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Acrylamide Assessment in meat products sold in Al-Qalubyia, Egypt, and the impacts of different cooking methods on their levels

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Abstract

The current study aimed to estimate acrylamide (AA) levels in protein-rich foods collected from Al-Qalubia restaurants, Egypt, and to determine the effects of processing methods on acrylamide formation. A total of sixty RTE meat were estimated using high performance liquid chromatography equipped with a UV-detector. In general, there were no significant variations in acrylamide levels between the meat samples at the meat-categories level ($P > 0.05$). However, there were significant variations across the twelve products examined ($P < 0.05$). The highest mean acrylamide concentration was found in chicken meat (1.74 µg/100gm), followed by fish (1.31 µg/100gm), and red meat (0.98 µg/100gm). Furthermore, substantial differences in acrylamide concentrations were found across five beef products, and four chicken products, while no differences were found among fish products. In beef products, the maximum acrylamide concentrations were in rice-kofta and escalope-panee, with 3.103 and 1.89 µg/100gm, respectively. Within chicken-products, the highest acrylamide concentrations were in strips, nuggets, and drumsticks, at 5.19, 4.29, and 4.15 µg/100gm, respectively. When compared to shrimp kofta, 0.39 µg/100gm, the highest acrylamide levels were in fish burger (2.42 µg/100gm) and fillet (1.13 µg/100gm). According to cooking method, the 40 samples prepared by deep frying had significantly higher mean acrylamide levels (1.70) than the 20 grilled samples, 0.54 µg/100gm ($P < 0.05$). Fortunately, current estimated acrylamide contents in estimated products were less than the daily toxic and carcinogenic levels previously determined by concerned authorities; however, more stringent regulations and control interventions are still needed to reduce exposure and determine permissible limits.

Keywords: *Acrylamide; RTE meat products; Al-Qalubia, Egypt; deep frying; grilling.*

1. Introduction

In today's world, frying is one of the most preferred ways to prepare food. Fried meals are popular, particularly among the younger generation, because of their appealing flavour, colour, and crisp texture **[1]**. During food processing, such as frying **[2]**, changes in heat, light, oxygen, solvent pH sensitivity, or the combined effect of these parameters may damage food nutrients such as minerals, carbohydrates, lipids, proteins, vitamins, enzymes, and other nutrients.

Another potential health problem arising from food frying is the creation of toxic compounds such as Acrylamide. Acrylamide can also be transformed to glycidamide, which is a mutagen and reproductive toxin **[3, 4].**

Based on the substance's carcinogenicity in rats, the International Agency for Research on Cancer (IARC) classified acrylamide as a "potential carcinogen for humans" in the "2A group," and the WHO Consultation in 2002 approved this classification. Acrylamide has recently been found to be neurotoxic **[5]**, carcinogenic in rats, and a probable carcinogen in humans **[6]**. It causes gene mutation and DNA damage. Though researchers are unsure of the exact mechanisms by which acrylamide forms in foods, the formation of acrylamide has been linked to higher temperatures, above 120°C and low humidity, reached during the heating, frying, baking, roasting, or grilling of carbohydrate-rich foods to achieve desired colour, flavour, and aroma of Maillard, where nonenzymatic browning reactions which including asparagine and glucose occur [**7].**

Regardless of the fact that high-carbohydrate diets have been correlated with high AA levels, high-temperature cooking of lipid-protein-rich meals like meat can also trigger AA formation, reaching levels of up to 300 µg/kg **[8]**. Excessive consumption of processed meat products has indeed been linked to an increased risk of cancer in humans (e.g., pancreatic, colorectal, prostate, ureter, and breast cancer) due to the high cooking temperatures used in their production, which can result in high levels of carcinogenic compounds like acrylamide **[9]**.

Food composition and processing conditions are the two most critical elements that determine AA production. The kind of amino acids (mostly asparagine), the presence of carbohydrates, the pH of the food, and the moisture level of the food are all factors in food composition **[10]**.

The Food and Agriculture Organization of the United Nations (FAO/WHO) stated that daily AA intake from food can range between 0.3 and 0.8 µg/kg [**11]**. A daily consumption of 1–10 mg/kg AA from food that exceeds this value can be harmful to humans. The tolerated daily intake (TDI) of acrylamide for neurotoxicity is 40 mg/kg per day and 2.6 mg/kg per day for carcinogenicity **[12]**. In general, the more acrylamide is generated, the deeper the colour of the food product **[13]**.

