Mariola Sansano Tomás; Castelló Gómez, ML.; Heredia Gutiérrez, AB.; Andrés Grau, AM. (2016). Protective effect of chitosan on acrylamide formation in model and batter systems. Food Hydrocolloids. 60:1-6. doi:10.1016/j.foodhyd.2016.03.017

PROTECTIVE EFFECT OF CHITOSAN ON ACRYLAMIDE FORMATION IN MODEL AND BATTER SYSTEMS

Sansano, M.*; Castelló, M.L.; Heredia, A.; Andrés, A.

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

* Corresponding author. Tel.: +34 963877365

E-mail address: masantom@upvnet.upv.es

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 fried batter systems. The results showed that chitosan is capable of inhibiting the formation of acrylamide in model systems and in fried batters. 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 ± 6 % with a chitosan concentration of 0.27% in batter formulations. Double concentrations of chitosan (0.54 %) did not considerably improve the inhibition capacity.

Keywords: Acrylamide, Chitosan, Model systems, Batter systems

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, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002). Acrylamide is formed during Maillard reactions, and mainly between the reaction of asparagine and reducing sugars at high temperatures (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Becalski, Lau, Lewis, & Seaman, 2003). 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 (Troncoso & Pedreschi, 2009; Barutcu, Sahin, & Sumnu, 2009; Sansano, Juan-Borrás, Escriche, Andrés, & Heredia, 2015). 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 (Pedreschi, Kaack, & Granby, 2004, Jung, Choi, & Ju, 2003, Zeng et al., 2009, Gökmen & Şenyuva, 2007, Rydberg et al., 2003).

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

78 humans, so it is considered to be a dietary fiber; which binds lipids and helps in reducing
79 cholesterol (Muzzarelli, 1996), and it is protective, fungistatic and antibacterial (El Ghaouth,
80 Arul, Ponnapalam, & Boulet, 1991; Tsai & Su, 1999). Moreover, chitosan is a molecule
81 which is rich in amino groups, this being the main characteristic leading to our hypothesis:
82 amino groups of chitosan would compete with amino groups of asparagine to bind to
83 carbonyl group of reducing sugars and thus, would modulate acrylamide generation
84 (Lindsay & Jang, 2005). If this hypothesis is confirmed chitosan would be proven to have
85 another function: protecting against acrylamide formation. The main purpose of this study
86 was to analyze the addition of chitosan as a way to mitigate the generation of acrylamide
87 during frying processes in model systems and fried batter systems. The effect of pH of the
88 reaction, the type of reducing sugars (glucose and/or fructose) present in the model system
89 and the temperature were also evaluated.
90
91 **2. Materials and methods**
92 *2.1. Chemicals and consumables*
93 Asparagine, glucose and fructose were purchased from Sigma-Adrich Company (St.
94 Louis, MO, USA). Chitosan (Poly (D-glucosamine)*Deacetylated chitin) was also
95 purchased from Sigma- Adrich (St. Louis, MO, USA). Chitosan was used in coarse
96 ground flakes and powder, presented a deacetylation degree superior to 75% with a high
97 molecular weight (lot: MKBH5816V). Formic acid, acetonitrile and magnesium sulfate
98 were purchased from VWR-Prolabo (Fontenay-sous-Bois, France), methanol and
99 hexane were obtained from Panreac (Barcelona, Spain). Acrylamide standard (> 99%)
100 was purchased from Merk (Darmstadt, Germany), sodium chloride was obtained from
101 Scharlab (Barcelona, Spain) and Primary secondary amine (PSA) was purchased from
102 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 μm) 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 25mL threaded Pyrex tube which contained 5 μmol of asparagine and 5 μmol of reducing sugars, and 100 μl 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 180ºC) and the total heating time for the samples was 30 minutes. After the reaction time, the tubes were immediately cooled in an ice-water bath for 5 minutes.

