Effect of Rocha Pear Peel Extracts Added to Wheat and Rye Bread Formulations on Acrylamide Reduction and Sensory Quality Maintenance

Cátia Morgado[1(✉)], Patrícia Bernardo[1], Inês Henriques[1], Susana Jesus[2], Andreia Rego[2], Inês Delgado[2], Inês Coelho[2], Isabel Castanheira[2], Nélson Félix[1], António Fernandes[1], Manuela Guerra[1], and Carlos Brandão[1]

[1] Food Science Department, Estoril Higher Institute for Tourism and Hotel Studies, Estoril, Portugal
{catia.morgado,nelson.felix,manuela.guerra,carlos.brandao}@eshte.pt, patriciabernardo@gmail.com, ines.a.l.h@gmail.com

[2] Food and Nutrition Department, National Health Institute Doutor Ricardo Jorge, Lisbon, Portugal
{Susana.jesus,andreia.rego,ines.delgado,ines.coelho,Isabel.Castanheira}@insa.min-saude.pt

Abstract. Pear peels are seen as potentially valuable for their low-cost beneficial components content such as polyphenols. These may reveal acrylamide (AA) mitigation effect and thus their application in a susceptible food matrix, such as bread, should be considered. Aiming to assess the AA reduction potential of Rocha pear peels in bread and the effects on its sensory quality, two types of bread highly consumed in Portugal - wheat (WB) and rye (RB) – were assayed with the extract of these by-products, in two forms aqueous [a] and dry [d]. Eight bread batches were produced (4 WB; 4 RB); each composed of one control sample and five replicates added with extract. The process included controlled fermentation, and cooking in a traditional oven (TO) and convection oven (CO). Hedonic evaluation was made to samples of each batch. Overall, slight differences were observed for WB and RB hedonic evaluation between the control sample and those with both extract forms. Lower scores were observed in both bread types baked in CO, with [d] comparing with the control; for bread with [a], oven influence varied; higher scores for WB in CO and for RB baked in TO, comparing with the control. Regarding AA reduction, the highest mitigation rate was accomplished by the [d] in WB cooked in a CO, 27.3%. However, for RB the best formulation was obtained with [d] in the TO, 19.2%. These results support the importance of selecting the best baking process according to the varieties of bread and AA reduction.

Keywords: Rocha pear peels · Acrylamide (AA) · Bread sensory quality

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1 Introduction

During the thermal process of food several chemical reactions occur, such as the Maillard Reaction, caramelization and lipid oxidation, which can generate undesired substances on food, like acrylamide (AA) [1–4]. This compound was identified in foodstuffs for the first time in 2002 and has been ever since a concern to the European Food Safety Authorities (EFSA) [5]. AA is largely formed by the reaction between an amino acid, asparagine, and a reducing sugar, glucose or fructose [6]. Still, it can also derive from lipids in the absence of glucose, where in this case the acrolein and acrylic acid, from the lipid oxidation, can react with asparagine and produce the contaminant. This organic compound is formed in food products containing high starch and carbohydrates such as cereal, coffee and potato products [7–11].

AA has been included on the list of priority substances of the project TDS exposure (Total Diet Studies) [7] and is classified by the European Union as carcinogen (category 1B), mutagen (category 1B) and reproductive toxicant (category 2, fertility) [12].

In 2015, EFSA concluded that the level of AA present in food is a concern for public health [5]. Following, in 2017 the European Commission established a regulation for AA levels in foodstuffs and AA mitigation measures by all food business operators along the food chain [13]. Therefore, it is important to understand the mechanisms of AA formation and develop reduction strategies in processed products.

In the particular case of bread, which is a staple food from the gastronomical, nutritional and economical points of view of a country, with an annual intake recommended by the World Health Organization (WHO) of 60 kg/capita [14]. However, according to FAO/WHO bakery products (bread and rolls) contribute between 10 and 30% to AA exposure in people's diet [15], making it fundamental to reduce the AA content in this matrix.

Previous studies have demonstrated that AA formation in heat-processed food depends on many factors, such as the initial concentration of the precursors, the processing methods, the processing conditions, additives, pH, water activity, and type of matrix [15–17].

