humans and animals. Observations have been made in a variety of laboratory animals, including cats, rats, mice, rabbits and monkeys. After administration of 0.5 to 50 mg acrylamide/kg/day, limb movement disorders and muscle weakness could be observed in all animals **[6]**.

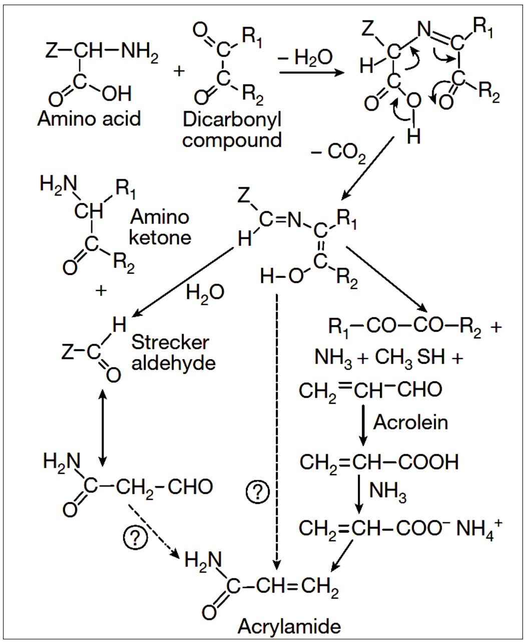

Figure 1. Outline of acrylamide formation [4]

2.2. Acrylamide in coffee

The acrylamide content of coffee is formed during roasting. In an extensive study, Guenther et al. found that it is produced in the highest amount (more than 7 mg/kg) during the initial stage of roasting, and then the amount decreases towards the end of the process. Towards the end of the roasting cycle, acrylamide is increasingly eliminated, with both physical and chemical losses **[7]**.

Kinetic models and other experiments with isotopically labeled acrylamide have shown that more than 95% of the acrylamide formed is degraded during the entire roasting process. This means that the acrylamide content of lightly roasted coffees with a shorter roasting cycle is much higher than that of dark roasted beans **[7]**.

The authors of the study also explained that green coffee beans contain very low concentrations of asparagine (0.2–1.0 g/kg), which is only negligibly higher in the case of Robusta species. Thus, it was found that the amount of asparagine and the acrylamide concentration showed a weak correlation, and even no correlation was found in Robusta beans. This is due to the fact that the rate of acrylamide loss far exceeds the rate of its formation **[7]**.

Alves et al. studied how the acrylamide content in brewed espresso coffee changes, as in their opinion it most often enters consumers' body in this form. Acrylamide is highly soluble in water, so it is extracted easily from coffee during brewing. The chemical properties of brewed coffee are influenced by many factors, such as the type of coffee (Arabica, Robusta, or a certain mixture of the two), the degree of roasting, or the amount of water used to make a given amount of coffee, which varies by individual taste. According to some studies the acrylamide content of different coffee beverages ranged from 2 to 25 μg/l **[8]**.

3. Objective

The main objective of my work was to investigate the acrylamide content of different types of capsule coffees by HPLC-MS/MS measurement.

Based on literature data, it was assumed that the acrylamide content of beverages brewed from capsule coffees is higher than the acrylamide content of the ground coffee extracted from the capsules, as it dissolves easily in the water during brewing. The goal was to examine and confirm this with the measurements.

Another objective was to compare different coffee machines. Coffee machines have different parameters (e.g., temperature, pressure, amount of water used), which may affect the amount of acrylamide released from capsule coffees.

Literature data are also available showing how the roasting technology of coffee affects the acrylamide content in the final product. The acrylamide content of so-called light-roasted coffees, roasted for a shorter period of time, is higher than that of dark-roasted coffees, roasted for a longer period of time. This influencing factor was also checked.

Given that decaffeinated coffees are produced by a different technology, some of these types were also examined.

4. Materials and methods

4.1. Chemicals, equipment and instruments used

During my work, analytical grade chemicals, HPLC grade solvent (methanol, acetic acid (anhydrous), n-hexane) and distilled water were used, as well as the following: acrylamide and 10 μg/ml acrylamide-$^{13}C_3$ as internal standard.

In addition to standard laboratory equipment, Biotage ISOLUTE® Multimode 1g/6ml and Biotage ISOLUTE® ENV+500mg/6ml SPE columns were used for sample preparation. For coffee brewing from capsule coffees, the following coffee machines were used: Nespresso Essenza Mini, Krups KP120H31, Tchibo Caffissimo and Martello Smart.

