



Tara pod (*Caesalpinia spinosa*) extract mitigates neo-contaminant formation in Chilean bread preserving their sensory attributes



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ABSTRACT

"Hallulla" is a highly consumed type of Chilean bread, which may contain considerable amounts of some Neo-Formed Contaminants (NFCs). The objective of this research was to study the effect of tara (*Caesalpinia spinosa*) pod polyphenolic extract (TPPE) on the mitigation of acrylamide (AA) and hydroxymethylfurfural (HMF) in "hallulla" bread without affecting its sensory attributes. The effect of different TPPE concentrations was evaluated (0–3000 mg kg⁻¹) over NFC formation during "hallulla" baking and its impact on sensory attributes. AA was mitigated by ~90% at 1500 mg/kg TPPE added to the bread pieces. A similar descriptive profile of the final product, with significant changes only in the crumb color was observed. HMF was mitigated by ~85% in bread pieces, when the highest concentration (3000 mg/kg) of TPPE was used. Our results highlight the potential of using polyphenolics from tara pod extracts to reduce the exposure of consumers to dietary neo-formed contaminants.

1. Introduction

Chile is the second largest consumer of bread in the world and the first one in Latin America, reaching 96 kg per capita of bread per year. In addition, nearly 59% of the population consumes bread at least 3 times per week, being indispensable in the Chilean food basket (United States Department of Agriculture USA, 2013). Chilean consumer preferences include foods that may contain considerable amounts of Neo-Formed Contaminants (NFCs) such as acrylamide (AA) and hydroxymethylfurfural (HMF) which have been classified as potential human carcinogens (International Agency for Research on Cancer, 1995).

Tara (*Caesalpinia spinosa*) is a leguminous tree indigenous to South America, having 8–10 cm long red or pale yellow pods (Chambi, Chirinos, Pedreschi, Betalleluz-Pallardel, Debaste & Campos, 2013; Romero, Fernández, & Robert, 2012). Tara pods and seeds have been used as a source of tannins and gum having a high potential for medical, industrial and food uses (Bravo, 2010). Tara pods (without seeds) represent ~65% of the total fruit weight and contains the highest percentage of tannins (~40%–60% of the total content). Tannic acid

obtained from tara pods is widely used in the pharmaceutical, chemical and paint industries, among others, and it is especially useful in the tannery industry (Chambi et al., 2013).

The successful replacement of some synthetic food antioxidants by safe natural antioxidants has fostered intensive search for new vegetable sources of antioxidants. In this sense, Skowrya, Falguera, Gallego, Peiró, and Almajano (2014) studied and quantified the phenol and flavonoid content of extracts of tara pods. Additionally, Aguilar-Galvez, Noratto, Chambi, Debaste, and Campos (2014) studied and quantified gallotannins obtained from tara pod extracts and from the products obtained after acid hydrolysis for 4 and 9 h. The antioxidant potential of tara pod extracts rich in gallotannins submitted to chemical hydrolysis was evaluated showing an increase in the release of gallic acid from the tara pod extracts during the hydrolysis process and an increased antioxidant activity compared to the non-hydrolyzed samples (Chambi et al., 2013).

Maillard reaction (MR) is one of the most important chemical reactions taking place during food processing at high temperatures (frying, baking, roasting or extrusion). Specifically, MR in

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protein–carbohydrate mixtures is important for the development of attractive sensory food attributes (color and flavor). However, the major concern arising from heating processes is the formation of toxic compounds that are not naturally present in foods (NFCs), but that may develop during heating processes and that reveal harmful effects such as mutagenic, carcinogenic and cytotoxic effects (Birlouez-Aragon, Morales, Fogliano, & Pain, 2010).

Acrylamide (AA) and hydroxymethylfurfural (HMF) are considered potentially carcinogenic to humans or might be metabolized by humans to potentially carcinogenic compounds. AA and HMF are mainly formed through MR and can be regarded as the most important heat-induced contaminants occurring in bread bakery products (Capuano & Fogliano, 2011). AA is produced as by-product of the MR in starchy foods processed at high temperatures ($> 120^{\circ}\text{C}$) (Pedreschi, Mariotti, & Granby, 2014). AA is known as a neurotoxin in humans and it is classified as a probable human carcinogen by the International Agency of Research on Cancer (International Agency for Research on Cancer, 1995). HMF is a furanic compound formed as an intermediate in MR when carbohydrates are heated in the presence of amino acids or proteins or, alternatively, by thermal dehydration of a sugar under acidic conditions (Capuano & Fogliano, 2011; Murkovic & Pichler, 2006). Abraham, Berg, Heinemeyer, Lampen and Appel (2011) found no relevance for humans concerning carcinogenic and genotoxic effects; however, HMF exposure resulting from caramel colors used as food additives needs to be further evaluated (Janowski, Glaab, Samimi, Schlatter, & Eisenbrand, 2000).