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

128 samples were kept for at least 30 minutes at room temperature before frying. The frying
129 step was carried out in a commercial deep-fat fryer with a capacity of 2 L (model: FM
130 6720 Ideal 2000 Professional, Solac) at 180±2ºC. Samples (11.5 ± 0.1g) were placed in
131 an aluminum cylindrical instrument and then introduced in the fryer in order to obtain
132 homogenous ring shaped fried samples (height: 11±1mm; outer diameter= 65mm±2 and
133 inner diameter= 25±1mm). Triplicate samples (n = 3) were fried for 2, 4 and 7 minutes
134 for the three formulations tested. The excess oil was removed with paper on both sides
135 for 20 seconds after taking the samples out of the fryer.

136 *2.4. Analysis of acrylamide*

137 2.4.1. *Extraction of acrylamide from pyrolysates (model systems)*

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

142 *2.4.2. Extraction of acrylamide from the fried batter systems*

143 The acrylamide content was determined by means of dispersive solid phase extraction
144 (QuEChERS) according to Mastovska and Lehotay (2006) with some modifications.
145 The standard addition was used rather than the traditional calibration curve in order to
146 remove the matrix effect, fortifying at five different levels (10, 20, 50, 100 and 300 µg
147 kg^{-1}), with six replicates for each level (n=6). Fried batter systems were subjected to a
148 previous acrylamide extraction as follows: three samples were ground in a blender and a
149 sub-sample (1 g) was placed in a 50mL Falcon tube, following which 5 mL of n-hexane
150 were added. The tube was shaken in a vortex for 30 seconds, after which10 ml
151 bidistilled water, 10 mL acetonitrile, 4 g MgSO4 and 0.5 g NaCl were added and stirred
152 in the vortex for one minute. The suspension was then centrifuged at 2026 RCF

(Centronic BL II (Selecta, Spain)) for 5 minutes, following which the hexane layer (upper phase) was discarded. 1mL of the acetonitrile phase, containing the acrylamide, was then transferred to a 2 mL polypropylene tube containing 50 mg PSA and 150 mg MgSO4, and stirred for 30 seconds. The homogenate was centrifuged at 2697 RCF (Labofuge 200 (Heraeus, Germany)) for 1 minute and the supernatant was transferred to a vial for acrylamide analysis.

2.4.3. LC/MS/MS analysis

The acrylamide analysis was performed with an Agilent 1200 Series HPLC system coupled to an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies Inc., CA, USA) with an electrospray type ionization source. The column used in this study was a Zorbax Eclipse XDB C-18 (2.1mmx50mm, 1.8µm). The mobile phase used consisted of 2.5% methanol/ 97.5% of 0.1% formic acid (A) and methanol (B). The elution gradient was as follows: 0-3 min 100% of A; 3.1-3.5 min 70% A; 3.6 min 100% A, with 1 min post-time to equilibrate the column. The column oven temperature was set at 30°C, the flow was maintained at 0.4 mL/minute and the injection volume was 10µL. The electrospray was operated in positive ion mode. The conditions used in the ionization source were: 350ºC at 12 L/min for the drying gas (N_2), a nebulizer pressure of 40 psi and a capillary voltage of 4000 V. Identification and quantification of acrylamide in the samples was performed using the multiple reaction monitoring mode (MRM), and the ion m/z 72 > 27 and m/z 72 > 55.2 were studied respectively.

2.5. Water content determination

Water content was analyzed by vacuum drying at 60ºC until constant weight was achieved (20.103, AOAC, 1980).

2.6. Statistical analysis

Statistical analysis of variance (ANOVA) was performed by Statgraphics Centurion to estimate the effect of process variables (pH, reaction temperature and time, reducing sugars and chitosan content) on the obtained results. Evaluations were based on 95 and 99% significance levels.

