Elsevier Editorial System(tm) for Journal of Food Engineering
Manuscript Draft

Manuscript Number: JFOODENG-D-14-00374R2

Title: Effect of water activity in tortilla and its relationship on the acrylamide content after frying

Article Type: Research Article

Keywords: Acrylamide; Tortilla chips; Minimum integral entropy

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Highlights

- Water activity affects the acrylamide content in tortilla chips.
- Minimum integral entropy is a reliable indicator to establish the most appropriate moisture conditions to get a reduction of acrylamide in tortillas chips.
- Tortilla dehydration to the minimum integral entropy reduces the acrylamide content.
- Products obtained from stored tortilla showed an acceptable texture and appearance.

Effect of water activity in tortilla and its relationship on the acrylamide content after frying

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Abstract

The objective of this study was to relate the tortilla minimum integral desorption entropy with acrylamide content during processing of tortilla chips. Tortilla pieces were stored at 30 ˚C at a_w of 0.11-0.84 for 4 days and fried later in soybean oil at 180 ˚C for 25 s. The lowest acrylamide content was observed in tortilla chips made of non-stored tortilla (a_w=0.98) as well as in those prepared from tortilla stored in the minimum integral entropy (a_w=0.53). In addition, the color and texture values were similar in both cases. These results suggest that the reduction of the acrylamide content during processing of tortilla chips and other tortilla based foods thermally processed might be modified by factors such as moisture content, a_w, and the physical state of water in the tortilla. Thus, the minimum integral entropy showed to be a reliable indicator to establish the most appropriate moisture conditions to obtain tortilla chips with reduced level of acrylamide when tortilla is dehydrated.

Keyword: Acrylamide, Tortilla chips, Minimum integral entropy

Chemical compounds studied in this article

Acrylamide (PubChem CID: 6579)

1. Introduction

The Maillard reaction is the main responsible of the generation of flavor and color in thermally processed foods (Ames, 1990). However, this reaction has been also linked to the acrylamide formation, specifically with the presence of carbonyl compounds with groups capable of forming a Schiff Base with the amino acid asparagine (Hidalgo et al. 2009; Mottram et al. 2002; Stadler et al. 2002). Acrylamide, a neurotoxic compound (Spencer & Schaumburg, 1974) and probable human carcinogen (IARC, 1994), has attracted the attention of the scientific community in recent years due to it has been found in high concentrations in thermally processed foods (Tareke et al. 2002).

The effect of water activity (a_w) on acrylamide formation has been widely studied. Depending on the water activity range studied, positive or negative correlations between water activity and acrylamide content have been observed. De Vleeschouwer et al. (2007) showed very slight promoting effect of water activity ($0.34 \leq a_w \geq 0.92$) on acrylamide amount in an asparagine–glucose model system. Similar results were reported by De Vleeschouwer et al. (2008) ($0.88 \leq a_w \geq 0.99$). The same tendency was observed in a potato model system ($0.11 \leq a_w \geq 0.97$) where the acrylamide content was rather dependent upon the moisture content than upon the water activity (Mestadgh et al. 2006). However, an opposite trend was found in aqueous glycerol asparagine model system ($0.33 \leq a_w \geq 0.71$) (Hedegaard et al. 2007) and plantain paste ($0.43 \leq a_w \geq 0.97$) (Bassama et al. 2011), revealing a decrease in acrylamide formation with increasing water activity.

The above-mentioned studies have in common that the food model showed a sorption isotherm typical of sugar-rich products (Mathlouthi, 2001) where a small variation in

moisture content corresponds to a large variation in a_w for water activities lower than 0.9. For water activities higher than 0.9, a steep increase in the corresponding moisture content can be observed (De Vleeschouwer et al. 2007). In contrast, most foods are a mixture of different compounds (for example, carbohydrate, protein, fat, minerals, vitamins, salts and others). Hence, real foods tend to have a sigmoidal moisture sorption isotherm due to the presence of solutes with very strong affinity for water that could maintain a larger amount of bound moisture at lower humidity than solutes with less affinity.

Water activity influences on the stability and chemical reaction rates in foods (Labuza et al. 1972). Thus the mobility of acrylamide precursors and as a consequence, the acrylamide formation is affected by the physical state of the water sorbed during thermal processing. In this context, it is expected that the final acrylamide content will be mainly affected by the moisture content (monolayer value) where strong bonds between the water (adsorbate) and the food (adsorbent) occur.

In an attempt to understand the influence of water activity of real foods on acrylamide content, the level of acrylamide was compared in tortilla chips prepared from tortilla equilibrated at different water activities, including that corresponding to the monolayer value. The monolayer was calculated from tortilla desorption isotherms by using both, GAB equation and the minimum integral entropy criteria.