Previous Egyptian studies focused on the estimation of acrylamide in thermally treated popular foods that are carbohydrate-rich, such as potatoes, kataief, Shearea, Noodle for soup (lesan elasfour), Fried rice, Baked Sweet potato, Fried onion, Fried tammia, White sauce (bashamiel), Outer layer of fried chicken, outer layer of fried fish **[14, 15].** However, acrylamide estimations in protein-rich foods are lacking. Furthermore, the present situation of Acrylamide bench levels in Egyptian Standards is unclear. As a result, the current study's goal is to estimate acrylamide levels in three categories meat products, including red meat, chicken, and fish, as prepared and served in local restaurants in Al-Qalubia, Egypt, under various cooking methods.

2. Material and Methods

2.1. Collection of samples

Ready to eat beef, chicken, and fish products, especially those requiring high cooking temperatures, were gathered for assessment. A total of 60 samples (5 of each product, 12 produvts,100 g) were purchased from local restaurants in Al-Qalubia governorate, Egypt.

Beef products such as fried rice kofta, escalope panne, sausage, grilled kofta, and burger are among the samples. Fried panee, fried nuggets, grilled drumsticks, and fried wings are among the chicken products. Finally, there are fried fish fillets, fried shrimp kofta, and pan-fried fish burgers among the fish items. The collected samples were kept frozen at -18^{O}C before sent to the laboratory to be evaluated for acrylamide content.

2.2. Assessment of Acrylamide by HPLC.

2.2.1. Preparation of samples

The meat samples only preparation technique includes defatting with dichloromethane and ethanol, extraction of acrylamide with acetone, and cleaning with solid phase extraction cartridges following modified approach of H. Wang, Feng, Guo, Shuang, and Choi **[16].** Finely ground and homogenised meat sample (10.0 g) was defatted twice in a butterfly shaker, first with 50 mL dichloromethane and 5 mL ethanol, then with 50 mL dichloromethane alone for 2 hours at room temperature. The mixture was centrifuged at 3500 rpm for 20 minutes at 15^{O}C. The supernatant was discarded, and the precipitate was dried at 40°C in a vacuum oven.

2.2.2. Extraction of Acrylamide

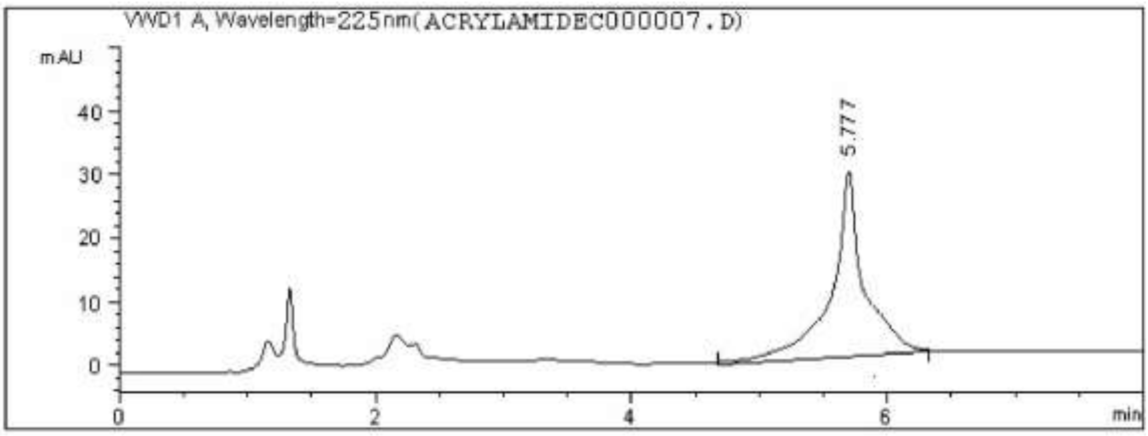
A 20 mL acetone was added to the defatted meat sample for Acrylamide extraction. For 30 minutes, the samples were forcefully agitated with a butterfly shaker. Samples were put in an ultrasonic water bath at 40°C for about 20 minutes, then centrifuged for 25 minutes at 15°C at 3000 rpm. A filter paper was used to filter the solvents (Whatman No.1). The filtrate was evaporated to dryness using a flash evaporator. 2 mL HPLC grade water: acetonitrile (40:60) was added to the mobile phase and carefully agitated to dissolve the residue. Using Solid Phase Extraction tubes, the aqueous solution was filtered before being fed into the column through a 20 µl injection loop [**17**].

