Effect of Precursors and Thermal Processing on Acrylamide Formation in Synthetic Potato Models. Validation with potato cultivars

Nivine Samir Bachir

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Spain Advisor

Dr. Montserrat Pujola

Spain Co-advisor

Dr. Francesc Sepulcre

Lebanon advisor

Dr. Amira Haddarah

Author

Nivine Samir Bachir

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Summary-English

Acrylamide is a toxic compound and is classified as a probable human carcinogen. In recent decades, international organizations have established levels of safety for ingestion due to its harmful effects endangering human health. It is found in a variety of commonly consumed foods, such as bread, baby snacks/food, and French fries/potato chips. The present thesis focused on formulating simple synthetic potato-based models with different combinations of amino acids and sugars to study in depth the interaction of acrylamide's precursors, the effect of temperature (170 and 190 °C) and the method of cooking (deep frying *vs* air frying) on acrylamide formation after thermal treatment. Acrylamide, asparagine, and sugars were quantified using GC-FID, K-ASNAM kit, and HPLC; respectively. Results showed that reducing sugars (fructose and glucose) and asparagine were the major contributors to acrylamide formation after thermal processing. In addition, the synthetic prototype model of composition: (39-41%) water, (60%) potato starch, (0.07%) glucose, (0.04%) fructose, (0.11%) sucrose, (0.25%) glutamine, (0.6%) asparagine, (4.8%) sodium alginate, and agar-agar (1.2%) had the healthiest combination of sugars and amino acids after heat treatment since it does not contain acrylamide. We also highlighted the mitigation role played by glutamine in the synthetic models, which was also confirmed in our prototype model. All glutamine-based models as well as our prototype did not contain acrylamide, hence, glutamine was not considered a major contributor in Maillard reaction compared to other precursors. Moreover, sodium alginate showed to decrease the formation of acrylamide through the interference of sodium cation found in sodium alginate in the Maillard reaction steps. On the other hand, temperature did not have any statistical significance on acrylamide formation; contrarily to cooking technique where air frying significantly decreased the production of acrylamide compared to deep frying. Additionally, we applied the results obtained from simple models on potato cultivars (Agria, Kennebec, and Monalisa) in order to better understand these interactions but in real food matrix. Results revealed that all potato cultivars produced acrylamide after frying in different concentrations with the least amount detected in Monalisa. Following that, for the purpose of facilitating the prediction of acrylamide for the food industry sector, we opted at adopting NIR spectroscopy after validating the results with GC-FID, and allocating acrylamide peaks in potato products. Hence, peaks at around 4439-4477 cm^{-1} were found to be present in common amongst all the samples, hence corresponding to acrylamide peak (standard acrylamide peak: 4439 cm^{-1}). Moreover, the relationships between acrylamide and acrylamide precursors in the raw form (before thermal treatment) were illustrated for the three potato cultivars for example in **Monalisa potato** cultivar the linear regression equation was $\left(Acrylamide\ concentration\ \left(\frac{\mu g}{kg}\right) = Glutamine\ (80.28) + Asparagine\ (58.57) + \mathrm{Glucose}\ (38.25) + \mathrm{Sucrose}\ (107.36) + \mathrm{fructose}\ (204.83) + (-284.98)\right)$.

Finally, the proposed potato-starch based prototype might be considered as a healthy promising potato snack, along with the glutamine-based models; yet future work is needed to concentrate on their organoleptic characteristics.

Resumen- Español

La acrilamida es un compuesto tóxico y está clasificado como probable carcinógeno humano. En las últimas décadas, organismos internacionales han establecido niveles de seguridad para la ingestión. Se encuentra en una variedad de alimentos de consumo común, como pan, refrigerios/alimentos para bebés y patatas fritas. La presente tesis se ha centrado en formular modelos simples a base de patata para estudiar en profundidad la interacción de los precursores de la acrilamida (azucares y aminoácidos), el efecto de la temperatura (170 y 190 °C) y el método de cocción (fritura tradicional *vs* fritura al aire). Acrilamida, asparagina, glutamina y azúcares (glucosa, fructosa y sacarosa) se cuantificaron mediante GC-FID; kit K-ASNAM y HPLC; respectivamente. Los resultados obtenidos en los diferentes modelos utilizados han mostrado que los azúcares reductores (fructosa y glucosa) y la asparagina han sido los principales contribuyentes a la formación de acrilamida después del procesamiento térmico; y que en la combinación usada de azucares y aminoácidos en el modelo sintético prototipo de composición: (39-41%) agua, (60%) almidón de patata, (0,07%) glucosa, (0,04%) fructosa, (0,11%) sacarosa, (0,25%) glutamina, (La asparagina al 0,6 %), el alginato de sodio (4,8 %) y el agar-agar (1,2 %) no ha permitido detectar acrilamida. Destacando el papel de mitigación que desempeña la glutamina en nuestros modelos, ya que en ninguno de las formulacione s se ha podido detectar acrilamida; por lo tanto, atendiendo a estos resultados no se la ha considerado un contribuyente importante en la reacción de Maillard en comparación con los otros precursores. Además, el alginato de sodio ha demostrado reducir la formación de acrilamida a través de la interferencia del catión sodio en algunos de los pasos de la reacción de Maillard. Las temperaturas utilizadas (170-190 ºC) no han tenido significancia estadística sobre la formación de acrilamida; al contrario de la técnica de cocción, donde la fritura al aire disminuyó significativamente ($p<0.05$) la producción de acrilamida en comparación con la fritura en aceite. Al aplicar los resultados obtenidos de modelos sintéticos simples a cultivares de patata (Agria, Kennebec y Monalisa), los resultados han indicado que todos los cultivares utilizados han producido acrilamida en diferentes concentraciones, siendo la variedad Monalisa la que ha producido menos cantidad de acrilamida. Posteriormente, con el fin de facilitar la predicción de acrilamida, optamos por aplicar la espectroscopia NIR después de validar los resultados con GC-FID encontrando que los picos de alrededor de 4439-4477 cm^{-1} presentes en todas las muestras, se correspondían con el pico de acrilamida patrón (4439 cm^{-1}). Además, se han encontrado ¡relaciones entre la acrilamida y sus precursores antes del tratamiento térmicopara los tres cultivares de patata. , La ecuación de regresión lineal para la variedad Monaliza fue $\left(\text{Concentración de acrilamida} \left(\frac{\mu g}{kg}\right) = Glutamina\ (80.28) + Asparagina\ (58.57) + \text{Glucosa}\ (38.25) + \text{Saccarosa}\ (107.36) + \text{fructosa}\ (204.83) + (-284.98)\right)$. Finalmente, el prototipo propuesto basado en fécula de patata podría considerarse como un tentempié de patata prometedor y saludable, junto con los modelos basados en glutamina; sin embargo, se necesita trabajo futuro para concentrarse en sus características organolépticas.

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Chapter 1: Introduction

1. Background

Acrylamide "CH_2=CHCONH$_2$" (Figure 1) is so called 2-propenamide by the international system "IUPAC". Another synonyms which are ethylene carboxyamide, acrylic acid amide, vinyl amide, propionic acid amide. Acrylamide has a molecular weight of 79.08 g.mol^{-1} and a melting point 84.5 ˚C. Acrylamide has a high boiling point of 125 ˚C at 25 mmHg and 192.6 ˚C at atmospheric pressure.

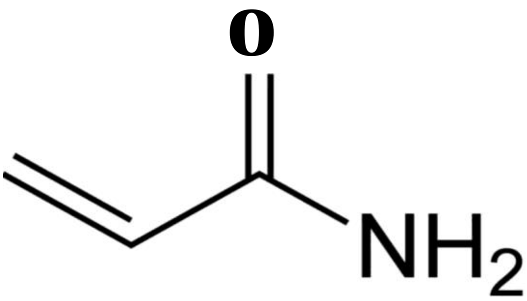

Figure 1: Schematic representation of acrylamide's structure (Hans Lingnert et al., 2002).

a. Occurrence and acrylamide Benchmark Dose Lower Confidence Limit set by International organizations

The Joint FAO/WHO Committee announced in its 64th report on food additives the national and regional estimates of acrylamide exposure. The mean dietary exposure to acrylamide for the general population, including children is 1 μg.kg^{-1} BW per day. According to a risk assessment study done by the European Food Safety Authority "EFSA" on acrylamide, results showed that acrylamide and its metabolite glycidamide are genotoxic and carcinogenic. As it is proven by the literature that any exposure to a genotoxic substance could potentially damage DNA and lead to cancer. Therefore, EFSA's scientists concluded that they cannot set a tolerable daily intake (TDI) of acrylamide in food but instead, they estimated the dose range within which acrylamide is likely to cause a small but measurable tumor incidence other potential adverse effects (neurological, pre- and post-natal development and male reproduction). EFSA's experts came up with the lower limit of this range and named it the Benchmark Dose Lower Confidence Limit "BMDL10" (Joint Expert Committee on Food Additives, 2006). The CONTAM Panel assessed 43 419 analytical results from food commodities in Parma, Italy. Acrylamide was found at the highest levels in solid coffee substitutes and coffee, and in potato fried products. Based on surveys across age groups, they found that the Mean and 95th percentile dietary AA exposures were estimated at 0.4 to 1.9 μg.kg^{-1} body weight per day and 0.6 to 3.4 μg.kg^{-1} body weight per day, respectively. They also concluded that the main contributor to total dietary exposure was Potato fried products except for potato crisps and snacks. The data from human studies were inadequate for dose-response relationship assessment. The CONTAM Panel selected BMDL10 values of 0.43 $mg.kg^{-1}$ BW per day for peripheral neuropathy in rats and of 0.17 $mg.kg^{-1}$ BW per day for neoplastic effects in mice. Results of the Panel revealed that the current levels of dietary exposure to AA are not of concern with respect to non-neoplastic effects. Yet, even though the epidemiological associations have not clearly showed that AA is a human carcinogen, the margins of exposure (MOEs) indicate an alarming concern for neoplastic effects based on animal evidence (Scientific Opinion on Acrylamide in Food, 2015).

The content of acrylamide varies between different food products and within the same food product. It also varies within the same manufacturing facility at different times and between manufacturers (Lineback et al., 2012). General indication on acrylamide contents in some food are presented in table 1.

Table 1: General indication on acrylamide content in some food (Rifai & Saleh, 2020)

Food	**Acrylamide content (** $\mu g.\ Kg^{-1}$)
Potato crisps	325 $\mu g.\ Kg^{-1}$
Breakfast cereals	<62–803 $\mu g.\ Kg^{-1}$
Carbohydrate-rich food	< 20–2,528 $\mu g.\ Kg^{-1}$
Pizza, minced meat, and fried bacon	0–1,480 $\mu g.\ Kg^{-1}$
Espresso coffee	11.4–36.2 $\mu g.L^{-1}$
Chocolate	102 $\mu g.\ Kg^{-1}$
Green tea	30-56 $\mu g.\ Kg^{-1}$
Baby foods	2-516 $\mu g.\ Kg^{-1}$

b.Toxicity of acrylamide

Acrylamide compound has been classified to be “probably carcinogenic to humans” (Group 2 A) and studies showed that it has a wide spectrum of toxic effects including neurotoxin effects (Mesias et al., 2019). Recently an alarming concern has emerged due to this classification and right after the announcement of the Swedish National Food Administration and the University of Stockholm regarding the presence of considerable amounts of acrylamide in processed foods especially those who are rich carbohydrates and treated at temperature above 120 ˚C in 2002 (Svensson et al., 2003). The World Health Organization 2006 presented in its 64th report on food additives the acute and lethal dose of this toxic compound. The single oral dose produces acute toxic effects only at a dose of > 100 $mg.kg^{-1}$ of body weight, and the median lethal doses (LD50s) are generally >150 $mg.kg^{-1}$ of body weight. Many studies were conducted to demonstrate the toxic effects of acrylamide on health. Table 2 shows several toxic effects from experiments performed on mice and rats when exposed to different doses of acrylamide.

Table 2: Toxicological effects to different doses of acrylamide in rats (Pundir et al., 2019)

Toxicity	Experiments/Observations	Effects	References
Reproductive	Exposing mice oocytes to acrylamide.	Acrylamide was converted into glycidamide that caused severe toxicity to the oocytes.	**Duru Zeynep, Sinan, Alp, & Ozgur, 2017**
Carcinogenicity	Exposing male and female $B6C3F_1$ mice to drinking water that contains acrylamide for two years	Gland and lung tumors in male and female mice. Increased incidence of fore-stomach tumors in male mice. Increase in mammary gland, ovary, and skin tumors in females.	**Beland *et al*., 2013**
Neurotoxicity	Feeding weaning rats with variable acrylamide concentrations for four consecutive weeks.	Rats from the moderate- and high-dose acrylamide groups had an abnormal gait. Biochemical tests in these rats showed significant reduction in glutamate concentration and significant increase in γ-aminobutyric acid content depending on acrylamide dose.	**Tian *et al*., 2015**
Neurotoxicity	Persons working in food industries get exposed continuously to acrylamide.	Damage of both peripheral and central nervous system.	**Huang et al., 2011; Pennisi et al., 2013**
Cardiac developmental toxicity (CDT)	Intake of acrylamide either in the form of dietary foods or from the environment.	Introduction of acrylamide after fertilization shrinked the heart and its abnormal morphological development. During atrioventricular valve development, acrylamide suppress the expression of genes like my17, vmhc, myh6, bmp4, tbx2b and notch1b leading to the cardiac developmental toxicity (CDT).	**Huang, Jiao, Wang, Xia, and Zhang, 2018**

2. Dietary acrylamide: absorption, carcinogencity and an update on health chronic risks

Every person is exposed to acrylamide on a daily basis due to its presence in many everyday foods. When ingested acrylamide is well absorbed from the stomach and gastrointestinal tract and it is distributed with the blood to all tissues and organs (Mesias et al., 2019). Thus acrylamide has potential to cause adverse side effects throughout the whole body. Acrylamide is a soft nucleophile that can easily bind with other soft nucleophiles such as cysteine residues of proteins. In our body, proteins often contain cysteine residues and have diversity of functions such as antibodies, receptors, hormones, and enzymes. Cysteine residues consist of intra- and inter-molecular disulfide bonds, important for protein stability and bonding, or of free thiol groups (S-H). Free thiols are part of the catalytic activity sites of enzymes. So, acrylamide by binding to these cysteine residues can potentially affect many proteins and interfere with their functions which lead to the disturbance of various physiological processes in our body (Mesias et al., 2019). Moreover, acrylamide by itself has low reactivity towards hard nucleophiles such as DNA. On the contrary to its epoxide metabolites, glycidamide has the ability to alkylate DNA, which can lead to DNA point mutations. Hence, initiating tumors by changing the DNA sequence potentially activating oncogenes and inactivating tumor suppressing genes (Mesias et al., 2019). In addition, glyciamide is a clastogen which can cause breakage in DNA that in turn can lead to cancer development.

Many other studies have shown that acrylamide is a carcinogenic compound and have neurological and reproductive effect in rodent toxicology studies (Friedman & Levin, 2008). As a result acrylamide has been classified as a Group 2A, probably carcinogenic to humans, as reported by the International Agency for Research on Cancer (Muttucumaru et al., 2014). The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) Joint expert committee on Food Additives (JECFA) recently concluded that the presence of acylamide in the human diet is of concern. To date, however, the results of the epidemiological studies have been controversial to firmly state the carcinogenicity of acrymalide. For example, a recent meta-analysis of epidemiological data led the authors to conclude that there was no relationship between dietary acrylamide intake and cancer (Lipworth et al., 2012), while a later Danish study revealed a link between acrylamide exposure and breast-cancer development (Olsen et al., 2012). In French fry varieties, which contain higher concentrations of sugars, acrylamide formation also correlated with free asparagine concentration, demonstrating the complex relationship between precursor concentration and acrylamide-forming potential in potato. Storage of the potatoes for 6 months at 9 °C had a significant, variety-dependent impact on sugar and amino acid concentrations and acrylamide. The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) Joint expert committee on Food Additives (JECFA) recently concluded that the presence of acylamide in the human diet is of concern. To date, however, the results of the epidemiological studies have been controversial to firmly state the carcinogenicity of acrymalide. For example, a meta-analysis of epidemiological data led the authors to conclude that there was no relationship between dietary acrylamide intake and cancer (Lipworth et al., 2012), while a later Danish study revealed a link between acrylamide exposure and breast-cancer specific mortality

(Olsen et al., 2012). In animal studies, when acrylamide is administrated orally it is directly absorbed by the gastrointestinal tract and widely distributed to tissues, as well as fetus. It has also shown that acrylamide was found in human milk. In the body, Cytochrome P450 E21 (CYP2E1) metabolizes acrylamide to a chemically reactive epoxide glycidamide. The reactive glycidamide is much more reactive with DNA than acrylamide. Acrylamide was classified as mutagenic and clastogenic in mammalian cells in vivo and in vitro.

The mutational spectra produced by glycidamide and acylamide in transgenic mouse cells are consistent with the formation of promutagenic purine DNA adducts in vivo (Joint Expert Committee on Food Additives, 2006).

Table 3: Different approaches to mitigate acrylamide formation in potatoes

Technology and products	Main findings	Reference
Use of Thyme, Cumin and Anise in Bakery Products.	Thyme, cumin and anise decreased and mitigated the formation of acrylamide in bakery products.	Tesby et al., 2018
Ultrasound pretreatment in fried potatoes.	Ultrasound was responsible for 90 % acrylamide content reduction when compared to potatoes fried directly.	Antunes-Rohling et al., 2018
Pulsed electric fields in potato crips.	Pulsed electric field technique could reduce acrylamide formation in deep fried potato crisps.	Genovese et al., 2019
L-asparaginase from *Aspergillus terreus* BV-C strain.	L-asparaginase from A. terreus showed to alleviate acrylamide formation the food.	Paul & Tiwary, 2020
Asparaginase and high pressure technology in fried potatoes	The combibation of the addition of asparaginase enzyme and High Pressure Processing showed to reduce acrylamide content in fried potatoes while with asparaginase or HPP alone there was no significant reduction.	Dourado et al., 2020

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Chapter 2: Bibliography

Formation, Mitigation and Detection of acrylamide in foods

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Abstract

Acrylamide is classified as a toxic food contaminant and it is formed in carbohydrate rich food when heated > 120˚C through the Maillard reaction. The main parameters that affect acrylamide formation in foods are the composition of the raw food, and time-temperature of food processing. International organizations have warned against the existence of acrylamide in foods and mandated to limit its consumption to prevent its adverse health effects. This review, summarizes some of the innovative and the conventional mitigation techniques. The innovative mitigation techniques are based on the use of lactic acid bacteria, yeasts, and cell extracts used alone or in combination with electrical pulses, while the conventional techniques are blanching, microwave and the addition of food ingredients in which their effectiveness is related to the control of the temperature and the cooking time. Although LC-MS and GC-MS with or without derivatization are cumbersome procedures, they give accurate results and reduce the interferences of compounds. On the other hand, the current trend for acrylamide detection in food matrices is using simple, fast and inexpensive techniques such as the filtration-assisted approach for optical detection and the PHYTON imaging program. Yet, their application needs an exhaustive validation.

Keywords: precursors, Maillard reaction, temperature, time, mitigation, optical detection

1. Introduction

Over the years, researchers shed light on acrylamide safety in humans due to its potential neurotoxicity and probable carcinogenicity (Hariri et al., 2015). Consequently, it is essential to identify the amount of acrylamide in food and to reduce its concentrations in commonly consumed food (M. Huang et al., 2018). Many studies have shown that, at high temperature exceeding 120˚C, low water activity and depending on the type of cooking, acrylamide is produced via the Maillard reaction in food like baked products, breakfast cereals, coffee, and potatoes (Capuano & Fogliano, 2016; Stadler et al., 2002). Additionally, many studies have shown that acrylamide presence in processed food like potatoes is influenced by various factors such as the types of cultivar, storage practices, and frying time and temperature (De Wilde et al., 2005; Pedreschi et al., 2006; Romani et al., 2008). According to De Wilde et al. (2005), potatoes stored at 4℃ enhanced acrylamide formation due to an increase in the concentration of reducing sugars. Other important factor in acrylamide formation is frying temperatures. Pedreschi et al. (2006), observed that as frying temperatures of potatoes decreased from 190℃ to150℃, acrylamide formation was also reduced. Moreover, Romani et al. (2008), showed that as frying time and temperature increase leading to an exponential increase in acrylamide formation. Lee et al., (2020) observed that air frying technique decreased acrylamide and polycyclic aromatic hydrocarbons in meat chicken as compared to deep frying method.

On the other hand, the European Commission Regulation 2017-2158 instituted mitigation standards and benchmark levels for decreasing the presence of acrylamide in food (750 $\mu g.kg^{-1}$ acrylamide) (**Table 1**). According to a Spanish study the effectiveness of the mitigation measures since the release of the regulation on potato crisps showed that in year 2019 acrylamide level was lower by 55.3% when compared to year 2004, and 10.3% lower compared to 2008 and level remained from 2014. However, a study conducted on different types of Romanian suppliers showed that acrylamide level was lower than the benchmark set by these Regulation in soft bread crackers and biscuits (Altissimi et al., 2017). These authors observed that the highest acrylamide levels in food streets are found in French fries and pizza, although the levels were within the regulated limits. Other studies confirm these results of acrylamide content: potato products>fried foods>bakery products>breakfast cereals > meat> egg products (Branciari et al., 2020; Khaneghah et al., 2020). Therefore, it is shown that the highest acrylamide contents are related to potato-based foods. This suggestion seems to be confirmed with the study based on a meta-regression analysis, results showed that potato-based products presented the highest average concentrations of acrylamide potato-based products (740.33 $\mu g.kg^{-1}$)

and this finding is correlated to their high content of acrylamide precursors mainly free asparagine (Mousavi Khaneghah et al., 2020).

Table 1 : Summary of acrylamide data during the time period 2011-2015 (Abt et al., 2019)

Food category	*Acrylamide level (ug.K^{-1})*
Infant foods	165.1
Breads and bakery Products	14.7
Breakfast Cereals	207.3
Coffee (brewed)	5
Coffee (as powder, not brewed)	272.4
Cookies and granola bars	181.8
Crackers	200.3
French fries/other potato-based foods (consumer prepared)	206
French fries/other potato-based foods(restaurant prepared)	418.4
Potato chips	498.2
Pretzels	138.5
Tortilla chips	220.2

2. Acrylamide formation

2.1 Formation of acrylamide through Maillard reaction

Maillard reaction is a browning mechanism that occurs in heat treated food. Acrylamide in food is formed through the asparagine route of the Maillard reaction which is known to be the major route (Figure 1) (Blank et al., 2005; Mottram et al., 2002). The end products of the Maillard reaction give some distinctive characteristics for the baked, fried, and toasted foods like the brown color, crust, and tasty flavor (Gökmen et al., 2006). Disaccharides such as fructose when compared to glucose, increase acrylamide formation by 2 times (Eriksson, 2005; Granvogl et al., 2004; Stadler et al., 2003; Yaylayan et al., 2003). According to Eriksson (2005), the mechanism of action is pointed to the α-hydroxy group that facilitates the conversion of asparagine by decreasing the activation power in the Maillard reaction.

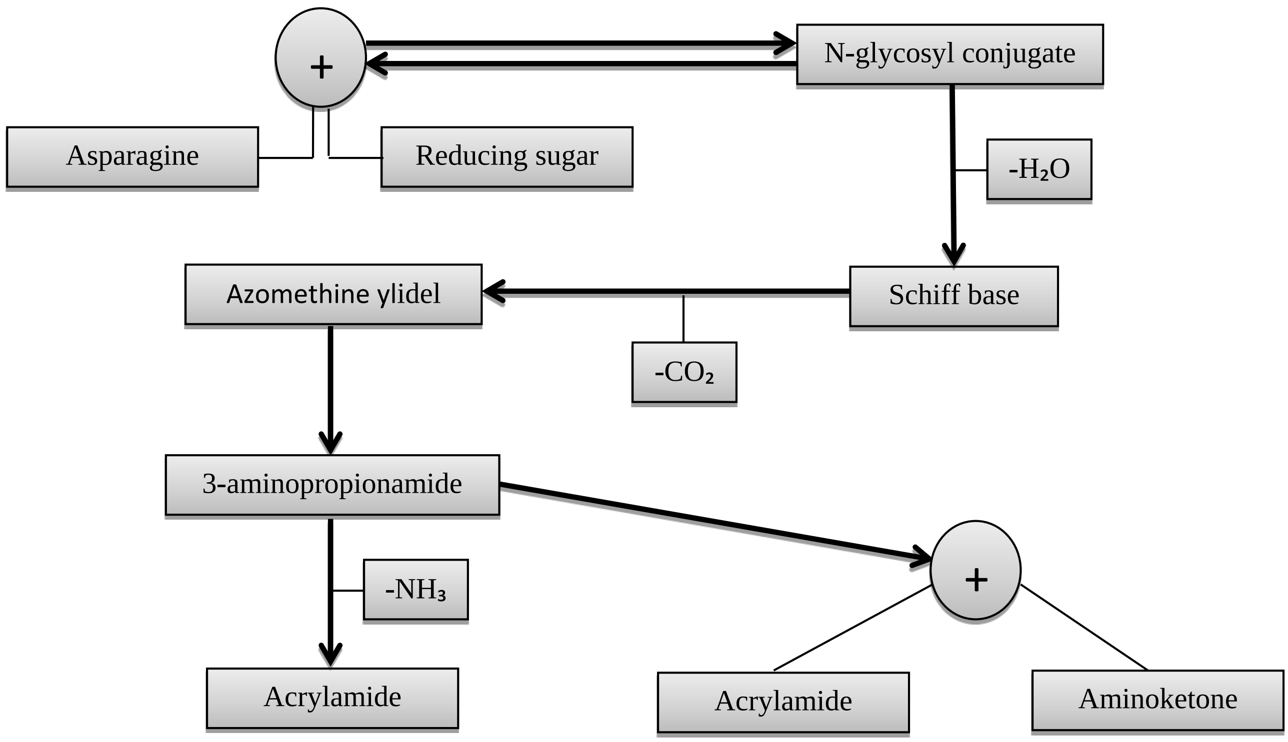

Figure 1: Acrylamide formation through Maillard reaction

2.2. Acrolein, carnosine, Beta-alanine, lactic acid, Cysteine/Serine and acrylic acid

According to many studies, acrylamide could be formed from acrolein in fatty foods **(Figure 2)**. When asparagine is free, acrolein provides its carbonyl group that favors its conversion into acrylamide in considerable amounts (Yasuhara et al., 2003). Further studies demonstrated that acrolein that is obtained from the breakdown of amino acids, lipid oxidation, and decomposition of carbohydrates promotes the formation of acrylamide throughout the Maillard reaction (Hans Lingnert et al., 2002; Mendel Friedman, 2003).