2. Materials and methods

2.1 Materials

Labeled [2,2,3-$^2H_3$]acrylamide was purchased from Sigma–Aldrich (St. Louis, MO). All other chemicals were analytical grade and purchased from Sigma (St. Louis, MO) or Merck

(Darmstadt, Germany). Soybean oil was obtained from local supermarkets in Querétaro, México.

2.2. Elaboration of nixtamalized corn flour and tortilla chips.

Nixtamalized corn flour was prepared with commercial corn variety named Pioneer 30P16 and commercial lime ($Ca(OH)_2$) (El Topo, Monterrey, N.L. Mexico), commonly used in the tortilla industry. This flour was prepared by cooking (8 kg) of whole corn kernels in a solution of 16 liters of water with 80 g of $Ca(OH)_2$, corresponding to 1.0 g/100 g of lime relative to the corn weight used. The corn was boiled in an aluminum pan for 25 min and steeped for 16 h at room temperature (22 ± 1°C). The steep liquor was removed. The cooked corn was washed with 16 liters of water, then ground into corn dough (FUMASA, M100, Querétaro, México), and finally dehydrated using a flash type dryer (Cinvestav-AV, M2000, Querétaro, México). The drying conditions were adjusted to have 250°C inlet air temperature and 90°C to the exhaust air to avoid burning the material. Before storage, the nixtamalized corn flour was milled using a hammer mill (PULVEX 200, México DF) equipped with a 0.5 mm screen.

For tortilla chips elaboration, nixtamalized corn flour (100 g) was rehydrated with enough water (118 mL) to provide fresh dough with proper consistency to make tortillas. The dough was shaped into thin disks (11cm diameter and 1.0 mm thickness) using a commercial tortilla roll machine (Casa Herrera, México, D.F.). The dough shaped into tortillas were cooked on both sides for around 1.0 min by using an iron hot plate (270 ± 10 °C) named “comal”. The resulting tortillas were cut into circular pieces with an average area of 10 cm^2 and its a_w and pH value (AACC International, 2010**)** determined. Two set of

tortilla pieces were obtained. The first set was fried immediately in soybean oil at 180°C for 25 s (control). The second set was used in the construction of desorption isotherms. After frying, tortilla chips were cooled on a paper towel to remove superficial oil and the color, fracture force and acrylamide content determined.

2.3. Water desorption isotherms

Water desorption isotherms were determined using a static equilibrium method. Tortilla samples (~4.0 g) (a_w =0.98) were weighed in triplicate into standard weighing dishes with a circular section on the bottom. Samples were placed in separate desiccators containing saturated salt slurries in the range of water activity from 0.11 to 0.85 using the a_w values reported by Labuza et al. (1985). The samples were held at 25, 30, and 35 °C until equilibrium was reached. Values of water activity were generated using equations reported in the same paper. A small amount of toluene was placed in each desiccator to prevent the growth of fungi. The equilibrium condition was attained within 7-10 days, when the differences among two consecutive weights were within 0.001 g. The dry matter content of the tortilla was determined by the drying in a conventional oven at 105 °C for 24 h. The Guggenheim-Anderson-De Boer (GAB) equation was used in modeling water sorption (Quirijns et al. 2005):

$$M = \frac{M_0\ C\ k\ a_w}{(1 - ka_w)(1 - ka_w + Cka_w)} \qquad (1)$$

Where a_w is water activity; M is water content of the sample on dry basis; M_0 is the monolayer water content; C is the Guggenheim constant, given by $C= c'exp\ (h_m-h_n)/RT$; where c' is the equation constant; h_m is the heat of sorption of the first layer; h_n is the heat of sorption of the multilayer; R is the gas constant; T is the absolute temperature; and k is

124 the constant correcting properties of multilayer molecules with respect to bulk liquid, and
125 given by $k = k' \exp (h_1 - h_n)/RT$; where k' is the equation constant; h_1 is the heat of
126 condensation of pure water. The parameters values of GAB equation (M_0, C and k) were
127 estimated by fitting the mathematical model to the experimental data, using non-linear
128 regression with the Kaleidagraph 4.0 package (Synergy Software, Perkiomen, USA).
129 Goodness of fit was evaluated using the average of the relative percentage difference
130 between the experimental and predicted values of the moisture content or mean relative
131 deviation modulus (P) defined by the following equation:

$$\mathrm{P}(\%) = \frac{100}{\mathrm{N}} \sum_{\mathrm{i}=1}^{\mathrm{N}} \frac{|\mathrm{Me_i} - \mathrm{Mc_i}|}{\mathrm{Me_i}} \qquad (2)$$

132 Where Me_i is the moisture content at observation i; Mc_i is the predicted moisture content at
133 that observations; and N is the number of observations. It is generally assumed that a good
134 fit is obtained when $P < 10\%$ (Lomauro et al. 1985).