The complexity of MR is the major hamper to the development of mitigation strategies aiming at reducing the concentration of potentially harmful MR products in food (Capuano & Fogliano, 2011; Mariotti et al., 2015; Mariotti, Granby, Rozowski, & Pedreschi, 2013). For instance, the use of natural antioxidants has been proposed as an eventual strategy to mitigate NFC formation and in some cases, the presence of polyphenols could influence the overall MR development (Zhang & Jin, 2015). In this sense, it has been shown that some phenolic compounds, such as (–)-epicatechin, rutin, hydroxycinnamic acid, curcumin behave as trapping agents of carbonyl limiting carbohydrates reactivity under such conditions (Totlani & Peterson, 2007). However, in many cases natural antioxidants were not effective or even promoted the formation of acrylamide (Bohn et al., 2015; Ou et al., 2010). The formation of NFCs could be mitigated by favoring the conditions under which MR is inhibited. Thus, the main objective of this research was to study the effect of different concentrations of tara (*Caesalpinia spinosa*) pod polyphenolic extract (TPPE) over the mitigation of AA and HMF in “hallulla” bread while preserving its sensory attributes.

2. Materials and methods

Red and yellow Tara pods (*Caesalpinia spinosa*) were characterized at the physico-chemical level and ground. Subsequently, raw material displaying the highest antioxidant potential was selected and extracted with water at different tara powder/water ratios. Then, the total polyphenol content (TPC) and antioxidant capacity (AOC) of all the extracts were evaluated. The extract displaying the TPC and AOC contents was added (0–4000 mg/kg) in “hallulla” bread to determine its effect on the mitigation of its neo-contaminants. Additionally, the sensory quality and profile of the “hallulla” breads containing the TPPE were assessed.

2.1. Raw materials

Tara pods harvested in March 2017 were kindly donated by the National Forest Corporation (CONAF) from the Valparaíso Region of Chile. Tara pods, received in a dried state, were manually cleaned, eliminating pods with fungal presence or damaged by insects. Pods were separated by color in red and yellow pods and their dimensions (length and wide) were measured (N = 50) and averaged. The seeds

were manually discarded and the pods were ground using a mixer machine (model 4169/4297, Braun AG, Kronberg, Germany) until obtaining a fine powder, which was sieved through a 0.65 mm mesh sieve and Tara powder was stored in sealed polyethylene bags at ambient temperature and in absence of light.

2.2. Chemicals and analytic reagents

Analytical grade reagents, standards and solvents were used in chemical analyses. Folin-Ciocalteu reagent, sodium carbonate, gallic acid, glucose, fructose, acrylamide and HMF standards, dimethylaminocinnamaldehyde (DMAC; F.W. 175.23), solvents (acetone, methanol, acetonitrile, formic acid, hydrochloric acid, acetic acid and ethanol), Carrez solution I, Carrez solution II and sodium hydroxide were purchased from Sigma Aldrich (Steinheim, Germany).

2.3. Liquid extraction of tara pods polyphenols

Red Tara pods were ground and subjected to conventional solid-liquid extraction according to a modified methodology of Bravo (2010). The extraction was carried out with water (60°C) and solid/liquid ratios as follow: 1:10, 1:30 and 1:60 during 35 min with constant agitation. Then, the extracts were centrifuged and the supernatants were concentrated (10 kPa) at 40°C and 60°C until dry. After that, all samples were chemically analysed.

2.4. Chemical analysis

2.4.1. Proximate analysis

The protein, crude fiber, ash and fat contents of red and yellow tara pods were determined according to the A.O.A.C (1995) methodology.

2.4.2. Total polyphenol content (TPC)

TPC of raw material and extracts were determined by Folin–Ciocalteu assay (Singleton, Rossi, & Rossi, 1965). For raw material, TPC were determined after triple extraction with water (60°C , 35 min, 1:60 solid/liquid ratio). Results were expressed as g of gallic acid equivalent (GAE) per g of dry tara pod and g of tannic acid equivalents (TAE) per g of dry tara pod.

2.4.3. Antioxidant capacity (AOC)

AOC was determined by spectrophotometry (Spectrometer UV 1240, Shimadzu, Kyoto, Japan) according to the free radical 2,2-diphenyl-1-picrylhydrazyl method (DPPH). For raw material, TPC were determined after triple extraction with water (60°C , 35 min, 1:60 solid/liquid ratio). Results were expressed as the efficient concentration of extracts (EC: mg/mL), which is the concentration necessary to inhibit the 50% of radical absorption of DPPH (Brand-Williams Cuvelier & Berset, 1995).

2.4.4. Determination of reducing sugar content

The reducing sugar contents of tara extracts was quantified according to a modified methodology of Mariotti-Celis, Martinez-Cifuentes, Huamán-Castilla, Pedreschi, and Pérez-Correa (2017).

