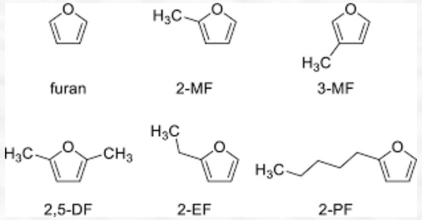
This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101060698

Design of Experiments combined with Mass Spectrometry Approaches to study the Fate of Mycotoxins and Furans during Food Processing

Michele Suman [a,b,c,*] & Alexandra Schamann [d] & Rudolf Krska [e***]**

[a] Barilla G. R. F.lli SpA, Advanced Laboratory Research, via Mantova 166, 43122, Parma, Italy
[b] Department of Food and Drug, University of Parma, Italy
[c] Department for Sustainable Food Process, Catholic University Sacred Heart, Piacenza, Italy
[d] FFoQSI GmbH - Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, Tulln, Austria
[e]BOKU UNIVERSITY, Universität für Bodenkultur, Vienna, Austria

* michele.suman@barilla.com ; michele.suman@unipr.it
** alexandra.schamann@ffoqsi.at
*** rudolf.krska@boku.ac.at



UNIVERSITÀ DI PARMA
onfoods Research and Innovation for Sustainable Food and Nutrition
FFoQSI
Barilla The Italian Food Company. Since 1877.

MYCOTOXINS: Aim of the study

- **Questions:**
  - What happens with mycotoxins during food processing? Are they stable?
- **Problem:**
  - Usually targeted analysis of mycotoxins in food → Modified forms might not be detected
- **Aim:**
  - Confirmation of the stability of mycotoxins
  - Or identification of new forms of myco-toxins formed during food processing

Part 1	Part 2
Gluten-free pasta	Wholemeal crackers
Maize flour	Wholemeal durum wheat flour
Aflatoxin B_1 Fumonisins B_1 + B_2	T-2 + HT-2 toxins

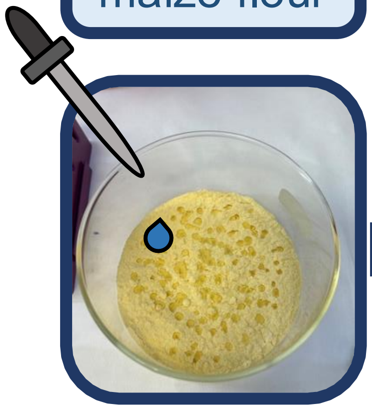
Fate of aflatoxin & fumonisin during pasta production

Spiking of maize flour

Producing pasta from spiked maize flour

Sample analysis

Data evaluation



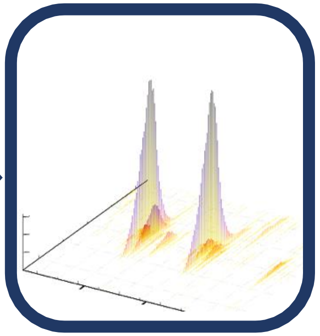
Unlabelled + ^{13}C labelled mycotoxins

Mixing & heating (75 °C)

Extrusion

Drying (≤ 80 °C) & cooking

Untargeted HRMS analysis



Identifying degradation products

No degradation products of aflatoxin B_1 & fumonisins B_1 + B_2 were detected

Fate of T-2 & HT-2 toxins during crackers production

- **Spiking** of wholemeal flour with unlabelled & ^{13}C-labelled mycotoxins
- Addition of further ingredients
- Kneading & **leavening** for 2 h at 25 °C
- Cracker shaping & **baking** for 6 min at 195 °C

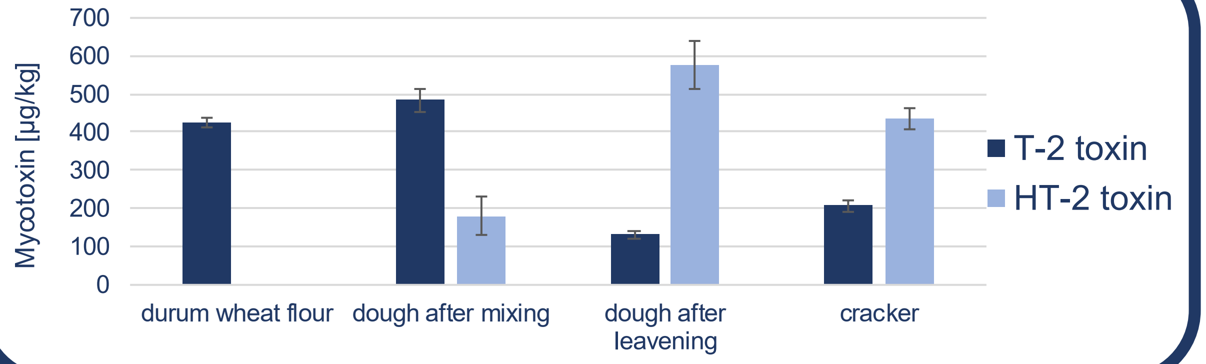


Fig.: T-2 and HT-2 toxin levels in samples collected during cracker production from wheat flour spiked with T-2 toxin

