Effect of Rocha pear peel extracts added to wheat and rye bread formulations on Acrylamide reduction and sensory quality maintenance

Morgado, C1; Bernardo, P1; Henriques, I1; Jesus, S2; Rego A2; Delgado I2; Coelho I2; Castanheira I2; Félix, N1; Fernandes, A1, Guerra, M1 and Brandão, C1

1Estoril Higher Institute for Tourism and Hotel Studies, Food Science Department, Estoril, Portugal;

2National Health Institute Doutor Ricardo Jorge, Food and Nutrition Department, Lisboa, Portugal

MISAGE

Mitigation Strategies of Acrylamide and Advanced Glycation End-Products in Bread

Projetos de Investigação Científica e Desenvolvimento Tecnológico (IC &DT)
Aviso 02/SAICT/2016

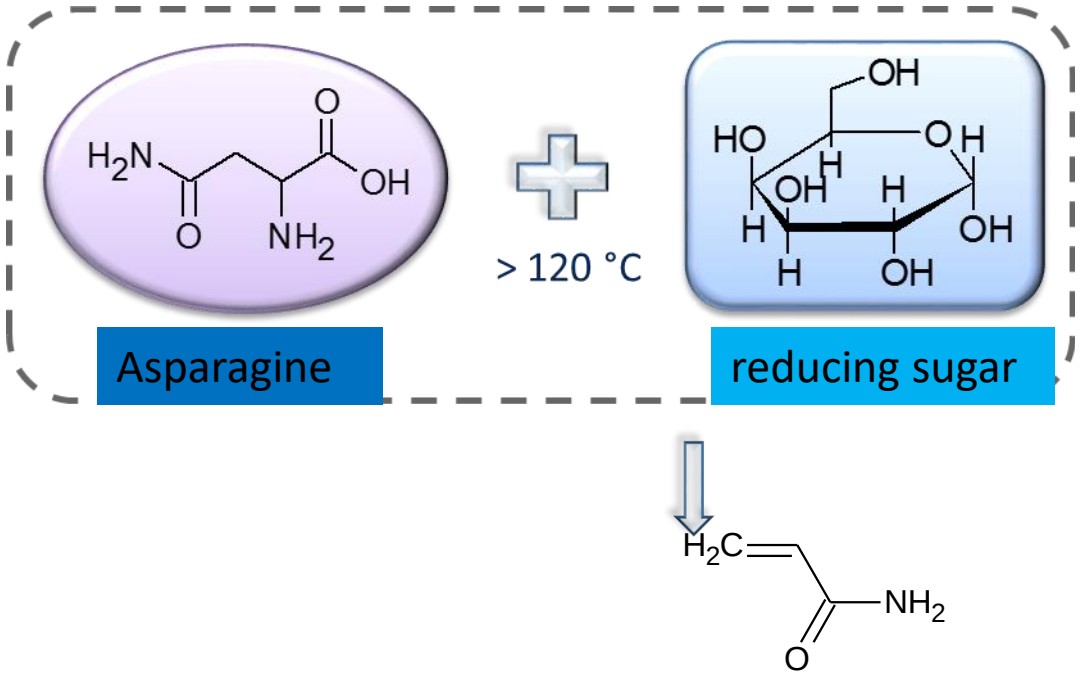
Consortium


Escola Superior de Hotelaria e Turismo do Estoril
• Food quality in catering
• Food innovation and development
• Sensory analysis
Instituto Politécnico de Santarém/ESA
• Rheological Assessment
Instituto Nacional de Saúde Doutor Ricardo Jorge
• Extraction of bioactive compounds
• Contaminant Analysis
• Antioxidant Analysis

Background – Acrylamide

- IARC refer acrylamide as a carcinogen for animals and probably for humans;

- Acrylamide is formed in a wide group of foods:
  - Potato Products
  - Breakfast Cereal
  - Coffee
  - Pastry & Bakery Products
  - Snacks



- Formed during thermal processing of food by caramelization and lipid oxidation, which can generate undesired substances on food, like acrylamide

Acrylamide - Toxicity and Classification

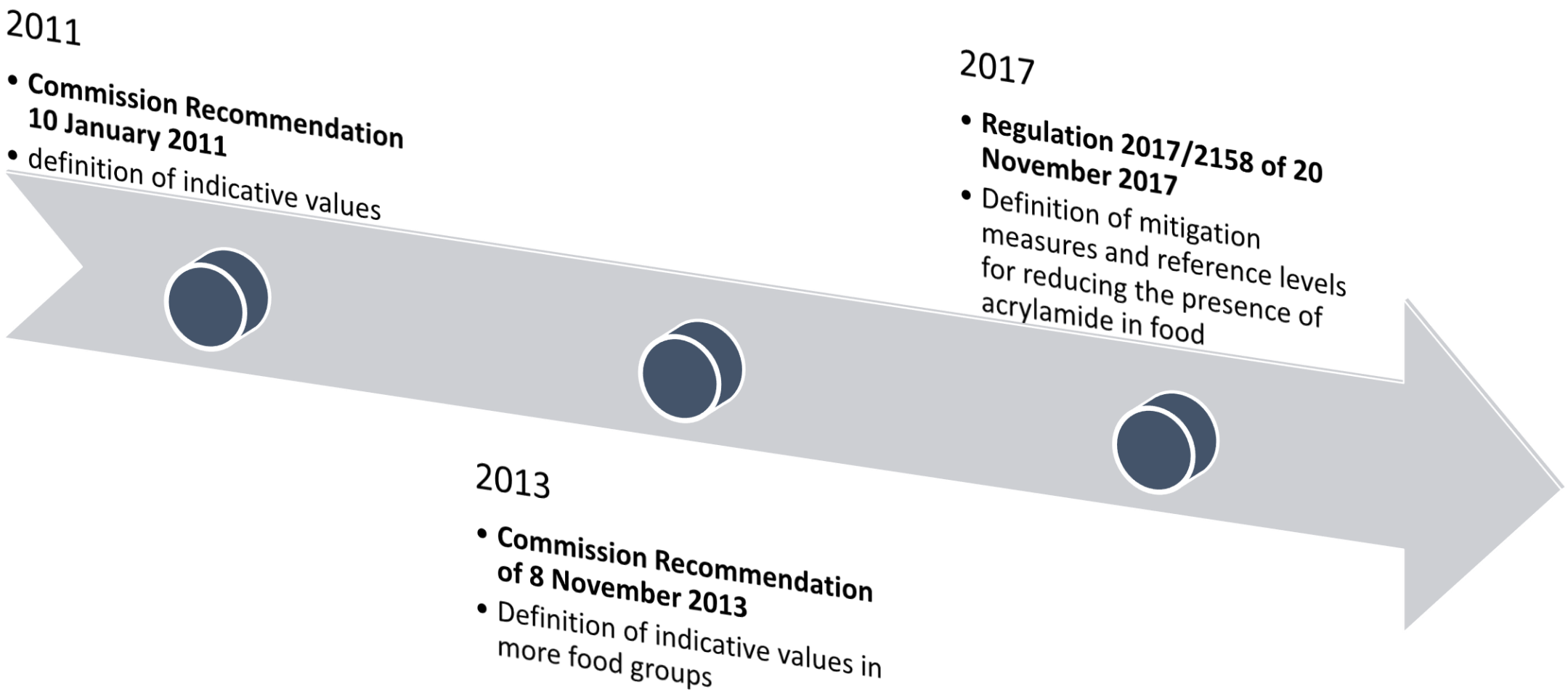
1994 IARC classified acrylamide as a carcinogen for animals and probably for humans (Group 2 A)

2002 European Commission classifies this compound as Category 2 for **carcinogenicity** and **mutagenicity**

Acrilamida – Toxicidade e metabolismo

- absorption in the gastrointestinal tract
- spread throughout the body
- Oxidation process begins through cytochrome P450 2E1, leading to transformation of acrylamide into **glycidamide** (genotoxic agent)
- Glycidamide reacting with DNA can give rise to **DNA adducts**.
- Most unprocessed acrylamide is excreted in the urine