3. Results and discussion

3.1. Effect of chitosan on acrylamide formation in model systems

The acrylamide content generated in model systems formulated with glucose, fructose and the equimolecular mixture of both sugars are shown in Figure 1. In all cases the higher the percentage of chitosan, the lesser the amount of acrylamide produced. Temperature was an important factor, considering that at 150ºC the acrylamide content grows gradually with heating time, but at 180ºC, after 10-15 minutes the amount of acrylamide generally reaches the maximum value and starts to decrease lightly with treatment time, likely due to polymerization (Stadler et al., 2004). The statistical analysis revealed that except pH, all the factors considered in this study (type of reducing sugars present in the system, the reaction time, the % of chitosan and the temperature) have a significant influence on acrylamide formation (Table 1). As the pH was not a significant factor, Figure 1 only includes results at pH 4. According to the F-Ratio values, the temperature and the percentage of chitosan are the most significant factors, as well as the interaction between them. Time is also a significant element, as has been proven in many studies, as well as the temperature and their interaction (Gertz & Klostermann, 2002).

The potential of generating acrylamide from suitable precursors has mainly been attributed to the concentration of asparagine, which directly provides the backbone of the acrylamide molecule. However, there is some indication in literature that the type of sugar, or in general

202 the carbonyl compound, may significantly affect the final amount of acrylamide generated
203 through the Maillard reaction. Some authors have speculated on the role of physical
204 properties of precursors and suggested that the melting point of sugars is a possible
205 parameter to consider (Stadler et al., 2004).
206 In figure 2, differences in acrylamide content depending on the type of reducing sugar in the
207 system can be appreciated. Higher amounts of acrylamide were produced with fructose than
208 with glucose. Other authors have stated that mixtures with fructose generate acrylamide
209 earlier, meaning at a lower temperature, than those containing glucose (at about 125 and
210 140ºC, respectively), which means the final content of acrylamide was higher with fructose
211 than with glucose (Robert et al., 2004).
212 Figure 3 shows the inhibitory effect of chitosan on acrylamide formation. In fact, adding
213 0.5% of chitosan led to an inhibition of acrylamide formation (according to the control) of
214 52% and 65% at 180 and 150ºC respectively, and 1% of chitosan, 75% and 76% at 180 and
215 150ºC respectively. The influence of the temperature on acrylamide formation is well known
216 but when the interaction between the temperature and the concentration of chitosan was
217 analyzed, it can be observed that chitosan drastically reduces the influence of temperature on
218 the acrylamide formation. This seems to indicate that there is likely to be a reaction of
219 chitosan with reducing sugars at temperatures below acrylamide formation temperatures. In
220 spite of the small concentration of chitosan, the resulting inhibitory effect was significant,
221 ranging between 40% and 84% (results not shown) depending on the reducing sugar and the
222 treatment temperature. The protective effect of chitosan is very high as compared to the
223 results reported by Zeng et al., (2010) , who used other hydrocolloids in model systems and
224 in which at the concentration of 1% none of the hydrocolloids showed a significant
225 inhibition of the formation of acrylamide, although around 60% was achieved when 2% of
226 alginic acid and pectin were tested.

227 *3.2. Effect of chitosan on acrylamide formation in fried batter systems*

228 As evidenced in the model systems, the effect of chitosan on acrylamide inhibition was
229 observed in real systems. The results showed that the concentration of chitosan was again
230 the most significant factor followed by the frying time (F-ratio: 44.38 and 33.43,
231 respectively) and their interaction (chitosan %-frying time). The ability of chitosan to
232 compete with asparagine to bind to reducing sugars was quite significant even at low
233 concentration and no significant differences are found between using 0.27 or 0.54% of
234 chitosan at 2 and 4 minutes (Figure 4). At 7 minutes, which is above the optimum frying
235 time, the reduction in acrylamide formation was dependent on the percentage of chitosan,
236 being more effective at 0.54 than 0.27%. It is generally known that water content is a key
237 factor that has to be considerate in fried products in terms of acrylamide formation. Chitosan,
238 as a hydrocolloid, joins water, but water content of fried samples was not a significant
239 variable in acrylamide formation in this study. Chitosan did not significantly modified water
240 content of fried samples (p-value= 0.9725), but, as was expected, frying time was a
241 significant factor (p-value=0.0000, F-ratio=60.99). Values of percentage of moisture content
242 at 2, 4 and 7 minutes were 20 ± 2^{a}, 17 ± 2^{b}, 5.8 ± 1.9^{c} for control samples; 21 ± 3^{a}, 16.3 ± 0.6^{b},
243 4.5 ± 1.8^{c} when 0.27% of chitosan was added, and 22 ± 2^{a}, 16 ± 2^{b}, 4.4 ± 0.2^{c} for 0.54% of
244 chitosan.

