Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 421–425
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

The formation of potentially harmful compounds in *churros,* a Spanish fried-dough pastry, as influenced by deep frying conditions

F.J. Morales *, G. Arribas-Lorenzo

Consejo Superior de Investigaciones Científicas, Instituto del Frío, José Antonio Novais 10, 28040 Madrid, Spain

Received 9 August 2007; received in revised form 19 November 2007; accepted 18 December 2007

Abstract

Colour, moisture, hydroxymethylfurfural (HMF) and acrylamide (AA) were investigated in traditional Spanish *churros*. Samples were deep-fried in sunflower oil at lab-scale temperatures of 180, 190 and 200 °C and for frying times of 2, 3, 5 and 7 min. Fresh made *churros* were also obtained from local producers. HMF ranged from 1.2 ± 0.02 to 221.4 ± 2.02 mg/kg for lab-scale experiments and an average of 74.3 ± 47.5 mg/kg was recorded in commercial samples. AA ranged from below the limit of quantitation to 90 ± 0.6 μg/kg for lab-scale experiments and an average of 46 ± 24.5 μg/kg was measured in commercial samples. Temperatures between 185 and 200 °C are commonly used to obtain *churros* with an acceptable palatability and a crispy surface. However, HMF and AA levels increased nearly two-fold from 190 to 200 °C at the same frying times, indicating that a more precise control of frying temperatures is required to minimize their formation.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Maillard reaction; Fried dough; Hydroxymethylfurfural; Acrylamide; Colour; *Churro*

1. Introduction

Churro is described as a deep-fried dough pastry which is very popular in Spain for breakfasts and snacks. The dough is prepared with wheat flour, water and salt, extruded and deep-fried as strips using sunflower oil as frying oil. Frying temperatures between 185 and 200 °C and frying times of 3–4 min are usually applied to obtain a crispy product. But the end point of this process, when fried-dough portions are removed manually from the fryers is not automatised. Generally, colour and crust formation are used as end point markers of the process but there is no specific information in any literature which discusses this fact.

The Maillard reaction (MR) is a complex chain of chemical reactions responsible for the brown colouring and many of the organoleptic properties of foods. Despite the beneficial impact of MR in the sensory appreciation of foodstuffs by consumers, nutritional consequences such as the impairment of lysine residues and the formation of potentially harmful compounds are to be expected (i.e. Friedman, 1996). The Maillard reaction has been shown to generate AA in thermally treated foods (Mottram, Wedicha, & Dodson, 2002; Stadler et al., 2002) and it is agreed that the main precursors are sugars and asparagine (Zyzak et al., 2003). AA has been classified as potentially carcinogenic to humans by IARC (1994). Hydroxymethylfurfural (HMF) is formed after 1–2 enolisation, dehydration and cyclization reactions from sugar caramelisation and Amadori product degradation at the advanced stage of the MR (i.e. Friedman, 1996; Kroh, 1994). Recently, some concern has arisen regarding the toxicological relevance of HMF, since in vitro studies of genotoxicity and mutagenicity have given controversial results (Janzowski, Glaab, Samimi, Schlatter, & Eisenbrand, 2000; Surh & Tannenbaum, 1994).

International organisations aim to evaluate exposure and risk assessment from dietary intake of AA, and two databases, one from the IRMM and another from the FDA, have been designed to compile results from laboratories. However, levels of AA in local foods which are largely

* Corresponding author. Tel.: +34 91 549 23 00; fax: +34 91 549 36 27.
E-mail address: fjmorales@if.csic.es (F.J. Morales).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.12.042

consumed in specific regions are lacking in most databases. Recently, Bermudo, Moyano, Puignou, and Galceran (2006) and Yusa, Quintas, Pardo, Marti, and Pastor (2006) reported average levels of 400 μg/kg for one fried dough but the number of samples included in those studies was limited and not characterised.

In contrast, levels of HMF in different foods have been extensively studied and published for decades. HMF has been used traditionally to mark the extent of the MR, product deterioration during storage, and as a marker of potential adulteration. HMF is largely present in processed foods ranging from 0.9 mg/kg to several g/kg for meat products and caramel products, respectively (Bachmann, Meier, & Känzig, 1977). However, HMF levels in *churros* have never been published, and only Ramirez-Jimenez, Garcia-Villanova, and Guerra-Hernandez (2000) reported levels of about 10.7 mg/kg HMF in a type of fried doughnut (160 °C for 30–40 s).