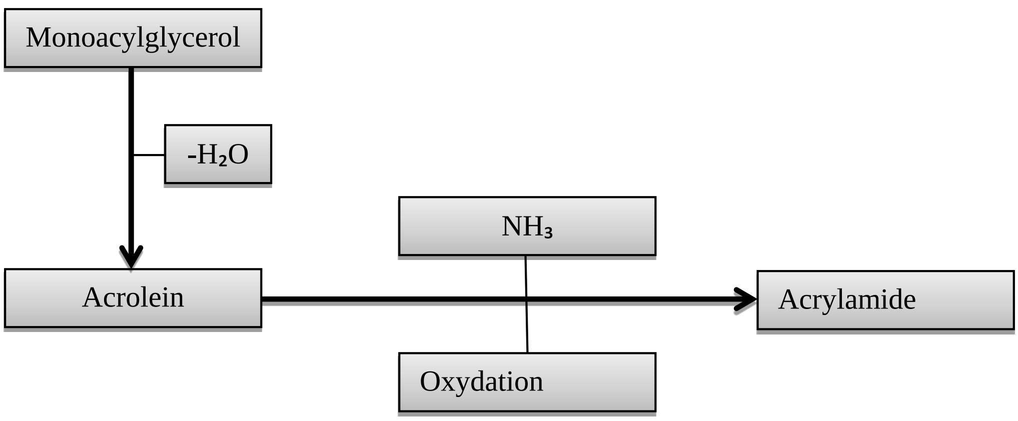

Figure 2: Acrylamide formation from Acrolein

On the other hand, during the thermal degradation of some products like aspartic acid, carnosine, and β –alanine, acrylamide can be formed through acrylic acid. In Further reaction, acrylic acid combines with ammoniac acid to form acrylamide (**Figure 3)** (Stadler et al., 2003; Yaylayan et al., 2005)

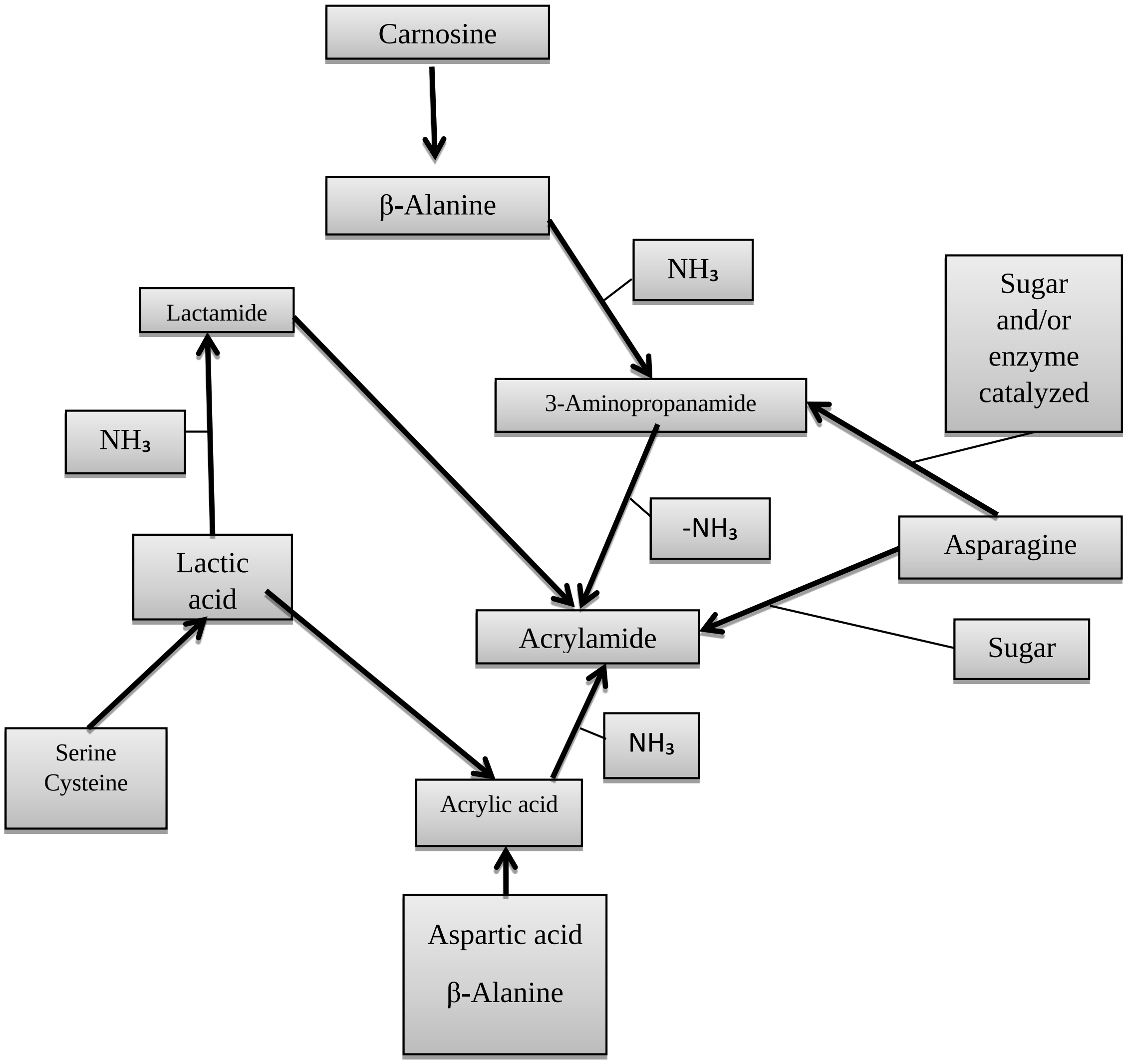

Figure 3: Acrylamide formation from Carnosine, Beta-alanine, lactic acid, Cysteine/Serine and acrylic acid

2.3.Aminopropionamide

3-Aminopropionamide was proposed to be a temporary transitional compound in acrylamide production, this happens when reducing sugars or aldehydes interact with asparagine obtained from thermal decomposition (Granvogl et al., 2004). It has been demonstrated by Granvogl et al. (2004), that 3-Aminopropionamide was found in stored potatoes of different cultivars in considerable concentrations and upon frying acrylamide was formed. Another study showed that 3-

Aminopropionamide is a key transitional chemical compound that leads to acrylamide formation through the Maillard reaction (Visvanathan R, 2014).

2.4 Pyruvic acid

Pyruvic acid can be generated from specific amino acids like serine and cysteine (Figure 4). Then pyruvic acid in its turn can be transformed into acrylic acid. The latter in the presence of ammonia generates acrylamide (Yaylayan et al., 2005). Another study also demonstrated that certain amino acids including pyruvic acid might produce acrylamide but in small amounts (Blank et al., 2005).

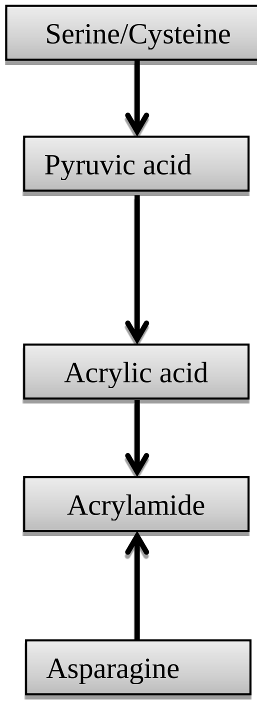

Figure 4: Acrylamide formation from Pyruvic acid

3. Inhibition processes of acrylamide formation

3.1 Innovative mitigation approaches

The World Health Organization and the European Food Safety Authority have been testing foods for acrylamide contamination and have circulated instructions to educate food production sectors about acrylamide mitigation approaches. A very recent study showed that upon the addition of pectin to a Glc/Asn model, the degradation of pectin at high temperature yielded to product that competed with glucose for asparagine in Maillard reaction resulting in an inhibition of acrylamide formation (P. Wang et al., 2022). Another study showed that lactic acid bacteria, cell extracts and yeast gave promising effects on acrylamide mitigation as compared to technological approaches. Lactic acid bacteria can either potentially bind or degrade food contaminants in food including acrylamide (Albedwawi et al., 2021). Moreover, the effect of an innovative pre-treatments of raw potato slices as a mitigation technique prior frying where results showed that dipping raw potato slices in yeast followed by pulsed electric fields gave a synergistic effect on decreasing the amount of acrylamide in the fried potatoes without affecting their quality (Schouten et al., 2020). This finding was also supported by another study which results showed that the combination of pulsed electric fields and yeast treatments of raw potato slices led to small decrease in acrylamide formation when compared to untreated potato chips (Schouten et al., 2020). Moreover, a non-thermal technology known as high pressure processing was combined with the use of asparaginase in order to decrease acrylamide formation in fried potatoes. Results showed that raw potato slices previously immersed in asparaginase enzyme followed by high pressure processing yielded a reduction of acrylamide levels by 26-47% (Dourado et al., 2020). Hence, the combination of the conventional mitigation methods with the emerging technique is more efficient than applying each technique alone on acrylamide concentration reduction in the final food product (Nematollahi et al., 2021). Moreover, another study showed that L-asparginase enzyme from *aspergillus terrus* species when combined to one or more conventional mitigation techniques can exhibit a synergistic effect on acrylamide mitigation resulting in 92.4% reduction on acrylamide formation (Paul & Tiwary, 2020). On the other hand, more research was invested in advanced mitigation techniques such as the addition of specific strains of probiotics especially *Lactobacillus casei* and *Lactobacillus reuteri* that possess asparaginase genes which could mitigate acrylamide in food. The mitigation can be achieved either through the binding of the peptidoglycan compounds of the bacteria to acrylamide or through the conversion of L-asparagine to L-asparatic acid and ammonia under the action of L-asparginase; hence preventing acrylamide from formation (Khorshidian et al., 2020). Moreover, other researchers showed that implementing the Partial Least Squares (PLS) and Discriminant Analysis (DA) known to be characteristic data reflecting high acrylamide, provide an indication of the probability of high acrylamide production in foods. Therefore, by using this data, the mitigation process can be occurred at early stages of production and could provide the food operators a good chance to make some modifications in the manufacturing process leading to decreasing acrylamide concentrations in the final product (Ledbetter et al., 2020). Another study showed that new baking technologies such as vacuum-baking technique might be considered as an alternative and safer technique while preparing potato chips. For instance, during vacuum baking of potato chips, the surface browning was significantly different, higher L* and lower a* were observed, and sensory analysis was enhanced when compared to other conventional techniques (Akkurt et al., 2021). Moreover, sequential soaking treatments of potato, beetroot and parsnip snacks by using two techniques cold soak/hot soak followed by blanching and soaking in $CaCl_2$ followed by blanching, where results showed that these techniques are effective in mitigating acrylamide and 5-hydroxymethylfurfural in vegetable species (Ledbetter et al., 2021).

3.2 Conventional Blanching process

Blanching might play an important role to inhibit acrylamide formation in processed food and this is performed by getting rid of the acrylamide precursors such as reducing sugars before frying (Pedreschi et al., 2004; Visvanathan R, 2014). Moreover, Bakhtiary et al. (2014) revealed that the blanching procedure has a significant role in decreasing acrylamide content in fried potatoes. The latter concluded that blanching causes an important reduction of around 60% in acrylamide concentration of potatoes when fried at 90˚C for 3 min. Another study supported this finding where results showed that blanching at 70˚C for a short period of time guaranteed less formation of acrylamide in food (Mestdagh, De Wilde, et al., 2008).

3.3 Microwave pre-thawing

Microwave frying has proven to decrease acrylamide concentrations and to give a lighter color for potato fried strips when compared to strips fried conventionally as demonstrated by (Belgin Erdogdu et al., 2007). Another study conducted showed that when using the microwave cooking technique, the frying time of pre-thawed frozen potato strips has reduced (EL-Saied et al., 2008). Consequently, this pretreatment had an influence on acrylamide amount in potato strips where acrylamide concentration was considerably decreased. Moreover, when potato strips were subjected to microwave pre-thawing before frying, acrylamide content was lower than control strips (Tuta et al., 2010).

3.4 Specific addition of food ingredients

The addition of specific food ingredients like antioxidants, chitosan, garlic, and many other natural compounds may lead to the decrease of acrylamide formation in the food according to (Ciesarová et al., 2008). A study detailed the powerful effect of some naturally occurring extracts of spices on decreasing the content of acrylamide in potatoes. Moreover, the inhibition power of these spices on acrylamide formation relies on their antioxidant capacities (Ciesarová et al., 2008). Furthermore the addition of natural antioxidants not only mitigated the formation of acrylamide in cookies but also enhanced the organoleptic attributes of cookies (Li et al., 2012). Additionally, results of a study concluded that after the addition of some natural extracts like polyphenols, and furans acrylamide formation was decreased in their model system (Oral et al., 2014).

3.5 Asparaginase enzyme

L-asparagine is naturally found in starchy food, and is known to be the key precursor of the Maillard browning reaction that leads to acrylamide formation (Mottram et al., 2002). L-asparaginase stops the Maillard reaction, hence, asparagine is converted into aspartic acid and ammonia ions. As a result, asparagine cannot undergo the Maillard reaction; therefore, acrylamide will not be formed (Kukurová, Ciesarová, et al., 2009; Visvanathan R, 2014). Results of a study showed that in aqueous medium, acrylamide formation and its mitigation with asparaginase enzyme were enhanced as compared to a fatty medium in short dough biscuits (Anese et al., 2011). Additionally, results of another study performed on a fried dough pastry model showed that asparaginase enzyme converted almost all asparagine to aspartic acid and the acrylamide level was reduced up to 90% (Kukurová, Morales, et al., 2009).

3.6 Addition of Amino acids

The addition of amino acids to baked food showed a modest effect on acrylamide production. Lysine, glycine, and cysteine have been demonstrated to have the most effect on acrylamide inhibition (Kim et al., 2005). Another study showed that upon adding Glycine or Glutamine to a starch model, acrylamide concentrations in potato products was decreased 30% when compared to samples where no addition of amino acids was performed (Bråthen et al., 2005). Moreover, amino acids addition to a fermented dough resulted in a decrease in acrylamide formation (Sadd et al., 2008). On the other hand, the addition of cysteine or lysine to the model significantly decreased the formation of acrylamide; contrarily, when glutamine were added acrylamide formation was promoted (Claeys et al., 2005).

4. Antioxidants' effect on acrylamide formation

Mediterranean diet is characterized by its richness in green leafy vegetables, nuts, legumes, and fruits all of which are high in minerals, vitamins and antioxidants (Maggi et al., 2021). Antioxidants might affect the acrylamide formation trend in many aspects. They can interfere with substrates, intermediates, or even end products of the Maillard reaction. However, some controversy is present concerning the role of antioxidants to decrease or increase acrylamide formation in food and that is due to the presence of carbonyl compound in some naturally occurring antioxidants. Hence, polyphenols can provide carbonyl groups leading to an acceleration of 3-aminopropionamide conversion to acrylamide; thus inhibiting acrylamide removal (Y. Liu et al., 2015). Ferulic acid, and vitamin C showed an inhibition of acrylamide and its precursor asparagine achieved by the inhibition of the carbonyl compounds formation (Ou et al., 2010). Moreover, the addition of some natural spices and herbs like cinnamon, thyme, wild oregano, and green tea to processed food might have a major effect in decreasing the amount of acrylamide. This inhibitory effect is mostly achieved when herbs and spices are added to the oil before frying potatoes. Hence, these herbs are considered potential free radicals scavengers that reduce the concentration of acrylamide formed in food (Morales et al., 2014; Tesby et al., 2018). Studies showed that plant-based food is an excellent source of phenols that possess antioxidant properties. Hence, based on the food pyramid guidelines, health practitioners and nutritionists always recommend the ingestion of plant origin food like fruits, vegetables, and legumes around 4-5 servings a day in order to protect the body from the damage of harmful contaminants including acrylamide. (Maggi et al., 2021).

5. Methods of Acrylamide quantification

Traditional quantification techniques have many advantages and are the most reliable due to their like high accuracy, reproducibility, sensitivity, and good stability; however, they need expensive types of equipment and are time-consuming (Pan et al., 2020). Therefore, more detection techniques have been developed based on sensor detection, immunoassay techniques and capillary electrophoresis. Furthermore, recently researchers came up with new, fast, inexpensive and reliable methods for acrylamide quantification for the favor of food production companies.

5.1 Innovative quantification techniques of acrylamide

A very recent approach has been adopted for rapid quantification of analytes which is the filtration-assisted method. This method is instrument-free, rapid, and inexpensive based on optical detection of acrylamide in foods. For instance, in this study acrylamide was detected at 1 $\mu g.ml^{-1}$ in biscuits and coffee based on the interaction of silver nanoparticles (Ag-NPs) and diethanethiol (EDT) platform, where the mixture of AgNPs and analytes are filtered and grey color intensity analysis is performed (Lin et al., 2021). Moreover, another recent study used PHYTON module to quantify acrylamide in potato chips through analyzing the browning parameters. In this study, the detection of acrylamide in potato chips was precise and ranged from 0.26 to 4.75 $\mu g.g^{-1}$, thus this method is cost-effective and can be utilized by food production companies to quantify acrylamide concentration in food products (Mahendran et al., 2021). A new perspective of acrylamide risk assessment in food was studied through the interaction of acrylamide and micelles in French fries and the aqueous extracts. Results showed that the diffusion rate of acrylamide is curbed by the French fries matrix and upon microfiltration the amount of acrylamide in the filtrate increased. Hence, that was due to the release of bound acrylamide from French fries micelles upon the shearing force of filtration then acrylamide content was quantified by UV absorbance at 200 nm (Ke et al., 2020).

5.2 Gas Chromatography

Gas chromatography is used to analyze compounds that can be vaporized without decomposition. Quantification of acrylamide or its metabolites in foods was performed by GC-MS with or without derivatization. The combination of GC-MS allows immediate isolation of the sample from the matrix and facilitates acrylamide quantification in food. Moreover, the most commonly used method of acrylamide derivatization is through its bromination before analyzing the sample. Therefore, the derivatization process reaction is of great importance to intensify the stability of acrylamide and to ameliorate the detection sensitivity of GC (Elbashir et al., 2014). It is worth mentioning that the major disadvantage of GC-MS without derivatization is the absence of specific ions in the mass spectrum found in the underivatized acrylamide and limitation is caused by the sample matrix composition. Consequently, the coupling of GC with MS favors a broader detection of acrylamide in studied samples and decreases the interference (Elbashir et al., 2014). Many researchers used GC for the detection of acrylamide in different food matrices (Cengiz & Boyacı Gündüz, 2014; Mastovska & Lehotay, 2006; Sun et al., 2012; Wenzl, 2003). Moreover, GC-FID has been used to quantify acrylamide in potatoes by Yang et al., 2016, and to quantify acrylamide in shrimp and chicken nuggets (Seilani et al., 2021).

Table 2: Determination of acrylamide in different food products using GC technique

Matrix	Extraction	Derivatization	Type of GC	Limit of Detection	Reference
Bread,potato chips and cookie	Extraction using water	Derivatization by hydro-bromic acid in the presence of ammonium peroxydisulfate	Gas-chromatography-electron capture detection	LOD: 0.60μg.L^{-1} 2.0 μg.L^{-1} 2.0–100.0 μg.L^{-1}	(Saraji & Javadian, 2019)
Potato chips and French fries	Extraction using (HS-SPME) using 10mL water per 1g of sample	Not applicable	Gas chromatography-flame ionization detection	LOD: 0.22 ug.g^{-1} 0.77 ug.g^{-1}	(Ghiasvand & Hajipour, 2016)
Bread	80 mL ethylene tetrachloride and 550 mL methanol	60 mL xanthydrol 5% in methanol and 2 mL of hydro-chloricacid	Gas chromatography-mass spectrometry	LOD: 0.54 ng.g^{-1}	(Norouzi et al., 2018)
Bread, biscuit, cracker, cake, and cookie	Extraction using 200 µL of acetamide solution and 10 mL of KOH: ethanol (80:20)	Derivatization by 0.05 g of xanthydrol powder in 1 mL methanol	Gas chromatography-mass spectrometry	LOD: > 100 ng.g^{-1}	(Nematollahi et al., 2019)
Potato products and onion	Samples mixed with water and hexane	Derivatization by xanthydrol	GC-MS	LOD: For potatoes:4-10 ng.g^{-1} For onions:3-8 ng.g^{-1}	(Yoshida et al., 2017)

5.3 Liquid chromatography

The Liquid chromatography/tandem mass spectrometry technique was used for the determination of acrylamide and its metabolites in foods. LC was commonly used in the separation and quantification of water-soluble and nonvolatile molecules (Pan et al., 2020). Generally, samples are homogenized in water, then internal standards are added followed by fat extraction. The usage of internal standards ameliorates the accuracy of detection. Additionally fat extraction is occurred by adding hexane and cyclohexane. Simultaneously, other reagents are added to purify the samples from protein traces. In the end, a purification step is performed through evaporation and solid phase extraction (SPE) cartridges leading to acrylamide collection. The main advantages of HPLC are its sensitivity and efficiency for acrylamide quantification. However, HPLC has some drawbacks, it is time- consuming and requires expensive types of equipment (Nielsen et al., 2006). According to Nielsen et al. 2006, HPLC performed well on quantifying acrylamide and asparagine. Many researchers used HPLC to detect acrylamide in many food types (Becalski et al., 2003; Tareke et al., 2002; Wang et al., 2013). **Table 3** summarizes acrylamide detection in different food types using LC technique.

Table 3: Determination of acrylamide in food using LC technique

Matrix	Extraction	Type of LC	Limit of Detection	Reference
French fries, non-fried noodle, vermicelli, rice noodle, phở, dried vegetable, and rice cracker	Addition of methanol and Hexane double extraction	LC-MS/MS	LOD: 1 μg.Kg^{-1}	(Hai et al., 2019)
Turkish coffee	1 mL Carrez solution I and 1 mL Carrez solution II were added. Then water and acetonitrile (90:10)	LC-MS/MS	LOD: 4.6 μg.kg^{-1}	(Akgün & Arıcı, 2019)
Biscuits	Addition of water and dichloromethane	LC-MS with electrospray ionization and Orbitrap	LOD: 3.55 μg.kg^{-1}	(Fernandes et al., 2019)
Seafood	Addition to 50 mL fluorinate ethylenepropylene	HPLC-MS/MS	LOD: 0.67 ng.mL^{-1}	(Qin et al., 2017)
Baby food	Extraction with acetonitrile	LC-MS/MS	LOD: 10 μg.kg^{-1}	(Petrarca et al., 2017)

5.4 Capillary Electrophoresis

Capillary electrophoresis requires a small number of samples and is characterized by its quick analysis and efficient separation, the fact that makes it an effective method for the detection of trace components found in food such as acrylamide (LOD: 0.1mg.kg^{-1}) (Chen et al., 2011; Robledo & Smyth, 2009). According to Robledo and Smyth (2009), capillary electrophoresis showed promising results concerning to non-volatile compounds and can be a feasible alternative to gas chromatography. However, to obtain reproducible quantification and constant electrospray settings, some steps must be applied such as optimization of the gas pressure, composition and flow rate of the liquid, and temperature of the capillary. This technique was also used to detect the amount of acrylamide in potato chip extracts (LOD: 2.6 ng.mL^{-1}) (Tezcan & Erim, 2008). Moreover, the concentrations of different amino acids like proline, valine, glutamine, alanine, asparagine and serine were measured in various food types such as potato, eggplant, chickpeas, and wheat flour using capillary electrophoresis (LOD: 0.32–0.56 mg.L^{1}) (Omar et al., 2017). Additionally, capillary electrophoresis was applied to detect acrylamide concentrations in French fries, breakfast cereals and biscuits (LOD: 0.07g.mL^{-1}) (Bermudo et al., 2006).

5.5 Immunoassay method

Elisa has recently shown high specificity and sensitivity to acrylamide, and many other water-soluble compounds (Preston et al., 2008). Immunoassay is a rapid technique that is based on the specific recognition of antigen to its specific antibody. According to Preston et al., (2008), the ELISA has shown high specificity for acrylamide and can detect very small amounts of samples which means that it is very convenient to be used in a wide range of food products to detect acrylamide. Hence, the immunoassay technique proved to be a validated technique for the quantification of acrylamide in coffee, potato, crisp bread and milk chocolate. Moreover, researchers also assumed that Elisa compared to other currently utilized techniques has proven to be viable and gives rapid results when detecting small molecules like acrylamide. Moreover, ELISA was used in quantifying acrylamide in instant noodles, potato crisps, cakes, and biscuits (LOD: 18.6 $ng.mL^{-1}$) (Quan et al., 2011). Furthermore, ELISA has been used for the determination and quantification of acrylamide in potato fries and biscuits (LOD: 6 $ng.mL^{-1}$) (Zhou et al., 2008).

5.6 Fluorescent Sensing technique

Sensors can monitor when the tested sample holds together with the recognition element in the experimental setup, thus creating a signal that can be measured thereby the quantification of the studied compound occurs (Pan et al., 2020). This technique has been used and many studies were performed to quantify acrylamide in flour, and fried potato chips. Hence, fluorescent sensing method showed to be promising with regards its detection of acrylamide in potato crisps (LOD: $3.5*10^5$ $g.L^{-1}$ (Hu et al., 2014). Furthermore, this method showed good applicability on determining acrylamide concentrations in white bread crust samples (LOD: 8.1×10^{-7}M) (Wei et al., 2020). Moreover, it has been demonstrated that the sensing technique showed high selectivity toward acrylamide detection in potato fries (LOD: 1×10^{-8}M) (Asnaashari et al., 2018).