135 *2.4 Determination of minimum desorption integral entropy*

136 The determination of the integral (enthalpy and entropy) thermodynamic properties, and
137 the water activity-temperature conditions where the tortilla minimum integral entropy
138 occurred, considered as the point of maximum storage stability (monolayer value), was
139 established as indicated by Pascual- Pineda et al. (2013) and Vigano et al. (2012). These
140 authors have provided a thorough description of the procedure followed and equations used
141 for this purpose. Briefly, the integral enthalpy changes $(\Delta H_{int})_T$ (J/mol) at the water-tortilla
142 interface and, at different stages of the adsorption process, were determined using the
143 equation of Othmer (Othmer, 1940).

$$\frac{\mathrm{dlnP_v}}{\mathrm{dlnP_v^0}} = \frac{\mathrm{H_v(T)}}{\mathrm{H_v^o(T)}} \qquad (3)$$

Where: the desorbed substance is water; P_v(Pa) is the vapor pressure of water over the adsorbent; P_v^0 (Pa) is the vapor pressure of pure water at the temperature of sorption; $H_v(T)$ (J/mol) is the integral molar heat of sorption, and $H_v^0(T)$ (J/mol) is the heat of condensation of pure water. Since all these terms are temperature-dependent, the equation can be integrated:

$$lnP_v = \left[\frac{H_v(T)}{H_v^o(T)}\right]_\phi ln\, P_v^0 + A \qquad (4)$$

Where: A is the adsorption constant, and Φ (J/mol) is the pressure of diffusion or surface potential. A plot of lnP_v versus $ln\, P_v^0$ gives a straight line if the ratio $H_v(T)/H_v^0(T)$ is constant within the range of temperatures used.

The molar integral enthalpy $(\Delta H_{int})_T$ can be calculated using equation (5), at a constant pressure of diffusion(Nunes & Ronstein, 1991):

$$(\Delta H_{int})_T = \left[\frac{H_v(T)}{H_v^o(T)} - 1\right]_\phi H_v^o(T) \qquad (5)$$

$$\phi = \mu_{ap-}\,\mu_a = RT\frac{W_{ap}}{W_v}\int_0^{a_w} Md\ lna_w \qquad (6)$$

Where: μ_{ap} (J/mol) is the chemical potential of the pure adsorbent; μ_a (J/mol) is the chemical potential of the adsorbent participating on the condensed phase; W_{ap} (g/mol) is the molecular weight of the adsorbent, and W_v (g/mol) is the molecular weight of the water.

158 By calculating $H_v(T)/H_v^0(T)$ from equation (4) and substituting it into equation (5) it
159 becomes possible to calculate the integral enthalpy at different temperatures, provided that
160 a good means of estimating $H_v^o(T)$ is available, such as that proposed by Wexler, (1976):

161 $H_v^0(T)$ J/mol-K = 6.15 x 104 – 94.14 T + 17.74 x 10-2T2 – 2.03 x 10-4T^3 (7)

162 Using the values obtained for $(\Delta H_{int})_T$ changes, the molar integral entropy $(\Delta S_{int})_T$ can be
163 estimated using the following equation :

$$(\Delta S_{int})_T = S_1 - S_L = -\frac{(\Delta H_{int})_T}{T} - R\ln a_w \qquad (8)$$

164 where: $S_1 = S/N_1$ (J/mol K) is the integral entropy of water desorbed in the foodstuff; S
165 (J/mol K) is the total entropy of water desorbed in the foodstuff; N_1 is the moles of water
166 desorbed in the foodstuff, and S_L (J/mol K) is the molar entropy of pure liquid water in
167 equilibrium with vapor.

168 *2.5. Storage of tortilla before frying*

169 Since water retention capacity in tortillas is poor, the moisture loss in tortilla after
170 processing is often caused by starch retrogradation. Taking advantage of this phenomenon,
171 tortilla samples were left at room temperature (25 °C) during 4 h to reduce their a_w from
172 0.98 to 0.85 and therefore, reducing the moisture content and preventing spoilage reactions
173 during the time that the samples reached the desired a_w. The tortilla pH was monitored in
174 order to confirm that acrylamide levels were not affected by pH changes during storage.
175 Duplicate samples containing ca. 5 g of tortilla were placed in desiccators containing the
176 same saturated salt slurries referred in section 2.3 at 30°C. Tortilla samples were withdrawn
177 at day 5 to prepare tortilla chips.

