ACRYRED

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Acrylamide in Cereal Products: Progress and Prospects

June 3rd - 4th 2026

Splendid Hotel La Torre, Palermo, Italy

BANDIRMA ONYEDI EYLUL UNIVERSITY
FACULTY OF HEALTH SCIENCES
NUTRITION AND DIETETICS DEPARTMENT

Impact Of Air-Fryer Technology On The Formation Of Heat-Induced Contaminants In Cereal-Based Biscuits

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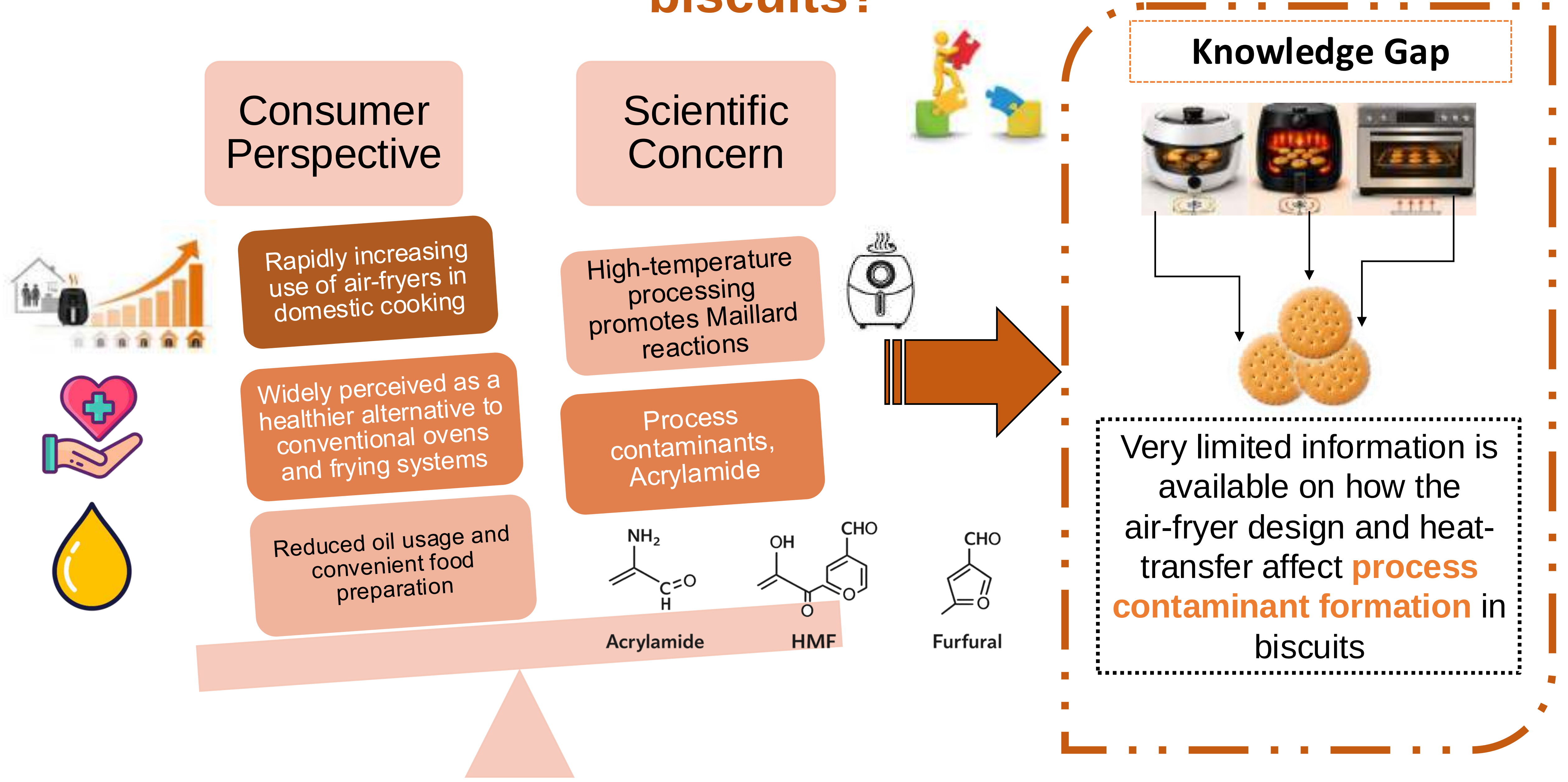
Why are air fryers attracting scientific attention for baking biscuits?



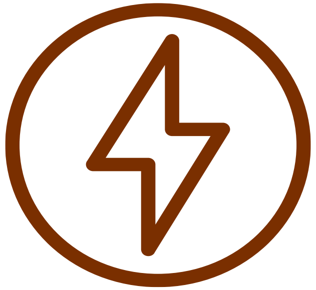
Widely consumed worldwide

appealing sensory properties

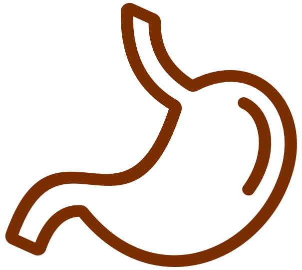
Convienence and long shelf life

popular snack across all age group

Nutritional potential



energy dense



quick satiety

added nutritional value

formulated with whole grains, fibre, and functional ingredients

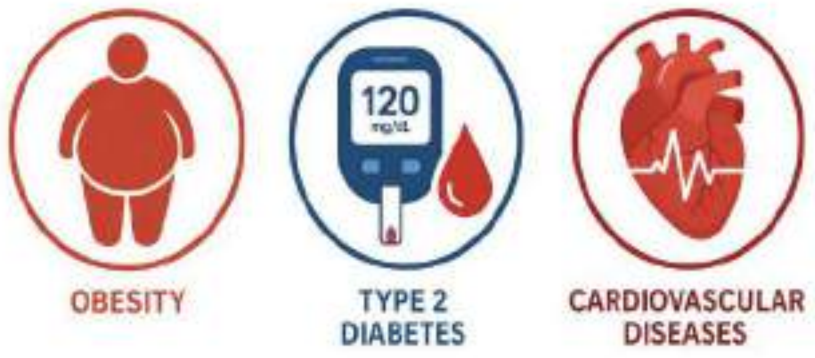
- Martins, A. P., Levy, R. B., Claro, R. M., Moubarac, J. C., & Monteiro, C. A. (2013). Increased contribution of ultra-processed food products in the Brazilian diet (1987–2009). Revista de Saúde Pública, 47(4), 656–665. https://doi.org/10.1590/S0034-8910.2013047004968
- Chavan, R. S., Sandeep, K., Basu, S., & Bhatt, S. (2016). Biscuits, cookies, and crackers: Chemistry and manufacture. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of food and health* (pp. 437–444). Academic Press.
- Goubgou, M., Songré-Ouattara, L. T., Bationo, F., Lingani-Sawadogo, H., Traoré, Y., & Savadogo, A. (2021). Biscuits: A systematic review and meta-analysis of improving the nutritional quality and health benefits. Food Production, Processing and Nutrition, 3(1), 26. https://doi.org/10.1186/s43014-021-00071-z
- Blasi, E., Rossi, E. S., Pietrangeli, R., Nasso, M., Cicatiello, C., Palombieri, S., & Sestili, F. (2024). Functional biscuits, a healthy addition to your coffee break—Evaluating consumer acceptability and willingness to pay. Foods, 13(11), 1731. https://doi.org/10.3390/foods13111731

Nutritional concerns

high in sugar

saturated fat

refined flours



During baking

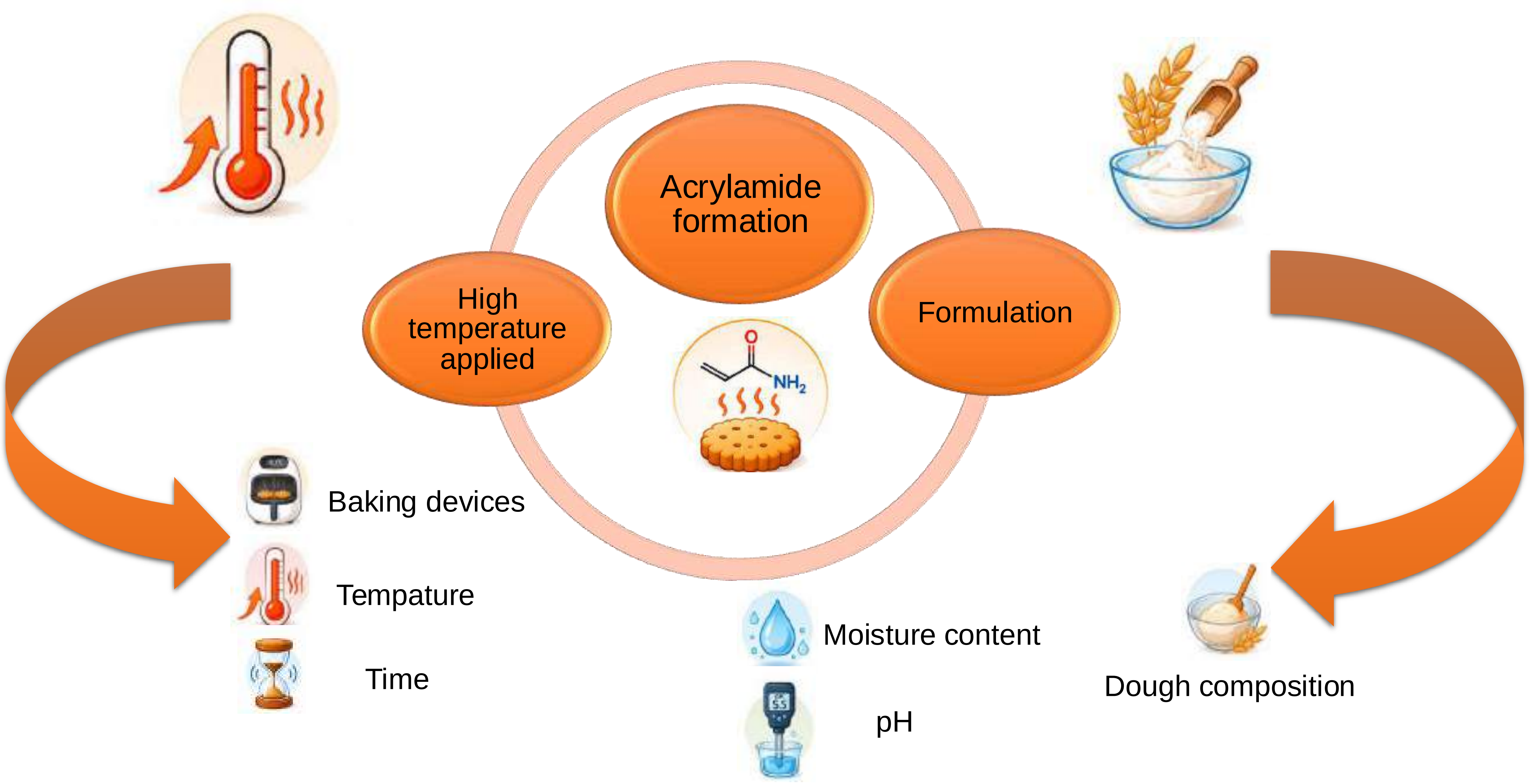
*High Temperature Applied
*Formulations
promote Maillard reactions

Acrylamide formation?