6. Which method is best for acrylamide quantification?

GC-MS and LC-MS remain the most sensitive methods for acrylamide quantification in food despite that they require time for sample preparation and analysis (Skinner et al., 2021). Fluorescent sensing, capillary electrophoresis and immunoassay showed to be applicable, rapid and easy to perform on foods to detect acrylamide. Recent methods such PHYTON programming and filtration-assisted methods were used and gave precise results for acrylamide concentrations and are cost-effective; however, the key factor on counting on such techniques remain in giving sensitive and reproducible results. Therefore, to control the formation of acrylamide in food scientists keep on searching for fast and cheap techniques that need to be validated in order to be implemented by the food production sector.

7. Future recommendations

Acrylamide, a toxic and ubiquitous contaminant, has been on the agenda of researchers and policy makers for the last few years. Many efforts should be exerted to minimize its presence, this can be achieved by either controlling the concentration of its precursors before processing or mitigation of its formation during the production. A possible idea could be using a sensing method of controlling the reducing sugars and precursors in raw materials. Moreover, the conventional methods like blanching might help in reducing acrylamide in foods. On the other hand, other mitigation techniques can be applied such as using lactic acid bacteria followed by pulsed electric field, antioxidants and enzymes, alone or combined together. The control of time and temperature of roasting/Frying along with the cooking treatment are important parameters that help reduce acrylamide formation. Moreover, the use of computer based programs might help to come up with a model system to predict the ideal conditions to reduce the acrylamide content.

8. Conclusion

Acrylamide is a toxic compound present in toasted and fried foods such as French fries, bread, cereals, and baby snacks, and its presence in food needs to be controlled and reduced. Acrylamide formation is confusing and depends on the processing conditions, especially the temperature-time factor and the concentration of the possible precursors that are found in the raw materials. Hence, different treatment and pre-treatment methods have been proposed before processing to reduce acrylamide such as the conventional mitigation techniques like microwave and blanching. On the other hand, techniques like the use of lactobacillus strains used alone or combined with pulsed electric field, and the use of asparaginase enzyme showed to give promising inhibitory effect on acrylamide. Conventional quantification systems were used to quantify acrylamide such as HPLC, LC-MS, and GC-MS. Despite the fact that filtration-assisted approach based on optical detection of acrylamide and Phyton imaging program gave good results, more efforts are needed to reduce the acrylamide content in foods. This can be achieved either through detection and reduction of their precursors, strict control of the production process conditions or through the use of fast and efficient quantification techniques online.

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Chapter 3: Objectives

Objectives

The aims of the study were to better understand the effect of precursors (amino acids and sugars) and thermal treatment on the formation of acrylamide in simple synthetic potato models and compare the results with potato cultivars.

Then, in order to fulfill the global objectives, several consecutive objectives were conducted to explain in-depth detailed objectives as follows:

1. Formulate ten synthetic potato-starch based models with different combinations of amino acids and/or sugars for the purpose of better understanding the interaction between acrylamide's precursors. The use of these models should serve to clarify whether sugars or amino acids had the greatest impact on acrylamide formation after thermal processing.

2. Evaluate the effect of precursors (glutamine, asparagine, glucose, fructose and sucrose) on the formation of acrylamide in French fries through the use of these potato models based on the composition of potato tubers. This helped to investigate the behavior and interaction of aminoacids (glutamine and asparagine) with sugars (glucose, fructose and sucrose) in synthetic potato models thermally treated on acrylamide formation. Moreover, to evaluate the effect of temperature (170 ˚C vs. 190 ˚C) and cooking treatment (deep frying vs. air frying) on acrylamide formation.

3. Compare the behavior of these simple models with potato tubers on acrylamide formation through investigating the relationship between sugars (glucose, fructose, and sucrose) and amino acids (asparagine and glutamine) in raw potato samples. The outcomes of the study could be of great relevance for potato industry to control acrylamide content while producing fried potato-based snacks.

4. Validate our findings with respect to acrylamide formed in the different types of synthetic potato models, and potato cultivars mainly Agria, Kennebec and Monalisa, using NIR technology spectroscopy with the results of GC-FID methods. Then, to derive a No significant risk level of acrylamide in potato based products that might help food production sector monitor the produced foods in terms of acrylamide content and mark them as safe products.

5. Develop a healthy potato snack that has the minimum amount of acrylamide using potato starch while retaining the umami flavor of potatoes. This can be accomplished by creating a product using the best combination of acrylamides' precursors (amino acids and sugars) along with sodium alginate that proved to have an inhibitory effect on acrylamide

Chapter 4: Experimental setup

To carry out this study, Five tasks have been proposed according to the objectives

Table 1: Overview of the experimental setup

Tasks	Material and treatments	Methodology and statistical methods
Task 1: Formulate ten synthetic potato-starch models with different combinations of amino acids and/or sugars to elucidate whether sugars or amino acids had the greatest impact on acrylamide formation after thermal processing. **Objective**: Using these models should help determine which amino acids or sugars had the greatest impact on the formation of acrylamide following the thermal processing. (1 and 2)	Materials: Raw potato starch, sugars, amino acids, agar-agar, sodium alginate, and water.	
Task 2: 1) Evaluate the effect of precursors (glutamine, asparagine, glucose, fructose and sucrose) on the formation of acrylamide in French fries through the use of these potato models based on the composition of potato tubers. 2) Study the effect of temperature (170 ˚C vs. 190) on the formation of acrylamide 3) Evaluate cooking treatment (deep frying *vs.* air frying) on the formation of acrylamide in the different models formulated. **Objective**: Studying the behavior and interaction of amino acids (glutamine and asparagine) with sugars (glucose, fructose, and sucrose) in synthetic potato models after thermal treatment would better	Materials: Raw potato starch, sugars, amino acids, agar-agar, sodium alginate, and water. Treatments: 1) Temperature of frying treatment: 170 and 190ªC 2) Cooking process: Deep and air frying	Methods used: 1) K-ASNAM (Megazyme kit) for the quantification of amino acids based on color change where absorbance was read at ʎ=340 nm 2) GC-FID for acrylamide quantification 3) HPLC for sugars quantification Statistical analysis: 1) Based on SPSS, Pearson correlation was conducted to examine the relation between the dependent variables and independent variables. 2) Simple and Multiple linear regression analyses were used to study the association between oil uptake, acrylamide concentration, acrylamide precursors, sugars, on

explain the formation of acrylamide in simple models. (1 and 2)		the proposed models adjusting for temperature and technique
Task 3: Compare the behavior of these simple models with potato tubers on acrylamide formation through investigating the relationship between sugars (glucose, fructose, and sucrose) and amino acids (asparagine and glutamine) in potato samples. **Objective**: The comparison with potato cultivars may clearly shed light on the effects of precursors on acrylamide formation after frying in potato cultivars, based on the conclusion drawn from the results of simple models. (objective 3)	Materials: 1) Raw potato starch, sugars, amino acids, agar-agar, sodium alginate, and water. 2) Three potato cultivars: Agria, Kennebec, and Monalisa. Treatments: 1) Temperature of frying treatment: 170 and 190ªC 2) Cooking process: Deep and air frying	Methods used: 1) K-ASNAM (Megazyme kit) for the quantification of amino acids based on color change where absorbance was read at ʎ=340 nm 2) GC-FID for acrylamide quantification 3) HPLC for sugars quantification Statistical analysis: 1) Pearson correlation and scatter plots were conducted to examine the relation between acrylamide concentration and the independent variables. 2) Simple and Multiple linear regression analyses were used to study the associations between acrylamide concentration and oil uptake and acrylamide precursors, adjusting for temperature and technique.
Task 4: 1) Validate our findings with respect to acrylamide formed in the different types of synthetic potato models, and potato cultivars mainly Agria, Kennebec and Monalisa, using NIR technology spectroscopy with the results of GC-FID methods. 2) Derive a No significant risk level of acrylamide in potato based products that might help food production sector monitor the produced foods in terms of acrylamide content and mark them as safe products.	Materials: 1) Raw potato starch, sugars, amino acids, agar-agar, sodium alginate, and water. 2) Three potato cultivars: Agria, Kennebec, and Monalisa. Treatments: 1) Temperature of frying treatment: 170 and 190ªC 2) Cooking process: Deep frying	Methods used: 1) K-ASNAM (Megazyme kit) for the quantification of amino acids based on color change where absorbance was read at ʎ=340 nm 2) GC-FID for acrylamide quantification 3) HPLC for sugars quantification 4) NIR-spectroscopy (wavelengths range 10,000–4000 cm^{-1}) Statistical analysis: 1) Multiple linear regressions were conducted to examine the relationship between acrylamide formation and acrylamide precursors after adjusting for temperature 2) Derivation of equations that permit to obtain acrylamide levels below the limit of quantification; thus,

Objective: Facilitating the detection of acrylamide in potato-based snacks while using NIR spectroscopy where the study's findings could have a significant impact on the potato industry's efforts to control acrylamide content in fried potato-based snacks. (objective 4)		indicating a "No significant risk level (NSRL) of acrylamide" in the produced potato snacks.
Task 5: Develop a healthy potato snack that has the minimum amount of acrylamide using potato starch while retaining the umami flavor of potatoes. **Objective:** Contributing in the potato production sector through fostering a healthy potato snack with least content of acrylamide subsequently diminishing its daily ingestion. (objective 5)	Materials: Raw potato starch, sugars, amino acids, agar-agar, sodium alginate, and water. Treatments: 1) Temperature of frying treatment: 170 ªC 2) Cooking process: Deep frying	Statistical analysis: 1) One-Way ANOVA test followed by Bonferroni Test, or Independent-Samples Kruskal-Wallis Test, as applicable to compare synthetic potato models and real potatoes. 2) Paired-Samples T-test or Wilcoxon signed-rank test, as applicable to compare between acrylamide and its precursors before and after thermal treatment. 3) Correlation of precursors (fructose, glucose, sucrose, glutamine, and asparagine) with acrylamide in the different models after thermal treatment was assessed using Pearson correlation or Spearman's Rank-Order Correlation

Chapter 5:

Study the interaction of amino acids, sugars, thermal treatment and cooking technique on the formation of acrylamide in potato models

- ***This article has been published as:***

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Abstract

This study unveiled the effect of the suspected precursors of acrylamide (asparagine, glutamine) combined/separated with different formulations of glucose, fructose, and sucrose. To better understand the interaction between acrylamide precursors, cooking technique (deep vs. air frying), and temperature (170 ºC vs. 190 ºC), seven potato models from starch, sugars, amino acids, water and hydrocolloids (alginate and agar) were formulated. In line with previous findings, the present results showed that asparagine, glucose and fructose played an important role in acrylamide formation in these synthetic potato models. Furthermore, glutamine and sodium alginate might have an inhibitory effect on acrylamide formation. A significant impact of frying technique was also revealed. On the other hand, GC-FID analysis detected acrylamide in only these three models, (glucose-fructose, sucrose and asparagine-glucose/fructose/sucrose models >LOD 333.33 µg.Kg^{-1}).

Key words: acrylamide, GC-FID, potato model, precursors, sodium alginate

1. Introduction

Acrylamide is a compound formed at temperature greater than 120 ˚C during several cooking processes like frying, roasting and baking in a wide range of foods (Ahn et al., 2002; Stadler et al., 2002). Many countries such as Canada, Australia, New Zealand in addition to the U.S. FDA always monitor foods for their acrylamide content, where coffee and potato fried products showed to contain the highest amount of acrylamide (Wei et al., 2020;Timmermann et al., 2021). French fries, breakfast cereals, toast, cookies, soft bread and coffee are the main sources of acrylamide in the diet of adolescents (Codex Alimentarius Commission, 2006). Acrylamide formation is related with many precursors and different metabolic routes. It has been proved that foods high in sugars and amino acids mainly free asparagine when subjected to heat contain acrylamide (Yaylayan & Stadler, 2005; Capuano & Fogliano, 2016;Bachir et al., 2022). According to Yasuhara et al., (2003), acrylamide can be formed from acrolein in fatty food especially when asparagine is free, because acrolein donates its carbonyl group hence favoring its conversion into acrylamide. Additionally, 3-aminopropionamide has shown to be a key transitional chemical compound that favors the formation of acrylamide through the Maillard reaction (Mottram et al., 2002; Visvanathan R, 2014). Certain amino acids like serine and cysteine are transformed into pyruvic acid which in its turn reacts with ammonia and produces acrylamide (Yaylayan & Stadler, 2005). Furthermore, in endothelial cell metabolism, glutamine provided nitrogen for asparagine to sustain proper cellular homeostasis (H. Huang et al., 2017). This finding reveals a novel link between glutamine and asparagine amino acids especially that asparagine is known to be the main precursor of acrylamide through the Maillard reaction. For that reason, acrylamide's precursors including glutamine and asparagine amino acids were examined and researchers worked on quantifying them in food matrices. Some factors affect the presence of acrylamide in potato products such as the potato cultivars, conditions and time of storing potato tubers and the cooking temperature and technique (De Wilde et al., 2005;Pedreschi et al., 2006; Romani et al., 2008; Ducreux et al., 2021). According to Yang et al. (2016), when frying temperatures increased from 150 ºC to 190 ºC, acrylamide formation increased, but unlike behavior was noticed among the different potato cultivars that were used in the experiment. Similarly, Romani et al., (2008) revealed that as frying temperature increased from 120 ºC to 140 ºC acrylamide formation increased. Another study conducted by Palazoglu et al.(2010), showed that acrylamide concentration in baked potato chips at 170 ˚C was higher by 2-fold as compared to chips fried at the same temperature; whereas acrylamide levels obtained from baking chips at 180 ˚C and 190 ˚C were lower than the fried ones. Moreover, they found out that acrylamide concentrations in the surface of French fries was higher to the center. According to Mesias et al. (2018), the 45.2% of samples taken from fried potatoes presented higher acrylamide concentrations as compared to the standard benchmark for French fries. The relationship between sugars and amino acids on acrylamide formation is not clear yet. Research has found that sugars are responsible for acrylamide formation and are considered the limiting factors in Maillard reaction. Fructose when mixed with asparagine led to acrylamide formation when compared to glucose (Robert et al., 2004;Ciesarová et al., 2011). Another study showed that glucose in the presence of asparagine produced higher amount of acrylamide (Amrein et al., 2004; X. Wang & Xu, 2014a). At high temperature, sucrose is hydrolyzed into glucose and fructose and it participated (Amrein et al., 2004; Wang & Xu, 2014) in acrylamide formation as demonstrated by (Wolfenden & Yuan, 2008; Yang et al., 2016b). On the other hand, studies have shown that asparagine is the limiting factor for maillard reaction and that was supported by (Mottram et al., 2002;Becalski et al., 2003;Yaylayan & Stadler, 2005).

Hence, in order to better understand the effect of precursors on the formation of acrylamide in French fries, seven potato models based on the composition of potato tubers are formulated. The main purpose of this study is to investigate the behavior and interaction of glutamine and asparagine with glucose, fructose and sucrose in potato models thermally treated on acrylamide formation. Moreover, we also aim to study the effect of temperature (170 ˚C vs. 190 ˚C) and cooking treatment (deep frying vs. air frying) on the formation of acrylamide in the different models formulated.

2. Materials and methods

2.1. Chemicals and equipment

Raw potato starch powder (Sigma-Aldrich), sodium alginate (emulsifier and stabilizer) (Sigma-Aldrich), agar-agar (thickening agent) (Scharlau) as fixed ingredients in specific weight then amino acids glutamine and asparagine (Chem- Lab, > 99% purity), glucose (Panreac, M 180.16 $g.mol^{-1}$), fructose (Merck, M 180.15 $g.mol^{-1}$), and disaccharide sucrose (Panreac, M 342, 30 $g.mol^{-1}$). Thermally treatment: deep Fryer (Mandine) and air Fryer (Tefal). Deep frying in which around 500 grams of synthetic potato strips are submerged in 2 liters of hot sunflower oil. Air frying works like a convection oven that stimulates deep frying without submerging the samples in hot oil.

2.2. Potato model

According to Amrein et al., (2003)**,** the composition of the synthetic potato models of the current study was created referring to the fresh weight ($g.kg^{-1}$) of sugars and free amino acids of different potato cultivars. The percent of potato starch (60%), sugars, amino acids (glutamine and asparagine, 0.25%, 0.6%, respectively), sodium alginate (4.8%) and agar-agar (1.2%) (responsible for the firmness and elasticity of the models) were fixed in all the models.

- Model 1 (Glutamine) (Gln): The ratio of glucose/fructose/sucrose to glutamine were 1:2, 1:3.5 & 1:1.4; respectively.
- Model 2 Asparagine (Asn): The ratio of glucose/fructose/sucrose to asparagine were 1:4.5, 1:7 & 1:3; respectively.
- Model 3 prototype of real potato (Gln/Asn) containing glucose, fructose, sucrose, glutamine and asparagine combined all together respecting the same ratios of the precedent models.
- Model 4 Sucrose (Sucr) glutamine and asparagine combined all together respecting the same ratios of the precedent models.
- Model 5 Glucose-Fructose (Glu/Fru) glutamine-asparagine combined all together respecting the same ratios of the precedent models.
- Model 6 Asparagine-Glucose-Fructose-Sucrose (Asn-GFS) all ingredients combined together respecting the same ratios of the precedent models
- Model 7 Glutamine-Glucose-Fructose-Sucrose (Gln-GFS) all ingredients combined together respecting the same ratios of the precedent models

After mixing all ingredients, water is added until obtaining a firm model ready for molding in the form of potato strips using a mold of 0.5*0.5*6 cm dimensions. Samples were divided into triplets and thermally treated using a DEEP fryer (Mandine) and an Air fryer (Tefal) at different time (4 minutes and 30 seconds and 3 minutes and 30 seconds with temperature intervals (170 ºC and 190 ºC). The % moisture and pH of the models were on average 39-41% and 6.2-6.4; respectively.

Table 1: Nomenclature of synthetic potato models

Model	Amino acid: Asparagine/ Glutamine	Sugar: Glucose /Fructose/ Sucrose
Model 1	Glutamine	Glucose Fructose Sucrose
Model 2	Asparagine	Glucose Fructose Sucrose
Model 3 prototype	**Asparagine and glutamine**	**Glucose, Fructose and Sucrose**
Model 4	Asparagine and glutamine	Sucrose
Model 5	Asparagine and glutamine	Glucose and Fructose
Model 6	Asparagine	Glucose, Fructose and Sucrose
Model 7	Glutamine	Glucose, Fructose and Sucrose

Note: In model 1, the ratio of Glutamine: Glucose=1:2, Glutamine: Fructose= 1:3.5, Glutamine: Sucrose=1:1.4. In model 2, the ratio of Asparagine: Glucose=1:4.5, Asparagine : Fructose= 1:7, Asparagine : Sucrose=1:3

2.3. Determination of acrylamide precursors: asparagine and glutamine

Asparagine and glutamine were quantified using the kit (K-ASNAM) of Megazyme international 2014 (Yang et al., 2016b). Samples were prepared as follows: 2 grams of the homogenized lyophilized fried sample, with 60 mL of distilled water was incubated in the oven at 60 ºC for 5 minutes, then transferred with distilled water until 100 mL. The solution was filtered using Whatman 1 filter paper. Then 0.1 mL of the filtrate were taken for the analysis. Samples were analyzed in triplicate.

For the kit analysis: 0.1 mL of the extract was mixed with 1.72 mL of distilled water, 0.3 mL of buffer solution pH 8 and 0.2 mL NADPH, then incubated for 5 minutes at room temperature, after that absorbance A1 was read at ʎ=340 nm. Then 0.02 mL of glutamate dehydrogenase suspension were added and incubated for 5 minutes at room temperature, then absorbance A2 was read at ʎ=340 nm.

Glutamine ($g.kg^{-1}$ DM) = $[(A_1\text{-}A_2)_{sample} - (A_1\text{-}A_2)_{blank}] * 0.5427$.

Then to continue the reaction, 0.2 mL buffer 1 (sodium azide as a preservative) pH 4.9, and 0.02mL glutaminase suspension were added to 0.1mL of the extract, then incubated for 5 minutes at room temperature. After that, 1.5 mL of distilled water, 0.3 mL of buffer 2 (2-oxaloacetate and sodium azide) pH 8, and 0.2 mL of NADPH were added, incubated for 5 minutes at room temperature. Then 0.02 mL of glutamate dehydrogenase suspension were added to the mix, and incubated for 5 minutes at room temperature. Finally, 0.02 mL of asparaginase were added, and incubated for 5 minutes at room temperature, then absorbance A3 was read at ʎ=340 nm.

Asparagine ($g.kg^{-1}$ DM) = $[(A_2 - A_3)_{sample} - (A_2 - A_3)_{blank}]*0.4949$.

Each sample was analyzed in triplicate.

2.4. Quantification of acrylamide by GC-FID method

The determination of acrylamide was conducted following the procedure of (Yang et al., 2016b) with slight modifications. 3 grams of the lyophilized fried samples was mixed with 20 mL 0.1% formic acid solution and centrifuged (1507 g * 10 min, 24 ˚C). Then, 3 mL aliquot of the clarified aqueous were passed through the SPE tube. The acrylamide residue in the SPE was eluted with 2 mL of acetone using gravity flow and collected for analysis. The GC analysis was performed on an Auto-System GC equipped with a flame ionization detector (FID) (Hewlett Packard 5890 series II) following the procedure by Sun et al., (2012). The column used was an Agilent HP-FFAP capillary (length = 25 m, i.d. = 0.2 mm, and thickness = 0.3 µm), and the analysis conditions were as follows: the initial column temperature was settled at 100 °C for 0.5 minute, then raised at a gradient of 10 $°C.min^{-1}$ to 200 °C; the temperatures of the injector and detector were set to 250 °C and 260 °C, respectively; helium was used as the carrier gas at a flow rate of 1 $mL.min^{-1}$ and a splitless of 1 minute, and the injection volume was 1 µm. The results were expressed as $\mu g_{acrylamide} \cdot kg^{-1}$ of (DW). All samples were done in triplicate.

2.5. Determination of Oil uptake

Two grams of lyophilized fried sample was put in a Soxhlet extractor for 4 hours using petroleum ether solution. Then the oil content was calculated and the results were expressed in $g.Kg^{-1}$ of DW (AOAC, 2005; Method 934.01). All samples were done in triplicate.

2.6. Determination of sugars by HPLC

Five grams of lyophilized fried samples with 10 mL of 80% ethanol were agitated for 15 minutes. After that, were centrifuged at 1200g for 10 minutes. The supernatant was collected and put aside. The process is repeated twice and new supernatant was added to the previous one. The extract was concentrated at 65°C for ethanol evaporation until reaching a total volume of 10 mL. For HPLC analysis, 3 mL of the extract was filtered (0.45 nm pore-size), then 20 µL of each filtrate was injected into a Hewlett Packard series 1100 injector with a Beckman 156 Refraction Index Detector. The separation was performed using a Tracer carbohydrates column (5 um, 250*4.6 mm) (Teknoroma). The mobile phase consisted of acetonitrile/water (75:25, v/v), and the flow rate was 1.4 $mL.min^{-1}$. Individual sugars were identified and quantified using external standards. Each sample was analyzed in triplicate. The sugar contents were expressed as $g.Kg^{-1}$ of DM (Yang et al., 2016).

2.7. Statistics

The data reported was the mean of triplicate readings of independent experiments. Statistical Package for the Social Science (SPSS) was used for all computations. Continuous variables were presented as means and standard deviation (STDEV). Pearson correlation was conducted to examine the relation between our dependent variables (oil uptake, acrylamide concentration, acrylamide precursors, and sugars) and the independent variables (temperature, technique and synthetic models). Simple and Multiple linear regression analyses were used to study the association between oil uptake, acrylamide concentration, acrylamide precursors, sugars, on the proposed models adjusting for temperature and technique. Results from the linear regression models were expressed as Beta coefficients (β) with 95% CI. All reported p-values were based on two-sided tests and were compared with a significance level of 5%.

3. Results and Discussion

3.1 Evaluation of Acrylamide formation in different models

Effect of temperature, time and models

Acrylamide was detected in only 3 (> LOD of standard acrylamide=333.33 $\mu g.kg^{-1}$) out of the 7 formulated models (Table 2a). Results from Pearson correlation showed a moderate significant association between technique and acrylamide concentration (r=0.581, p=0.009), (Table 2b). Specifically, deep frying increased acrylamide content compared to air frying. Moreover, no significant correlations were found between acrylamide concentration and temperature (170°C vs 190°C) (r=0.244, p =0.181), in models (5), (4) (6) (r=0.187, r=-0.131, and r=0.364, p=0.244, p=0.314, p=0.083, respectively) (Table 2a). Multiple linear regression showed that technique mainly deep frying was significantly associated with an increase in acrylamide concentration by 245 $ug.kg^{-1}$ (β=244.911, 95% CI: 60.096, 429.726, p=0.014). This finding was in line with similar studies (Yang et al., 2016b ; Mesias et al., 2018;Haddarah et al., 2021; Orsák et al., 2022), where results showed that frying yielded higher acrylamide concentration as compared to baking. In addition, the Glu-Fru model (5) revealed borderline significance with acrylamide concentration (β=258.286, 95% CI: -5.251-521.823, p=0.054). The decomposition of the mono-saccharides (fructose and glucose) may have occurred more rapidly than disaccharide (sucrose) hydrolysis leading to higher acrylamide content in potato tubers based on (Amrein et al., 2004; Henao Toro et al., 2022). On the other hand, Asn-GFS model (6) was significantly associated with an increase in acrylamide formation by 301 $\mu g.kg^{-1}$ (β=300.776, 95% CI: 41.595, 559.957, p =0.027). This was also found when asparagine was thermally treated in a different experiment conducted by Yaylayan et al., (2003), whereby asparagine was converted into maleimide then to acrylamide via deamination and decarboxylation. Similar findings were presented by (Yaylayan & Stadler, 2005; Rydberg et al., 2005; Hidalgo et al., 2009;Yang et al., 2016b;Henao Toro et al., 2022), explaining a strong positive correlation between the reducing sugars, asparagine on acrylamide formation.