This investigation is aimed at studying the formation of AA and HMF in *churros*, on a laboratory scale under controlled deep frying conditions.

2. Materials and methods

2.1. Chemicals

All chemical used were of an analytical grade and were obtained from Sigma–Aldrich (St. Louis, MO, USA), unless mentioned otherwise. $^{13}C_3$-Acrylamide (isotopic purity 99%) was from Cambridge Isotope Labs (Andover, MA, USA). Methanol and acetonitrile (HPLC grade) were from Scharlau (Barcelona, Spain). Oasis-HLB cartridges (30 mg, 1 mL) were from Waters (Milford, MA, USA).

2.2. Samples and frying conditions

Fresh made commercial *churros* were randomly purchased at local producers ($n = 10$). For kinetic studies under controlled frying conditions, frozen commercial ready-to use dough for *churros* was obtained from a local producer (Antonio y Ricardo, S.A., Madrid, Spain) and stored at −20 °C. The dough was allowed to defrost overnight at 8–12 °C before use. Dough strips 1.2 cm thick were rolled out to form a ribbon 10–11 cm length and 5.5–6.0 cm wide, and deep-fried at 180, 190 and 200 °C for 2, 3, 5 and 7 min in 3 l capacity domestic fryers (Solac, Model 513, 1850 watts, France). Experiments were done in triplicate. Four units of *churros* (containing 20–23 g of dough per unit) were placed in the basket to allow free movement but never below the oil surface during frying. The sample was turned every minute during the frying time. The sample:oil ratio was kept constant within a range of 0.025–0.030 g/g. High oleic content sunflower oil was used as frying oil as this oil is commonly used on an industrial scale. The temperature of the fryers had been calibrated previously, with external thermocouples (type K, 0.1 mm) being placed in the fryers and the temperature being continuously recorded by a datalogger. The oil was preheated at the selected temperature for 15 min and after eighteen frying sessions the oil was discharged. After frying, samples were dried on paper, cooled to room temperature, processed, distributed in containers and stored at −20 °C for analysis.

2.3. Moisture content

The moisture content was determined by a gravimetric method as described in AOAC-925.10 (AOAC, 1995). Samples were homogenized in a household cutter (Moulinette, Moulinex, Paris, France) and 2 g weighed into Chopin dishes. They were dried in a convection oven (Digiheat, JP-Selecta, Barcelona, Spain) till they reached a constant weight after three consecutive readings at 105 ± 1 °C. Analyses were carried out in duplicate.

2.4. Analysis of HMF and furfural

HMF and furfural determinations were performed according to Rufián-Henares, Delgado-Andrade, and Morales (2006).

2.5. LC-ESI-MS determination of acrylamide

A sample preparation as described by Şenyuva and Gökmen (2006) was used with minor modifications. A finely ground sample (0.450 g) was then weighed into a 10 mL centrifuge tube. The sample was spiked with 100 μL $^{13}C_3$-labelled acrylamide (10 μg/mL) for recovery, and 5 mL of Milli-Q water was added. Then the mixture was vortexed, kept for five minutes at room temperature and subsequently homogenized for 1 min (Ultraturrax). Then, 500 μL Carrez I and 500 μL Carrez II solution were added, vortexed and left to stand for 10 min. Tubes were centrifuged at 5000 rpm for 15 min at 4 °C. The clear supernatant (1 mL) was clarified onto a pre-conditioned HLB cartridge. The first drops were discharged and the rest of the eluate was collected in amberlite vials for analysis.