T-2 was partly de-acetylated to HT-2 during dough preparation

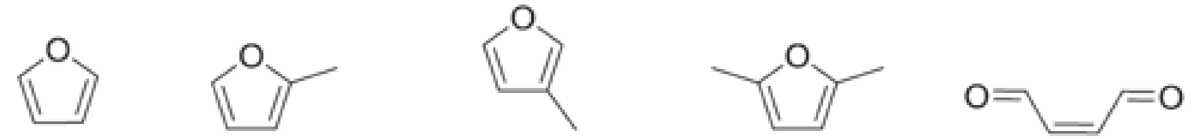
HT-2 toxin was stable

FURANS: WHAT?

Furans and furan derivatives are volatile highly relevant aroma compounds contribute to the characteristic flavor of many cooked foods, including canned and jarred foods, baby food containing meat, and various vegetables.

In addition to furan, other furan derivatives can arise from the same routes of furan formation in food. These compounds, **such as 2-alkylfurans**, 2-acetylfuran, furfural and furfuryl alcohol, have been recognized toxic for animals and humans and a **concern for their genotoxicity in humans was identified by EFSA in 2011**.

- **Occurrence in foods**
  - Furan is **widely detected across food categories**, with **particularly high levels in canned foods**, including sauces.
- **Toxicological classification**
  - In **1995**, the **IARC** classified furan as **“possibly carcinogenic to humans” (Group 2B)**, triggering long-term regulatory and scientific attention.
- **EU-level framework**
  - **Commission Recommendation (EU) 2022/495** calls for **reliable and comparable monitoring** of furan and alkyl-furans across Member States.
  - The Recommendation provides **guidance on sampling strategies and analytical performance criteria**, but does **not set legal limits**.
- **Emerging regulatory outlook**
  - **Non-official regulatory discussions** indicate possible **future guidance or limit values in the range of 100 ppb** (sum of furans).



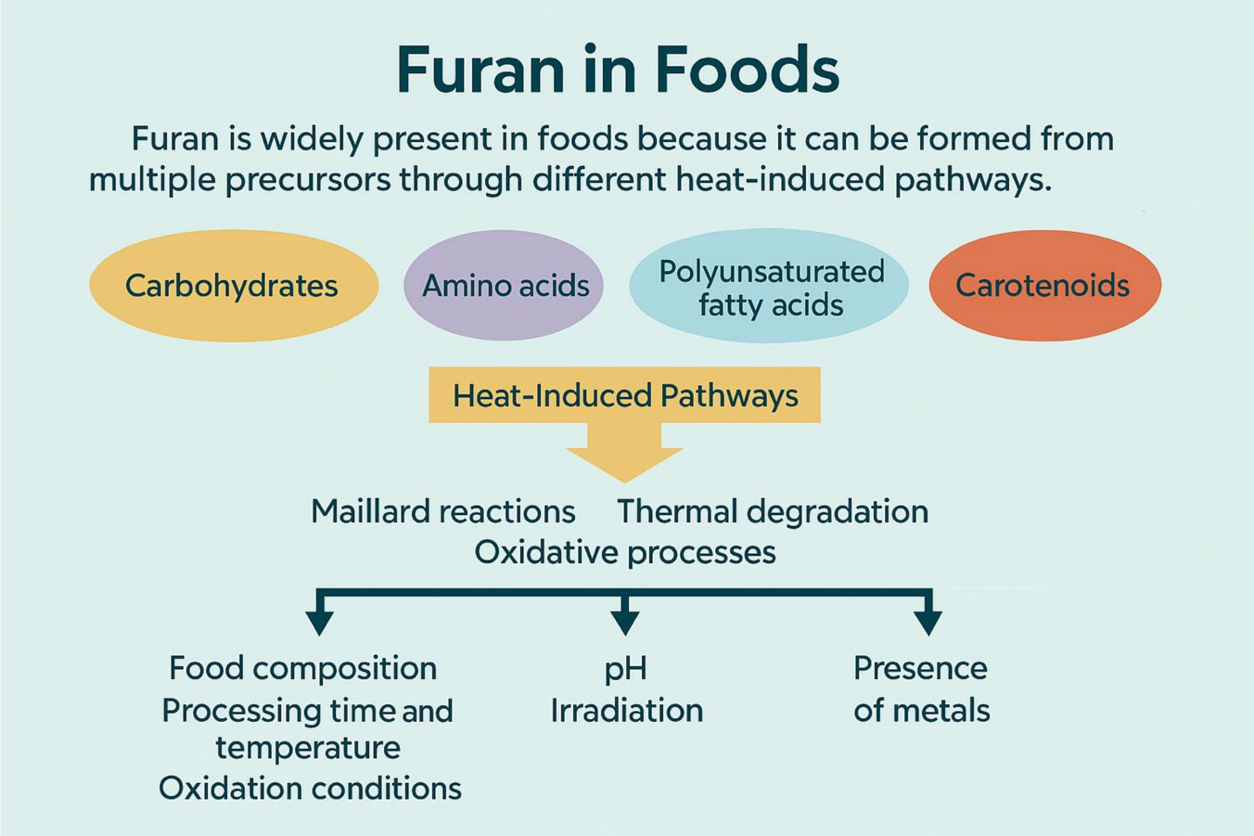
Furan | 2-Methylfuran | 3-Methylfuran | 2,5-Dimethylfuran | 2-Z-Butenedial

