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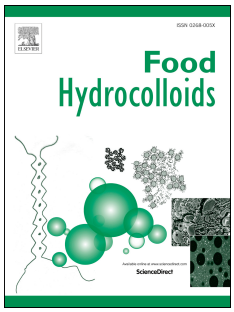

Acrylamide formation and quality properties of chitosan based batter formulations

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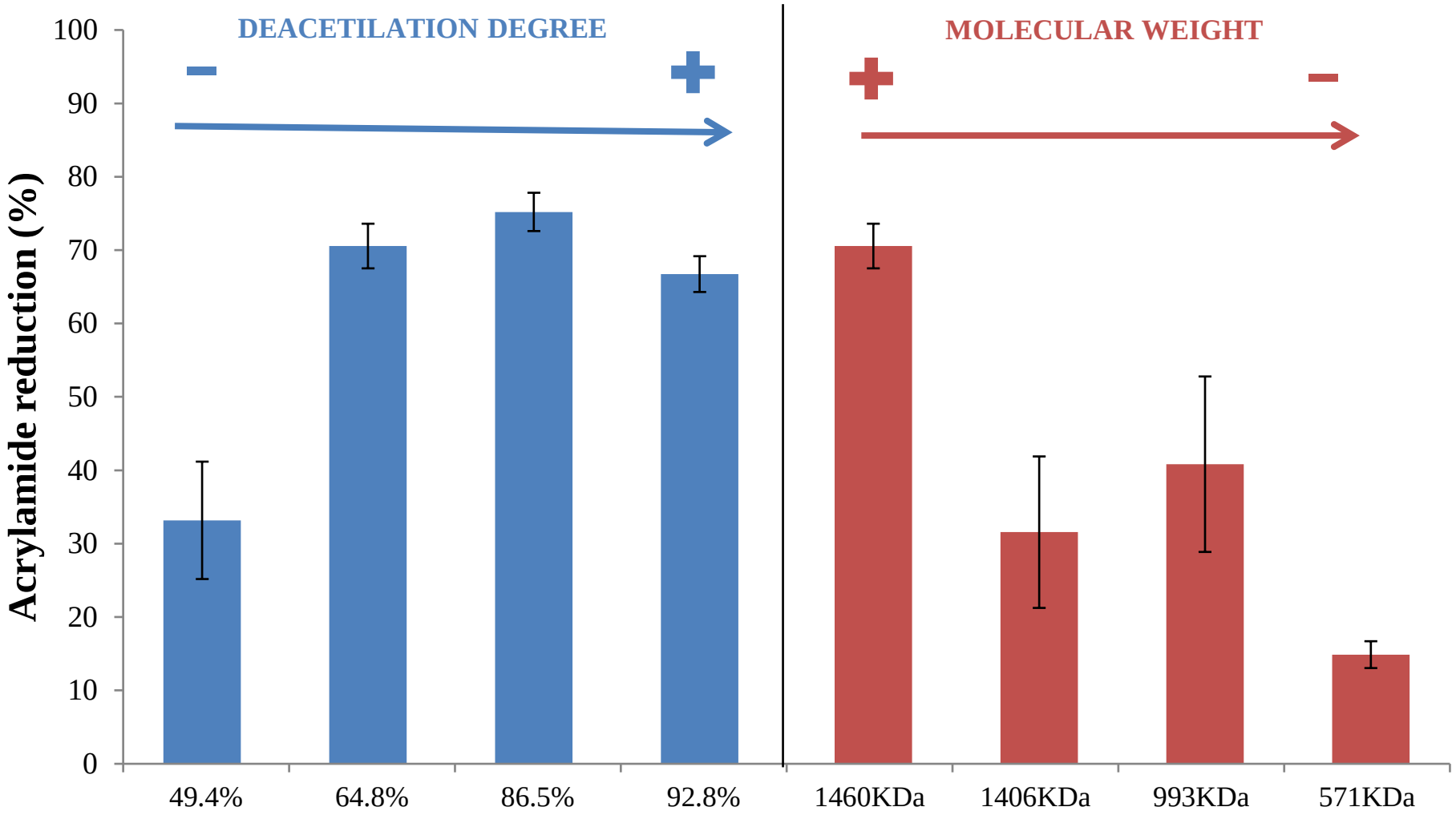
DEACETILATION DEGREE
MOLECULAR WEIGHT
Acrylamide reduction (%)
100
90
80
70
60
50
40
30
20
10
0
49.4%
64.8%
86.5%
92.8%
1460KDa
1406KDa
993KDa
571KDa

ACRYLAMIDE FORMATION AND QUALITY PROPERTIES OF CHITOSAN BASED BATTER FORMULATIONS

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ABSTRACT

The potential of chitosan to mitigate acrylamide formation has been already demonstrated. The two main objectives of this study were: 1) to select the most adequate degree of deacetylation (DD) and molecular weight (Mw) of chitosan based on acrylamide mitigation criteria and 2) to evaluate the influence of including chitosan in batter formulations on some important technological parameters of raw batters (flow behavior and water retention capacity) and on some quality properties of fried batters (oil uptake, color and texture). Results in model systems showed that chitosans with higher deacetylation degree (86.5 and 92.8%) achieved a decrease of acrylamide between 44 and 81%, depending on reaction time, compared to the control (without chitosan). Furthermore, acid hydrolysis process of chitosan was found to negatively affect its inhibitory effect on acrylamide formation independently of the molecular weight. Raw chitosan based batter formulations presented higher consistency and water retention capacity than the control; chitosan addition to batters reduced the hardening of the fried samples during the post-frying cooling period. No significant differences in water loss were observed between batters with or without added

chitosan; however, chitosan-batter formulations showed lower oil uptake during frying as compared to control samples.