- Rippe, J. M., & Angelopoulos, T. J. (2016). Relationship between added sugars consumption and chronic disease risk factors: Current understanding. Nutrients, 8(11), 697. https://doi.org/10.3390/nu8110697
- Gaesser, G. A. (2019). Perspective: Refined grains and health: Genuine risk, or guilt by association? Advances in Nutrition, 10(3), 361–371. https://doi.org/10.1093/advances/nmy104
- Sanders, L. M., Zhu, Y., Wilcox, M. L., Koecher, K., & Maki, K. C. (2021). Effects of Whole Grain Intake, Compared with Refined Grain, on Appetite and Energy Intake: A Systematic Review and Meta-Analysis. Advances in Nutrition, 12(4), 1177–1195. https://doi.org/10.1093/advances/nmaa178
- Capuano, E., & Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT–Food Science and Technology, 44(4), 793–810. https://doi.org/10.1016/j.lwt.2010.11.002

Air

Nicolosi, A., Laganà, V. R., & Di Gregorio, D. (2023). Habits, health and environment in the purchase of bakery products: Consumption preferences and sustainable inclinations before and during COVID-19. Foods, 12(8), 1661. https://doi.org/10.3390/foods12081661



- Taeymans et al. (2004). A review of acrylamide: An industry perspective on research, analysis, formation, and control. Critical Reviews in Food Science and Nutrition, 44(5), 323–347. https://doi.org/10.1080/10408690490478082
- Peivasteh-Roudsari, L., Marziyeh, K., Barzegar-Bafrouei, R., Samiee, S., Karami, H., Tajdar-Oranj, B., Mahdavi, V., Alizadeh, A. M., Sadighara, P., Oliveri Conti, G., & Mousavi Khaneghah, A. (2024). Toxicity, metabolism, and mitigation strategies of acrylamide: A comprehensive review. International Journal of Environmental Health Research, 34(1), 1–29. https://doi.org/10.1080/09603123.2022.2123907

Moisture loss

Surface Temperature

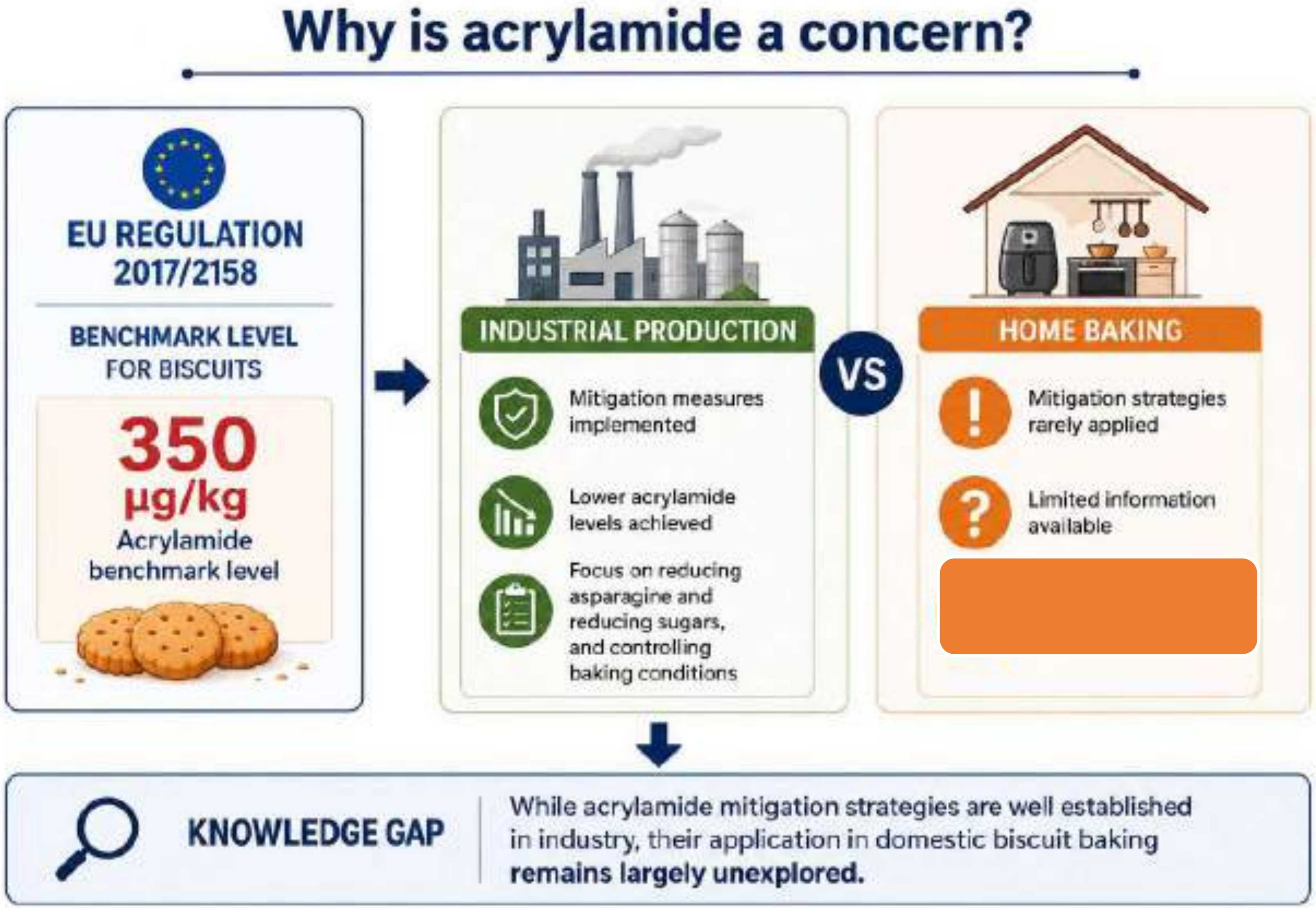
Maillard-driven contaminant formation

Potential Health Risk of Acrylamide

vary considerably across domestic cooking appliances

- **“Possibly carcinogenic to humans”** compound by the **International Agency for Research on Cancer (IARC)** in 1994 and classified in **group 2A.**
- The European Commission has classified acrylamide **in Category 1B as a carcinogen and mutagen**, and in **Category 2 as a reproductive toxicant**.
- The European Chemical Agency (2022) included acrylamide in the List of Substances of **Very High Concern**

- International Agency for Research on Cancer (IARC). ACRYLAMIDE (Group 2A). 1994. Available online: https://inchem.org/ documents/iarc/vol60/m60-11.html
- Hogervorst, J.G.; Schouten, L.J. Dietary acrylamide and human cancer; even after 20 years of research an open question. Am. J. Clin. Nutr. 2022, 116, 846–847.
- National Toxicology Program. Toxicology and carcinogenesis studies of acrylamide (CASRN 79-06-1) in F344/N rats and B6C3F1 mice (feed and drinking water studies). Natl. Toxicol. Program Tech. Rep. Ser. 2012, 575, 1–234.
- The European Chemical Agency. One Hazardous Chemical Added to the Candidate List. Available online: https://echa.europa.eu/-/one-hazardous-chemical-added-to-the-candidate-list
- European Commission. Commission Regulation (EU) 2017/2158 of 20 November 2017 Establishing Mitigation Measures and Benchmark Levels for the Reduction of the Presence of Acrylamide in Food. Available online: https://eur-lex.europa.eu/eli/reg/2017/2158/oj



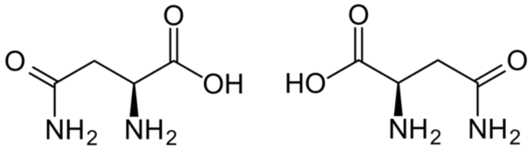
- EC (European Commission). (2017). Commission regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. Official Journal of the European Union, L304, 24–44. http://data.europa.eu/eli/reg/2017/2158/oj
- Mesías, M., Morales, F.J., & Delgado-Andrade, C. (2019). Acrylamide in biscuits commercialised in Spain: a view of the Spanish market from 2007 to 2019. Food & Function, 10, 6624. https://doi.org/10.1039/C9FO01554J

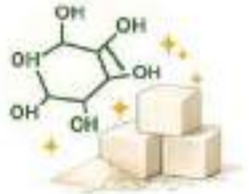
Acrylamide Levels in Home-Baked Biscuits?