FURANS: HOW CAN THEY BE FORMED?

Furan is widely present in foods because **it can be formed from multiple precursors—such as carbohydrates, amino acids, ascorbic acid, polyunsaturated fatty acids, and carotenoids.**

Through different heat-induced pathways, including Maillard reactions, thermal degradation, and oxidative processes.

Its formation is **influenced by several factors, notably food composition, processing time and temperature, oxidation conditions, pH, irradiation, and the presence of metals**.

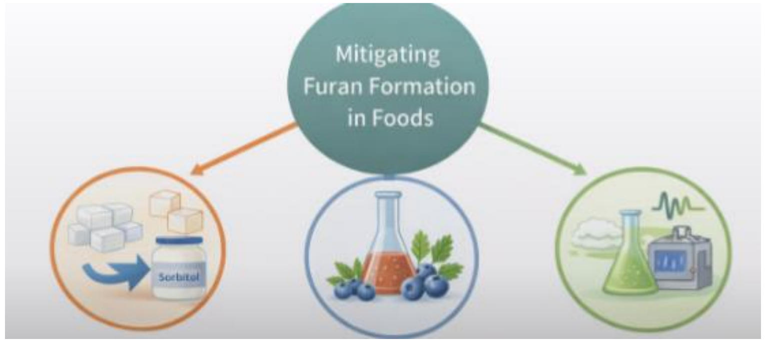


FURANS: MITIGATION STRATEGIES

Mitigation strategies for furan formation in foods **must carefully balance sensory quality, nutritional value, food safety, the potential formation of other processing contaminants, and economic feasibility.**

Currently, mitigation approaches are generally grouped into **three main categories: (i) removal or substitution of major furan precursors, (ii) addition of specific additives, particularly antioxidants, and (iii) modification of processing conditions** or implementation of alternative processing technologies.

Recent studies report the effectiveness of different strategies, including precursor reformulation, high-pressure thermal sterilization (HPTS), high hydrostatic pressure (HHP), ohmic heating, vacuum treatment, and antioxidant application.



Sugars can play a key role in furan formation, with fructose and glucose producing higher levels than sucrose in sterilized vegetable-based foods. Consequently, reducing monosaccharides or partially replacing them with sucrose or sugar alcohols has been proposed. Among sugar substitutes, sorbitol is the least efficient furan precursor.

Antioxidants such as polyphenols, chlorogenic acid, and reducing agents can further inhibit furan formation.

Additionally, reducing thermal load or applying **innovative technologies such as HHP, ohmic heating, or vacuum treatments has proven effective in lowering** furan levels, particularly in baby foods.