Concerning bakery products, in which bread is included, many studies have been developed for reducing AA using different strategies, such as the type of flour, the addition of enzymes, and modified the processing conditions [18–20]. More recently, many studies demonstrated a positive correlation between AA mitigation and the application of herbs extracts, spices and antioxidants [21–23]. Jesus [24] studied the effect of aromatic herbs and spices in bread with oat flour in which AA reduction reached 50 to 80%. Other author studied the effect of different antioxidants in a model system, which contained asparagine and glucose, and concluded that caffeic acid reduces AA formation [25]. Also, Levine and Smith obtained good results with the addition of ferulic acid to crackers [26]. In all these studies highest reductions were obtained by the addition of aromatic herbs and spices that contain ferulic acid, caffeic acid and/or gallic acid. The reduction process based on these additives depends on their origin [27] but is more affordable than using the asparaginase enzyme, which represents an expensive solution and therefore more natural sources of AA mitigants should be investigated.

Every year, food industries produce large amounts of by-products (or food wastes), which are further seen as potentially valuable not only for their low-cost beneficial components content but also for the environmental benefits their effective use may represent. Fruit peels are among the vegetable-derived food wastes, some of which known for their content of components with a health benefit, and thus with potential to be used as food additives [28, 29].

Pear production represents a significant economic activity to Portugal (c.a. 190,000 tons per year), being the cultivar Rocha, an exclusive Portuguese variety, accounting for 95% of the national production that is mainly concentrated in the West region of the country [30]. Many studies concluded that pear has a high concentration of polyphenols. Furthermore, Wang et al. concluded that the pear peel contains more nutrient components than in pulp, and it is an important source of polyphenols and triterpenes [15].

Reiland and Slavin [29] refer a study by Barbosa et al. [31] who investigated the phenolic- compounds in aqueous and ethanolic extracts of peel and pulp from 8 different pear varieties in the USA. The peel extracts had higher total soluble phenolic content and related antioxidant capacity than pulp extracts. Previously in Portugal, Salta et al. [30] studied the phenolic profile and the antioxidant activity of Rocha pear, compared with other commercially available pear varieties. Rocha pear (peel and pulp) presented the highest content of total phenolics, such as chlorogenic, syringic, ferulic and coumaric acids, arbutin and (-) epicatechin.

Considering these studies, the application of Rocha pear peel extracts in a food matrix, such as bread, should be considered as a potential AA mitigant. This strategy requires, the assessment of the effects of the pear peel extracts at different food parameters (toxicity, rheological, nutritional), including the sensory characteristics of the products, which are a main determinant consumption factor. In fact, 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. In these situations, sensory analysis is an approach of particular importance, which includes, among others, tools that allow the prediction of products acceptance and afterwards the measurement and interpretation of consumer behaviour [32].

The objective of this work is to preliminary assess the AA reduction potential of the Rocha pear peels extract in two types of bread highly consumed in Portugal - wheat and rye bread and the respective effects on the sensory quality of these products.

2 Materials and Methods

2.1 Plant Materials and Chemicals/Reagents

Rocha pears were obtained from local supermarkets in Lisbon, March 2018.

For this study, the following reagents were used: 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and gallic acid (Sigma-Aldrich), Folin-Ciocalteau reagent, sodium carbonate (Na_2CO_3), and methanol (Merck,), Acetonitrile (Merck gradient grid is liquid chromatography), formic acid (Group Carlo Erba Reagents, 99% for analysis), methanol

(Merck, hypergrade for LC-MS) and ultrapure water (captured from a Milli-Q water purification system). AA (99%) was purchased from Dr. Ehrenstorfer GmbH.

2.2 Preparation of the Extracts

Dry and aqueous extracts of Rocha pear peel were prepared in order to test their ability to mitigate AA in bread samples.

Aqueous extract ([a]) was prepared by adding water to the Rocha pear peel and left stirring for 60 min on a horizontal shaker at room temperature. The extract was then filtered by a 0.2 µm pore size membrane filter.

The dry extract ([d]) was performed by adding a solvent mixture of ethanol:water (6:30) to the Rocha pear peel and stirred for 60 min on a horizontal shaker at room temperature. The solution was centrifuge at 15000 rpm at 25 °C for 15 min and the supernatant was filtered by a 0.2 µm pore size membrane filter. Then, the extract was concentrated in vacuo at 40 °C using the rotary evaporator.

