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INTERRUPTED CASE STUDY: ESPRESSO ACRYLA-MACCHIATO

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Part I – Coffee Break

Flávio and André are undergraduate chemistry students. They take a break every day and leave the university in the afternoon for a cup of coffee at StarCoffees. Arriving at the cafeteria, they order a mocaccino for Flávio and a double espresso for André. Taking advantage of the moment during the break, they start talking about coffee.

– André, imagine how much acrylamide we are consuming every day. Did you stop to think about it?

– Are you talking about all meals or just our coffee?

– I was thinking specifically about that little coffee! Does the coffee shop know anything about acrylamide?

– I believe so; they are a worldwide chain!

– Shall we investigate? I have some ideas that can be useful for my Course Completion Work!

– Shall we send an e-mail to the company?

– Yes, help me write here.

Then Flávio and André sent the following e-mail:

Dear StarCoffees,

My name is Flávio. I am an undergraduate chemistry student. I came across a question and would like, if possible, a position from the company on the concentration of acrylamide in its products.

I believe you know that acrylamide is a by-product of the Maillard reaction, triggered at high temperatures. The reaction occurs between the carbonyl group of reducing sugars and the amino group of proteins, peptides or amino acids, obtaining products that give flavor, odor and, mainly, color to food. The golden appearance of foods after roasting or frying, such as grilled bread, french fries, and well-done meat, is due to the Maillard reaction. Acrylamide is considered a risk factor since it has carcinogenic potential for humans.

Could you provide me with some data that would help in an investigation on the subject? I want to study about it and propose solutions that benefit everyone.

Yours sincerely,

Flávio

After a few days, the company sends a reply:

Dear Flavio,

My name is Fátima, I am the director of Research and Development (R&D) at StarCoffees/Brasil. I was delighted with your e-mail, as the concentration of acrylamide is one of the cases discussed in the company.

Unfortunately, some data is confidential. However, I can provide technical data to assist you in your investigation. Our flagship is espresso coffee, and for the production of beverages, Arabica (Coffea arabica) and Robusta (Coffea canephora) coffee beans are used in different roasts under the conditions specified in Table 1.

Table 1. Arabica and Robusta coffee roasting times and temperatures used by StarCoffees in the production of espresso coffee.

Roast	Time (min)	Temperature (°C)
Low – (R1)	12	230
Medium – (R2)	14	240
High – (R3)	17	250

Yours sincerely,

Fátima Rodrigues

R&D Director's - StarCoffees/Brasil

Classroom activities

1. What do you already know about the case? That is, what have you read about similar situations? What experiences have you had that refer to the subject addressed in the case?

2. Based on the narrative, build hypotheses about the factors influencing acrylamide formation in the coffe at StarCoffees and justify their relevance.

3. Based on the narrative, propose a question to be investigated by the group. Attention: the question must consider the context of the narrative, and the possible answers must provide

information so that the characters can take action to solve the problem they are facing.

Homework activities

1. Search and select a review text that deals with the subject addressed in the case. Construct a summary, indicating the group's motivation for your choice and what information therein may help in understanding and/or solving the case, justifying the answer.

2. What do you believe would be interesting to know to solve the case in terms of content related to the area of natural sciences and content from other areas of knowledge?

3. Is there additional information about the case context that would allow the group to formulate more precise hypotheses and a more straightforward question?

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Part II – Preparing a Coffee

After receiving the reply from the e-mail sent to StarCoffees, Flávio told André the news.

– Andre they replied. Shall we analyze the concentration of acrylamide in this coffee?

– Let's go! But before that, open the game; what are you thinking?

– So, I want to quantify the acrylamide present in this coffee and, subsequently, try to reduce its concentration, obtaining a healthier product. Who knows, maybe it will be possible to sell this technique to the company later and make some money.

– I thought it was bold, but I loved it. Let's try!

First, Flávio and André return to the data provided in the R&D Director's e-mail (Table 1).

Table 1. Arabica and Robusta coffee roasting times and temperatures used by StarCoffees in the production of espresso coffee.