178 *2.6. Color determination in tortilla chip samples*

179 Color changes were determined using a colorimeter MiniScan XE, model 45/0-L (Hunter
180 Associates Laboratory, 11491 Sunset Hill Rd., Reston, Va., U.S.A.). Total color differences
181 (ΔE) at the different periods of time were calculated from the determined CIELAB L^* a^*
182 b^* values according to Hunter (1973): $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$; where L^* =
183 brightness or lightness (100 = perfect white, to 0 = black); a^* = greenness/redness [negative
184 (green) to positive (red)]; b^* = yellowness/ blueness [negative (blue) to positive (yellow)];
185 ΔL^*, Δa^*, and Δb^* = absolute differences of the values between the reference tile (white
186 porcelain) and sample values; ΔE = total difference between reference and sample color.
187 The reference values (calibration) were: L^* = 92.22, a^* = -0.82 and b^* = 0.62.

188 *2.7. Texture determination in tortilla chips*

189 The fracture force of the tortilla chips was evaluated using the Texture Analyzer TA-XT2
190 (Texture Technologies Corp., N. Y.). Fracture force was evaluated in freshly prepared
191 samples. The test was carried out using a 2.03 mm diameter stainless-steel probe and a
192 platform accessory with a hollow cylindrical base with 33.5 and 10 mm external and
193 internal diameters, respectively. The probe traveled at a velocity of 10 mm/s until it cracked
194 the sample (distance 6 mm).

195 *2.8. Acrylamide determination in tortilla chips*

196 Acrylamide was analyzed as the stable 2-bromopropenamide derivative by gas
197 chromatography–mass spectrometry (GC–MS) using a combination of the methods of
198 Andrawes et al. (1987) and Castle et al. (1991) as described previously (Salazar et al.
199 2012). Tortilla chips were ground in a mortar lab and powdered samples (~0.8 g) were

successively weighed in centrifugal tubes, spiked with 20 µL of internal standard solution (0.5 mg/mL of deuterium-labeled [2,2,3-$^{2}H_3$]acrylamide in acetonitrile), and stirred with 8 mL of distilled water and 10 mL of *n*-hexane at room temperature for 5 min. After centrifugation at 2000 x *g* for 10 min, organic phases were removed. Co-extractives from supernatants were precipitated with 30 µL of carrez I and 30 µL of carrez II solutions. Later, supernatants were centrifuged at 2000 x *g* for 5 min and filtered. These extracts (4 mL) were treated with 0.45 g of potassium bromide, 200 µL of sulfuric acid (10 mL/100 mL), and 300 µL of potassium bromate solution (0.1 mol/L). After 1 h in the dark at 4°C, the bromination reaction was terminated by adding of 1 mol/L sodium thiosulfate until solutions became colorless, and solutions were extracted with 5 mL of ethyl acetate/hexane (4:1). Organic layers were recovered after centrifugation at 2000 x g for 10 min, and were dried with sodium sulfate and evaporated to dryness under nitrogen. Each sample was dissolved in 50 µL of ethyl acetate, treated with 25 µL of triethylamine, and analyzed by GC–MS. The ions monitored for the identification of the analyte, 2- bromopropenamide, were $[C_3H_4NO]^+$ = 70, $[C_3H_4{}^{79}BrNO]^+$ = 149, and $[C_3H_4{}^{81}BrNO]^+$ = 151, using *m/z* 149 for quantification. The ions monitored for the identification of the corresponding derivative 2-bromo[$^{2}H_2$]propenamide were $[C_2{}^{2}H_2H^{81}Br]^+$ = 110 and $[C_3{}^{2}H_2H_2{}^{81}BrNO]^+$ = 153, using *m/z* 153 for quantification.

GC–MS analyses were conducted with a Perkin Elmer GC Clarus 500 coupled with a Perkin Elmer Clarus 560 MSD (Mass Selective Detector-Quadrupole type). In most experiments, a 30 m × 0.32 mm i.d. × 0.25 µm Elite-5MS capillary column was used. Working conditions were as follows: carrier gas helium (1 mL/min at constant flow);

injector, 250°C; oven temperature: from 50 (10 min) to 240°C at 5°C/min and then to 300°C at 10°C/min; transfer line to MSD, 280°C; ionization EI, 70 eV.

Quantification of acrylamide was carried out by preparing standard curves of this compound. Acrylamide content was directly proportional to the acrylamide/internal standard area ratio (r = 0.999, p < 0.0001). The coefficients of variation at the different concentrations were lower than 10%.

2.9. Statistical analysis

All results were expressed as mean± SD values (n=3). When significant F values were obtained, group differences were evaluated by the Tukey test. All statistical procedures were carried out using the JMP 9.0 package (SAS Institute Inc., Cary, NC). The significance level was $p < 0.05$ unless otherwise indicated.