Furthermore, acrylamide was not detected in our glutamine-based potato models (Glutamine-Glucose, Glutamine-Fructose, Glutamine-Sucrose and Glutamine-GFS) (7) when applying GC-FID technique (LOD <333.3 $\mu g.kg^{-1}$). Similarly, previous research showed that glutamine amino acid when added alone or mixed with other ingredients did not contribute to the formation of acrylamide (Amrein et al., 2003). Moreover, similar findings showed that Glutamine and Glycine amino acids caused the highest reduction in the formation of acrylamide (76%), followed by Lysine (43%) and alanine (14%) (Champrasert et al., 2022). Likewise, Glutamine was reported to decrease acrylamide formation after its addition in a homogenized potato model and heated in the oven at 180℃ for 25 minutes (Rydberg et al., 2005). It is worth mentioning that our potato models contain sodium alginate as firming compound, hence, research showed that this molecule has inhibitory effect on acrylamide formation in both conventional and microwave heating due to its high emulsion stability. The Na^+ in sodium alginate could inhibit acrylamide by preventing the formation of asparagine and related intermediates (Lindsay & Jang, 2005; Gökmen & Şenyuva, 2007;Champrasert et al., 2022).

Additionally, sucrose model showed lower levels of acrylamide formation when compared to Glu-Fru (5) model albeit not significant (β=110.776, 95% CI: -148.405-369.957, p=0.36). This may be possibly due to the fact that by sugar cane species contain high sucrose content and are less likely to participate in the Maillard reaction to form acrylamide (Henao Toro et al., 2022).

Table 2 a: Concentrations of Acrylamide (µg.Kg-1) in potato models. Effect of temperature and frying technique

		acrylamide content (µg.Kg^{-1})
prototype Model	Agar AF 170 ℃	184.00 ± 17.34
	Agar DF 170 ℃	184.89 ±18.40
	Agar AF 190 ℃	193.11 ± 28.10
	Agar DF 190 ℃	261.55± 5.97
Model 5	Glu-Fru AF 170 ℃	211.11± 76.98
	Glu-Fru DF 170 ℃	600.00± 66.66
	Glu-Fru AF 190 ℃	(--)
	Glu-Fru DF 190 ℃	644.44± 38.49
Model 4	Sucrose AF 170 ℃	244.44± 19.24
	Sucrose AF 190 ℃	244.44± 38.49
	Sucrose DF 170 ℃	333.33± 33.33
	Sucrose DF 190 ℃	444.44± 76.98
Model 6	Asn-GFS AF 170 ℃	191.11± 36.71
	Asn-GFS AF 190 ℃	177.77± 50.03
	Asn-GFS DF 170 ℃	624.44± 82.82
	Asn-GFS DF 190 ℃	873.33± 81.92

(--) means not present

Table 2 b: β values of acrylamide concentrations, Pearson correlations and p-values

	Pearson Correlation	p-value	Acrylamide Concentration (μg.Kg^{-1}) β (95% CI)
Temperature	0.244	0.181	
170 ℃			
190 ℃			101.152,(-89.724-29.029), p=0.265
Technique	0.581	**0.009**	
AF			
DF			**244.911,(60.096-429.726),p=0.14**
Models			
Prototype Model			
Glu-Fru Model	0.187	0.244	**258.286,(-5.251-521.823),p=0.054**
Sucrose Model	-0.131	0.314	110.776,(-148.405-369.957),p=0.363
Asn-GFS Model	0.364	0.083	**300.776,(41.595-559.957),p=0.027**

Note: β of the dependent variable acrylamide concentration is presented with 95 % CI using simple linear regression. Significance at p<0.05
170 ℃ was taken as reference in the statistical analysis
Prototype model was taken as reference in the statistical analysis

3.2 Oil uptake by Glutamine, Asparagine and prototype models

Temperature, cooking technique and sugars effects

During thermal treatment, it is still not clearly understood how the oil is absorbed and penetrated into the samples, hence this is a complex phenomenon (Zhang et al., 2018). Oil absorption is closely related to the pore structure properties, volume of the pores and porosity of the potato chips and is affected by frying time (Zhang et al., 2018). In the present study, Pearson correlation showed a positive, strong and significant correlation between oil uptake and technique ($r=0.80$, $p<0.05$). A weak significant correlation was observed between oil uptake and agar mixed model (3) ($r=0.33$, $p=0.01$). Moreover, a borderline negative association was found between oil uptake and Glutamine-Glucose model ($r= -0.21$, $p=0.083$).

Simple linear regression showed that temperature was not significantly associated with oil uptake; however, technique and only agar mixed model (3) was found to be associated with oil uptake (Table 3, Figure 1). Deep frying significantly increased oil uptake by 29 percent compared to air frying (β: 29.0; 95% CI: 21.47, 36.53, $p<0.05$). Agar Mixed model (3) showed a significantly higher oil uptake in comparison to other models (β: 25.19; 95% CI: 5.51, 44.86), p=0.01). No significant differences were observed in oil uptake in all other models (p>0.05). Multiple linear regression revealed that technique and agar prototype model (3) remained significantly associated with oil uptake after adjusting for temperature and technique. Deep frying significantly increased oil uptake compared to air frying (β: 28.24; 95% CI: 20.62, 35.86). In agreement with our finding, Haddarah et al., (2021) found that the levels of oil uptake was higher in deep fried samples as compared to air fries ones 23% and 7.49% ; respectively at 175 °C .

Agar mixed model (3) also showed a significant increase in oil uptake (β: 20.20; 95% CI: 5.12, 35.28), p=0.01). The value of R^2 is 0.581, this indicates that 58.1% of variability in oil uptake percentage is explained by temperature, technique, and different models.

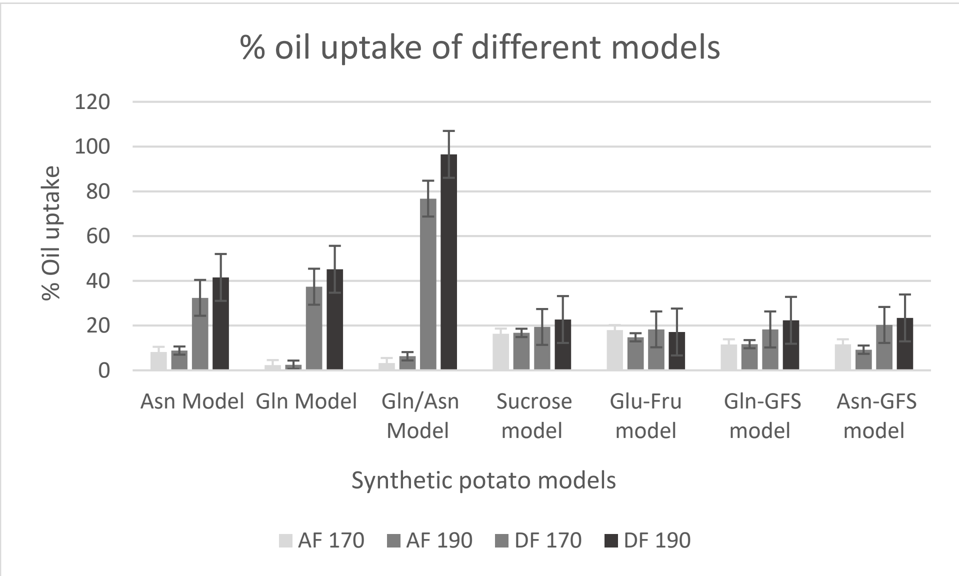

Figure 1: Percentage of oil uptake by the 7 synthetic potato models.

3.3 Evaluation of sugar content after frying

The initial content of sugars: Fructose, Glucose, and Sucrose in all models was 0.7 $g.Kg^{-1}$, 1.2 $g.Kg^{-1}$, and 1.8 $g. Kg^{-1}$), respectively. After frying, around 71% of Fructose, 75% of Glucose and 83% of sucrose reacted. Multiple linear regression revealed that technique and temperature were not associated with concentration of fructose, glucose, and sucrose (p>0.05). Sucrose model (4) yielded more fructose (β: 0.18; 95% CI: 0.1, 0.26, $p<0.001$). In addition, sucrose model (4) also generated acrylamide (at 170 °C, 333.33 $\mu g.Kg^{-1}$ ± 33.33, and at 190 °C, 444.44 $\mu g.Kg^{-1}$ ± 76.98). This may be due to the fact that sucrose at high temperature (>186°C) undergoes hydrolysis that led to the release of fructose (sucrose metabolite) which participates in Maillard reaction leading to the formation of acrylamide. Moreover, sucrose was responsible for the browning of the potato strips giving them a taste similar to potato chips.

In Asn-GFS (6) and Gln-GFS (7) models, results showed a significant decrease in glucose and sucrose concentration (see table 4). For example, Asn-GFS (6) model resulted in lower glucose amount (β: -0.31; 95% CI: -0.52, -0.110.1, 0.26, $p=0.006$). In Asn-GFS model (6), the reactant glucose amount (75%) led to the formation of acrylamide which might explain that glucose might be a main precursor among the sugars responsible for acrylamide formation. It was noted in the Gln-GFS (7) model that there was a significant reduction in glucose and sucrose with no formation of acrylamide. This further might indicate that the amino acid glutamine is not a major precursor for acrylamide formation in the Maillard reaction. It is well noticed that the behavior of the sugars when mixed all together (glucose-fructose-sucrose) is different than when only (Glu-Fru) are interacting together. In the present models, as shown in Table 4, the amount of glucose reactant is higher than the amount of fructose reactant possibly due to the fact that glucose is an essential reducing sugar and might be more involved in the Maillard reaction than fructose. Moreover, fructose also yielded high amount of acrylamide in fructose-asparagine model under high temperature due to the formation of the Schiff base as reported by (Knol et al., 2010).

In some of the potato models, sucrose reactant disappeared after frying and contributed to acrylamide formation after deep frying at high temperature (190 °C). In fact, sucrose content reacted in the studied potato models and it was converted through the non-enzymatic browning of the potato models responsible for the caramelization and the flavor of the potato strips (Amrein et al., 2003). The formation of acrylamide in some of the models may be attributed to the high temperature and to the enzymatic kinetics resulting from the spontaneous and fast hydrolysis of sucrose (Wolfenden & Yuan, 2008). Moreover, research showed that glucose and fructose had higher reactivity as compared to sucrose, hence, reducing sugars remain the main precursors for acrylamide formation in potato tubers (Amrein et al., 2004).

Table 3: Simple and multiple linear regression models: associations between oil uptake percentage and temperature, technique, and potato models, (n=52).

	Simple linear regression	**Multiple Linear regression**
	% Oil uptake β (95%CI)	**% Oil uptake β (95%CI)**
Temperature		
170 ˚C	0.0	0.0
190 ˚C	3.77(-7.34,14.87)	3.77(-3.45,10.98)
Technique		
AF	0.0	0.0
DF	**29.0(21.47,36.53),p<0.05**	**28.24(20.62,35.86), p<0.05**
Models		
Prototype model	**25.19(5.51,44.86),p=0.01**	**20.20(5.12,35.28), p=0.01**
Sucrose model	-3.95(-24.86,16.96)	-6.70(-21.77-8.40)
Glu-Fru model	-5.85(-26.72-15.01)	-8.45(-23.53-6.62)
Gln-GFS model	-7.02(-27.86-13.82)	-9.53(-24.60-5.55)
Asn-GFS model	-6.84(-27.69-14.00)	-9.37(-24.44-5.71)
Gln-Gluc model	-20.60(-49.02-7.82)	-8.74(-29.25-11.78)
Gln-Fru model	-3.34(-32.22-25.66)	-6.26(-26.17-13.66)
Gln-Suc model	-0.94(-29.95-28.07)	-3.95(-23.87-15.96)
Asn-Glu model	-12.70(-41.49-16.08)	-1.14(-21.66-19.37)
Asn-Fru model	-5.67(-34.64-23.30)	-8.50(-28.41-11.42)
Asn-Suc model	-7.34(-36.27-21.60)	-10.10(-30.02-9.81)
		R^2=0.581

Note: β of the dependent variable acrylamide concentration is presented with 95 % CI using simple linear regression. Significance at p<0.05
17 0C was taken as reference in the statistical analysis
AF was taken as reference in the statistical analysis

Table 4: Multiple linear regression models: associations between concentration of fructose, glucose and sucrose versus temperature and technique in 4 potato models (n=20).

	Multiple linear regression		
	Fructose ($g.Kg^{-1}$) β (95%CI)	**Glucose ($g.Kg^{-1}$) β (95%CI)**	**Sucrose ($g.Kg^{-1}$) β (95%CI)**
Temperature			
170 ˚C	0.0	0.0	0.0
190 ˚C	0.00(-0.001,0.001)	0.00(-0.002,0.001)	0.00(-0.001,0.001)
Technique			
AF	0.0	0.0	0.0
DF	-0.01(-0.06,0.04)	0.09(-0.04,0.21)	-0.001(-0.043,0.041)
Models			
Glu-Fru	0.03(-0.06,0.11)	0.03(-0.18,0.23)	0.003(-0.07,0.07)
Sucrose	**0.18(0.1,0.26), p<0.001**	0.09(-0.12,0.29)	-0.02(-0.09,0.05)
Asn-GFS	0.04(-0.04,0.13)	**-0.31(-0.52, -0.11), p=0.006**	**-0.16(-0.23, -0.09),p<0.0001**
Gln-GFS	0.02(-0.07,0.1)	**-0.24(-0.44, -0.03), p=0.027**	**-0.2(-0.27, -0.13),p<0.0001**

Note: β of the dependent variables sugars concentration is presented with 95 % CI using simple linear regression. Significance at p<0.05
170ºC was taken as reference in the statistical analysis
AF was taken as reference in the statistical analysis

3.4 Evaluation of acrylamide precursors

Multiple linear regression revealed that technique and temperature were not associated with concentration of potential acrylamide precursors (ammonia, glutamine, and asparagine) (p>0.05). Asparagine potato model with fructose and sucrose sugars resulted in lower ammonia concentration in comparison to remaining models (β: -5.56; 95% CI: -9.84, -1.28 and β: -11.67; 95% CI: -15.95, -7.39, p=0.013 and p<0.0001, respectively). At high temperature, glutamine is not stable and is degraded leading to the release of ammonia that is responsible for acrylamide formation (Amrein et al., 2004; X. Wang & Xu, 2014a). Several potato models were found to significantly increase glutamine concentration including agar mixed, Asn-GFS (6), Gln-GFS (7), Glutamine-Glucose (1.1), Glutamine-Fructose (1.2), Glutamine-Sucrose (1.3), Asparagine-Fructose (2.2), and

Asparagine-Sucrose (2.3). For instance, Asn-GFS (6) and Asparagine-fructose/sucrose potato models generated glutamine (β: 9.18; 95% CI: -0.12, 18.49, β: 11.18; 95% CI: 1.42, 20.94, β: 13.74; 95% CI: 3.98, 23.5, p=0.053, p=0.026 and p=0.007, respectively). In addition, we observed that all potato models resulted in decrease in asparagine concentration after frying but without statistical significance except for agar mixed model (β: -4.47;95% CI: -5.66, -3.28, p<0.0001). It is important to note that potato models that originally did not contain glutamine in its composition yielded glutamine concentration after frying. This fact might be explained by the theory that the degradation of asparagine is followed by the appearance of glutamine and vice versa, hence, there is an inter-conversion between glutamine and asparagine during the thermal treatment. In general, the treatment of glutamine at high temperature causes their degradation and the release of ammonium (source of amine group) and at the same time, promotes the oxidative deamination of glutamate which results in the formation of α-ketoglutarate which forms the backbone of Asparagine (one of the intermediates of the citric acid cycle (Krebs cycle). Another suggested pathway for the formation of the Asparagine backbone is from the intermediates of the glycolytic pathway (Litwack, 2018; Puigserver, 2018). However, since the models are synthetic and the reactions are performed in-vitro, the suggested interpretation of the inter-conversion between asparagine ($C_4H_8N_2O_3$) and glutamine ($C_5H_{10}N_2O_3$) within the same model might be due to the methylation/de-methylation made by glutamate dehydrogenase enzyme used during the experiment where NADPH is converted to $NADP^+$ and CO_2 is released and the CH_3 group is donated from glutamine to asparagine and vice versa.

Results showed that prototype model generated glutamine and reduced asparagine level, which is a precursor for acrylamide, suggesting that model has a healthy composition in comparison to other models. In addition, most studied potato models increased glutamine concentration (e.g., Glutamine-Glucose, Asparagine-Fructose, and Asparagine-Sucrose. This may be explained by a number of factors: 1- potato models originally containing glutamine as reactants (such as Gln-GFS (7)), 2- inter-conversion of glutamine and asparagine mediated by enzymatic reaction under the effect of thermal treatment (e.g., Asparagine-Sucrose). Furthermore, prototype model did not produce any acrylamide concentration after frying.

Potato models (6), (5) and (4) did not yield asparagine (Table 5). However, (6), (5), and (4) potato models generated acrylamide (β: 300.776, 95% CI: 41.595-559.957, β: 258.286, 95% CI: -5.251-521.823, β: 110.776, 95% CI: -148.405-369.957, p=0.027, p=0.054 p=0.363, respectively). This may be potentially due to the fact that asparagine reacted with reducing sugars in these models to produce acrylamide via the Maillard reaction.

Table 5: Multiple linear regression models: associations between concentration of ammonia, glutamine, and asparagine versus temperature and technique in 7 potato models (n=44).

	Multiple linear regression		
	Ammonia ($g.Kg^{-1}$) β (95%CI)	**Glutamine ($g.Kg^{-1}$) β (95%CI)**	**Asparagine ($g.Kg^{-1}$) β (95%CI)**
Temperature			
170 ˚C	0.0	0.0	0.0
190 ˚C	-0.01(-0.02,0.01)	-0.01(-0.03,0.02)	-0.001(-0.004,0.001)
Technique			
AF	0.0	0.0	0.0
DF	0.62(-0.49,1.74)	-0.89(-3.43,1.66)	0.163(-0.162,0.49)
Models			
Agar-mixed	1.14(-2.93,5.22)	**12.16(2.86,21.47), p=0.012**	**-4.47(-5.66, -3.28), p<0.0001**
Asn-GFS	0.97(-3.11,5.05)	**9.18(-0.12,18.49), p=0.053**	0.36(-0.83,1.55)
Gln-GFS	1.25(-2.83,5.33)	**14.19(4.88,23.49), p=0.004**	-0.33(-1.52,0.86)
Glu-Fru	1.05(-3.03,5.13)	5.25(-4.05,14.56)	-0.61(-1.8,0.58)
Sucrose	1.27(-2.81,5.35)	4.37(-4.93,13.68)	0.34(-0.85,1.53)
Glutamine-Glucose	1.42(-2.14,4.98)	**12.48(4.36,20.61), p=0.04**	-0.16(-1.2,0.88)
Glutamine-Fructose	1.08(-3.09,5.24)	**10.95(1.45,20.44), p=0.025**	-0.47(-1.69,0.74)
Glutamine-Sucrose	0.74(-3.13,4.6)	**9.29(0.48,18.09), p=0.039**	-0.83(-1.95,0.3)
Asparagine-Glucose	-1.08(-4.06,1.9)	-3.78(-10.58,3.02)	-0.61(-1.48,0.26)
Asparagine-Fructose	**-5.56(-9.84, -1.28), p=0.013**	**11.18(1.42,20.94), p=0.026**	-0.36(-1.6,0.89)

Note: β of the dependent variables sugars concentration is presented with 95 % CI using simple linear regression. Significance at p<0.05
170 ˚C was taken as reference in the statistical analysis
AF was taken as reference in the statistical analysis

4. Conclusion

The present study showed that prototype model is healthy as it has a similar composition to real potato with no acrylamide production after thermal treatment. Deep frying technique significantly ($p<0.05$) increased acrylamide concentration and oil uptake percentage in potato models compared to air frying. Temperature did not significantly affect sugar concentration, precursor formation or oil uptake percentage in the studied potato models. Increasing glucose and asparagine content in the synthetic potato strips increased the acrylamide content. The results showed that the largest amount of acrylamide was produced in the Asn/GFS > Glu-Fru > Sucrose model systems. The interaction of sugars with asparagine yielded to high acrylamide formation in Glucose- Fructose, Sucrose, and Asn-GFS models. In contrast, the role of glutamine in potato models did not affect acrylamide formation regardless of the type and combination of the sugars that were initially present in these glutamine-based models. Moreover, the use of sodium alginate as a firming compound in the potato models might have an inhibitory effect on acrylamide formation. From here, we conclude that the composition of the potato models in terms of type and quantity of main precursors is of great importance in order to mitigate the formation of acrylamide after thermal treatment. Hence, agar mixed model was formulated to serve as a prototype to real potato that naturally contains reducing sugars and amino acids in optimal amounts that do not react to produce acrylamide. Further research is needed to elucidate in depth the role of glutamine and sodium alginate on acrylamide mitigation. Findings from the present study may help food production companies to develop healthy acrylamide-free potato snacks.

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Chapter 6:

Impact of amino acids and sugars after thermal processing on acrylamide-formation in synthetic potato models and real potatoes

- ***This article has been submitted as:***

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Abstract

Amino acids and sugars along with the thermal processing are considered main parameters to control acrylamide formation in fried potatoes. To evaluate which of these parameters had the greatest influence, ten synthetic potato-starch based models formulated in different amino acids and/or sugars combinations and three potato cultivars were assigned. Megazyme kit, high-performance-liquid chromatography, gas chromatography flame-ionized-detector were applied to quantify amino acids, sugars and acrylamide. Results showed that reducing sugars and sucrose significantly increased acrylamide formation amongst all potato samples. Synthetic potato models Asn-GFS contained the highest amount of acrylamide compared to Glu-Fru and real potatoes (Agria, and Kennebec). Thus, sugars were considered critical factors for acrylamide formation in potatoes and remained the most practical way of reducing its production.

Keywords: acrylamide, amino acids, potato cultivar, potato models, reducing sugars, sucrose

1. Introduction

Acrylamide, formed in thermally heated foods particularly starchy foods, impose a major health problem (Bachir et al., 2022). It is formed via Maillard reaction, a non-enzymatic browning that happens in foods when amino acids chemically react with carbohydrates mainly reducing sugars (Zyzak et al., 2003). Acrylamide is classified as a probable carcinogen and it can cause neurological and reproductive problems (Tareke et al., 2002; Mendel Friedman, 2003). French fries, potato chips, and breakfast cereals contain the highest amount of ingested acrylamide and are considered essential contributors to dietary intake across Europe (Per Rydberg & Margareta Tornqvist, 2006; Muttucumaru et al., 2014). The association between the concentrations of glucose and fructose, with asparagine in potatoes and acrylamide formation during frying is complicated. Some studies concluded that reducing sugar concentration had a major role in acrylamide formation while others showed that free asparagine concentrations might be the determining factor in Maillard reaction (Amrein et al., 2003; Becalski et al., 2003; Shepherd et al., 2010). Studies have found that sugars are the limiting factors in the Maillard reaction and are responsible for acrylamide formation in carbohydrate rich food (Amrein et al., 2003, 2004; Knight et al., 2021). For instance, acrylamide production has been examined in model systems containing amino acids and reducing sugars. Correlations have been shown between fructose and acrylamide formation when combined with asparagine (Mottram et al., 2002; Stadler et al., 2002; Becalski et al., 2003; Zyzak et al., 2003; Robert et al., 2004; Ciesarová et al., 2011). Whereas, other studies showed that glucose produced higher amount of acrylamide when mixed with asparagine (Amrein et al., 2004; X. Wang & Xu, 2014a). Sucrose hydrolysis at high temperature led to the formation of acrylamide as demonstrated by (Wolfenden & Yuan, 2008; Yang et al., 2016b). On the other hand, further studies showed that free asparagine can be a major precursor for Maillard reaction, thus it increased acrylamide formation in food (Mottram et al., 2002). This finding was also supported by many other researchers (Becalski et al., 2003; Yasuhara et al., 2003; Zyzak et al., 2003; Yaylayan et al., 2005; Oddy et al., 2022).

The objectives of the present study were to formulate ten synthetic potato-starch based models with different combinations of amino acids and/or sugars for the purpose of better understanding the interaction between acrylamide’s precursors. Moreover, to elucidate whether sugars or amino acids had the greatest impact on acrylamide formation after thermal processing. Then to compare the behavior of these simple models with potato tubers on acrylamide formation after frying through investigating the relationship between sugars (glucose, fructose, and sucrose) and amino acids mainly asparagine and glutamine amongst all potato samples. This comparison might help to better understand which precursor had the greatest impact on acrylamide formation in fried potatoes; hence might also assist potato industry to control acrylamide content while producing fried potato-based snacks.

2. Materials and methods

2.1. Sample

Fresh Potato tubers

Three potato cultivars (Solanum. *tuberosum* L.): Agria, Kennebec and Monalisa were selected and purchased from Mercabarna (Mercados de Abastecimientos de Barcelona S.A., (Barcelona, Spain)). The average weight of the potato tubers ranged from 175.09 to 337.60 g. Approximately 6 kg of each potato cultivar of similar size and weight were selected, washed with tap water and dried on paper towels. Potato tubers were then cut into strips (1 * 1 *6 cm) with a stainless steel slicer. Sunflower oil was used in the frying. Each cultivar was fried in triplicate under the same frying conditions. After frying, the samples were lyophilized using a Cryodos-45 freeze-drying instrument (UPC, Spain), packed in falcon tubes.

Synthetic Potato Models

According to Amrein et al., (2003)**,** the composition of the synthetic potato models was created and was as such: potato starch (60%), sugars, amino acids (glutamine and asparagine, 0.25%, 0.6%, respectively), sodium alginate (4.8%) and agar-agar (1.2%) (responsible for the firmness and elasticity of the models). All ingredients were mixed with distilled water until obtaining a dough-like product easy to cut using a stainless steel mold. The ten synthetic potato models were formulated in different combinations of amino acids and/or sugars and were named as follows:

- Prototype Model/ Control model: contained glutamine, asparagine with glucose, fructose, and sucrose.
- Asn-GFS/ model 1: contained only asparagine with glucose, fructose, and sucrose.
- Gln-GFS/ model 2: contained only glutamine with glucose, fructose, and sucrose.
- Glu-Fru/ model 3: contained glutamine, asparagine with glucose, and fructose.
- Sucrose/ model 4: contained glutamine, asparagine with sucrose only.

- Gln-Glu/ model 5: contained glutamine with glucose only.
- Gln-Fru/ model 6: contained glutamine with fructose only.
- Gln-Suc/ model 7: contained glutamine with sucrose only.
- Asn-Glu/ model 8: contained asparagine with glucose only.
- Asn-Fru/ model 9: contained asparagine with fructose only.
- Asn-Suc/ model 10: contained asparagine with sucrose only.

