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Investigating the Formation and Mitigation of Heterocyclic Amines and Dicarbonyl Compounds in Food Products

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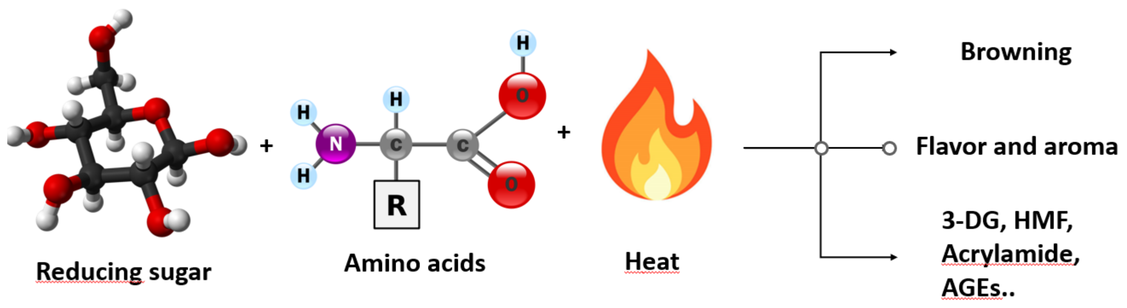
1.State-of-the-Art

In the last century, several studies have shown that chemical substances (polycyclic aromatic hydrocarbons - IPA, heterocyclic amines - HCAs, dicarbonyl compounds α-DC, AGEs...) are generated during cooking process which could represent a risk to human health. (Barzegar *et al*., 2018, Cincotta *et al.*, 2021). High temperatures and extended cooking times significantly contribute to the formation of these substances. The International Agency for Research on Cancer (IARC) has classified some heterocyclic amines, such as IQ, as probable carcinogens (group 2A), while others like PhIP, MeIQ, AαC, and MeAαC are possibly carcinogenic (group 2B) (IARC, 1993). The Council of Europe has set a daily limit of 1 µg for HCAs (Sinha et al., 1995). Their formation process is influenced by the characteristics of the food item such as pH, aw, and moisture, but also by the amount of free amino acids, sugars, lipids, creatine and creatinine. Antioxidant compounds can inhibit their formation. Due to their low concentration in foods (ng/g), efficient extraction methods are crucial (Nadeem et al., 2021). Recently, the QuEChERS method, commonly used for pesticides, has been effectively applied for HCAs extraction (Hsiao et al., 2017). High-performance liquid chromatography (HPLC) is the primary technique for HCAs quantification, often paired with detectors like DAD, MS, fluorescence, and UV-vis (Barzegar et al., 2018).
Several studies have shown that high-temperature cooking methods such as grilling, frying and barbecuing tend to produce higher levels of HCAs than low-temperature cooking methods such as boiling or steaming. So, by increasing the time and cooking degree, the formation of HCAs also increases (Polak *et al.*, 2020). A higher amount was found in red meat than in white meat, presumably due to the higher content of free amino acids, precursors of heterocyclic amines. However, a high amount was found in the skin of chicken, which is usually consumed (Solyakov & Skog 2002). Various natural or added substances can exert a variable inhibitory action on the formation of HCAs. Natural substances, such as spices, herbs, and plant extracts, can significantly reduce HCAs production due to their antioxidant content, including polyphenols, flavonoids, and anthocyanins (Nadeem et al., 2021).
α-Dicarbonyl compounds (α-DCs) are intermediates formed in heat-treated foods through reactions like the Maillard reaction. While they contribute to the aroma, flavor, and color of foods, α-DCs can also negatively affect human health by reacting with proteins and DNA. Their formation is influenced by factors such as sugars, amino acids, heat intensity, and processes like maturation, fermentation, and storage (Fallico et al., 2022).

The detection of α-DCs involves three analytical steps: deproteinization to remove insoluble proteins, derivatization using diamine derivatives, and HPLC analysis with UV detection (Cincotta et al., 2021). This PhD thesis aims to analyze these harmful compounds in various food matrices and develop effective strategies to reduce their formation, with a focus on sustainability and food safety.

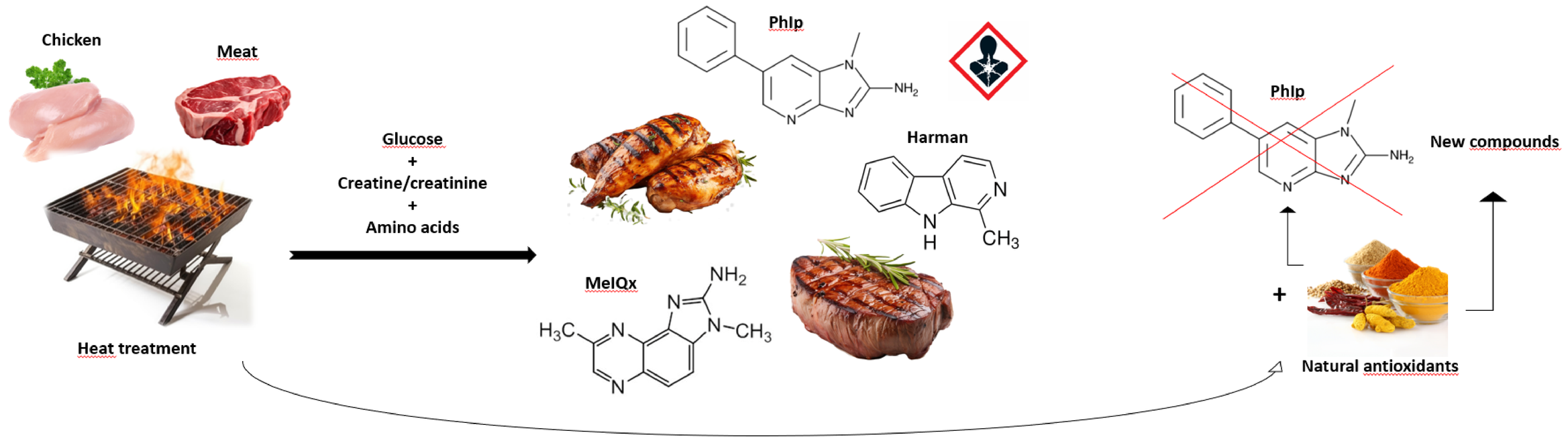


2. PhD Thesis Objectives and Milestones

The project, which will take place during the 3 years of the doctoral school, will follow the steps illustrated below:

1) **Bibliographic research:** Deepen knowledge on HCAs and α-DCs regarding toxicity, analysis methods, quantities in foods, comparisons between food matrices and reduction strategies. Identify knowledge gaps and define specific research objectives.
2) **Methodological development**: Development of an efficient and specific extraction method for HCAs and α-DCs. Validation of the instrumental method for their quantification.
3) **Sample analysis**: Preparation of white and red meat samples with different cooking methods, and the preparation of bakery products, like cookies. Determination of HCAs and α-DCs levels in the prepared samples. Comparison of the levels found in the matrices with the same cooking conditions and evaluation of the evolution of these substances over time.
4) **Development of reduction strategies**: Evaluate the effect of different variables on the formation of HCAs and α-DCs: test various cooking methods (grilling, frying, baking, boiling, microwave, sous vide etc.), act by changing the temperature and cooking times; vary the food formulation (addition of antioxidants, marinades, spices and flavourings, etc.)
5) **Dissemination of results:**

- ❑ Drafting of the doctoral thesis.
- ❑ Publication of scientific articles and reviews.
- ❑ Presentation of results at conferences and seminars
- ❑ Creation of informative posters for scientific dissemination



3. Selected References

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