The reducing sugar contents of both raw and purified extracts were quantified by High performance Liquid Chromatography coupled with a Refraction Index Detector (HPLC-IR) according to a modified methodology of Mariotti-Celis et al. (2017). The extract was dissolved in MilliQ water (1:30 w/v) and mixed in a vortex (Heidolph Reax top, Nuremberg, Germany) during 60 s. The diluted samples were centrifuged (Hettich Zentrifugen, MIKRO 220 R, Massachusetts, USA) at 4032 g during 10 min at 4°C . Then, 2 mL of supernatant was filtered with a 0.22 μm nylon syringe filter. Subsequently, 300 μL of filtered solution were mixed with 700 μL of acetonitrile to be injected in an HPLC-IR system (Thermo Scientific Dionex Ultimate 3000, Massachusetts, USA) equipped with a normal phase Li ChroCART[®] 250-4 Purospher[®] STAR

Table 1
“Hallulla” bread formulation.^a

Ingredients	Weight (g)
Wheat flour	100
Warm water at 30 °C	50
Lard	5
Freeze dried yeast	2
Partially skimmed milk powder	0.6
Sugar	0.5

^a Based on 100 g of flour.

(5 µm) at 40 °C. Chromatographic separation was carried out at isocratic conditions. The mobile phase, flow rate and injection volume were: acetonitrile solution (70% v/v), 1 mL/min and 20 µL, respectively. Under these operating conditions the fructose and glucose retention time were 4.6 and 5 min, respectively. Analyses were performed in triplicate and results were expressed in mg of reducing sugar (fructose/glucose) per gram of dried weight.

2.4.5. Determination of the acrylamide content

The acrylamide content of “hallulla” breads was determined by GC-MS according to the methodology described by Mariotti-Celis, Martínez-Cifuentes, Huamán-Castilla, Pedreschi, and Pérez-Correa (2017).

2.4.6. Determination of the HMF content

HMF concentrations of “hallulla” breads were measured according to the method of Toker, Dogan, Ersoz, and Yilmaz (2013).

2.5. “Hallulla” bread production

Bread pieces were made according to the optimized formulation of Plaza (2015) with some modifications (Table 1). Dough was formed in two stages. (i) An initial mixed (58 rpm for 2 min) of the dough ingredients, flour, sugar, salt, semi-skimmed milk powder and yeast, previously dissolved in warm water at 30 °C. Dissolved tara powder was added when corresponding. (ii) A kneaded (110 rpm for 4 min) was done after melted lard (50 °C) was incorporated to the mix. Both stages were done in a kitchen mixer (KitchenAid Classic, model 5K45SS, USA). Once the dough was formed, dough sheets (6 mm) were formed by using an electric laminator (Supermaq, model 1001, Brasil) and circular dough pieces (7 cm diameter) were cut using a stainless steel circular mold. Formed pieces were perforated in 3 central points at their surface and then placed in a fermenter (Garbin, model 4333, Italy) at 30 °C for 30 min. Then, fermented dough pieces were baked in an electric oven (Garbin, model 4333, Italy) at 200 °C for 20 min. Finally, hallulla breads were cooled at ambient temperature and stored in sealed plastic bags (low-density polyethylene). For NFCs quantification, breads were stored at –18 °C for three days and for sensory evaluation the samples were stored at ambient temperature for 24 h.

2.6. Sensory evaluation

Training of the sensory panel was performed in the Sensory Evaluation Laboratory of the Universidad de Chile. Fourteen assessors with experience in sensory testing were selected, 9 women and 5 men, all students of Food Engineering with ages between 23 and 26 year old. Their training process consisted in one initial session to generate descriptors and five 1-h-sessions for assessing samples with different polyphenol concentrations and baking time. Reference samples for the different descriptors were presented at each session.

A descriptive analysis on a 10 cm non-structured linear scale was used considering the following descriptors (anchors between parenthesis): crust color (very pale – dark gold), violet gray color of bread crumb (absent – intense), fermented aroma (absent – intense), toasted aroma (absent – intense), tactile hardness (soft – hard), elasticity of the

crumb (none elastic – very elastic), hardness in the mouth (soft – hard), fermented flavor (absent – intense), toasted flavour (absent – intense). The panel was considered trained when it did not present significant differences among assessors in any attribute ($p > 0.05$).

The performance of the sensory panel was validated following the guidelines of the standard ISO 11132:2012 for the key attributes crust color, grey violet color, roasted aroma, hardness in the mouth and roasted flavor, by testing three significantly different samples, using the following baking times and tara extract concentrations, respectively: (i) 10 min and 200 mg TPPE/kg; (ii) 20 min and 1000 mg TPPE/kg; (iii) 30 min and 0 mg TPPE/kg. Samples were evaluated in triplicate in separate sessions.

The effect of the extract concentration in the final bread was assessed through the evaluation of bread samples containing 0 (control), 600, 750, 1000 and 1500 mg of tara pod extract per kg of wheat flour, respectively, by the previously trained and validated sensory panel in three sessions. A three factor (assessor, sample, session) ANOVA test was performed, followed by Tuckey's multiple difference test ($p \leq 0.05$), using StatGraphics Centurion XVI.I program (StatPoint Technologies, 2007).