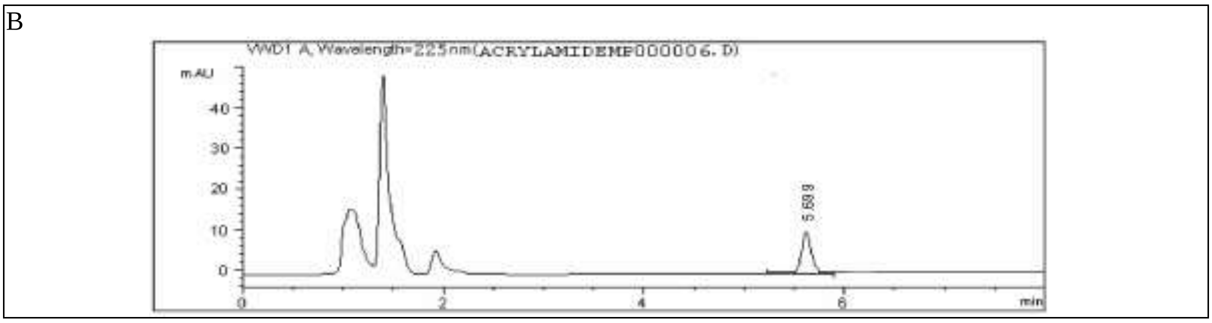
2.2.3. Condition of chromatography

The current work used HPLC (Agilent HP 1200 Series Apparatus, USA) with a UV-Visible variable wavelength detector (2486) set at 225 nm to estimate the acrylamide level (supplementary figure 1). All separations were performed on a Spherisorb ODS-2 column

(250 x 4.6 mm 5 m) with a 30^{O}C thermostat. Throughout the studies, an ultrasonic water bath was used to extract acrylamide from the sample matrix, remove air bubbles from the mobile phase, and separate acrylamide using the mobile phase. HPLC grade water and acetonitrile were filtered using a vacuum pump, and acetonitrile and formic acid were evaluated for resolution. The flow rate of the solvent was 0.8 mL/min, and the detection wavelength was 210 nm **[18 and 16**].

A





Supplementary figure 1 shows the acrylamide peaks of the standard (A) and one of the estimated samples (B) at 225 nm wavelength.

2.2.4. statistical Analyses

A one-way variance analysis (ANOVA) was used to analyse the data using SPSS (version 20; IBM, Chicago, IL, USA). On the figures, the acquired data and associated standard errors of the mean are shown as means and maximums. The individual acrylamide levels served as the experimental unit within each product, within and between the groups based on products origin or species, products (commercial name, n=12), and cooking techniques for examining variations. One fixed effect variable was included in the statistical model. Multiple comparisons were made using Tukey's tests to evaluate the differences between groups, products, and cooking techniques [19]. A $P < 0.05$ was used to define significant differences, and trends were reported.

3. Results

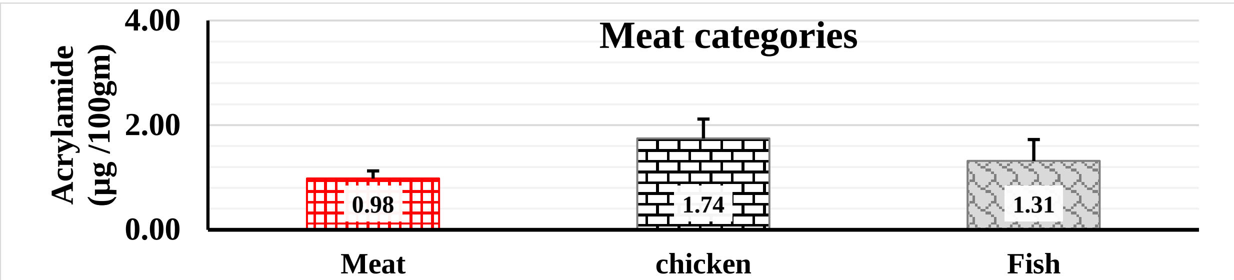


Fig (1). The amounts of acrylamide in meat products are depicted based on the meat categories

The figure depicts the levels of acrylamide in meat categories based on their species of origin. Poultry-derived meat products had the highest mean acrylamide levels compared to fish and beef products, but the difference was not statistically significant ($P > 0.05$).