Variations in cooking appliances

Differs in the Thermal environment and Moisture dynamics

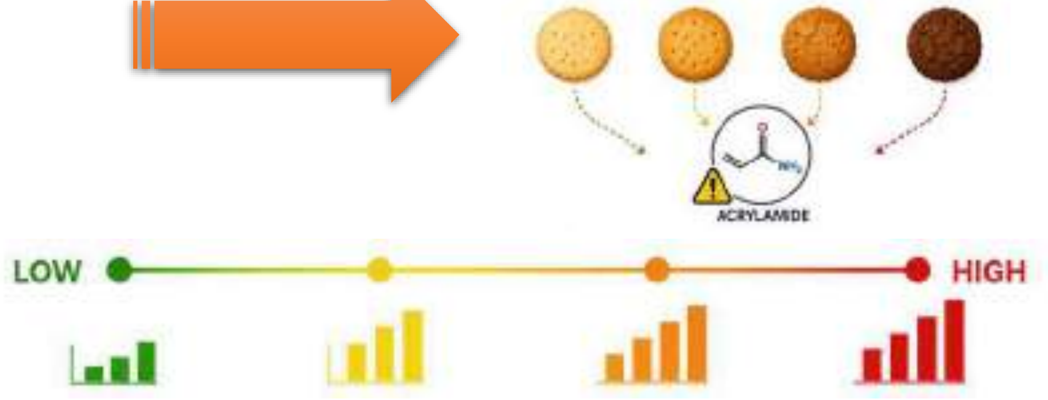
Choice of raw material





Whole grain cereals
Pseudocerals flours

Free asparagine + Reducing sugar



Variable in acrylamide levels

- Verma, V., Singh, Z., & Yadav, N. (2025). Optimization of the combination of different flours to minimize the acrylamide and hydroxymethylfurfural and evaluation of physicochemical properties in biscuits by using a D-optimal mixture design. Food and Humanity, 5, 100631. https://doi.org/10.1016/j.foohum.2025.100631
- Taeymans et al. (2004). A review of acrylamide: An industry perspective on research, analysis, formation, and control. Critical Reviews in Food Science and Nutrition, 44(5), 323–347. https://doi.org/10.1080/10408690490478082
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- Çelik, E. E., & Gökmen, V. (2020). Formation of Maillard reaction products in bread crust-like model system made of different whole cereal flours. European Food Research and Technology, 246(6), 1207–1218. https://doi.org/10.1007/s00217-020-03481-4.
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- Téllez-Morales, J. A., Rodríguez-Miranda, J., & Aguilar-Garay, R. (2024). Review of the influence of hot air frying on food quality. Measurement: Food, 14, 100153. https://doi.org/10.1016/j.meafoo.2024.100153

Objective of Study

To evaluate the formation of **acrylamide** in **cereal-based biscuits** baked using different **air-fryer technologies** and a **conventional oven** under conditions that mimic typical domestic practices

Can air fryers really help us prepare safer biscuits at home?

The overarching goal was to identify potential risks associated with air-fryer use and to provide consumers with evidence-based recommendations for preparing healthier, safer biscuits at home.

Raw Material

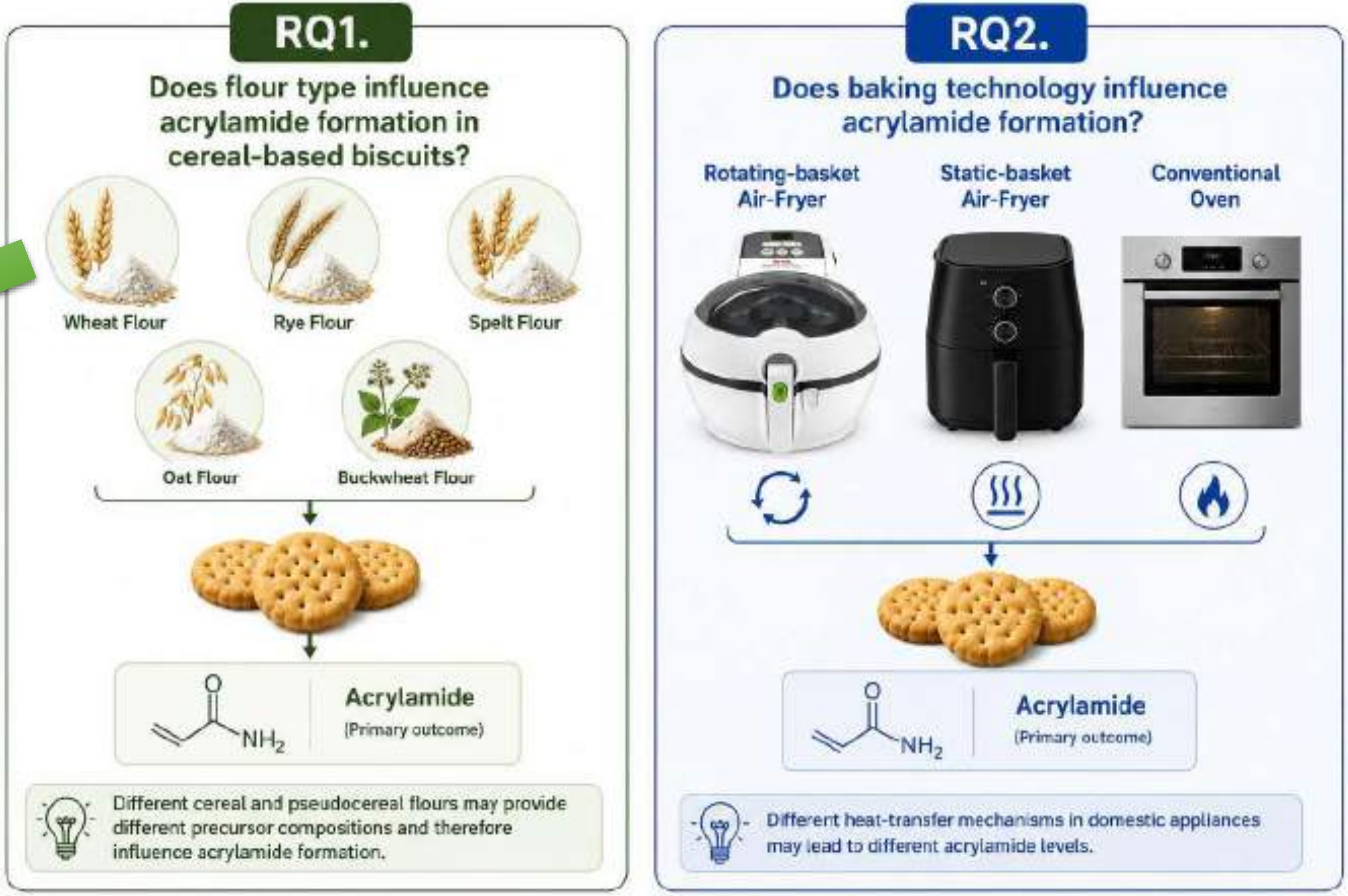
Processing Technology

Refined Wheat (Triticum aestivum)

Whole Wheat Flour (Triticum aestivum)

<u>Whole Grains</u>

- Rye (Secale cereale),
- Spelt (Triticum spelta),
- Oat (Avena sativa),
- Buckwheat (Fagopyrum esculentum)



Study Design and Biscuit Preparations

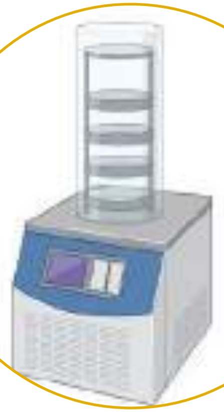
Formulation (AACC Method 10–54) Dough Composition (per batch)

- 64 g shortening
- 70 g granulated sugar
- 2 g salt
- 1.6 g sodium bicarbonate $NaHCO_3$
- 0.8 g ammonium bicarbonate NH_4HCO_3
- 30 g distilled water
- 160 g sifted flour (variable)

Processing Conditions

- Mixed with an **artisan KitchenAid (model 5KSM125)** stand mixer under controlled conditions
- **Rested** at room temperature: **30 min**
- **Rolled out and cut into uniform disc**
- **Standardised size**: 5 cm diameter × 3 mm thickness (experimental consistency)
- Identically scaling for all samples



Lyophilization

Samples were first lyophilised to ensure

(i) moisture removal

(ii) stabilise the matrix for subsequent chemical analyses

Key Maillard Precursors analysis

High-Performance Anion-Exchange Chromatography With Pulsed Amperometric Detection (HPAEC-PAD)

Reducing Sugars

- Reducing sugars (glucose, fructose, and sucrose) were measured as carbonyl precursors.

ANALYSIS OF SUGARS:
SAMPLE PREPARATION AND HPAEC-PAD QUANTIFICATION

1 EXTRACTION

For each sample, **200 mg** were vortexed for 30 min with **9 mL** of Milli-Q water at **50 °C and 1 mL** of sorbitol (0.5 mg/mL) used as internal standard.

9 mL Milli-Q water | 1 mL Sorbitol (0.5 mg/mL) | 50 °C

2 INCUBATION

The mixture was incubated in a Vortemp (UniEquip) for **1 h at 50 °C** and **900 rpm**.

50 °C | 900 rpm | 1 h

3 REFRIGERATION

The samples were allowed to rest for **30 min** in refrigeration.

30 min

4 CENTRIFUGATION

The samples were centrifuged (**9000×g** for **20 min**) at **4 °C**.

9000×g | 20 min | 4 °C

5 FILTRATION

The resulting supernatant was filtered through a **0.2 µm** filter and collected in an HPLC vial.

0.2 µm

6 HPLC VIAL

The filtrate was transferred to an HPLC vial for HPAEC-PAD analysis.

HPAEC-PAD ANALYSIS

INSTRUMENTATION

High-Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection (HPAEC-PAD)

Ion chromatography system (Metrohm AG, CH) equipped with 945 Professional Detector Vario, Metrohm AG, CH.

COLUMN

Metrosep Carb 2 column (250 × 4 mm µm, Metrohm AG, CH)

MOBILE PHASE

300 mM sodium hydroxide and 1 mM sodium acetate

OPERATING CONDITIONS

- Flow rate: **0.5 mL/min**
- Injection volume: 10 µL
- Detection: Pulsed Amperometric Detection (PAD)

QUANTIFICATION

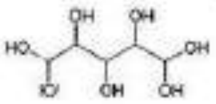
Glucose

Fructose

Sucrose

Maltose

Glucose, fructose, sucrose and maltose were quantified by an internal standard method using the sugar/sorbitol responses.