Both extracts were then stored at 4 °C until application in bread samples and characterization of their antioxidant activity.

2.3 Preparation of Bread Samples

Eight distinct batches were produced, corresponding to two types of flour - wheat and rye and two types of extracts – [d] and [a] for reducing AA. The selected flour formulas for each type of bread were mixed followed by controlled fermentation, division of units, and cooking in a TO and CO. 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 extract (Fig. 1).

2.4 Analytical Methods

Scavenging Effect on 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical

The antioxidant capacity of the dry and aqueous extracts was determined by the DPPH radical (DPPH•) method. First, the decrease in absorbance as a function of time was monitored at intervals of 5 min at 517 nm to determine the inhibition time in order that radical inhibition curves could be outlined. After in the second stage, it was added the DPPH solution to different extract concentration and the reaction mixture was kept in the dark for 40 min at room temperature. Then, the absorbance of each extract was measured at 517 nm against methanol blank. The half inhibitory concentration (IC_{50}) of DPPH radical was calculated based on linear regression of the inhibition percentage of DPPH as a function of the extract concentration. The results were expressed as µg of extract per mL of the reaction mixture (µg/mL).

Determination of Total Phenolic Content (TPC)

The total phenolic content of the extracts was determined by the Folin-Ciocalteau reagent method, using the Folin-Ciocalteau's reagent and the gallic acid as a standard. An aliquot of diluted extracts was mixed with 250 µL of Folin-Ciocalteau reagent and

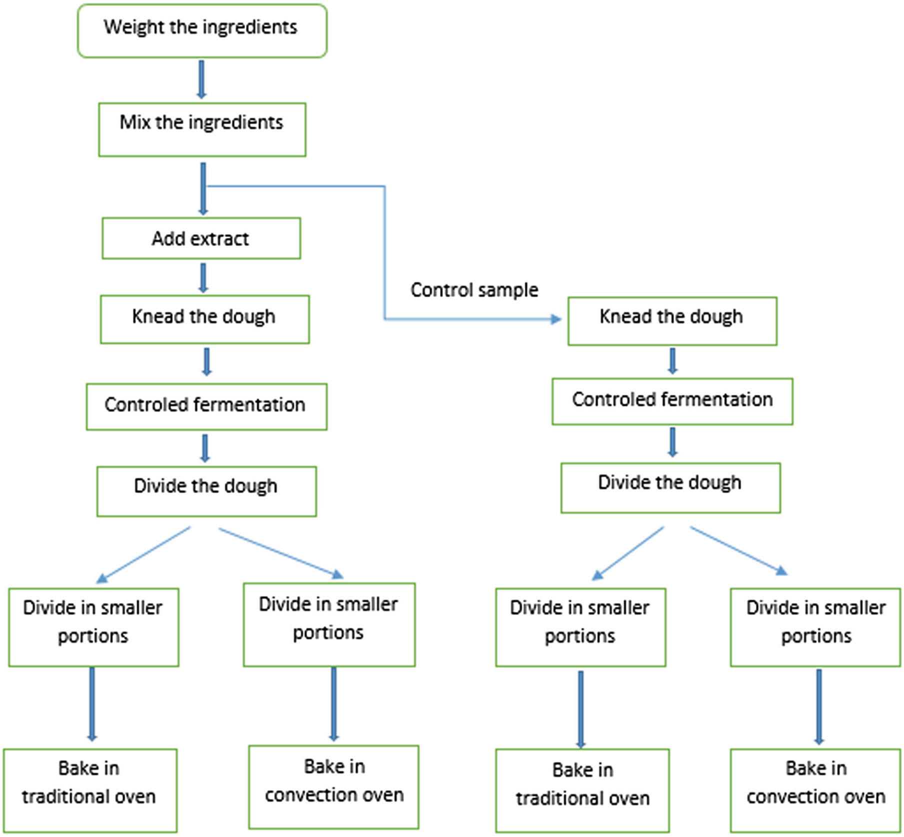

Fig. 1. Diagram for bread production.