KEY WORDS: Acrylamide, chitosan, deacetylation degree, molecular weight, batters

1. INTRODUCTION

Consumers are becoming more health-conscious and demand high quality food products, binding the food industry to take measures to provide these needs. Healthier fried products, with low-fat content and/or free of acrylamide, could be examples of this growing demand. Acrylamide is a probable carcinogenic for humans (Group 2A) according to the IARC classification that could be formed in foods, especially in starchy foods such as potatoes or cereals, submitted to processes taken place at temperatures above 120 ºC, being an intermediate-product of Maillard reactions (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Becalski et al., 2003). On 4 June 2015, European Food Security Administration (EFSA) published its first full risk assessment of acrylamide in food reconfirming that acrylamide in food potentially increases the risk of developing cancer for consumers in all age groups. FoodDrinkEurope consortium annually updates to the Acrylamide Toolbox rapport which recompiles different strategies from scientific literature addressed to Food Industry, policy-makers and consumers to inhibit the acrylamide generation. Most of these are addressed to reduce the acrylamide precursors (asparagine or reducing sugars) in the food matrix and/or stablish mechanisms to avoid its generation interfering in Maillard reactions (Medeiros Vinci, Mestdagh, & De Meulenaer, 2012; Morales, Capuano, & Fogliano, 2008). In the last years, the influence of hydrocolloids in acrylamide formation has been tested in model and fried systems. Certain

hydrocolloids (pectin and alginic acid) have demonstrated good potential to mitigate up to 50% of acrylamide content in foods when they are incorporated above of 5% in crackers (Zeng et al., 2010). Recently, the capacity of chitosan to limit acrylamide generation has been proved in model and fried batter systems (Sansano, Castelló, Heredia, & Andrés, 2016; Chang, Sung, & Chen, 2016). Concretely, the addition of small amounts of chitosan (~ 0.5%) to model systems and batters reduces acrylamide generation in 58±23 and 61±7 %, respectively. The action mechanism is based on the free amino groups present in chitosan which are able to compete with asparagine in binding to the reducing sugars, which implies a reduction in acrylamide formation. Chitosan is a polysaccharide obtained by deacetylation of chitin, an abundant polysaccharide in nature. Chitosan has been found to be biocompatible, biodegradable, biofunctional, and has strong antimicrobial and antifungal activities (Aider, 2010). Some of the current applications of chitosan are based on its antioxidant character (Darmadji & Izumimoto, 1994), antitumor (Tsukada et al., 1990), anti-cholesterolemic, anti-ulcer and its antiuricemic properties, which are related to the capacity of chitosan to bind specifically fatty acids, bile acids, phospholipids and uric acid (Muzzarelli, 1996). It should be highlighted that those biological properties of chitosan (antimicrobial, antioxidant and anti-cholesterolemic) mainly depend on its deacetylation and polymerization degrees (Aranaz et al., 2009). Therefore, these properties might affect its potential to reduce acrylamide formation as well. For this reason, these properties should also be considered to select the specific chitosan to be added as ingredient with this specific purpose. The influence of deacetylation degree (DD) and molecular weight (Mw) of chitosan on acrylamide reduction needs to be correlated. Chang et al., (2016) reported the effect of chitosan with low molecular weight on acrylamide generation in model systems, indicating a statistically significant

capacity to reduce acrylamide formation of 50-190 KDa-chitosan compared to the control, but there are no scientific papers published of the effect of deacetylation degree of chitosan on acrylamide mitigation.

Batters are used as coatings for fried products such as onion rings, tempura products (vegetables, prawns...), battered squid rings, chicken or fish nuggets, and they are composed basically of flour, water, salt and leavenings. It is well known that the selection of batter ingredients determines the visual appearance, color, flavor, crispiness, adhesion and therefore consumer acceptance (Hsia, Smith, & Steffe, 1992). In this sense, the use of chitosan as ingredient in batter formulations with the main goal of inhibits acrylamide could have an impact on different properties of raw and fried batters as well.

For this reasons, the main objective of this study was to evaluate the influence of deacetylation degree and molecular weight of chitosan on the acrylamide generation in model systems in order to select the most appropriate chitosan to be included in batter formulations. The influence of chitosan addition on the rheological parameters and water retention capacity of raw batter, as well as the influence on water loss and oil uptake during frying were also evaluated. Finally, fried samples were also compared in terms of color and texture.

2. MATERIALS & METHODS

2.1. Reagents

Reducing sugars (glucose and fructose), asparagine, chitosan (Poly (D-glucosamine) *Deacelyled chitin, high molecular weight) and acetic anhydride were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Acetic acid, sodium hydroxide, lactic acid, methanol were from Panreac (Barcelona, Spain). Formic acid was purchased

from VWR-Prolabo (Fontenay-sous-Bois, France). The standard of acrylamide (≥ 99%) was from Merck (Darmstadt, Germany) and the internal standard, 13C3-labelled acrylamide (99%) from Cambridge Isotope Laboratories (Andover, MA). All chemicals were analytical grade, except those used for chromatographic analysis (HPLC grade). The bidistilled water was obtained by a purification process of water (Milli-Q, Millipore Corp., Bedford, MA).