245 Figure 5 shows that at similar heating times, 85±9% of reduction was the highest inhibitory
246 rate found in model systems when 1% of chitosan was present in the medium. In fried batter
247 systems the inhibition rate reached by adding chitosan to batter formulation was about
248 60±7% regardless of the percentage of chitosan tested (0.27 and 0.54%). These are excellent
249 results as compared to other hydrocolloids tested in real foods, i.e., the maximum inhibitory
250 rate reported by Zeng et al. (2010) that tested the addition of different hydrocolloids to the
251 formulation of a cracker, was 43% when 5% of pectin was incorporated to the formula.

4. Conclusion

Adding small amounts of chitosan in model and fried batter systems has been proven to be a new way to mitigate the generation of acrylamide. In fried batters, 0.27% of chitosan was capable of reducing the content of acrylamide in the final product by 59% and in model systems, the reduction depended largely on the reducing sugar tested, but to an even greater extent, on the percentage of chitosan, especially when fructose was present in the medium (up to 85%). The proposed mechanism of acrylamide reduction is based on the richness of amino groups of chitosan, which compete with asparagine amino groups to bind carbonyls (e.g. reducing sugars), the first stage of acrylamide formation. For this reason, chitosan has a high potential to provide consumers with healthy food products (lower acrylamide content) if it is incorporated into batters on a commercial scale.

Acknowledgements

The authors would like to thank the Generalitat Valenciana (GV/2012/072) for the financial support given to this investigation.

References

AACC. (1995). Approved methods of the American Association of cereal chemists. *9ª Ed. The Association. St Paul. MN*.

Albert, S., & Mittal, G. S. (2002). Comparative evaluation of edible coatings to reduce fat uptake in a deep-fried cereal product. *Food Research International*, *35*, 445–458.

AOAC. (1980). Official methods of analysis of the Association of Official Analytical Chemists. *13th ed.Association of Official Analytical Chemists, Washington DC (1980)*.

Balasubramaniam, V. M., Chinnan, M. S., Mallikarjunan, P., & Phillips, R. D. (1997). The effect of edible film on oil uptake and moisture retention of a deep-fat fried poultry product. *Journal of Food Process Engineering*, *20*(1), 17–29.

Barutcu, I., Sahin, S., & Sumnu, G. (2009). Acrylamide formation in different batter formulations during microwave frying. *LWT - Food Science and Technology*, *42*(1), 17–22.

Becalski, A., Lau, B. P.-Y., Lewis, D., & Seaman, S. W. (2003). Acrylamide in Foods: Occurrence, Sources, and Modeling. *Journal of Agricultural and Food Chemistry*, *51*(3), 802–808.

Blank, I. (2005). Current status of acrylamide research in food: measurement, safety

assessment, and formation. *Annals of the New York Academy of Sciences*, *1043*, 30–40.

El Ghaouth, A., Arul, J., Ponnapalam, R., & Boulet, M. (1991). Chitosan Coating Effect on Storability and Quality of Fresh Strawberries. *Journal of Food Science*, *56*(6), 1618–1620.

Gertz, C., & Klostermann, S. (2002). Analysis of acrylamide and mechanisms of its formation in deep-fried products. *European Journal of Lipid Science and Technology*, *104*(11), 762–771.

Gökmen, V., & Şenyuva, H. Z. (2007). Acrylamide formation is prevented by divalent cations during the Maillard reaction. *Food Chemistry*, *103*(1), 196–203.

Holownia, K. I., Chinnan, M. S., Erickson, M. C., & Mallikarjunan, P. (2000). Quality Evaluation of Edible Film-Coated Chicken Strips and Frying Oils. *Journal of Food Science*, *65*(6), 1087–1090.

IARC, I. A. for R. on C. (1994). Monographs on the Evaluation of Carcinogenic Risks to Humans; Lyon, France. *IARC*, *60*, 389–433.