(Fardella et al., 2021; Kettlitz et al., 2019; Limacher et al., 2008; Owczarek-Fendor et al., 2012; Palmers, Grauwet, Buve et al., 2016; Grembecka, 2015; Owczarek-Fendor et al., 2012; Anese & Suman, 2013)

FURANS: data (ppb) - Benchmarking

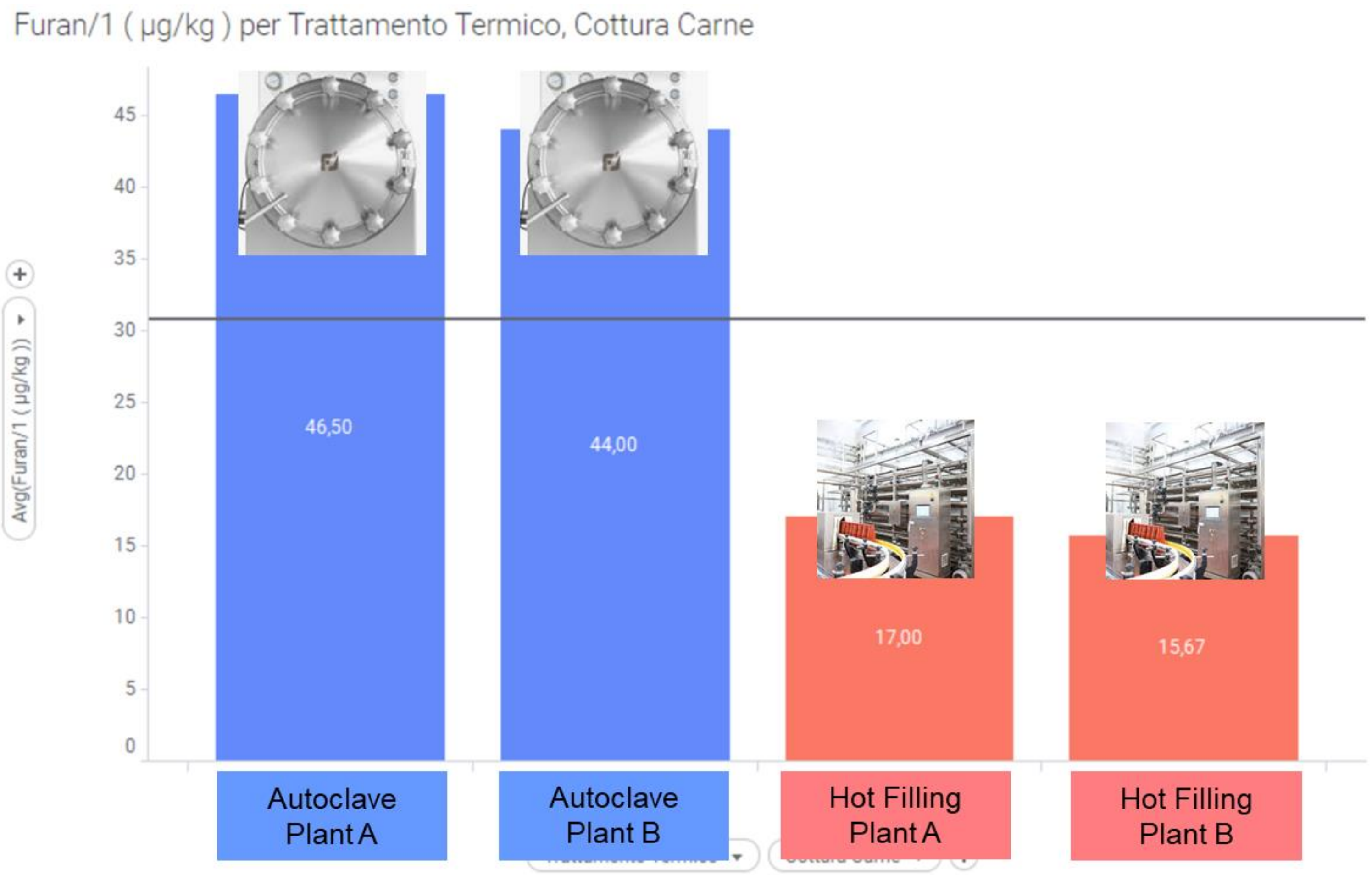
Bolognese Sauce Market Samples

Tomato Sauce Market Samples

	Bolognese Sauce Market Samples		Tomato Sauce Market Samples	
	furan	furans sum	furan	furans sum
average:	52	97	10	15
max:	71	131	14	20

*values in ppb

FURANS in RAGU: Preliminary Design of Experiments Outcomes



- **Significantly higher furan content after autoclaving**

- **Sugar content and pre-cooking step for meat does not seem to have a decisive impact** on the development of furan

FURANS in RAGU: Preliminary Design of Experiments – Key Learnings

- Main contributor is **sterilization (T°/t)**
(seems to affect more furan & 2-methylfuran)

- **Low influence** of **sugar** addition and **meat cooking** (seems to affect more 3-methylfuran)

- **Smaller jar**: right direction in furan mitigation
(- 30%: 300gr vs 400 gr)

FURANS in RAGU: Focus on size/jar/treatment

Lower values if:
tin can; smaller jar; acidified+hot filling

A **consistent reduction of furan and alkyl-furans** is observed when moving from **400 g** to **300 g** formats for most products.