Instrumental analysis of the samples were performed on a Thermo Scientific™ Dionex UltiMate™ 3000 HPLC system with a Phenomenex Kinetex® C18 2.6 μm 100 Å 150x4.6 mm column and a Thermo Scientific™ TSQ Quantis™ triple quadrupole MS detector.

4.2. Sample preparation

Sample preparation and the measurements were carried out as described in standard MSZ EN 16618:2015 Food analysis. Determination of acrylamide in food by liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS).

The samples obtained from commercial sources were caffeinated (25 pcs) and decaffeinated (8 pcs) of capsule coffees from different manufacturers. Measurements were performed on both the ground coffee in the capsules and the brewed coffees. ***Table 1*** shows the sample nos. of the examined coffees and the coffee machines used.

Table 1. Coffees and coffee machines

Coffee type	Nespresso	Dolce Gusto	Caffissimo	Martello
Sample nos. of caffeinated coffees	1, 5, 7, 8, 10, 16, 20, 21, 23	3, 4, 6, 9, 15	17, 25, 26, 27, 28, 29	13, 30, 31, 32, 33
Sample nos. of decaffeinated coffees	2, 11, 12, 19, 22	24	18	14

IBM SPSS Statistics software was used for the statistical evaluation of the results.

5. Results

5.1. Acrylamide content

Acrylamide content measurement results of the coffee samples are summarized in ***Table 2***. Both the results measured in the ground coffees and the results of the corresponding brewed coffees are listed.

Table 2. Measurement results

Sample no.	Ground (μg/kg)	Brewed (μg/kg)	Sample no.	Ground (μg/kg)	Brewed (μg/kg)
1	224.0	170.7	18	215.1	205.9
2	237.7	230.0	19	205.6	242.2
3	176.6	184.1	20	271.1	279.9
4	168.8	179.4	21	195.7	212.6
5	274.8	243.0	22	128.8	126.5
6	201.6	231.9	23	223.1	231.7
7	161.6	183.8	24	203.8	198.3
8	189.7	207.4	25	284.9	250.2
9	231.2	232.4	26	399.8	389.9
10	287.9	280.3	27	121.4	124.2
11	107.0	128.7	28	130.2	119.0
12	240.3	118.6	29	174.3	159.4
13	*446.5*	*427.4*	30	189.8	200.2
14	353.7	351.0	31	224.9	255.6
15	204.9	221.6	32	263.3	276.2
16	270.7	156.1	33	*416.2*	*407.9*
17	274.0	240.0			

The results obtained were not in all cases in line with the reference level of 400 μg/kg for roasted coffee set out in Commission Regulation (EU) 2017/2158, as the acrylamide content of some caffeinated samples (nos. 13 and 33) exceeded this level. It is likely that the higher acrylamide level in the case of coffee sample no. 13 was due to the fact that the sample contained Robusta coffee with a higher intensity of acrylamide formation, according to the literature. The result of sample no. 33 can be explained by the fact that is was a hazelnut-flavored mixture. Given that a Robusta variety was added to the Arabica coffee variety, this may have been the reason for the higher results, to which the roasted hazelnut flavor could also have contributed. On average, the acrylamide content of the ground coffees was higher, or in some cases almost identical to the results of the brewed coffees. There were also samples in the case of which the brewed coffees contained more acrylamide than the ground coffees, but most of these values were within the 10% measurement uncertainty.

Based on my statistical (ANOVA) calculations, there was no significant difference between the measurement results of ground and brewed coffees at the 95% confidence level ($p > 0.05$).

5.2. Effect of brewing on acrylamide content

My objective was to investigate the extent to which the acrylamide content in ground and brewed coffees could vary depending on which coffee machine was used for brewing. Thus, I was looking to answer whether the coffee machines worked with different efficiencies. There was no significant difference between capsule and brewed coffees for any of the coffee machines ($p > 0.05$).

However, the results showed that, in the case of Martello type capsules, the measured values of both brewed and ground coffees were in a higher range than the results of the other types.

For the Martello type, this range was between 200 and 450 µg/kg (***Figure 2***), while for the other types (for example, for Nespresso, see ***Figure 3***), typical values were between 100 and 250 µg/kg.