Acrylamide - European Legislation



Acrylamide - Regulation 2017_1

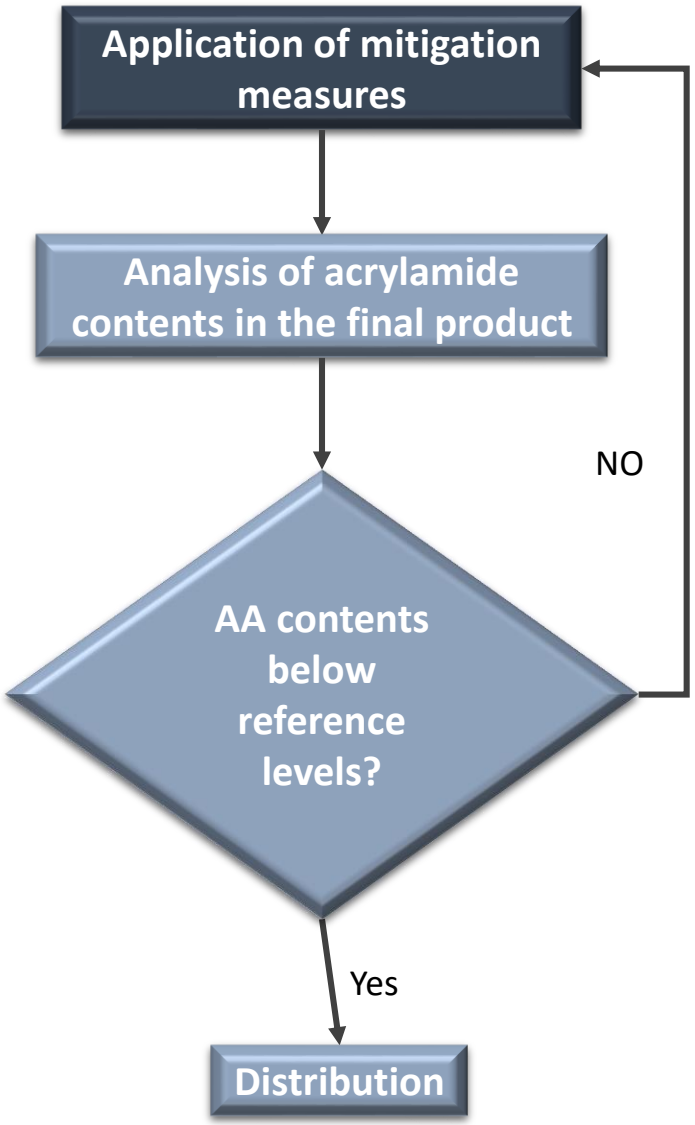
Approach to mitigating Acrylamide levels

- Implementation of good hygiene practices
- Application of procedures based on HACCP principles

Scope of application

- French fries
- Bread
- Breakfast Cereal
- Bakery and Pastry Products
- Coffee
- COFFEE Substitutes
- Baby food

Acrylamide – Regulation 2017_2



Potato Products

Agronomy
- Selecting Low Precursor Potato Varieties

Storage and transport conditions
- Temperature

Processamento
- Potato bleaching

Bakery and fine pastry products

Agronomy
- Avoid high asparagine levels - balance sulfur levels

Recipe and Formulation
- Flour change
- Ammonium Baking Replacement

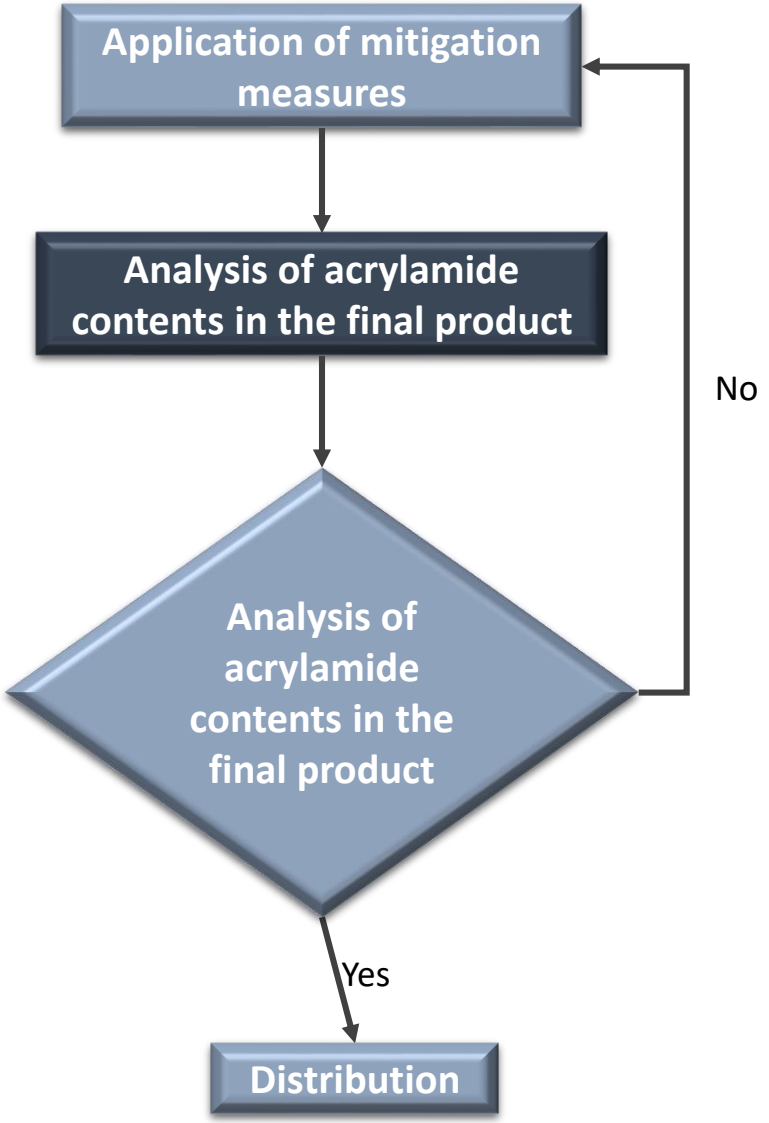
Processamento
- Increase moisture content