2.2 Acetylation and deacetylation of commercial chitosan

Commercial chitosan was used to obtain chitosan with different deacetylation degree (DD) by acetylation and deacetylation processes. Acetylation process was performed according to Kiang, Wen, Lim, & Leong, (2004). Briefly, 15 g chitosan were dissolved in a solution of 2% acetic acid (300 mL), distilled water (400 mL) and methanol (800 mL), and stirred for 20 minutes. Then, 2 mL of acetic anhydride were added into the solution and the mixture was stirred for 12 hours. To end the reaction, 1M NaOH was added to the solution in order to precipitate the chitosan, which was washed several times until neutral pH with distilled water and dried under vacuum at 60°C. Deacetylation was done according to Zhou et al., (2008) with slight modifications: chitosan (10 g) was dissolved in 100 mL of NaOH solution (ratio of 1:2 (w/v)) for 30 minutes at 100ºC, washed repeatedly with distilled water and dried at 60ºC. This process was considered as a one cycle deacetylation process, and was applied one or twice to obtain different DD.

The titration method described by Wang et al. (2006) with minor modifications was used to determine the deacetylation degree (DD) of the chitosan obtained from the above described process. 0.2 g of chitosan was dissolved in 20 mL of HCl 0.1 M under stirring for 4 h. Measurements were performed with a solution of NaOH 0.1 M

by using a Metrohm's high-end titrator. The DD of chitosan was calculated as follows (equation 1):

$$DD = \frac{\Delta V \cdot C_{NaOH} x\ 10^{-3} \cdot 16}{M \cdot 0.0994} \qquad (I)$$

where ΔV is NaOH volume of between two inflexion points, C_{NaOH} is concentration of NaOH solution, M is the mass of the sample, and 16 and 0.0994 are the molecular weight and theoretical amount of amino groups, respectively.

2.3. Depolymerization of commercial chitosan by acid hydrolysis

Chitosan with different molecular weight (Mw) were obtained by acid hydrolysis according to the method described by Zhou et al., (2006) with minor modifications. Commercial chitosan (2g) was dissolved in 2% acetic acid (100mL), stirred and heated at 70ºC for different times (2, 4 and 8 hours) in order to obtain chitosan with different molecular weights. After that, the reaction mixture was neutralized with NaOH. Absolute ethanol was added (70 mL per liter of solution) in order to completely precipitate the chitosan. The samples were filtered, washed with distilled water, and dried at 60ºC.

The molecular weight of the chitosan (M_v) was determined by viscosimetry (Bof, Bordagaray, Locaso, & García, 2015). The measurements were performed using an Ubbelohde capillary viscometer No. 2121R, (Ø= 0.4 mm) equipped with a thermostat bath at 25.0° C ± 0.01ºC. Chitosan was dissolved in 0.1M acetic acid/0.2M NaCl, into different concentrations: $5.0 \cdot 10^{-4}$, $6.5 \cdot 10^{-4}$, $8.5 \cdot 10^{-4}$ y 10^{-3} g/mL, being filtered (0.45µm) before viscosity determinations. Draining times of a fixed volume of chitosan solutions (t) and pure solvent (t_0) were measured. From these, relative viscosity (η_r) and specific viscosity (η_{sp}) of were calculated using the following equations *II* and *III*:

$$\eta_r = \frac{\eta}{\eta_0} = \frac{t}{t_0} \qquad (II)$$

$$\eta_{sp} = \eta_r - 1 \qquad (III)$$

where η is chitosan solution viscosity and η_0 is viscosity of the pure solvent, and their corresponding draining times (t and t_0).

The reduced viscosity (η_{red}) was calculated from the specific viscosity (η_{sp}) and the concentration of chitosan solution (*equation IV*):

$$\eta_{red} = \eta_{sp}/C \qquad (IV)$$

where C is concentration of chitosan solution (g/mL).

The intrinsic viscosity $[\eta]$ was determined graphically, extrapolating values of reduced viscosity (η_{sp}/C) to zero concentration. The intrinsic viscosity was used to determine the viscosity average molecular weight (M_v) from Mark-Houwink-Sakurada-Staudinger equation (*equation V*):

$$[\eta] = K_m \cdot (M_v)^a \qquad (V)$$

where *Km* and *a* are two constants dependent on the particular polymer-solvent system ($1.81 \cdot 10^{-3}$ and 0.93, respectively) (Roberts & Domszy, 1982).

2.4. Acrylamide generation in model systems with chitosan

Chemical model reactions were carried out following the method described by Sansano et al., (2016). The reaction was carried out at pH = 4 using a 25 mL threaded Pyrex tube which contained 5 µmol of asparagine and 5 µmol of a mixture of glucose-fructose 1:1 (w/w), and 100 µl of a 0.5% acid lactic solution containing 0 (control samples) and 1% (w/w) of each one of the chitosan with different DD and molecular weight. Tubes kept closed along experiments. Samples were subjected to 180ºC in an oil bath (model: FM 6720 Ideal 2000 Professional, Solac with a nominal power of 2,000 W) during 5, 10 and 15 minutes, in triplicate. During heating processes only the

bottom of the tubes was covered with oil. After time of reaction, the tubes were immediately placed on ice for 5 min. Two mL of Milli-Q water were added and tubes were vortexed for 1 min. The mixture was filtered with Nylon filter (0.45 µm) and transferred to a vial for chromatographic analysis of acrylamide. 100 ng of $^{13}C_3$-acrylamide were added to each sample as internal standard.