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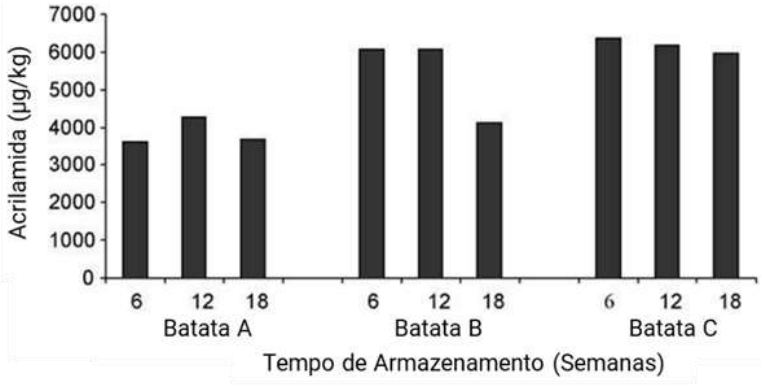
Then, the two get samples of the coffees used by StarCoffees and find ways to quantify acrylamide in the literature. They analyze the samples, using 2 g of roasted and ground coffee, then perform the extraction with 40 mL of ultrapure water, with 400 µg of acrylamide (internal standard), stirring for 60 min in a mechanical shaker. After centrifugation, samples were prepared by solid phase extraction (SPE) and quantified by LC-MS. The results obtained are shown in Figure 1.

Figure 1. Acrylamide concentration (µg/kg) in roasts (R1: 230 °C for 12 min / R2: 240 °C for 40 min / R3: 250 °C for 17 min) of Arabica and Robusta coffee used at StarCoffees.



Classroom activities

1. How does the data obtained by Flávio and André help the group to answer the question posed in Part I? Can the hypotheses built in Part I be corroborated or refuted with the new data?

2. Establish relationships between the data presented so far and then elaborate statements that can be provided to the characters in the case to clarify the quality of the coffee from StarCoffee. The greater the number of statements, with due justification, the more satisfactory the group's response will be.

Homework activities

1. Helping Flávio and André mitigate the concentration of acrylamide in the coffee is also one of the group's missions. Research at least two ways that can be used to mitigate acrylamide formation and argue for one of them as being the most appropriate. In order to build the group's argument in favor of the chosen method, it is suggested that the following aspects be considered: procedure complexity, cost, amount of sample needed to carry out the analyses, analysis time, organoleptic changes, reproducibility on an industrial and home scale, among others that the group deems interesting.

2. What are the main sectors or groups of society directly affected by acrylamide formation in food? Look for information that corroborates your statements, such as data related to the population's eating habits and the pertinence of using the Maillard reaction in gastronomy.

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Part III – The One-in-a-Million Idea

With the acrylamide concentration data in the coffee samples in hand, Flávio and André are faced with reducing the said concentration in the beans. After studying, they decided to use an enzymatic treatment with asparaginase on coffee beans before roasting, as asparaginase can hydrolyze asparagine, which is one of the precursors of the Maillard reaction.

The treatment was carried out for both species of coffee beans, according to the following experimental procedure: for pore opening, 150 g of green coffee beans were pre-treated with steaming in an autoclave for 45 minutes at 100 °C. Then, the beans were mixed with 103.5 mL of water heated to 60 °C containing doses of the enzyme asparaginase with the following enzymatic loads: 2000, 4000 and 6000 ASNU[1]/kg of dry coffee. Samples were incubated for 60 minutes at 60°C with shaking. After about 45-60 minutes, most of the water has been absorbed by the grains. Then, the grains were transferred to the desiccator for the final sample weight of 150 g. Finally, acrylamide was quantified using the same previously described experimental procedure (SPE/LC-MS).

[1] ASNU = Asparaginase Unit

Classroom activities

1. From the debate that took place between groups that studied the same case study: a) What relationships (similarities, differences, curiosities, among others) are possible to establish between Flávio and André 's proposal and the one chosen by your group for mitigating acrylamide in coffee? b) What criteria did they use that were not contemplated by your group and vice versa? c) Given what was discussed in the debate, do you think it is appropriate to change the proposed procedure? Argue in favor of the answers.

Homework activities

1. Considering the question posed by your group in Part I of the case study, what trends can be predicted for acrylamide analysis after the treatment? Argue in favor of the answers.

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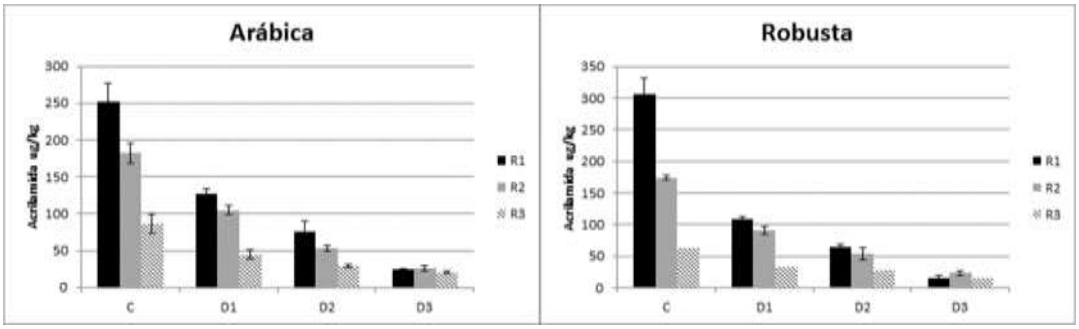
Part IV – May We Not Lack Coffee!