- ***Bolognese based recipe***: *10–20% reduction*
- ***Amatriciana based recipe:*** *≈ 20–30% reduction*
- ***Sausages based recipe***: *≈ 30–40% reduction*

Inference

- **Container size / thermal history is a dominant driver** of furan formation.
- Smaller formats likely experience **shorter effective heat load or better heat dissipation**, outweighing formulation effects.

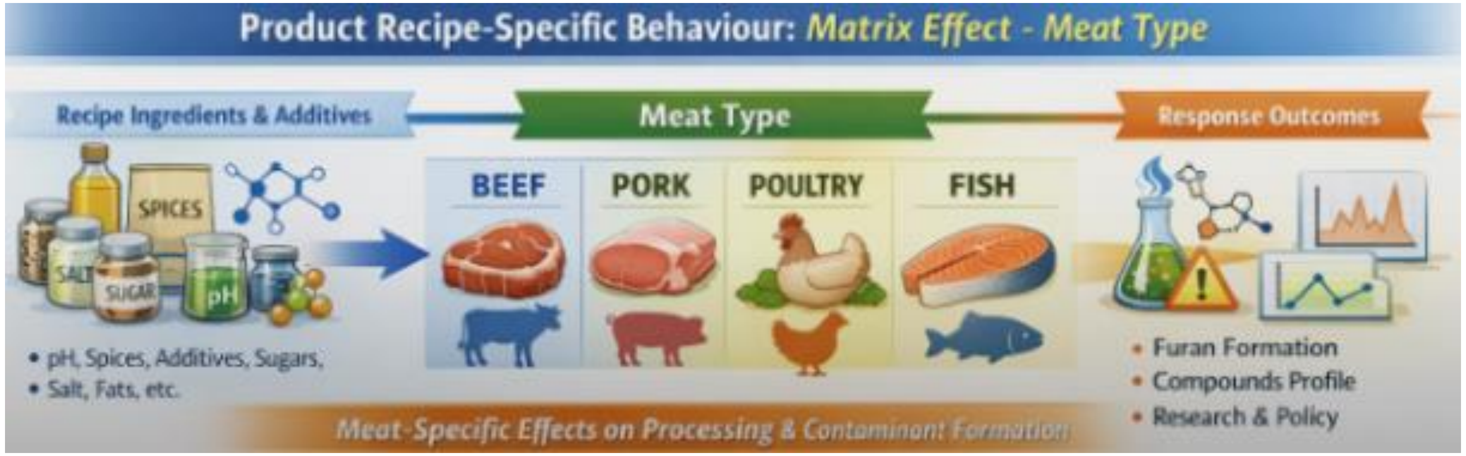
FURANS in RAGU: Product/recipe-specific behaviour (matrix effect)

Absolute furan levels differ by recipe:

- **Sausages based recipe** repeatedly show **higher absolute sums**, especially in larger formats
- **Bolognese** products show more **moderate but variable values**