It was found that capsules made for Martello type coffee machines contained ground coffees that typically had a higher acrylamide content. The Martello type capsule coffees tested contained Robusta coffee or a mixture of Robusta and Arabica, which explains the higher acrylamide content, as Robusta-types coffees have higher acrylamide levels than Arabica varieties. One of the Martello type coffee capsules was roasted hazelnut flavored, which also may have contributed to the higher result.

Based on my measurement results, it can be stated that there was no significant difference between the effects of the different coffee machines. However, as the Martello type coffee capsules, on the whole, contains ground coffee with a higher acrylamide concentration compared to the other types, it caused a significant difference between the measurement results of the ground coffees in the capsules.

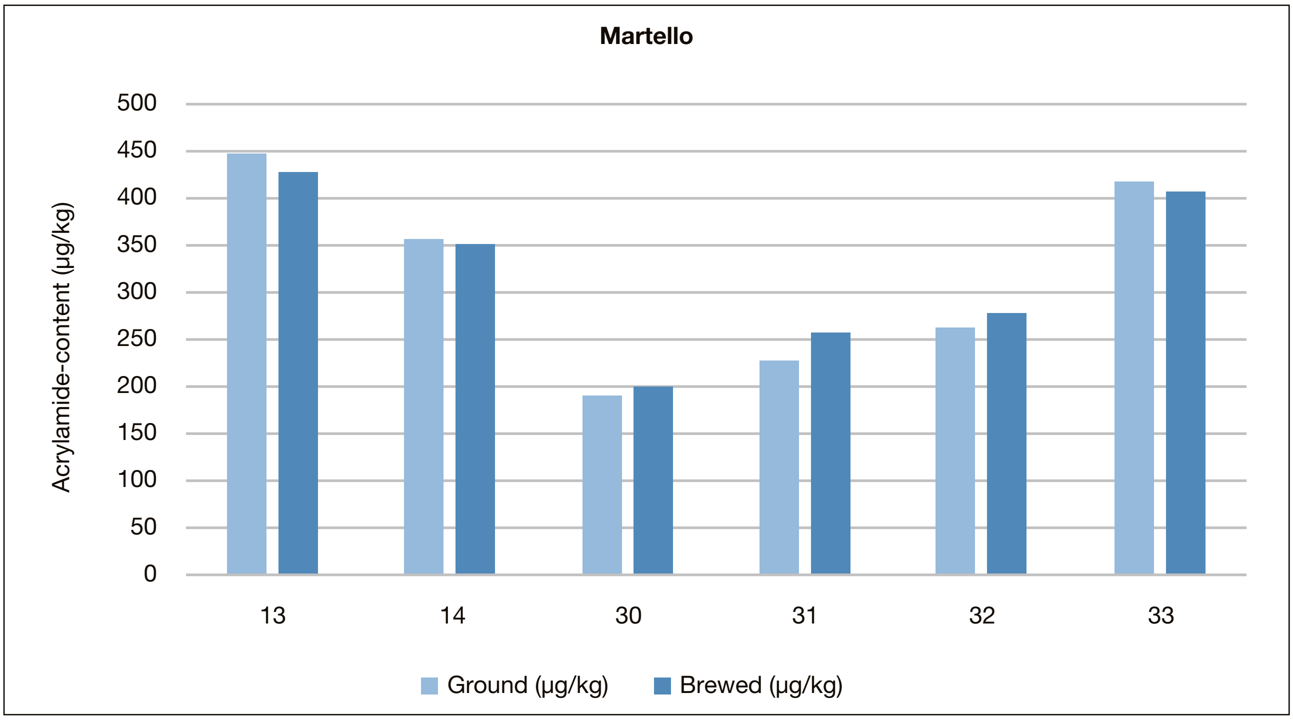

Figure 2. Measurement results of coffee beverages brewed with a Martello coffee machine