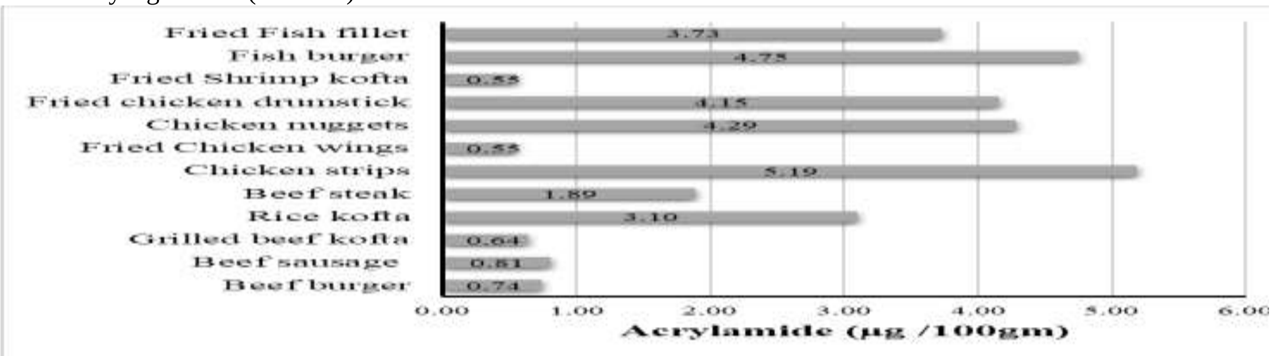


Fig (2). The maximum acrylamide levels found in meat products studied.

The maximum acrylamide levels recorded in the meat products analyzed are revealed that, there was a statistically significant difference between the twelve items evaluated ($P < 0.05$). Chicken strips and fish burgers had the greatest levels of acrylamide, followed by chicken nuggets, drumsticks, and finally fish fillet and beef rice kofta.

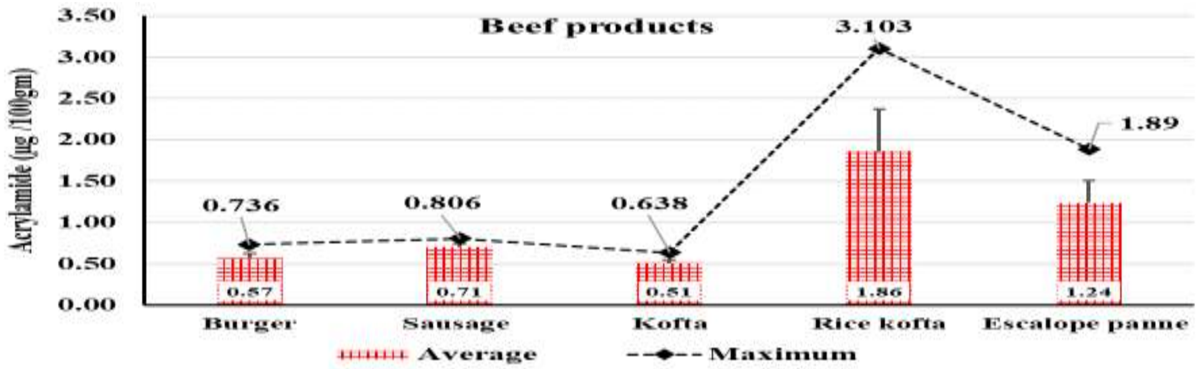


Fig (3). depicts a comparison of the mean and maximum acrylamide levels in beef-derived products.

Figure 4 compares the mean and maximum acrylamide levels in beef-derived products. Among the beef-derived RTE products evaluated, rice kofta had the highest mean and maximum concentrations. Mean acrylamide levels in beef burger and rice kofta, beef sausage and rice kofta, and meat escalope pane were all found to be different ($P < 0.05$)