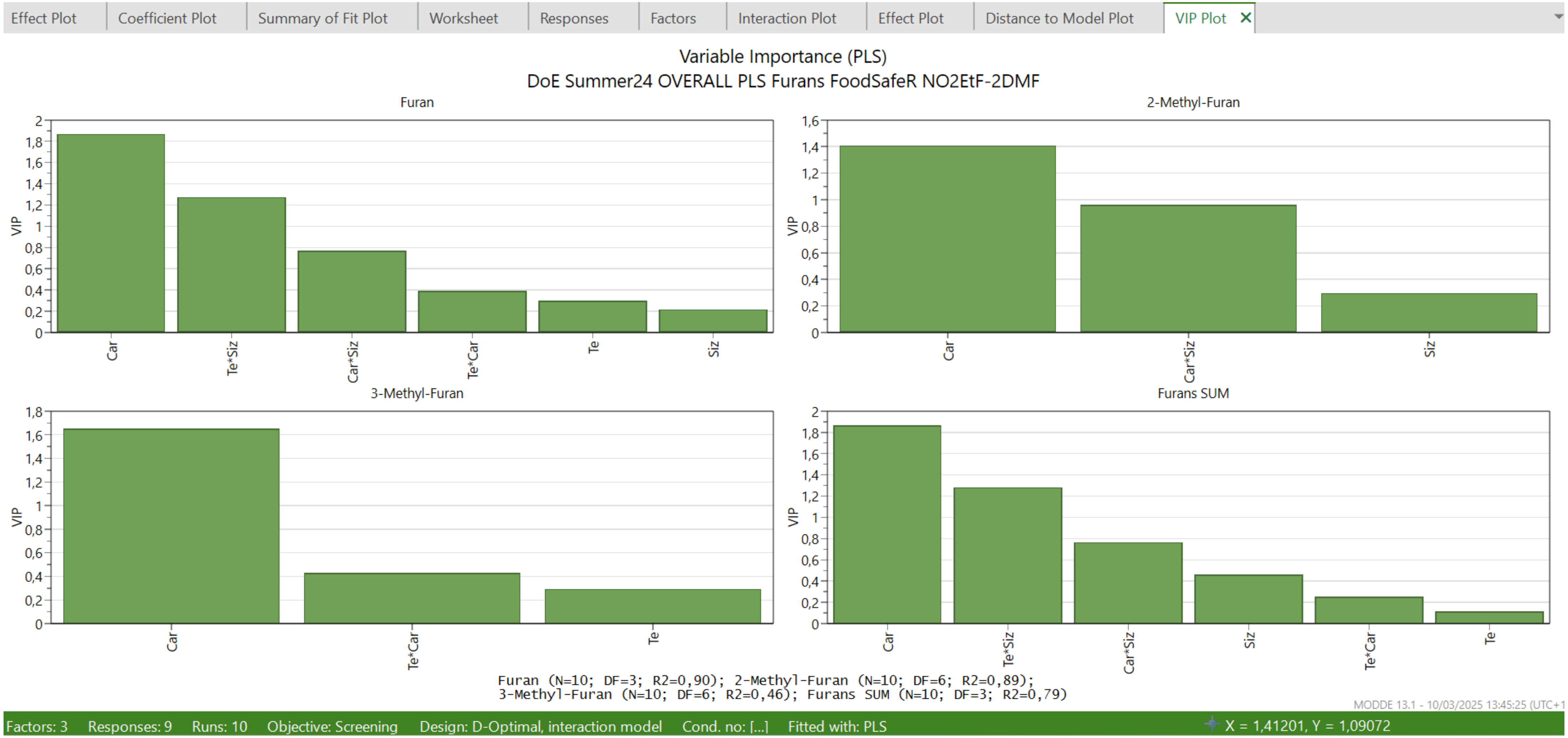
Inference

- **Fat-rich, meat-intensive matrices** appear more prone to furan formation.
- Presence of **complex meat/fat systems** likely enhances precursors (lipid oxidation + Maillard intermediates).
- “Leaner” or simpler recipes show lower baseline risk even before format mitigation.