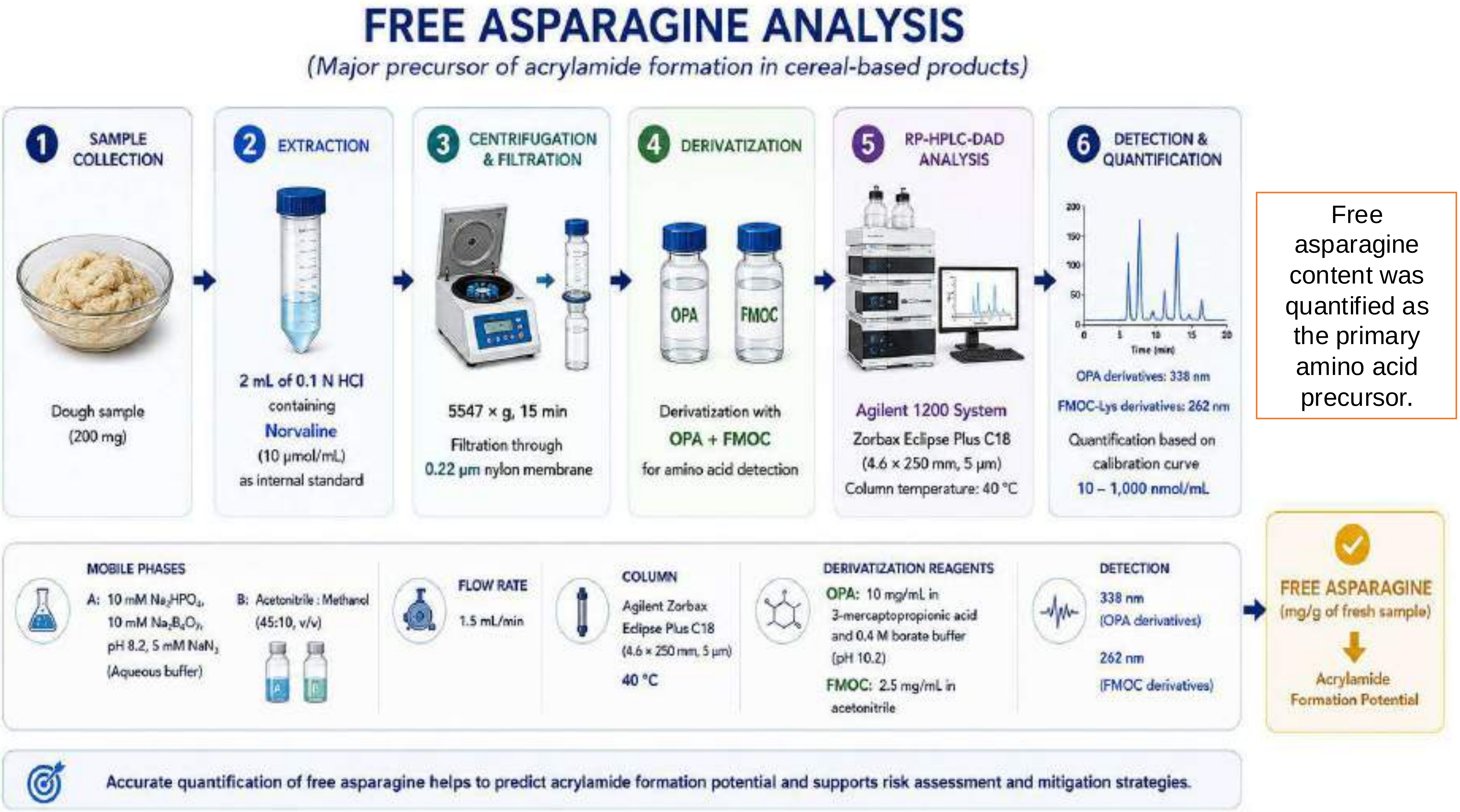


Sorbitol

RESULTS EXPRESSION AND CALCULATIONS

- Results were expressed as mg/g fresh sample.
- **Free reducing sugars** were calculated as the sum of glucose and fructose.
- **Total reducing sugars** also included sucrose and maltose.

PERFORMANCE | The limit of quantitation (LOQ) was set at **0.20 mg/g.** | Results were expressed as mg/g fresh sample.



Free asparagine content was quantified as the primary amino acid precursor.

1

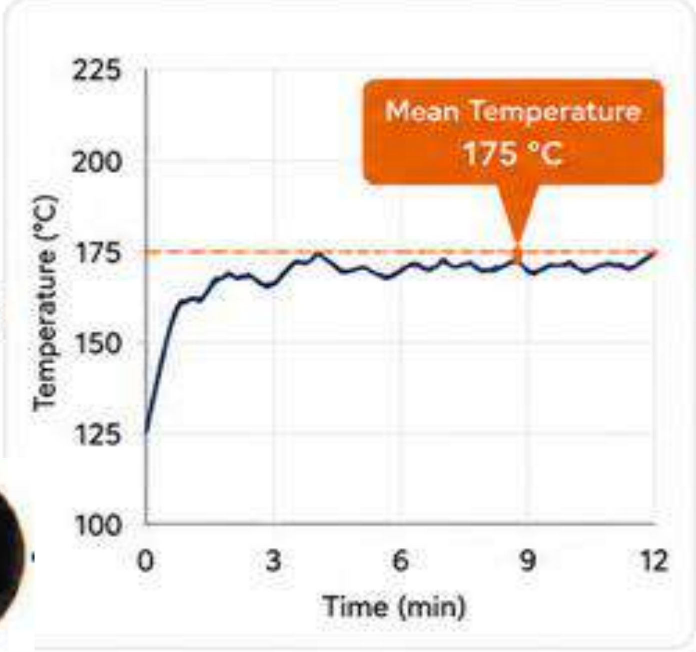
TEMPERATURE ASSESSMENT
Rotating Basket Air-Fryer

2

TEMPERATURE MEASUREMENT
External Probe Monitoring

3

AVERAGE OPERATING TEMPERATURE ESTABLISHED



The operating temperature of the rotating basket air-fryer was initially assessed using an external probe to estimate the mean working temperature of the device.

Temperature was monitored during the preheating and cooking phase. The average (mean) operating temperature was calculated.

Based on these measurements, an average operating temperature of 175 °C was established for biscuit preparation.

preheated to the target temperature before placing the samples inside

Rotating-basket air-fryer, top-mounted heating element, 1400 W, no allowed temp control

Static-basket air-fryer fixed upper resistance and high-velocity airflow, 1500 W, adjustable temp control

Convective, air-forced, electric oven, 53 L internal capacity

Eight biscuits were prepared in duplicate

Color Determination

a portable Konica Minolta CM-3500D spectrophotometer (Konica Minolta, Osaka, Japan)

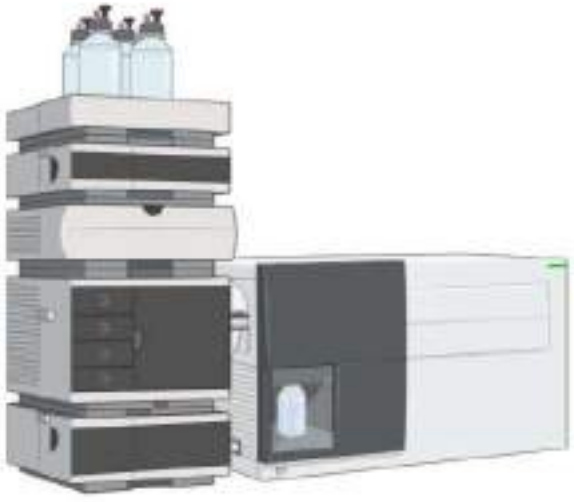
operating in the CIELAB colour space

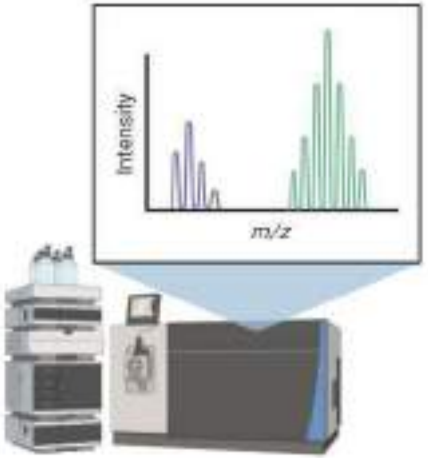
- Measured on the surface in both doughs and biscuits (top and bottom)
- Four separate measurements of
- L* (lightness),
- a* (redness) and
- b* (yellowness) were taken at different spots on the food surfaces to evaluate colour distribution.
- E index calculated as= $(L^*2 + a^*2 + b^*2)^{1/2}$

Acrylamide determination by LC-ESI-MS/MS

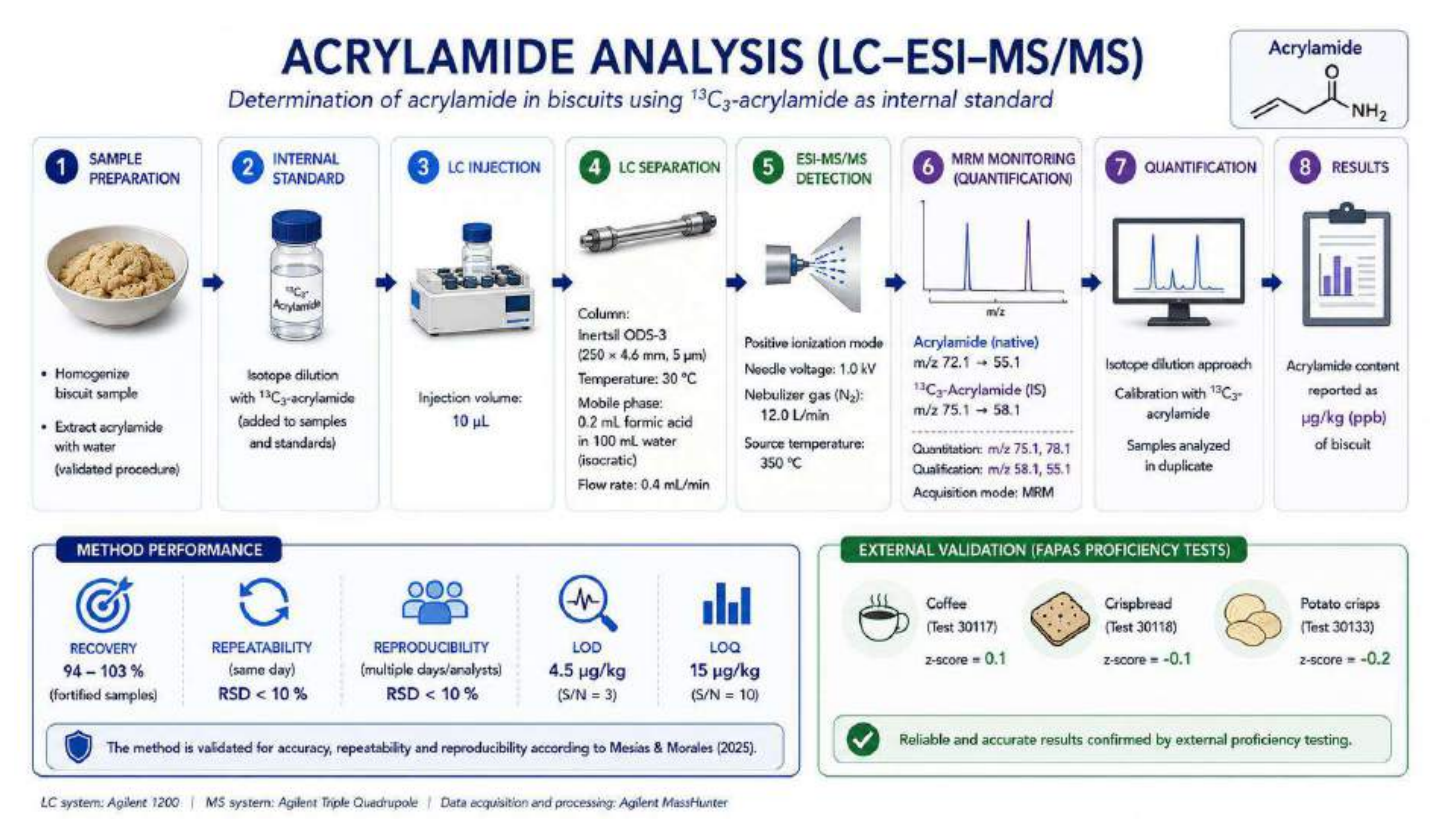
**Determination of acrylamide by LC-ESI-MS/MS (liquid chromatography coupled with electrospray ionization tandem mass) spectrometry using $^{13}C_3$-acrylamide as Internal Standard