Based on the experimental procedure indicated in Part III of the case study, Flávio and André obtained the results shown in Figure 1. After studying, they decided to use an enzymatic treatment with asparaginase on the coffee beans before roasting.

Figure 1. Effect of enzymatic treatment with asparaginase on the acrylamide content (µg/kg) of roasted Arabica and Robusta coffee: Control without enzyme (C), load of 2000 ASNU/kg (D1), load of 4000 ASNU/kg (D2) and 6000 ASNU/kg (D3). From Gottschalk et al. (Congresso Brasileiro de Pesquisas Cafeeiras 2018).



For a better evaluation of the data presented in Figure 1, Flávio and André searched for international parameters of maximum permitted concentrations of acrylamide in food since Brazilian legislation does not have such reference values.

Table 1 shows the reference values they found determined by the European Union to evaluate the acrylamide concentration.

Table 1. Reference levels for the presence of acrylamide in some coffee-related foodstuffs.

Foodstuff	Reference Level (µg/kg)
Roasted coffee	400
Instant coffee (soluble)	850
Coffee substitutes exclusively from cereals	500

Source: European Union (2017).

In addition to the information obtained so far, Flávio and André carried out some more research on the formation of acrylamide in coffee and discovered the following: During roasting, the Maillard reaction is responsible for producing most of the volatile compounds in coffee, around 1000 compounds, mostly furans, pyrazines and thiols (sulfur-containing compounds). More specifically, the low roast accentuates the perception of sweet, chocolate and almond flavors, while the medium roast accentuates the differences in geographical origin. On the other hand, the high roast displays burnt, acid, oxidized and pungent characteristics.[2] Regarding the reaction precursors, asparagine levels become relevant when working with unripe fruits, as its concentration is higher in immature coffee fruits. The peeling of unripe fruits makes it possible to reduce the levels of asparagine and,

consequently, the formation of acrylamide.

[1] EUROPEAN UNION. Jornal Oficial da União Europeia, v. 304, p. 24-44, 2017.

Classroom activities

1. Establish relationships between the data presented in Figure 1 and then elaborate statements that can be provided to Flávio and André to help them in their purpose, mentioned in Part II of the case.

2. Compare the acrylamide concentration values obtained by Flávio and André, illustrated in Figure 1, with the maximum concentrations allowed in the legislation, mentioned in Table 1, and present conclusions about it.

3. What can you say about the trends you explained in the exercise in Part III for analyzing acrylamide after treatment? Justify the answer.

Homework activities

1. Construct and complete the summary table below, which summarizes the actions and results obtained in all stages of the study.

Problem situation: This item presents the existing problem in the case. That is, it is necessary to briefly describe the narrative and the problem that afflicts the main characters.
Problem: This item presents the problem/issue to be resolved. As every problem must start with a question, the statement must be formulated in such a way as to end with a question mark.
Hypotheses: This item presents the hypotheses formulated to solve the problem. Hypotheses are assumptions issued as preliminary answers to the problem at hand. The same problem can have many hypotheses, which are possible solutions for its resolution. In this perspective, indicate your basic hypothesis, which is the explanation you have chosen as being the main one for the proposed problem. Then, indicate the secondary hypotheses, which are complementary statements and/or other possible answers to the problem. These can cover in detail what the basic hypothesis states in general and aspects not specified in the basic hypothesis.
Mitigation planning: This item describes the steps and resources you used to propose a mitigation route to evaluate your hypotheses. Add pictures to make your answers more straightforward. Finally, conclude the answer by pointing out whether you changed the initial planning when confronted with the planning presented by the case characters or colleagues in Part III (Debate).
Data: This item presents empirical data or data from other sources (including data provided during classes) with which you worked that allowed you to evaluate your hypotheses and the range of responses to the problem. Add pictures to make the answer more straightforward.
Conclusions: This item presents the final conclusion of the group regarding the problem explained in the case and all the other conclusions reached during the resolution of the case.
Justifications: This item presents the justifications that support the explicit final conclusion and those that support the partial conclusions mentioned above. Try to gather the largest number of justifications and present them so that they are supported by available data (in this case, add images to make the answer more straightforward) and knowledge reported in the literature.