LC-ESI-MS analyses were performed as described by Morales, Rufian-Henares, and Arribas-Lorenzo (2007) using an Agilent 1100 HPLC system (Waldbronn, Germany) consisting of a quaternary pump, an autosampler and a temperature-controlled column oven, coupled to an Agilent 1100 MS detector equipped with an electrospray ionization interface. The analytical separation was performed on an Inertsil ODS-3 column (250 × 4.6 mm, 5 μm) using an isocratic mixture of 0.2% aqueous solution of formic acid at a flow rate of 0.6 mL/min at 25 °C. Data acquisition was performed, with a delay time of 8 min, in a selected ion monitoring (SIM) mode using the following interface parameters: a drying gas (N2, 100 psig) flow of 12 L/min, nebulizer pressure of 45 psig, drying gas temperatures 350 °C, a capillary voltage of 3 kV and a fragmenter voltage of 70 eV. Monitored ions were 72.1 *m/z* for acrylamide and 75.1 *m/z* for $^{13}C_3$-labeled acrylamide. Full scan analyses were performed in the mass range 50–200 for the

spectral identification of acrylamide in samples apart from the retention time. An acrylamide calibration curve was built in the range of 2–100 μg/L and a quantitation limit was determined at 18 μg/kg.

2.6. Colour determination

Colour was measured as reflectance in a Konica Minolta CM-3500d reflectance spectrophotometer (Konika Minolta Sensing INC, Japan) using the CIE Lab colour system, where L^* is lightness, the a^* parameter is redness and the b^* parameter yellowness (CIE Colorimetric Committee, 1974). Samples (3 g) were defatted three times with hexane (6 mL). Colour was measured in defatted samples to avoid any interference with oil absorbed components on the *churro* surface. The colour difference (ΔE) between processed and unprocessed samples was determined according to the following equation:

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

ΔL^* being the brightness difference, Δa^* the redness difference and Δb^* the yellowness difference (Francis & Clydesdale, 1975). Results give the average from six readings.

2.7. Statistical Analysis

Analyses were performed using Statgraphics Plus, version 5.1, 2001 (Statistical Graphics Corp, Rockville, MD, USA). At least, two independent analyses were carried out per sample.

3. Results and discussion

Deep frying at 190 °C for 3–4 min is usually applied by local producers based on their practical experience since the process has not been automatised and still remains artisanal. Fig. 1 shows the changes in the moisture content of *churros* deep-fried at different combinations of temperatures (180, 190, and 200 °C) and time of frying (2, 3, 5 and 7 min). The loss of moisture is progressive relative to the frying time. When fried for the same length of time, the frying temperature was seen to have no significant impact on moisture content. However, lengthening the frying time proved critical regardless of the temperature. Moisture content ranged from 32% to 10–5% for 2 min and 7 min, respectively. During deep frying, the quantity of steaming water released from within the sample was determined by the frying conditions. Commercial *churros* are fried at high temperatures for a short period of time to avoid excessive oil penetration in the product and served a few minutes after frying to avoid subsequent hydration. Moisture content in freshly prepared industrial samples was 25.3 ± 4.8 ($n = 10$).

One of the most important physical changes during frying of *churros* is the darkening of the crust which is mainly a consequence of the MR. CIE-Lab* colour parameters were measured in defatted and ground portions of a sample to prevent a false reading produced by oil incorporated into the sample. In a cross-section, a brown surface ring of crust can be observed but the core still remains pale yellow and moist. The surface of the sample was neither uniform nor large enough to perform precise and representative readings. Furthermore, as it was not possible to remove the surface efficiently, the whole sample was ground and colour readings are related to the whole sample.

Fig. 2 depicts the evolution of the colour index for *churros* deep-fried at lab-scale. Contrary to what happened with moisture content, temperature has a more critical effect on the final colour than the processing time. Decrease in lightness is attributed to the formation of coloured pigments during the advanced and final stages of the MR in the crust and this process is enhanced at 200 °C. An average ΔE-value of 67.0 ± 2.8 ($n = 10$) was obtained in commercial samples. It is noteworthy that the coefficient of variation

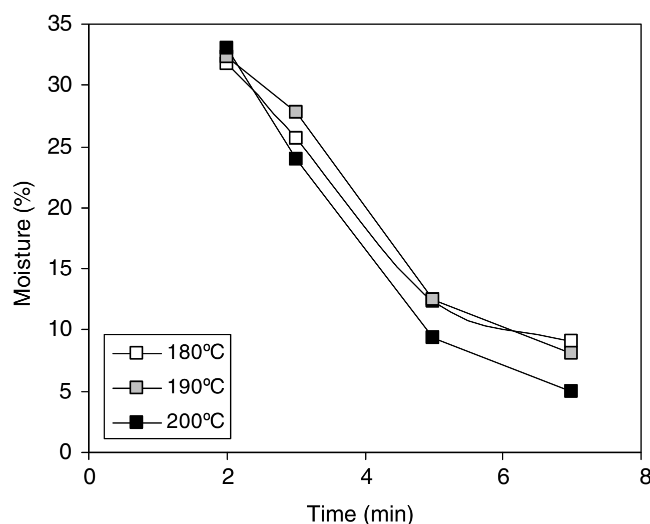

Fig. 1. Changes in the moisture content of *churros* during deep frying at different times and temperatures.