3. Results and discussion

3.1. Tara pods characterization

Ripe tara pods can present red or pale yellow coloration (Chambi et al., 2013). This color difference was used to classify pods, finding a mayor proportion of red pods, close to 70%. Tara pod dimensions were statistically different ($p < 0.05$) for red and yellow pods. Red pods had length of 7.45 ± 0.70 cm and wide of 1.97 ± 0.18 cm; while yellow pods had length of 8.05 ± 0.65 cm and wide of 2.06 ± 0.17 cm. Color and dimensions of tara pods were according with previous studies (Chambi et al., 2013).

The chemical characterization of tara pods aimed to determine the best raw material to start the extraction of polyphenols. The antioxidant activity and reducing sugar content of tara were considered as the determining responses (Mariotti-Celis et al., 2017). For the first case, it was necessary to use the tara pod with the highest values since during the thermal treatment both TPC and AOC decreased due to thermal oxidation of polyphenols (Huamán-Castilla, Mariotti-Celis, & Perez-Correa, 2017). Contrary, both glucose and fructose should be at the lowest possible concentrations because reducing sugars are reactive precursors of Maillard reaction products (Mariotti et al., 2013).

The proximate analysis of red and yellow tara pods showed only significant differences in total fat (Table 2). Additionally, the obtained values are in agreement with those previously reported by other authors (Chambi et al., 2013) indicative that the drying process of tara pods was applied in a correct form, allowing to obtain a homogenous raw material.

In terms of reducing sugars, yellow tara pods presented significantly higher concentrations of glucose (~13%) than red tara pods. Although, red tara pods exhibited significantly higher AOC (~19%), its TPC was similar to the red pods (Table 2). It could be attributed to differences in their polyphenol profile, suggesting that some antioxidants compounds of red tara pods would present a higher antioxidant capacity than those contained in yellow pods. According to the obtained results, red tara pods were selected as the best raw material for extraction experiments since they presented the highest AOC and the lowest reducing sugar content (Table 2).

3.2. Phenolic extracts of tara pods

Extraction of polyphenols from plant materials is usually carried out using organic solvents, with the subsequent environmental and human risks. In this scenario, it is desirable to use “green solvents” such as water, for polyphenol extraction in order to maximize the safety of the

Table 2
Chemical characterization of tara pods (both red and yellow).

	Red pods	Yellow pods
Proximate analysis (%) ^a		
Moisture content	7.93 ± 0.12 ^a	7.93 ± 0.12 ^a
Ash	3.23 ± 0.06 ^a	3.33 ± 0.06 ^a
Proteins	2.90 ± 0.00 ^a	2.92 ± 0.06 ^a
Total fat	0.40 ± 0.10 ^a	0.60 ± 0.00 ^b
Nitrogen-free extract	81.93 ± 0.31 ^a	81.50 ± 0.17 ^a
Crude fiber	3.60 ± 0.10 ^a	3.67 ± 0.06 ^a
Reducing sugars (%) ^a		
Glucose	7.9 ± 0.4 ^a	8.9 ± 0.1 ^b
Fructose	1.1 ± 0.1 ^a	1.2 ± 0.1 ^a
Total phenolics (mg/g dry solids) ^a		
Tannic acid equivalent	627.4 ± 23.8 ^a	585.5 ± 39.5 ^a
Gallic acid equivalent	697.9 ± 33.7 ^a	677.5 ± 69.6 ^a
Antioxidant capacity (µg extract/mL) ^a		
DPPH EC ₅₀	1.9 ± 0.1 ^a	1.6 ± 0.1 ^b

^a Values are the mean of three replicates with red and yellow tara pod analysis duplicated, followed by the standard error. Values in each row followed by the same superscript letter are not significantly different at $p > 0.05$.

Table 3
Effect of the operating conditions on the antioxidant properties of red tara pod extracts.

Concentration temperature (°C)	40		
Solid:liquid ratio	1:10	1:30	1:60
	Total phenolics (mg/g dry solids) ^a		
Tannic acid equivalent	523.1 ± 25.6 ^a	558.1 ± 53.9 ^a	672.8 ± 34.3 ^b
Gallic acid equivalent	689.3 ± 28.7 ^a	727.3 ± 60.2 ^a	753.2 ± 59.2 ^a
Concentration temperature (°C)	40		60
Solid:liquid ratio	1:60		
	Antioxidant capacity (µg extract/mL) ^a		
DPPH EC ₅₀	1.86 ± 0.15 ^a		1.72 ± 0.19 ^a

^a Values are the mean of three replicates for different extraction conditions of tara pod antioxidants, followed by the standard error. Values in each row followed by the same superscript letter are not significantly different at $p > 0.05$.

functional ingredient and minimize the environmental impact (Kumar, Yadav, Kumar, Vyas, & Dhaliwal, 2017). The use of GRAS solvents in pressurized liquid extraction (PLE) has shown to be a feasible alternative for the recovery of these compounds. However, the high temperature applied during PLE produce the generation of hydroxymethyl furfural (Marioti-Celis et al., 2017). Considering this background, red tara pods were extracted at atmospheric conditions and 60 °C varying the solid:liquid ratio and concentration temperature (Table 3). The best tara pods:water ratio was 1:60 because the highest tannic acid content was attained (~673 mgTAC/g).