3.70 mL of water. The reaction mixture was left to react for 5 min at room temperature. Then, the solution was neutralized with 1 mL of 15% (m/v) Na_2CO_3 and incubated for 30 min at 40 °C in a water bath. After incubation, the solution was left cooling at room temperature for 10 min, and the absorbance was measured at 760 nm.

The results were expressed as mg of GA equivalent per g of sample. The working range was 10 to 200 µg/mL, and the linear equation had a correlation coefficient greater than 0.9997.

Sensory Evaluation

Samples of each batch were sensory evaluated on the same day, following cooling to room temperature, by a panel of 6 experienced panellist members of the Food Science department of ESHTE. Panellists were asked to evaluate each sample for 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.

For the analysis of the hedonic evaluation, the Wilcoxon non-parametric test was used to verify the existence of differences between samples using the control and the replicates. Since the panel consisted of 6 experts in sensory evaluation, the exact values of p-value of this test were used. Statistical analysis was performed using the IBM SPSS Statistics Software, v.25, with $\alpha = 0.05$.

Sample Preparation and AA Determination by UPLC-MS/MS
Bread samples were prepared after the reception in the laboratory. Each sample was grinded using a high-speed grinder (Knife mill GRINDOMIX GM), homogenized and stored in vacuum bags at −80 °C. Then, 2 g of homogenized sample was weighed into a centrifuge tube, and 20 ml water with 0.1% of formic acid was added. The solution was stirred in a vortex for 2 min, followed by continuous agitation for 30 min in an oscillating shaker at 70 oscillations per minute. After, it was centrifuged at 10,000 rpm for 15 min at room temperature. For the clean-up of the extracts, an Oasis HLB SPE cartridges (Waters) were used. The cartridges were conditioned with methanol and equilibrated with acidified water. All samples were prepared and analysed in three replicates.

The stock solution of AA standard (1 mg/ml) was prepared by dissolving in ultrapure water with 0.1% of formic acid. The working standard solutions for the linear calibration were prepared by diluting the stock solution to the concentration sequences of 1, 10, 50, 100, 150, 250 ug/l. The stock and working solutions were kept at 4°C until injection in the system.

The quantification of AA was performed by Ultra Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-MS/MS) with electrospray ionization source (ESI), in the positive ion mode. For the analytical separation an UPLC BEH C18 column (2.1 × 50 mm) was used with isocratic elution with 90% water and 10% acetonitrile at a flow rate of 0.2 ml/min.

3 Results and Discussion

In the present study, the antioxidant capacity of the pear peel extracts was analysed. The results are reported as IC_{50}, which correspond to the required amount of extract to inhibit 50% of DPPH. For the extracts, IC_{50} of 0.2 mg/ml was obtained which are in agreement with Salta et al. [30]. In relation to the TPC, the results were lower than described in the literature, 0.7 mg eq AC/g sample, however for this analysis there aren't data available for this variety in particular.

In terms of global hedonic evaluation, in WB, it was verified that the addition of the [a] in a TO resulted in a lower score than the control (7.7/7.4); while in the CO it was higher than the control (7.1/7.4). Regarding the [d], it was observed that in comparison to the control, the score increased (7.7/7.8) in the TO; while in the CO it decreased (7.1/6.6) (Table 1) (Fig. 2).

It is thus evident that the only batch to show a decrease in terms of hedonic valuation was the [d] in the CO, with a lower texture score, especially the breakability, important factors in the consumer acceptance [33].

Regarding the RB, it was observed that the addition of the [a] in a TO resulted in a higher score than the control (7.8/8.2); while in the CO it was the contrary (7.3/6.8). Regarding the [d], no difference was observed with the control (7.8/7.8) in the TO; while in the CO there was a score decrease (7.3/7.2) (Table 1) (Fig. 3).