Jung, M., Choi, D., & Ju, J. (2003). A novel technique for limitation of acrylamide formation in fried and baked corn chips and in French fries. *Journal of Food Science*, *68*(4), 1287–1290.

Lindsay, R. C., & Jang, S. (2005). Chemical Intervention Strategies for Substantial Suppression of Acrylamide Formation in Fried Potato Products. In *Chemistry and Safety of Acrylamide in Food* (pp. 393–404).

Mastovska, K., & Lehotay, S. J. (2006). Rapid Sample Preparation Method for LC−MS/MS or GC−MS Analysis of Acrylamide in Various Food Matrices. *Journal of Agricultural and Food Chemistry*, *54*(19), 7001–7008.

Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Acrylamide is formed in the Maillard reaction. *Nature*, *419*(6906), 448–449. Retrieved from

Muzzarelli, R. A. A. (1996). Chitosan-based dietary foods. *Carbohydrate Polymers*, *29*(4), 309–316.

Pedreschi, F., Kaack, K., & Granby, K. (2004). Reduction of acrylamide formation in potato slices during frying. *LWT - Food Science and Technology*, *37*(6), 679–685.

Robert, F., Vuataz, G., Pollien, P., Saucy, F., Alonso, M.-I., Bauwens, I., & Blank, I. (2004). Acrylamide Formation from Asparagine under Low-Moisture Maillard Reaction Conditions. 1. Physical and Chemical Aspects in Crystalline Model Systems. *Journal of Agricultural and Food Chemistry*, *52*(22), 6837–6842.

Rudel, R. a, Ackerman, J. M., Attfield, K. R., & Brody, J. G. (2014). New exposure biomarkers as tools for breast cancer epidemiology, biomonitoring, and prevention: a systematic approach based on animal evidence. *Environmental Health Perspectives*, *122*(9), 881–95.

Rydberg, P., Eriksson, S., Tareke, E., Karlsson, P., Ehrenberg, L., & Tornqvist, M. (2003). Investigations of Factors That Influence the Acrylamide Content of Heated Foodstuffs. *Journal of Agriculture and Food Chemistry*, *51*(24), 7012–7018.

Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: a critical review. *Journal of Food Science and Technology*, *47*(6), 587–97.

Sansano, M., Juan-Borrás, M., Escriche, I., Andrés, A., & Heredia, A. (2015). Effect of

Pretreatments and Air-Frying, a Novel Technology, on Acrylamide Generation in Fried Potatoes. *Journal of Food Science*, *80*(5), T1120–T1128.

Sanz, T., Salvador, A., & Fiszman, S. M. (2004). Effect of concentration and temperature on properties of methylcellulose-added batters Application to battered, fried seafood. *Food Hydrocolloids*, *18*(1), 127–131.

Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., … Riediker, S. (2002). Acrylamide from Maillard reaction products. *Nature*, *419*(6906), 449–450.

Stadler, Robert, F., Riediker, S., Varga, N., Davidek, T., Devaud, S., … Blank, I. (2004). In-Depth Mechanistic Study on the Formation of Acrylamide and Other Vinylogous Compounds by the Maillard Reaction. *Journal of Agricultural and Food Chemistry*, *52*(17), 5550–5558.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Tornqvist, M. (2002). Analysis of Acrylamide , a Carcinogen Formed in Heated Foodstuffs. *Journal of Agricultural and Food Chemistry*, *50*(17), 4998–5006.

Troncoso, E., & Pedreschi, F. (2009). Modeling water loss and oil uptake during vacuum frying of pre-treated potato slices. *LWT - Food Science and Technology*, *42*(6), 1164–1173.

Tsai, G.-J., & Su, W.-H. (1999). Antibacterial activity of shrimp chitosan against Escherichia coli. *Journal of Food Protection*, *62*(3), 239–243.

