

Assessment of Acrylamide Levels in Selected Chocolate Samples Commercialized in Al-Bayda, Libya, via HPLC-UV Technique

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تقييم مستويات الأكريلاميد في عينات مختارة من الشوكولاتة المُتداولة في مدينة البيضاء، ليبيا، باستخدام تقنية HPLC-UV

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Abstract:

An investigation into acrylamide levels in chocolates available in the Libyan marketplaces was conducted using HPLC UV analysis. This study examined six chocolate products, including milk chocolates, plain chocolates, and those with additional ingredients, to determine their acrylamide content. Results revealed that Lindt Excellence contained the highest acrylamide levels (average 4.48 $\mu\text{g kg}^{-1}$), followed by Loacker at 2.82 $\mu\text{g kg}^{-1}$ and Ward chocolate at 2.46 $\mu\text{g kg}^{-1}$. Notably, all chocolate samples analyzed had acrylamide concentrations significantly lower than the European Commission's indicative value of 1000 $\mu\text{g kg}^{-1}$.

Keywords: Acrylamide, Cancer, Chocolate, HPLC, Libya.

المخلص:

تم إجراء دراسة للتحري عن مستويات الأكريلاميد في أنواع الشوكولاتة المتوفرة في الأسواق الليبية باستخدام تقنية الكروماتوغرافيا السائلة عالية الأداء المزودة بكاشف الأشعة فوق البنفسجية (HPLC-UV). شملت هذه الدراسة ستة منتجات من الشوكولاتة، بما في ذلك الشوكولاتة بالحليب، والشوكولاتة الداكنة، وتلك التي تحتوي على مكونات إضافية، وذلك بهدف تحديد محتواها من الأكريلاميد. أظهرت النتائج أن شوكولاتة Lindt Excellence سجلت أعلى مستويات من الأكريلاميد بمتوسط قدره 4.48 ميكروغرام/كغ، تلتها شوكولاتة Loacker بمتوسط 2.82 ميكروغرام/كغ، ثم شوكولاتة Ward بمتوسط 2.46 ميكروغرام/كغ. ومن الجدير بالذكر أن جميع عينات الشوكولاتة التي تم تحليلها أظهرت تراكيز من الأكريلاميد أقل بكثير من القيمة الاسترشادية التي حددتها المفوضية الأوروبية والبالغة 1000 ميكروغرام/كغ.

الكلمات المفتاحية: الأكريلاميد، السرطان، الشوكولاتة، الكروماتوغرافيا السائلة عالية الأداء (HPLC)، ليبيا.

Introduction:

Acrylamide is a process-induced compound that can form in certain foods, including chocolate products, particularly those with high carbohydrate content, when they are subjected to thermal processing methods such as frying, baking, or roasting at temperatures above 100°C (Krishnakumar

& Visvanathan, 2014). The primary method of producing the compound is through the Maillard reaction, which involves the asparagine, amino acid and carbonyl compounds, for instance glucose and fructose. This compound has two forms: polyacrylamide, a safe polymer, and acrylamide, a toxic monomer that has various industrial applications and is found in cosmetics (Adebo et al., 2017). According to studies, exposure to may contribute to several harmful health effects, notably neurotoxicity, carcinogenicity, and reproductive toxicity (Zamani et al., 2017). Given these potential health risks, it is crucial to monitor acrylamide levels in widely consumed food products, such as chocolate.

Chocolate is widely consumed worldwide in substantial quantities. However, concerns regarding its potential health risks have arisen due to the presence of acrylamide, a contaminant that may be present in chocolate (Vinci et al., 2012).

Studies have indicated that acrylamide is present in chocolates and is produced during the roasting stage. Nevertheless, there is insufficient information about acrylamide levels in chocolate products sold in local markets of Al Bayda City, Libya. Consequently, this investigation aims to determine the acrylamide level in chocolate samples obtained from local supermarkets in Al Bayda city, Libya, utilizing HPLC UV analysis.