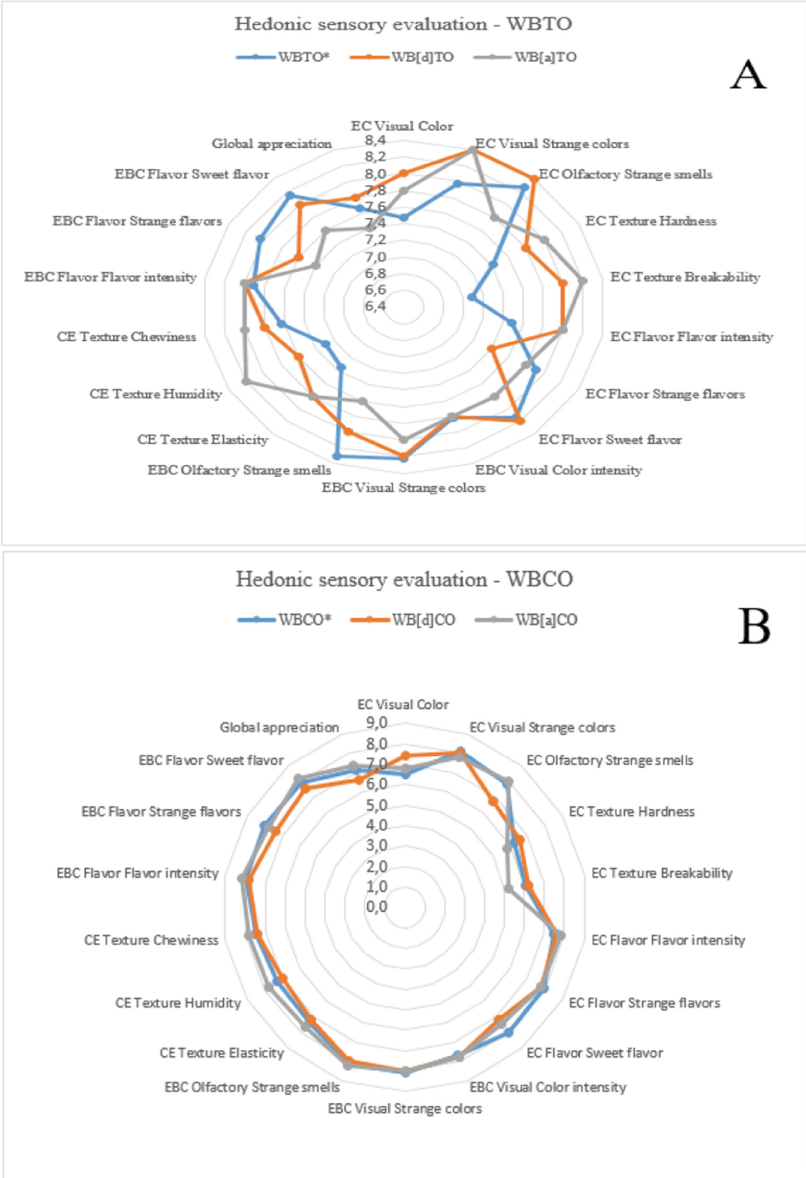

Fig. 2. Hedonic sensory evaluation of Wheat Bread (WB), (A): cooked in a traditional oven (TO) and (B): convection oven (CO); 9-point scale where 9 was excellent and 1 extremely unsatisfactory.