3. Results and discussion

Figure 1 shows the desorption isotherms of the tortilla used to make tortilla chips at 25, 30 and 35 °C. Sigmoidal isotherms described as type by Brunauer et al. (1940) were determined. Since desorption is an endothermic process, the increment of the temperature at constant a_w enhances the moisture desorbed from the tortilla. Similar desorption isotherms have been reported for starchy products such as potato (McLaughlin & Magee, 1998), maize (Samapundo et al. 2006) and banana (Yan et al. 2008), chestnut flour (Chenlo et al. 2011) and Japanese noodle (Inazu et al. 2001). **Table 1** gives the estimated parameters obtained by fitting the GAB equation to experimental data. As can be seen from the table, the GAB equation describes adequately the experimental data over the whole measured range of a_w. Thus the values of r^2 were very close to unit and the P value was ≤ 10 % under

244 the studied conditions, which it indicates a good fit (Lomauro et al. 1985). As expected,
245 maximum water desorption by strongly binding sites (monolayer) predicted for GAB
246 equation decreased with increasing temperature. The moisture content in the monolayer
247 (*Mo*) was 10.12, 9.46 and 8.96 g water/100 g dry solid (corresponding to a_w of 0.32, 0.31
248 and 0.30) for 25, 30 and 35 °C, respectively. It is assumed that the value of *Mo* is a
249 parameter for estimating the amount of water bound to specific polar sites in dehydrated
250 food and in this water content; a storage product should be stable against degradation
251 reactions (Rahman & Labuza, 1999). The constant *C* is a measure of the attraction force
252 between the water and the sorption sites. The values obtained are higher than those reported
253 by Polou et al. (1997) in commercial tortilla chips. In this study, the *k* values increased with
254 increasing temperature. The value of *k* provides a measure of the interaction of the
255 molecules in the multilayers with the adsorbent, and tends to fall between the energy value
256 of the molecules in the monolayer and the liquid water. When $k = 1$, the properties of the
257 water present in the multilayer are similar to those of the free water (Perez-Alonso et al.
258 2006).

259 Variations in the differential and integral entropy with respect to a_w of the tortilla used to
260 make tortilla chips are represented in **Figure 2**. The intersection of the curves is found in
261 the water content and a_w corresponding to the minimum integral entropy. It could be
262 observed that as tortilla desorbed moisture, their integral entropy fell to a minimum.
263 Integral entropy can be directly related to the order-disorder of water molecules sorbed on
264 food, and therefore it is a useful function to study the effect of drying method on the
265 stability of the product. The minimum integral entropy is expected where strong bonds
266 between the adsorbate and the adsorbent are (Nunes & Rotstein, 1991), and hence water

molecules are less available to participate in spoilage reactions. This point has been proposed as the most suitable for storage and it is considered as the water content corresponding to the monolayer (Dominguez et al. 2007; Hill et al. 1951).

Although the value of minimum entropy may be unique, there are food products with zones in which this minimum does not vary appreciably in a defined range of moisture. As it can be observed in **Figure 2**, the minimum integral entropy at 30 °C for tortilla corresponds to a_w value of 0.53 (13.20 g water/100g dry solids). It should be noted that although the minimum integral entropy occurs in a given a_w, this minimum does not vary appreciably in a defined range of water activities (0.45 to 0.62). Differential entropy had a minimum at 7.3 g water/100 g dry solids (a_w=0.18). This parameter does not mean order or disorder of the total system. The differential entropy represents the algebraic sum of the integral entropy at a particular hydration level, plus the change of order or disorder after new water molecules were desorbed by the system at the same hydration level. If the values of moisture content corresponding to minimum integral entropy and minimum differential entropy are different, this particular hydration level at the minimum differential entropy cannot be considered as the maximum stability point, because not all available active sites have been occupied at that particular water content, and therefore it is possible to obtain after this point lower differential changes that provide a better ordering of the water molecules desorbed on food (Beristain et al. 2002).

The minimum integral entropy approach has been verified in several studies (see for example: Beristain et al. 1994; Bonilla et al. 2010; Carrillo -Navas et al. 2011; Dominguez et al. 2007; Rascon et al. 2011).

On the other hand, frequently the thermodynamic analysis is not in accordance with the monolayer obtained with the GAB model (Beristain & Azuara, 1990; Beristain et al. 2002; Nunes & Rotstein, 1991). In this study, the minimum integral entropy point is higher than the calculated *Mo* value with GAB equation. Since the BET theory, which assumes that the surface of the adsorbent is energetically uniform and does not take into account horizontal interactions between the molecules in the adsorbed layer (Dollimore et al. 1976), is also present in the GAB equation, the GAB monolayer concept has a limited applicability.