Table S1 summarized the composition of the ten synthetic potato models and the type of potato cultivars used in this study before thermal treatment. The weight of the ingredients was expressed $g.kg^{-1}$ of dry matter. Samples were divided into triplets and thermally treated using a deep fryer (Mandine) and air frying (Tefal). Around 500 grams of potato samples were submerged in 5 liters of hot sunflower oil and deep fried at 170 ºC and 190 ºC. The % moisture, and pH of the models were on average 195-205 $g.kg^{-1}$ and 6.2-6.4, respectively. The Dry matter, and pH of the potato tubers were on average 199-205 $g.kg^{-1}$ and 5.89-6.3, respectively (Table 1).

Table S 1: Composition of synthetic potato models and potato cultivars before thermal treatment: Weight of ingredients ($g.Kg^{-1}$ DM) in the studied samples

Raw weight ($g.Kg^{-1}$ DM)	Glutamine Models (Gln)			Asparagine Models (Asn)			(Gln/Asn) Prototype Model	Glu/Fru Model 3	Sucrose Model 4	Asn/GFS Model 1	Gln/GFS Model 2	Potato cultivars		
	(Gln-Glu) Model 5	(Gln-Fruc) Model 6	(Gln-Sucr) Model 7	(Asn-Glu) Model 8	(Asn-Fruc) Model 9	(Asn-Sucr) Model 10						Agria	Kennebec	Monalisa
Glutamine	2.5	2.5	2.5	0	0	0	2.5	2.5	2.5	0	2.5	0.5	0.43	0.6
Asparagine	0	0	0	5.7	5.7	5.7	5.7	5.7	5.7	5.7	0	2.25	0.93	0.8
Glucose	1.2	0	0	1.2	0	0	1.2	1.2	0	1.2	1.2	1.2	4.6	0.43
Sucrose	0	0	1.8	0	0	1.8	1.8	0	1.8	1.8	1.8	1.25	1.03	1.2
Fructose	0	0.7	0	0	0.7	0	0.7	0.7	0	0.7	0.7	0.8	2.53	0.37
Agar	18.3	18.76	18.94	18.51	17	18	17.1	17.1	17.1	17.1	17.1	(--)	(--)	(--)
Water	655	641.7	663.1	715	765.6	669.5	707.3	700	700.2	701.1	702.1	(--)	(--)	(--)

Note: (--) means not present

2.2. Determination of amino acids: asparagine and glutamine

Asparagine and glutamine were quantified using the kit (K-ASNAM) of Megazyme international 2014 (Yang et al., 2016b). Two grams of the homogenized lyophilized fried sample were mixed with 60 mL of distilled water and incubated in the oven at 60 ºC for 5 minutes, then filled with distilled water until the mark 100 mL The solution was filtered using Whatman 1 filter paper. Then 0.1 mL of the filtrate were taken for the analysis. Samples were analyzed in triplicate.

For the kit analysis (discussed in details in our previous research, data not shown): 0.1 ml of the extract was mixed with series of enzymes then the absorbance A1 was read at ʎ=340 nm. Then the reaction continues absorbance A2 was read at ʎ=340 nm. Glutamine concentration was calculated as follows: Concentration of glutamine ($g.kg^{-1}$ DM) = $[(A_1\text{-}A_2)_{sample}\text{-}(A_1\text{-}A_2)_{blank}]$* 0.5427.

To calculate asparagine concentration, the reaction continues after adding other enzymes, then absorbance A3 was read at ʎ=340 nm. Concentration of asparagine ($g.kg^{-1}$ DM) = $[(A_2\text{-}A_3)_{sample}\text{-}(A_2\text{-}A_3)_{blank}]$*0.4949. Ammonia absorbance was obtained as follows: Δ ammonia = $(A1\text{-}A2)_{ammonia\ sample}$ - $(A1\text{-}A2)_{ammonia\ blank}$. To calculate ammonia concentration: $[(A1\text{-}A2)_{ammonia\ sample}$ - $(A1\text{-}A2)_{blank}]$*0.06325. Each sample was analyzed in triplicate.

2.3. Quantification of acrylamide by GC-FID method

According to Yang et al., (2016b) procedure with some modifications, the concentration of acrylamide was determined. Three grams of the lyophilized fried samples was mixed with 20 mL 0.1% formic acid solution and centrifuged (1507 g for 10 minutes, 24˚C). Then, 3 mL aliquot of the clarified aqueous were passed through the SPE tube. The acrylamide residue in the SPE was eluted with 2 mL of acetone using gravity flow and collected for analysis. The GC analysis was performed on an Auto-System GC equipped with a flame ionization detector (FID) (Hewlett Packard 5890 series II) following the procedure by Sun et al., (2012). The column used was Agilent HP-FFAP capillary (length = 25 m, i.d. = 0.2 mm, and thickness = 0.3 µm), and the analysis conditions were as follows: the initial column temperature was settled at 100°C for 0.5 min, then raised at a gradient of 10 °C per minute to 200 °C; the temperatures of the injector and detector were set to 250°C and 260 °C, respectively; helium was used as the carrier gas at a flow rate of 1 $mL.min^{-1}$ and a splitless of 1 min, and the injection volume was 1 µm. The results were expressed as $\mu g_{acrylamide}\cdot kg^{-1}$ of sample (DW). All samples were done in triplicate.

2.4. Determination of sugars (glucose, fructose and sucrose) by HPLC

Ten mL of 80% ethanol with 5 grams of lyophilized fried samples were agitated for 15 minutes. Then, samples were centrifuged at 1200 g for 10 minutes. The extraction was performed three times.

First, the supernatant was collected and put aside. Then, the process was repeated twice and new supernatant was added to the previous one. The extract was concentrated at 65°C for ethanol evaporation until reaching a total volume of 10 mL For HPLC analysis, 3 mL of the extract was filtered (0.45 nm pore-size), then 20 µL of each filtrate was injected into a Hewlett Packard series 1100 injector with a Beckman 156 Refraction Index Detector. The separation was performed using a Tracer carbohydrates column (5 um, 250*4.6 mm) (Teknoroma). The mobile phase consisted of acetonitrile/water (75:25, v/v), and the flow rate was 1.4 $mL.min^{-1}$. Individual sugars were identified and quantified using external standards. Each sample was analyzed in triplicate. The sugar contents were expressed as $g.Kg^{-1}$ of sample DM (Yang et al., 2016).

2.5. Statistics

Data were entered and analyzed using Statistical Package for the Social Science (SPSS) version 24. Continuous variables were presented as means and standard deviation (STDEV). Pearson correlation and scatter plots were conducted to examine the relation between acrylamide concentration and the independent variables (temperature, technique, synthetic models, and potato tubers). Simple and Multiple linear regression analyses were used to study the associations between acrylamide concentration and oil uptake and acrylamide precursors, sugars, on the proposed models and potato tubers adjusting for temperature and technique. Simple and multiple logistic regression analysis was performed to examine the likelihood of exceeding the threshold of acrylamide (below versus above threshold) for acrylamide precursors, sugars, on the proposed models and potato tubers adjusting for temperature and technique. Results from the linear regression models were expressed as Beta coefficients (β) with 95% CI. Results from the logistic regression models were expressed as Odds Ratio (OR) with 95% CI. All reported p-values were based on two-sided tests and were compared with a significance level of 5%.

3. Results and Discussion

3.1. Comparison of acrylamide formation in synthetic potato models and potato tubers

Effect of sample composition on acrylamide content

Table 1. represented a summary of mean acrylamide, acrylamide precursors (amino acids and sugars) after thermal treatment in synthetic potato models and potato cultivars. In synthetic potato

models, the prototype model contained the least concentration of acrylamide (205.9 µg.Kg^{-1} DM) produced after frying. With regards to models, the concentration of acrylamide was found to be the highest in Asn-GFS model (516.7 µg.Kg^{-1}DM) > Glu-Fru model (438.9 µg.Kg^{-1}) > Sucrose model (316.7 µg.Kg^{-1}DM). The focus of the present study was on the samples that produced acrylamide after thermal treatment.

Table 1: Comparisons of mean acrylamide, acrylamide precursors (amino acids and sugars) after thermal treatment and moisture and pH by synthetic potato models and potato cultivars.

	Acrylamide (µg. Kg^{-1} DM)	Glutamine (g.Kg^{-1} DM)	Asparagine (g.Kg^{-1}DM)	Fructose (g.Kg^{-1}DM)	Glucose (g.Kg^{-1}DM)	Sucrose (g.Kg^{-1}DM)	Moisture (g.Kg^{-1})	pH
				Mean±SD				
Synthetic potato models								
Prototype control model	205.9±37.3	2.2±1.6	4.8±1.0	0.2±0.0	0.1±0.0	(--)	192±9.2	6.22±1.6
Asn-GFS (Model 1)	516.7±29.67	5.2±2.2	(--)	0.1±0	0.4±0.3	0.2±0.0	195±8.9	6.30±1.9
Gln-GFS (Model 2)	(--)	0.2±0.1	0.7±0.3	0.2±0	0.3±0.1	0.2±0.1	190±9.5	6.29±2.7
Glu-Fru (Model 3)	438.9±29.78	9.1±1.6	1.0±0.2	0.2±0.1	0.1±0.0	(--)	197±8.5	6.25±1.4
Sucrose (Model 4)	316.7±94.9	10.0±1.4	(--)	(--)	(--)	(--)	205±7.9	6.27±2.1
Glutamine-Glucose (Model 5)	(--)	3.7±0.7	0.7±0.4	(--)	(--)	(--)	200±9.1	6.30±1.8
Glutamine-Fructose (Model 6)	(--)	3.1±0.5	1.0±0.7	(--)	(--)	(--)	191±7.7	6.32±2.3
Glutamine-Sucrose (Model 7)	(--)	3.0±1.3	0.9±0.5	(--)	(--)	(--)	200±8.8	6.34±2.0
Asparagine-Glucose (Model 8)	(--)	6.1±4.8	1±0.5	(--)	(--)	(--)	198±8.2	6.38±1.5
Asparagine-Fructose (Model 9)	(--)	10.1±1.7	0.8±0.5	(--)	(--)	(--)	203±9.3	6.36± 1.8
Asparagine-Sucrose (Model 10)	(--)	2.4±4.5	0.9±0.8	(--)	(--)	(--)	200±5.7	6.40± 2.2
Potato cultivars								
Kennebec	338.7±6.5	8.4±8.5	8.1±1.6	(--)	(--)	0.7±0.0	205±7.2	5.89± 0.9
Monalisa	205.6±19.4	8.0±3.1	1.47±2.4	1.4±0.1	0.3±0.4	0.7±0.1	202±9.6	6.2±2.1
Agria	441.6±20.8	2.4±2.7	7.8±0.3	0.5±0.8	(--)	1.0±0.1	199±8.3	6.3±1.4

Note: (--) means not present.

- *Synthetics models*

The concentration of asparagine in the synthetic potato models after frying ranged from 0.0 to 4.8 $g.Kg^{-1}$. Whereas, the concentration of glutamine in the models after frying ranged from 0.2 to 10.1 $g.Kg^{-1}$. In glutamine-based models (5, 6, and 7), acrylamide was not detected and that could be due to two reasons: 1-) glutamine is not a major contributor for Maillard reaction, 2-) sugars in these models were added separately with glutamine; hence confirming that sugar when put alone cannot react with glutamine to form acrylamide. Moreover, even when the three sugars were added together with glutamine as in Gln-GFS (model 2), acrylamide was likewise not detected and formed. Hence, glutamine-based models might additionally prove that glutamine is not a key contributor for acrylamide development. Our finding was also supported by (Amrein et al., 2003;
Per Rydberg & Margareta Tornqvist, 2006, Champrasert et al., 2022) . On the other hand, in asparagine-based models (8, 9, and 10) when asparagine was added with only one sugar, acrylamide was not formed. However, in Asn-GFS (model 1) when asparagine was mixed with glucose, fructose and sucrose altogether, huge amount of acrylamide (516.7 $\mu g.Kg^{-1}$). This finding was also proved by (Robert et al., 2004; Knol et al., 2005, 2010). In Asn-GFS (model 1), sucrose presence along with the reducing sugars might have increased acrylamide formation due to its hydrolysis (Amrein et al., 2004; Wolfenden & Yuan, 2008; Wang & Xu, 2014; Yang et al., 2016b). It is worth mentioning that when formulating glutamine-based models and asparagine-based models, glutamine was not added to asparagine-based models and vice versa. However, after frying the synthetic potato strips, asparagine was detected in glutamine-based models and glutamine was detected in asparagine-based models, this can be explained by the theory that there is an inter-conversion between glutamine and asparagine during the thermal treatment. In general, glutamine at high temperature degrades and releases ammonium (source of amine group) and at the same time, promotes the oxidative deamination of glutamate which results in the formation of α-ketoglutarate that forms the backbone of Asparagine. Another proposed pathway for the formation of the asparagine backbone is from the intermediates of the glycolytic route (Litwack, 2018; Puigserver, 2018). Nevertheless, since the potato models are synthetic and the reactions of the quantification of amino acids are performed in-vitro, the proposed explanation of the inter-conversion between asparagine ($C_4H_8N_2O_3$) and glutamine ($C_5H_{10}N_2O_3$) within the same model might be due to the methylation/de-methylation. This reaction is mediated by glutamate dehydrogenase enzyme added during the experiment where NADPH is converted to $NADP^+$ and CO_2 is released and the CH_3 group is donated from glutamine to asparagine and vice versa.

- *Potato cultivars*

In potato cultivars, Agria produced higher acrylamide concentration (441.6 µg.Kg^{-1}) > Kennebec (338.7 µg.Kg^{-1}) > Monalisa (205.6 µg.Kg^{-1}). In real potatoes, asparagine concentration ranged from 7.8 to 14.7 g.Kg^{-1}. As for glutamine concentration, it ranged from 2.4 to 8.4 g.Kg^{-1}. Sugar concentration in all potato samples decreased after frying the fact that proved their interaction and participation in Maillard reaction.

3.2 Effect of thermal processing on amino acids and sugar content in synthetic potato models and potato cultivars

After thermal treatment, acrylamide was only formed in the following samples (Agria, Kennebec, sucrose, Glu-Fru and Sucrose models). Therefore, the analysis was conducted on the previously mentioned samples in order to investigate the behavior of amino acids and sugars on acrylamide formation. In potato cultivars, acrylamide was produced in high amounts in Agria and Kennebec, and in very low amounts in Monaliza. When studying the percentage of reacted acrylamide's precursors as shown in table 3, sucrose and asparagine were found to be the main reactants leading to acrylamide formation. In Agria, 20% of reacted sucrose, 71.1% of reacted asparagine, and 89.5% of reacted glutamine yielded 441.6 µg. Kg^{-1} of acrylamide. Furthermore, in Kennebec, 32% of reacted sucrose and 88 % of reacted asparagine yielded 338.7µg. Kg^{-1} of acrylamide (Table 2). The higher amount of acrylamide produced in Agria as compared to Kennebec might be explained by the synergistic effect between glutamine and asparagine on acrylamide formation and that was also elaborated in another study (Devleeschouwer et al., 2009). On the other hand, in Monaliza, when all acrylamide's precursors reacted in different percentages among each other, acrylamide was formed in a very small amount. Hence, in potato cultivars, sucrose and asparagine seemed to play a major role on acrylamide formation. With respect to synthetic potato models, reducing sugars showed to have a main impact on acrylamide formation. This finding was also supported in previous study (X. Wang & Xu, 2014a). For instance, when all the amount of reducing sugars reacted with all asparagine in the models, explaining their total participation in the Maillard reaction, and as a result favoring the formation of Maillard's byproducts. Hence, glucose, fructose and asparagine appeared to significantly increase acrylamide formation. In sucrose model, when sucrose reacted 100% with glutamine 74% and asparagine this led to the formation of acrylamide but in a less amount as compared to Asn-GFS and Glu-Fru models. Therefore, these findings confirm that reducing sugars, sucrose, and asparagine were the major contributors in the Maillard reaction, thus leading to acrylamide formation.

Table 2: Percentage of reactant acrylamide's precursors after thermal processing

Potato samples	% reactant sucrose	% reactant Fructose	% reactant glucose	% reactant glutamine	% reactant asparagine
Potato cultivars					
Agria	20	25.23	0	89.46	71.12
Monalisa	41.7	73.43	29.51	90.94	94.43
Kennebec	32	0	0	0	88.17
Potato models					
Sucrose model	100	0	0	73.92	100
Glu-Fru	0	100	100	73.25	100
Asn-GFS	83.2	100	100	18.52	100

3.3 Relation between acrylamide concentration potato samples, amino acids and sugars

Simple linear regression of acrylamide in all potato samples showed that Asn-GFS (model 1) and Glu-Fru (model 3) synthetic potato models significantly increased acrylamide formation (β= 390.2, 95%CI: 182.5-597.93, $p<0.0001$, β= 305.66, 95%CI: 86.32, 524.99, $p=0.007$), respectively (Table 3). In multiple linear regression, these synthetic potato models remained statistically significant ($p<0.001$). In addition, sucrose synthetic model showed increased formation in acrylamide (β=290.93, 95%CI: 152.10, 429.75, $p<0.001$). It is important to note that in sucrose model, sugars disappeared after frying, the fact that proved their participation in Maillard reaction, hence high acrylamide concentration was detected. With respect to real potatoes, simple linear regression showed that Agria potatoes were associated with acrylamide formation (β=295.76, 95%CI: -20.34, 611.86, $p=0.06$, borderline significance). Results from multiple logistic regression showed that both Agria and Kennebec potato species produced higher amount of acrylamide compared to other models (β=415.87, 95%CI: 225.08, 606.670, and β=321.98, 95%CI: 152.10, 429.75, $p<0.001$) (Table 3). It is worth mentioning that the amount of sugars decreased as acrylamide concentration increased.

The highest amount of acrylamide was formed in the synthetic potato models in the following order: Asn-GFS > Glu-Fru > Sucrose. This finding supported the significant role of sugars as major precursors on acrylamide formation as likewise demonstrated by (Amrein et al., 2003; Henao Toro et al., 2022). In line with our finding, Parker et al., (2012) showed that the ratio of fructose: glucose had a great impact on acrylamide formation in French fries. With respect to potato cultivars, acrylamide concentration was found to be highest in Agria > Kennebec > Monalisa. This result is in line with Yang et al., (2016b) finding. The low concentration of acrylamide in Monalisa cultivar could be due its low content of sugars mainly fructose as compared to Agria and Kennebec. For instance, low sugar levels limit the formation of acrylamide and favor the competition of asparagine with other amino acids (Knight et al., 2021). It is also worth stating that acrylamide formation in potato tubers was found to be cultivar-specific where the concentration of acrylamide varied (Dite Hunjek et al., 2021; Liyanage et al., 2021). Moreover, results showed that temperature did not have any statistical significance on acrylamide formation in the studied samples contrarily to other studies findings (Palazoglu et al., 2010; Yang et al., 2016b). Furthermore, air frying showed to decrease acrylamide in the potato samples which was in line with previous findings (Lee et al., 2020; Haddarah et al., 2021).

As such we illustrated the relation between our main dependent variable (acrylamide concentration), and our independent variables (potato samples, amino acids and sugars) in a linear regression equation 1 below:

$$\boldsymbol{y = ax1 + bx2 + cx3 + dx4 + ex5 + fx6 + constant}$$

Where **y** axis denotes for acrylamide concentration, x axis refers to the type of potato model and a to f represent the beta coefficients (i.e. rate of change).

The obtained parameter estimates for the linear regression are presented below in equation 1:

$$Acrylamide\ concentration\ \left(\frac{\mu g}{kg}\right) = \mathrm{Asn - GFS}(490.93) + \mathrm{Glu - Fru}(413.15) + \mathrm{Sucrose}(290.93) + \mathrm{Agria}(415.87) + \mathrm{Kennebec}(321.98) + \mathrm{Monalisa}(179.81) + 25.74 \qquad \textbf{\textit{(equation1)}}$$

Table 3: Simple and multiple linear regression of associations between acrylamide concentration and potato samples of different composition, (n=50).

	Simple linear regression	Multiple Linear regression
	Acrylamide concentration ($\mu g.kg^{-1}$DM) β (95%CI)	Acrylamide concentration ($\mu g.kg^{-1}$DM) β (95%CI)
Potato Models		
Asn-GFS (model 1)	**390.2(182.5-597.93), p<0.0001**	**490.93(-20.54,72.01),p<0.001**
Glu-Fru (model 3)	**305.66(86.32,524.99),p=0.007**	**413.15(274.33,551.97),p<0.001**
Sucrose (model 4)	172.80(-58.41,404.02),p=0.14	**290.93(152.10,429.75),p<0.001**
Potato cultivars		
Agria	295.76(-20.34,611.86),p=0.06	**415.87(225.08,606.670,p<0.001**
Kennebec	188.60(-134.36,511.52),p=0.25	**321.98(152.10,429.75),p<0.001**
Monalisa	49.87(-277.36,377.09),p=0.76	179.81(-10.97,370.61),p=0.064
Temperature		
170 °C	9.8(-117.6-137,3),p=0.88	9.0(-95.16-113.12),p=0.26
190 °C	0	0
Technique		
Air frying	-76.05(-212.05-59.94),p=0.26	-56.66(-168.13-54.81),p=0.31
Deep frying	0	0

Note: β of the dependent variable acrylamide concentration is presented with 95 % CI using simple and multiple linear regression. Significance at p<0.05.
190 °C and deep frying were taken as reference in the statistical analysis.

3.4. Rate of change in amino-acids and sugars on acrylamide formation

Effect of precursors on acrylamide content

When looking at amino-acids and sugars separately, we found that fructose increased borderline acrylamide formation in all the samples (r=0.23, p=0.05) (Table 4). This result was in line with previous studies which showed that reducing sugars react to produce high levels of acrylamide (Surdyk et al., 2004; Gökmen & Şenyuva, 2006; Ciesarová et al., 2011). Hence, through the Amadori rearrangement, aldose is transformed to a ketose sugar derivative. It is supposed that in the reaction between ketoses (fructose) and amino groups, ketosylamines are formed, then followed by the Heyns rearrangement to form 2-amino-2- deoxyaldoses (Brands, 2002). Sucrose increased acrylamide significantly where acrylamide content may be obtained from the hydrolysis of sucrose (r=0.36, p=0.06) (Table 5) (Amrein et al., 2004; Halford et al., 2012; Yang et al., 2016b ; Henao Toro et al., 2022; Orsák et al., 2022). Figure 1 represented a scatter plot of sucrose concentration versus acrylamide concentration for all potato models. The figure depicted a positive correlation between sucrose and acrylamide (y= 299.26x + 119.86, R^2=0.1267). This means that sucrose explained about 13% of the change in acrylamide concentration. Moreover, in adjusted models only sucrose was found to significantly have an impact on acrylamide (β= 371.02, 95%CI (4.89, 737.14), p=0.047). For every one unit ($g.kg^{-1}$) increase in sucrose at the end of the frying process there is an increase in acrylamide concentration by 371.02 $\mu g.kg^{-1}$, p=0.047. It is worth saying that from the present study, it was concluded that the impact of sugars on acrylamide formation is much more significant than amino-acids (asparagine and glutamine). This finding is in line with previous research (Amrein et al., 2003, 2004; Yang et al., 2016b; Knight et al., 2021).

Table 4: Pearson correlations and linear regression analyses of associations between acrylamide concentration and acrylamide precursors in all potato samples

Parameters	Pearson Correlation	p-value	Acrylamide Concentration Simple regression ($\mu g.Kg^{-1}DM$) β (95% CI)	Acrylamide Concentration Multiple regression ($\mu g.Kg^{-1}DM$) β (95% CI)
Sugars				
Glucose	0.14	0.16	189.73(-192.34,570.61),p=0.32	7.49(-426.82,441.81),p=0.97
Fructose	0.23	**0.05***	169.14(-34.30,372.58),p=0.1	66.86(-272.13,405.85),p=0.69
Sucrose	0.36	0.06	**299.26(71.23,527.30),p=0.01***	**371.02(4.89,737.14),p=0.047***
Amino-acids				
Glutamine	0.13	0.18	5.73(-6.25,16.78),p=0.36	4.98(-6.60,16.55),p=0.39
Asparagine	0.20	0.80	14.29(-5.80,34.37),p=0.16	-15.86(-53.09,22.18),p=0.40

Note: β of the dependent variable acrylamide concentration is presented with 95 % CI using simple and multiple linear regression. Significance at p<0.05.

3.5. Study the odds ratios of acrylamide content in potato models

Sample composition effects

A new categorical dichotomous variable was created based on the cut-off of acrylamide level for each type of potato models, where "0" indicates below the threshold level and "1" indicates above the threshold level. Multiple logistic regression results showed that Asn-GFS (model 1) and Gluc-Fru (model 3) synthetic potato models increased almost 11 times the risk of exceeding acrylamide the threshold level (OR=10.80, 95%CI: 1.01,115,43), p=0.05 (Table 5). These conclusions concurred that the composition of the potato model had a major impact on acrylamide formation. Hence, sugar content played a major role in producing acrylamide along with amino-acids mainly asparagine and glutamine. It is important to note that potato cultivars produced acrylamide after frying, however mean acrylamide concentration was lower than the content of acrylamide formed in the synthetic models. Results showed that potato cultivars reduce the likelihood of exceeding acrylamide. Kennebec and Agria decreased the odds by 20% (OR=0.82, 95%CI, 0.095, 7.02, p=0.85). Monalisa was shown to greatly minimize the risk of exceeding acrylamide content (OR=0.22, 95%CI, 0.02, 2.53, p=0.23). This may be explained by the fact that real potatoes contain several phytochemicals such flavonoids, and carotenoids, known as bioactive compounds, that are beneficial to human health. These phytochemicals might act as protective factors and decrease acrylamide formation in potato pulp (71 µg acrylamide·kg^{-1} dry matter) (Trabert et al., 2022). Another interpretation could be due to the low content of sugars that are initially present in Monalisa cultivar (Knight et al., 2021; Yang et al., 2016b).

Table 5: Multiple logistic regression of the likelihood of exceeding acrylamide concentration in different potato samples

	Acrylamide Concentration (µg.Kg^{-1}DM) Odds Ratios(95% CI), P-value
Potato Models	
Asn-GFS (model 1)	**10.94(1.07,120,43),p=0.05**
Glu-Fru (model 3)	**10.80(1.01,115.43),p=0.05**
Sucrose (model 4)	3.20(0.40,25.31),p=0.27
Potato cultivars	
Agria	0.82(0.09,7.02),p=0.85
Kennebec	0.82(0.09,7.02),p=0.85
Monalisa	0.22(0.02,2.53),p=0.23

Significance at p<0.05.