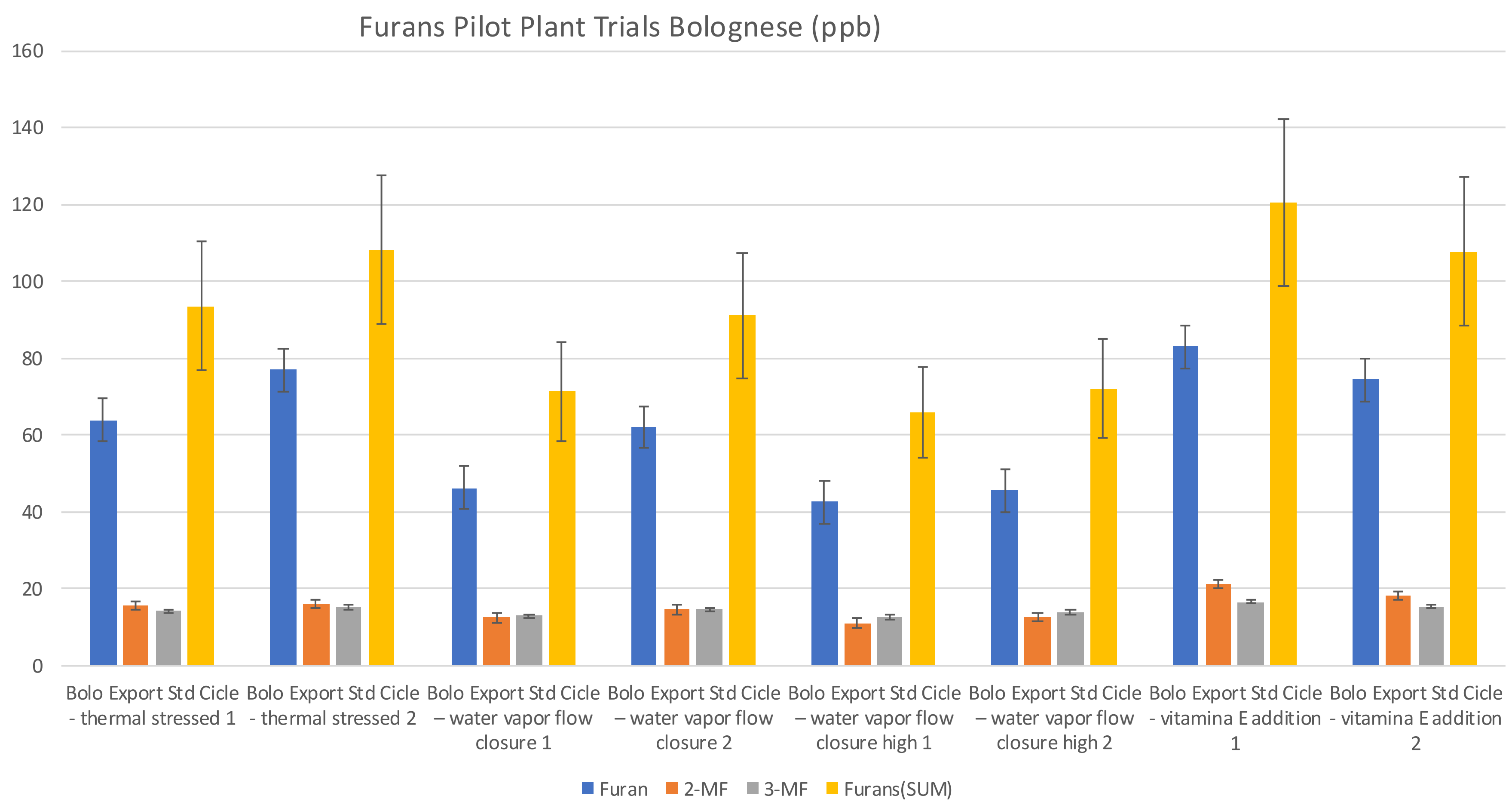
FURANS in RAGU: Final Design of Experiments Outcomes

- ***Changing the ratio between sterilization temperatures and times only brings partial benefits*** *because, to remain within process standards, at the minimum acceptable value of the corresponding F0 there is still sufficient energy to activate furans generation*
- ***Carrots play a pivotal role*** *in the recipe*
- ***Size of the jar is confirmed as relevant*** *in terms of overall compromise between energy vs microbial stabilization/furans generation*