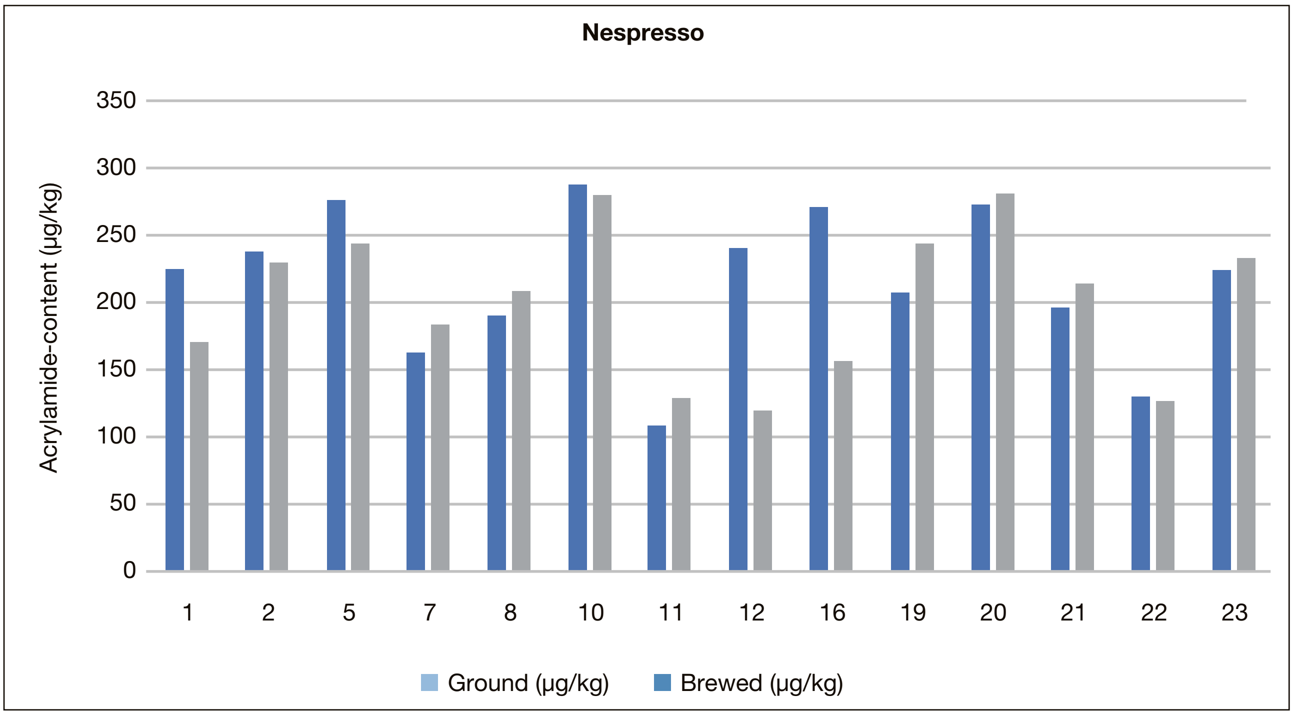

Figure 3. Measurement results of coffee beverages brewed with a Nespresso coffee machine

5.3. Effect of roasting on acrylamide content

It was also examined how different roasting levels affect the amount of the acrylamide formed. In ***Table 3***, coffee samples are grouped according to roasting levels. The ground and brewed samples were marked with separate hues. ***Figure 4*** shows the measured acrylamide amounts according to the different roasting levels. Light roasted samples typically yielded similar of higher results than dark roasted coffees. According to the literature, acrylamide levels of dark roasted coffees are lower than those of light roasted coffees, and this was confirmed by our results.

However, when performing statistical analyses, it was found that there was no significant difference in the amount of acrylamide formed between the results of either ground or brewed coffees at the 95% confidence level ($p > 0.05$).

The analyses were also performed for the different coffee machines, as the measured values of the coffees brewed with the different machines were typically in different ranges, so this grouping results in a more accurate comparison. However, there was no significant difference between the roasting levels this way either.

Table 3. Acrylamide levels of coffee samples according to roasting levels (see Figure 4. for resolution of hues)

	Light roast	Medium roast	Dark roast
Sample no.	2, 10, 13	14, 20, 27, 29, 32, 33	1, 6, 7, 11, 19, 22, 25, 26, 28, 30, 31