2.5. Acrylamide determination by chromatography

Acrylamide was analyzed by triplicate according to Sansano et al. (2015) with minor modifications using an Agilent 1200 Series HPLC system coupled to an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies Inc., CA, USA), in positive electrospray ionization mode. A Zorbax XDB C-18 column (2.1mmx50mm, 1.8µm) was used. Six different levels (20, 50, 100, 200, 300 and 500 µg/L), with six replicates for each level (n=6) were studied, being 20 µg/L the limit of quantification. The column temperature was set at 30ºC, the sample injection volume was 5µL and flow rate was maintained at 0.4mL/minute. The mobile phase consisted of 2.5% methanol/ 97.5% of 0.1% formic acid (A) and methanol (B), and 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. The ionization source was nitrogen at 350ºC at 12 L/min and 40 psi of nebulizer pressure and 4000V of capillary voltage. Multiple reactions monitoring mode (MRM) was used to identify and quantify acrylamide. The MRM transition 72>55.2 was used to quantify and 72>27 was also monitored for acrylamide and 75>58 for $^{13}C_3$-acrylamide (internal standard).

2.6. Batter formulations

The batter formulations were prepared from a commercial dry mix for battering products (Yolanda®, Murcia, Spain), consisting on wheat and rice flours, acidity regulator (E-334), bulking agent (E-500ii) and coloring (E-160b). Batters were

prepared according to Sansano et al., (2016). Flour basis was added in a water-to-dry-mix proportion of 1.2:1 (w/w), with addition of 2.5% of table salt. Chitosan batters were prepared in the same way but replacing the water by a chitosan solution at 1% dissolved in 0.5% lactic acid at pH=4. The final chitosan content in the batters formulation was 0.54 % (final pH= 5.7) and batters without chitosan were used as control. Protein and fat contents of the commercial dry mix were 10.0 and 1.4%, respectively (data supplied by manufacturers); moisture (11.5%) and ash contents (1.8%) were measured following AACC methods (1995). Average particle size (78.0 µm) was analyzed with a Mastersizer 2000 coupled with Scirocco 2000 module for dry measurement (Malvern Instruments, Germany).

Batters were kept for 30 minutes at room temperature before frying.

2.7. Flow properties and water retention capacity of raw batters

Apparent viscosity of raw batter formulations was determined using a Haake Rheostress 1 rheometer (Thermo Electric Corporation, Germany) equipped with a plate-plate (60 mm of diameter) at 10, 20, 30 and 40 ºC. Apparent viscosity (Pa·s) was measured as a function of shear rate (γ) from 0 to 100 s^{-1} after 5 min of stabilization time. Rheological constants *K* (consistency index, Pa·s^n) and *n* (flow behavior index) were adjusted to the Ostwald-De Waele model (*equation VI*):

$$\tau = K \cdot \gamma^n \qquad (VI)$$

Water Retention Capacity of raw batters was analyzed as follows: 18 g of each batter formulation were weighed in a 30 mL centrifuge tube, tempered at different temperatures (10, 20, 30 and 40 ºC) and centrifuged at 17300 RCF for 10 minutes. The supernatant was removed and weighed to calculate the WRC (*equation VII*).

$$WRC = \frac{(W_s \cdot x_w) - W_w}{(W_s \cdot x_w)} \cdot 100 \qquad (VII)$$

where W_s is the total sample mass (g); x_w is water mass fraction of batter (g w/ g batter) and W_w is the supernatant mass (g).

Both determinations were done by triplicate.

2.8. Frying process

Frying of batters was carried out in a commercial deep-fat fryer of 2 L of capacity (model: FM 6720 Ideal 2000 Professional, Solac) at 180±2ºC. Samples (11.5 ± 0.1g) were placed in an aluminum cylindrical cake mold and introduced in the fryer in order to obtain homogenous ring shaped fried samples (height: 11±1mm; outer diameter= 65mm ±2 and inner diameter= 25 ±1mm). Three samples (n = 3) were fried for each frying time (2, 4 and 7 minutes). The excess of oil was removed with paper on both sides for 20 seconds after taking out the samples from the fryer.

After frying, the following determinations were carried out by triplicate (n=3) at each time of frying, except for mass fluxes, color and texture (n=5 for each frying time).

Water content was analyzed by vacuum drying at 60ºC until constant weight was achieved (20.103, AOAC, 1980). Total oil content was determined by solvent extraction using the Soxhlet method (AACC, 1995), performing the extraction procedure with petroleum ether.

Net changes of components (ΔM^i_t) (concretely, oil uptake (ΔM_t^{oil}) and water loss (ΔM_t^{w}) during frying were obtained according to *equation VIII*

$$\Delta M_t^i = \frac{\left(M_t \times x_t^i\right) - \left(M_0 \times x_o^i\right)}{M_0} \qquad (VIII)$$

where M_0 is the total mass of the sample at initial time (g), M_t is the total mass at time *t* (g), x_0^i is the mass fraction of component (water or oil) at initial time (g/total g) and x_t^i is the mass fraction of component (water or oil) at time *t* (g/total g). Superscript *i* is "*oil*" or "*w*" for oil and water component, respectively.

2.9. Texture properties of deep-oil fried batters

Texture changes over frying time were evaluated by a puncture test using a Texture Analyser (mod. TA-XT PlusAname, Spain) equipped with a 50 Kg load cell. Texture test was performed twice, just after frying but tempering the samples at a consuming temperature (55ºC) and after cooling the samples at room temperature (25ºC). The plunger used for the test was a cylinder with a flat base of 2 mm diameter. Samples were placed on a holed platform to ensure a total sample perforation. The crosshead speed was 1 mm/s. The maximum shear force F_{max} (N) necessary to perforate each sample was recorded from the force-deformation curve.

