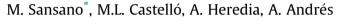
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Protective effect of chitosan on acrylamide formation in model and batter systems



Institute of Food Engineering for Development, Universitat Politècnica de València, P.O. Box 46022 Valencia, Spain

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ABSTRACT

In recent years high contents of acrylamide, a potentially carcinogenic substance, have been found in a wide range of fried and baked foods. For this reason, the health authorities together with the food industry have carried out research to find ways to minimize the presence of acrylamide during food processing. The addition of chitosan may be an excellent alternative for achieving this goal because due to their richness in amino groups, they would interfere with the Maillard reaction that unleashes the formation of acrylamide. The main aims of this study were to analyze the addition of different concentrations of chitosan in model systems as a new way of mitigating generation of acrylamide during frying processes, while evaluating the influence of pH, reducing sugars (glucose and fructose) present in the system and frying temperature, and to determine the functionality of adding chitosan in model systems. The results showed that chitosan is capable of inhibiting the formation of acrylamide in model systems, a reduction in acrylamide ranging from 49 to 85% was achieved for 1% of chitosan, the maximum inhibition taking place in asparagine—fructose model systems and the lowest in asparagine—glucose model systems. In fried batter, acrylamide was mitigated by $59 \pm 6\%$ with a chitosan concentration of 0.27% in batter formulations. Double concentrations of chitosan (0.54%) did not considerably improve the inhibition capacity.

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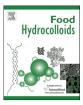
1. Introduction

It is well-known that food processing can improve nutrition, quality and safety. However, toxic substances such as acrylamide can sometimes be formed through the interaction of food compounds, from natural and added ingredient. According to some epidemiological studies, acrylamide is potentially carcinogenic compound for humans (IARC, 1994), not only due to its consumption, but also to its role as a precursor in the development of other compounds during hepatic metabolism such as glycidamide (Blank, 2005). Acrylamide is mainly used in industrial processes used to make paper, dyes, plastics and treating drinking water. However, it can also be present in small amounts in food packaging, some adhesives and cigarette smoke (Rudel, Ackerman, Attfield, & Brody, 2014). Acrylamide was also found to be formed in some starchy foods, especially potato products, during high-temperature cooking and under low moisture conditions, such as frying, baking and roasting, formation being lower in protein-rich foods (Tareke,

* Corresponding author. E-mail address: masantom@upvnet.upv.es (M. Sansano). Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). Acrylamide is formed during Maillard reactions, and mainly between the reaction of asparagine and reducing sugars at high temperatures (Becalski, Lau, Lewis, & Seaman, 2003; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002). Several studies have proven the importance of temperature, time, levels of precursors, pH, nature of the matrix, etc. on acrylamide formation in food. Consequently, a wide range of strategies have been developed in the last decade to reduce the final content of acrylamide in model systems and foods processed at high temperatures. Some strategies based on controlling processing conditions such as time and temperature (Tareke et al., 2002), as well as frying in low pressure conditions or novel frying techniques, such as, microwave or air frying have achieved a significant inhibition of acrylamide formation (Barutcu, Sahin, & Sumnu, 2009; Sansano, Juan-Borrás, Escriche, Andrés, & Heredia, 2015; Troncoso & Pedreschi, 2009). It is also advantageous to apply treatments before frying, such as blanching, or soaking the food products in acids, vitamins, cations or amino acids in order to reduce acrylamide precursors, and to interfere with and modify Maillard reactions triggering acrylamide formation (Gökmen & Şenyuva, 2007; Jung, Choi, & Ju, 2003; Pedreschi, Kaack,







& Granby, 2004; Rydberg et al., 2003; Zeng et al., 2009).

Hydrocolloids are hydrophilic polymers that modify the functional properties of food systems, such as thickening, gelling and emulsifying properties (Saha & Bhattacharya, 2010). Some studies have tested the use of hydrocolloids to control moisture diffusion and consequently, oil absorption during frying. Lower contents of fat were obtained when including hydrocolloids such as soy protein isolate, whey protein isolate, methylcellulose and hydroxypropyl methylcellulose as an edible film coating before frying (Albert & Mittal, 2002; Balasubramaniam, Chinnan, Mallikarjunan, & Phillips, 1997) or, what seems to be most effective, introducing them as an ingredient in batter formulation (Holownia, Chinnan, Erickson, & Mallikarjunan, 2000; Sanz, Salvador, & Fiszman, 2004). Zeng et al. (2010) tested some hydrocolloids (agar, alginic acid, carrageenan, carob gum, gelatin, hydroxyprpyl distarch phosphate, pectin and xanthan gum) in acrylamide formation in model and real systems. They found positive results mainly for pectin and alginic acid, but these hydrocolloids did not significantly change the water content of the fried potatoes strips. Therefore, they are unlikely to modulate the formation of acrylamide due to their property of water retention. These authors suggested that the formation of surface coatings might also modulate heat transfer from the surrounding oil to the product.