**Validated isotope-dilution LC/MS-MS method with external proficiency verification



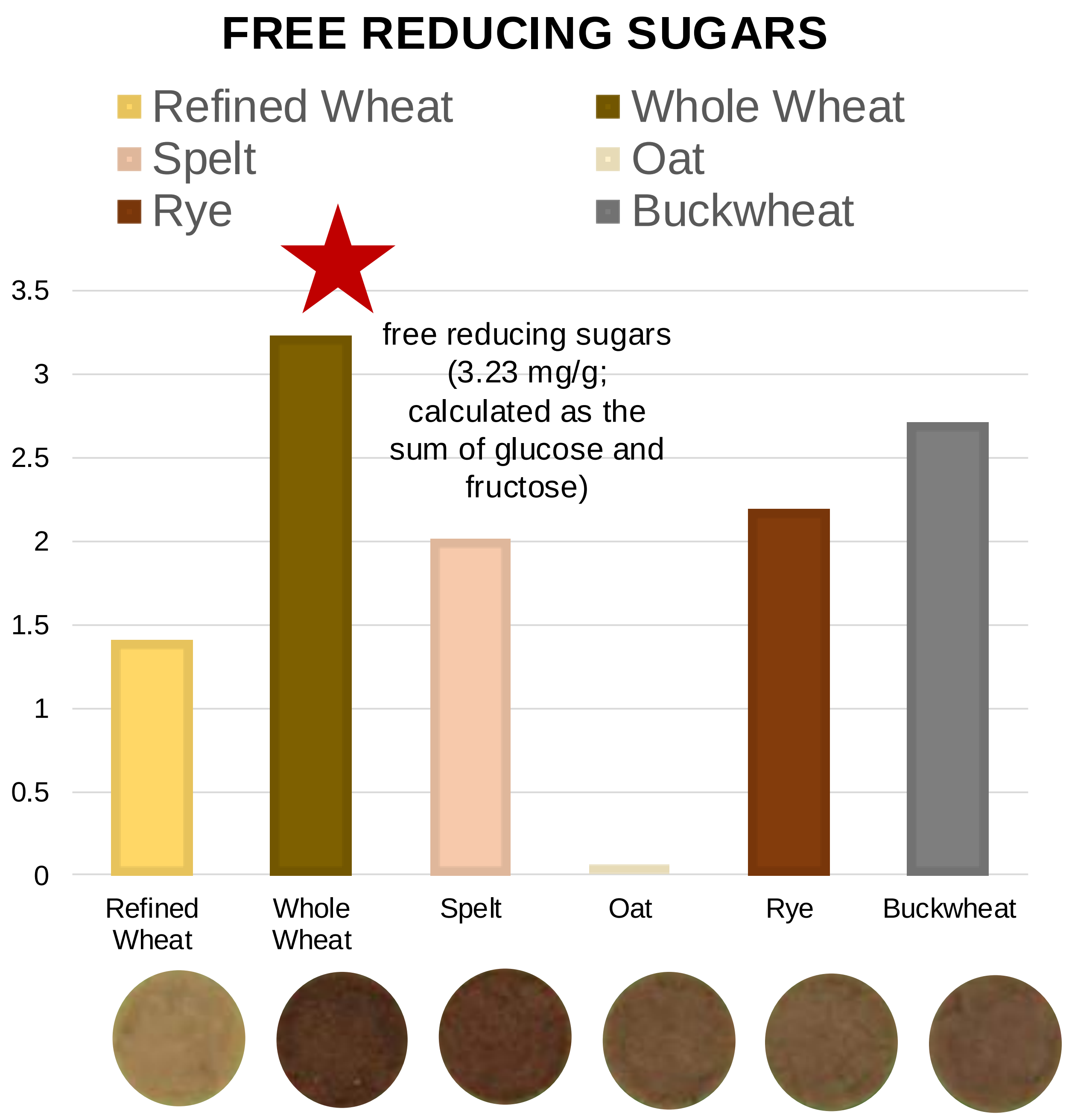


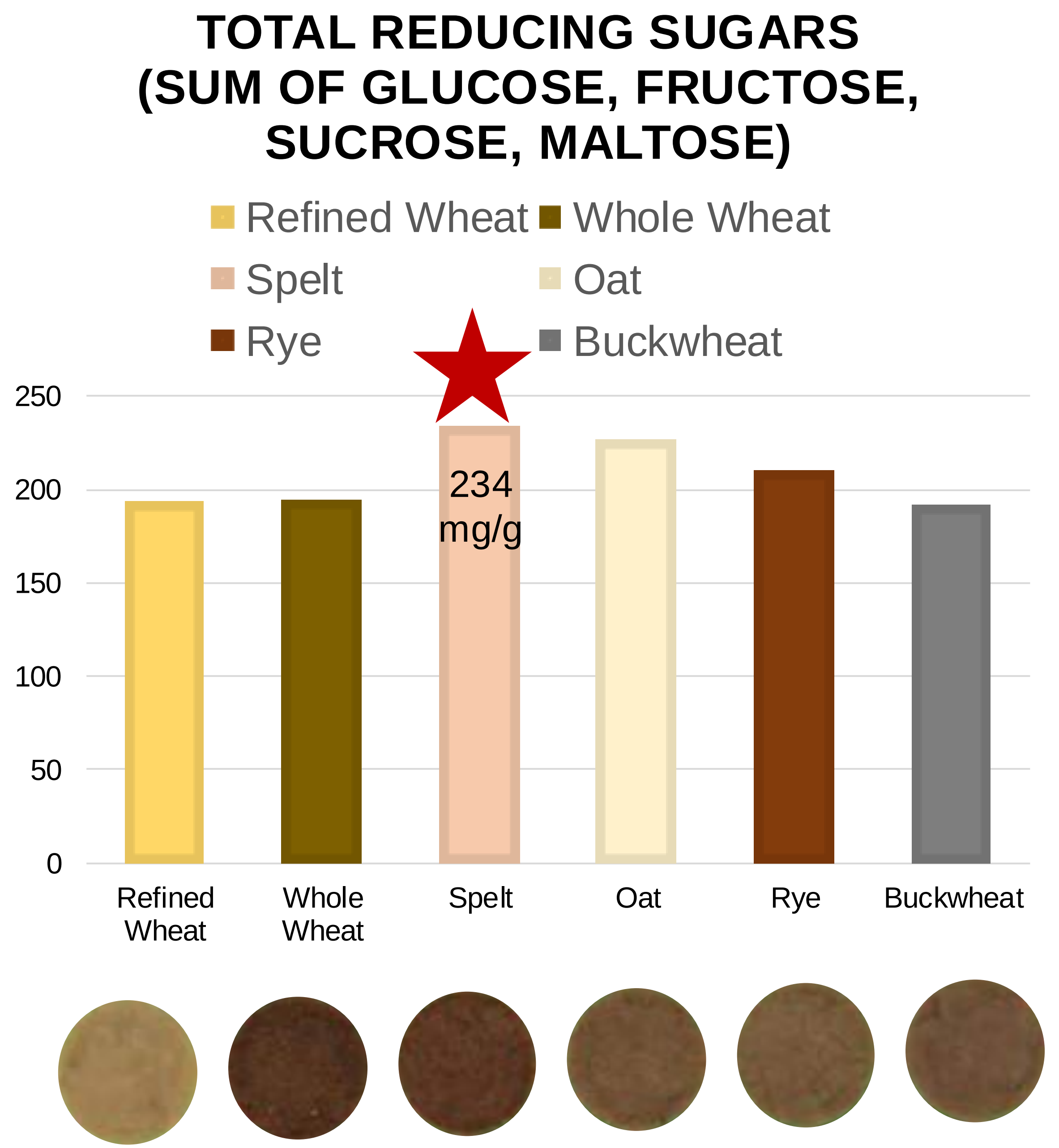
Mesias, M., & Morales, F. J. (2025). Acrylamide- and hydroxymethylfurfural-forming capacity of alternative flours in heated dough systems. Foods, 14(9), 1597. https://doi.org/10.3390/foods14091597