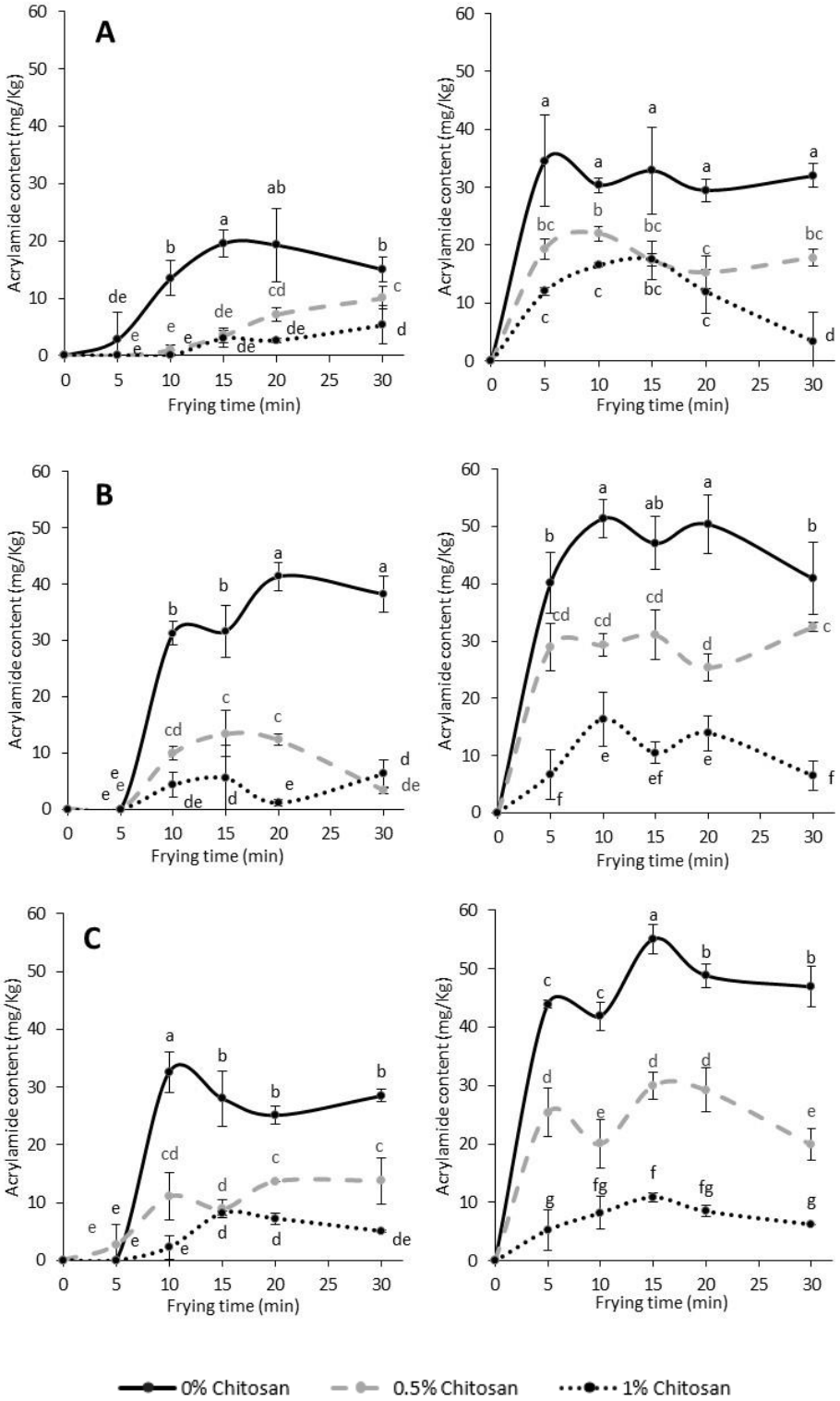
Zeng, X., Cheng, K.-W., Du, Y., Kong, R., Lo, C., Chu, I. K., … Wang, M. (2010). Activities of hydrocolloids as inhibitors of acrylamide formation in model systems and fried potato strips. *Food Chemistry*, *121*(2), 424–428.