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

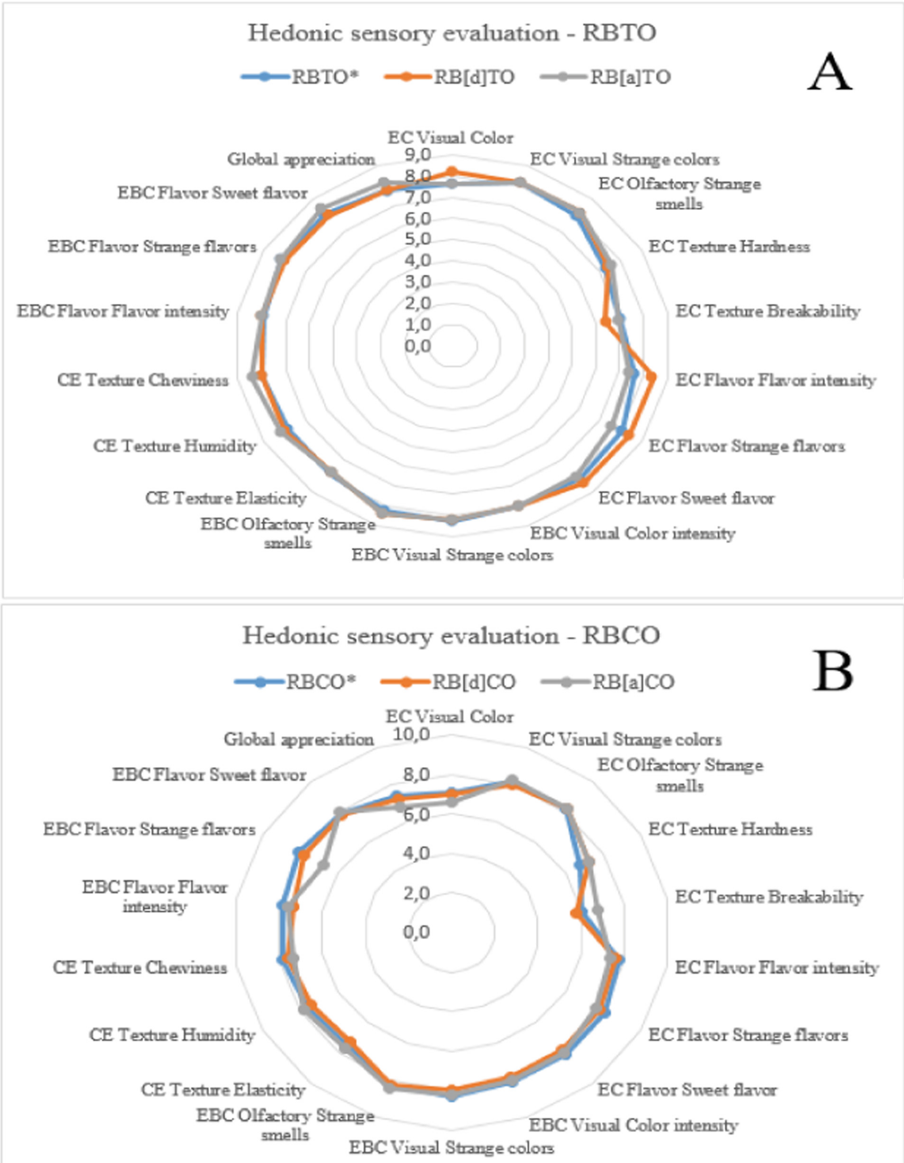

Fig. 3. Hedonic sensory evaluation of Rye Bread (RB), (A): cooked in a traditional oven (TO) and (B): convection oven (CO); 9-point scale where 9 was excellent and 1 extremely unsatisfactory.

The hedonic evaluation demonstrated that there is only a higher score when products are baked in the CO and added with [a] (Table 1).

From the sensory point of view, there were only two out of eight combinations tested showing to be not applicable, namely: WB/CO/[d] and RB/CO/[a].

The Mann-Whitney test reveals that the testers did not find differences between the control samples, which will allow an analysis of the significance of the results. No significant differences were observed between the follow control and the replicates: WB[d]TO and WBTO*, $Z = -0.447$, $p = 1$; WB[d]CO and WBCO*, $Z = -1.342$, $p = 0.5$; WB[a]TO and WBTO*, $Z = -3442$, $p = 0.5$; WB[a]CO and WBCO*, $Z = -3442$, $p = 0.375$; RB[d]TO and RBTO*, $Z = -0.577$, $p = 1$; RB[d]CO and RBCO*, $Z = -0.447$, $p = 1$.

The AA content in control samples for WB and RB, shown in Fig. 4, present a range of 497–1178 μg/kg and 1000–1510 μg/kg, respectively. These results were lower than the values published by EFSA in 2009 and 2011 [34]. Also, Mojska et al. found similar levels, in a range of 65 to 1271 μg/kg in crisp bread [35]. However, some studies reported lower AA concentrations in bread products [15, 36]. Furthermore, it was shown that the AA content in the RB was higher than in the WB, which is in agreement with the studies performed by Przygodzka et al. [37] and Capuano et al. [38]. These authors concluded that there is an impact of flour type on AA formation, where the RB has a higher content, then the spelt bread and at last the wheat bread. Comparing the two types of the oven in both bread (WB and RB) was observed that, in general, in the TO AA content is lower than in the CO.