Materials And Methods:

Sampling:

Chemicals, reagents, and solutions:

All of the chemicals, reagents, solvents, and analytical standards employed in this investigation were of high purity and analytical grade. 99.0% pure acrylamide was acquired from No-Vachim in Tunisia. Other substances and solvents included hexane (95% purity; Sigma-Aldrich, Germany) and acetonitrile (99.9% purity, HPLC grade; Carlo Erba Reagents S.A.S., France). Anhydrous magnesium sulphate was acquired from FLUKA, Germany, while formic acid (98–100% purity) was provided by Riedel-Dehañ AG, Seelze, Hannover. VARIAN (USA) provided the primary secondary amine, while PANREAC QUMICA (Spain) supplied the 96% acetic acid. A local company provided the distilled water (Shiromi et al.).

A stock solution of acrylamide:

The acrylamide stock solution ($100 \mu\text{g mL}^{-1}$) was prepared by dissolving 0.01 g of acrylamide in 100 mL of distilled water. Working standard solutions at concentrations of 2.5, 5.0, 10, 15, and 20 $\mu\text{g mL}^{-1}$ were then prepared by serial dilution of the stock solution using distilled water.

2.4. Sample Extraction
The extraction of acrylamide from chocolate samples was carried out through a multistep procedure. First, a 50 mL centrifuge tube was filled with 7.5 g of the homogenised sample. Then 5 mL of hexane, 0.75 mL of acetic acid, and 7.5 mL of acetonitrile were added. The mixture was vortexed for 1 min to ensure adequate dispersion of the sample in the solvent system. The tube was then centrifuged at 3700 rpm for 5 min using a Sorvall RT6000B centrifuge, after which the hexane layer was discarded. The remaining supernatant was moved into a centrifuge tube, and 375 mg of anhydrous MgSO_4 together with 125 mg of primary secondary amine (PSA) were added. The tube was immediately capped, shaken vigorously for 30 s, and centrifuged again at 3700 rpm for 5 min. The collected supernatant was subsequently evaporated to dryness using a rotary vacuum evaporator. Lastly, before HPLC analysis, the dry residue was reconstituted in two ml of acetonitrile (Anastassiades et al., 2003). Recovery was evaluated by fortifying 7.5 g of thoroughly homogenized chocolate sample with 5 mg of acrylamide standard, followed by extraction and drying. Subsequently To achieve a concentration of $100 \mu\text{g mL}^{-1}$, 10 mL of acetonitrile was added. Using the following formula, percentage recoveries were determined:

Percentage of recovery = $[\text{CE}/\text{CM} \times 100]$:

where CM is the spiking concentration after extraction and CE is the useful concentration obtained from a standard acetonitrile solution containing $100 \mu\text{g mL}^{-1}$ of each ingredient. (Chowdhury et al., 2012).

Chromatographic determination was conducted using an HPLC setup fitted with a Thermo Series P2000 pumping unit, an automated Series 200 injector, and a Series 200 UV/Vis detector with an operating range of 190 to 1000 nm. Sample introduction was achieved through a 20 μL loop. The separation step was carried out on a Brownlee Bio C18 column ($250 \times 4.6 \text{ mm}$; $5 \mu\text{m}$). For elution, a binary mobile phase was used, consisting of aqueous 0.1% formic acid and acetonitrile. The system was operated under a flow rate of 1.0 mL min^{-1} , and analyte monitoring was performed at a wavelength of 200 nm.

Results and Discussion:

Acrylamide determination in samples:

Table 1 displays the samples' highest and lowest mean acrylamide concentrations was related to the Lindt Excellence chocolate samples (4.48 $\mu\text{g/g}$) and the Dairy milk chocolate samples (1.71 $\mu\text{g/g}$), respectively.

Quantification of Acrylamide in selected Chocolate samples:

Six chocolate products—Lindt Excellence, Locker, Dairy milk, today milk, Ward chocolate, and Ritter sports dark—were analysed using the validated acrylamide method (Table 1). For qualitative confirmation, sample chromatograms were evaluated against the chromatogram of an acrylamide standard solution (20 $\mu\text{g mL}^{-1}$), using retention time as the identification criterion. The adopted extraction protocol, which did not include solid-phase extraction, enabled clear recognition of the acrylamide peak and prevented interference from co-eluting compounds. This simplified treatment also resulted in a cleaner UV chromatogram with fewer non-target peaks at 200 nm.