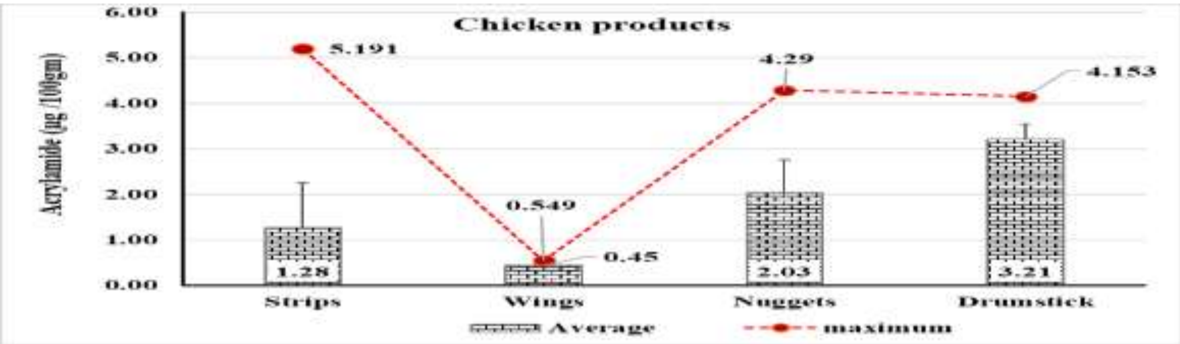


Fig (4). The mean and maximum acrylamide levels in the ready-to-eat (RTE) poultry-derived meat products investigated.

The mean and maximum acrylamide levels between the studied ready to eat (RTE) products within the poultry-derived meat declared that there was a substantial difference between the studied items, especially between the high amount detected in chicken drumsticks and the lowest level found in wings ($P < 0.05$).

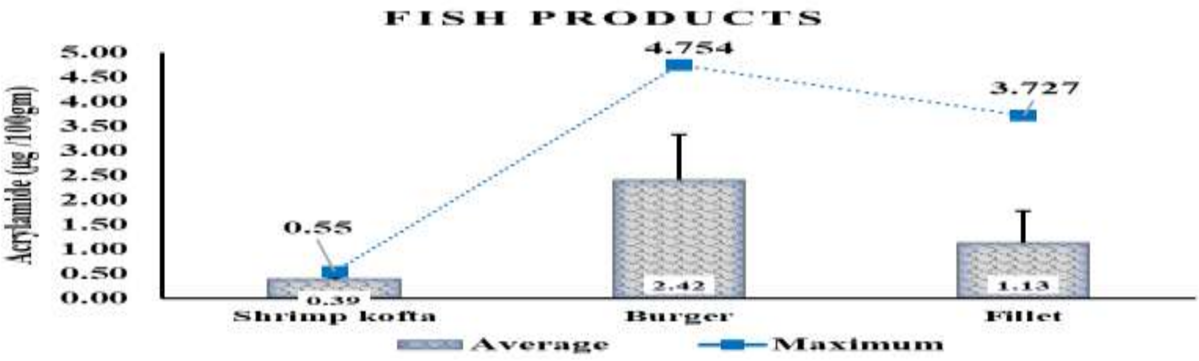


Fig (5). depicts the mean and maximum acrylamide levels in fish-derived products.

The mean and maximum acrylamide levels in fish-derived products are shown in Figure 5. There was no significant difference between the mean acrylamide concentrations of the three fish-derived RTE dishes ($P > 0.05$), although there was a difference between the maximum values ($P < 0.05$). Burgers contained the highest mean and maximum concentrations, followed by fillet dishes, when compared to shrimp kofta.

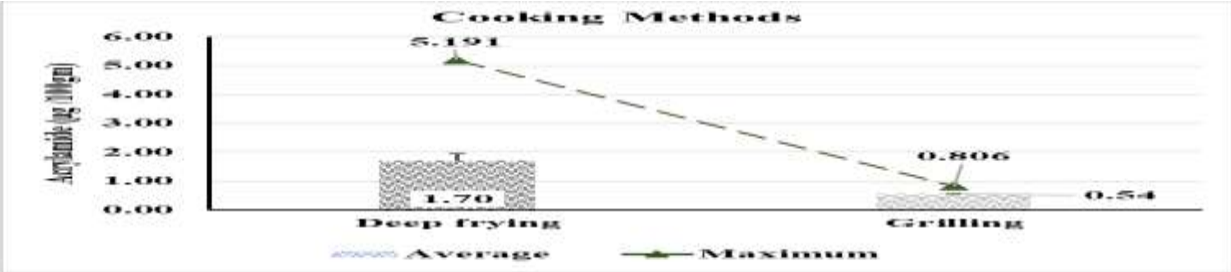


Fig (6). illustrates the impact of cooking method on the mean and maximum acrylamide levels in RTE dishes.