2.10. Optical properties of deep-oil fried batters

Optical properties of the fried samples were determined by using a spectrocolorimeter (MINOLTA, mod. CM-3600d). The color space coordinates CIEL*a*b* were obtained from the absorption spectrum between 380 and 770 nm by reflectance with the reference system: D_{65} illuminant and 10 ° observer, and a 12 mm lens. Chroma ($C_{ab}^*=(a^{*2}+b^{*2})^{1/2}$) and hue ($h_{ab}=\arctan(b^*/a^*)$), as well as total color changes ($\Delta E=[(L^*-L^*_{control})^2+(b^*-b^*_{control})^2+(a^*-a^*_{control})^2]^{1/2}$) of fried batters were calculated.

2.11. Statistical analysis

The influence of degree of deacetylation and the molecular weight of chitosan on acrylamide generation in model systems, as well as the effect of chitosan on the analyzed physicochemical properties of raw and fried batters were analyzed using Statgraphics Centurion XVI. Analysis of variance was carried out with a multifactorial ANOVA, obtaining a significance level of 95%.

3. RESULTS AND DISCUSSION

3.1. Influence of deacetylation degree and molecular weight on acrylamide formation in model systems

The estimated deacetylation degree was 64.8±0.8 % for commercial chitosan, 49.4±0.3 % for the chitosan obtained from the acetylation process, and 86.5±0.6 % and 92.80±0.12 % for the chitosan obtained from 1 or 2 deacetylation cycles, respectively. Briefly, it can be observed that the application of two cycles of deacetylation did not substantially increase the degree of deacetylation of chitosan compared to the DD achieved after 1 cycle.

The inhibitory capacity of chitosan on acrylamide generation in model systems was confirmed independently of the deacetylation degree (DD) (reductions between 44-74% with respect to the control) (Fig.1). Nevertheless, results did not show a clear relationship between chitosan-DD and the degree of the acylamide mitigation. At short-time of reaction (5 min), the lowest DD (49.4%) seemed to be the least effective against acrylamide generation, while no difference on acrylamide reduction was observed between those with higher deacetylation degree. At longer reaction times (10 and 15 min) similar acrylamide generation was found in model systems with acetylated chitosan (DD 49%) and commercial chitosan (DD 64.8%). A lightly higher reduction of acrylamide formation was observed when chitosan obtained from one cycle deacetylation process (DD 86.5 %) was added to the model system. However, the results obtained with 92.8%-DD chitosan does not allow to conclude that the higher the DD the higher the inhibitory capacity of chitosan. The method used to obtain this DD, a 2-cycle deacetylation process, could be co-responsible of these results, but it should be confirmed. Apparently, two consecutive deacetylation cycles could not only modify the deacetylation degree, but also affect other properties. Tsai, Su, Chen, & Pan, (2002) reported that chitosan with a high level of deacetylation tends to form

aggregates in aqueous solutions which contribute to form intermolecular interactions that might reduce available sites on the chitosan molecule to inhibit acrylamide formation.

In addition to the DD of chitosan, the influence of molecular weight of chitosan on acrylamide formation was explored using the same model systems (Fig.2). Table 1 shows the viscosity average molecular weight (M_v) measured on the commercial chitosan (0 h) and on those obtained by hydrolysis process of different duration (2, 4 and 8 hours). Chang et al. (2016) recently reported the effect of chitosan with low molecular weight on acrylamide generation in model systems, indicating a statistically significant acrylamide reduction capacity of 90-150 KDa-chitosan compared to the control, but no statistical effect of including chitosan with 190-310 KDa or 310-375 KDa in model systems on acrylamide generation. The range of molecular weight evaluated in the present studied was above the interval studied by Chang et al. (2016), being the lowest M_v achieved in this study 571 ± 21 KDa with the longest hydrolysis time (8 h). The longer the duration of the hydrolysis process the greater the reduction on molecular weight of chitosan, but in any case it is possible to reduce the formation of acrylamide at levels lower than those achieved with the commercial chitosan (Fig.2). These results can be explained if they are considered as the overall result of two opposite effects: the first one related to structural changes induced by the acid hydrolysis with a negative influence on inhibiting acrylamide formation and a positive second one related to the decrease of molecular weight. In fact, Kumar (2000) recommended the enzymatic hydrolysis against the acid one because of the better control of the process and the absence of chemical modifications of the structure. Furthermore, the hydrolysis can promote changes in the chain conformation of chitosan and new intermolecular bonds (Rege & Block, 1999). The negative effect of

hydrolysis is observed independently of the duration of the process because of commercial chitosan (1460 KDa) was more effective inhibiting acrylamide formation than chitosan with similar Mv but submitted to a 2 hours acid hydrolysis process (1406 KDa) (Fig.2). This effect is slightly countered by the decrease of Mv in chitosan of 993 KDa while it seems to be the predominant effect in model systems with 571 KDa chitosan.

3.2. Quality properties of chitosan based batter formulations

The above results together with those published by the same authors in a previous study (Sansano et al., 2016) were the base to select the commercial chitosan (64.8% DD and 1460 KDa) as ingredient in batter formulations and study the main quality properties of the new formulation. In this study, commercial chitosan was used to formulate batters according to Sansano et al., (2016) in order to evaluate the effect of this hydrocolloid on the rheological behavior and water retention capacity of raw batters as well as water loss and oil uptake during frying, and color and textural changes of fried samples.