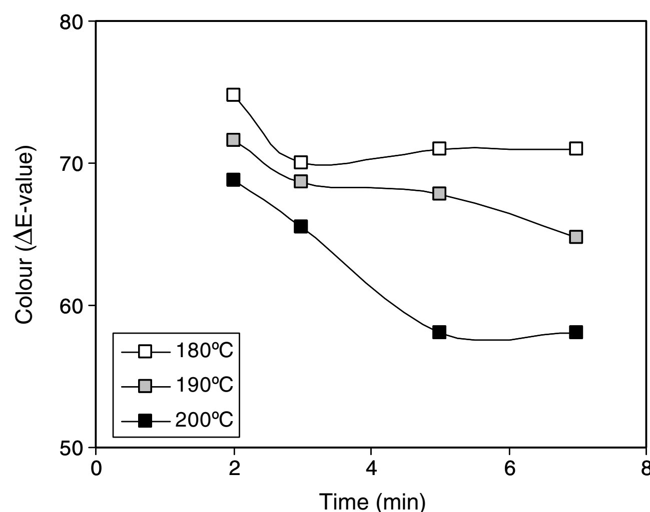

Fig. 2. Total colour changes (ΔE) in *churros* during deep frying at different times and temperature.

obtained was 4.1%, thus indicating that the final colour of the product is one of the most important parameters for defining the end of the frying process by local producers.

Accumulation of HMF was quantitatively more important when compared with furfural whose traces (<1 mg/kg) were barely detectable under severe frying conditions. Fig. 3 shows the evolution of HMF formation in *churros* during deep frying under controlled conditions. Before HMF formation, a lag-phase of up to 3 min can be clearly observed and is more relevant at lower temperatures. HMF marks the advanced stage of the MR and precursors should have formed previously. The average HMF content in commercial samples was 74.3 ± 47.5 mg/kg ($n = 10$) indicating an important variability among samples. Furfural was not detected in commercial samples. Results agree with the range of HMF obtained at laboratory scale. HMF formation was closely related to moisture content increasing exponentially at moisture levels lower than 10% (data not shown).

To our knowledge, there are no published results dealing with the AA content of *churros* and the influence of frying conditions. Fig. 4 plots the formation of AA in *churros* for different frying times and temperatures. AA only formed under the most severe frying conditions where an induction period of from 3 to 5 min, depending on the frying temperature, was observed. Frying temperature has a critical effect on the formation of AA. In the range of frying times commonly used by local producers, an increase of just 10 °C implies a two-fold increase in the levels of AA. For potato chips, a reference temperature of 175 °C has been calculated, but a higher temperature is necessary for *churros* in order to meet the product quality demanded by consumers. The average AA content in commercial samples was 46 ± 24.5 μg/kg ($n = 10$). In this investigation, potential AA formed from frying oil and incorporated into the sample was not considered since it has recently been confirmed that frying oil used in deep frying would not contaminate foodstuffs with AA (Totani, Yawata, Takada, & Moriya, 2007).

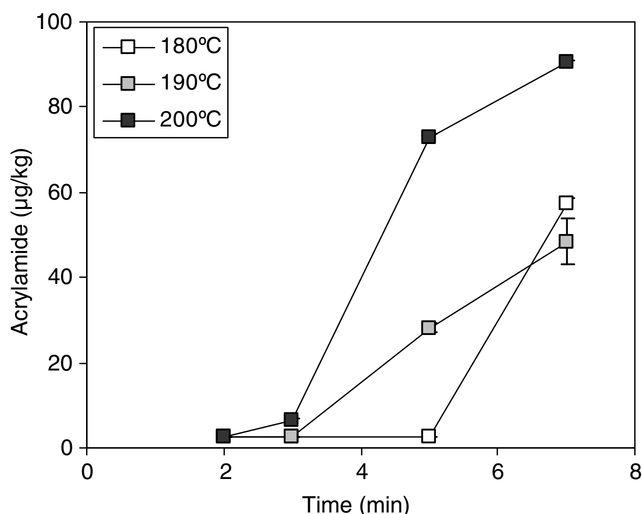

Fig. 4. Acrylamide formation in *churros* during deep frying at different times and temperatures.