The consequences of cooking type on the mean and maximum acrylamide levels in RTE dishes is shown in Figure 6. The gathered products were divided into two categories based on their cooking methods: forty samples were deep fried and the remaining twenty were grilled. Grilled meat products had much lower mean and maximum acrylamide levels than deep-fried meat products ($P < 0.05$).

4. Discussion

In Egypt, 134,632 new cancer cases and 89,042 cancer-related deaths were reported in 2018 **[20].** Cancer is the second leading cause of death (14%) after cardiovascular disease (46 %) **[21]**. According to the National Cancer Registry Program (NCRP), age-standardized cancer incidence rates per 100,000 in Egypt were 166.6 (both sexes) at the national and regional levels, and by 2050, a 3-fold rise in incident cancer was predicted compared to 2013 **[22]**. Egypt still needs additional data on dietary factors that contribute to the appearance of more cancer cases, such as rates of dietary acrylamide from RTE meat-derived foods, for cancer prevention. The goal of this study was to determine the amounts of acrylamide (AA) in sixty meat products from three animal species: beef, chicken, and fish, which were purchased from local markets in Al-Qalubia, Egypt.

Previous reports for acrylamide concentrations in Egypt or globally did not comprehensively evaluate all recently tested meat products. Furthermore, every three years, a repeated screening investigation is recommended to illustrate contents and update benchmark levels as well as mitigation methods **[23].**

The current findings revealed that chicken and fish products expose customers to higher quantities of acrylamide than beef dishes, but their concentrations remained below the indicated neurotoxic and carcinogenic levels, (100-1000 µg /100gm) **[11, 12]**. Acrylamide levels in processed meat and seafood products assessed in the Hong Kong region were similarly quite low, less than 3 µg /kg **[24]**. The amount of AA in high protein foods meals was less than 10.0 µg/100 gm in studies conducted around the world, but increase to more than 10.0–400.0 µg/100 gm in high carbohydrate foods **[25].** Acrylic acid formation, which results from the degradation of lipids, carbohydrates, or free amino acids; dehydration/decarboxylation of organic acids (malic acid, lactic acid, and citric acid); and direct formation from amino acids are three possible mechanisms precursors for AA formation in food during high-temperature cooking, in addition to Pathways based on Maillard browning **[26].**

In Egypt, meat-rice kofta is a well-known, delicious, and popular local meal **[27]**. It was observed that among the beef products analysed, beef rice kofta had the highest mean and maximum acrylamide amounts. This could be attributed to the use of rice flour in such recipes, where previous studies found acrylamide concentrations ranging from 18 to 24.5 µg / 100 gm. In Europe, the acrylamide benchmark level for maize, oat, spelt, barley, and rice-based products was set at no more than 150 µg /kg **[23]**. It should be mentioned, however, that rice is advised to replace other grains with high levels of asparagine, such as rye, oats, wheat, and maize, because it has the lowest levels **[23]**. Despite the fact that it is a breaded recipe with primarily wheat flour, escalope panne ranks second to rice kofta in terms of acrylamide concentration. Earlier research, however, found that the quantity of acrylamide created was less than half of what was observed when egg and bread crumbs were present in the product and heat-processed combined, but the inclusion of wheat flour increased the acrylamide level in final products by roughly 8.5 µg /100 gm **[28]**. The amount of acrylamide consumed in breaded food ranged from 1.7 µg in fine breadcrumbs with egg to 13.3 µg in coarse breadcrumbs with egg and flour. Compared to earlier estimates **[29])** of AA levels in beef burger (1-1.6 µg /100gm), the current study range was lower. Previously, the presence of acrylamide was linked to the presence of soya and grains in meat products such as burgers because they contain more glucose and asparagine amino acids, which are more prone to generating acrylamide **[30].** Sausage formulations created to contain fat and rusk **[31],** which under high temperature could be plausible acrylamide routes, but the small quantities found here may necessitate further research into the food product's ingredients and processing conditions. One probable explanation is that the outer sausage shell reduces dehydration of the packed substance and has a temperature impact.