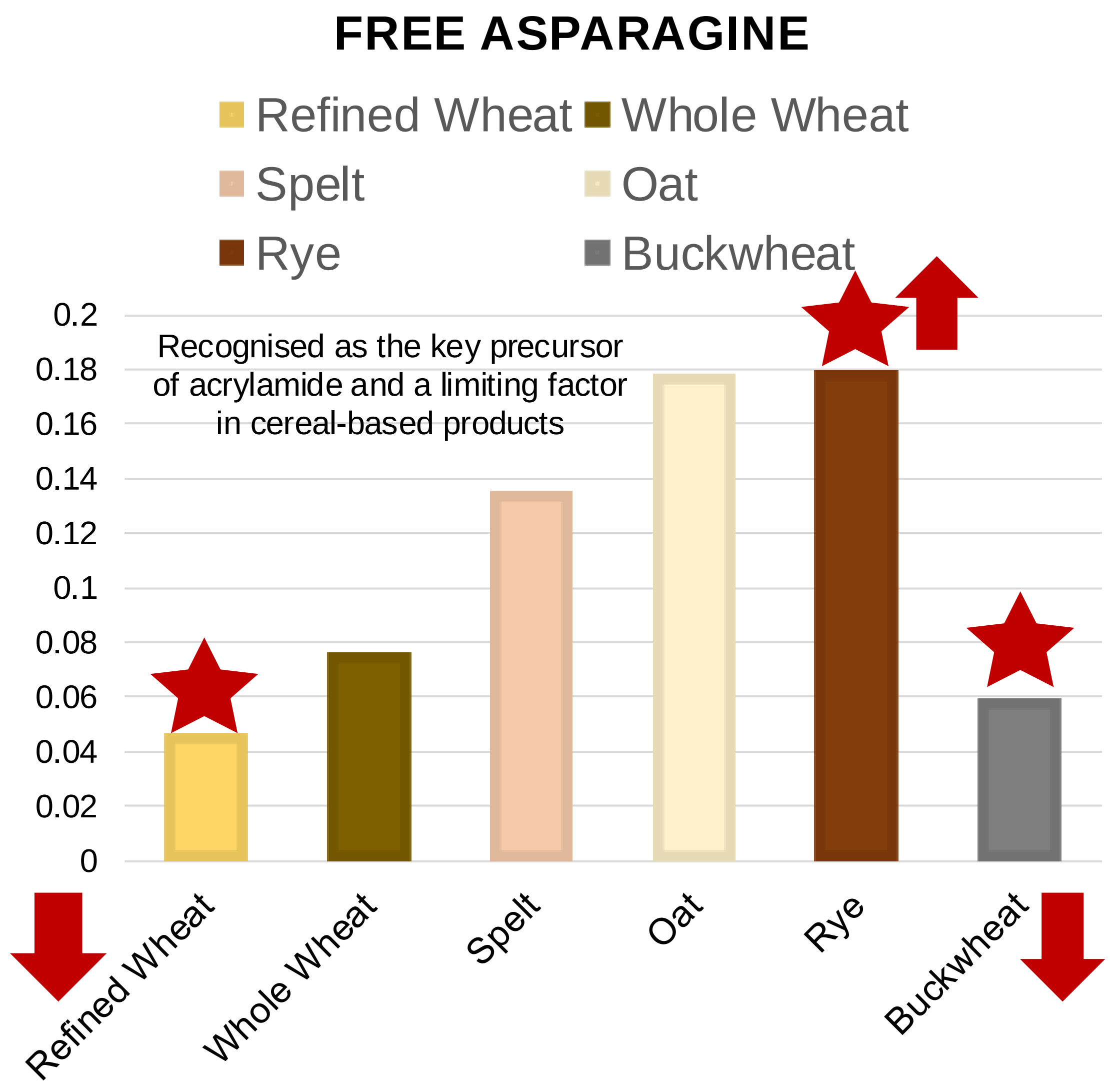
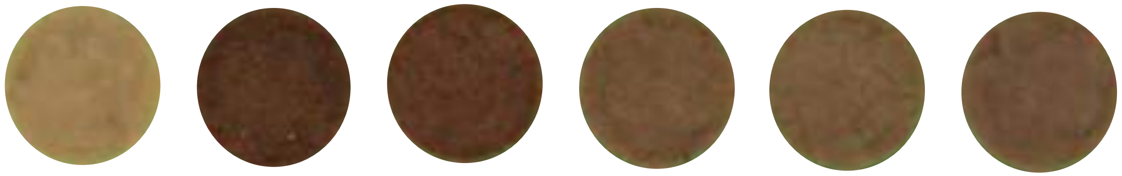
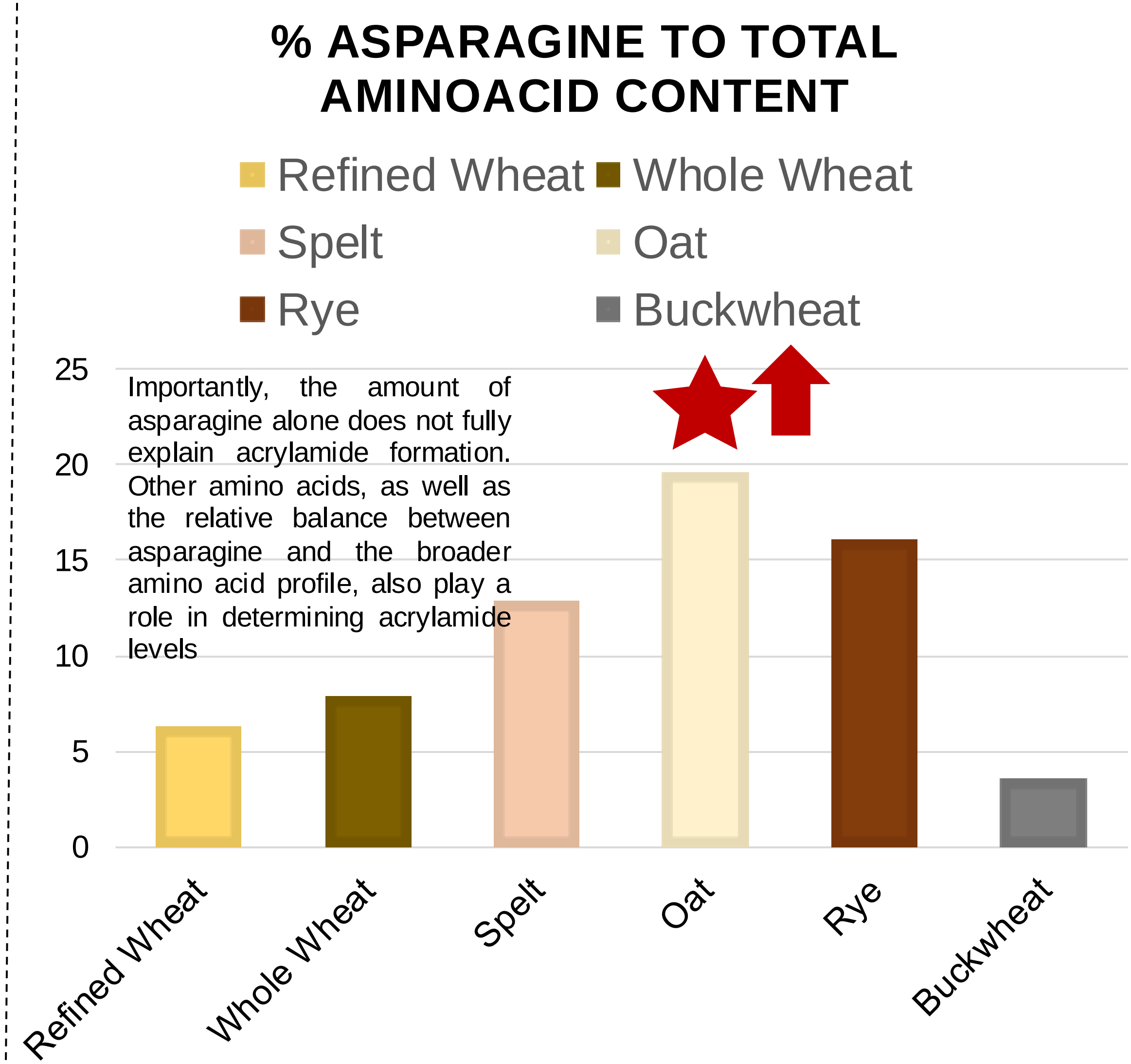
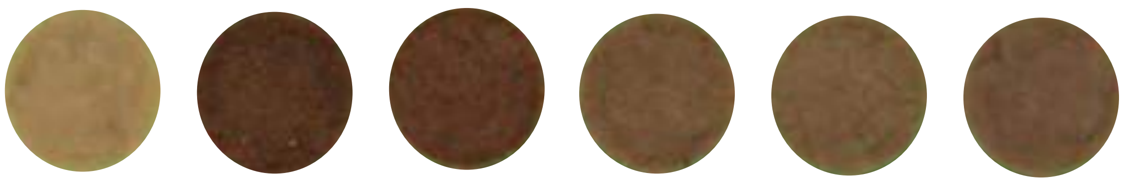

ACRYLAMIDE ANALYSIS (LC–ESI-MS/MS)
Determination of acrylamide in biscuits using $^{13}C_3$-acrylamide as internal standard
Acrylamide
O
NH_2
1 SAMPLE PREPARATION
• Homogenize biscuit sample
• Extract acrylamide with water (validated procedure)
2 INTERNAL STANDARD
$^{13}C_3$-Acrylamide
Isotope dilution with $^{13}C_3$-acrylamide (added to samples and standards)
3 LC INJECTION
Injection volume: 10 µL
4 LC SEPARATION
Column: Inertsil ODS-3 (250 × 4.6 mm, 5 µm)
Temperature: 30 °C
Mobile phase: 0.2 mL formic acid in 100 mL water (isocratic)
Flow rate: 0.4 mL/min
5 ESI-MS/MS DETECTION
Positive ionization mode
Needle voltage: 1.0 kV
Nebulizer gas (N_2): 12.0 L/min
Source temperature: 350 °C
6 MRM MONITORING (QUANTIFICATION)
m/z
Acrylamide (native) m/z 72.1 → 55.1
$^{13}C_3$-Acrylamide (IS) m/z 75.1 → 58.1
Quantitation: m/z 75.1, 78.1
Qualification: m/z 58.1, 55.1
Acquisition mode: MRM
7 QUANTIFICATION
Isotope dilution approach
Calibration with $^{13}C_3$-acrylamide
Samples analyzed in duplicate
8 RESULTS
Acrylamide content reported as µg/kg (ppb) of biscuit
METHOD PERFORMANCE
RECOVERY 94 – 103 % (fortified samples)
REPEATABILITY (same day) RSD < 10 %
REPRODUCIBILITY (multiple days/analysts) RSD < 10 %
LOD 4.5 µg/kg (S/N = 3)
LOQ 15 µg/kg (S/N = 10)
The method is validated for accuracy, repeatability and reproducibility according to Mesias & Morales (2025).
EXTERNAL VALIDATION (FAPAS PROFICIENCY TESTS)
Coffee (Test 30117) z-score = 0.1
Crispbread (Test 30118) z-score = -0.1
Potato crisps (Test 30133) z-score = -0.2
Reliable and accurate results confirmed by external proficiency testing.
LC system: Agilent 1200 | MS system: Agilent Triple Quadrupole | Data acquisition and processing: Agilent MassHunter