Zeng, X., Cheng, K.-W., Jiang, Y., Lin, Z.-X., Shi, J.-J., Ou, S.-Y., … Wang, M. (2009). Inhibition of acrylamide formation by vitamins in model reactions and fried potato strips. *Food Chemistry*, *116*(1), 34–39.

Table 1. Multifactor ANOVA for Acrylamide content (mg/Kg) of main effects and their interactions in model systems.

MAIN EFFECTS	*Df*	*F-Ratio*
pH	1	0.13 (NS)
Reducing Sugar	2	54.86**
Time (min)	5	175.00**
Chitosan (%)	2	618.45**
Temperature (ºC)	1	624.55**
INTERACTIONS	*Df*	*F-Ratio*
pH- Reducing sugar	2	6.01**
pH- Time	5	4.18**
pH-Chitosan	2	4.43*
pH- Temperature	1	1.39 (NS)
Reducing sugar-Time	10	5.26**
Reducing sugar-Chitosan	4	17.61**
Reducing sugar-Temperature	2	2.56 (NS)
Time- Chitosan	10	29.79**
Time -Temperature	5	41.27**
Chitosan-temperature	2	77.67**

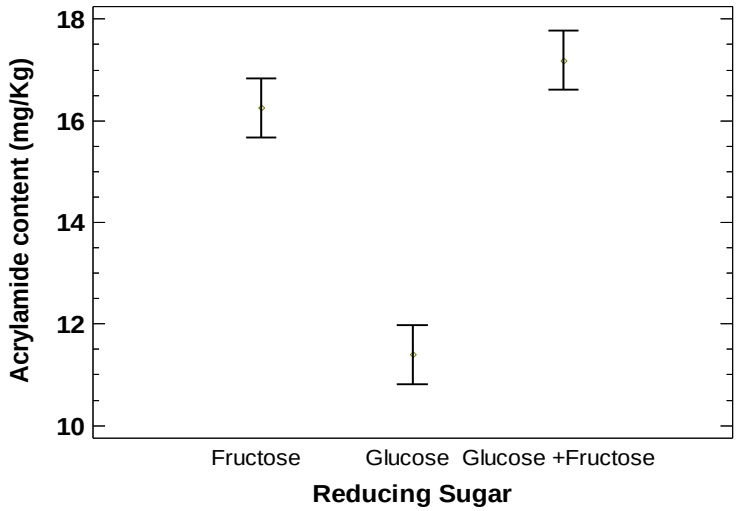
**Statistical significance ≥99% (p-value ≤ 0.01); *Statistical significance ≥95% (p-value ≤ 0.05); NS (not statistical significance, p-value > 0.05). Df: degrees of freedom



363

364

365 **Figure 1.** Average acrylamide content (mg/Kg) generated in model systems with 0, 0.5 and 1% of
366 chitosan, pH 4 at 150ºC (left) and 180ºC (right) after 5, 10, 15, 20 and 30 minutes of frying. (A)
367 asparagine-glucose; (B) asparagine-fructose; (C) asparagine-glucose-fructose. Error bars represent
368 standard deviations (n=3). Homogeneous groups are represented by the same letter.

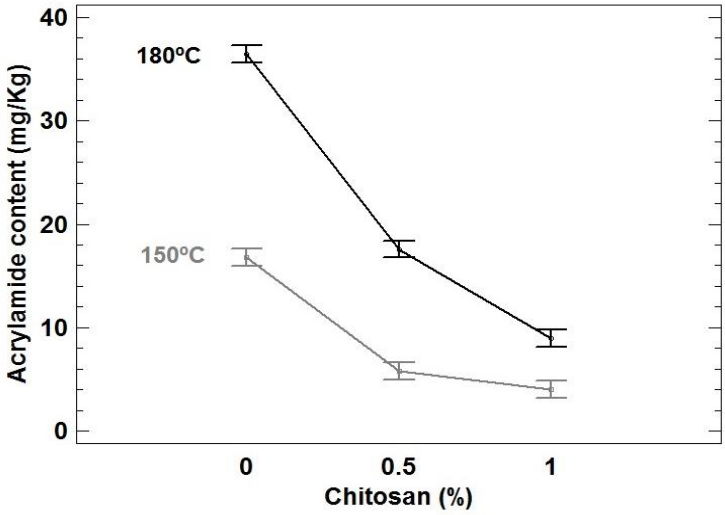


369

370 **Figure 2.** Influence of the type of reducing sugars tested on acrylamide formation (mg/Kg) in model

371 systems. Error bars represent 95% LSD (Least significance difference).