Table 2 shows the rheological parameters, consistency (K) and flow behavior index (n) of batters with and without chitosan, their apparent viscosity at 20 s^{-1} (reference shear stress), as well as their water retention capacity (WRC). For the entire range of studied temperatures (from 10 to 40 ºC), the addition of chitosan resulted in a significant increase of both the consistency (K) and the apparent viscosity at 20 s-1 of the studied batters. The increase of consistency of batters when chitosan is added will need to be considered for future applications because of its influence on batter pick-up, yield and crispiness (Sanz, Salvador, & Fiszman, 2004).

As refers to flow behavior index, it was not affected by chitosan addition at 10 and 20ºC and only a slight decrease was observed at 30 and 40ºC. Flow behavior index (n)

was highly dependent on temperature regardless of chitosan addition. This fact indicates that the control of temperature is a key-variable during the line production of batters formulation at industrial scale (Baixauli, Sanz, Salvador, & Fiszman, 2003). Similar results have been reported by other authors for other hydrocolloids such as methylcellulose, guar gum or xanthan gum (Hsia et al., 1992; Sanz et al., 2004).

In a similar way than in other hydrocolloids, such as methylcellulose (Sanz et al., 2004), the WRC was higher in the raw batters containing chitosan than in the control (0% of chitosan), thus showing the ability of chitosan polymeric chain to retain water.

Do Amaral et al., (2015) and Sayas-Barberá et al., (2011) reported that using chitosan as an ingredient in formulations of sausages and hamburgers contributed to increase water retention after oven cooking at 150ºC (internal temperature in the product 72ºC), compared to the control indicating that chitosan encouraged water retention after thermal processing. However, chitosan based batters presented lower WRC at 30 and 40ºC than at 10 or 20ºC, probably due to very different interface conditions as compared with sausages.

Additionally to the evaluation of the above-parameters in raw batter formulations, other relevant properties were analyzed during and after the frying process, since the impact of chitosan addition (~ 0.5%), on acrylamide mitigation in fried batters has been recently published by the same authors (Sansano et al., 2016) (average reductions of 32, 60 and 59% after 2, 4 and 7 min of frying, respectively). Table 3 shows the results of water loss and oil uptake along frying and the texture after tempering the fried samples at 55 and 25ºC. No statistically significant differences were found on water loss during frying between batters with and without chitosan. It suggests that water retention capacity of chitosan in raw batters gets lost during frying. In this sense, Sayas-Barberá et al., (2011) did not found significant differences of moisture contents

of fried burgers with chitosan (up to 1%) and the control. However, Do Amaral et al., (2015), who incorporated a higher chitosan percentage (1-2%) than in this study, reported a higher retention of moisture in sausages compared to the control. Likewise, Ansarifar et al., (2015) obtained samples with higher water and fat contents in fried cheese nuggets when chitosan was incorporated at 0.5 and 1.5%. All these results point out that the effect of chitosan on the final moisture content of the fried product, and then on water loss, is quiet dependent on the type of matrix and the cooking method. On the other hand, some hydrocolloids, such as wheat and soy protein isolates, methylcellulose or hydroxypropylmethylcellulose, have been proved to reduce oil uptake and water loss during frying (Albert & Mittal, 2002; (García, Ferrero, Bértola, Martino, & Zaritzky, 2002). In this study, chitosan seems to slightly limit the oil uptake in batters after 2 and 4 min of frying as compared to the control; while Usawakesmanee, Wuttijumnong, Chinnan, Jangchud, & Raksakulthai, (2005) did not find significant differences between using chitosan as an ingredient of fried breaded potato and the control in terms of final fat content of fried products. Once again, the influence of chitosan in the fat retention will be different depending on the food matrix composition. Some studies showed that including dextrin or dried egg combined or not with chitosan reduced breaking force compared to the control (Baixauli et al., 2003) and some others showed that low molecular chitosan increased the hardness of fried batters (Ansarifar et al., 2012; 2015).

Concerning to the chitosan influence on the colour and texture of fried batters, results from texture test showed an increase of maximum force with frying time, mainly due to the decrease of water content at the surface, meaning an increase of crispness because of the crust formation. The addition of chitosan did not imply significant differences in maximum force values, i.e. in hardness of the external crust, of fried

batters at 55ºC (serving temperature). However, after cooling the samples at 25ºC, the maximum force registered in samples without chitosan was higher than in samples with chitosan, except for over-fried samples (7 min). This result reveals that the presence of chitosan protects tightening during cooling, maybe because its capability of binding water. Finally, Table 4 includes the optical parameters of fried batters with or without chitosan at each frying time. As can be observed, lightness (L*) and hue (h_{ab}) of batters were not affected by chitosan addition. However, both parameters decreased as expected with frying time due to brownish. According to ANOVA results, chitosan addition provided lower C_{ab}* values at 2 minutes compared to control samples, but it increased the color saturation (C_{ab}*) of fried samples at 4 and 7 minutes. Other hydrocolloids, such as guar gum or xanthan gum, contributed to a lower color development, while gum Arabic included in batters for chicken nuggets increased darkness, probably due to the reduction of protein content, at replacing flour by a hydrocolloid (Sahin, Sumnu, & Altunakar, 2005). The increase of darkness when chitosan is present in the food system may be due to Maillard reactions progress as a consequence of the reaction between the amino groups of this polysaccharide and carbonyl groups of glucose at high temperature (Rao et al., 2011; Phisut & Jiraporn, 2013). Color differences (ΔE values) were similar for all frying times and low, which implies that probably, consumers will not perceive color changes promoted by chitosan addition.