4. Conclusion

The present comparative study demonstrated that acrylamide content was affected mainly by the composition of sugars rather than amino acids, where glucose, fructose and sucrose can be considered as main contributors in the Maillard reaction. Moreover sucrose showed to have a major impact on acrylamide formation in all the samples (synthetic models and potato cultivars). Consequently, sugars were considered key and critical factors for acrylamide formation in potatoes and remained the most practical way of reducing its production in potato products. Thus, this might be achieved either by optimization of cultivars or controlling the storage conditions of potatoes. This comparison might help potato industry to control acrylamide content while producing fried potato snacks.

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Chapter 7:

Derivation of a No significant risk level (NSRL) of acrylamide in potato-based models and validation by Near Infrared Radiation spectroscopy

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Abstract

The composition of raw potato-based products mainly sugars and amino acids plays a vital role in acrylamide formation after thermal treatment. Megazyme kit, High Performance Liquid Chromatography, and Gas chromatography Flame-Ionized-Detector were applied to measure sugars, amino acids, and acrylamide. Findings of the present study revealed that sucrose and asparagine had a significant impact on acrylamide. NIR spectroscopy was opted to validate GC-FID with respect to acrylamide detection in synthetic potato models and real potatoes. Peaks at 4439.33-4477.9 cm^{-1} were assigned to indicate acrylamide presence in all potato samples. Prototype model unveiled the best combination amongst all potato models regarding the least acrylamide formation (Agria > Monalisa > Kennebec >Prototype). Mathematical equations were derived to calculate acrylamide concentration that should be below the threshold LOD < 166.6 $\mu g.kg^{-1}$, thus, indicating a "No significant risk level (NSRL) of acrylamide" in the produced potato snacks if Agria and Monalisa cultivars were used.

Keywords: Acrylamide, Amino acids, Potato models, NIR spectroscopy, Sugars

1. Introduction

Acrylamide has been detected in most commonly consumed foods such as French fries, Potato chips, breakfast cereals and coffee contain the highest amount of acrylamide (Bachir et al., 2022; Per Rydberg & Margareta Tornqvist, 2006). Food and Drug Administration warned people of significant exposures to acrylamide. For instance, in certain countries, Business companies were urged to either reformulate the products to lower acrylamide levels below the level set by the country or to post a warning sign on the nutrition label to warn citizens (Guidance for Industry: Acrylamide in Foods, 2016). Currently, the most popular methods for acrylamide detection are the conventional techniques based on the principle of chromatography and mass spectrometry such as HPLC, GC-MS, GC-FID give sensitive and accurate results. These techniques are cumbersome and time-consuming (Pan et al., 2020). Yet, they are used to quantify acrylamide in food. For instance, acrylamide was quantified in bread, potato chips and cookies using GC electron capture detection, where LOD ranged 0.60 $\mu g.L^{-1}$ - 100.0 $\mu g.L^{-1}$ (Alshuiael & Al-Ghouti, 2020). Moreover, acrylamide levels where detected in bread, biscuit, cracker, cake, and cookie using Gas chromatography-mass spectrometry, where LOD > 100 $ng.g^{-1}$ (Nematollahi et al., 2019). Additionally, acrylamide was quantified in bread using GC- MS , where LOD: 0.54 $ng.g^{-1}$ (Norouzi et al., 2018). On the other hand, acrylamide was quantified using LC-MS/MS in French fries, non-fried noodle, vermicelli, rice noodle, dried vegetable, and rice cracker, LOD 1 $\mu g.Kg^{-1}$ (Hai et al., 2019). Moreover, acrylamide was also quantified in Turkish coffee and biscuit using LC-MS/MS and LC-MS with electrospray ionization and Orbitrap, respectively, LOD were 4.6 $\mu g.kg^{-1}$, and 3.55 $\mu g.kg^{-1}$, respectively (Akgün & Arıcı, 2019; Fernandes et al., 2019). Given the fact that foods are complex matrices and the conventional quantification techniques take time, this urged researchers to come up with easy and rapid methods; yet many challenges might be faced. Recently, a novel acrylamide sensing method of quantifying acrylamide in foods by integrating thiolene Michael addition kit with gold nanoparticles mediated by catalytical oxidation, was applied and showed an excellent detection of acrylamide in the range of 0.5–175 μM with a detection limit of 0.16 μM (Zhuang et al., 2022). Moreover, a rapid and sensitive 3D origami paper-based analytical device achieved the quantitative analysis of acrylamide in foods with a limit of detection of 1.13 $\mu g.L^{-1}$ within 120 minutes (Yan et al., 2021). Food applications such as quality assurance and food safety have made use of infrared spectroscopy, which offers useful details about the biochemical composition of the samples, particularly in the fingerprint region. NIR has been recently used in the study of foods because it is straightforward, quick, extremely specific, and requires little to no sample preparation. NIR has been applied to qualitatively detect acrylamide in potato products (Shiroma & Rodriguez-Saona, 2009; Ayvaz & Rodriguez-Saona, 2015; Skinner et al., 2021). The objectives of the present study are to validate our findings with respect to acrylamide formed in the different types of synthetic potato models, and potato cultivars mainly Agria, Kennebec and Monalisa, using NIR, with the results of GC-FID methods. Then, to derive a No significant risk level of acrylamide in potato based products that might help food production sector monitor the produced foods in terms of acrylamide content and mark them as safe products.

2. Materials and methods

2.1.Sample description

Four synthetic potato models that contained acrylamide after thermal treatment in addition to the three cultivars are as follow:

- ASP/ asparagine model (Asn-GFS), this model contains asparagine with glucose, fructose, and sucrose.
- RS /reducing sugars model (Glu-Fru), this model contains asparagine, glutamine with glucose, and fructose.
- SUC/ Sucrose model, this model contains asparagine, glutamine with sucrose.
- C/ control model / Prototype, this model contains asparagine, glutamine with glucose, fructose, and sucrose.
- And 3 types of potato cultivars (Kennebec, Agria, and Monalisa).

The composition of the models and potato cultivars are explained in Table 1. The methodology of sample preparation and thermal treatment (cooking technique and temperature) are explained in details according to (Bachir et al., 2022).

Table 1: Values of acrylamide precursors (asparagine, glutamine, fructose, glucose, and sucrose) in raw potato samples (synthetic potato models and potato cultivars) before thermal treatment. Mean values and standard deviations of acrylamide in the cooke

		Content of precursor before frying (g.Kg^{-1}DM)				
Samples	Acrylamide (µg.Kg^{-1}DM)	Asparagine	Glutamine	Fructose	Glucose	Sucrose
Potato cultivars			Mean±SD			
Kennebec	338.7±6.5	0.93±0.3	0.43±0.1	2.53±1.7	4.60±1.9	1.03±0.7
Agria	441.6±20.8	2.25±1.6	0.50±0.2	0.80±0.3	1.20±0.8	1.25±0.9
Monalisa	205.6±119.4	0.8±0.1	0.6±0.1	0.37±0.1	0.43±0.1	1.20±0.5
Synthetic Potato Models			Mean±SD			
Asn-GFS model	873.33± 81.92	5.60	(--)	0.75	1.28	1.80
Glu-Fru model	644.44± 38.49	5.60	2.56	0.75	1.28	(--)
Sucrose model	444.44± 76.98	5.60	2.56	(--)	(--)	1.80
Prototype (Control Model)	261.55± 5.97	5.60	2.56	0.75	1.28	1.80
Note: (--) means not present						

2.2 Quantification of acrylamide by Gas Chromatography Flame Ionized Detector

Acrylamide concentration was determined according to Yang et al., (2016b) procedure with some modifications. 3 grams of the lyophilized fried samples was mixed with 20 mL 0.1% formic acid solution and centrifuged (1507 g for 10 minutes, 24 ˚C). Then, 3 mL aliquot of the clarified aqueous were passed through the SPE tube. The acrylamide residue in the SPE was eluted with 2 mL of acetone using gravity flow and collected for analysis. The GC analysis was performed on an Auto-System GC equipped with a flame ionization detector (FID) (Hewlett Packard 5890 series II) following the procedure by Sun et al., (2012). The column used was Agilent HP-FFAP capillary (length = 25 m, i.d. = 0.2 mm, and thickness = 0.3 μm), and the analysis conditions were as follows: the initial column temperature was settled at 100°C for 0.5 min, then raised at a gradient of 10 °C per minute to 200 °C; the temperatures of the injector and detector were set to 250°C and 260 °C, respectively; helium was used as the carrier gas at a flow rate of 1 mL.min^{-1} and a splitless of 1 min, and the injection volume was 1 μm. The results were expressed as $\mu g_{acrylamide} \cdot kg^{-1}$ of (DW). All samples were done in triplicate.

2.3. Assignment of acrylamide peak in potato samples using NIR

NIR Spectra Acquisition

To identify the presence of acrylamide in our potato samples (synthetic potato models and real potato cultivars) qualitatively, we used NIR spectroscopy that was found to be easy fast and can be used as a complementary technique. Since NIR spectra tend to have linear baseline increase, second derivative is applied to enhance the separation of overlapping peaks.

Around 5 grams of potato samples (potato model samples and French fries samples) were homogenized using a blender then sealed in an XDS mini sample cup and scanned 32 times to obtain an average spectrum in ambient laboratory condition (20°C,65% relative humidity). Spectra were captured using FT-NIR Antaris II (Thermo Fisher©), using a spinner module. The spectrometer is designed for the non-destructive fast analysis of samples in different states (solid, powder, slurries, and liquid). It captured spectral data in reflectance mode in the range of 10,000–4000 cm^{-1} with a resolution of 8 cm^{-1}, and it was displayed in terms of absorbance. The absorbance spectrum was obtained by rationing the sample spectrum against that of a blank optical path (reference spectrum). Infrared spectra of reference blanks and samples were observed on a personal computer using Win-IR Pro Software 3.0 (Varian Inc., Palo Alto, CA).

2.4. Statistics

The studied data were analyzed using Statistical Package for the Social Science (SPSS) Asparagine, glutamine, fructose, glucose, sucrose, and reducing sugar of potato samples before frying (raw form) are summarized using mean and standard deviation. Multiple linear regressions were conducted to examine the relationship between acrylamide formation and acrylamide precursors (sugars and amino acids) adjusting for temperature for potato tubers and synthetic potato models. Results from the linear regression models were expressed as Beta coefficients (β) with 95% CI. All reported p-values were based on two-sided tests and were compared with a significance level of 5%.

3. Results and Discussion

3.1. Evaluation of Acrylamide formation in potato samples (synthetic potato models vs. potato cultivars)

In synthetic potato models after deep frying at 190℃, acrylamide concentration was higher in Asn-GFS than Glu-Fru and Sucrose model with 873.33 µg.Kg^{-1}DM > 644.44 µg.Kg^{-1}DM > 444.44 µg.Kg^{-1}DM, respectively. The acrylamide content in prototype C did not contain acrylamide the fact that makes it the healthiest in terms of composition. With respect to potato cultivars, acrylamide concentration was found to be higher in Agria followed by Kennebec, then Monalisa (441.6 µg.Kg^{-1}DM > 338.7 µg.Kg^{-1}DM > 205.6 µg.Kg^{-1}DM, respectively) (Table 2).

Multiple linear regression showed that asparagine increased significantly acrylamide formation in Asn-GFS model, sucrose model, potato models as well as in Kennebec, Agria, Monalisa potato cultivars after adjusting for other acrylamide precursors and temperature (β=32.32, 95% CI: 4.58, 70.07, β=46.47, 95% CI: 7.18, 85.75, β= 32.67, 95% CI: 5.30,60.06, β= 52.24, 95% CI: 21.48,83.00, β= 58.57, 95% CI: 24.49,92.65, respectively, $p<0.05$) (Table 2). It is important to note that when asparagine concentration is low in raw potato samples (cultivar and model) it is shown to slightly increase acrylamide (Muttucumaru et al., 2014). Glutamine also was found to increase acrylamide in Kennebec and Agria potatoes (β= 65.55, 95% CI: 14.57, 116.53, and β= 74.53, 95% CI: 15.31, 133.74, respectively, $p<0.05$) (Table 3).

With respect to the sugars, glucose increased acrylamide in Kennebec (β= 172.50, 95% CI: 63.83, 281.12, $p<0.05$). Moreover, sucrose resulted in significantly higher acrylamide level in Sucrose model (β= 106.98, 95% CI: 13.20, 200.76, p=0.03, as well as in Kennebec, Agria, Monalisa potato cultivars (β= 158.30, 95% CI: 85.96, 230.64, and β= 99.08, 95% CI: 22.02, 176.14, and β= 107.36, 95% CI: 28.49, 186.23, respectively, $p<0.05$) (Table 3). Fructose content was found to significantly increase acrylamide in Kennebec and Monalisa (β= 324.61, 95% CI: 158.21, 527.10, and β= 204.83, 95% CI: 9.98, 399.68, respectively, $p<0.05$). The value of R^2 which represents the proportion of variance in the dependent variable (acrylamide) that can be explained by the independent variables (glutamine, asparagine, glucose, sucrose, and fructose) in regression models. For instance, in synthetic potato models R^2 ranged from 27.2% to 46%. In potato cultivars, R^2 values ranged from 48.1% to 62.3%. The highest values were obtained in Asn-GFS model (46%) and Kennebec (62.3%), which means that the % of change in our dependent variable acrylamide can be explained up to 46% and 62.3% by the studied factors, hence, it is a good fit (Table 3). Similar to previous studies results showed that the association between acrylamide formation and sugar concentration (fructose >sucrose > glucose) was stronger than that observed between acrylamide and free glutamine and asparagine concentration in all potato samples (Amrein et al., 2003; De Wilde et al., 2005; Halford et al., 2012; Muttucumaru et al., 2014)**.** Sucrose has been shown to contribute in acrylamide formation at high temperature as it is hydrolyzed to fructose and glucose which in part participate in Maillard reaction and form acrylamide. Sucrose is also responsible for the caramelization (non-enzymatic browning) of the potato samples (synthetic potato models and French fries) giving a distinctive flavor. Hence, the formation of acrylamide in the samples may be attributed to the high temperature and to the enzymatic kinetics resulting from the spontaneous and fast hydrolysis of sucrose (Wolfenden & Yuan, 2008; Whittaker et al., 2010; Henao Toro et al., 2022). In line with our findings, Amrein et al., (2004); and Wang & Xu, (2014) found that glutamine increased acrylamide. Other studies showed mixed findings on the association of glutamine with acrylamide formation; whereby some studies showed a negative correlation with acrylamide formation (Rydberg et al., 2005; Champrasert et al., 2022), particularly in the chipping potatoes, but no significant correlation in the French fry varieties (Halford et al., 2012; Muttucumaru et al., 2014). Results revealed that fructose had highest potential to form acrylamide than glucose. Similarly, positive correlations have been found between fructose and acrylamide formation when combined with asparagine (Robert et al., 2004; Ciesarová et al., 2011). The reactivity of fructose is due to the formation of ketosylamines then followed by the Heyns rearrangement to form 2-amino-2-deoxyaldoses (Brands, 2002).

All sugars were found to significantly increase acrylamide levels in all potato samples. It is important to mention that there are several factors that influence acrylamide formation: raw content of acrylamide precursors, temperature and cooking method. Previous studies showed that the type of potato cultivar as well as the initial concentration of sugars and amino acids in raw form are of great importance to impact the acrylamide content after frying (Amrein et al., 2003; De Wilde et al., 2005; Halford et al., 2012). After analyzing the concentration of the raw acrylamide precursors and their impact on acrylamide we found that the composition of Agria cultivar (raw) might be recommended in terms of glutamine, asparagine, glucose, fructose and sucrose concentrations as shown (Table 1) since it slightly increased acrylamide with all sugars and amino acids combined all together.

Table 2: Acrylamide concentrations (μg.Kg^{-1}DM) in potato cultivars and synthetic potato models

Samples		**Acrylamide ($\mu g.Kg^{-1}DM$)**
Potato cultivars	Kennebec	338.7±6.5
	Agria	441.6±20.8
	Monalisa	205.6±119.4
Synthetic potato models	Asn-GFS	873.33± 81.92
	Glu-Fru	644.44± 38.49
	Sucrose	444.44± 76.98
	Control model	(--)

Note: No statistical significant difference was observed using Anova test in mean acrylamide concentrations among all potato samples. (--) means not present

The association between acrylamide and acrylamide precursors in the raw form (before thermal treatment) can be presented for **Agria potato** cultivar in a linear regression equation below:

$$y = ax1 + bx2 + cx3 + dx4 + ex5 + constant$$

Where **y** axis denotes for acrylamide concentration, x axis refers to the type of potato model and a to e represent the beta coefficients for acrylamide precursors (i.e. rate of change).

The obtained parameter estimates for the linear regression are presented below:

$$Acrylamide\ concentration\ \left(\frac{\mu g}{kg}\right) = Glutamine\ (74.53) + Asparagine\ (52.24) + \text{Glucose}\ (38.10) + \text{Sucrose}\ (99.08) + \text{fructose}\ (173.43) + (-232.86)$$

Similar to this study, when detecting acrylamide using GC in potato samples results showed that Monalisa produced the least amount as compared to Agria potatoes (Yang et al., 2016; Knight et al., 2021). In our previous work, it was found that when analyzing ingredients of Monalisa potato cultivar, we had the least increment in acrylamide content compared to all other samples. The relations between acrylamide and acrylamide precursors in the raw form (before thermal treatment) can be illustrated for **Monalisa potato** cultivar in a linear regression equation below as such:

$$y = ax1 + bx2 + cx3 + dx4 + ex5 + constant$$

Where **y** axis denotes for acrylamide concentration, x axis refers to the type of potato model and a to e represent the beta coefficients for acrylamide precursors (i.e. rate of change, slope).

The obtained parameter estimates for the linear regression are presented below:

$$Acrylamide\ concentration\ \left(\frac{\mu g}{kg}\right) = Glutamine\ (80.28) + Asparagine\ (58.57) + \text{Glucose}\ (38.25) + \text{Sucrose}\ (107.36) + \text{fructose}\ (204.83) + (-284.98)$$

Moreover, the relations between acrylamide and acrylamide precursors in the raw form (before thermal treatment) can be illustrated for **Kennebec potato** cultivar in a linear regression equation below as such:

$$y = ax1 + bx2 + cx3 + dx4 + ex5 + constant$$

Where **y** axis denotes for acrylamide concentration, x axis refers to the type of potato model and a to e represent the beta coefficients for acrylamide precursors (i.e. rate of change, slope).
The obtained parameter estimates for the linear regression are presented below:

$$Acrylamide\ concentration\ \left(\frac{\mu g}{kg}\right) = Glutamine\ (65.55) + Asparagine\ (32.67) + \text{Glucose}\ (172.48) + \text{Sucrose}\ (158.30) + \text{fructose}\ (342.61) + (-309.13)$$

It is worth mentioning that when applying these proposed equations, the concentration of acrylamide obtained should be below the threshold < 166.6 $\mu g.kg^{-1}$, thus, indicating a "No significant risk level (NSRL) of acrylamide" in the produced potato snacks.

On the other hand, in synthetic models, results demonstrated that the impact of sugars mainly sucrose increased the rate of change of acrylamide in sucrose model. With respect to amino acids potential on acrylamide formation, asparagine showed more powerful influence on acrylamide formation as compared to glutamine. Thus, potato snack food industry are recommended to use these equations to predict acrylamide concentration in the final product before placing it in the market using the suggested raw levels of acrylamide precursors in Agria, Monalisa, and Kennebec.

3.2. Interpretation of NIR peaks indicating the presence of acrylamide in potato samples

Figure 1, and Figure 2, showed that the characteristic NIR spectrum of potato samples with bands representative of specific functional groups associated mainly to their oil, amino acids (asparagine, and glutamine), sugars (glucose, fructose, and sucrose), and acrylamide. The NIR reflectance spectra of the absorption bands in the following ranges (4400-4033 cm^{-1}), and (4350-4150 cm^{-1}) were associated with C-H structural combination of aliphatic groups of oil (fatty acids) (Shiroma & Rodriguez-Saona, 2009). Bands in the 5000–4550 cm^{-1} region have been related to amide functional groups (McClure et al., 1996; Liu et al., 2009; Shiroma & Rodriguez-Saona, 2009; Rady & Guyer, 2015) with bands near 4850 and 4600 cm^{-1} assigned to combinations of amide A/I and amide B/II, respectively (P. Robert et al., 1999; Shiroma & Rodriguez-Saona, 2009). With respect to acrylamide bands, according to Adedipe et al., (2016), the assigned bands were in the range of (1400-1600 nm) that correspond to (7143-6250 cm^{-1}), (1650-1850 nm) that correspond to (6061-5405 cm^{-1}), and (1900-2200 nm) that correspond to (5263-4545 cm^{-1}). In our potato samples (synthetic potato models and real potato cultivars), peaks at around 4439-4477 cm^{-1} were found to be present in common amongst all the samples, hence corresponding to acrylamide peak.

Table 3: Multiple linear regression of associations between acrylamide precursors (glutamine, asparagine, glucose, sucrose, and fructose) and potato samples (raw synthetic models and raw potato cultivars) of different composition

			Acrylamides' precursors			
			Acrylamide concentration ($\mu g.kg^{-1}$ DM) β (95%CI)			
	R^2	**Glutamine ($g.kg^{-1}DM$)**	**Asparagine ($g.kg^{-1}DM$)**	**Glucose ($g.kg^{-1}DM$)**	**Sucrose ($g.kg^{-1}DM$)**	**Fructose ($g.kg^{-1}DM$)**
Potato Models						
Asn-GFS model	46%	(--)	**32.32(4.58,70.07), p=0.03**	49.46(-50.38,149.31), p=0.32	70.18(-18.92,159.30) ,p=0.12	130.10(-71.10,331,21), p=0.20
Gluc-Fru model	42.5%	30.76(-54.57,116.10), p=0.47	33.11(-7.13,73.35), p=0.10	25.20(-80.05,130.46),p=0.63	(--)	170.40(-29.54,370.35), p=0.09
Sucrose model	40.3%	63.12(-14.84,141,08) p=0.11	**46.47(7.18,85.75), p=0.02**		**106.98(13.20,200.76), p=0.03**	(--)
Prototype (Control Model)	27.2%	1.34(-55.33, 58.02), p=0.96	21.58(-5.12, 48.29), p=0.11	**90.24(28.51, 151.96) p<0.05**	**78.83(-0.351,158.00), p=0.05**	**140.01(18.63, 261.38), p=0.02**
Potato cultivars						
Kennebec	62.3%	**65.55(14.57,116.53), p=0.01**	**32.67(5.30,60.06) p=0.02**	**172.50(63.83,281.12),p<0.05**	**158.30(85.96,230.64), p<0.05**	**324.61(158.12,527.10), p<0.05**
Agria	51%	**74.53(15.31,133.74), p=0.01**	**52.24(21.48,83.00), p<0.05**	38.10(-58.09,134.30), p=0.42	**99.08(22.02,176.14), p=0.01**	173.43(-14.25,361.11), p=0.07
Monalisa	48.1%	80.28(17.23,143.34) ,p=0.08	**58.57(24.49,92.65), p<0.05**	38.25(-60.53,137.04), p=0.43	**107.36(28.49,186.23), p<0.05**	**204.83(9.98,399.68), p=0.04**

Note: β of the dependent variable acrylamide concentration is presented with 95 % CI using simple and multiple linear regression. Significance at p<0.05. (--) means not present.

Another justification of acrylamide's peak was also demonstrated by Skinner et al., (2021) who showed that acrylamide's peaks were found in these ranges (5000–5500 cm^{-1}) and (6000–6500 cm^{-1}) which are close to our findings with slight shifts. Moreover, similar range of peaks indicating acrylamide presence were revealed by Ayvaz & Rodriguez-Saona, (2015), where acrylamide peaks were assigned as such: 4501–5406 cm^{-1} associated with 2^{nd} stretching overtone of C-O (5208 cm^{-1}), N-H stretching/N-H bending combination (5025 cm^{-1}), symmetrical N-H stretch/amide I combination (4866 cm^{-1}), N-H 2^{nd} overtone bending (4587 and 4854 cm^{-1}) and C-H stretch/C-O stretching combination (4546 and 4673 cm^{-1}) (Ayvaz & Rodriguez-Saona, 2015). With respect to our samples, when comparing the spectral plots of standard acrylamide with those of the synthetic potato models and real potatoes, the common peaks corresponding to acrylamide were as follows: 4439 cm^{-1} in standard acrylamide, 4477 cm^{-1} in real potato tubers, and 4466 cm^{-1} in synthetic potato models which all fall in almost the same range of acrylamide peaks as demonstrated by previous studies (Figure 1, Figure 2, and Figure 3).

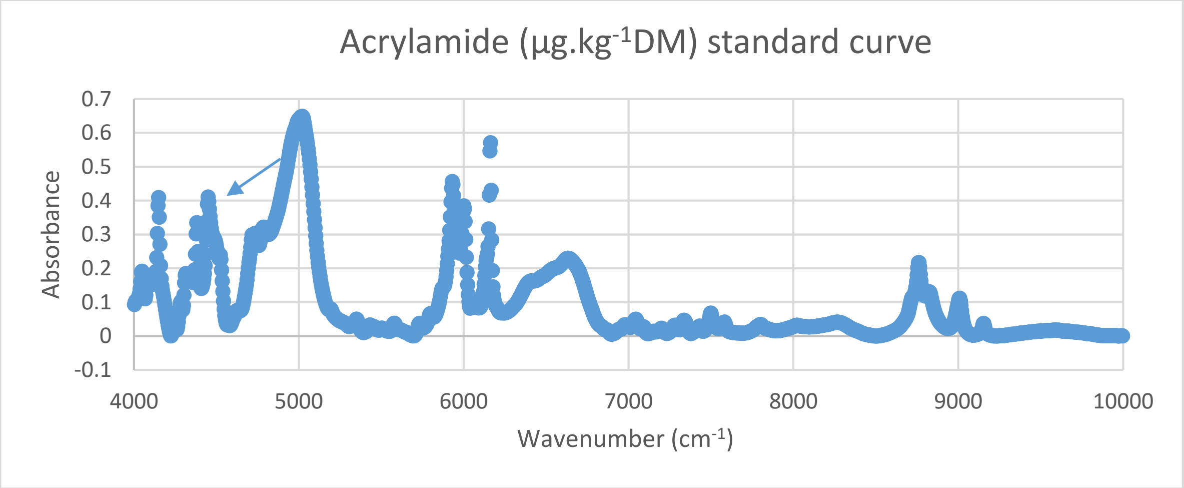

Figure 1:The common peak in the standard acrylamide spectra common with the spectra of synthetic potato models and real potatoes is at 4439 cm^{-1}.