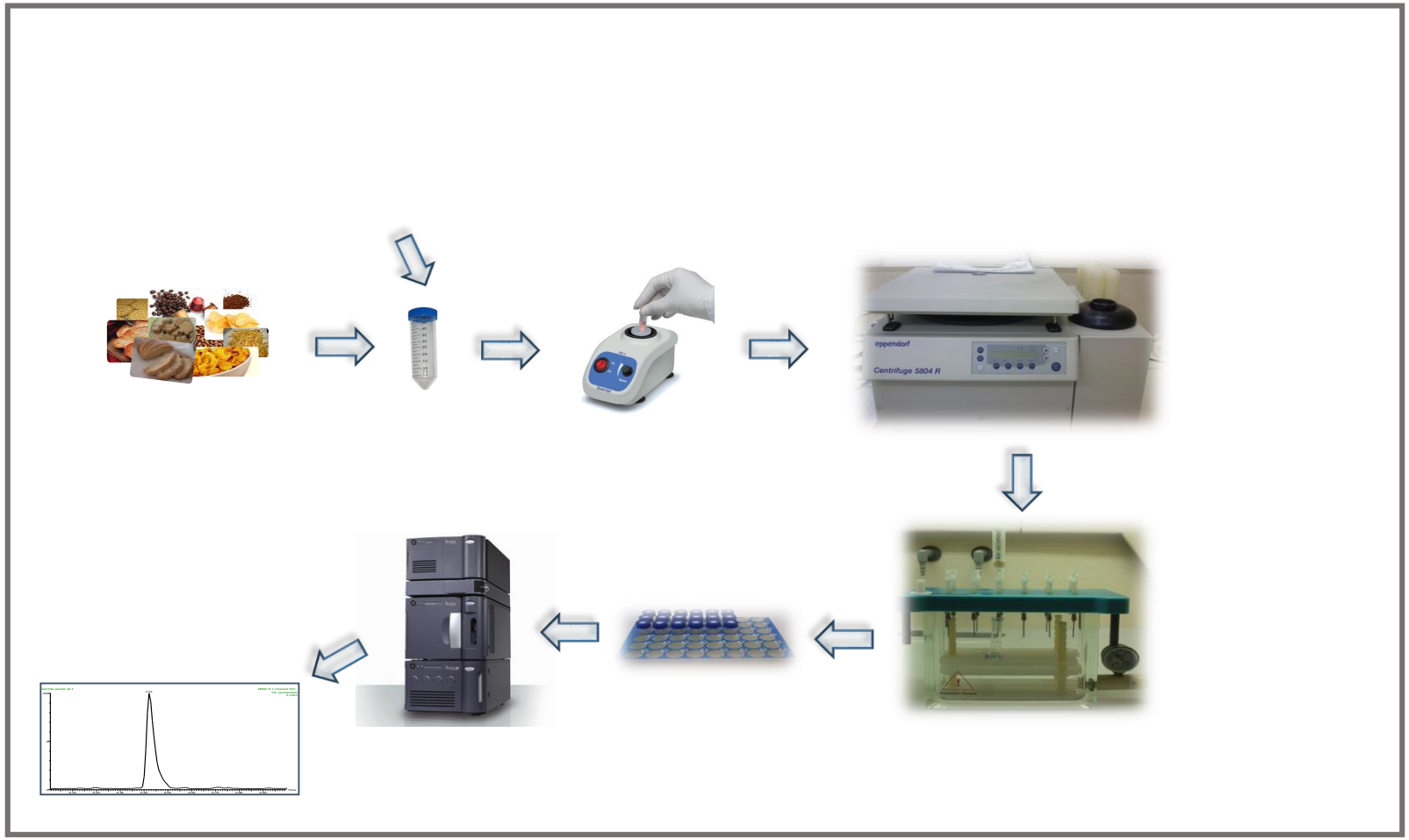
Breakfast Cereal

Agronomia
- Maintenance of sulfur levels

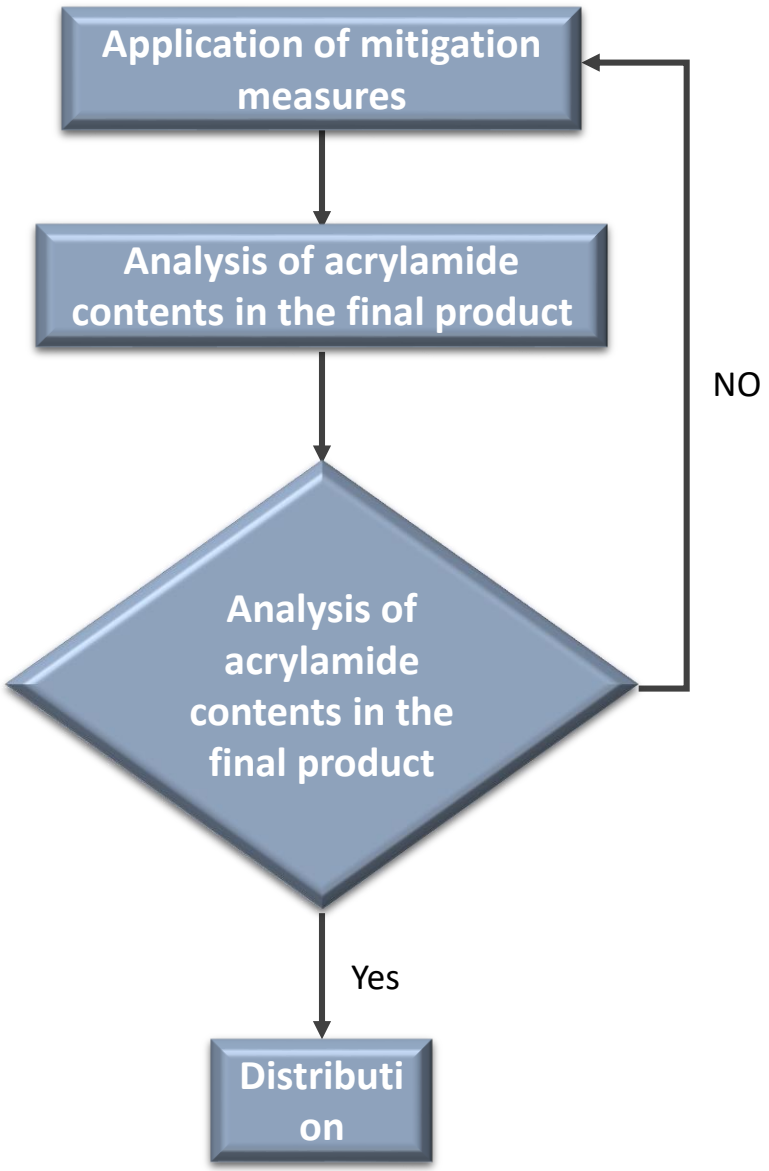
Recipe
- Consider using corn and rice
- Control the addition of sugars

Acrylamide – Regulation 2017_5



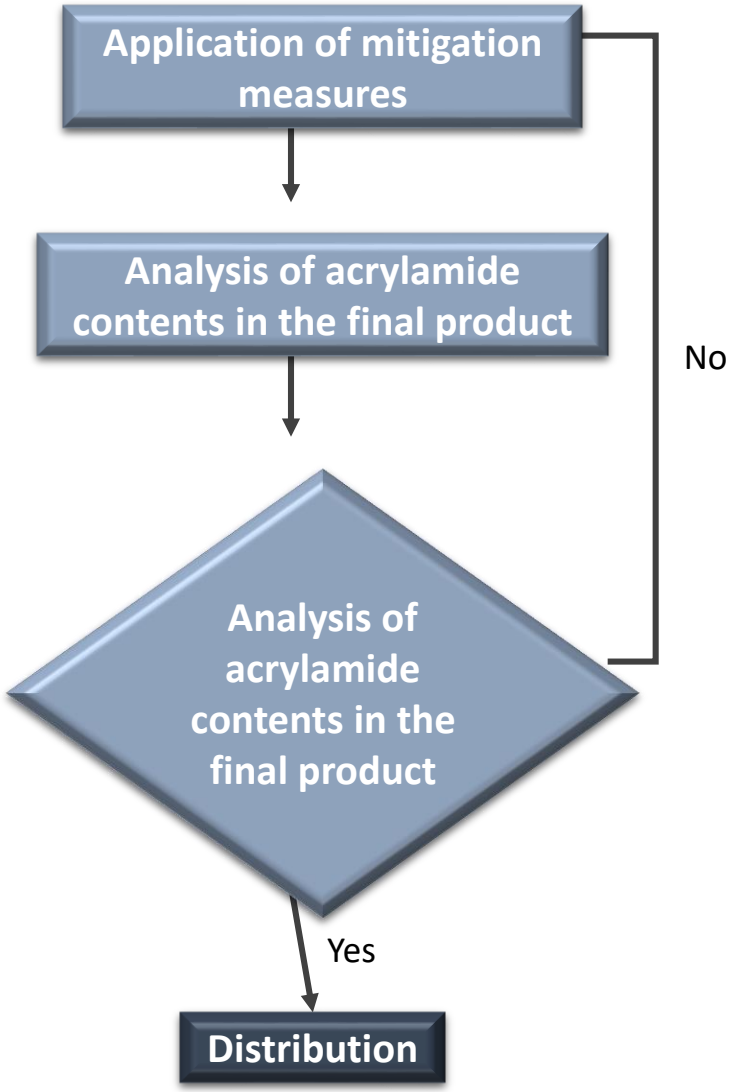


Acrylamide – Regulation 2017_6



Food	Benchmark level [µg/kg]
French fries (ready-to-eat)	500
Potato crisps from fresh potatoes and from potato dough Potato-based crackers Other potato products from potato dough	750
Soft bread	
(a) Wheat based bread	50
(b) Soft bread other than wheat based bread	100
Breakfast cereals (excl. porridge)	
— bran products and whole grain cereals, gun puffed grain	300
— wheat and rye based products (1)	300
— maize, oat, spelt, barley and rice based products (1)	150
Biscuits and wafers	350
Crackers with the exception of potato based crackers	400
Crispbread	350
Ginger bread	800
Products similar to the other products in this category	300
Roast coffee	400
Instant (soluble) coffee	850
Coffee substitutes	
(a) coffee substitutes exclusively from cereals	500
(b) coffee substitutes from a mixture of cereals and chicory	(2)
(c) coffee substitutes exclusively from chicory	4 000
Baby foods, processed cereal based foods for infants and young children excluding biscuits and rusks (3)	40
Biscuits and rusks for infants and young children (3)	150

Acrylamide – Regulation 2017_7



Consumers

The packaging of final products shall have recommended cooking instructions and shall comply with Regulation (EU) No 1169/2011 of the European Parliament and of the Council.