Table (1): HPLC-UV analysis of the acrylamide concentration of chocolate chosen from the Libyan market:

No	Product Name of chocolate	Analytical sample repetition	Acrylamide contents ($\mu\text{g/g}$)
			Mean \pm SD
1.	Lindt Excellence	3	4.48 \pm 0.29
2.	Locker	3	2.82 \pm 0.03
3.	Dairy milk	3	1.71 \pm 0.16
4.	Today milk	3	2.59 \pm 1.06
5.	Ward chocolate	3	2.64 \pm 1.02
6.	Ritter sport dark	3	2.09 \pm 0.03

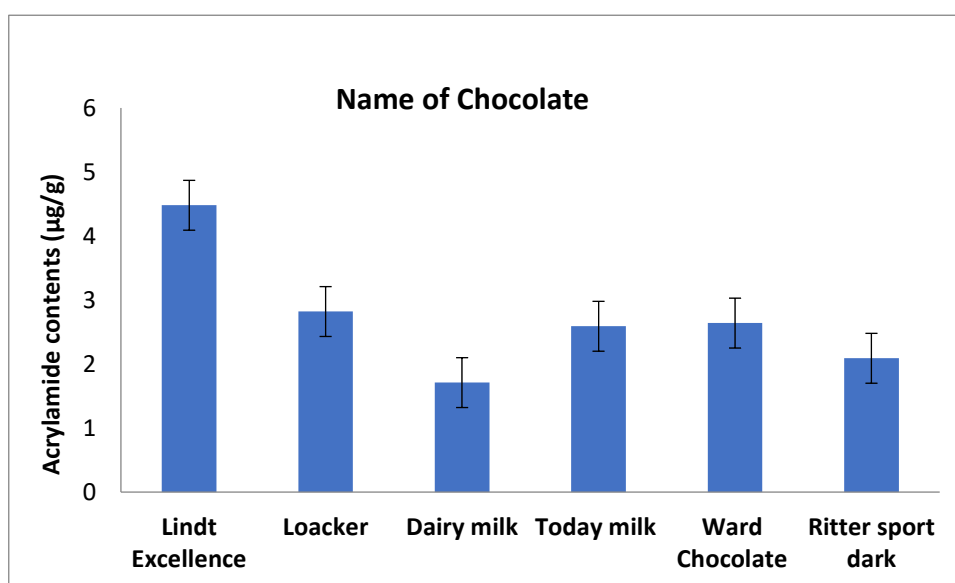


Figure (1): Acrylamide concentration ($\mu\text{g/g}$) of certain chocolates sold on the Libyan market.

The analysis of acrylamide levels in various chocolate samples revealed significant variations. The maximum mean concentration of acrylamide was found in Lindt Excellence chocolate samples, with a value of 4.48 $\mu\text{g/g}$. In contrast, the Dairy Milk chocolate samples exhibited the lowest mean acrylamide concentration of 1.71 $\mu\text{g/g}$. These findings suggested that the manufacturing process and composition of chocolate samples may have a substantial impact on the final acrylamide levels (Arvanitoyannis and Dionisopoulou, 2014).

The higher acrylamide content in Lindt Excellence chocolate samples could be attributed to the roasting and processing methods employed in their production. Acrylamide is primarily formed during the Maillard reaction, which occurs when decreasing sugars and amino acids are subjected to high temperatures. It is possible that the Lindt Excellence chocolate line employs more rigorous roasting or

processing methods, resulting in greater acrylamide production compared to Dairy Milk chocolate samples (Mottram et al., 2002).

Furthermore, the ingredient composition of chocolate samples may also play a role in the observed acrylamide levels. Factors, such as the type and proportion of sugars, amino acids, and other additives, can influence acrylamide formation during the manufacturing process.(Govindaraju et al., 2024). Dairy Milk chocolate samples may have a more balanced ingredient profile that mitigates acrylamide production, resulting in lower concentrations.

These findings highlight the importance of monitoring and controlling acrylamide levels in chocolate products to ensure consumer safety and compliance with the regulatory guidelines. Manufacturers should optimize their production methods and ingredient formulations to minimize acrylamide formation, while also considering the potential trade-offs between product quality, taste, and acrylamide content.

Conclusion:

Acrylamide was successfully quantified in selected chocolate products marketed in Al-Bayda using HPLC-UV, with concentrations ranging from 1.71 to 4.48 µg/g. Although variability among samples was observed, all values were markedly below established international benchmark levels, indicating no immediate safety concern. Nonetheless, the differences detected highlight the influence of processing conditions and reinforce the importance of continued monitoring and mitigation strategies to minimize acrylamide formation in chocolate products.

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