In this context, the Maillard reaction (main pathway acrylamide formation pathway) is a reaction that requires the interaction of two reagents. Therefore, those factors which affect the concentration and mobility of the reactants will have a significant impact on the reaction rate (Bell, 2007). Thus, the plasticizing effect of water significantly influences on the rate of the Maillard reaction (Bell et al. 1998; White & Bell, 1999). In the minimum integral entropy point, water is less reactive to carry out spoilage reactions in the food, so the mobility of acrylamide precursors is reduced, affecting it formation during thermal processing.

Figure 3 shows acrylamide content in tortilla chips prepared from tortillas stored at different water activities. All water activities induced the formation of acrylamide in the tortilla chips, but tortilla chips made of no-stored tortilla (a_w=0.98) always contained less acrylamide (688.55±65 μg/kg) than those made of tortilla stored at different water activities ($p < 0.05$). This suggests a dilution of acrylamide precursors caused by high moisture content. This effect is similar to that reported for the Maillard reaction in which a reduction in reaction rate is observed in a_w close to 1 because of the reagent dilution as well as the inhibitory effect that water exerts in this reaction (Ames, 1990; Acevedo et al. 2008). Thus,

312 a reduction in the acrylamide content was observed in the minimum integral entropy point.
313 The acrylamide content of tortilla chips prepared from tortilla stored at a_w=0.53 (741.85±88
314 μg/kg) was similar to those prepared from no-stored tortilla ($p < 0.05$). This point is
315 characterized by strong water-food interactions causing a reduction in the diffusion and
316 mobility of the system and affecting to the development of chemical reactions during frying
317 such as the acrylamide formation. On the other hand, an increase on acrylamide content on
318 tortilla chips proportional to reduction of a_w (0.43-0.11) on tortilla was observed. This
319 increase may be linked to the concentration of the precursors as well as the evaporation of
320 water which takes place during the start of frying. At this stage, the temperature of the
321 tortilla does not exceed 100 ˚C until a certain amount of water is evaporated. Since
322 acrylamide is not formed at temperatures below 100 ˚C, an increase on acrylamide
323 formation is observed when frying is carried out at low a_w where a rapid increase of the
324 internal temperature of the food occurs (Mestdagh et al. 2006).

325 Foods are heterogeneous and dynamic mixtures of macromolecules, solvent and solutes,
326 therefore, the positive or negative correlation between water activity and acrylamide
327 content are probably due to differences in composition and structure of used food systems.
328 According to Viveros- Contreras et al. (2013) and Vigano et al. (2011), food with the same
329 chemical composition and different microstructure could show different moisture sorption
330 behavior, and therefore the a_w where spoilage reactions occurs, could be modified . This
331 fact suggests that the microstructure and physical structure of the water in the food is able
332 to restrict the mobility of the precursors responsible for the formation of acrylamide to a
333 specific humidity level during thermal processing and it explains the decrease of the
334 acrylamide content of tortilla elaborated from tortillas equilibrated at the a_w corresponding

335 to minimum integral entropy zone. Thus, the influence of a_w on the acrylamide formation
336 will be different depending on each particular food system.

337 Texture and color are considered the most important parameters of quality and acceptability
338 of fried products. **Figure 4** shows the effect of tortilla water activity on the parameters of
339 tortilla chips above mentioned. The color has been correlated with the acrylamide
340 generation in thermally processed foods (Gökmen & Şenyuva, 2006; Lukac et al. 2007;
341 Majcher & Jelen, 2007; Pedreschi et al. 2007). In this study, the appearance of the tortilla
342 chips (**Figure 4a**) remained constant in the range of a_w from 0.43 to 0.84 and increased
343 proportionally to a_w reduction (0.43-0.11). It should be emphasized that this change was
344 observed in the water activity zone corresponding to the minimum integral entropy. The
345 fracture force (**Figure 4b**) showed similar values over the whole range of water activities
346 studied. The obtained fracture force values are close to those found (8.73–9.63 N) by Tseng
347 et al. (1996), and higher than those (5.33–6.17 N) described in commercial tortilla chips
348 (Lujan-Acosta & Moreira, 1997).

349 **4. Conclusions**

350 The effect of a_w on the acrylamide content of tortilla chips could be related to the moisture
351 sorption behavior. The minimum integral entropy in conjunction with the corresponding a_w
352 obtained from the desorption isotherm provided a reliable indicator to establish the most
353 appropriate moisture conditions to obtain tortilla chips with reduced level of acrylamide
354 when tortilla was dehydrated. Furthermore, the acrylamide content reduction was similar
355 when tortilla chips were prepared from non-stored tortilla with a moisture content
356 corresponding to water activities close to 1. It should be noted that a partial tortilla

dehydration to a water activity different to the minimum integral entropy results in tortilla chips with higher acrylamide content. Although a detailed sensory evaluation of tortilla chips prepared is needed, preliminary results showed that the products obtained from tortilla stored in the minimum integral entropy a_w did not show any significant change in the appearance of the food product. Therefore, the results suggest that the control of a_w, moisture content and the physical state of water in the tortillas are factors to reduce the formation of acrylamide during processing of tortilla chips. This is of the upmost importance, because it provides the food manufacturer with information of which a_w is more likely to get a low acrylamide content product over a wider range of processing conditions.