Acrylamide Mitigation Strategies_2

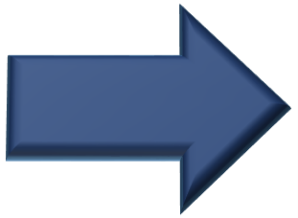
- **Recipe**

Potatos

- Addition of Amino Acids
- Use of rosemary extract in frying oil
- Ascorbic acid and green tea treatments
- Addition of calcium salts, citric acid, vanadium salts or acetic acid
- Radiofrequency application of heating after drying

Cereals

- Ammonium bicarbonate replacement with sodium bicarbonate
- Glycine and Spice Addition
- Application of bamboo leaves or tea polyphenols
- Addition of pure phenolic compounds



It is important that mitigation strategies do not affect sensory and rheological properties.

Acrylamide Mitigation Strategies_3

- **Processing**

Potatos

- Decrease frying temperature
- Reduce frying time
- Use of asparaginase enzyme

Cereals

- Increase fermentation process time
- Use of asparaginase enzyme

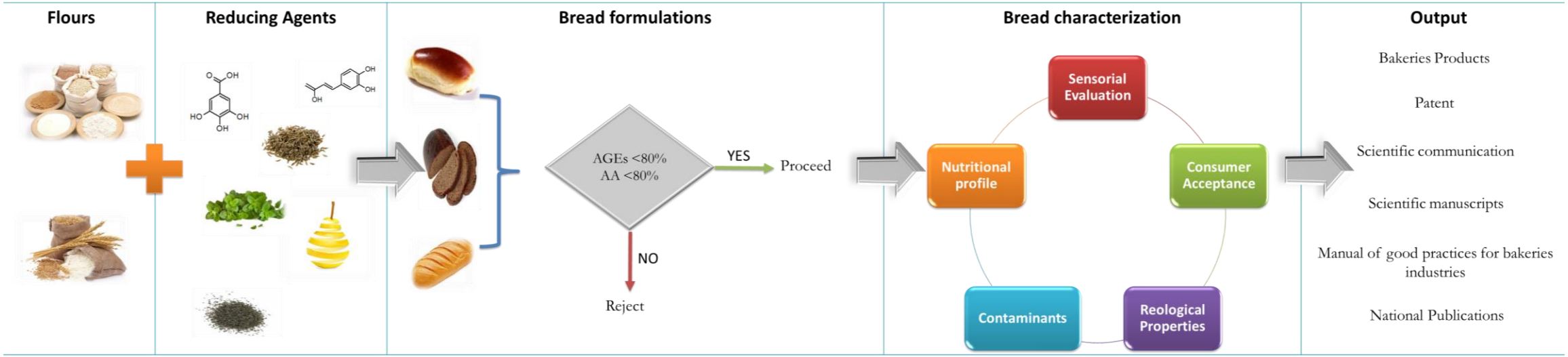
Coffe

- Increase roasting temperature
- Increase roasting degree
- Coffee dilution
- Supercritical CO_2 extraction in the processing phase
- Cysteine Addition Heat Treatment

It is important that mitigation strategies do not affect sensory and rheological properties

Project MISAGE - Goal

- Development of different combinations of national ingredient and by-product extracts to reduce the concentration of acrylamide and advanced final glycolysis products.



Project MISAGE – the challange

Sensory properties

(ESHTE)

preparation of extracts and determination of contaminants

(INSA)

Processing

(ESHTE/INSA)

Rheological properties

(IP Santarém)

Project MISAGE – Activities

1. Preparation of extracts and definition of precursors (INSA / ESHTE)

- Task 1.1 - Determination of Maillard Reaction Precursors
- Task 1.2 - Preparation of reducing agents and determination and quantification of antioxidant activity

2. Production Test (ESHTE / INSA)

Task 2.1 - Pre-preparation of breads followed by sensory acceptance tests and determination of acrylamide and advanced glycolysis products.

Task 2.2 - Bread production (variables: reducing agent concentration, baking process

Task 2.3 - Sensory characterization and determination of consumer acceptance

3. Determination of rheological characteristics (IP Santarém)

Task 3.1 - Evaluation of the rheological characteristics of bread produced in activity 2

4. Bromatological analysis of final products (INSA)

Task 4.1 - Determination of contaminants (acrylamide and AGEs)

Task 4.2 - Nutritional Characterization (protein and fiber)

5. Data Collection and Processing (INSA / ESHTE / IP Santarém)

Task 5.1 - Data analysis to select the best extract formulations

Acrylamide formation in food

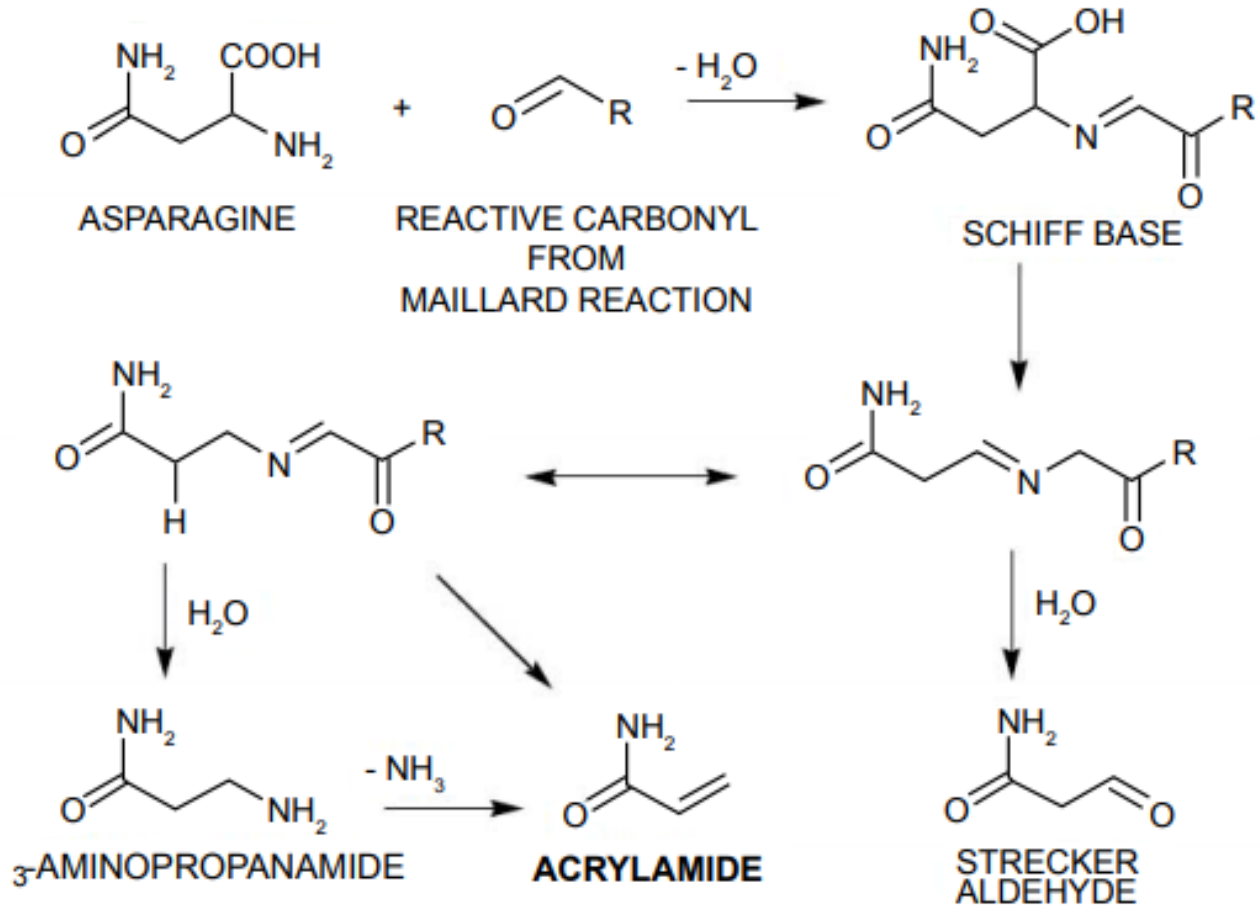
Acrylamide is formed when foods are heated to temperatures above 120 ° C for moderately long periods under the limited presence of water.

The cooking process, in particular, **frying or roasting** at high temperatures, induces a higher degree of acrylamide formation.

Acrylamide formation in food

The primary route of acrylamide formation is the **Maillard Reaction**, between asparagine and reducing sugars (fructose or glucose, for example).