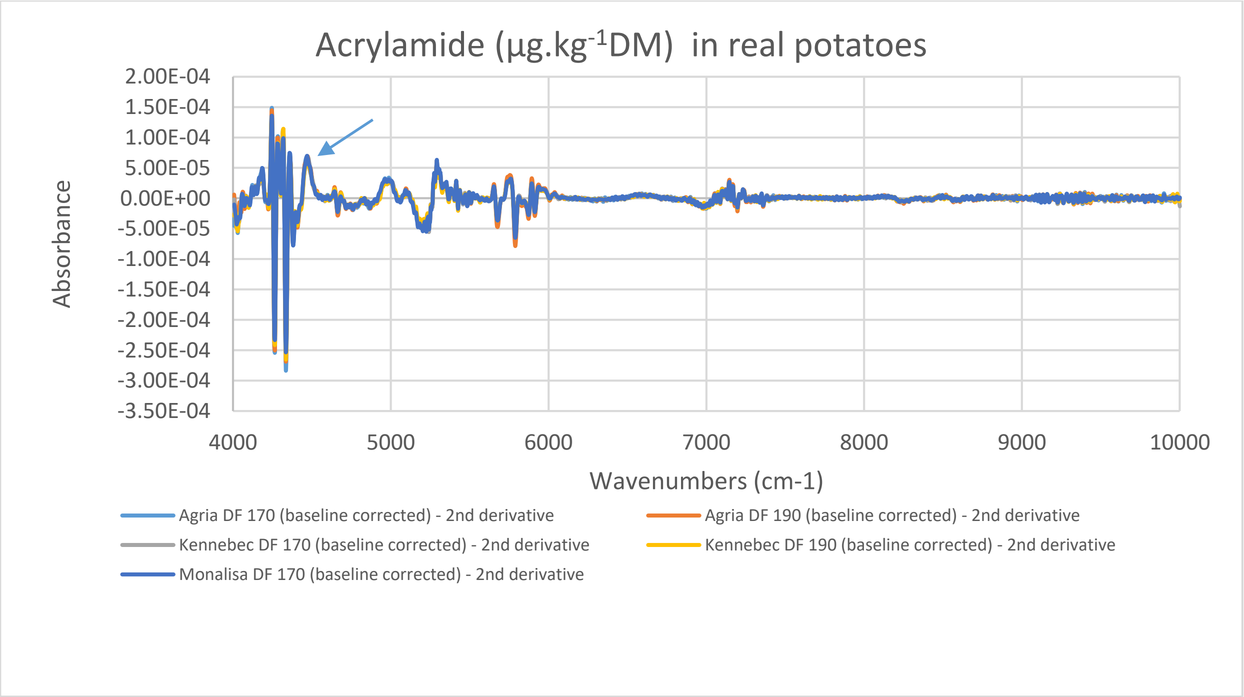

Figure 4: The common peak in the standard acrylamide spectra common with the spectra of synthetic potato models and real potatoes is at 4466 cm^{-1}.

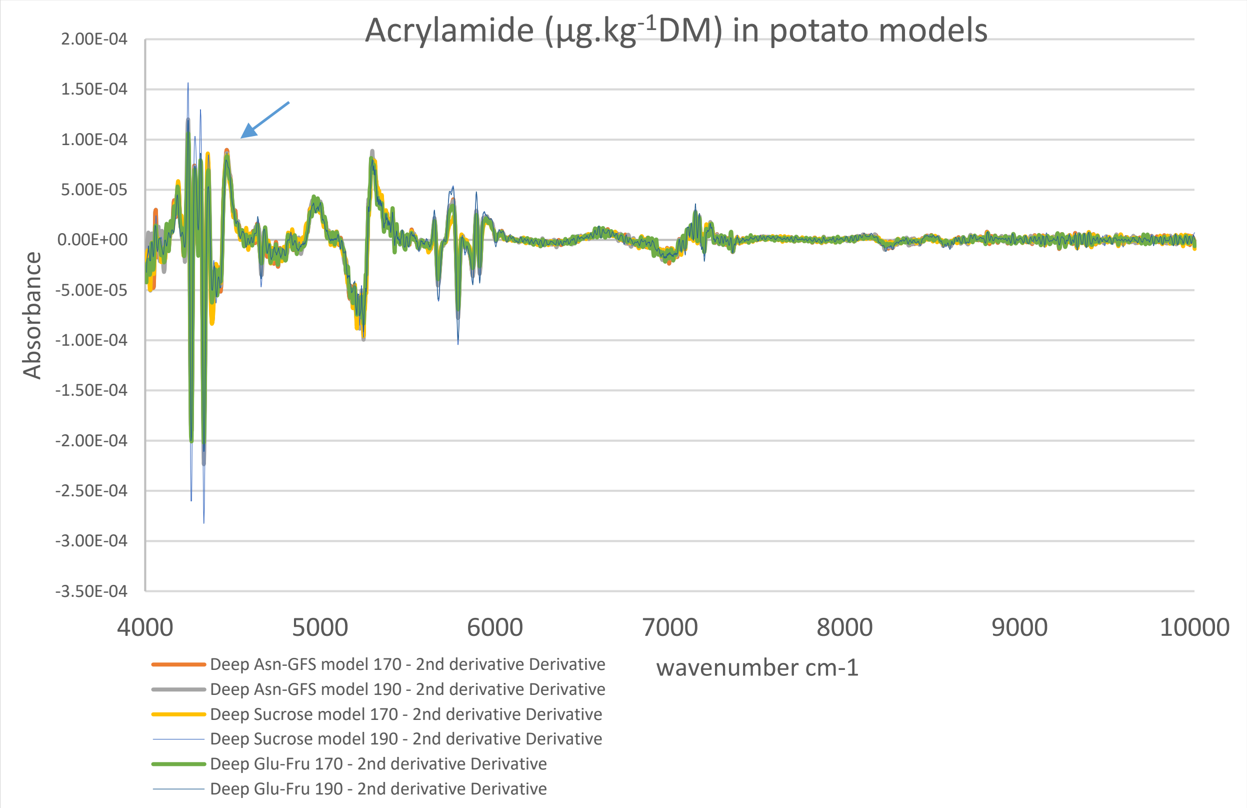

Figure 3: The common peak in the standard acrylamide spectra common with the spectra of synthetic potato models and real potatoes is at 4477 cm^{-1}.

4. Conclusion

Results of the present study showed that NIR presented a fast, practical, and convenient complementary technique to qualitatively detect acrylamide content in fried potatoes. Hence, our paper might be the first conducted study that managed to link the allocated peaks corresponding to acrylamide in the potato samples with actual values of acrylamide obtained from GC-FID. The significance and practicality of using NIR spectroscopy to detect acrylamide in potato-based snacks may be clarified by this validation. Moreover, findings of the present study showed that in the synthetic potato models, the prototype model was the best representative model in terms of composition for acrylamide mitigation. With respect to potato cultivars, Agria and Monalisa were also highly recommended to reduce acrylamide formation. It is worth mentioning that Kennebec in its raw form contained higher concentrations in sugar, the fact that makes it less recommended as compared to other cultivars. In terms of acrylamide's precursors, sucrose and asparagine played a major role in Maillard reaction compared to glutamine, glucose and fructose. Outcomes of this study may help potato based food industry to produce healthy potato chips with least acrylamide formation after frying provided that acrylamide's precursors' levels in the raw form of potatoes were similar to those of Monalisa or prototype model.

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Chapter 8: **Future Product development** ***(a preliminary study)***

Chapter 8:

Future Product development *(a preliminary study)*

Acrylamide-Free potato starch-based prototype: A healthy substitute to potato snacks?

1. Introduction

Potato-based products contribute largely to the daily ingestion of acrylamide. The European Commission has issued a list of foods that should be monitored for their acrylamide content (González-Mulero et al., 2023). Since acrylamide in present in a large number of processed foods, researchers, food producers and stakeholders worldwide have put ample efforts to mitigate or reduce acrylamide formation (Kumari et al., 2022). Hence, several actions must be taken on all levels from farm to fork. For instance, at the agronomical level, several parameters must be taken into account such as potato varieties, fertilizers used, irrigation, postharvest storage conditions (time and temperature), in addition to the content of acrylamide precursors (reducing sugar, asparagine). At the farm level, some approaches can be done to reduce acrylamide level. For example, a good selection of fertilizers, water management to avoid water stress induced on the cultivars, harvesting tubers at maturity, gentle handling of potatoes and good storage conditions to minimize cold induced sweetening and finally ensuring sprout inhibition throughout storage. On the other hand, numerous studies have been conducted to significantly reduce acrylamide content in potato-based snacks like conventional blanching, incorporation of mono and di-valent cations in potatoes (Cirit et al., 2023). Furthermore, the use of asparaginase enzyme (Kumari et al., 2022), and polyphenols (Y. Liu et al., 2015). Other steps can be made at the processing level that include manipulation of acrylamides' precursors (amino acids and reducing sugars), soaking raw potatoes in additive solutions like salt and citric acid. Controlling the time and temperature of cooking process, in addition to avoid deep frying in oil many times as the level of polyunsaturated fatty acids increase. It is worth mentioning that nowadays, the food production section has shifted to replace potatoes by other food components to reduce acrylamide content; for instance, based on the literature, there was a worldwide great consumption of corn-based snacks. Surprisingly, this contributed to a high dietary acrylamide intake, for example, the content of acrylamide in corn/tortilla chips, popcorn, and corn flakes, was reported to be between 5 and 6360 $\mu g.kg^{-1}$, and higher intake was observed in younger generation (Žilić et al., 2022). Moreover, acrylamide content was found to be higher in beetroot and carrot based vegetable chips as compared to sweet potatoes and parsnip (Oellig et al., 2022). A recent study showed that new cassava breeding had not only economic benefits, but also can be an alternative free of acrylamide chips. The willingness of consumers to buy such a healthy product was assessed and it was found that they preferred to buy it despite its slightly high cost (Chancharoenchai & Saraithong, 2021).

2.1 Sample

✓ *Prototype and potato cultivars composition*

According to Amrein et al., (2003)**,** the composition of the prototype in the current study was created referring to the fresh weight ($g.kg^{-1}$) of sugars and free amino acids of different potato cultivars. The percent of potato starch (60%), sugars (glucose, fructose and sucrose (0.07%, 0.04%, 0.11%, respectively) , amino acids (glutamine, and asparagine, 0.25%, 0.6%, respectively), sodium alginate (4.8%) and agar-agar (1.2%) (responsible for the firmness and elasticity of the prototype), and water (39-41%) (Table 1)

✓ *Selected potato cultivars*

Three potato cultivars (Solanum. *tuberosum* L.): Agria, Kennebec and Monalisa. The average weight of the potato tubers ranged from 175.09 to 337.60 g. Approximately 6 kg of each potato cultivar of similar size and weight were selected, washed with tap water and dried on paper towels. Potato tubers were then cut into strips (1 * 1 *6 cm) with a stainless steel slicer.(Table 1)

Table 1. Percent composition of prototype and potato cultivars

% Compounds	prototype	Agria cultivar	Monalisa cultivar	Kennebec cultivar
Starch	60	70	70	70
glucose	0.07	0.1	0.04	0.06
fructose	0.04	0.08	0.04	0.15
sucrose	0.11	0.13	0.12	0.10
Agar-agar	1.2	(--)	(--)	(--)
alginate	4.8	(--)	(--)	(--)
asparagine	0.6	0.26	0.1	0.1
Glutamine	0.25	0.15	0.16	0.14
Water	33	22	21	22

Note (--) means ingredient not present

✓ *Thermal treatment used*

Deep Fryer (Mandine) was used. Deep frying in which around 500 grams of synthetic potato strips and real potato strips were submerged in 2 liters of hot sunflower oil at 170℃. After frying, the samples were lyophilized using a Cryodos-45 freeze-drying instrument (UPC, Spain), packed in falcon tubes.

2.2. Determination of compounds studied

In table 2, you can find the summary of the techniques used to quantify the precursors (amino acids and sugars) and acrylamide. In the previous chapters, the procedures are described in details.

Table 2. Showing the compouds analyzed and the analytical techniques used in the study

Compound analyzed	**Analytical technique**
Asparagine and glutamine	K-Asnam Megazyme kit
Glucose, fructose and sucrose	HPLC
Acrylamide	GC-FID

2.3. Statistics

The data were analyzed using SPSS, version 28. Normal distribution of the data was checked using Shapiro-Wilk test. Continuous variables were summarized as means (SD) and skewed variables were summarized as medians (IQR). Comparison of variables in prototype model and potato cultivars after thermal treatment was conducted using One-Way ANOVA test followed by Bonferroni Test, or Independent-Samples Kruskal-Wallis Test, as applicable. Comparison of concentrations of acrylamide, amino acids, and sugars in prototype model and potato cultivars before and after thermal treatment was conducted using Paired-Samples T-test or Wilcoxon signed-rank test, as applicable. Correlation of precursors (fructose, glucose, sucrose, glutamine, and asparagine) with acrylamide in the different models after thermal treatment was assessed using Pearson correlation or Spearman's Rank-Order Correlation. The correlation coefficient of |0.00–0.10| was interpreted as negligible correlation, |0.10–0.39| as weak correlation, |0.40–0.69| as moderate correlation, |0.70–0.89| as strong correlation, and |0.90–1.00| as a very strong correlation. *p*-value less than 0.05 was considered significant (Schober et al., 2018).

3. Results and discussion

3.1. Mitigation effect of sodium alginate on acrylamide in prototype

Sodium alginate played an important role in acrylamide mitigation in our developed product. This is achieved through the interference of Na^+ cation with intermediates of acrylamide in Maillard reaction. Moreover, sodium alginate showed no hardening effect on the texture of the biscuits and increased the reducing power compared to chitosan and pectin (Fang et al., 2022). Similarly, coating potato chips with sodium alginate decreased oil uptake and mitigated acrylamide formation (H. Liu et al., 2020). Likewise, alginate reduced acrylamide formation in a reaction model made up of glucose and asparagine solution heated at 170 °C for 3 minutes; where the ratio of alginate:solution was (1:0.5) (Champrasert et al., 2022).

3.2. Acrylamide, and its precursors' concentrations in potato samples after thermal treatment

3.2.1 Descriptive interpretation of the samples

Table 3. represented a summary of mean of acrylamide and its precursors (amino acids and sugars) before and after thermal treatment in prototype synthetic models and three types of potato cultivars. The formulated prototype did not contain acrylamide which is below the LOQ of GC-FID used in this work. The potato cultivars, Agria produced the highest amount of acrylamide, followed by Kennebec, then Monalisa: 442.18 $\mu g.Kg^{-1}DM$> 337.10 $\mu g.Kg^{-1}DM$> 203.30 $\mu g.Kg^{-1}DM$; respectively, all values were above the LOQ. With respect to acrylamide's precursors, glucose seemed to react the most among other sugars as its concentrations in all potato samples were found to be negligible after frying. Hence, this is in line with previous studies stating that glucose is one of the major contributors to acrylamide formation (Becalski et al., 2003; De Wilde et al., 2005; Hwang & Kwon, 2022). On the other hand, it is worth mentioning that after frying asparagine concentration in Monalisa is higher than Agria and Kennebec Hence, asparagine in Monalisa did not fully react with sugars to produce acrylamide; contrarily to other potato cultivars. Based on previous studies, asparagine was considered a limiting factor (Muttucumaru et al., 2017; N. Sun et al., 2020; Halford et al., 2022).

Table 3. Comparison of variables in prototype model and potato cultivars **after** thermal treatment (n=12)

	Prototype (n=3)	**Agria (n=3)**	**Monalisa (n=3)**	**Kennebec (n=3)**	**p-value**
			Mean(SD)		
Acrylamide	**<333.33[a,b]**	442.18(14.75)[a,c]	203.30(84.53)[c,d]	337.10(5.40)[b,d]	**<0.001***
Asparagine	4.66(0.14)[a]	7.77(0.34)[b]	13.82(2.04)[a,b,c]	7.52(1.30)[c]	**<0.001***
			Median(IQR)		
Fructose	0.18(-)	Absent	1.30(-)	Absent	0.206^
Glucose	0.09(-)	Absent	Absent	Absent	0.269^
Sucrose	Absent	0.95(-)	0.65(-)	0.68(-)	0.277^
Glutamine	2.30(-)	4.56(-)	Absent	2.54(-)	0.462^

SD: Standard deviation; IQR: Interquartile range

Acrylamide and asparagine are of normal distribution. Fructose, glucose, sucrose, and glutamine are of skewed distribution

*The significance level is 0.050 based on One-Way ANOVA

^ The significance level is 0.050 based on Independent-Samples Kruskal-Wallis Test

[a,b,c,d] represent a statistically significant difference based on Bonferroni Test

sig. p-values are highlighted in Bold

3.2.2. Effect of samples' composition on acrylamide formation

Table 3 showed the comparison of variances of acrylamide and asparagine in the potato samples. With respect to acrylamide, results showed that there is a statistical significance based on Bonferroni test between prototype-Agria, and prototype-Kennebec (p<0.001). Hence, acrylamide in the prototype was significantly lower compared to Agria and Kennebec. Similarly a statistical significance was reported between Agria-Monalisa, and Agria-Kennebec (p<0.001) explaining that the concentration of acrylamide is higher in Agria compared to Kennebec and Monalisa. Moreover, acrylamide was statistically higher in Kennebec compared to Monalisa .Based on the literature, the initial amount of precursors mainly sugars in the potato cultivars had a major impact on acrylamide formation and this was supported by (Amrein et al., 2003; Halford et al., 2012; Wang & Xu, 2014; Orsák et al., 2022). Table 2 represented the composition of the potato samples before and after thermal treatment, Kennebec had the highest amount of sugars, on the other hand Agria potato is the one with most amount of acrylamide formed. With respect to the formulated prototype, the sugar content is relatively close to that of Monalisa; hence it has no acrylamide formed after frying.

Despite the fact that the mean of sugars was not statically significant across all models as shown in Table 3, however there was a strong negative correlation between reducing sugars and acrylamide in prototype model (Table 3). With respect **to prototype**, Pearson correlation revealed a strong negative association ($p<0.05$) between glucose and fructose and acrylamide formation. Moreover, paired t-test results showed a significant decrease in reducing sugars amount in prototype model ($p<0.05$) after thermal treatment (Table 3). This was also shown in the scatter plots (Figure 1). The acrylamide-free prototype status might be explained due to the presence of sodium alginate in the prototype that might have interfered in the Maillard reaction at the level of reducing sugars. It is worth mentioning that this hypothesis needs to be further validated in future research. Consequently, the no-acrylamide content in the prototype might be attributed to two main ingredients that were initially added to the mixture. The first ingredient is glutamine (2.56 $g.Kg^{-1}DM$) that was higher than the amount of glutamine in potato cultivars of average of 0.5 $g.Kg^{-1}DM$. Glutamine showed to have mitigation effect on acrylamide formation and that was previously shown that when it is added alone or mixed with other ingredients did not contribute to the formation of acrylamide (Amrein et al., 2003). Moreover, other results showed that certain amino acids like glutamine and glycine caused the highest reduction in the formation of acrylamide (76%), followed by lysine (43%) and alanine (14%) (Champrasert et al., 2022). Likewise, glutamine was reported to decrease acrylamide formation after its addition in a homogenized potato model and heated in the oven at 180°C for 25 minutes (Rydberg et al., 2005). The second ingredient is sodium alginate, a firming compound that was originally added to the synthetic prototype mix to give it proper elasticity and firmness. Hence, research showed that this molecule has inhibitory effect on acrylamide formation in both conventional and microwave heating due to its high emulsion stability. The Na^+ in sodium alginate compound could have inhibitory power by preventing the formation of asparagine and subsequent intermediates (Lindsay & Jang, 2005; Gökmen & Şenyuva, 2007; H. Liu et al., 2020; Fang et al., 2022; Champrasert et al., 2022).

With respect **to potato cultivars**, a strong positive association was found between sucrose and acrylamide formation in Kennebec ($p<0.05$) (Table 5). This might explained by the fact that the initial concentration of sucrose (before frying) was higher than Agria followed by Monalisa, (4.60>1.25>0.2 $g.Kg^{-1}DM$); respectively. Our findings were in line with previous studies where Agria and Kennebec cultivars produced high amount of acrylamide (Wang & Xu, 2014b; Yang et al., 2016b). The mean concentration of glutamine was found to be the same across all the models and no statistical significance was shown (Table 5). Moreover, there was no association between glutamine and acrylamide formation ($p>0.05$). This might point out that glutamine might not be a major contributor to acrylamide formation as supported by (Becalski et al., 2003; De Wilde et al., 2005; Hwang & Kwon, 2022). Contrarily, asparagine content was significantly lower in prototype, Kennebec, and Agria compared to Monalisa ($p<0.001$) (Table 4). On the other hand, the low content of asparagine (after frying) in Agria and Kennebec is attributed to its reaction with sugars leading to acrylamide formation. Consequently, this finding highlighted the role of asparagine as a major contributor to acrylamide production as also demonstrated by (Mottram et al., 2002; Becalski et al., 2003; Zyzak et al., 2003; Yasuhara et al., 2003; Yaylayan et al., 2005; Oddy et al., 2022).

Based on the results of our previous chapter 7, the proposed equations derived from the linear regression analyses taking into account the content of acrylamide's precursors in the potato-based models can be applied where NIR can also be used as a control technique (to measure the content of sugars and AA in raw potatoes).

Finally, considering the results obtained, the maximum amount of sugars and asparagine present in a cultivar mainly **Monalisa** to minimize the acrylamide content would be in the order of: 0.04 g glucose.100 g^{-1} potato, 0.04 g fructose.100 g^{-1}, 0.12 g sucrose.100 g^{-1}, and 0.1 g asparagine.100 g^{-1} (expressed in DM)

Table 4. Mean (SD) concentrations of acrylamide, amino acids, and sugars in prototype model and potato cultivars **before** and **after** thermal treatment

	Prototype (n=3)		**Agria (n=3)**		**Monalisa (n=3)**		**Kennebec (n=3)**	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Acrylamide ($\mu g.Kg^{-1}$DM)	(--)	(--)	(--)	442.18(14.75)	(--)	203.30(84.53)	(--)	337.10(5.40)
Fructose) ($\mu g.Kg^{-1}$DM)	0.77(0.01)	0.18(0.02)*	0.80(0.14)	0.47(0.06)	1.2(0.05)	0.93(0.01)	2.53(0.30)	(--)
Glucose ($\mu g.Kg^{-1}$DM)	0.18(0.02)	0.08(0.01)*	1.20(0.10)	(--)	0.43(0.01)	0.2(0.01)	4.60(0.89)	(--)
Sucrose ($\mu g.Kg^{-1}$DM)	1.80(0.22)	(--)	1.25(0.31)	0.67(0.02)	0.2(0.01)	0.47(0.04)	1.03(0.65)	0.48(0.01)
Glutamine ($\mu g.Kg^{-1}$DM)	2.56(0.78)	2.00(0.19)	0.50(0.08)	3.17(0.50)	0.6(0.03)	7.04(1.03)	0.43(0.02)	5.50(0.98)
Asparagine ($\mu g.Kg^{-1}$DM)	5.27(1.24)	4.66(0.14)	2.25(0.99)|	7.77(0.34)*	0.43(0.02)	5.50(0.98)*	0.93(0.01)	7.52(1.30)

Note. SD: Standard deviation. *The difference is significant based on Paired-Samples T-test
(--) means not present

Table 5. Correlation of precursors (fructose, glucose, sucrose, glutamine, and asparagine) with acrylamide in the different models **after** thermal treatment

	Fructose	**Glucose**	**Sucrose**	**Glutamine**	**Asparagine**
			Correlation with acrylamide		
Prototype	-1.00*	-1.00*	0	0	0
Kennebec	0	0	1.00*	0	0
Monalisa	0	0	0	0	0
Agria	0	0	0	0	0

*Correlation is significant at $p<0.05$ based on Pearson

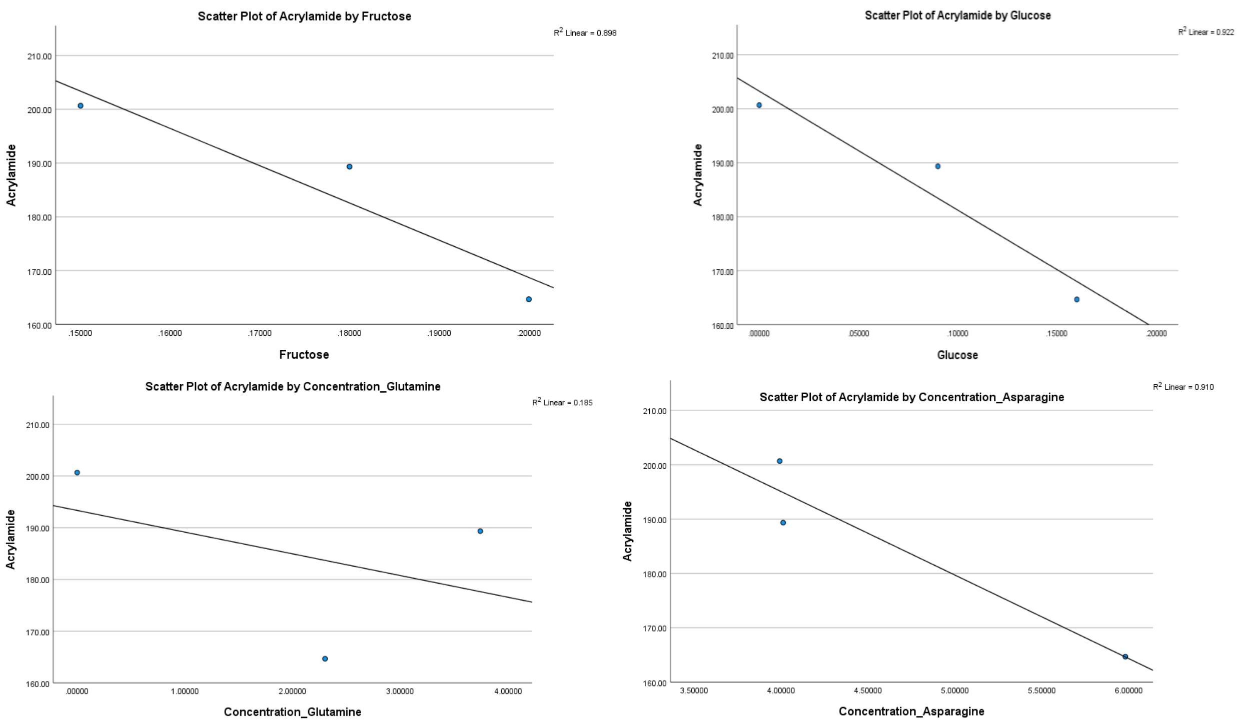

Figure 1. Scatter plots showing the correlation between acrylamide and its precursors in the prototype model **after** thermal treatment

3.2.3 ***Can the suggested prototype be a promising, healthy potato product?***

Given that the suggested potato-starch based prototype (Figure 2) had an acrylamide content that is lower than LOQ of the technique used in this study, it may be categorized as healthy as it might also be considered as a promising healthy potato snack. In fact our study presented several strengths. It is the first study to attempt to formulate a potato-starch product with characteristics that are somewhat similar to those of potatoes. It is worth mentioning that the crucial stage in making the prototype acrylamide-free is the use of an ideal combination of sugars and amino acids, along with the addition of sodium alginate. As a matter of fact, previous studies largely looked at how various factors in laboratory-based systems affected the production of acrylamide. However, based on our earlier research, that we thoroughly examined the impact of acrylamide's precursors in various potato-based models, in this current study, we intended to shed light on our prototype, which may become a promising replacement for unhealthy potato snacks. Our upcoming effort will concentrate on examining the prototype's organoleptic characteristics in order to create a healthy snack that everyone would enjoy. It is important to note that the current study's sample

size is modest, however we will take this into account in our future work.