Among the different hydrocolloids, chitosan, a polycationic polymer and waste product from the sea food processing industry, is an abundant natural resource that has, as yet, not been fully utilized. The advantages of this polymer include availability, low cost, high biocompatibility, biodegradability and ease of chemical modification. Chitosan has many applications in several sectors because of its multiple properties: it is not digestible by humans, so it is considered to be a dietary fiber; which binds lipids and helps in reducing cholesterol (Muzzarelli, 1996), and it is protective, fungistatic and antibacterial (El Ghaouth, Arul, Ponnapalam, & Boulet, 1991; Tsai & Su, 1999). Moreover, chitosan is a molecule which is rich in amino groups, this being the main characteristic leading to our hypothesis: amino groups of chitosan would compete with amino groups of asparagine to bind to carbonyl group of reducing sugars and thus, would modulate acrylamide generation (Lindsay & Jang, 2005). If this hypothesis is confirmed chitosan would be proven to have another function: protecting against acrylamide formation. The main purpose of this study was to analyze the addition of chitosan as a way to mitigate the generation of acrylamide during frying processes in model systems and fried batter systems. The effect of pH of the reaction, the type of reducing sugars (glucose and/or fructose) present in the model system and the temperature were also evaluated.

2. Materials and methods

2.1. Chemicals and consumables

Asparagine, glucose and fructose were purchased from Sigma-Adrich Company (St. Louis, MO, USA). Chitosan (Poly (D-glucosamine)*Deacetylated chitin) was also purchased from Sigma-Adrich (St. Louis, MO, USA). Chitosan was used in coarse ground flakes and powder, presented a deacetylation degree superior to 75% with a high molecular weight (lot: MKBH5816V). Formic acid, acetonitrile and magnesium sulfate were purchased from VWR-Prolabo (Fontenay-sous-Bois, France), methanol and hexane were obtained from Panreac (Barcelona, Spain). Acrylamide standard (>99%) was purchased from Merk (Darmstadt, Germany), sodium chloride was obtained from Scharlab (Barcelona, Spain) and Primary secondary amine (PSA) was purchased from Supelco (Bellefonte, USA). Double distilled water was prepared for chromatographic use (Milli-Q, Millipore Corp., Bedford, MA). All chemicals used were analytical grade, and those used for chromatographic analysis were HPLC grade. To test the effect of chitosan in a real system, a commercial formulation was used (Yolanda, Murcia, Spain). This formulation consists of wheat and rise flours, an acidity regulator (E-334), bulking agent (E-500ii) and coloring (E-160b). Moisture and ash contents (11.5% and 1.8%, respectively) were measured using AACC methods (1995), protein and fat contents (10.0% and 1.4%, respectively) were supplied by manufacturers, and particle size (78.0 μ m) was analyzed with the Mastersizer 2000 (Malvern Instruments, Germany) coupled with the Scirocco 2000 module for dry measurement.

2.2. Preparation of reaction mixtures for pyrolysis

In order to confirm our hypothesis, we carried out chemical model reactions following the method proposed by Gökmen and Şenyuva (2007) with some minor modifications. The reaction was carried out using a 25 mL threaded Pyrex tube which contained 5 μ mol of asparagine and 5 μ mol of reducing sugars, and 100 μ l of acid lactic solution on which chitosan was previously dissolved at 0, 0,5 or 1%. Eighteen different model systems were formulated depending on the type of sugar used: glucose, fructose or an equimolecular mixture of both; the pH (4 and 5) and the concentration of chitosan (0, 0.5 and 1%).

The samples were placed in an oil bath previously preheated at the two temperatures tested (150 and 180 $^{\circ}$ C) and the total heating time for the samples was 30 min. After the reaction time, the tubes were immediately cooled in an ice-water bath for 5 min.

2.3. Preparation of batters systems for frying

Batter formulations consisted of the commercial formulation with chitosan solutions (at 0, 0.5 and 1%) at pH = 4 with 2.5% of salt in a water-to-dry-mix proportion of 1.2/1. The final chitosan contents in the formulations were 0, 0.27 and 0.54% respectively. Batter samples were kept for at least 30 min at room temperature before frying. The frying step was carried out in a commercial deep-fat fryer with a capacity of 2 L (model: FM 6720 Ideal 2000 Professional, Solac) at 180 \pm 2 °C. Samples (11.5 \pm 0.1 g) were placed in an aluminum cylindrical instrument and then introduced in the fryer in order to obtain homogenous ring shaped fried samples (height: 11 \pm 1 mm; outer diameter = 65 mm \pm 2 and inner diameter = 25 \pm 1 mm). Triplicate samples (n = 3) were fried for 2, 4 and 7 min for the three formulations tested. The excess oil was removed with paper on both sides for 20 s after taking the samples out of the fryer.

2.4. Analysis of acrylamide

2.4.1. Extraction of acrylamide from pyrolysates (model systems)

Two mL of Mili-Q water were added to the pyrolisates obtained and tubes were agitated in a vortex for 1 min. The tube content was filtered (0.22 μ m Nylon filters) and transferred to a vial for the following acrylamide content determination, studied in triplicate (n = 3).

2.4.2. Extraction of acrylamide from the fried batter systems

The acrylamide content was determined by means of dispersive solid phase extraction (QuEChERS) according to Mastovska and Lehotay (2006) with some modifications. The standard addition was used rather than the traditional calibration curve in order to remove the matrix effect, fortifying at five different levels (10, 20, 50, 100 and 300 μ g kg⁻¹), with six replicates for each level (n = 6). Fried batter systems were subjected to a previous acrylamide extraction as follows: three samples were ground in a blender and a

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