Food Hydrocolloids 81 (2018) 113–119

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Pectic polysaccharides as an acrylamide mitigation strategy – Competition between reducing sugars and sugar acids

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ARTICLE INFO

Article history:

Keywords:
Acrylamide
Pectic polysaccharides
Galacturonic acid
Pectate
Tartaric acid
Biscuits

ABSTRACT

The main route for acrylamide formation is the reaction between reducing sugars and asparagine. As foods pH modulates acrylamide formation, cooking under the lowest pH is a possible mitigation strategy, as long as it does not compromise the expected foods sensorial characteristics. Polymeric acid compounds, such as pectin or pectate, which are acidic polysaccharides, can provide acidity without significant interference in taste or colour. To prove this hypothesis, biscuits were prepared with tartaric acid (control to acidic effect), and galacturonic acid in monomeric (GalA), oligomeric, and polymeric forms (pectin or pectate). While 1% GalA leads to 95% increase in acrylamide, biscuits prepared with 5% pectin had a 67% decrease. Replacing sucrose with fructose increased the acrylamide levels in control biscuits above the benchmark level of 150 μg/kg recommended by EFSA for children. However, addition of pectin/pectate had an effective acrylamide mitigation by lowering its content below these indicative values.

1. Introduction

Cooking adds taste and colour, improving digestibility and consumer acceptance to the food products. Both colour and flavour compounds are generated during thermal processing mainly by the Maillard reaction, along with some toxic compounds such as acrylamide (Mottram, Wedzicha, & Dodson, 2002; Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002), classified as probably carcinogenic to humans (class 2A). Infants, toddlers, and other children are the most exposed group to acrylamide, estimated on average between 0.5 and 1.9 μg/kg b.w. per day (EFSA, 2015). This exposure may even be higher if considering a higher acrylamide availability after gastric digestion (Sansano, Heredia, Peinado, & Andrés, 2017). Considering that biscuits are highly consumed among children, the amount of acrylamide in these commodities should be below 150 μg/kg (EU, 2017).

Maillard reaction has been reported as the main route for acrylamide formation by reaction between sugars, mainly glucose and fructose, and the amino acid asparagine. In addition, acrylamide formation in foods is directly influenced by processing factors such as temperature, heating time, water activity, and pH (Mestdagh et al., 2008a). Mitigation strategies should, however, be done without affecting the final sensorial quality of the foods (Mestdagh, De Wilde, Delporte, Van Peteghem, & De Meulenaer, 2008b). One example is the adjustment of the binomial temperature and heating time, which showed effectiveness on lowering acrylamide formation, although the insufficient browning level observed under extreme conditions resulted in non-acceptable products (Capuano et al., 2009). Controlling moisture, and therefore the water activity, prevents Maillard reaction (de Vleeschouwer, Plancken, Van Loey, & Hendrickx, 2007). As acrylamide formation is maximum at pH 8 (Low et al., 2006), a large set of pH-lowering agents has been proposed (Amrein, Schönbächler, Escher, & Amadò, 2004; Graf et al., 2006; Mestdagh et al., 2008a; Rannou, Laroque, Renault, Prost, & Sérot, 2016; Zeng, Cheng, Du, Kong, Lo, Chu, et al., 2010). The use of hydrocolloids such as chitosan, a polysaccharide composed by galactosamine residues, was also proposed as an acrylamide mitigation strategy, due to the availability of the chitosan amino groups to compete with the amino group of asparagine (Sansano, Castelló, Heredia, & Andrés, 2016). Nevertheless, the pH-lowering effect of the acidic solutions in which chitosan is solubilized should also be taken into account when determining the mitigation mechanism (Mogol & Gökmen, 2016). To be effective, foods should be treated with a pH-

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https://doi.org/10.1016/j.foodhyd.2018.02.032
0268-005X/

lowering agent in concentrations of at least 0.02% of weight, resulting in a pH reduction of at least 0.5 units than the intrinsic pH of the food (Low et al., 2006; Mestdagh et al., 2008a). However, because low pH may adversely affect the taste of foods, the sensorial properties of the final product should also be considered. Small molecules, like tartaric acid, a grape component, or citric acid, from citrus fruits, have been presented as food additives to reduce pH value, and consequently mitigate acrylamide formation (Amrein et al., 2004; Graf et al., 2006; Mestdagh et al., 2008b; Rannou et al., 2016). However, their sour taste and faded colored products may represent a drawback, as observed by the addition of 0.5% of citric acid to gingerbread (Amrein et al., 2004).

Among other hydrocolloids, pectin was successful applied as a coating layer in a fried potato model for acrylamide mitigation (Zeng et al., 2010), although the mechanism is not yet clear. Pectin is the linear region from pectic polysaccharides, consisting of units of (α1→4)-D-Gal*p*A residues, also known as homogalacturonan or polygalacturonic acid. The Gal*p*A residues can also carry methyl ester groups and/or be acetylated at the galacturonan backbone (Ferreira, Passos, Madureira, Vilanova, & Coimbra, 2015). When pectin loses the methyl ester groups, it is named pectate. In order to evaluate the possibility of using pectin as an acrylamide mitigation strategy in biscuits, these were prepared with 1 and 5% of addition to the dough (w/w, in relation to the flour content) of a polymeric commercial pectin obtained from citrus (CP), using a sucrose-based formulation. Biscuits were also prepared using a pectic oligomeric sample (HP) partially hydrolysed from the initial pectin, as well as galacturonic acid (GalA), the correspondent monosaccharide repeating unit. The use of the monomeric, oligomeric, and polymeric forms also allowed to study the relevance of the anomeric carbon (reducing sugars) on the acrylamide mitigation effect of the acid moiety of the sugar derivatives. To test the hypothesis, the polymeric form was also applied on the modified fructose-based recipe, in which the high acrylamide contents were of concern. To maximize the availability of the acid moiety while reducing the percentage of addition required, which is an industrial requirement for its applicability, 2% pectate instead of 5% pectin, representing an equivalent molar ratio of acidic groups, was also tested.