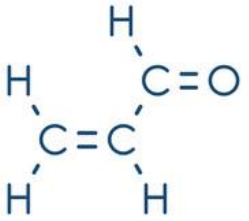
The first step is the formation of a Schiff Base, which after decarboxylation is hydrolyzed to form 3-aminopropionamide (3-APA), a potent precursor of acrylamide, or can directly form acrylamide.



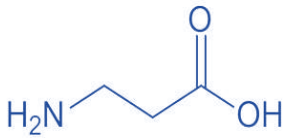
Acrylamide formation in food

➢ Secondary reaction route

Acrolein is an unsaturated aldehyde produced from triglycerides through an intense heat treatment that also leads to the formation of **acrylamide**.

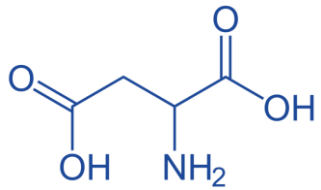


acrolein



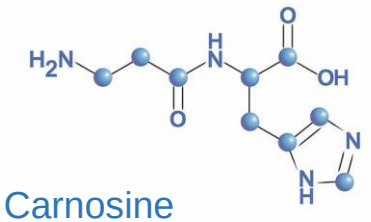
β-alanine

Amino acids, such as **β-alanine** and **aspartic acid**, can also lead to the formation of acrylamide.



Aspartic Acid

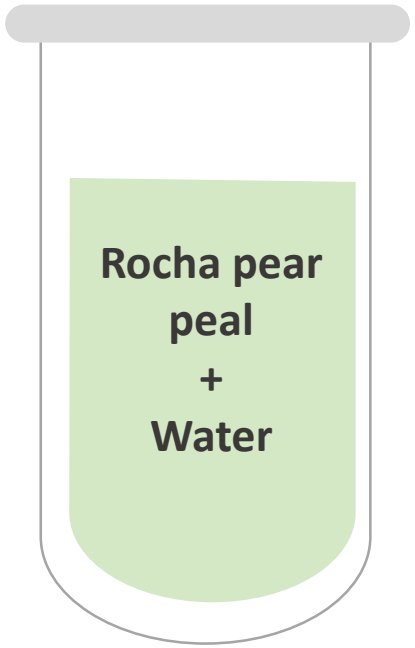
Another secondary route for acrylamide formation that only occurs in certain food groups, such as meat, is the **carnosine pyrolysis reaction**.



Carnosine

Extraction Preparation

➢ Aqueous Extract



Agitation
60 min

Filtration

Extraction Preparation

➢ Dry Extract

Rocha pear peal
+
Ethanol:Water

Agitation

60 minutes

Centrifuge

15000 rpm for 15 minutes

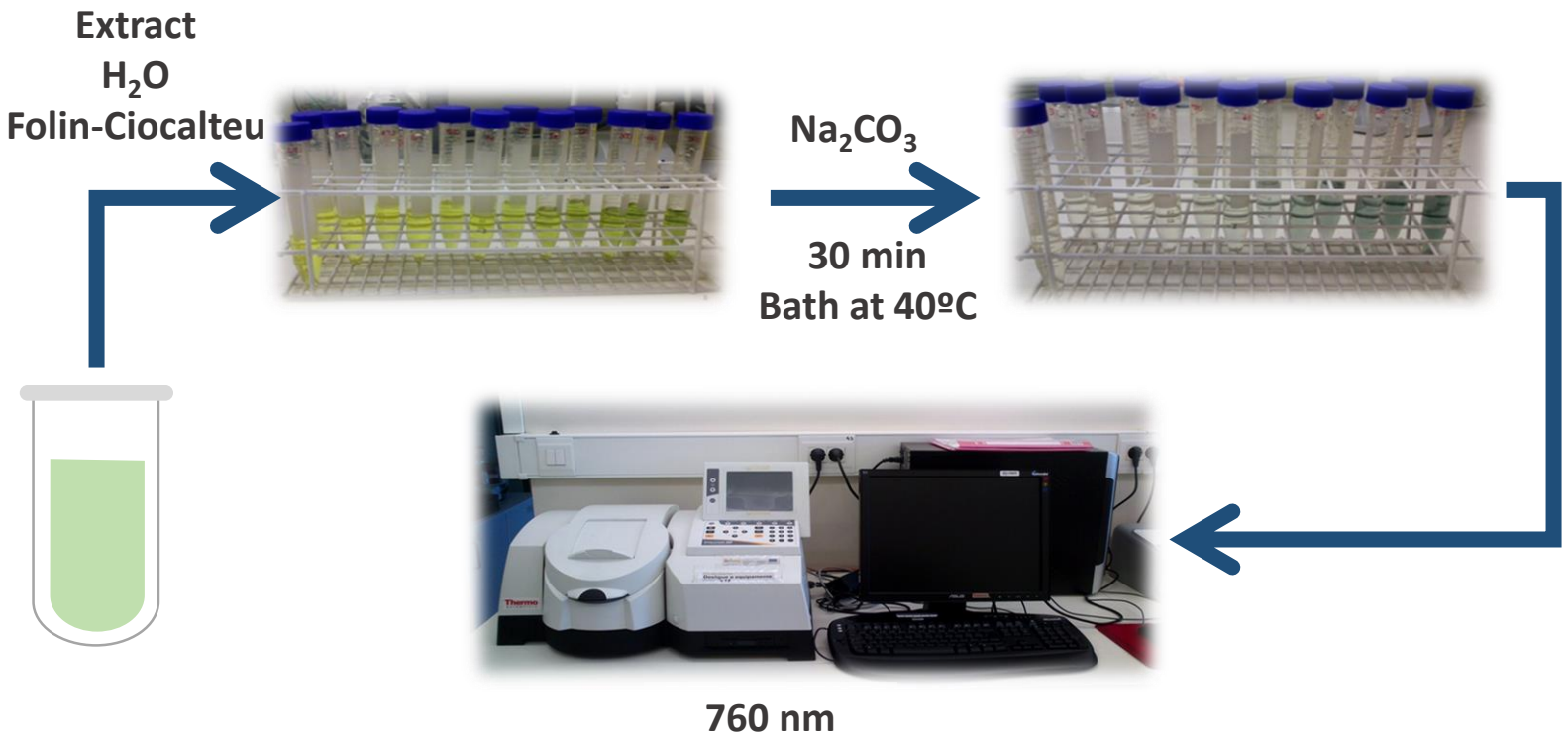
Filtration

Evaporation of the ethanol

40°C

Analytical procedure

➢ Total Phenol Content (TPC)