HMF and AA content of deep-fried samples at lab-scale were correlated (Fig. 5). For both chemicals, an induction period was observed after which the formation of HMF and AA followed the same upward trend reaching values of about 90 mg/kg, and 60 μg/kg for HMF and AA, respectively. Optimal quality features for *churros* lie in this intermediate region of the graph. However, under more severe frying conditions, the two trends are modified and the reduced gradient of HMF formation is enhanced in comparison with that of acrylamide (Fig. 5, dotted line). This region consisted of over-processed *churros* where loss of colour quality by excessive darkening was noticeable. It is also plausible that evaporation loss or destruction of acrylamide at 200 °C becomes more relevant and subsequently the net formation of AA is reduced. Frying times of over 3 min at

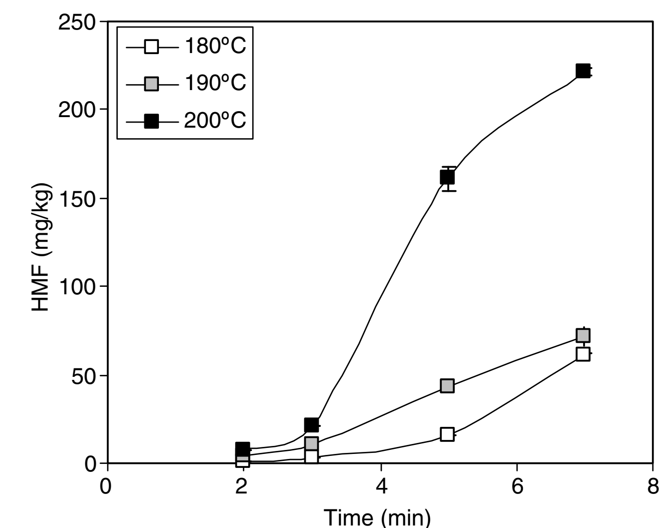

Fig. 3. HMF formation in *churros* during deep frying at different times and temperatures.

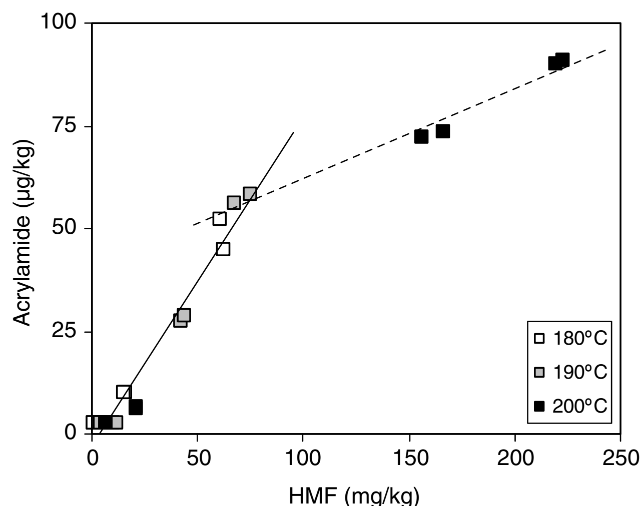

Fig. 5. The relationship between acrylamide and HMF in lab-scale samples deep-fried under controlled conditions.

200 °C cause excessive moisture loss and browning resulting in a loss of product quality.

Dehydration of the surface while maintaining a moist interior imparts a desirable taste and texture to *churros* but also involves MR and subsequently HMF and AA formation. Frying temperatures between 185 and 200 °C are commonly used to produce highly palatable *churros* with a crispy surface by local producers, but, HMF and AA levels nearly duplicate from 190 °C to 200 °C for the same frying times. It is critical to determine the end point of the frying process to minimise the formation of potentially harmful compounds in *churros*. Based on this experimental data, it should be possible to manufacture *churros* with reduced levels of AA and HMF by controlling the frying temperature and subsequently reducing their contribution to the dietary intake of the Spanish population.