The higher the solid:liquid ratio, the driving force does not decrease which favors the mass transfer of polyphenols from the tara pods to the extraction solvent. Contrary, the AOC of TPPE did not present significant differences at the two different temperatures applied (Table 3). In order to prevent the thermo-oxidation ($T \geq 50$ °C) of some polyphenols such as anthocyanins (Monrad, Howard, King, Srinivas, & Mauromoustakos, 2010) the tara pod extracts were concentrated at 40 °C before their application in the “hallulla” bread elaboration.

3.3. Acrylamide and HMF mitigation using tara pod extracts (FP)

Acrylamide values for control hallullas were significantly lower to those reported in bread by EC in 2011 (~150 µg/kg) and slowly higher

than the levels reported in bread by European Food Safety (FSA) (2012) in the range of 30–75 µg/kg. Recent baking experiments with hallulla bread indicated acrylamide values around of 108 ± 6.1 µg/kg (Plaza, 2015), where more than 99% of this acrylamide was located in the bread crust as it was previously reported by Surdyk, Rosén, Andersson, and Åman (2004).

The highest temperatures during baking are reached in bread surface generating a surface dried level in which Maillard reaction takes place. It originates changes in the color, new flavors and aromas, among others, giving as a result the crust formation of the bread. On the other hand, the temperature of the core or inner part of the bread piece has an average temperature during all the baking process very slightly higher than 100 °C, giving as a result the formation of the crumb of the bread.

When compared with other kinds of starchy foods processed at high temperatures, the average acrylamide content of hallulla is relatively low. However, the consumption of this kind of bread is extremely high in Chile.

This issue makes that bread be one of the most critical sources responsible of a high dietary exposition to AA of the Chilean population (Pedreschi et al., 2014).

Addition of the tara pod extract to hallulla bread diminished significantly ($p \leq 0.05$) AA concentration for all extract concentrations tested as can be seen in Fig. 1. When the tara pod extract was added in hallulla formulation in different concentrations ranging from 500 to 1500 mg/kg of wheat flour (0.05–0.15%), AA concentration after baking was reduced significantly ($p \leq 0.05$); following a non-linear trend which was extract concentration dependent. The highest the concentration extract in hallullas, the highest the reduction in AA formation in hallullas after baking. This effect has also been observed in other foods containing low polyphenol concentrations, which ranged between 0.25% and 4% such as cookies by the addition of liquid extracts of cinnamon, clove, cumin and turmeric (Zhu, Cai, Ke, & Corke, 2011). Similarly, Zhang, Chen, Zhang, Wu, and Zhang (2007) used antioxidant extracts from bamboo leaves and from green tea in model systems to model Maillard reaction. Their results suggested that AA formation is not only influenced by the type of antioxidant but also by the concentration (Zhang & Jin, 2013; Zhang, Chen et al., 2007). Addition of 750 mg/kg of tara pod extract to hallulla bread, resulted in AA formation reduction of almost 50%. Moreover; when 1500 mg/kg of tara pod extract was added to hallulla, AA formation was reduced in 97.12%, reaching AA levels below the detection limit (LOD = 2,38 µg/kg). Similarly, Hedegaard, Granby, Frandsen, Thygesen, and Skibsted (2008) reported 62% AA reduction in wheat buns after being baked at 225 °C for 20 min, when they added 10 g/kg of rosemary extract (~40 mg EAG) during the formulation step. Additionally, Zhang and Zhang (2007), reported AA reductions in bread sticks fried at 180 °C of 82,9% and 72,5% when they added concentration of 10 g/kg bamboo leave antioxidants and concentration of 1 g/kg antioxidants of green tea extract (~98% flavonoids). Finally, Li et al. (2012) after adding individually 200 mg/kg bamboo leave antioxidant extract (25.51% flavonoids and 8.25% polyphenolic acids), 100 mg/kg of tea polyphenol extract (~98% flavonoids) and 100 mg/kg of vitamin E (98% α -tocopherol), in processed cookies baked at 190 °C for 7 min, reached AA mitigation percentages of 63.9, 71.2 and 43%, respectively.

But, Açar and Gökmen (2009) reported that the antioxidants extracted from grape seeds did not have any effect over AA formation in a bread model system simulating bread crust. Due to the structure diversity and functional groups in different antioxidants and to the complexity of food matrixes, phenolic compounds might participate at different stages during the occurrence of Maillard reaction and, thus, perform multiple effects at the same time (Liu et al., 2015; Zhang & Jin, 2013). Significant AA reduction could be attributed to the capacity of tara polyphenols of reacting with the AA precursors, the intermediary compounds of the reaction or with AA (Xu et al., 2014; Zhang & Jin, 2013).