In order to determine the reduction rate of AA, the average of the values obtained in the five replicates was calculated, being this value compared to the value of AA content obtained in the control sample. Therefore, relatively to the effect of the pear peel extracts, it was concluded that AA reduction varies between 27.3% (WB[d]CO) and 4.26% (RB[a]TO) (Fig. 4. For the RB the best reduction was accomplished with [d] in the TO, 19.2%. Comparing to literature, Levine and Smith obtained higher reduction rates with the addition of ferulic acid, which is a phenolic compound present in the pear [26]. Also, Zhu et al. found that the addition of proanthocyanins, such as catechin, epicatechin, in a starch-base model system result in a reduction around 31% and 62% [39].

One of the advantages of using pear peel extract as a mitigating agent for AA, when compared to the use of other compounds, is that it is a natural agent [19]. Also, in comparison with other studies, it has good sensory acceptance [40]. Another comparative advantage is that the results have been 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 [41]. Nonetheless, other authors accomplished much higher reduction values, using additives such as sodium hydrogen carbonate, reaching reduction levels of about 70% [42].

These results reinforce the idea that various combinations of variables must always be assessed and validated, such as formulas; composition of extract and type of oven that influence the mitigation effect on AA as well the acceptability of bread, although slightly.

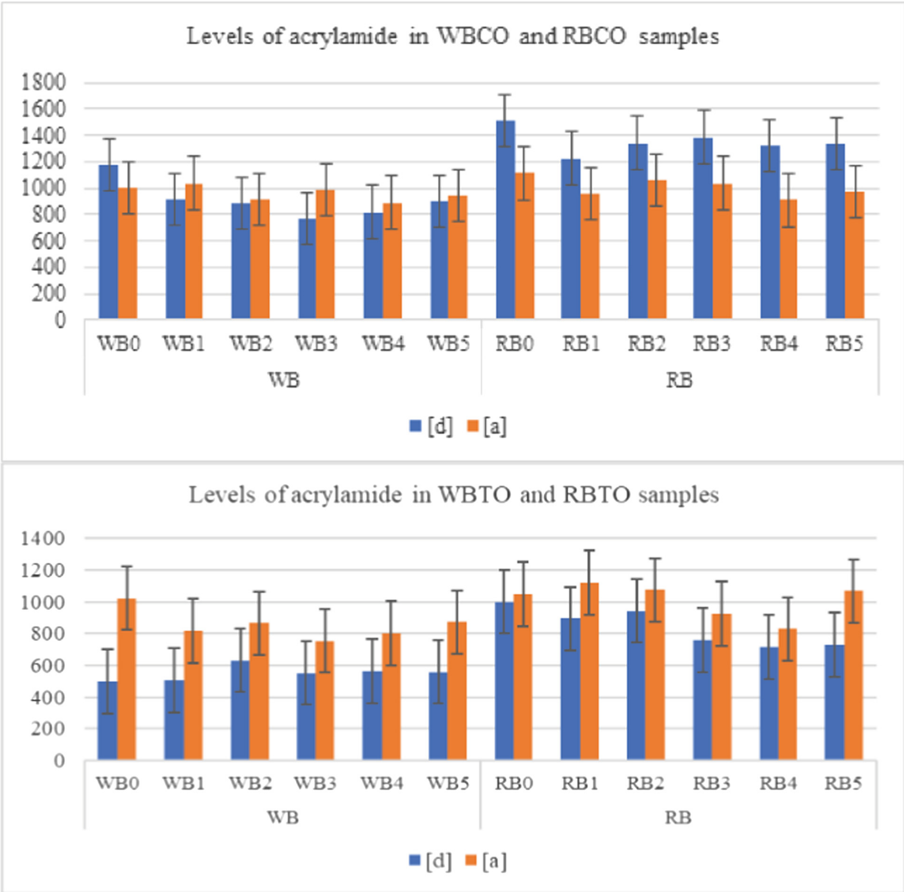

Fig. 4. Levels of AA (µg/kg) - determined in (A): Wheat Bread (WB) and Rye Bread (RB) cooked in a convention oven (CO); and (B): Wheat Bread (WB) and Rye Bread (RB) cooked in a traditional oven (TO); [d] – dehydrated extract; [a] – aqueous extract.

4 Conclusion

In all different types of assays, the best results were found in 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.

The effect of extracts varies with the matrix, type of oven and also with the interactions of phenolic compounds, reinforcing the importance of understand the acrylamide (AA) formation and mitigation in each matrix.

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.

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