Acknowledgements

This research was partly supported by a Scientific Research programme of the Comunidad de Madrid (ANALISYC Program, S-505/AGR-0312) and a European Collective Research Project (516415) supported by the Sixth Framework Programme for Research and Technological Development. G. Arribas-Lorenzo has a grant from the Consejería de Educación y Ciencia (Comunidad de Madrid). Ms. E. Ruiz and D. Gómez are thanked for their technical assistance.

References

Association of official analytical chemists (1995). *Official methods of the association of official analytical chemists* (16th ed.). Washington, DC: AOAC.

Bachmann, S., Meier, M., & Känzig, A. (1977). 5hydroymethyl-2-furfural (HMF) in Lebensmitteln. *Lebensmittelchemie, 51*, 49–50.

Bermudo, E., Moyano, E., Puignou, L., & Galceran, M. T. (2006). Determination of acrylamide in foodstuffs by liquid chromatography ion-trap tandem mass-spectrometry using an improved clean-up procedure. *Analytica Chimica Acta, 559*, 207–214.

CIE Colorimetric Committee (1974). Technical notes: Working program on colour differences. *Journal of the Optical Society of America, 64*, 896–897.

Francis, F. J., & Clydesdale, F. H. (1975). *Food colorimetry theory and applications*. Wesport, CT: AVI (pp. 131–224).

Friedman, M. (1996). Food Browning and its prevention: An overview. *Journal of Agricultural and Food Chemistry, 44*, 631–653.

IARC (1994). *IARC Monographs on the evaluation of carcinogenic risks to humans some industrial chemicals* (vol. 60). Lyon: International Agency for Research on Cancer (pp. 389–433).

Janzowski, C., Glaab, V., Samimi, E., Schlatter, J., & Eisenbrand, G. (2000). 5 Hydroxymethylfurfural: Assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food Chemistry and Toxicology, 38*, 801–809.

Kroh, L. W. (1994). Caramelisation of food and beverages. *Food Chemistry, 45*, 1570–1573.

Morales, F. J., Rufian-Henares, J. A., & Arribas-Lorenzo, G. (2007). Acrylamide content of selected Spanish foods: Survey of biscuits and bread derivatives. *Food Additives and Contaminants, 24*, 343–350.

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

Ramirez-Jimenez, A., Garcia-Villanova, B., & Guerra-Hernandez, E. (2000). Hydroxymethylfurfural and methylfurfural content of selected bakery products. *Food Research International, 33*, 833–838.

Rufián-Henares, J. A., Delgado-Andrade, C., & Morales, F. J. (2006). Application of a fast HPLC method for simultaneously determination of furanic compounds and glucosylisomaltol in breakfast cereals. *Journal of AOAC International, 89*, 161–165.

Şenyuva, H. Z., & Gökmen, V. (2006). Interference-free determination of acrylamide in potato and cereal-based foods by a laboratory validated liquid chromatography–mass spectrometry method. *Food Chemistry, 97*, 539–545.

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

Surh, Y., & Tannenbaum, S. (1994). Activation of the Maillard reaction product 5-(hydroximethyl)furfural to strong mutagens via allylic sulfonation and chlorination. *Chemical Research Toxicology, 7*, 313–318.

Totani, N., Yawata, M., Takada, M., & Moriya, M. (2007). Acrylamide content of commercial frying oil. *Journal of Oleo Science, 56*, 103–106.

Yusa, V., Quintas, G., Pardo, O., Marti, P., & Pastor, A. (2006). Determination of acrylamide in foods by pressurized fluid extraction and liquid chromatography-tandem mass spectrometry used for a survey of Spanish cereal-based foods. *Food Additives and Contaminants, 23*, 237–244.

Zyzak, D. V., Sanders, R. A., Stojanovich, M., Tallmadge, D. H., Eberhart, B. L., & Ewald, D. K. (2003). Acrylamide formation mechanism in heated foods. *Journal of Agricultural and Food Chemistry, 51*, 4782–4787.