Acknowledgments

We are indebted to Juan Veles, Edmundo Gutierrez, Carlos Alberto Ávila, Araceli Mauricio and Veronica Flores from CINVESTAV Querétaro for their technical assistance.

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Table 1

Estimated GAB parameters for tortilla desorption isotherms

	25°C	**30°C**	**35°C**
***M*o (g H_2O/100 g d.s.)**	10.11	9.46	8.96
C	14.67	15.72	14.26
k	0.66	0.67	0.70
r^2	0.99	0.99	0.99
P (%)	0.96	1.36	1.58

Figure captions

Figure 1. Moisture desorption isotherms of tortilla used to make tortilla chips at 25 (●), 30 (□) and 35 (△) °C. The continuous lines are the fitted points using the GAB model.

Figure 2. Differential (■) and integral (○) entropy changes as a function of water activity for tortilla at 30 °C.

Figure 3. Effect of tortilla water activity on acrylamide content in tortilla chips fried at 180°C for 25 s.

Figure 4. Effect of tortilla water activity on: color (**a**) and fracture force (**b**) in tortilla chips fried at 180°C for 25 s.

Figure 1

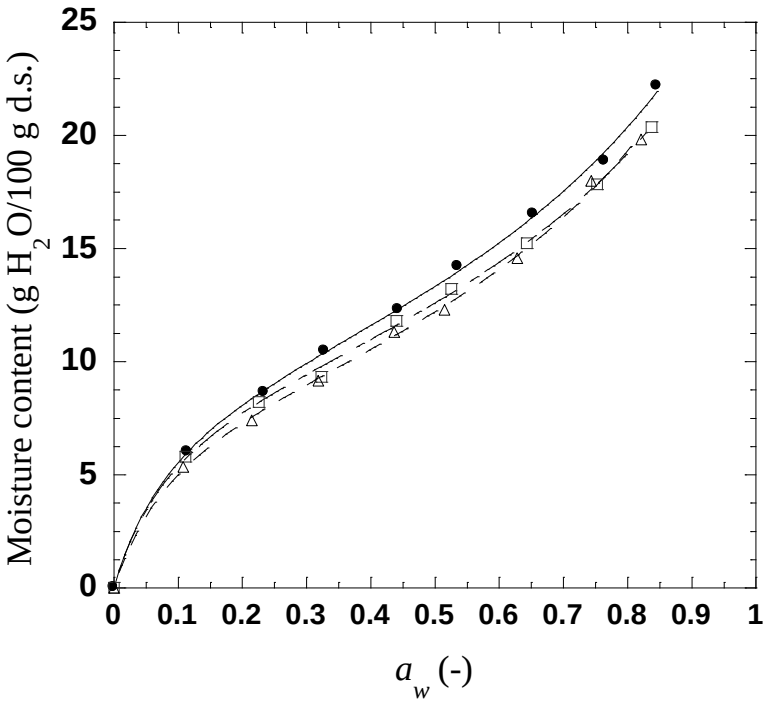

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Figure 2

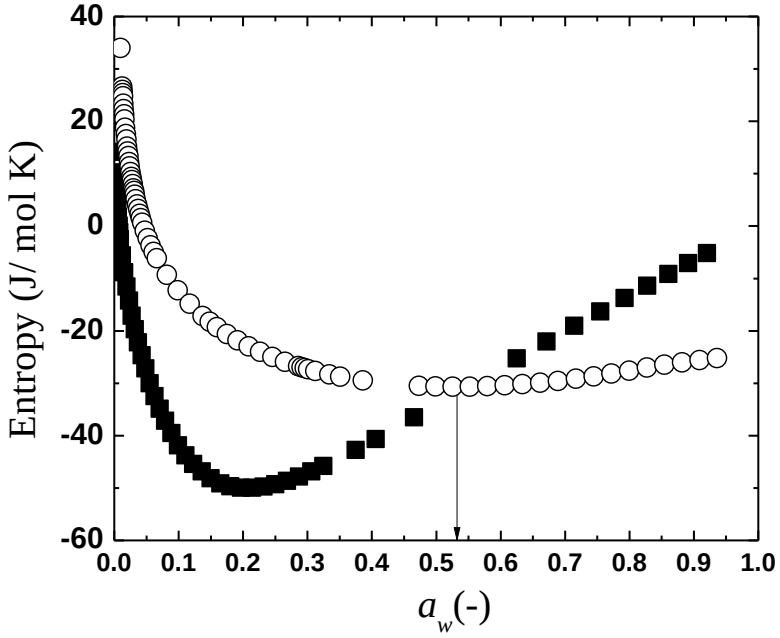

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Figure 3

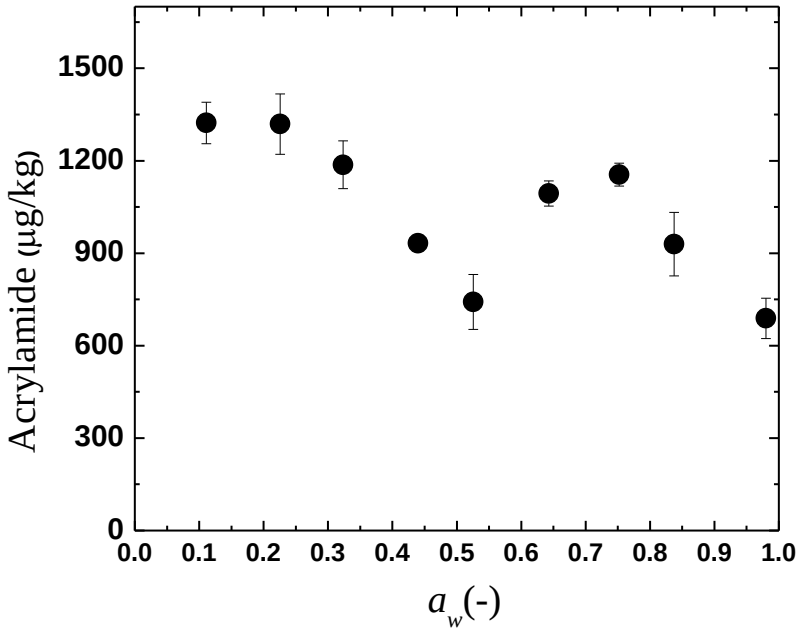

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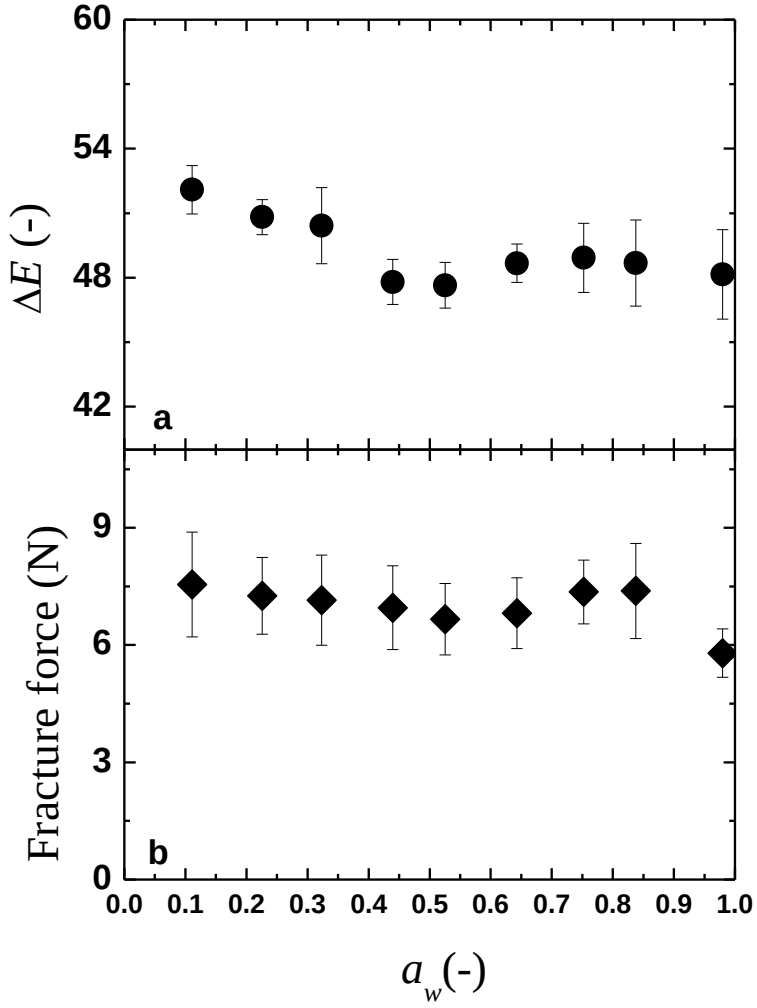
Figure 4



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