The use of antioxidants has been proposed as an eventual strategy to

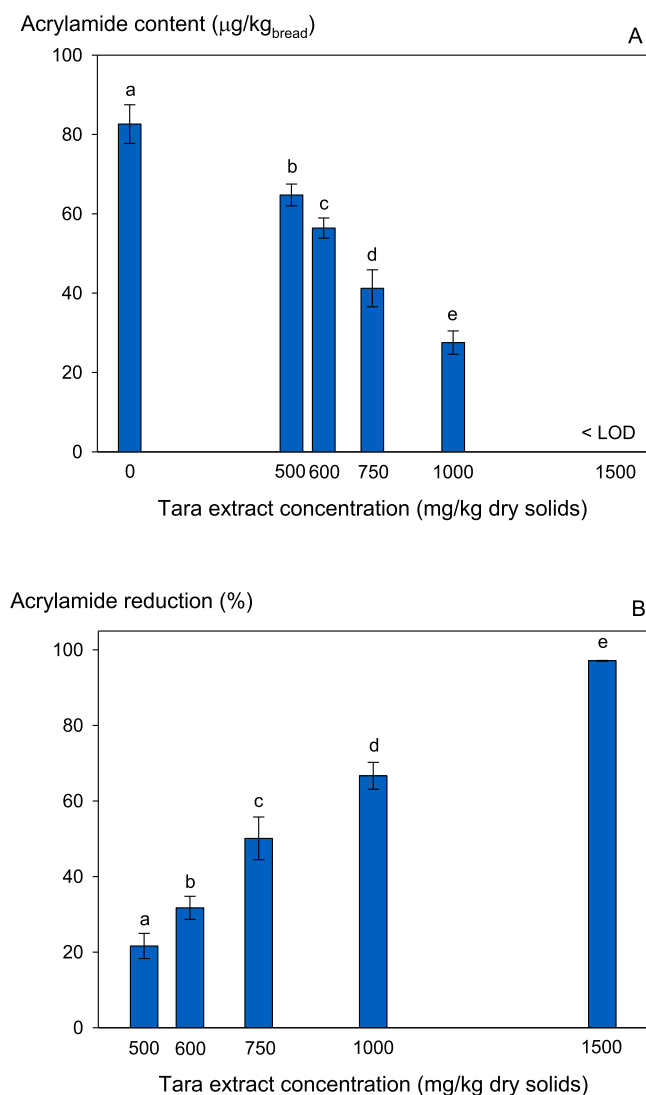


Fig. 1. Acrylamide content (A) and reduction (B) in hallulla-type bread using a Tara pod phenolic extract. LOD means limit of detection (2.38 µg/kg). Bars with the same letter are not significantly different at $p > 0.05$.

mitigate the formation of AA and in some cases, the presence of polyphenols could influence the overall MR development (Troise & Fogliano, 2014). In this sense, polyphenol effect over AA formation relies on chemical structure, concentration, antioxidant capacity and reaction conditions (Kahkeshani, Saeidnia, & Abdollahi, 2015; Liu et al., 2015; Zhang & Jin, 2013). Liu et al. (2015) has reported that reactive carbonyl groups are key places where antioxidants react to form AA (Liu et al., 2015). For instance, some phenolic antioxidants such as tannins could form complex products with amino acids leading to asparagine precipitation and avoiding its conversion to AA. Finally, formed AA has a double vinyl deficient in electrons, which can react with some antioxidants by Michel addition leading to AA mitigation (Zhang & Jin, 2013).

Terminal functional groups of the side chain such as hydroxyls and aldehydes will be crucial in the phenolic compound capacity to interrupt or to favor certain stages in AA formation route (Zhang & Jin, 2013). For instance, *o*-dihydroxyphenolic structures very linked to the antioxidant capacity could be very efficient for AA mitigation (Liu et al., 2015; Zhang & Jin, 2013). On the other hand, aldehydic groups of the side chain of phenolic compounds could react with asparaginase favouring AA formation (Zhang & Jin, 2013).