372



373

374 **Figure 3**. Influence of process temperature (150 and 180ºC) and chitosan content (0, 0.5 and 1%) on

375 acrylamide formation (mg/Kg) in model systems. Error bars represent 95% LSD (Least significance

376 difference).

377

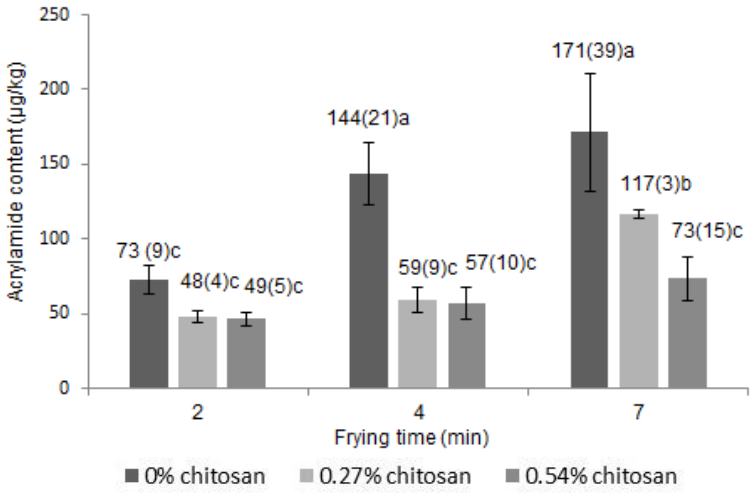


Figure 4. Acrylamide content (mean and standard deviation) in fried batter systems with 0, 0.27 and 0.54% of chitosan at 2, 4 and 7 minutes. Homogeneous groups are represented by the same letter.

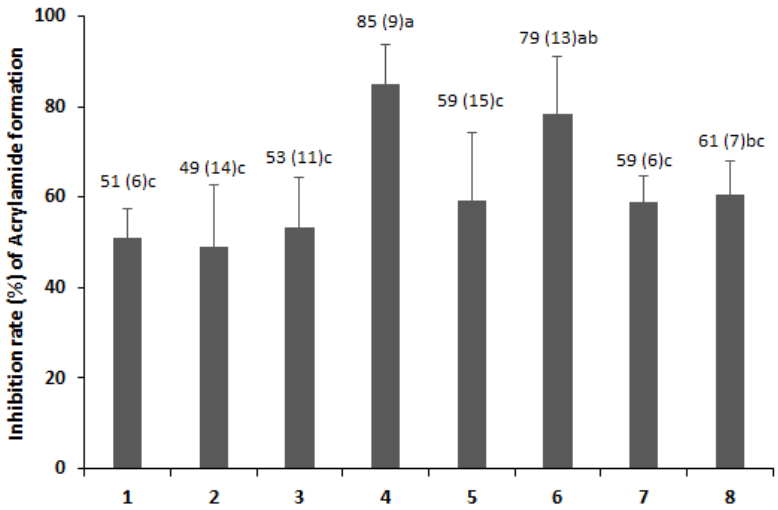


Figure 5. Inhibitory effect of chitosan (%) (mean and standard deviation (n=3)) on acrylamide formation in model systems (after 5 minutes of reaction time) and fried batter systems (after 4 minutes of frying) at 180ºC. X-axis legend: (1) (2) asparagine-glucose and 0.5 or 1% of chitosan; (3) (4) asparagine-fructose and 0.5 or 1% of chitosan; (5) (6) asparagine-glucose-fructose and 0.5 or 1% of chitosan; (7) (8) fried batters with 0.27 or 0.54% of chitosan. Homogeneous groups are represented by the same letter.