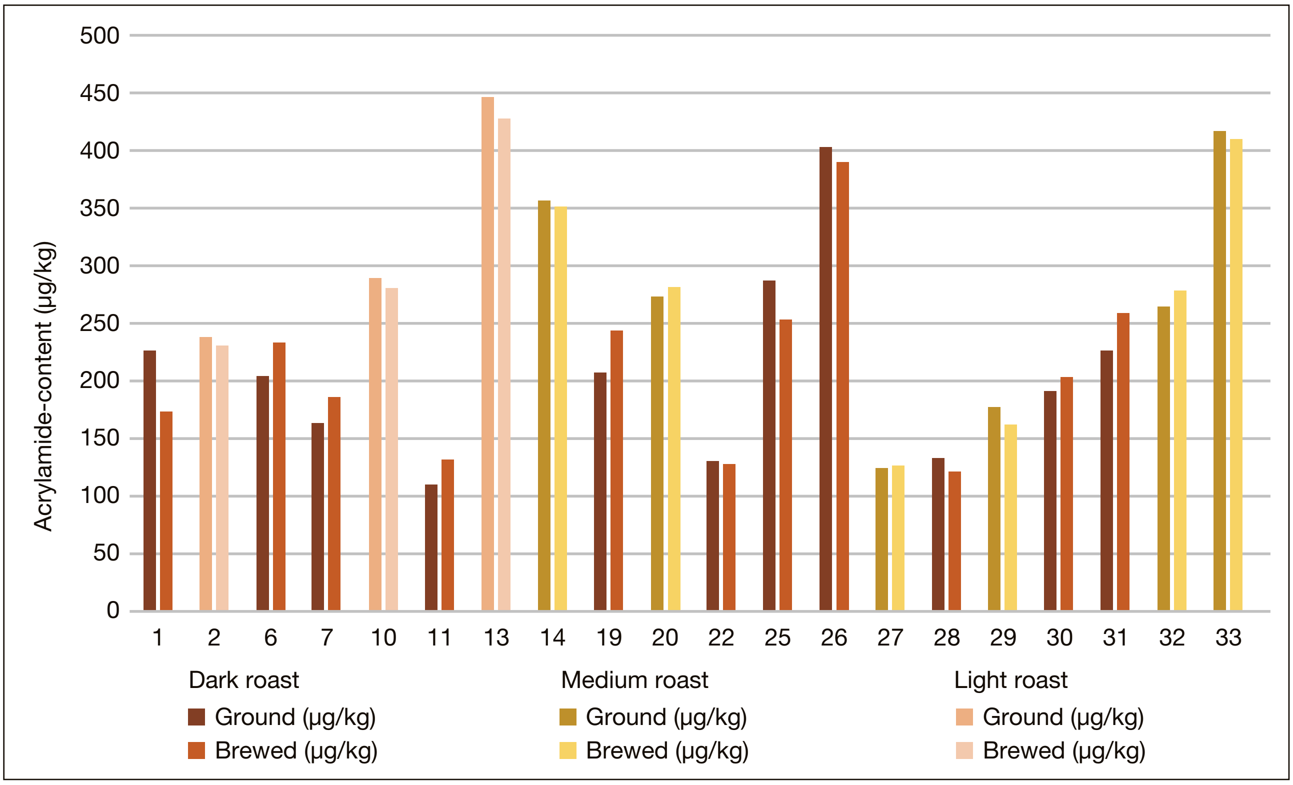

Figure 4. Acrylamide levels of coffee samples according to roasting levels

5.4. Results of caffeinated and decaffeinated coffees

Significantly different techniques are used for the production of decaffeinated coffees, therefore the acrylamide content results of caffeinated and decaffeinated coffees were also compared. The measured values of decaffeinated coffees were in a similar range as the values of caffeinated samples. It was found that there was no significant difference between the different types of samples in terms of acrylamide content.

To confirm this, ANOVA analyses were performed when examining the results of both ground and brewed coffees. At the 95% confidence level, there was no significant difference between the types in either case ($p > 0,05$).

6. Conclusions

Based on literature data, it was hypothesized that the acrylamide content of the beverages brewed from capsule coffees was higher than the acrylamide content of the ground coffee extracted from the capsules. Based on my measurements, it was found that the acrylamide content of the ground coffees was on average higher than or in some cases similar to the acrylamide levels of the brewed coffees. However, in some cases, brewed coffees did contain more acrylamide. Nevertheless, based on statistical calculations, the difference between the results was not significant. Based on these results, the claims in the literature could not be substantiated unequivocally.

Based on my results, it can be stated that there was no significant difference between the brewed coffees and their ground coffee counterparts in the case of any of the coffee machines in terms of the measured amount of acrylamide. It was found that the acrylamide levels of Robusta type coffees are higher than those of Arabica varieties. The Martello type capsule coffees contained Robusta coffee or a mixture of both, which may explain their higher acrylamide content.

Light roasted samples typically yielded similar or higher acrylamide content results than dark roasted coffees. There was no significant difference in the results of either ground or brewed coffees between the roasting levels.

It was found that there was no significant difference between caffeinated and decaffeinated coffee samples. The acrylamide content of coffee is not significantly affected by the decaffeination processes used.

7. References

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