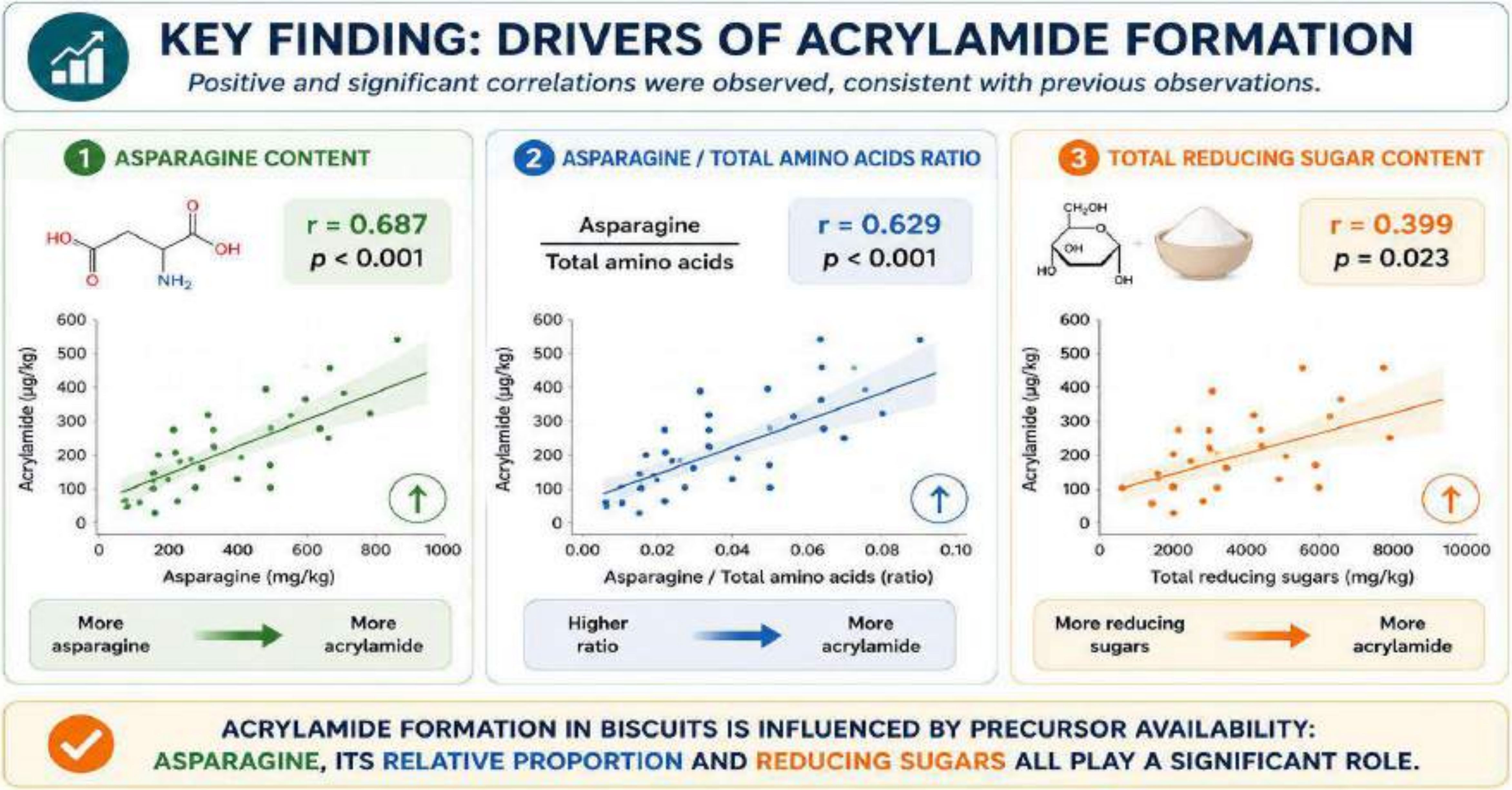
Statistical Analysis

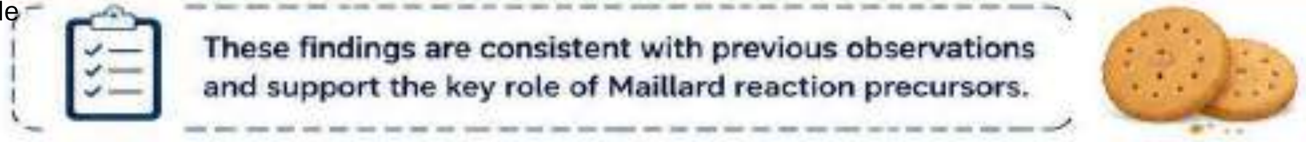
- All statistical analyses were conducted using IBM SPSS 25.0 (SPSS IBM Inc., IL, USA) and Statgraphics Centurion XVI.I software.
- Results were expressed as means and standard deviations (SD).
- The normality of data distribution was assessed using the Shapiro-Wilk test and homogeneity of variances was verified using Levene’s test.
- Differences between the mean values were evaluated using one-way analysis of variance (ANOVA) followed by the Tukey’s post-hoc test at a 95% significance level.
- Relationships between the parameters analyzed were explored using Pearson’s linear correlation coefficients, considering statistical significance at $p < 0.05$.


FREE REDUCING SUGARS
Refined Wheat
Whole Wheat
Spelt
Oat
Rye
Buckwheat
free reducing sugars (3.23 mg/g; calculated as the sum of glucose and fructose)
3.5
3
2.5
2
1.5
1
0.5
0
Refined Wheat
Whole Wheat
Spelt
Oat
Rye
Buckwheat

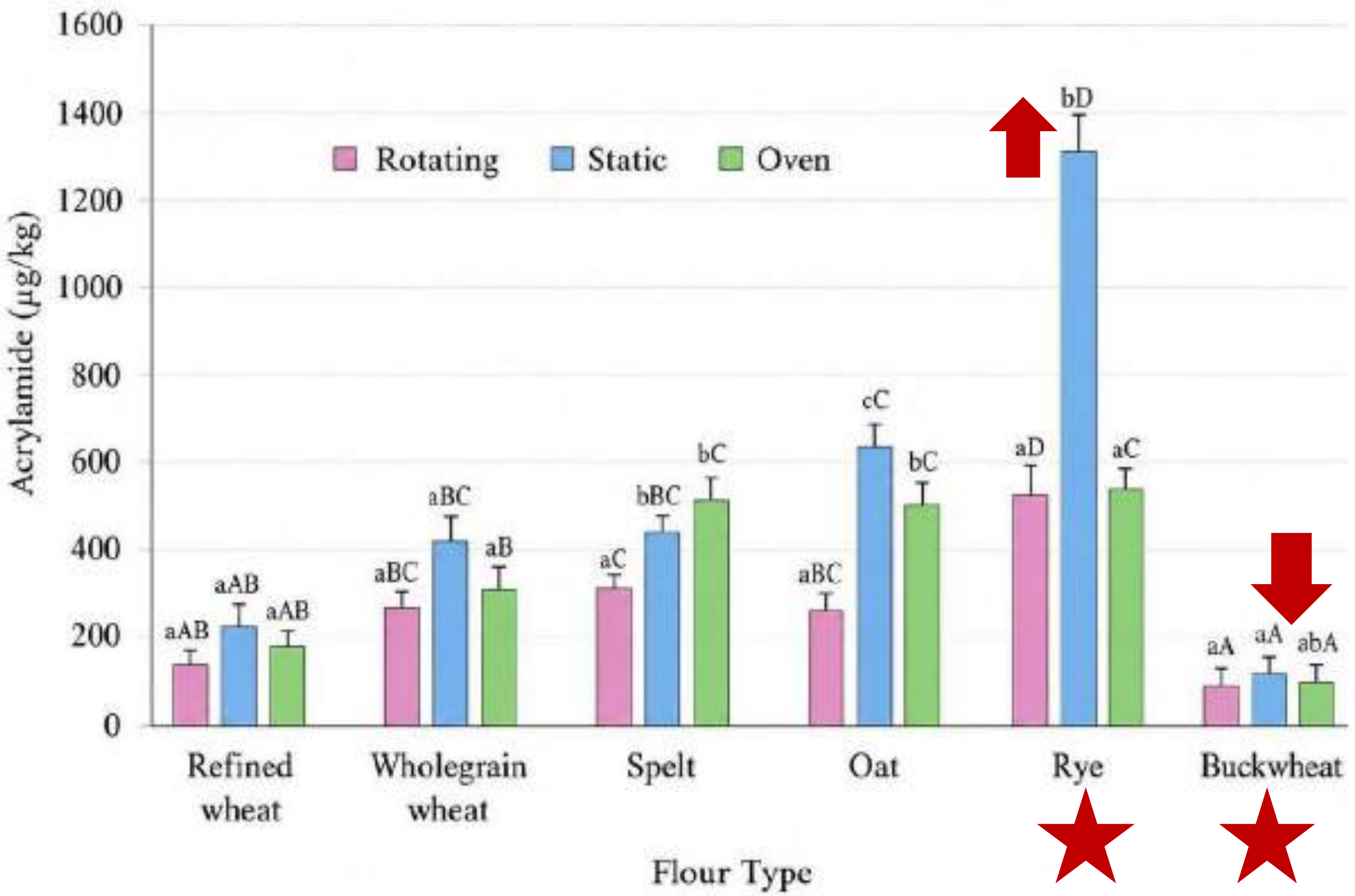

TOTAL REDUCING SUGARS (SUM OF GLUCOSE, FRUCTOSE, SUCROSE, MALTOSE)
Refined Wheat
Whole Wheat
Spelt
Oat
Rye
Buckwheat
234 mg/g
250
200
150
100
50
0
Refined Wheat
Whole Wheat
Spelt
Oat
Rye
Buckwheat