In chicken products, strips had the greatest acrylamide maximum values, whereas nuggets and drumsticks had the highest mean levels. In average, AA levels in crumbed or battered poultry or game ranged from 1-6.4 µg / 100g **[32]**. During 2003, 2004, and 2006, the FDA conducted studies on acrylamide levels in meat, poultry, and fish products as part of a comprehensive research program. Similar to the trend of current study, fast-food fried Chicken breast and Chicken nuggets had the greatest acrylamide levels (1.1-2.6 µg /100 gm) in 2003, followed by fish (Fish sticks 1.2 µg /100 gm) and red meat (not detected) **[33]**. Acrylamide levels in the same Chicken products grew to 3 µg /100 gm in 2004, followed by fish (Fish sticks 1.3 µg /100 gm) and the rank remained unchanged. In comparison to 2002–2006, acrylamide levels in selected food categories did not decrease considerably in 2011–2015 [**34**]. In the current investigation, the mean (1.74 µg / 100gm) and maximum (5.91 µg / 100 gm) acrylamide levels found in chicken products were still within the later ranges. In an Egyptian research, acrylamide levels in the outer layer of fried chicken strips reached 3.8 µg/100 gm **[14].** In other investigations, acrylamide levels in chicken strips and drumsticks, and commercial nuggets were found to be as high as 7.2-8.2, and 13.5-15.9 µg/100gm, respectively **[8; 35, 36]**. In comparison to other products in the same category, chicken wings produced the least amount of acrylamide. Lee, Han, Jung, Lee, and Chung [**37**] found the lowest concentration, 0.491–0.619 µg/100 gm, however [**38**] found a greater concentration of 16.4–22.5 µg/100 gm. The amount and content of the batter, the cooking procedure employed, and the diets of the birds may all play a role in acrylamide levels in different chicken products [**39**]. To illustrate, acrylamide levels reached 91.0–97.0 µg/100gm after pre-cooked breaded filleted chicken breast and leg were coated in a batter of beaten eggs, and breadcrumbs. These results were significantly greater than those found during the flour breading. The interactions between carbohydrates in breadcrumbs and free amino acids in the egg and chicken tissue were blamed for this **[39].**

The amounts of acrylamide in fish and seafood (breaded, fried, baked) from various countries were estimated to range from 2.5-23.3 µg/100 gm **[17].** According to **CODEX [32**], acrylamide AA levels in crumbed and battered fish and seafood products ranged from 0.2 to 3.9 g/100 gm, which is similar to the current study findings. In Egypt, [**14**] also found that the outer layer of fried fish had 2.0 µg/100 gm of acrylamide in their investigation on acrylamide levels in Egyptian food.

The current study's second goal was to determine the effects of different processing methods on the acrylamide levels of evaluated meat products. The forty deep-fried samples had considerably greater mean and maximum acrylamide values than the twenty grilled samples. The chemical Millard reaction, high temperatures applied, and low access to water in food materials, in addition to the composition of the deep-fried product, such as reducing sugars, cereal-based breading material that includes different types of flour, starch, and breadcrumbs, are the main explanations for significant levels of AA formed in deep fried products estimated during this study. The coating of fried chicken drumsticks manufactured in a high-pressure fryer, for example, had a high acrylamide content of 28.5 µg /100 gm [**8**]. Also, fish slices fried following an Indonesian style contained 9.3 µg /100 gm of acrylamide in the Hong Kong region [**24**]. Moreover, the discrepancies in acrylamide levels amongst products in the same category were earlier attributed mainly to variances in heat treatments used during preparation **[40].**

In general, the findings show that the measured acrylamide levels are safe and comparable to previous research in the United States [**24, 33**]. The European Food Safety Authority (EFSA) established benchmark standards for the presence of acrylamide in foodstuffs, with the lowest levels being 4 and 5 g/100 gm, respectively, for baby foods, processed cereal-based foods for infants and young children, and soft bread. [**23**]. Only three poultry and one fish sample (6.66%) surpassed benchmark level when compared to these thresholds. Dietary acrylamide exposure is influenced by the amount of acrylamide in food, the portion size consumed, the frequency of consumption, as well as cooking, storage, and cultural differences in dietary practices [**26**]. In Egypt, however, rigorous screening and mitigation studies are still required to establish a benchmark level in tested products and to lower acrylamide levels.

5. Conclusions

The current study provides the Authority's database with the most recent acrylamide incidence data in twelve ready-to-eat meat products available in Al-Qalubia, Egypt, which will help to identify the benchmark levels, as well as the implementation of all essential mitigating measures

6. Acknowledgement

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