4. CONCLUSIONS

The results showed that the higher the deacetylation degree of chitosan, the higher the reduction in acrylamide compared to the control samples (without chitosan). However,

418 the two cycles deacetylation process provokes additional changes that results in less
419 efficiency in acrylamide mitigation. Additionally, the acid hydrolysis was found to be
420 a process that not only decrease the molecular weight, which would contribute to a
421 high inhibition of acrylamide formation, but also promote changes with negative effect
422 on chitosan capacity to reduce acrylamide. Therefore, commercial chitosan with high
423 molecular weight and DD was found to be the most appropriate to evaluate quality
424 properties of a new batter formula.
425 Chitosan as ingredient in battering formulations in a concentration of ~ 0.5%, which
426 implies an acrylamide reduction between 32 and 69% depending on frying time,
427 increased both the consistency and the water retention capacity of raw batters without
428 significant modifications of the final color or texture of fried batters. Therefore, batters
429 formulations with chitosan (~ 0.5%) can be used to largely mitigate acrylamide
430 without prejudice to the main quality properties valued by the consumers.
431

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435

436 **6. REFERENCES**

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541

Table 1. Intrinsic viscosity and viscosity average molecular weight (M_v) corresponding to hydrolyzed chitosan samples. 0 h corresponds to commercial Chitosan.

Hydrolysis time (h)	**[ŋ] intrinsic viscosity (mL/g)**	**M_v (KDa)**
0	902 (3)d	1460 (5)d
2	868 (5)c	1406 (3)c
4	613 (7)b	993 (11)b
8	353 (13)a	571 (21)a

Table 2. Rheological parameters: consistency (*K*) and flow index behavior (*n*), apparent viscosity at 20 s^{-1}, and water retention capacity (WRC (%)) of batter formulations with and without chitosan at 10, 20, 30 and 40°C. Homogeneous groups are represented by the same letter.

	T[a] (ºC)	***K* *(Pa s^n)***	***n***	**Apparent viscosity, shear rate= 20s^{-1} (Pa·s)**	**WRC (%)**
Without chitosan	10	2.63 (0.07)d	0.73 (0.00)a	1.10 (0.04)c	34.4 (0.1)d
	20	2.44 (0.08)e	0.71 (0.01)b	0.96 (0.05)d	34.9 (0.2)d
	30	2.01 (0.01)f	0.70(0.00)c	0.784 (0.009)e	34.6 (0.5)d
	40	1.92 (0.02)f	0.69 (0.01)d	0.708 (0.004)e	36.1 (0.6)c
With chitosan	10	4.16 (0.03)a	0.73 (0.00)a	1.73 (0.02)a	44.3 (0.2)a
	20	3.15 (0.12)bc	0.71 (0.00)b	1.23 (0.05)b	43.9 (0.3)a
	30	3.36 (0.01)b	0.69 (0.01)d	1.21 (0.03)b	39.6 (0.3)b
	40	3.01 (0.16)c	0.67 (0.00)e	1.06 (0.07)c	40.3 (0.1)b

Table 3. Average values and standard deviations of water loss and oil uptake (n=3) and F_{max} at 55 and 25 ºC (n=5) of fried batters with or without chitosan at different frying times. Homogeneous groups are represented by the same letter.

	Frying time (min)	**Water loss (ΔM_w)**	**Oil uptake (ΔM_{oil})**	**F_{max} (N) at 55ºC**	**F_{max} (N) at 25ºC**
Without chitosan	2	-0.37 (0.04)a	0.24 (0.02)a	2.06 (0.03)b	1.1 (0.4)d
	4	-0.44 (0.09)b	0.28 (0.08)a	5 (3)b	5 (3)c
	7	-0.51 (0.04)c	0.30 (0.05)a	14.5 (1.1)a	23 (4)a
With chitosan	2	-0.35 (0.05)a	0.21 (0.04)b	2 (2)b	1.2 (0.8)d
	4	-0.424 (0.014)b	0.203 (0.012)b	3.6 (0.8)a	3.5 (1.4)cd
	7	-0.525 (0.017)c	0.24 (0.04)a	12 (0.9)a	17 (3)b

Table 4. Average values and standard deviations (n=5) of chromatic parameters L*, C*, h* and ΔE of fried batters with or without chitosan at different frying time. Homogeneous groups are represented by the same letter.

	Frying time (min)	**L***	**C_{ab}***	**h_{ab}**	**ΔE**
Without chitosan	2	61.5 (1.4)a	34.4 (1.5)b	89.1 (0.8)a	-
	4	53 (2)b	34.0 (1.3)b	79.9 (0.7)b	-
	7	47 (3)c	30.4 (1.1)c	69.9 (1.8)c	-
With chitosan	2	60.9 (1.5)a	31.4 (1.7)c	92.3 (1.4)a	5.2 (1.6)b
	4	50 (3)b	37.0 (0.9)a	79.7 (1.7)b	6.3 (1.7)ab
	7	44 (3)c	37.2 (0.9)a	67.9 (1.4)c	6.7 (1.2)a