FREE ASPARAGINE
Refined Wheat
Whole Wheat
Spelt
Oat
Rye
Buckwheat
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Recognised as the key precursor of acrylamide and a limiting factor in cereal-based products
Refined Wheat
Whole Wheat
Spelt
Oat
Rye
Buckwheat


% ASPARAGINE TO TOTAL AMINOACID CONTENT
Refined Wheat
Whole Wheat
Spelt
Oat
Rye
Buckwheat
25
20
15
10
5
0
Importantly, the amount of asparagine alone does not fully explain acrylamide formation. Other amino acids, as well as the relative balance between asparagine and the broader amino acid profile, also play a role in determining acrylamide levels
Refined Wheat
Whole Wheat
Spelt
Oat
Rye
Buckwheat






Žilić, S., et al. (2020). Acrylamide formation in biscuits made of different wholegrain flours, depending on their free asparagine content and baking conditions. Food Research International, 132, 109109. https://doi.org/10.1016/j.foodres.2020.109109



- ✓ Acrylamide levels varied substantially by flour type and heating technology, **ranging from 81 to 1320 µg/kg.**
- ✓ The **static-basket air-fryer produced** the highest acrylamide **(up to 1320 µg/kg)**, whereas the **rotating-basket air-fryer** yielded levels comparable to those of **conventional oven baking (up to 515 µg/kg and 540 µg/kg**, respectively).
- ✓ **Buckwheat-based biscuits** consistently showed the **lowest contaminant levels** among other cereal types.
- ✓ Even with **identical dough formulations** and **nominal temperature–time settings**, the design of the heating element and resulting **heat-distribution patterns** within each device are critical determinants of **acrylamide formation.**

Acrylamide (µg/kg) levels in biscuits formulated with different flours and baked using different devices.
Values are mean ± SD. Different lowercase letters indicate significant differences among devices within the same flour type ($p < 0.05$), whereas different uppercase letters indicate significant differences among flours within the same device ($p < 0.05$).

Biscuits	Dough	Rotating basket air fryer 1400 W – Top	Rotating basket air fryer 1400 W – Bottom	Static basket air fryer 1500 W – Top	Static basket air fryer 1500 W – Bottom	Oven convective, air-forced – Top	Oven convective, air-forced – Bottom
Refined wheat	60.2 ±0.2^{e}	68.9 ±1.9	70.6± 1.5	57.3 ±2.4	67.0 ±2.5	69.9 ±1.9	65.9 ±2.0
Whole wheat	44.6 ± 1.0^{a}	53.7 ±2.2	52.8 ±0.9	45.4 ±2.9	52.4 ±1.8	55.1 ±2.0	50.5 ±2.2
Spelt	47.1 ±1.0^{b}	55.8 ±1.8	56.1 ±1.0	43.7 ±1.5	53.4 ±2.2	53.8 ±1.5	47.6 ±2.4
Oat	52.9 ± 0.5^{d}	65.9 ±1.4	64.6 ±2.0	47.1 ±1.1	57.0 ±2.5	60.2 ±1.8	56.0 ±1.2
Rye	53.0 ± 0.3^{d}	64.8 ±1.4	65.8 ±2.0	49.1 ±1.6	60.3 ±2.2	67.3 ±2.1	64.1 ±1.7
Buckwheat	49.7± 0.3^{c}	58.3 ±1.2	58.1 ±1.7	46.6 ±2.6	57.6 ±1.5	58.1 ± 2.7	55.3 ±2.4

E index overall color intensity

✓ No significant correlation between E-index and acrylamide

✓ Darker color ≠ higher acrylamide

✓ Flour composition strongly influenced color development

✅ Device design and flour type influenced browning intensity.

Color Development Findings

✅ Color development (E index) followed **similar trends** to acrylamide formation, supporting its use as a rapid indicator of thermal processing intensity.

✅ **Static-basket air-frying** showed **darker and more heterogeneous surfaces** than those baked in the **rotating-basket air-fryer** or **conventional oven.**

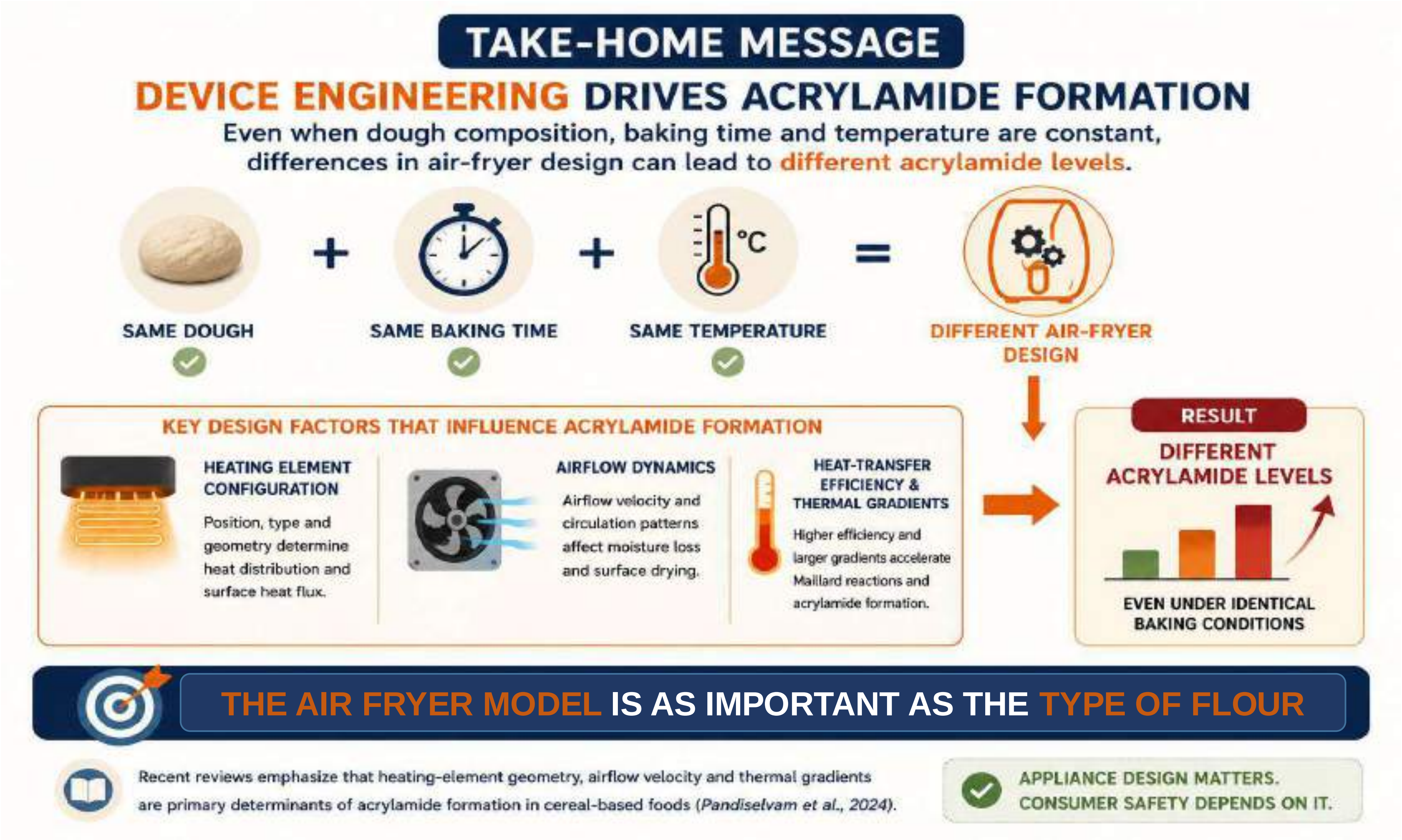
✅ Flour type remained the primary determinant of biscuit colour, with **wholegrain formulations** exhibiting distinct browning patterns compared with **refined wheat biscuits.**

✅ **Buckwheat biscuits** exhibited moderate colour development together with **the lowest acrylamide levels** among the tested formulations.

✅ **Device design** significantly influenced **surface browning**, highlighting the role of **air-distribution geometry** in thermal reaction pathways.

Main Findings

- ✓Acrylamide ranged from **81 to 1320 µg/kg,** depending **on the flour and the device.**
- ✓**Static-basket air-frying** promoted the **highest acrylamide formation** among the evaluated technologies.
- ✓Air-fryer **air-distribution geometry** significantly dictates acrylamide formation.
- ✓**Buckwheat biscuits** showed the greatest mitigation potential, because they yielded the **lowest contaminant levels** across all devices.
- ✓While **rye** was the **most susceptible flour** to acrylamide formation.


TAKE-HOME MESSAGE
DEVICE ENGINEERING DRIVES ACRYLAMIDE FORMATION
Even when dough composition, baking time and temperature are constant, differences in air-fryer design can lead to different acrylamide levels.
SAME DOUGH
+
SAME BAKING TIME
+
°C
SAME TEMPERATURE
=
DIFFERENT AIR-FRYER DESIGN
KEY DESIGN FACTORS THAT INFLUENCE ACRYLAMIDE FORMATION
HEATING ELEMENT CONFIGURATION
Position, type and geometry determine heat distribution and surface heat flux.
AIRFLOW DYNAMICS
Airflow velocity and circulation patterns affect moisture loss and surface drying.
HEAT-TRANSFER EFFICIENCY & THERMAL GRADIENTS
Higher efficiency and larger gradients accelerate Maillard reactions and acrylamide formation.
RESULT
DIFFERENT ACRYLAMIDE LEVELS
EVEN UNDER IDENTICAL BAKING CONDITIONS
THE AIR FRYER MODEL IS AS IMPORTANT AS THE TYPE OF FLOUR
Recent reviews emphasize that heating-element geometry, airflow velocity and thermal gradients are primary determinants of acrylamide formation in cereal-based foods (Pandiselvam et al., 2024).
APPLIANCE DESIGN MATTERS. CONSUMER SAFETY DEPENDS ON IT.

Future Directions

- ✓Air-frying **is not inherently a safer** alternative to conventional baking.
- ✓Future mitigation strategies should consider **not only recipe reformulation** but **also appliance design and heat-transfer dynamics.**

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Thank you for your attention ! ☺