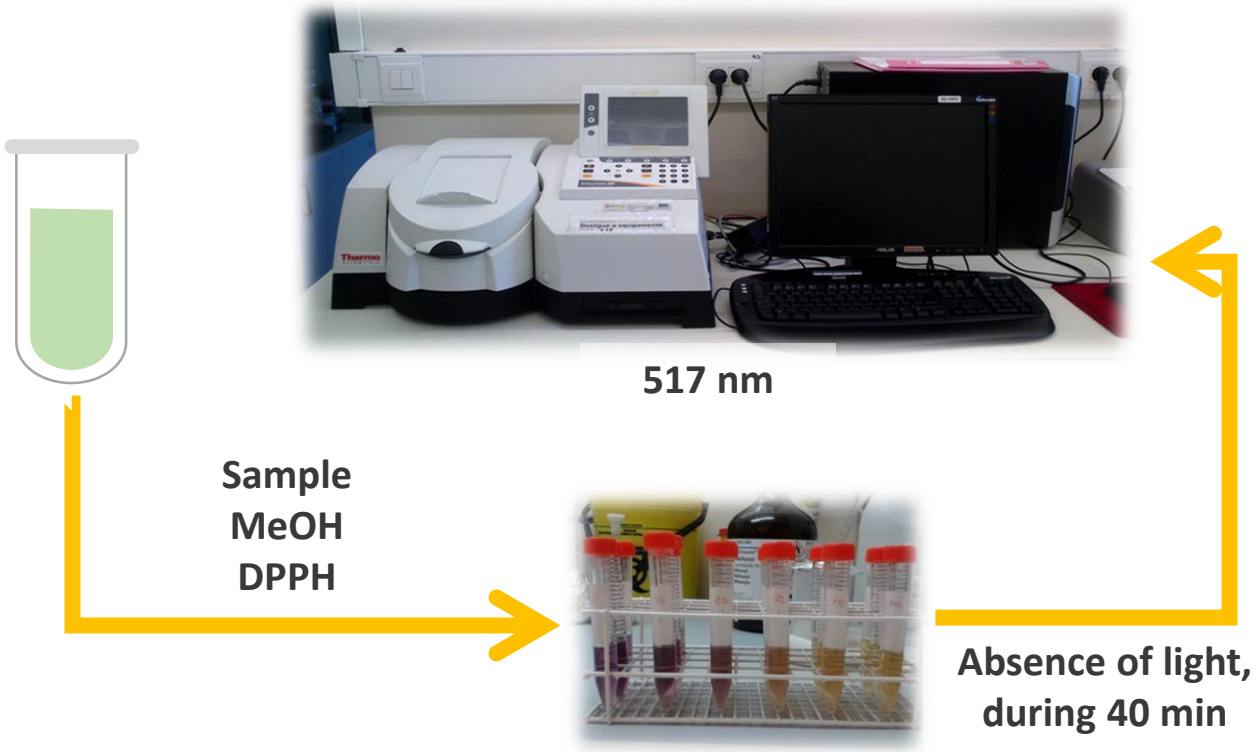


TPC was determined by the Folin-Ciocalteau method. A colorimetric with absorbance read at 760 nm. A calibration curve was performed with gallic acid. Results are expressed in mg equivalents of gallic acid per g sample (mg eq. GA / g_{sample}).

Analytical procedure

➢ Scavenging effect on DPPH radical

The antioxidant capacity was analyzed by the elimination of free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH•). The absorbance of each solution was measured at 517 nm.



Analytical procedure

➢ Sample preparation and AA determination by UPLC-MS/MS

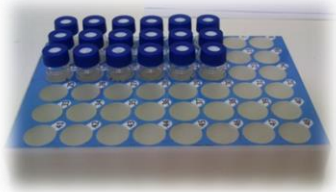
Grind Sample

Extraction
Water + Sample

Agitation

Centrifugation

Solid Phase
Extraction



UPLC-MS/MS
Acrylamide Determination

Production tests

- Eight distinct batches were produced:
  - two types of flour – wheat and rye
  - Aqueous extract (a) and Dry Extract (d)
  - Traditional Oven (A) vs Convection Oven (B)

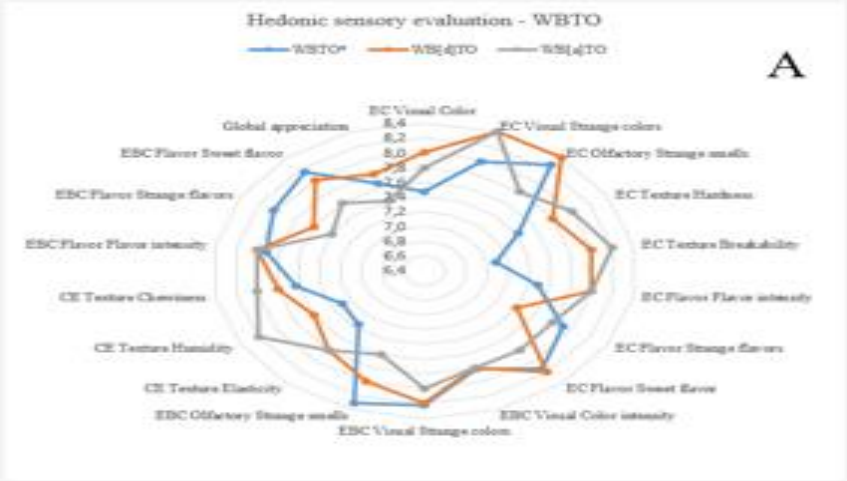
- All variables were defined and controlled
  - (fermentation time, cooking time, cooking temperature and homogeneity of premixes).
  - Each batch was composed of one control sample and five replicates with the addition of the extract.

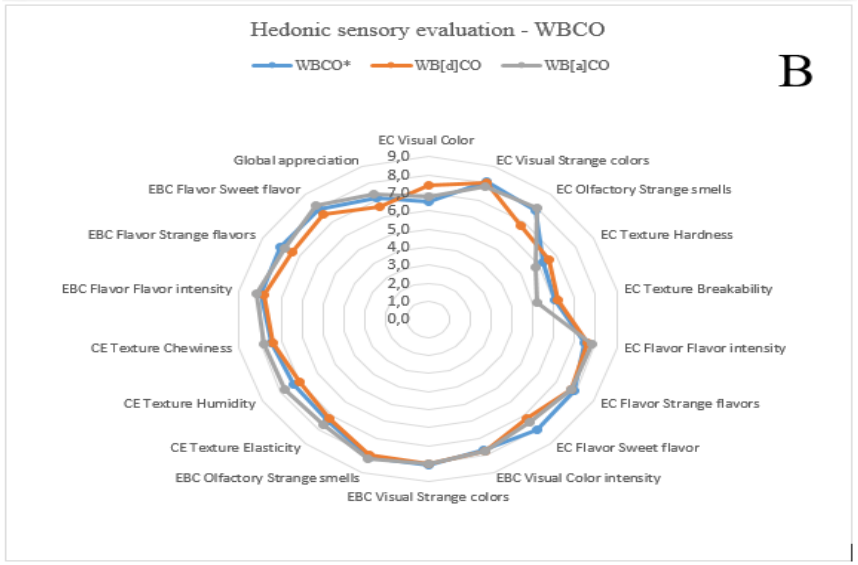
Sensorial evaluation

- appearance, odour, texture, taste, colour, and overall acceptability for both bread crumb and crust.
- For hedonic evaluation a 9-point scale was used where 9 was considered excellent and 1 extremely unsatisfactory.
- verify the existence of differences between samples using the control and the replicates.

Results

➢ Wheat bread hedonic evaluation (Global)





Hedonic sensory evaluation of Wheat Bread (WB), (A): cooked in a traditional oven (TO) and (B): convection oven (CO); 9-point scale where 9 was excellent and 1 extremely unsatisfactory.

The addition of the extract in **aqueous form (a):**

- ❑ in the **Convection Oven (B)** had a higher score than the **control** (7.1 / 7.4)

- The addition of the extract in **dry form (d):**

- ❖ in a **Traditional Oven (A)** had a higher score than the **control** (7.7 / 7.8)

Evidence:

- The only batch to show a **decrease** in terms of hedonic valuation was: wheat bread with **dry extract in the Convection Oven -** with a lower **texture score**, especially the breakability, important factors in the consumer acceptance

Results

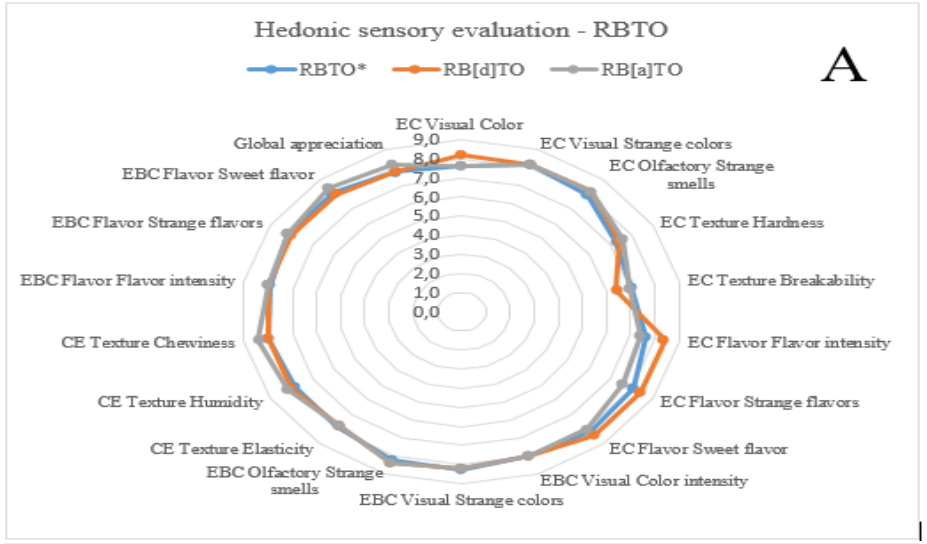
➢ Rye bread hedonic evaluation (Global)