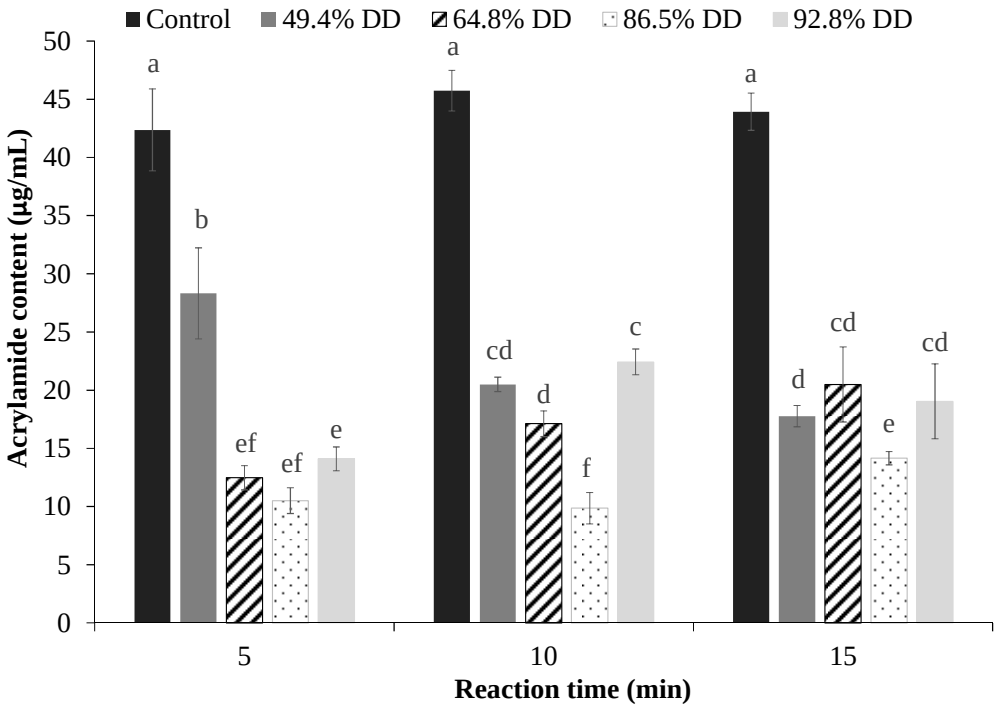

Figure 1. Acrylamide content (µg/mL) versus reaction time generated in different model systems consisting of 5µmol of asparagine, 5µmol glucose:fructose (1:1) and 0 (control) and 1% of chitosan with different deacetylation degree (DD). Homogeneous groups are represented by the same letter.

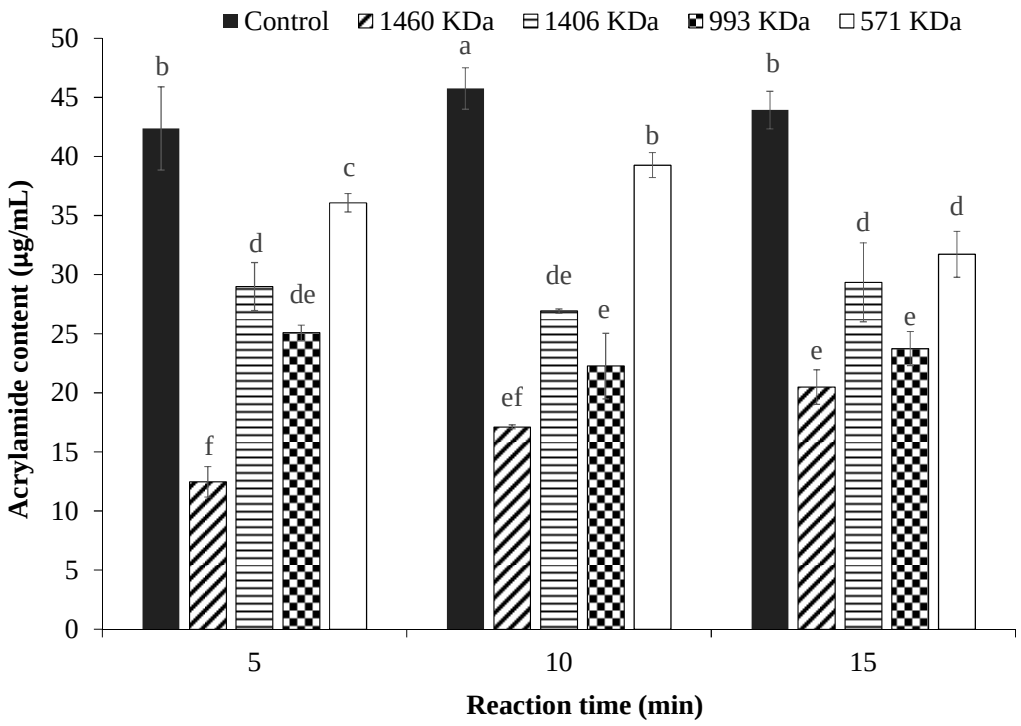

Figure 2. Acrylamide content (µg/mL) versus reaction time generated in different model systems consisting of 5µmol of asparagine, 5µmol glucose:fructose (1:1) and 0 (control) and 1% of chitosan with different molecular weight (M_v). Homogeneous groups are represented by the same letter.

HIGHLIGHTS

-The higher the deacetylation degree of chitosan the greater the AA reduction.

- CH increased consistency and water retention capacity of raw batters

- CH addition to batters reduced oil uptake and acrylamide formation during frying

-Incorporation of CH in batters did not affect color and texture of fried batters

ABBREVIATIONS: AA: Acrylamide; CH: Chitosan