As in the case of AA, HMF formation in hallullas decreased as the

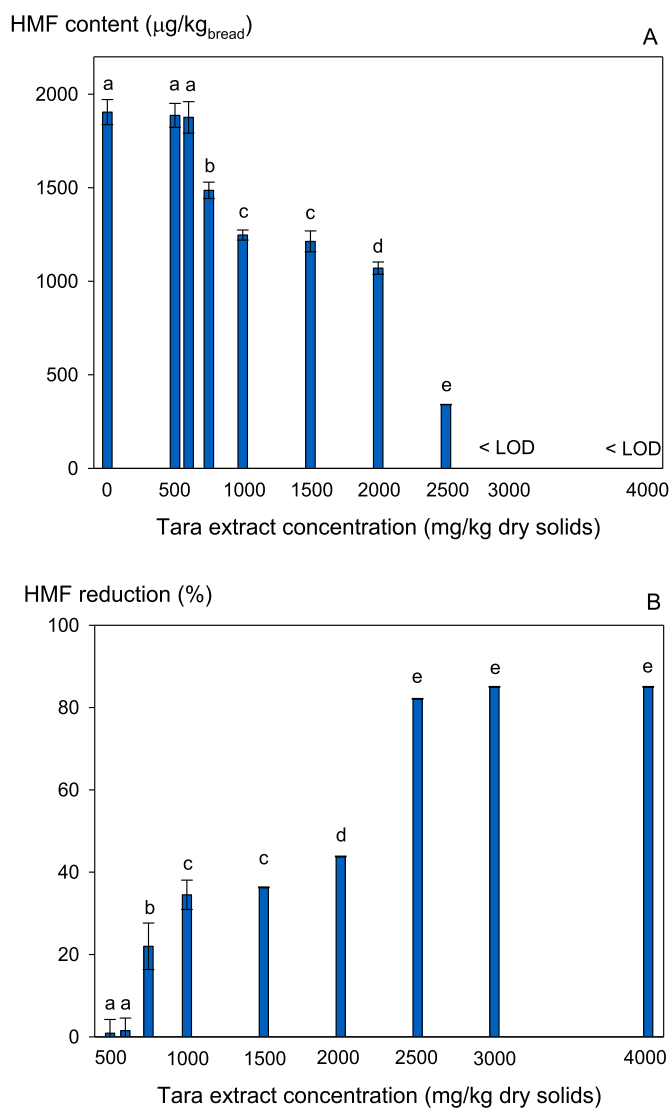
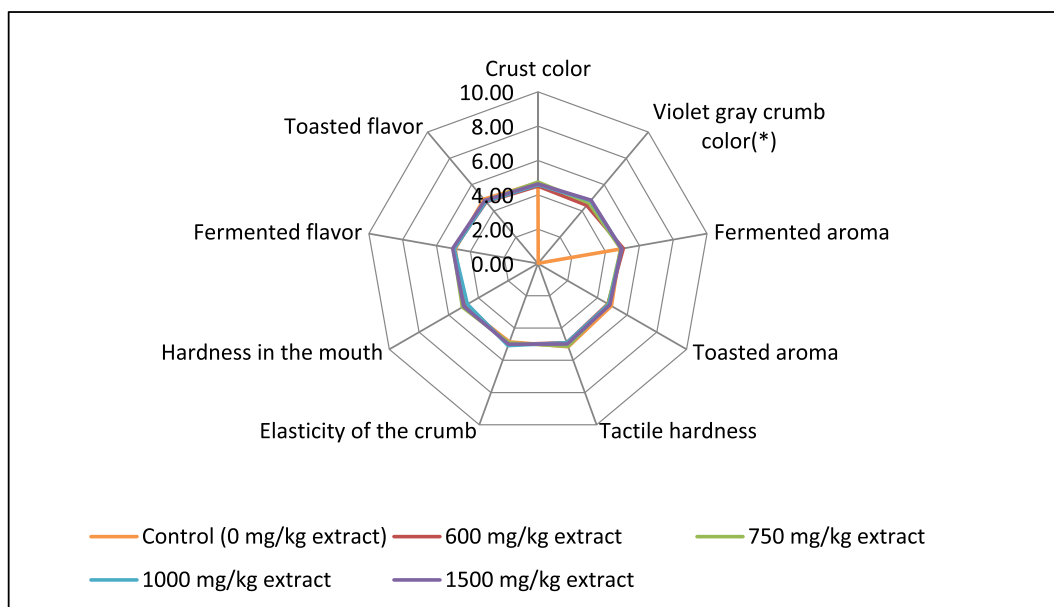


Fig. 2. HMF content (A) and reduction (B) in hallulla-type bread using a Tara pod phenolic extract. LOD means limit of detection (1.5 µg/kg). Bars with the same letter are not significantly different at $p > 0.05$.

polyphenol concentration extract added to the formulation was increased from 0 to 4000 (mg/kg of wheat flour). However, the effect of the concentration of the added extract was more significant in mitigating AA content than HMF content. Mitigation of AA and HMF in hallulla bread clearly differed since they are probably acting at different stages and ways to Maillard reaction. It is worth to mention that HMF began to be mitigated significantly in hallulla at tara extract concentrations higher than 600 mg/kg of wheat flour; extract additions lower than this value did not mitigate HMF in hallullas.

When 1500 mg/kg of wheat flour of tara pod extract was added to hallulla bread, AA formation was reduced in almost 97% in hallulla while HMF was mitigated only in 36% (Figs. 1 and 2).