Figure 2. Prototype model before and after thermal treatment

4. Conclusion

Prototype model contained no acrylamide after thermal treatment as compared to potato cultivars. Reducing sugars in the prototype showed a strong negative association with acrylamide formation and that might be attributed to the interference of the Na^{+} (sodium alginate) at some steps in the Maillard reaction. Glutamine might have inhibitory effect on acrylamide; hence lowering acrylamide content in our prototype. Hence, these models might also be classified as safe and can be used as healthy prototypes. Finally, the proposed potato-starch based prototype might be considered as a healthy promising potato snack, yet future work is needed to concentrate on its organoleptic characteristics.

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Chapter 9: GENERAL DISCUSION

1. **Keywords: Mitigation, detection, acrylamide**

Acrylamide is present in many foods that are consumed on a daily basis like bread, baby snacks, French fries and coffee (Mesias et al., 2019, 2022; Strocchi et al., 2022). The fact that urged EFSA, European Commission Regulation, and many other international organizations to set a daily intake limit level of acrylamide. The European Commission has issued a list of foods that should be monitored for their acrylamide content (González-Mulero et al., 2023). This has forced researchers to conduct studies to better understand the causes (condicitions of process, implicated precursors and chemical reaction) implicates of acrylamide formation through the major route Maillard reaction. Since acrylamide is present in a large number of processed foods, researchers, food producers and stakeholders worldwide have put ample efforts to mitigate or reduce acrylamide formation (Kumari et al., 2022) spanning from farm to fork., postharvest storage conditions and control to the content of acrylamide precursors. On the other hand, numerous studies have been conducted to significantly reduce acrylamide content in potato-based snacks like conventional blanching, incorporation of mono and di-valent cations in potatoes (Cirit et al., 2023). Furthermore, the use of asparaginase enzyme (Kumari et al., 2022), and polyphenols (Liu et al., 2015). Other steps can be made at the processing level that include manipulation of acrylamides' precursors (amino acids and reducing sugars), soaking raw potatoes in additive solutions like salt and citric acid. Controlling the time and temperature of cooking process, in addition to avoid deep frying in oil many times as the level of polyunsaturated fatty acids increase (Žilić et al., 2022). A recent study showed that new cassava breeding had not only economic benefits, but also can be a good alternative to french fries to obtain acrylamide free chips (Chancharoenchai & Saraithong, 2021).

Another aspect to considering the acrylamide evaluation is the detection and/or quantification techniques: **Which Method Is Best for Acrylamide Quantification?** The answer is unfortunately not decisive yet. It is well known over the past years that GC–MS and LC–MS were the gold standard for accurate quantification of acrylamide in food. However, several studies have shown that compounds available concomitantly to acrylamide in some products can interfere with the signal of acrylamide if LC–MS/MS analytical conditions are not appropriate, leading to overestimation (Delatour et al., 2022).

Hence, it is not conclusive which analytical method is better and this remains specific to the conducted study where all factors and parameters must be controlled and taken into account to maximize the results obtained and minimize overestimation of acrylamide.

Further to note that using LC-MS/MS some compounds were found to interfere with acrylamide detection such as valine, alanine, lactanamide and 3-aminopropionamide leading to overestimation of acrylamide from interferences where it can reach 40% in coffee or 20-fold in cocoa powder (Şenyuva & Gökmen, 2005). From here, the importance of finding a fast and easy detection technique such as NIR, FTIR and the use of different sensory approaches, is key to quantify the precursors in the raw material in order to predict their possible formation..

2. **Keywords: potato models, precursors (asparagine, glutamine, reducing sugars and sucrose), temperature, acrylamide**

To better understand the interaction of precursors (amino acids and sugars) on acrylamide formation. Synthetic potato models were formulated based on potato starch according with (Amrein et al., 2003) with a little modifications (Table S1, chapter 6). Results showed that acrylamide was detected in only 3 out of the 7 formulated synthetic potato models

For instance, after adjusting for temperature and frying process, only the following potato models,

Asn-GFS (β: 300.776, 95% CI: 41.595-559.957), p=0.027,

Glu/Fru (β: 258.286, 95% CI: -5.251-521.823), p=0.054),

Sucrose (β: 110.776, 95% CI: -148.405-369.957), p=0.363, have generated acrylamide.

Asparagine had been classified as a major contributor to acrylamide formation. This was explained when asparagine was thermally treated, whereby asparagine was converted into maleimide then to acrylamide via deamination and decarboxylation (Yaylayan et al., 2003). Similar findings were presented by (Yaylayan & Stadler, 2005; Rydberg et al., 2005; Hidalgo et al., 2009;Yang et al., 2016b; Henao Toro et al., 2022), explaining a strong positive correlation between the reducing sugars, asparagine on acrylamide formation. Our results showed supported previous findings where Asn-GFS, Glu-Fru, and sucrose potato models generated acrylamide (Halford et al., 2022; Hwang & Kwon, 2022; Orsák et al., 2022).

Based on our results, glutamine amino acid had shown not to be one of the major contributors to acrylamide formation. Furthermore, in the present study, acrylamide was not detected in all glutamine-based potato models (Glutamine-Glucose, Glutamine-Fructose, Glutamine-Sucrose and Glutamine-GFS) (7) Hence, this finding was supported by research that showed that glutamine amino acid when added alone or mixed with other ingredients did not contribute to the formation of acrylamide (Amrein et al., 2003). Moreover, similar findings showed that Glutamine and Glycine amino acids caused the highest reduction in the formation of acrylamide (76%), followed by Lysine (43%) and alanine (14%) (Champrasert et al., 2022). On the other hand, in endothelial cell metabolism, glutamine provided nitrogen for asparagine to sustain proper cellular homeostasis as demonstrated by Huang et al., (2017), the fact that shed the light on the indirect contribution of glutamine on acrylamide formation when present in a certain ratio when found concatinately with asparagine. For instance, in our work we have concluded that the degradation of asparagine is followed by the appearance of glutamine and vice versa, hence, there is an inter-conversion between glutamine and asparagine during the thermal treatment.

According to Halford et al.,(2022), glucose and fructose are the predominant reducing sugars in potato tubers, with very little maltose, and the ratio of glucose to fructose is also important. It is worth-mentioning that the ratio of glucose:fructose in real potatoes and synthetic models was 1:0.63, and 1:0.60; respectively. Both of these sugars contribute to the formation of acrylamide, but fructose has been shown to flavor the production of acrylamide over colour compounds during the cooking of French fries, in comparison with glucose. This is consistent with predictions obtained from our study where when all sugars mixed together (glucose-fructose-sucrose) had a different behavior than only when two sugars mixed together as in the case of (Glu-Fru) synthetic potato model. In the synthetic models, the amount of glucose reactant (0.1-0.4 $g.Kg^{-1}$DM) is higher than the amount of fructose (0.1-0.2 $g.Kg^{-1}$DM) reactant possibly due to the fact that glucose is an essential reducing sugar and might be more involved in the Maillard reaction than fructose (Figure 1). For instance, in our synthetic models after frying, around 71% of Fructose, 75% of Glucose and 83% of sucrose reacted. Sucrose model generated high acrylamide and this may be due to the fact that sucrose at high temperature (>186°C) undergoes hydrolysis that led to the release of fructose (sucrose metabolite) which participates in Maillard reaction leading to the formation of acrylamide. In Asn-GFS model, the reactant glucose amount (75%) led to the formation of acrylamide which might explain that glucose might be a main precursor among the sugars responsible for acrylamide formation. In some of the potato models, sucrose reactant disappeared after frying and contributed to acrylamide formation after deep frying at high temperature (190°C). Consequently, in line with previous finding, the contribution of sucrose to acrylamide formation remain restricted as compared to the powerful effect of reducing sugars. Therefore, glucose and fructose had higher reactivity as compared to sucrose, hence, reducing sugars remain the main precursors for acrylamide formation in potato tubers.

With respect to asparagine amino acid, in asparagine-based models, when it was added with only one sugar, acrylamide was not formed. However, in Asn-GFS when asparagine was mixed with glucose, fructose and sucrose altogether, huge amount of acrylamide (516.7 $\mu g.Kg^{-1}$DM). This finding was also proved by (Robert et al., 2004; Knol et al., 2005, 2010). In Asn-GFS (model 1), sucrose presence along with the reducing sugars might have increased acrylamide formation due to its hydrolysis (Amrein et al., 2004; Wolfenden & Yuan, 2008; Wang & Xu, 2014; Yang et al., 2016b).

Based on the literature, the frying temperature showed to have a significant role on acrylamide formation; however, in our present study, temperature has no significant correlation ($p>0.05$) with acrylamide formation in the synthetic models.

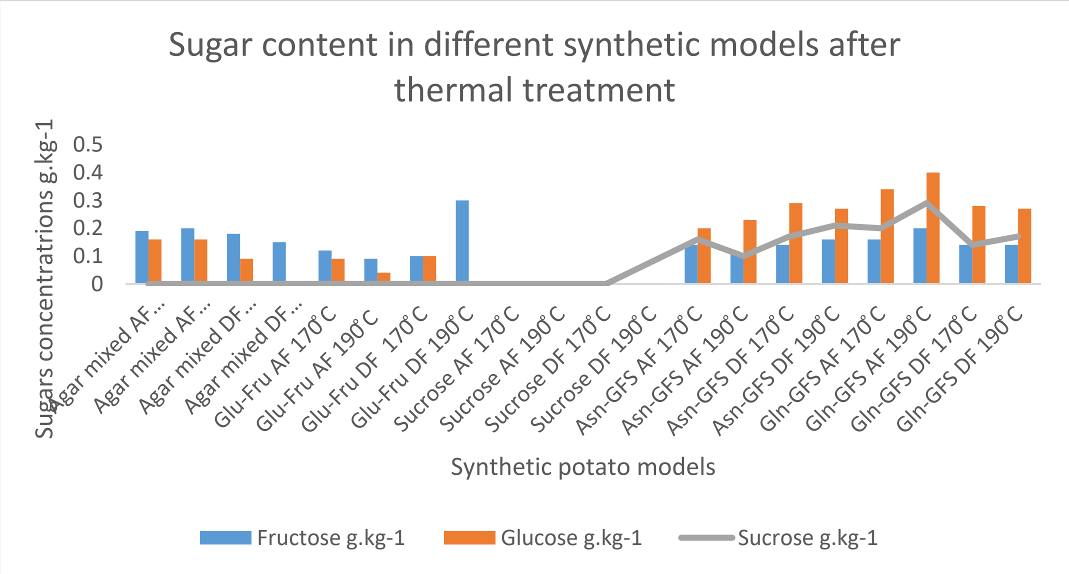

Figure 1. Shows the sugar content in the synthetic potato samples after thermal treatment

In the present study, the potato-starch prototype model was formulated using sodium alginate (4.8%) as a firming agent. Figure 2 in chapter 8, showed the final form of the prototype before and after thermal treatment. We developed an acrylamide-free prototype, and studied the association between acrylamide's precursors in the prototype and three potato cultivars (agria, Kennebec and Monalisa) and acrylamide formation. We believed that sodium alginate played an important role in acrylamide mitigation in our developed product. For instance, polysaccharides were reported to have inhibitory effect on acrylamide in food systems such as sodium alginate, pectin, and chitosan (Fang et al., 2022). This is achieved through the interference of Na^+ cation in sodium alginate with intermediates of acrylamide in Maillard reaction. Moreover, sodium alginate showed no hardening effect on the texture of the biscuits and increased the reducing power compared to chitosan and pectin (Fang et al., 2022). Similarly, coating potato chips with sodium alginate decreased oil uptake and mitigated acrylamide formation (Liu et al., 2020). Likewise, alginate reduced acrylamide formation in a reaction model made up of glucose and asparagine solution heated at 170 °C for 3 minutes (Champrasert et al., 2022).Therefore, sodium alginate may greatly reduce the formation of acrylamide in fried potato chips. Consequently, it might be a new approach to reducing acrylamide formation in industrially produced fried foods without compromising the primary consumer-valued quality attributes. Moreover, as demonstrated in our previous published paper, all glutamine-based synthetic models did not contain acrylamide after frying, this could explain that glutamine alone when mixed with sugars did not contribute to acrylamide formation; hence, it might not play alone a major role in Maillard reaction. Furthermore, all the synthetic models contained sodium alginate that proved to have inhibitory effect on acrylamide. Therefore, glutamine-based models along with the prototype had the healthiest combination of precursors leading to no formation of acrylamide after frying. However, the advantage of the prototype over the glutamine-based models was that it originally contained all the ingredients mixed together (sugars and amino acids) and had a very similar composition to real potoates, whereas, in glutamine-based models, asparagine was not added to the mixture and sugars were added separately.

3. Keywords: potato cultivars, precursors, temperature, thermal treatment, acrylamide

After studying the impact of precursors on acrylamide formation in **simple** synthetic potato starch-based models, we aimed at applying this on **complex food** potato cultivars. The main reason of this application was to associate the studied parameters in the simple models to the behavior of acrylamide's precursors in three potato cultivars. Hence, in line with our previous study,

Which potato cultivar produced more acrylamide?

Based on our results, Agria produced higher acrylamide concentration (441.6 $\mu g.Kg^{-1}DM$) > Kennebec (338.7 $\mu g.Kg^{-1}DM$) > Monalisa (205.6 $\mu g.Kg^{-1}DM$). This finding is consistent with previous results (Yang et al., 2016a, Orsák et al., 2022). (Figure 2)

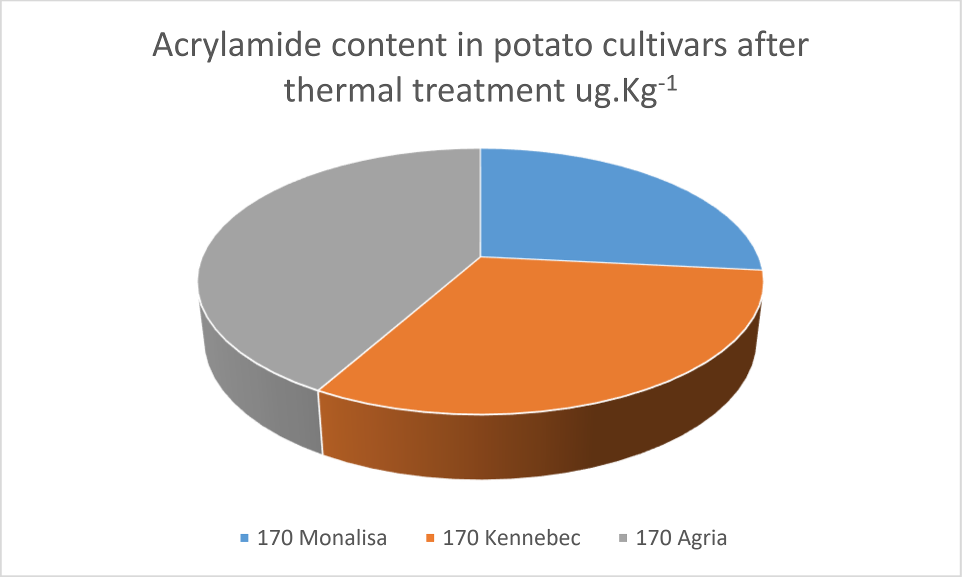

Figure 2: Acrylamide content (µg.Kg^{-1}DM) in potato cultivars after thermal treatment at 170°C, deep frying

Regarding the effect of sugars , it was concluded that in Agria, 20% sucrose, 71.1% of asparagine, and 89.5% of glutamine reacted . Furthermore, in Kennebec,only 32% of sucrose and 88 % of asparagine reacted. The higher amount of acrylamide produced in Agria as compared to Kennebec might be explained by the synergistic effect between glutamine and asparagine on acrylamide formation and that was also elaborated in another study (Devleeschouwer et al., 2009). On the other hand, in Monalisa, when all acrylamide's precursors reacted in different percentages among each other, acrylamide was formed in a very small amount. Hence, in potato cultivars, sucrose and asparagine seemed to play a major role on acrylamide formation (Figure 2). This finding was also supported in previous study (Wang & Xu, 2014). For instance, acrylamide content was studied in different potato cultivars than the ones we studied, yet results were in line with ours confirming the important role that sugars played in acrylamide formation (De Wilde et al., 2005). Similarly, another study performed on potato cultivars, showed that sugars (fructose, glucose, and sucrose) were strongly correlated with acrylamide formation (Orsák et al., 2022). Furthermore, it has been shown that the sugar content of potato cultivars correlated significantly as demonstrated by (Mesias et al., 2019).

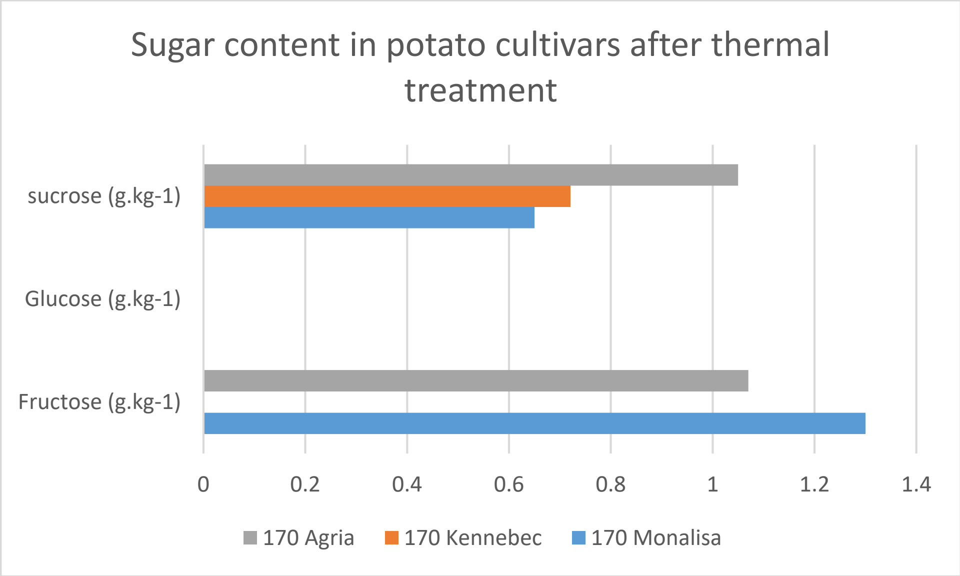

Figure 3: Sugar content (g.Kg^{-1}DM) in potato cultivars after thermal treatment (170 ºC). Glucose was not detected in the cultivars after deep frying, it reacted all.

4. Keywords: NIR, GC-FID, acrylamide, validation

Although the techniques normally used to quantify acrylamide have been GC-MS and LC-MS, as already mentioned, they are long and expensive techniques normally used in research laboratories or laboratories dedicated to food analysis. The use of non-destructive techniques such as NIR and obtaining results in a short time (although its calibration is laborious) can be a great solution for the food industry as a process control system or raw materials.

In order to simplify the detection of acrylamide in potato based snacks in potato production companies, we thought of validating the results obtained from GC-FID with NIR spectrophotometry. Based on our results, NIR spectra showed that in our potato samples (synthetic potato models and real potato cultivars), peaks at around 4439-4477 cm^{-1} were found to be present in common amongst all the samples, hence corresponding to acrylamide peak. Another justification of acrylamide's peak was also demonstrated by Skinner et al., (2021) who showed that acrylamide's peaks were found in these ranges (5000–5500 cm^{-1}) and (6000–6500 cm^{-1}) which are close to our findings with slight shifts Moreover, similar range of peaks indicating acrylamide presence were revealed by. With respect to our samples, when comparing the spectral plots of standard acrylamide with those of the synthetic potato models and real potatoes, the common peaks corresponding to acrylamide were as follows: 4439 cm^{-1} in standard acrylamide, 4477 cm^{-1} in real potato tubers, and 4466 cm^{-1} in synthetic potato models which all fall in almost the same range of acrylamide peaks as demonstrated by previous studies. In the past, for instance, NIR was used to detect sugars in potatoes; results showed a strong correlation for sucrose and a weaker correlation for glucose (Rady & Guyer, 2015). However, the primary objective of our study was to employ this method to locate acrylamide in potato samples. Our study might be the first to use NIR spectroscopy to compare and detect acrylamide in real and synthetic potato models. The comparison of the synthetic potato models formulated based on the same composition of potatoes (Amrein et al., 2003) with potato cultivars assisted with better recognition of acrylamide utilizing the proposed methods. Then, to determine a No risk level of acrylamide in potato based items that could be useful to the food production sector to screen acrylamide content of the delivered food sources and label them as healthy items. To conclude, our findings might open the door to other scientists to further validate the results; hence, facilitating the detection of acrylamide in potato based snacks at the production level in order to protect consumers' health from its detrimental effects.

Chapter 10: General Conclusion

Based on the experimental results, the general conclusions are summarized below:

1) **Effect of precursors, temperature and thermal process in synthetic potato models**

- Deep frying technique significantly ($p<0.05$) increased acrylamide concentration and oil uptake percentage in potato models compared to air frying.
- Temperature (170ºC -190ºC) did not significantly affect ($p<0.05$) sugar concentration, precursor formation or oil uptake percentage in the studied potato models. Increasing glucose and asparagine content in the synthetic potato strips increased the acrylamide content.
- The interaction of sugars with asparagine yielded to high acrylamide formation in Glucose-Fructose, Sucrose, and Asn-GFS models. In contrast, the role of glutamine in synthetic potato models did not affect acrylamide formation regardless of the type and combination of the sugars that were initially present in these glutamine-based models.
- The use of sodium alginate as a firming compound in the potato models might have an inhibitory effect on acrylamide formation. From here, we conclude that the composition of the potato models in terms of type and quantity of main precursors is of great importance in order to mitigate the formation of acrylamide after thermal treatment.
- Agar mixed model was formulated to serve as a **prototype** to real potato that naturally contains reducing sugars and amino acids in optimal amounts that do not react to produce acrylamide

2) **Comparation between synthetic potato models *vs* potato cultivar**

- Acrylamide content was affected mainly by the composition of sugars rather than amino acids, where glucose, fructose and sucrose can be considered as main contributors in the Maillard reaction. Moreover sucrose showed to have a major impact on acrylamide formation in all the samples (synthetic models and potato cultivars).
- Sugars were considered key and critical factors for acrylamide formation in potatoes and remained the most practical way of reducing its production in potato products. Thus, this might be achieved either by optimization of cultivars or controlling the storage conditions of potatoes.

3) Identification and quantification of acrylamide

- The GC-FID technique proved to be an alternative method that produced reliable results for acrylamide detection in potatoes. The results obtained through GC-FID fell within the range of acrylamide concentrations that were typically found in actual potatoes, implying that this method can be effectively used as a substitute for the traditional techniques. Overall, GC-FID provided an accurate and efficient method for the detection of acrylamide in potatoes. Its availability as a testing technique for acrylamide detection can allow for more widespread monitoring of this potentially harmful chemical, to ensure food safety and reduce health risks for consumers.
- NIR (Near Infrared) spectroscopy was found to be a rapid, efficient, and convenient technique for qualitative detection of acrylamide levels in fried potatoes. It is considered a suitable complementary method for monitoring acrylamide levels during production. However, to ensure its robustness, further repetitions using a wider range of cultivars would be required. Overall, NIR presents a promising potential as an effective control technique for acrylamide detection in the food industry.

4) Healthy snack products with minimum acrylamide content

- The results of this study can be valuable for the potato-based food industry in producing healthier potato chips with minimal acrylamide formation during the frying process, under the condition that the levels of acrylamide precursors in the raw potatoes are comparable to the Monalisa and prototype model. By utilizing this information, food manufacturers can reduce the amount of acrylamide in their products, providing healthier options for their consumers.

5. Future perspectives

- This thesis tackled the major parameters that affect acrylamide formation in synthetic potato starch based models and real potatoes after frying. The formulation of the simple potato models facilitated the study of the major factors leading to acrylamide development, hence, applying the outcomes on real potatoes.
- The prototype/control model was found to be the healthiest among all models and even potato cultivars, the fact that makes it a potential product to be developed in the future as it did not contain acrylamide after frying.
- NIR-spectroscopy presented a fast, practical, and convenient complementary technique to qualitatively detect acrylamide content in fried potatoes. Therefore, its application in potato snack industry might facilitate the detection of acrylamide in the final products with the least complications for optimum results.