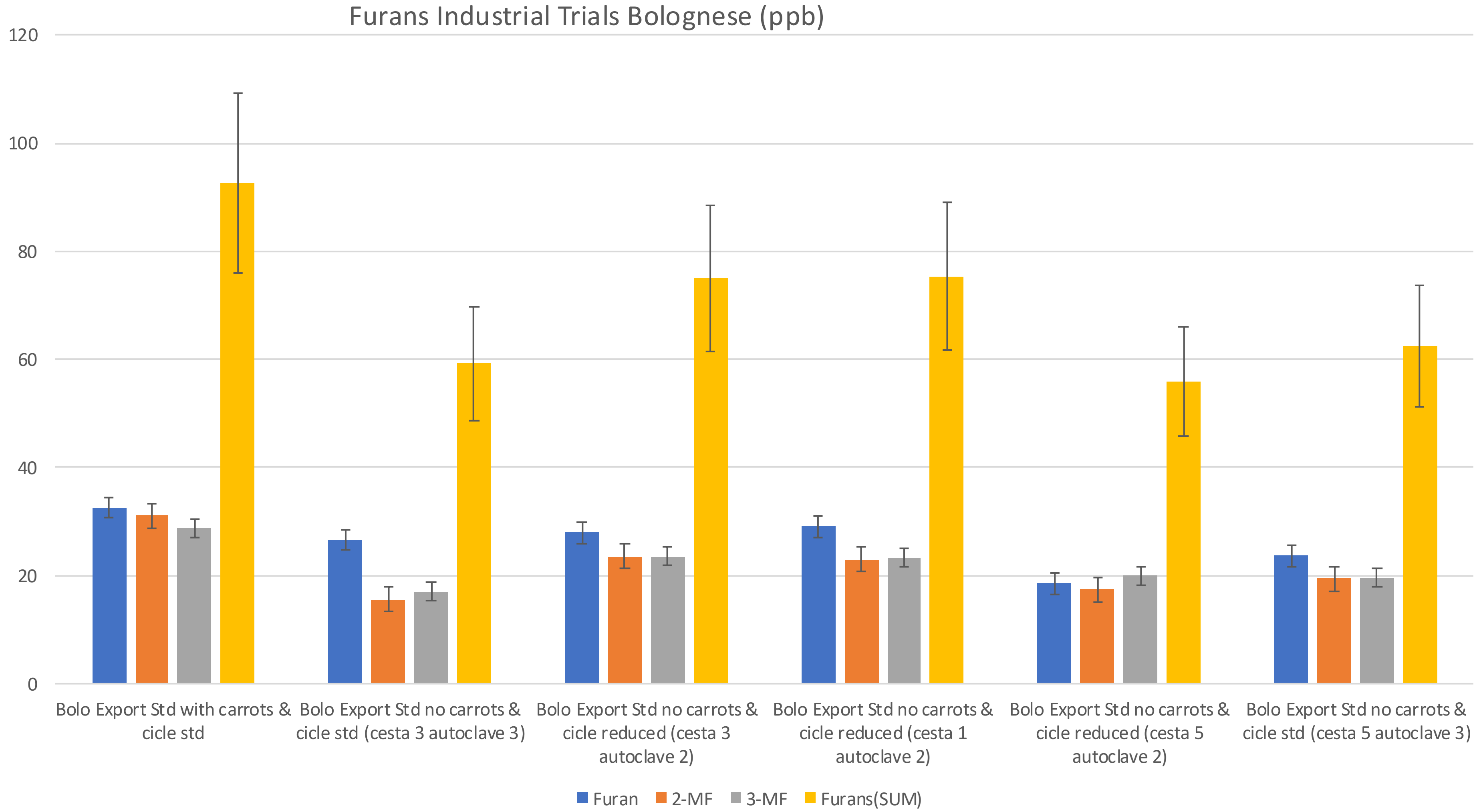
Car: Carrots
Te: Temperature
Siz: Size

FURANS – Bolognese recipes: Final Pilot Plant Trials Inferences



- The addition of **Vitamin E has no effects**
- The different management of the **steam blade (in an attempt to reduce the oxygen involved) does not bring any effects whatsoever**
- As expected, **reducing the cooling phase (i.e. increasing the "thermal stress") shows an increasing tendency**, especially for furan

FURANS – Bolognese recipes: Final Industrial Trials Inferences



- There are **not so relevant differences whatsoever between std and reduced thermal sterilization cicles**
- The presence of **carrots impacts furans, especially 2-MF and 3-MF**
- There are **no significant differences in the industrial line positions**

Strategic Take-Home Messages

Carotenoid Removal

Eliminating carotenoids, carrots specifically in these meat sauce recipes, effectively reduces furans during the sterilization phase without complex process modifications.

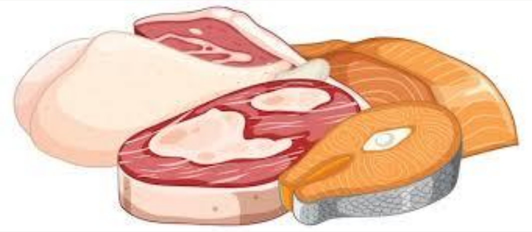
Carrot removal is a simple, safe, and sustainable intervention with limited impacts on industrial production.

Optimization of the sterilization process

Small variations in the sterilization process have a limited impact on furan generation alone and require complex and not always industrially sustainable validation, if obviously combined with ensuring microbiological safety.

Fat variability and fleshy matrix

The variability of fats and the fleshy matrix is difficult to control and does not represent an immediate lever, although it could be actionable.

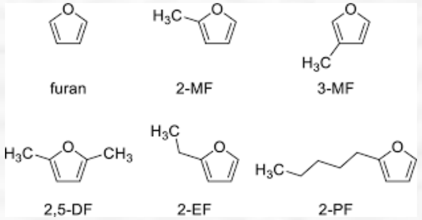


This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101060698

Michele Suman [a,b,c,*] & Alexandra Schamann [d **]

[a] Barilla G. R. F.lli SpA, Advanced Laboratory Research, via Mantova 166, 43122, Parma, Italy
[b] Department of Food and Drug, University of Parma, Italy
[c] Department for Sustainable Food Process, Catholic University Sacred Heart, Piacenza, Italy
[d] FFoQSI GmbH - Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, Tulln, Austria

* michele.suman@barilla.com ; michele.suman@unipr.it
** alexandra.schamann@ffoqsi.at



UNIVERSITÀ DI PARMA
onfoods Research and Innovation for Sustainable Food and Nutrition
FOODSAFER EVERYBODY'S BUSINESS
FFoQSI
Barilla The Italian Food Company. Since 1877.