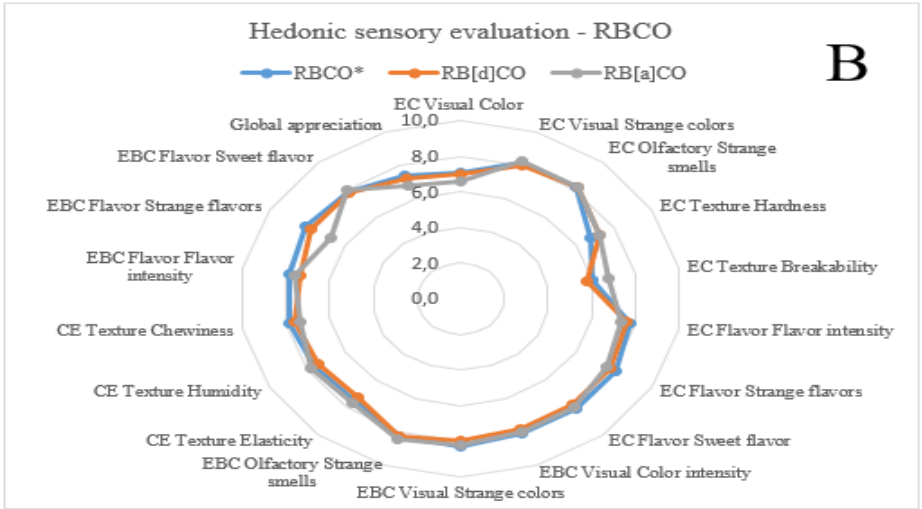
The addition of the **aqueous extract (a)**

- ❑ in a **Traditional Oven (A)** had a higher score than the **control** (7.8 / **8.2**)

The addition of the **dried extract (d)**

- ❖ in the **Traditional Oven (A)** had the same score as the **control** (**7.8 / 7.8)**





Hedonic sensory evaluation of Rye Bread (RB), (A): cooked in a traditional oven (TO) and (B): convection oven (CO); 9-point scale where 9 was excellent and 1 extremely unsatisfactory

Results

➢ Hedonic evaluation (Global)

Table 1 – Results of the hedonic evaluation of wheat and rye bread with and without the addition of Rocha pear peer extract cooked in two types of oven.

	Crust evaluation - Visual		Crust evaluation - Olfactory	Crust evaluation - Texture		Crust evaluation - Flavor			Bread crumb evaluation - Visual		Bread crumb evaluation - Olfactory	Bread crumb evaluation - Texture			Bread crumb evaluation - Flavor			Global
	Color	Strange colors	Strange smells	Hardness	Breakability	Flavor intensity	Strange flavors	Sweet flavor	Color intensity	Strange colors	Strange smells	Elasticity	Humidity	Chewiness	Flavor intensity	Strange flavors	Sweet flavor	
WBTO*	7,5	8,0	8,3	7,4	7,1	7,5	7,9	8,1	7,8	8,2	8,3	7,4	7,3	7,6	7,9	8,0	8,2	7,7
WB[d]TO	8,0	8,4	8,4	7,8	8,0	8,0	7,4	8,2	7,8	8,2	8,0	7,8	7,6	7,8	8,0	7,6	8,0	7,8
WB[a]TO	7,8	8,4	7,8	8,0	8,2	8,0	7,8	7,8	7,8	8,0	7,6	7,8	8,2	8,0	8,0	7,4	7,6	7,4
WBCO*	6,5	8,1	7,9	6,3	6,1	7,4	8,0	8,0	7,7	8,0	8,1	7,4	7,3	7,4	8,0	8,0	8,0	7,1
WB[d]CO	7,4	8,0	6,8	6,6	6,2	7,6	7,8	7,2	7,8	8,0	8,0	7,2	7,0	7,4	7,8	7,4	7,6	6,6
WB[a]CO	6,8	7,8	8,0	5,8	5,2	7,8	7,8	7,4	7,8	8,0	8,2	7,6	7,8	7,8	8,2	7,8	8,2	7,4
RBTO*	7,6	8,2	7,9	7,3	7,1	7,6	8,0	8,2	8,0	8,2	8,3	7,8	7,8	8,0	7,9	8,2	8,1	7,8
RB[d]TO	8,2	8,2	8,2	7,4	6,4	8,4	8,4	8,4	8,0	8,2	8,4	7,8	8,0	8,0	8,0	8,0	8,0	7,8
RB[a]TO	7,6	8,2	8,2	7,6	7,0	7,4	7,6	8,0	8,0	8,2	8,4	7,8	8,2	8,4	8,0	8,2	8,4	8,2
RBCO*	7,0	8,2	8,2	6,8	6,0	7,8	8,1	8,1	8,1	8,3	8,2	7,4	7,6	7,8	7,8	8,1	7,9	7,3
RB[d]CO	7,0	8,0	8,2	7,2	5,8	7,6	7,8	7,8	7,8	8,0	8,2	7,2	7,4	7,6	7,4	7,8	7,8	7,2
RB[a]CO	6,6	8,2	8,2	7,2	6,8	7,4	7,6	8,0	8,0	8,2	8,4	7,6	7,8	7,4	7,6	6,8	8,0	6,8
SD	0,552	0,175	0,417	0,640	0,880	0,301	0,259	0,342	0,120	0,111	0,230	0,245	0,377	0,301	0,210	0,416	0,234	0,456

Legend: WB- Wheat Bread; RB- Rye Bread; TO – Traditional Oven; CO – Conventional Oven; [d] – dehydrated extract; [a] – aqueous extract; * - Control Sample; SD – Standard deviation

Evidence:

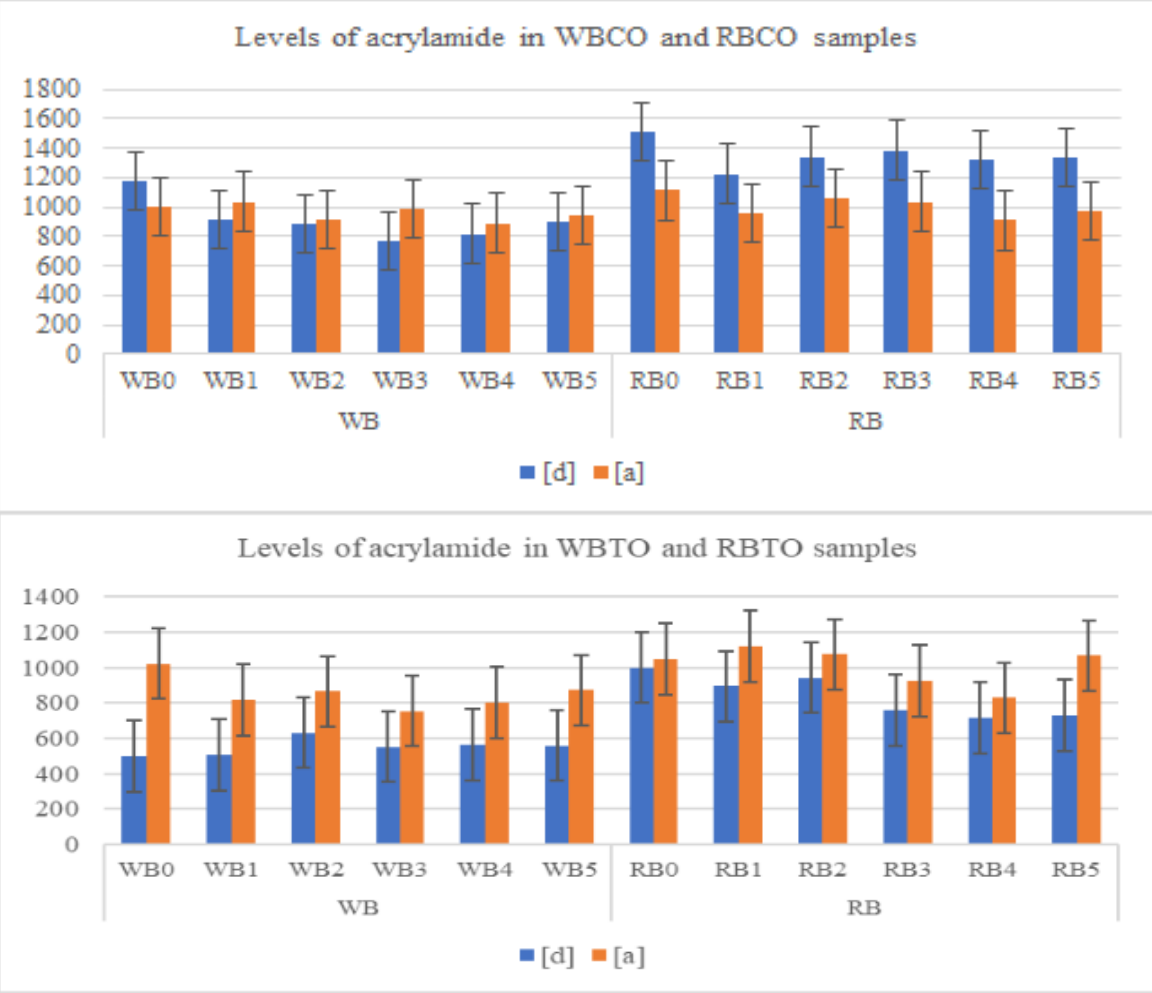
- there were only **two out of eight combinations** tested showing to be **not applicable**, namely:

- Wheat Bread / Convection Oven / dried extract [d]
- Rye Bread / Convection Oven /aqueous extract [a]

No significant differences were observed between the control and the replicates

Results

➢ Acrylamide content



Levels of AA (µg/kg) - determined in (A): Wheat Bread (WB) and Rye Bread (RB) cooked in a convention oven (CO); and (B): Wheat Bread (WB) and Rye Bread (RB) cooked in a traditional oven (TO); [d] – dehydrated extract; [a] – aqueous extract

The AA content in control samples for:

Wheat Bread (WB0) - ranged from 497 – 1178 µg/kg
Rye Bread (RB0) - ranged from 1000 – 1510 µg/kg

- These values are:

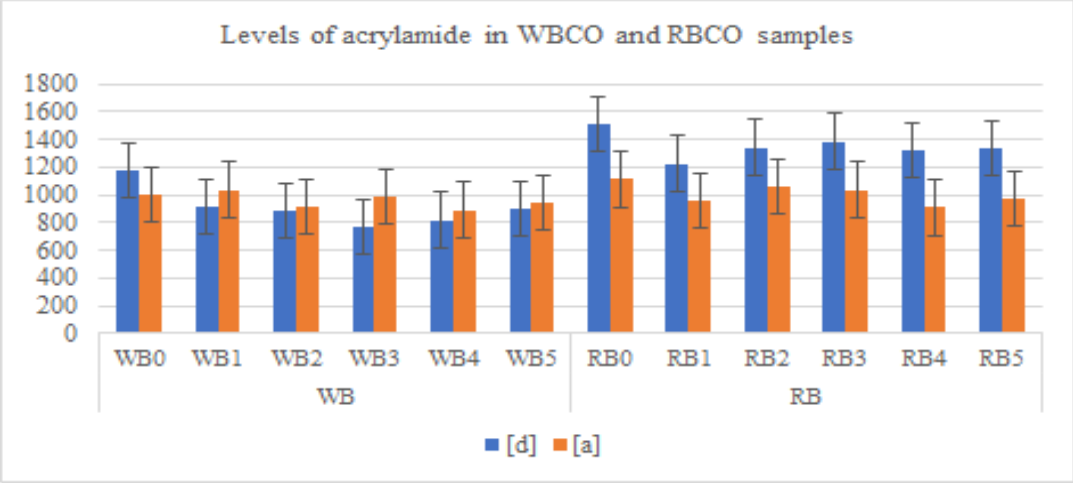
- lower than others (EFSA in 2009 and 2011)
- similar, in a range of 65 to 1271 µg/kg in crisp bread (Mojska et al, 2010)
- higher, some studies reported lower AA concentrations in bread products (Mojska et al., 2010; Wang et al. 2016)

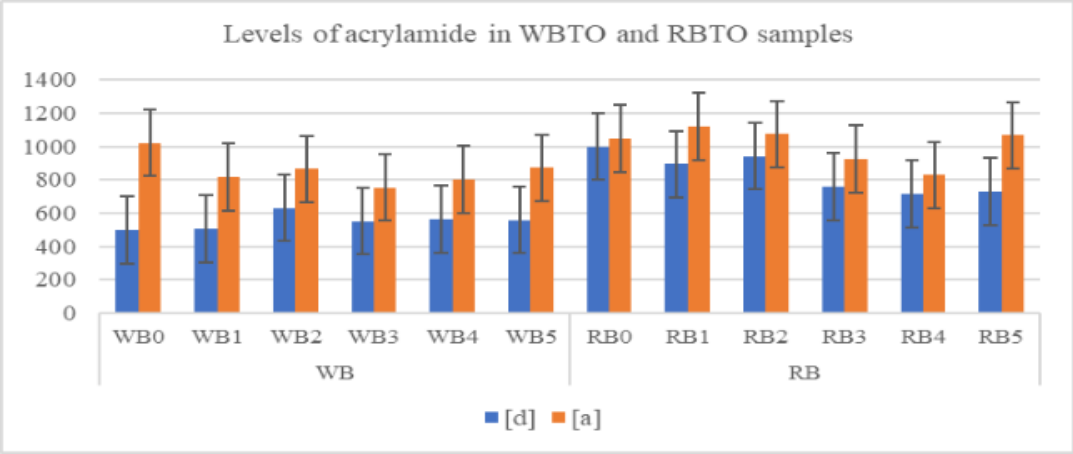
- **It was shown that the AA content in the Rye Bread was higher than in the Wheat Bread**, which is in agreement with other studies

- Comparing the two types of the oven in both bread (WB and RB) was observed that, in general, **in the Traditonal Oven AA content is lower than in the Convection Oven**

Results

➢ Acrylamide content





Levels of AA (µg/kg) - determined in (A): Wheat Bread (WB) and Rye Bread (RB) cooked in a convention oven (CO); and (B): Wheat Bread (WB) and Rye Bread (RB) cooked in a traditional oven (TO); [d] – dehydrated extract; [a] – aqueous extract

Reduction rate of AA

The average of the values obtained in the five replicates was calculated

this value was compared to the value of AA content obtained in the control sample

Effect of the pear peel extracts:

- AA reduction varies between

- 27.3% (Wheat Bread; dried extract (d) in Convention Oven) - 4.26% (Rye Bread; aqueous extract [a] in Traditional Oven)

- For the Rye Bread the best reduction was accomplished with dried extract [d] in the Traditional Oven: 19.2%.

Conclusions

The effect of extracts varies with:

- the matrix,
- type of oven
- with the interactions of phenolic compounds

Reinforcing the importance of understanding the acrylamide (AA) formation and mitigation in each matrix

Best results

- wheat bread (WB) baked in a convection oven (CO) and in which the dry extract ([d]) was added

- To use a traditional oven (TO), the best results would be achieved in: wheat bread added with aqueous extract.

(since the remaining combinations in this type of oven showed little expressive mitigation values)

Conclusions

Various combinations of variables must always be assessed and validated

- Formulas
- Composition of extract
- Type of oven
- The acceptability of bread

Advantages of using pear peel extract as a mitigation agent for AA:

- When compared to the use of other compounds, is that it is a natural agent
- In comparison with other studies, it has good sensory acceptance
- Good results achieved with a bakery product, such as bread, and not in products with more ingredients such as cookies, biscuits or cakes, for which it is easier to mask the taste.

- Depending on the acrylamide (AA) mitigation effects, these results enable us to select the best baking process according to the varieties of bread and oven
- One of the major challenges in the development of new formulations of high consumption products, such as bread or bakery products, relates to the acceptance of the innovated products by its usual consumers.
- Therefore, further studies are already planned to determine the acceptance of the selected formulas by potential consumers

Effect of Rocha pear peel extracts added to wheat and rye bread formulations on Acrylamide reduction and sensory quality maintenance

Morgado, C1; Bernardo, P1; Henriques, I1; Jesus, S2; Rego A2; Delgado I2; Coelho I2; Castanheira I2; Félix, N1; Fernandes, A1, Guerra, M1 and Brandão, C1

1Estoril Higher Institute for Tourism and Hotel Studies, Food Science Department, Estoril, Portugal;

2National Health Institute Doutor Ricardo Jorge, Food and Nutrition Department, Lisboa, Portugal

MISAGE

Mitigation Strategies of Acrylamide and Advanced Glycation End-Products in Bread

Projetos de Investigação Científica e Desenvolvimento Tecnológico (IC &DT)
Aviso 02/SAICT/2016