2. Experimental

2.1. Reagents and materials

A commercial citrus low-methoxyl pectin, with an average (viscosimetric) molecular weight of 5.13×10^4 (Cardoso, Coimbra, & Lopes da Silva, 2003), supplied by HP Bulmer (Hereford, UK), was used. D-galacturonic acid (97%) was purchased from Sigma-Aldrich (Steinheim, Germany). Acrylamide (2-propenamide) (≥99% purity) was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA), and d_3-acrylamide (2-propenamide-2,3,3-d_3) were obtained from Cambridge, Isotope Laboratories (Maryland, USA). DNS reagent was prepared by slowly dissolving 5 g of dinitrosalicylic acid in 100 mL of 2 M NaOH. To this solution, 250 mL of potassium and sodium tartrate tetrahydrated (0.6 g/L) solution was added, performing 500 mL DNS solution by adding distilled water. Dinitrosalicylic acid was purchased from Panreac (Barcelona, Spain), NaOH from José Manuel Gomes dos Santos (Odivelas, Portugal), and both potassium and sodium tartrate tetrahydrated were purchased from Panreac (Barcelona, Spain). Ethanol was purchased from Carlo Erba Reagents S.r.l. (Parc des Affaires Val de Reuil, France). All other reagents used were of analytical grade or higher available purity.

Wheat flour extra soft (Penam, Nitra, Slovak Republic), fine granulating white sugar (Castello, Nemšová, Slovak Republic), brown sugar (Považský cukor, Trenčianska Teplá, Slovak Republic), shortening Akobake Soft (AarhusKarlshamm, Malmo, Sweden), salt (Solivary, Prešov, Slovak Republic), non-fat powder milk (Foodpack, Nitra, Slovak Republic), and fructose (Vaspress, Prešov, Slovak Republic) were purchased in a local market. The total amino acids content determined in the flour was 527 mg/kg with an individual asparagine contribution of 31.24 ± 0.92 mg/kg.

2.2. Carbohydrates preparation

To prepare the partially hydrolysed pectin sample (HP), approximately 10 g of the pectin were suspended in 400 mL of water and heated at 80 °C for 1 h. The polysaccharides were then subjected to a saponification treatment in 0.57 M NaOH solution during 1 h at 25 °C, stopping the reaction by neutralisation with HCl (Nunes, Rocha, Saraiva, & Coimbra, 2006). The sample was then submitted to a partial acid hydrolysis with 0.09 M HCl at 80 °C for 24 h (Renard, Lahaye, Mutter, Voragen, & Thibault, 1997). The solution was then filtered on a G-1 sintered glass filter, adjusted to pH 6 by addition of 2 M NaOH, and left overnight at 4 °C. As no precipitate was formed, the total solution was concentrated by rotary evaporation at 40 °C to about 10% of the initial solution volume. To the concentrated solution, absolute ethanol was added gradually until a precipitate was formed (when 50% ethanol (v/v) was reached). The precipitated material was then separated by centrifugation at 15,000 rpm for 15 min at 4 °C, re-dissolved in water, rotary evaporated at 40 °C to completely remove the ethanol, and then frozen, freeze-dried and stored under anhydrous atmosphere.

To prepare the pectate sample, an aliquot of 3 g of pectin was suspended in 120 mL of water and saponified with 48 mL of 2 M NaOH during 1 h at 25 °C and then neutralized with48 mL of 2 M HCl (Nunes et al., 2006). The solution was diluted to 500 mL and purified at room temperature on an ultrafiltration module - Lab-scale TFF System (Millipore), using a pellicon XL ultrafiltration ultracel membrane with cut-off 5 kDa working among 10 and 20 psi transmembrane pressures. The system, starting with 500 mL in the reservoir, was concentrated to 50 mL with control of permeate conductivity. Water was then added to fill the 500 mL reservoir and the process of concentration started again. This process was repeated three times until the permeate conductivity was <20 us.cm^{-1}. The retentate material was frozen and freeze-dried.

2.3. Neutral, free, and reducing sugars determination

The individual neutral sugars were determined by their derivatization to alditol acetates, and analysis by GC-FID as described by Passos and Coimbra (2013). To determine the free monosaccharides content, the samples were submitted to the same derivatization procedure, with the omission of the acid hydrolysis step. The reducing sugars content was determined using the 3,5-dinitrosalicylic acid (DNS) assay (Miller, 1959). An aliquot of each sample (150 μL, 1 mg/mL) was mixed with 150 μL of the DNS reagent in a test tube and the mixture was incubated in a boiling water bath for 5 min. After cooling to room temperature, the absorbance of the supernatant at 540 nm was measured. A calibration curve was prepared using D-galacturonic acid (0–0.20 mg/mL).

2.4. Determination of the degrees of methyl-esterification (DM) and acetylation (DA)

The degrees of methyl-esterification and acetylation were determined by quantification of methanol and acetic acid, respectively released by saponification of methyl ester and acetyl groups, acidification of solution, using headspace solid phase microextraction (HS-SPME) and analysis by gas chromatography with a flame ionization detector (GC-FID), as described by Nunes et al.