Polyphenol extract additions between 2500 mg/kg to 4000 mg/kg of wheat flour were required to mitigate HMF formation in hallullas in more than 80%. Indeed, reduction of HMF in food is a challenging issue due to the difficulty to find effective ways applicable at the industrial level (Anese & Suman, 2013). As HMF formation is concomitant to that of color and flavor of heated foods, it is very difficult to mitigate their formation without compromising the food sensory acceptability. HMF forms through MR and caramelization, which mostly contribute to desired color, taste and aroma of heated foodstuffs. Unfortunately, HMF formation follows the same pathways leading to brown and flavor



(*) Significant differences ($P < 0.05$) between samples with tara pod phenolic extract and the control sample

Fig. 3. Sensory Profile of hallulla-type bread with Tara pod phenolic extract.

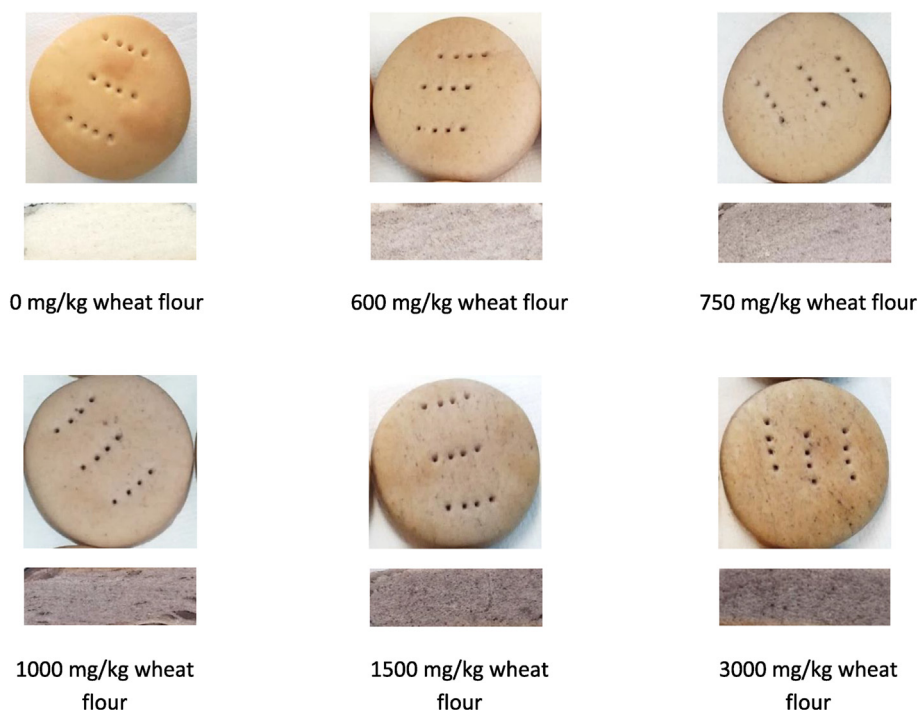


Fig. 4. Color changes in bread crust and crumb by the addition of tara pod extracts. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

compounds. For instance, a high correlation between HMF content and browning development has been repeatedly reported (Capuano et al., 2009).

3.4. Sensory evaluation

Six panel training sessions with 14 judges were completed, at the end of results indicated significant differences between samples ($p \leq 0.05$) and absence of significant differences between assessors ($p > 0.05$) (results not shown). During the validation of the panel carried out subsequently, 3 assessors were eliminated, and the final

panel was constituted by 11 assessors who demonstrated good overall panel performance (homogeneity and reproducibility) and also individual assessor performance (discrimination, repeatability and consistency).

According to the sensory profile (Fig. 3) only the color of the crumb was affected by the addition of tara extract (Fig. 4). Similar coloration for all the tested tara extract concentrations was obtained (between 4.4 and 4.8 in a 10-cm-scale) and all were significantly different from the control without extract ($p \leq 0,05$). No significant differences were obtained for the rest of the descriptors, neither for sessions nor assessors. Color changes in crumb and crust of bread by the addition of

antioxidant extracts have also been reported by Danza et al. (2014) by adding yellow pepper flour, and by Zhu, Sakulnak, and Wang (2016) by adding black tea polyphenols, attributing the color change to phenolic pigments, subjected to oxidation and caramelization reactions with the participation of sugars, during the baking process.

4. Conclusions

Tara (*Caesalpinia spinosa*) pods polyphenolic extract successfully mitigates AA and 5-HMF formation in hallulla bread preserving their attractive sensory attributes. Water extraction at low temperature and atmospheric condition allows to obtain polyphenol extracts from tara pods with high antioxidant capacity (IC₅₀: 1,72 µg extract/mL) and total polyphenol content (617,84 mg EAT/g dry solid). The higher the amount of the added concentration extract, the higher the reduction of AA and HMF formation in hallulla bread. At an extract concentration of 1500 mg/kg of wheat flour, AA and HMF formation in hallulla was mitigated in 97% and 40%, respectively, without affecting negatively their sensorial attributes.

Conflicts of interest

The authors declare that they have no conflict of interest.

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